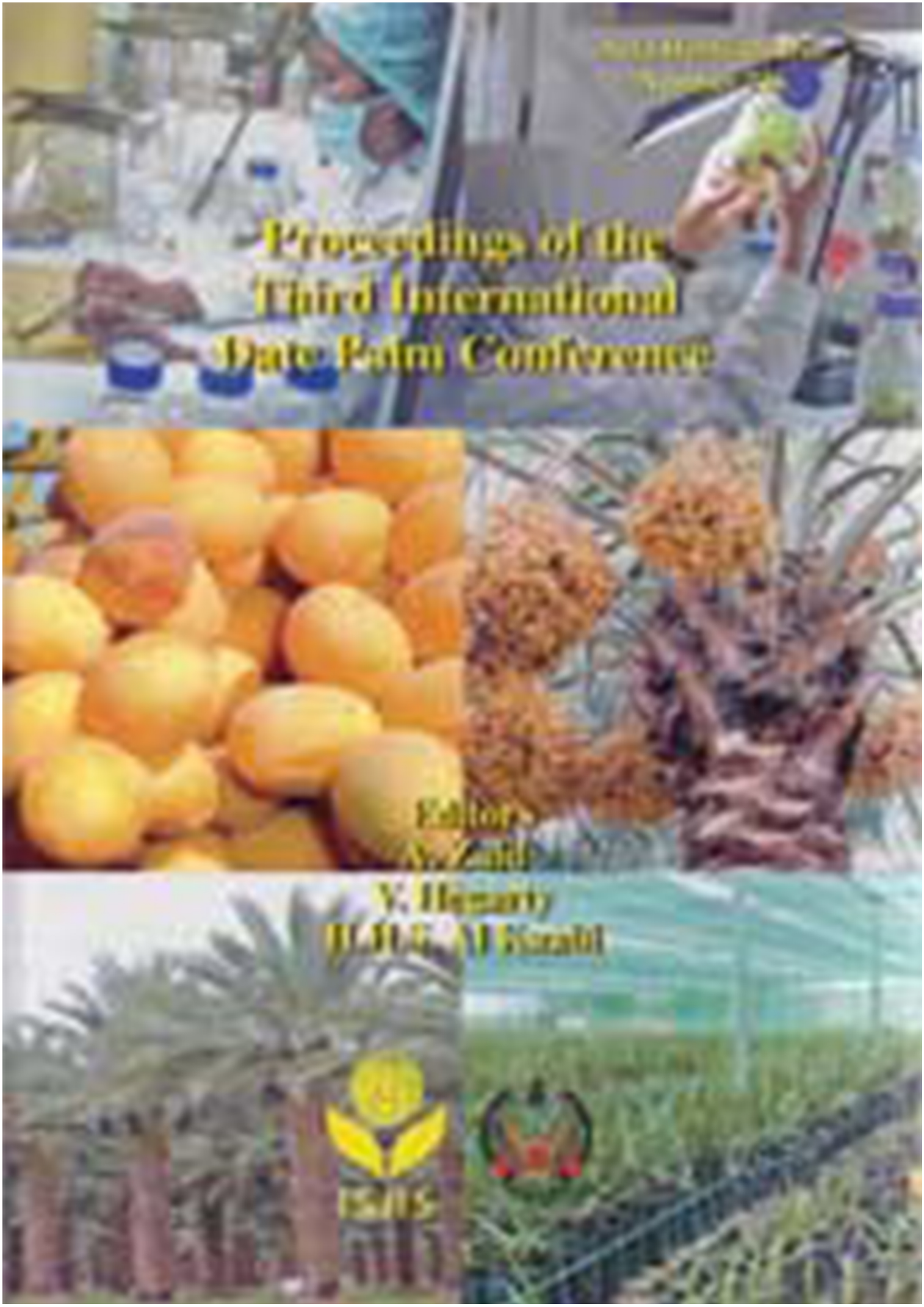
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Past and Present Date Varieties in the United States

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Abstract

A study was carried out to identify past and present commercial date varieties in the United States. The date industry began in the 1890s with the first importation of named variety offshoots from North Africa and the Middle East. From 1890 to 1929, the U.S. Department of Agriculture imported 1,076 lots of date offshoots containing about 20,000 individuals of standard date varieties. Other introductions were made by private growers. Medjool was one of the last varieties to be introduced, in 1927. Imported offshoots were grown primarily in Arizona and California. Commercial production commenced in 1912 with the first harvesting and marketing of Deglet Noor fruits. California emerged as the major producing state owing to more favorable climatic conditions. In 1950, Nixon described 160 imported date varieties in the United States. The current study found only 16 imported commercial varieties, originating from four countries: Algeria, Egypt, Iraq and Morocco. Most prominent are Barhee, Deglet Noor, Halawy, Khadrawy, Medjool, Thoory and Zahidi. In 1955 Nixon also described 40 American varieties that had been selected and reproduced from the imported varieties; since that time a few more American varieties have been added. On a small scale, nine American varieties currently are grown commercially, the three most important are Empress and Honey, both derived from Thoory; and Blond Beauty, derived from Deglet Noor. Date palm germplasm is preserved in four collections, two in California and two in Arizona; together they include nearly all of the current imported commercial varieties, but are inadequate for the American commercial varieties. Date production in 2003 amounted to 16,662 mt on a total of 2,145 ha. Deglet Noor is the most important commercial date variety, representing about 70-75% of production; Medjool is second with 20-25%; all other commercial varieties account for only a few percent of total production. From a broad base of a total of about 200 imported and American varieties, the United States date industry is now narrowly focused on two primary and 23 secondary commercial varieties.

EARLY HISTORY AND DATE OFFSHOOT IMPORTATIONS

What is now California and Arizona was part of Mexico when the first date palms were grown from seeds planted at Spanish religious missions established beginning in 1769. Mission gardens in warm, dry locations produced acceptable date fruits and provided an example to settlers to cultivate the palm. Many seedling dates were planted in both California and Arizona after the Mexican War ended in 1848, when control of the area passed to the United States. A leading book on California fruit culture described growing dates from seed (Wickson Hela, 1889). Late in the 19th century, it was recognized that it would be necessary to import offshoots of named, standard, commercial varieties to establish a successful commercial date industry. The United States Department of Agriculture took the lead and made an initial importation of offshoots in 1890/1891, which was a failure, but interest in a date industry persisted and led to the first successful importation of Deglet Noor and Rhars from Algeria in 1899/1900. There followed a series of government expeditions to obtain offshoots of numerous varieties from Algeria, Baluchistan (Pakistan), Egypt, Iraq and Tunisia. Among the noteworthy varieties introduced were Areshty, Halawy, Hayany, Khadrawy, Kustawy, Saidy and Zahidi. Government agricultural experiment stations were established in Tempe, Arizona, near Phoenix, and Mecca in the Coachella Valley, California, to test the imported varieties. The last major introduction occurred in 1927 when Medjool offshoots were

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brought in from Morocco. Over the period 1890-1929, the USDA by itself imported 1,076 lots of date offshoots, numbering some 20,000 individuals, and representing 160 standard varieties (Nixon, 1950, 1971; Popenoe, 1913; Swingle, 1904; Toumey, 1898).

Commercial importation of date offshoots paralleled the USDA program. From 1903 to 1922, about 15 private expeditions were sent to Algeria, Egypt and Iraq to bring in additional quantities of offshoots of those varieties that had been identified as showing promise. These efforts brought in about 43,320 date offshoots to California and Arizona, and permitted a more rapid expansion of the fledgling industry (Nixon, 1950, 1971).

COMMERCIAL DATE VARIETIES

The United States date production currently is focused on two geographic areas. The Coachella Valley, California, Riverside County, which includes the City of Indio, is preeminent. It is the center for Deglet Noor growing, as well as most of the other lesser commercial varieties. The second focus of production is in fact two adjoining areas separated by the Colorado River: the Bard Valley, Imperial County, California and Yuma County Arizona. Formerly, the area of Tempe, Arizona, now a suburb of Phoenix, was an early center for dates, and many of the imported varieties were first tested there. It once had a small commercial production. However, climatic conditions in that area are less than ideal and date growing became most prominent in California.

Date growers in California and Arizona were surveyed to determine the relative prominence of particular date varieties in overall production. This was necessary because date production statistics currently are not reported by variety. Additional information came from published sources (Johnson et al., 2002; Karp, 2002).

The criterion used to designate a commercial variety was that it was offered for retail sale by any means, including by the grower, without regard for the quantity marketed. The intent was to capture all varieties in the industry to provide a full picture of the varietal diversity and to serve as a basis for assessing germplasm resources.

Commercial date varieties can be broken down into three groups: major imported commercial varieties, minor imported commercial varieties and American commercial varieties, all of which are minor.

Major Imported Commercial Varieties

Together, the Deglet Noor, Medjool, Barhee and Zahidi varieties account for more than 90% of the United States date production.

1. Deglet Noor. This is the most widely planted and commercially important date variety in the United States. It originated in Algeria and was first introduced in 1900, initially to Arizona then to California. It was soon evident that Deglet Noor produced better quality fruits in California and demand for offshoots led to several large importations in the period 1911-1921. At present, Deglet Noor accounts for about 70-75% of total production. The semidry brown fruits have an excellent flavor and ripen late. Entire fruit bunches may be harvested resulting in a saving of labor costs. The fruit's firm texture minimizes damage during packing, handling and storage. This is the only commercial variety grown that is pitted mechanically. Palms in full production yield 91-136 kg per season. Deglet Noor produces 8-12 offshoots per palm. The center of Deglet Noor production is the Coachella Valley, California.

2. Medjool. A late introduction from Morocco in 1927, the large size, soft flesh and attractive appearance of the fruit has made it very popular in recent decades among growers and consumers. Medjools command the highest market prices, which helps to recover the additional production costs. The number of inflorescences, which may reach 30, is reduced to 22, evenly spaced around the crown. As fruits develop, they are thinned by hand, removing entire strands and individual fruits, to allow maximum fruit size and superior quality. Galvanized spreader rings are inserted into the bunch before fruits reach the *khalal* stage to improve ventilation and reduce checking and blacknose. Typically growers also place a mesh bag over the bunch to prevent bird damage. Fruits are harvested by hand as they ripen. Medjool production represents about 20-25% of the

United States total. Fruit yields range from 68-91 kg per season. Medjool is a prolific offshoot producer. Bard Valley, California and nearby Yuma, Arizona represent the chief Medjool production area, which has optimal climatic conditions for the variety.

3. Barhee. This soft date is unusual because of its unique fruit which has excellent eating qualities in the *khalal*, *rutab* and *tamar* stages; in the ripening process changing color from a brilliant yellow to amber and finally to a reddish brown. The fruit is crisp and only slightly astringent in the *khalal* stage; becoming ever softer as it ripens further until is almost liquid, with a rich, pleasant flavor. It is described as ripening late, but that is in reference to the *tamar* stage. Introduced from Iraq in 1913 Barhee dates are not widely known in the United States, but are found in season (late August into September) in Middle Eastern grocery markets, sold in the *khalal* stage. Some growers in the Coachella Valley, where Barhee cultivation is concentrated, sell by mail order to retail customers. *Khalal* fruits are harvested by cutting bunches, leaving the fruits attached. It is estimated that Barhee represents about 1% of total date production. Barhee is a high-yielding variety, averaging 113-136 kg per season. It is not a prolific offshoot producer, seldom having more than six to eight per palm.

4. Zahidi. A semidry date, Zahidi was introduced from northern Iraq in 1913. The reddish brown fruits ripen in midseason. Harvesting of entire bunches is done when fruits are about three-quarters ripe, finishing the less mature fruits in maturation chambers. Zahidi accounts for about 1% of total date fruit production. The Zahidi palm has a characteristic compact crown and is vigorous and hardy, yielding 91-136 kg of fruit per season; it is a prolific offshoot producer, 15-25. It is most commonly grown in the Coachella Valley.

Minor Imported Commercial Varieties

Table 1 lists the 12 imported varieties which each account for some minimal commercial production. Most widely grown are Halawy and Khadrawy. In some instances the variety is represented by only a few trees maintained by a particular grower for apparently historic reasons, rather than for any future development plans. The overall industry trend toward specializing in only a few varieties does not bode well for the future of minor varieties. The most significant obstacle to expanded growth and production of minor varieties is the need to educate both consumers and wholesalers.

American Commercial Varieties

Several California and Arizona date growers maintain a few of the 40 American varieties (Table 2), originally described by Nixon (1955). Typically, the number of bearing trees is small and fruit production is low. Shields Date Garden in Indio is an exception for it represents the largest grower of American varieties, with their exclusive Blond Beauty and Brunette Beauty dates, having 433 and 153 producing trees respectively. The company has built and maintained their reputation through the uniqueness of these two American varieties.

It is noteworthy that all of the American varieties listed in Table 2 produce soft fruits, which are preferred by domestic consumers. Most of the noncommercial American varieties described by Nixon (1955) have been lost.

CURRENT PRODUCTION LEVELS

In 2003, date production in California amounted to 16,662 mt on an area of 2,145 ha. Riverside and Imperial counties account for nearly all dates grown in California. Arizona's production is difficult to determine because dates are a relatively minor crop in the state and an indeterminable amount of the production in Yuma County is transported the short distance across the Colorado River for processing in Bard; several growers have orchards in both Bard and Yuma. The significant expansion of date production in the United States is taking place in Bard and Yuma, and most of the new areas are for Medjool production.

GERMPLASM RESOURCES

Date palm germplasm exists in four collections in the United States (Table 3), which represents an essential genetic resource for the date industry. Altogether, the four collections include all of the imported commercial varieties with the exception of Zagloul. In addition, 14 currently noncommercial varieties are represented. As for commercial American varieties, the collections lack Blond Beauty, Brunette Beauty and McGill's No. 1 varieties, but include Haziz and Peggy Ann, which are not commercial.

CONCLUSION

Commercial date growing in the United States began with the importation of about 160 different varieties, chiefly from North Africa and the Middle East.

In addition, 40 American varieties were described. Despite this broad diversity, the date industry today is quite narrowly focused on two primary commercial varieties (Deglet Noor and Medjool) and 23 decidedly minor imported and American varieties. Over the 20th century, about 75% of the date varieties once in the United States have been lost.

In recent years, the industry trend has been to cultivate Medjools in new date areas, and to promote expanded markets for what has been described as the finest date in the world. The diminution of varietal diversity in the United States date industry carries with it the risk of serious losses should a particular major variety be stricken with a disease or insect plague. Crop diversity is one of the best forms of crop insurance, something the date industry should consider.

To conclude on a positive note, a recent article proposes that growers consider promoting and marketing a wider number of dates at the *khalal* stage (Nave, 2005). In addition to the familiar Barhee, the article suggests that the imported varieties Halawy, Hayany, Maktoom, Saidy, Samany and Zagloul; and American varieties Desert Gem, Mariana (both undescribed) and Tabarzal have similar potential. The challenge is to induce consumers to sample and acquire a taste for fresh, crisp date fruits. Developing a market for these *khalal* dates could provide an economic incentive for growers to diversify the date varieties they grow.

Note: This article is based on materials gathered for a forthcoming book:
Hodel, D.R. and Johnson, D.V. Growing Imported and American Varieties of Dates (*Phoenix dactylifera*) in the United States, Based on the Work of Roy W. Nixon. University of California Division of Agriculture and Natural Resources, forthcoming 2006.

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Tables

Table 1. Minor imported commercial varieties.

Variety	Country of Origin	Fruit Characteristics (color at *tamar* stage)	Notes
Amir Hajj, Amir Haj, Mirhage	Iraq	Soft, reddish brown, but relatively small fruit.	Ripen* midseason; vigorous grower and good yields.
Dayri, Dairee, Dairi	Iraq	Semidry, pale purplish brown.	Ripen midseason; very hardy variety.
Halawy, Hallawi, Hellawi	Iraq	Soft, golden brown.	Ripen early; good yield prolific offshoot producer.
Hayany, Hayani	Egypt	Soft, nearly black; usually sold fresh.	Ripen early, but do not cure well.
Iteema, Itima, Itime, Ytima, Yatimeh	Algeria	Soft, reddish brown.	Ripen midseason; fruits susceptible to checking.
Khadrawy, Khadrawi, Khadhrawi, Khudrawee	Iraq	Soft, reddish brown.	Ripen early; fruits cure and keep well.
Khisab, Khasab	Iraq	Soft, nearly black.	Ripen very late; fruit mediocre.
Maktoom, Maktum	Iraq	Soft, reddish brown, fairly large.	Ripen late; fruits very resistant to checking and splitting.
Samany, Samani, Samiani, Rashedi	Egypt	Soft, brown, large.	Ripen midseason; fruits sour easily.
Sayer, Sayir, Saiar, Sai, Ista'amran	Iraq	Soft, reddish brown.	Ripen midseason; peduncles tend to break.
Thoory, Thusi, Tsuri, Thauri	Algeria	Dry, light brown.	Ripen late; vigorous and robust palm.
Zagloul, Zaglul	Egypt	Soft, nearly black, large.	Ripen midseason; fruits subject to checking and souring. Not a promising variety.

* Time of ripening is for the Coachella Valley and Imperial Valley, California; and Yuma, Arizona. Early = ripening about 15 August and lasting 6-10 weeks. Midseason = ripening about 1 September and lasting 6-10 weeks. Late = ripening about 15 September and lasting 8-12 weeks.
Source: Nixon, 1950.

Table 2. American commercial varieties.

Variety	**Origin**	**Fruit Characteristics (color at *tamar* stage)**	**Notes**
Abada	Unknown; palm resembles Deglet Noor	Soft, black; subject to checking.	Early ripening; breeding potential.
Blond Beauty	Deglet Noor	Soft, reddish brown.	Midseason ripening; variety exclusive to single grower.
Brunette Beauty	Deglet Noor	Soft, nearly black.	Late ripening; variety exclusive to single grower.
Empress	Thoory	Soft, reddish brown, large.	Midseason ripening; from same seed lot as T-R; potential for breeding.
Honey	Deglet Noor	Soft, amber colored.	Broad midseason ripening, beginning before Empress and continuing longer.
McGill's No. 1	Unknown; palm resembles Kustawy.	Soft, reddish brown.	Season unknown; fruits resemble Khalasa.
Sphinx; Black Sphinx	Unknown; possibly Hayany.	Soft, black.	Late ripening; high yielding and prolific offshoot producer; slow vertical growth.
Tabarzal	Unknown	Very soft, reddish brown, large.	Early ripening; after Khadrawy.
T-R	Thoory	Soft, melting, reddish brown.	Early ripening; from same seed lot as Empress.

Source: Nixon, 1955.

Table 3. Date germplasm resources (X = female palm present in the collection).

Imported Varieties / Origin	1. Thermal, California	2. Brawley, California	3. Tempe, Arizona	4. Yuma, Arizona
Amir Hajj, Iraq	X	X		
Ashrasi, Iraq	X	X	X	
Badrayah, Iraq	X	X	X	
Barhee, Iraq	X	X	X	X
Bentamoda, Sudan	X		X	
Braim, Iraq			X	
Dayri, Iraq	X	X	X	
Deglet Beida, Algeria	X		X	
Deglet Noor, Algeria	X	X	X	X
Halawy, Iraq	X	X	X	
Hayany, Egypt			X	X
Hilali, Algeria	X	X	X	
Horra, Algeria	X		X	
Iteema, Algeria			X	
Khadrawy, Iraq	X	X	X	X
Khalasa, Saudi Arabia	X	X	X	
Khir, Saudi Arabia	X	X	X	
Khisab, Iraq	X		X	
Maktoom, Iraq			X	
Medjool, Morocco	X	X	X	X
Menakher, Tunisia			X	
Rhars, Algeria			X	
Saidy, Egypt	X		X	
Samany, Egypt	X			
Sayer, Iraq	X	X	X	X
Tadala, Algeria			X	
Tazizoot, Algeria		X		
Thoory, Algeria	X	X	X	X
Zahidi, Iraq	X	X	X	
American Varieties				
Abada	X	X	X	
Empress	X			
Haziz	X	X	X	
Honey	X		X	
Peggy Ann			X	
Sphinx	X		X	X
Tabarzal	X		X	
T-R	X			

1, 2. USDA-ARS National Germplasm Repository.

3. Arizona State University.

4. Yuma Mesa Agricultural Center, Somerton AZ.

Source: compiled from records of the collections.

An Overview of the Changing Date Industry in the United States

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Abstract

The commercial date industry in the United States is located primarily in the Sonoran Desert of southeast California and southwest Arizona. The industry comprises about 3800 hectares, of which 78% is found in California and the rest in Arizona. While date palms were introduced to the United States by the Spaniards, small quantities were imported for experimental purposes beginning in the late 1800's, and commercial quantities were imported in the early 1900's. During this period, most imported offshoots originated from Algeria, Egypt, Tunisia and Iraq. Important varieties that were imported include 'Deglet Noor', 'Khadrawi', 'Zahidi', 'Hayany' and 'Halawy'. More recently, the 'Medjool' from Morocco was introduced. This variety is becoming increasingly popular because of its large size and high sugar content. Date palm operations are moving from areas that are under pressure from urbanization to more remote locales. Low volume drip and microjet irrigation is beginning to replace the tradition flood and basin irrigation methods. Some dates are produced using organic methods because of consumer demand. Farm operations begin in January when the trees are dethorned. Operations that occur later in the year include pollination, training the fruit arms, strand thinning, fruit thinning, supporting the arms, spreading the strands, bagging the developing fruit and harvest. Individual growers are increasingly forming cooperatives to pack the fruit at a centrally located packinghouse. At the house, fruit are graded, then packed, and placed in storage until shipment. Dates from the region are marketed by individual growers, and by the grower cooperatives, and sold to customers around the world. Palm trees are also sold for landscape purposes to customers across the United States.

INTRODUCTION

The United States date industry is facing many changes as we move into the 21st century. Among these are changes in the location and area of date plantings, changes in the popularity of the primary cultivars grown, changes in production practices, changing uses for the trees themselves and changing markets and marketing of the fruit.

LOCATION OF THE INDUSTRY

Date palms may be grown in several areas in the US, including coastal Georgia and South Carolina, the Florida peninsula, the coastal regions of the Gulf of Mexico, the lower Rio Grande Valley, southern Arizona, southern Nevada, and California as far north as San Francisco. Additional trees exist elsewhere in protected locations. However, because of insufficient heat necessary to mature the fruit and/or the incidence of high humidity and rain during the period of fruit maturation, most of these locations are unsuitable for a commercial date industry. Only Southwest Arizona and the deserts of Southern California provide the ideal locations to grow date fruit.

Virtually all the industry exists within the Sonoran Desert, an area characterized by high summer temperatures and low rainfall. Even within this desert, there are areas where it is too cool to grow dates. For example, summer temperatures in Tucson, Arizona in July reach an average of 37°C, and there are 1050 mm of rain annually, much of it in the late summer. These conditions make the city unsuitable for a commercial date industry. However, in the lower desert, these climactic conditions are met.

The industry exists today in three zones. The oldest zone is within Riverside County, and is found in the Coachella Valley. This Valley contains the city of Palm Springs and several other areas that are popular winter vacation spots. The second zone is in far Southeast California, in Imperial County, around the town of Bard, California. This second zone is on the west side of the Colorado River, across from Yuma Arizona. Also included in this area are a few date orchards on the south side of the Salton Sea. The third zone is on the east side of the river, surrounding the city of Yuma, Arizona. Also included in this area are a few groves as far east as 150 km east of Yuma.

ORIGIN OF THE INDUSTRY

Date seeds were first brought to the United States by Spaniards in the 1700's as they established a series of settlements in Southern California and Arizona (Tate and Hilgeman, 1971). Date offshoots were planted in the Yuma area as early as the 1860's, but in the 1890's the United States Department of Agriculture (USDA) imported 75 palm offshoots from Egypt and Algeria and planted them in Arizona, California and New Mexico. All died later. Undaunted, the USDA imported the first 'Deglet Noor' offshoots from Algeria, and planted them in Tempe, Arizona in 1900. Following this, there were many importations of offshoots by private individuals, beginning in 1903. The year 1913 saw the importation of 20,000 offshoots by private individuals. By 1922, the industry in Coachella/Riverside County was well established. Another important milestone occurred in 1927 when just 11 'Medjool' offshoots were imported by the USDA from Morocco, and planted in southern Nevada. The final importation of date offshoots by the USDA occurred in 1929. Commercial plantings were established in Arizona in the early 1900's, but disappeared slowly over about 50 years due to urbanization. Date orchards were first planted in Bard/Imperial County in 1957 (D. Mansheim, pers.comm.).

CURRENT AND FUTURE EXPANSE OF THE INDUSTRY

Today, the industry comprises about 3800 hectares. Coachella/Riverside County includes 2500 hectares, about a five-fold increase since 1925 (Riverside County Agriculture Commissioner, 2005). Bard/Imperial County includes about 500 ha (Imperial County Agriculture Commissioner, 2005), and Arizona comprises about 800 ha (G. Nuñez, pers. comm.), which is a sharp increase since 2000 (Fig. 1). Today, Coachella/Riverside County boasts 66% of the entire area planted to dates in the US, while Bard/Imperial County comprises 12%, and Arizona includes 22%.

These areas are not likely to remain static for long. There are tremendous urbanization pressures affecting all farming in the Coachella Valley. Population projections for the cities of Coachella and La Quinta, both very close to many date orchards in the area, show a potential 50% increase over the next 20 years (Southern California Association of Governments, 2004). As a result, land costs today range from USD $150,000 to $250,000 per hectare, and much of the land has been purchased for current or future development (P. Mauk, pers. comm.). Additionally, there is little additional land in the area that is suitable for date production due to lack of available water. Thus, some old date orchards are being removed, and the trees used for landscape purposes as farmers sell the land to developers. There is no doubt that the area planted to dates will decrease in the Coachella/Riverside County area over the next ten years.

Production area is decreasing in Bard/Imperial County for different reasons. There is some urbanization pressure, and land costs are increasing, but in most cases, the orchards are getting old, and trees are being sold for landscaping. Also, taxes and workmen's compensation insurance costs in California are high, compared with neighboring Arizona (G. Vandevoort, pers. comm.).

Therefore, most new plantings are being established in Arizona. All of these plantings are 'Medjool'. Again, urbanization and population increase threaten established groves in some areas, but there is ample, relatively inexpensive land that is flat, and water quality and quantity is good. Although most dates have historically been planted in silty or clay soil near rivers, these new plantings in Arizona (over 600 ha) are in the sand.

Rather than using the traditional flood irrigation, these plantings use water from wells, and employ pressurized drip systems.

Another area of increased planting is Mexico. Some producers from the Coachella Valley are moving their operations to the area surrounding Mexicali, Mexico. This area affords ample land, good quality water and inexpensive labor. The only threat to these lands is political and economic instability. It is not easy for foreigners to own land in Mexico; most farms are leased. These leases are occasionally broken by the governmental authorities in Mexico (D. Manscheim, pers. comm.).

CULTIVARS AND PRODUCTION PRACTICES OF THE INDUSTRY

Another area of change is in the cultivars used. Coachella/Riverside County has historically planted the 'Deglet Noor', a cultivar which still comprises about 65% of the total. Only recent plantings have included the 'Medjool' (about 25%). Quality of 'Medjool' is not as high here as in Bard/Imperial County and Arizona, there is more skin separation problems with 'Medjool' grown here. Other varieties comprise 10% of the dates planted here, including 'Amir Hajj', 'Barhee', 'Empress', 'Halawy', 'Khadrawy', 'Sayer', and 'Zahidi' (D. Nelson, pers. comm.).

More than 95% of Bard/Imperial County and Arizona production is the 'Medjool'. Less than 5% of the production is 'Barhee', 'Deglet Noor', 'Halawy', 'Khadrawy', 'Sayer' and 'Zahidi'. 'Medjool' dates must be dethorned, pollinated, trained, thinned, and harvested like all other cultivars, but some of these operations are somewhat different. 'Medjool' dates are thinned only to 20 to 22 fruiting arms per tree, and there are 30 to 40 strands per arm, and 13 to 20 fruit per strand. Fruit clusters are bagged with nylon bags, rather than paper, to protect the fruit from birds, insects and rain, and to provide warmth to hasten fruit ripening. Fruit are harvested several times, rather than just once, and only the ripe fruit that falls into the bottom of the bag when the cluster is shaken is removed. Some trees can produce 100 kg of fruit (Paulsen, 2005)

Once harvested, 'Medjool' dates are dried to 24 to 28% moisture content, rather than dried on the tree as are the 'Deglet Noor' dates. Thus the 'Medjool' becomes a much more desirable product for the consumer, with a greater cost and return for the grower. However, because of the cost of 'Medjool', the purchase of them is considered a luxury and will decrease if the economy of the purchasing country is poor.

Another change is that several date producers in the United States are growing their dates organically. Fertilizers provided to the trees are from organic sources and no pesticides are used. Organic dates from all areas are certified by the California Certified Organic Farmers (CCOF), or the National Organic Standard (NOS). Some producers are working toward getting their fruit Eurepgap certified. Organic fruit commands a USD $0.25 price premium per kilogram. (D. Nelson, pers. comm.).

PACKING AND MARKETING OF THE DATES

Bard/Imperial County and Arizona growers have formed a grower's cooperative: The Bard Valley Medjool Date Grower's Association. As a group, they have developed a series of grades that are more stringent than those of the USDA. "Jumbo" dates are of the highest quality and have between 35 and 42 dates per kg. "Large" dates are of the same high quality, but have between 43 and 51 dates per kg. "Fancy" dates can be of any size, but must show only minor blemishes, slight skin separation, and must be moist. "Choice" dates can be of any size, and can show some dryness, skin separation, and may be broken. (D. Nelson, pers. comm.).

While all Coachella/Riverside County growers continue to pack their dates individually, the Bard/Imperial County and Arizona growers pack most of their fruit through the Datepac LLC packinghouse. The packinghouse provides the advantages of reduced labor costs, improved product uniformity, and a year-round supply of fruit for the purchaser. Datepac is also actively investigating automatic sorting technology for dates.

Because of the changes, there are research needs to be met. Growers would like to see improved fruit set, particularly for young trees. Improving yield is also important, and

a comprehensive study of the degree of fruit thinning and its effect on yield is needed. Use of plant growth regulators for thinning and improving fruit size should be studied. Fertility and irrigation rates and timing, particularly on sandy soils using drip irrigation should be investigated. Also, investigations of the effects of pre- and post-harvest cultural treatments on skin separation are needed.

Dates from the United States are marketed around the world (Fig. 2). The most important market is Canada, followed by various Northern and Central European countries, Australia, the United Kingdom and Mexico (National Agricultural Statistics Service, 2005). About 55% of the dates produced in the United States are sold within the US or in Canada. Since 1997, production of dates in the US has stayed fairly constant; about 20 million tons annually (Fig. 3). The value of the crop has risen as producers are paid more for their product. Producers expect to see increasing competition within those markets from dates from other countries.

DATE PALMS AS LANDSCAPE PLANTS

Date palms are becoming increasingly popular for use as landscape plants in the United States. Palms now surround new shopping malls, hotels and housing developments. Most of these are taken from commercial palm orchards in California and Arizona. Palms are first wrapped so that no offshoots can form, then they are dug, the fronds are tied, and they are loaded onto flatbed trucks and transported to the buyer. Upon arrival, they are set into the ground, each with their own source of irrigation water. Often, each palm is located next to a light, so the tree can be illuminated at night. Fronds are untied once trees have started growing in their new location.

Older trees are likely to be sold if they have grown too tall to harvest. Younger trees can be sold if “the price is right”. Some growers are now planting orchards at reduced spacing with the intention of harvesting the fruit while the trees are young, then selling them when they reach the appropriate height. The price for a mature palm can reach USD $600.00 per meter of height (G. Vandevoort, pers. comm.). In 2004, 40 hectares of trees were sold in Bard/Imperial County, and additional trees were sold in Coachella/Riverside County.

CONCLUSION

The date palm industry in the US is changing. Urbanization is forcing production to move, the main cultivars are changing, cultural and packing practices are evolving, new research is needed, markets are changing and now date palms are being used for landscaping purposes. Through all this, the US date industry will continue to produce and market a quality product.

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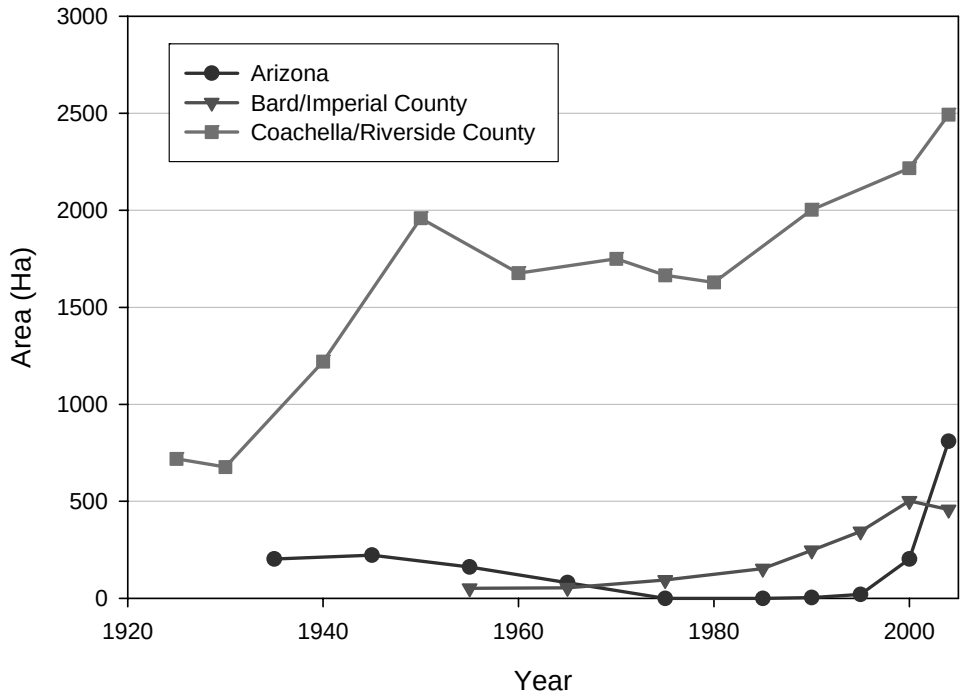

Fig. 1. Area of the three date palm production zones in the United States, beginning in 1925. Source: Riverside County Agriculture Commissioner, 2005; Imperial County Agriculture Commissioner, 2005.

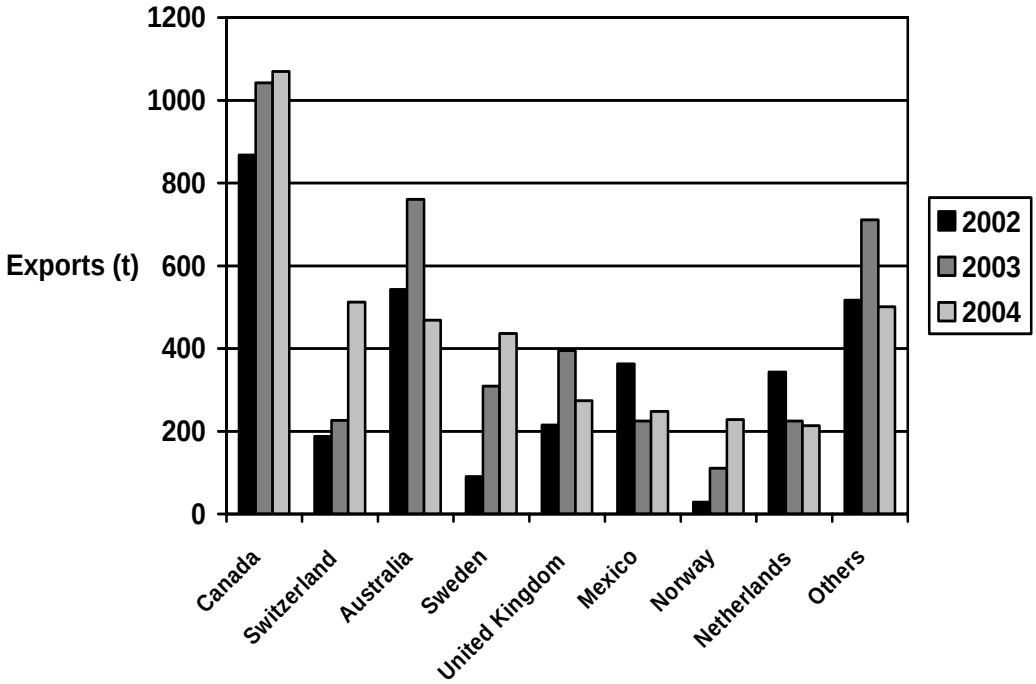

Fig. 2. Exports of date fruit to selected markets from 2002 through 2004. Source National Agricultural Statistics Service, 2005.

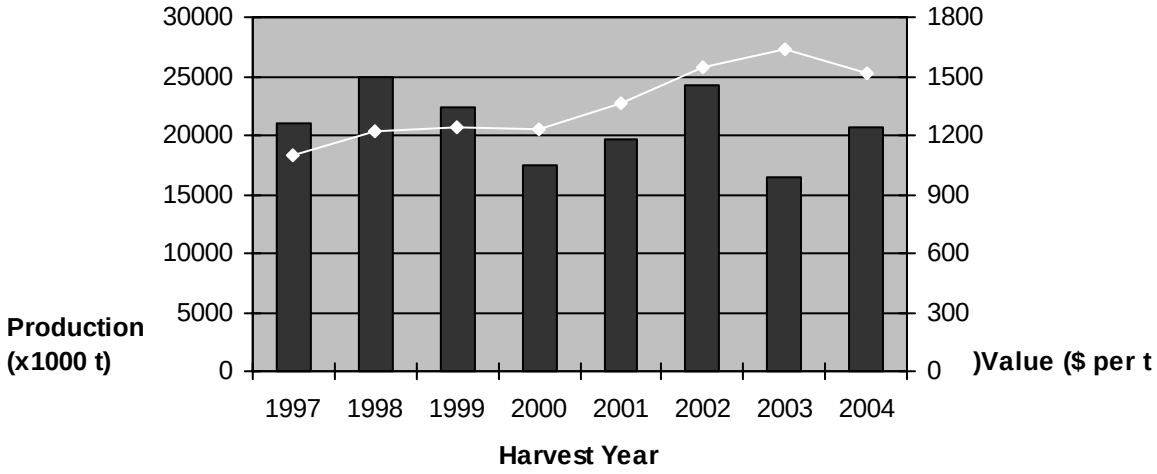

Fig. 3. Annual production (bars) and value (line) of dates produced in the United States from 1997 through 2004. Source: National Agriculture Statistics Service, 2005.

The South African Date Palm Industry - Strengths and Weaknesses

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Keywords: *Phoenix dactylifera*

Abstract

The date palm industry in the Republic of South Africa is a new comer to date palm production. It is geographically far from the known date palm production areas of the world. For this reason, it has been isolated from the technology available and the markets. Will the date palm industry survive in the Republic of South Africa? The strengths and weaknesses of date palm production and marketing are highlighted and discussed under the following sections: Location and Growing Areas, Cultivar Selection, Climatic Conditions, Labour versus Mechanization, Production and Harvesting, Marketing and Economic Viability and Sustainability.

INTRODUCTION

The first date palms (*Phoenix dactylifera* L.) in the Republic of South Africa were planted from seed only about a century ago. The first commercial cultivar plantation was established 27 years ago in the Northern Cape region from Medjool offshoots shipped in from Yuma in the United States. The date palm industry in South Africa is therefore still young.

Although South Africa is far from the main date palm growing areas in the world, its location in the Southern Hemisphere gives it an advantage in producing out-of-season fresh fruit. The production of date fruit in South Africa is still not sufficient to supply the local demand. There is therefore potential for expansion. However, there are still some problems facing the local growers.

To date only a few studies have been made on the date palm industry in South Africa and there are few references from which to draw data (McCubbin, 2003). This study therefore aimed to conduct a current survey on the date palm industry in the Republic of South Africa with specific emphasis on the weaknesses and strengths.

MATERIALS AND METHODS

The date palm growing areas are divided into 2 main regions: the Northern Cape and the Limpopo (Fig. 1). Data was collected from 2 date palm growers in the Northern Cape region and 2 date palm growers in the Limpopo region. The data collected covered problems associated with:

a) Cultivar selection
b) Climatic conditions
c) Pests and diseases
d) Production and harvesting
e) Labour and mechanization
f) Marketing
g) Planting schedules for future

RESULTS AND DISCUSSION

Northern Cape Region

1. Location and Climatic Conditions. The Northern Cape region is situated far from the export ports: approx 800 km from Durban to the east and approx 500 km from Cape Town in the south. Johannesburg, from where the majority of goods are airfreighted, is also more than 500 km away. Therefore, whether for export or for local markets, the transport costs are significant, especially taking into consideration the rising oil prices

over the past year. This is a drawback for this region.

On the other hand, the climatic conditions are ideal for the production of good quality date palm fruit, especially Medjool. The date palm plantations in this region draw their water mainly from the Orange River. The rainfall is in winter and thus not during the fruiting season. Conditions are hot and dry and a light brown even colouration is obtained with no or little loose skin on the fruit. The higher humidity levels in some countries cause the skin on the fruit to loosen. Good export prices are obtained for the high quality fruit.

About 2 years ago there were more than 10 commercial date palm growers on farms near Pofadder, Upington, Henkries, Kakamas, Grobelaarshoop and as far as Ceres. Due to financial constraints and various hardships, many farmers and companies have been taken over or are now jointly managed by one large company in the region that exports not only dates, but grapes and other agricultural produce. The expertise obtained by this large company has been used to manage the smaller plantations on a profitable basis, while at the same time utilizing the packing facilities at one central site. About 90% of the commercial date palm plantations in this region is now owned or managed by this one company.

2. Cultivar Selection. The highest percentage of plantings is of the Medjool variety (75%) (Fig. 2). This variety was chosen for its large fruit and export potential. Barhee (15%) was chosen due its popularity and potential for harvest in both the fresh and dry state. A small percentage (5%) of seedling palms and other varieties such as Khadrawy, Deglet Noor and Khalas still remain. These cultivars and the seedlings are gradually being replaced by Medjool off-shoots as they become available.

3. Production and Harvesting. With the phasing out of seedling plants, which are being replaced with Medjool off-shoots, and some new plantings of Medjool tissue culture plants by other farmers, there is still a large percentage of palms that are not yet in full production. The largest date palm grower manages over 200 ha of date palms. There is approximately another 85 ha of date palms grown in the region, totaling 285 ha in all. In the previous year approximately 1000 tons of Medjool fruit and 300 tons of Barhee were harvested. A major strength in this area is that there is little or no disease found. Pollen is harvested from over 450 male palms for pollen distribution throughout the country and good pollination practices are observed. Three mechanical harvesters are used for the harvesting of fruit from tall date palms.

4. Marketing. The marketing of date palm fruit is the main strength of this region as 90% of the date palm fruit produced here is marketed and packaged by one company. In addition, the company also markets fruit produced from a plantation in Namibia at Naute. The date palm fruit is sorted and graded and the best quality is exported. Approximately 60% of total Medjool fruit is exported and the remaining 40% is used for the local market. The Medjool fruit was exported to Europe this past year. The fruit is sold locally as edible loose dates packaged in punnets for supermarkets in three major cities. Although the export markets are still lucrative, profitability is not increasing. The stronger South African Rand and higher oil prices have lessened profitability.

South Africa still imports more than 250 tons of fruit each year and the local demand for fruit is growing as more people become familiar with the fresh fruit. Farmers do not store fruit until the next season as all fruit is sold within the year of production.

5. Planting Schedules for the Future. The main focus has been on the removal of cultivars other than Medjool and Barhee and replacing these with Medjool off-shoots. Off-shoots from their own plantation are preferred to introducing tissue cultured plants due to reports of off-types. However, new plantations of tissue culture plants have been established near Loriesfontein. Of the 3 date palm producing tissue culture laboratories that were operating in South Africa 5 years ago, none exists today. Plants have to be sourced from outside the country.

Limpopo Region

1. Climatic Conditions and Location. Temperatures in this region reach 38-40° C. The

Limpopo River provides enough water for the commercial plantations. This region has a summer rainfall where rain occurs from September to February. This normally corresponds with fruit set, resulting in rotting of the fruit. This is a major drawback for the region. The Limpopo region is about 400 km away from Johannesburg where the fruit is sold locally or can be exported. Governmental land claims in the region have not affected the date palm farms.

2. Cultivar Selection. Besides a small 2 ha trial block with various clones of tissue cultured plants planted 20 years ago, the date palms planted in this region are mainly of the Medjool variety. Forty hectares of date palms were established here. The choice of cultivar has been a weakness, as it ripens when the rains come. The high humidity causes the fruit to sour and ferment. A variety such as Barhee would have been preferable, but in a country where the Barhee fruit are not known, the marketing would have been difficult.

3. Production and Harvesting. Only one farm continues to harvest their fruit in this region. Less than 20 tons was harvested. The occurrence of the African Palm Weevil, *Rhynchophorus phoenicis* F. (Zaid et al., 1999) (Fig. 3) continues to destroy the date palms in the region and most farmers are removing their date palms. The black scorch disease was also found to be present here. Manual labour is used for the harvesting of fruit.

4. Marketing. As mentioned, there is very little produce in this region. The fruit obtained is specially dried in tobacco ovens and marketed locally in small punnets. No specialized packhouses have been set up for packaging and marketing.

5. Planting Schedule for the Future. Farmers in this region are not optimistic after years of soured fruits caused by rain. They do not intend to expand their plantings in the future.

CONCLUSIONS

Will the date palm industry in South Africa survive? It is quite clear that in the Limpopo region it will continue to be a struggle with the elements, and expansion will not occur unless technologies are developed to reduce the souring of fruit. Alternatively, as markets for cultivars that ripen during the Khalal stage, eg. Barhee, are explored and opened, this cultivar may be produced successfully in this region.

In the Northern Cape however, the industry has strengthened substantially and has grown to be a competitive enterprise in the export and local markets. Its strengths have been its location in the Southern Hemisphere and, through good management of one company, its high quality fruit for export. The centralization of packing through one packhouse has ensured that the quality is maintained. The one major company has promoted the use of its packing facilities to date palm farmers. Newcomers will need to erect good packhouses or be encouraged to tap into existing infrastructure and resources.

Date palms remain a long term investment which only a few can afford. Other lucrative agricultural crops such as grapes and pecans are also being planted in the region. But for those who have the capital and are willing to maintain the high quality standards, the date palm industry in South Africa looks likely to survive for many years to come.

ACKNOWLEDGEMENTS

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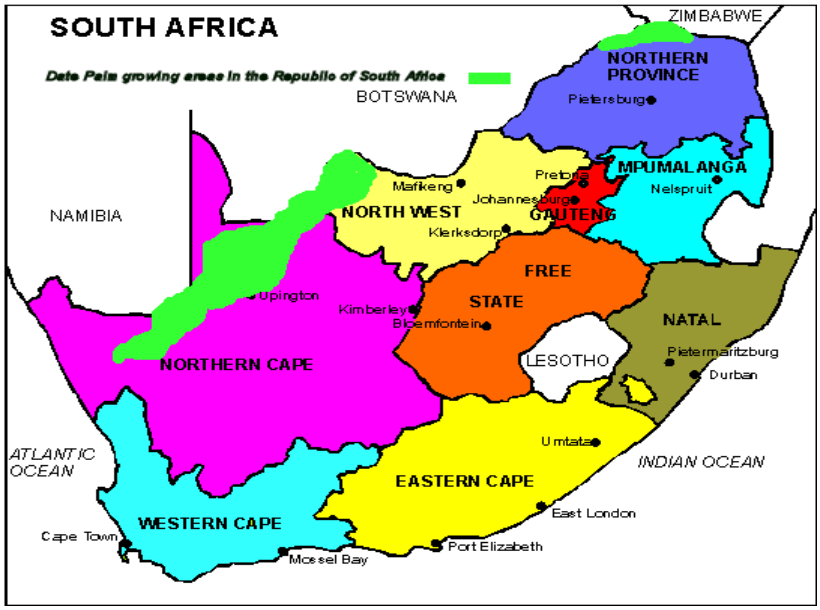

Fig. 1. Map of the Republic of South Africa depicting the two main date palm growing regions: Northern Cape and the Limpopo.

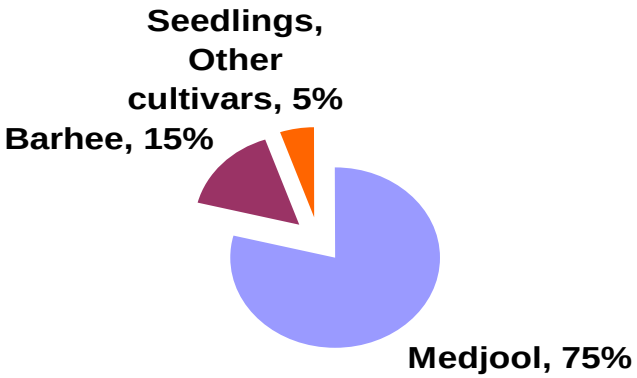

Fig. 2. Percentage date palm plantings per cultivar in the Northern Cape region.

Fig. 3. The African Palm Weevil, *Rynchophorus phoenicis* F. (Zaid et al., 1999).

The Effect of Climatic Conditions and Geographical Distribution on the Success of New Date Palm Varieties (*Phoenix dactylifera* L.) in The Gaza Strip

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Abstract

In 1998 four date palm varieties were introduced to the Gaza Strip: Hallawi, Zehedi, Ameri and Berhi. Varieties were randomly distributed over three geographic areas (North, Middle and South) to a number of farmers. Sites were selected after comprehensive study of official records to find common climatic conditions in the different locations in the Gaza strip. The readings included maximum and minimum temperatures and relative humidity. The average cumulative thermal units were then calculated from the beginning of May till the end of October. Also included were the average rain trends. Fruit set and production rates were recorded as well as the average production for each variety. The average cumulative thermal units during the period of 1990-2001 were 1792 thermal units. The data indicated that Gaza is suited to the introduction of semidry varieties. The period of fruiting and ripening goes from May until October when it is dry (no rain). This time is also characterized by a mild increase in relative humidity which facilitates increase in fruit setting as well as rate of production.

Rainfall in Gaza ranges from 200-400mm annually It increases in the north and decreases towards the south. There was a clear effect of climatic conditions as well as geographic location on the rate of fruit set of the introduced varieties. Al Hayani, the commonly grown variety, performed the best over the 3 regions wih an average fruit set of 87.6%. Al Hallawi had the highest off-shoot production (7-offshoots) compared to the other varieties. Alberhy produced the lowest number of offshoots and this was attributed to the varieties themselves. It was concluded from the study that the common climatic conditions in Gaza strip are suitable for planting new varieties along with Alhayani, the traditional variety. Also semidry varieties could be introduced. The possibility of success for the dry varieties under the conditions of middle and south districts would depend on specific treatment.

INTRODUCTION

Gaza lies on the eastern coast of the Mediterranean Sea. It has a total area of 363.06 square kilometers. Limited area and increasing population have made Gaza the most crowded place in the world. Population density is up to 2653 persons per square kilometer. Gaza lies north of the equator at a lattitude of 31.13 to 31.36° N. This is responsible for its good climate all year round.

The total area of historical Palestine is 27 009 square kilometers. However the suggested Palestinian state in Gaza and West Bank covers only 6209 square kilometers, which is equal to 22.95% of the area of historical Palestine: 21.6% West Bank and 1.35% Gaza Strip.

Geographic Location

1. Palm Trees. Palm trees are grown predominantly in the middle and southern areas of Gaza, where about 3000 "Donoms" are grown (The Donom equals 0.10 hectare). Ninety percent of these palm trees are Al Hayani species while the other 10% are species such as Bent Al Eish.

Palm tree cultivation has developed rapidly in recent years. About 10000 palm trees have been planted during the last 8 years. (Reality of Palm Trees in the Territory/ Albanna, Mofeed Fayez PARC).

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
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Climate is the most important factor and determines the areas for successful palm tree cultivation. In the summer certain degrees of temperature must be available (called effective heat units) to help physiological changes to take place in the fruits. On the other hand, the warmth of winter days helps the growth of young bunches and their early flowering (Chandler, 1950). It is noted that palm trees growng in areas where temperatures in the shade are less than 18°C do not produce fruits. Fruits are obtained only when the temperature is 25°C in the shade (Decandolle, 1855).

2. Humidity. A standard level of humidity is required during the red date stage (khelal) and the black date stage (rutab) because the fruit loses a lot of its moisture before reaching the dry date stage. A rise in atmospheric humidity leads to physiological disorders in the development of fruit, which leads to a delay in ripening and the appearance of some physiological diseases. Fruit may even fall, which would mean commercial loss.

3. Rain. Even with the availability of the required heat for ripening of fruit (Fathi Hussein et al., 1979, 1950), rainfall is usually considered a very important factor in the cultivation of palm trees.

Therefore, climate elements throughout the year must be well studied. Such studies are useful to predict the likelihood of successful cultivation of certain varieties of palm trees when introduced into another area or country. (Fathi et al., 1979)

It is known that certain varieties of palm tree require similar temperatures if grown in different areas. Generally, areas that experience heat units over 18°C (1150 T.H.U.) are suitable for soft early species. On the other hand, areas which have a total of 1800-2600 T.H.U. are more suited to the growth of species characterized by their dry and semi-dry dates (Zaid and de Wet; Hussein et al. ,1979. Albaker, Al-Behr, 1972; Al Juburi, 1993).

This study examined the effective heat units for the successful cultivation of date palm and the monthly changes in these units. Changes in the climate, especially during the time of fruit ripening in Gaza were also considered.

The aim was to investigate the possibility of introducing new varieties and their likelihood of success in Gaza conditions.

METHODS AND MATERIAL

This study was carried out in two stages.

The First Stage

Statistics were collected from a climate watch station which is situated in Gaza city. Average annual temperature was calculated from 1990–1995. This study was concluded in 1997. It led to the introduction of four new varieties (Berhi, Zuheidi, Ameri and Al Hallawi) to the area.

From 1990-2001, the average heat degrees were recorded. The following factors were considered:

1. Mean maximum temperature
2. Mean minimum temperature
3. Cumulative thermal units range
4. Mean relative humidity
5. Total rainfall in mms

Concerning the total cumulative thermal units, the mean maximum temperature was calculated for each month starting from May until the end of October every year for 12 years from 1990-2001.

The Second Stage

This stage involved the introduction of four new varieties: Berhi, Zuheidi, Ameri and Al Hallawi. They were cultivated in the northern area (Beitlahia), middle area (Deir albalah) and southern area (Rafah). These varieties were planted from 1998 – 2000. They were distributed at random to farmers in different numbers. Records were kept of growth, fruit set and fullness, percentage of fallen fruit, production quantities and the production

of offshoots. This took place during the fruit set and development period from April to August 2005. Results were taken from some farmers who had all experimental varieties. Average fruit set for each variety was determined by manually counting three strands from three bunches distributed at random on each palm tree. Eventually successful fruit fullness in addition to number of offshoots for each tree and each variety were recorded.

RESULTS AND DISCUSSION

First: Climate and Temperature

Temperature is considered one of the most important atmospheric elements that dominate the distribution of water on earth. It is also the criterion by which we measure the quantity of thermal energy that the air wins from sun rays or earthly temperature.

Daily temperatures increase from sunrise until 3 o'clock in the afternoon, before declining gradually and reaching their minimum at 3 o'clock in the early morning. Many factors influence the daily change in temperature such as clouds, rainfall and the nature of earth's surface.

In Palestine temperatures vary according to geographical location, lattitude and exposure to dominant winds and marine effects.

Tables 1 and 2 show that January is the coldest month and August is the hottest month. Average temperatures in November vary from one region to another but there is a general decline in temperatures in all parts of Palestine from November. There is a gradual increase in temperatures starting from March, and during Khamaseen wind time temperatures may rise to reach 40°C. This is because Gaza lies in the transitional zone between the climate of the Sinai Desert and the climate of moderate Mediterranean Sea. The average temperature in winter is 14.6°C and in summer it is 27.7°C. The temperature ranges from 18.1-30.4°C in summer and from 10.7 to 24.4°C in winter. It is noticed that the further from the coast, the higher the temperature (Palestinian Environmental Encyclopedia). Table 3 and Figures 2 and 3 show that the total thermal degrees vary from one year to another: 1990 experienced the least average thermal units (1655.5) and the most occurred in 1998 (1931.9). The average thermal units from 1990 to 2001 was 1792.

Conclusion

It is concluded that the total cumulative thermal units in the area is suitable for cultivating new varieties, especially the semi-dry varieties that are grown under specific conditions (Abduljabbar elbakr, 1982; Humaed Aljuboury Date Palm Trees, 1993).

Second: Rain

Rain quantities and averages in Gaza vary from year to year. Rainfall ranges from 200-400mm a year. From 1967 to 2001, the average rainfall in Gaza was 408mm. Rainfall in Gaza has surpassed recordings during the previous 30 years.

Rainfall decreases from north to south (Rafah),from 450 to 200 mm. Most rain falls from the middle of October to the end of May. The rest of the year is quite dry.

Conclusion

The conclusion is that the fruiting period from May to October is a dry period. This facilitates increase in fruit setting as well as rate of production (Hussain et al., 1950; 1979)

Third: Relative Humidity

Relative humidity is influenced by distance from the sea. Table 5 shows that the most humid months in Gaza are from May to August. In September relative humidity starts to gradually decline. The relative humidity in the coastal areas of Gaza ranges in the summer from 65% during the daytime to 80% at night. In the winter it ranges from 60% during the daytime and 80% at night.

Relative humidity decreases to 30% during the time of Khamasine winds. It

reaches its minimum percentage during the transitional months from September to October and from April to May. This is because of the blowing of desert winds during autumn and spring.

Conclusion

We conclude that relative humidity during the time of fruit set and production is moderate and suited especially to the stages of red dates (khelal) and black dates (rutab) (Alshurfa, moh-yoseef-1982).

Table 5 shows monthly relative humidity levels in Gaza.

Fourth: Ratios of Fruit Setting and Production

Table 6 reveals the variation in fruit set and production among species. Al Hayani gave the highest fruit set in all areas with an average was 87%. The next highest were Al Zuheidi (67.9%) and Al Berhi (63%). Al Halawi gave the highest fruit set in the southern area (81.3% mean), however it did not produce fruit in the middle area because there were many offshoots on the trees. The percentage of Al Hallawi fruit set in the north was 48.2%. Al Ameri achieved 50.2% fruit set in the middle area this year, but did not produce any fruit in the north or the south.

Table 8 shows the average number of bunches. Al Hayani gave the highest average with 7 bunches per tree. Al Zuheidi was next with an average of 4 bunches. Al Hallawi had the highest production in the southern area of 6 bunches and 4 bunches in the northern area. In the middle area, Al Hallawi did not produce any fruit this year. Al Ameri produced 5 bunches in the middle area. Production of Al Berhi varied, with 3-4 bunches in the middle area but only one in the southern area. This was due to weak trees and negligence.

Conclusion

It is clear that there is a great effect of climate and geography on the average fruit set and fruit fall. This average differs from one variety of palm tree to another and depends also on good service. These results indicate that there are various successes with different relative ratios according to the species that are included in the study.

Fifth: Average of the Production of Offshoots

Table 7 reveals the similarity in production of offshoots among Al Ameri, Al Hallawi and Al Zuheidi, with on average of 4 for each species noting the variation that occurred in the different geographical areas. Al Hayani, on the other hand, produced the highest average number of offshoots in all three areas. Al Berhi produced only one offshoot in the southern area.

Conclusion

The production of offshoots depends on the variety and the service it receives, and on the climate and geography in the area of cultivation (Ibraheem, Sonbul 1989; Humeed Aljubury, 1993).

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Tables

Table 1. Average annual temperatures from 1990 to 2001 in Gaza.

Years / Temp (°C)	90	91	92	93	94	95	96	97	98	99	2000	2001
max	24.9	24.5	25	24.7	23.9	24.1	24.9	23.7	24.7	24.3	23.5	24
min	17.9	16.2	18.1	17.4	16.9	17.1	17.3	16.7	17.7	17.6	17.2	18.9
average	21.5	22.3	21.2	22	20.9	20.6	20.7	20.2	21.2	21.1	20.6	21.1
grass	16.2	17.5	17.1	16.8	17	16	16.7	15.1	16.6	16.4	16.2	16.9
see	22.3	21.8	22.4	21.9	21.1	22.1	21.8	22	22.1	22.6	21.8	22.5

Table 2. Average daily maximum temperature (°C) in Gaza.

MONTH	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	AVRE
MAX	18.1	18.2	20.2	21.2	24.7	27.7	29.9	30.4	29.4	27.1	23.9	20.2	24.3
MIN	10.7	11.1	13.1	15.1	18.6	21.8	23.7	24.4	23.0	20.5	16.4	12.2	17.6
AVR.	14.6	14.7	16.9	18.4	22.0	24.8	26.9	27.7	26.7	24.1	20.2	16.2	21.1

Table 3. Thermal heat units (1990 -2001).

Month / Year	May	Jun	Jul	Aug	Sep	Oct	total
1990	159.1	250.1	327.5	339.8	297.5	281.5	1655.5
1991	187.1	247.8	311.8	338.3	296.7	280.8	1662.5
1992	166.1	259.7	325.4	333.2	388.1	278.7	1751.2
1993	190.7	295.9	337.5	351.4	313.9	310.4	1799.8
1994	217	269.9	337.2	358.7	356.3	354.4	1893.5
1995	179.8	315	365.8	378.2	333	251.1	1822.9
1996	229.4	273	356.5	368.9	336	272.8	1836.6
1997	186	285	362.7	341	309	263.5	1747.2
1998	210.8	273	359.6	430.9	360	297.6	1931.9
1999	207.7	291	368.9	384.4	342	282.1	1876.1
2000	179.8	285	375.1	372	321	232.5	1765.4
2001	210.8	261	337.9	368.9	321	263.5	1763.1
Total	193.7	275.5	347.2	363.8	331.2	280.7	1792.1

Table 4. Average rainfall in Gaza (1967-2001).

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Aver
Quantities	110.5	71.1	39.7	12.4	5.9	0	0	0	1.8	37.5	41.7	88.3	408.9

Table 5. Average relative humidity in Gaza (1990 – 2001).

MonthS	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Aver
Perc %	66.9	67.3	69.5	67	73.1	73	75	76.1	72	70.6	65.3	67.5	70.3

Table 4 6. Percentage fruit set of introduced varieties and AlHayani.

Loc Var	South	Middle	North	Average
Hayani	82.3	91.5	89.1	87.60%
Barhi	59.8	69.3	60.2	63.00%
Hallawi	81.3	0	48.2	43.20%
Zuhadi	62.2	78.3	63.2	67.90%
Amri	0	50.2	0	16.70%

Table 7. Number of offshoots produced.

Average	Territories Varieties	Average of the production of offshoots		
		North	Middle	South
7	Hayani	7	6	8
1	Barhi	2	0	0
5	Hallawi	2	5	10
4	Zahedi	3	2	6
4	Amri	1	1	10

Table 8. Average number of bunches produced.

Territories Varieties	Average of brunch production			Average
	South	Middle	North	
Hayani	7	7	8	7
Berhi	1	4	3	3
Hallawi	6	0	4	4
Zahedi	4	5	3	4
Amri	0	5	0	2

Figures

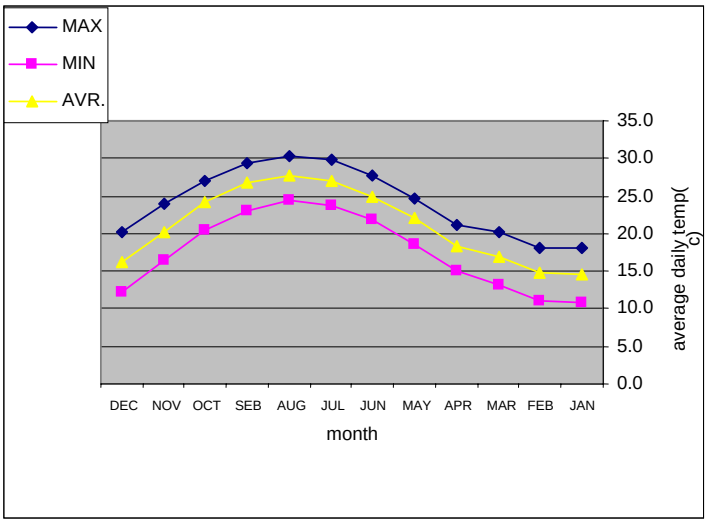

Fig. 1. Average daily and annual maximum temperature.

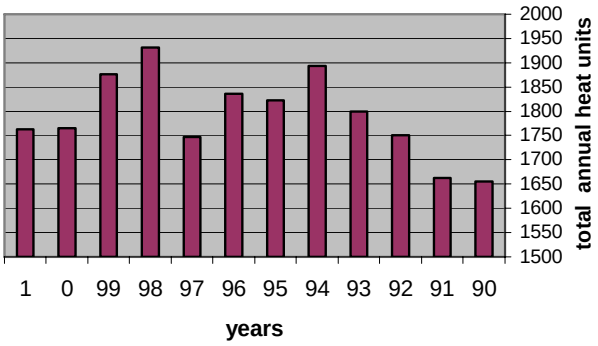

Fig. 2. Value of thermal heat units (°C).

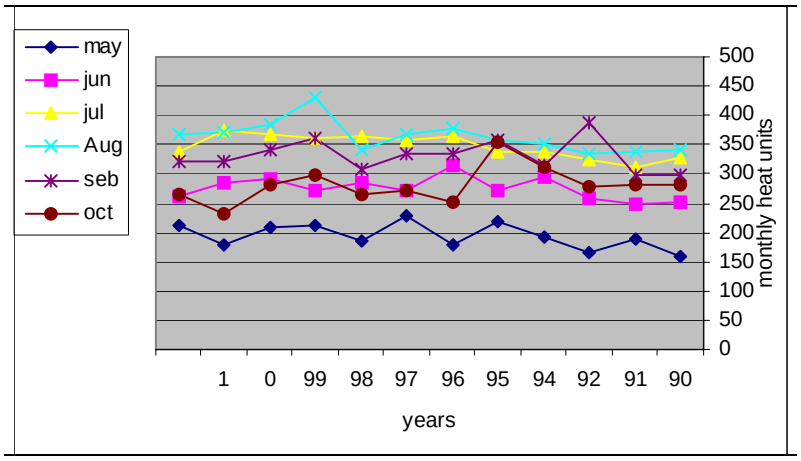

Fig. 3. Thermal heat units (1990-2001).

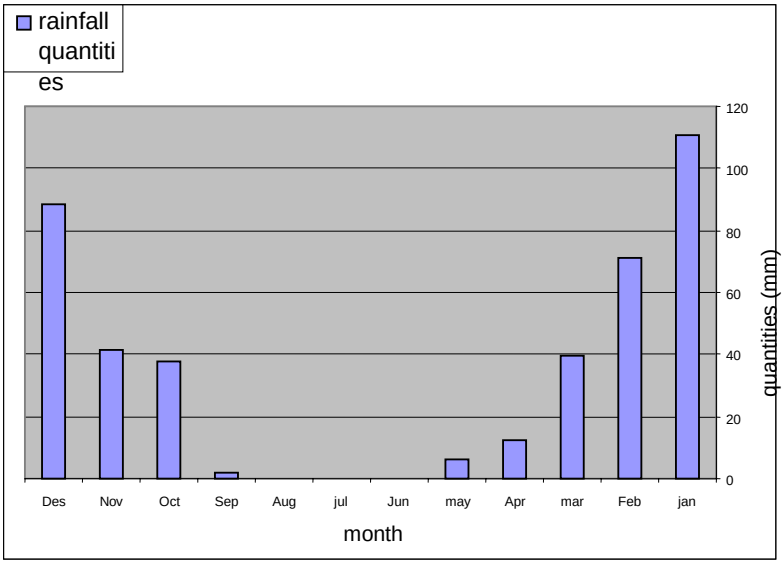

Fig. 4. Rainfall quantities (1967-2001).

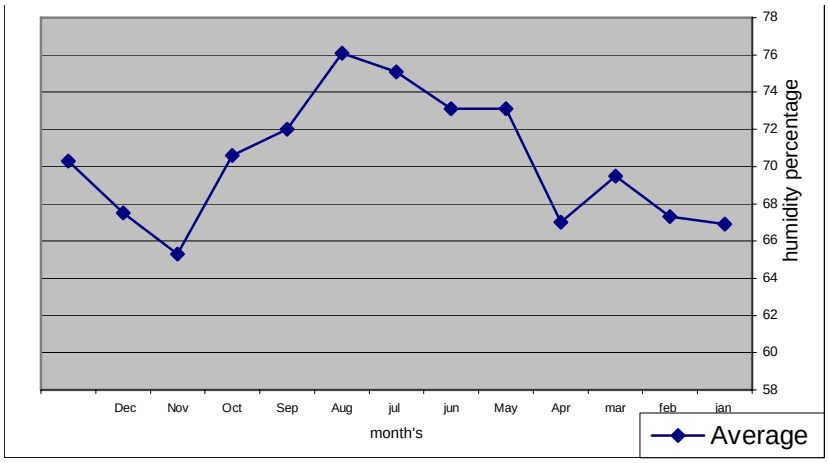

Fig. 5. Relative humidity.

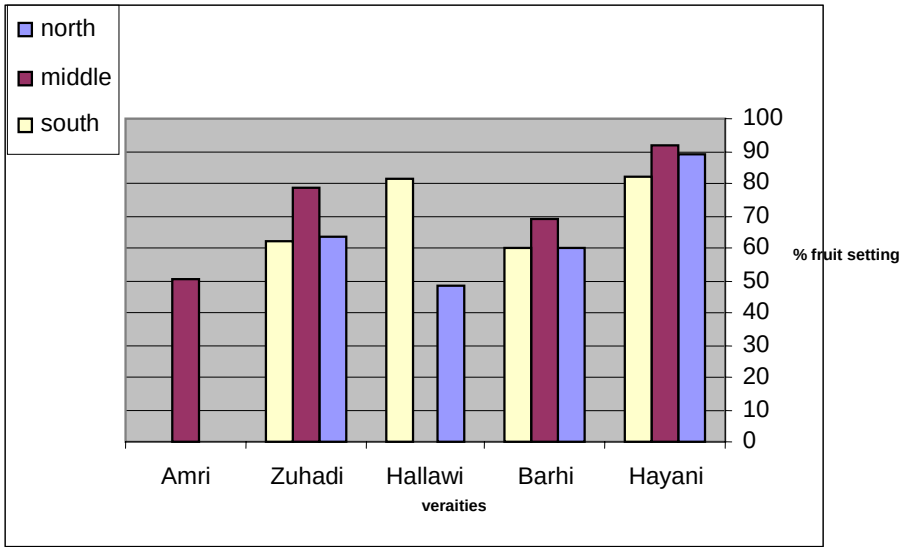

Fig. 6. Percentage of fruit set.

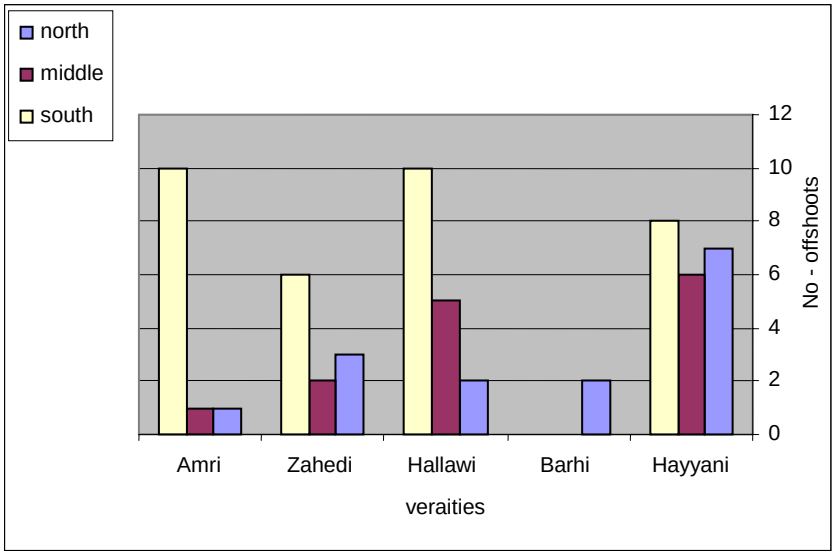

Fig. 7. Average production of offshoots.

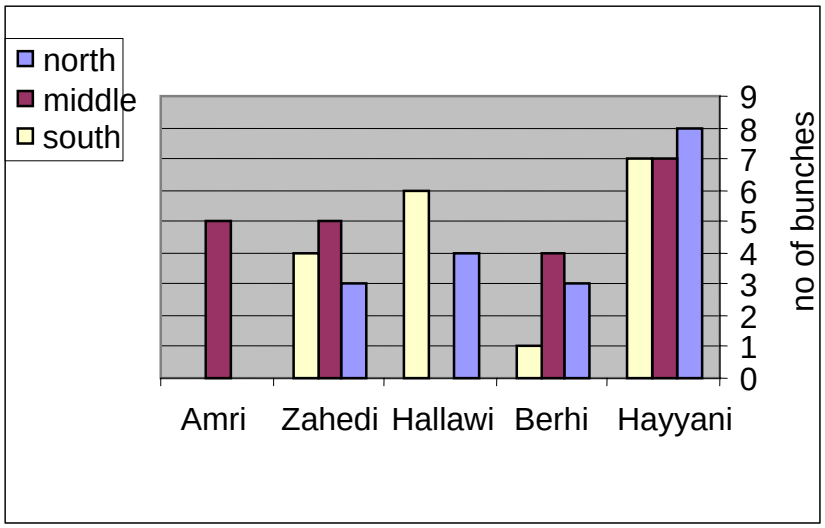

Fig. 8. Average number of bunches.

Date Palm Cultivation in Chile and Peru (South America): Current Status and Future Prospects for Development

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Abstract

This article analyzes the current situation and perspectives for the development of date palm (*Phoenix dactylifera* L.) cultivation in Chile and Peru. These two countries are situated on the western coast of South America on the Pacific Ocean, both are desert areas with a climate suited to date fruit production. Local varieties of dates have been derived from seed in Peru and Chile and developed along the length of the 2,300 km coastal strip and up to an elevation of 1,500 m. These seedling dates currently represent an unstudied natural germplasm bank that has adapted over the course of 400 years in geographic isolation and in the absence of date pests and diseases. They have developed within an environment of lower summer temperatures, accumulation of thermic units and higher relative humidity compared to the desert areas of North Africa and the Middle East. By contrast, they possess advantages to deal with optimal conditions of luminosity, active photosynthesis and the absence of rainfall and frost. As a result of these conditions there have developed multiple date palm genotypes, some with notably large fruit size of high quality. Flowering occurs between August and October, producing harvestable fruits during the months of March to August, with variation according to elevation and specific local conditions. Nevertheless, commercial date production is minimal and of a low level of technology, with minimal development of processing techniques. Some date palms are grown for ornamental purposes. Date fruit are almost unknown outside the production zones and date consumption does not exceed 2 g per person per year.

EARLY HISTORY OF THE DATE PALM IN SOUTH AMERICA

The precise time period when *P. dactylifera* was introduced to the New World is unknown, but it was almost certainly brought about by Spanish colonial administrators. The palm's propagation by seed and acclimatization were carried out by Spanish religious orders in the 16th and 17th centuries. Date seeds presumably came from Morocco and the initial area of their development was on the Central Coast of Peru. In 1612, the Jesuit scholar Bernabé Cobo (1964) wrote a detailed account of the date palm in the Viceroyalty of Peru, pointing out that 'the fruits from the date palms grown in the floodplain of the (Pisco) valley ripen as well as the date fruits brought in from the Barbary Coast of Africa.'

Date palms are frequently mentioned in official documents of the Jesuits that provide historical evidence of their establishment in 14 large landholdings during Peru's Colonial Period. It appears that the initial purpose for planting date palms was to provide a source of leaves for ceremonial use for Palm Sunday and other religious celebrations during the week before Easter. These festivities are similar to those still practiced in Elche, Spain. The cultivation by seed of date palms for edible fruits was important only in specific locations where proper fruit ripening could occur, such as Zaña, Pisco and Ica, in Peru. In 1767, when the Jesuit Order was expelled from Peru and elsewhere in Spanish South America, it can be speculated that the date palm had reached its greatest importance and extent. Shortly thereafter, the labors of 200 years of agricultural development by the Jesuits were abandoned to the desert and the date palms have survived to the present day primarily by natural reproduction in a feral state, receiving neither supplementary watering nor any care.

Given the considerable trade between the Peru and Mexico in the 16th and 17th

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centuries, Peru was possibly the source of date seeds carried to Mexico, especially to Baja California, where they were established and still persist, Likewise, the traditional cultivation practices in Coastal Peru and Baja California are similar (Aschmann, 1957). In all of these cases, the introduction and propagation of date palm was effected with seeds. As a result there currently exists a large number of varieties (cultivars) derived from natural selection, that are very heterogeneous and neglected, and consist of populations with equal numbers of male and female palms.

In Chile also there are varieties that are remnants of the Spanish Colonial Period. From 1965 to 1970, the government took action to introduce standard varieties of date palms for commercial fruit production. Offshoots obtained from California in the United States were established in the Tarapacá region. At the Esmeralda de Pica Experiment Station, 240 offshoots of the 'Zahidi,' 'Medjool' and 'Deglet Noor' varieties were planted. From this source, offshoots have been propagated and planted on a small scale at various locations near the cities of Arica, Pica and Iquique.

GEOGRAPHIC DISTRIBUTION IN CHILE AND PERU

In the coastal desert of Peru and Chile there are reports of various scattered locations of date fruit production over the wide latitudinal range of the Pacific Coast and the western slopes of the Andes Mountains. Date palms are found from Zaña in Chiclayo (7º S) to Pica in Chile (20º S), covering a long, narrow strip of 13 degrees of latitude and a distance of 2,300 km along the coast, extending inland no more than 120 km. It is worth noting that this latitudinal distribution, north to south, is the greatest in the world for *P. dactylifera*.

Likewise, *P. dactylifera* reportedly produces fruit over a broad range of elevation, from sea level up to 1,500 m elevation in the valleys of the western slopes of the Andes Mountains, such as in the Pisco and Ingenio valleys in Peru, and the Codpa and Tarapacá valleys in Chile. This distribution represents the greatest range of adaptation to elevation by date palm in the world (Fig. 1).

It is important to add that there are more geographic zones suitable for date palm cultivation, from the interior of the coastal valleys in Peru at higher latitudes to the south in Chile. According to reports of climatic suitability in Chile, it would be possible to adapt the date palm to locations some 1,000 km south of Iquique along the coastal strip. In addition to the locations listed in Table 1, there are suitable climatic conditions in the interior of the Taltal and El Salado valleys (26º 30´ S Lat.), Copiapó Valley (28º S Lat.) and at San Félix Valley in Vallenar (29º S Lat., at 1,600 m and about 125 km from the ocean).

CLIMATIC CONDITIONS

General Description

The Pacific Coastal desert of Peru and Chile is under the permanent influence of the Pacific anticyclone, which creates conditions of extreme atmospheric stability. This anticyclone is comprised of descending air currents which, upon reaching the ocean surface and because of the presence of the cold Peru (Humboldt) Current, which runs northward along the coast, creates a chilling of the atmosphere and the formation of a thermic inversion below 1,200 m in altitude. In this littoral zone a very dense fog cover is produced, resulting in condensation at about dawn, which then dissipates during the day.

The Andean Mountains that emerge above this thermic inversion, up to 6,880 m elevation (Ojos el Salado, Chile), represent a zone of receptivity to precipitation originating from the subtropical humid air masses of the Amazon Basin to the east. This produces orographic precipitation in the summer months (December to March) as the air moves up and over the eastern slopes of the Andes. These subtropical air masses in turn are forced to descend as subsiding air down the western slopes of the Andes which produces adiabatic heating by compression (the *foehn* effect). As a result, the relative humidity of the coastal desert is reduced to about 20% and this perpetuates conditions of

extreme dryness both day and night, lack of precipitation and leads to noticeably high levels of solar radiation.

The presence of the Pacific Ocean, which has relatively low water temperatures given the latitude, regulates and moderates to a considerable degree the thermic regime of the coastal desert of Chile and Peru. This is evidenced by the absence of cold periods and frost, a noticeably mild desert climate, which is the product of a daily cycle of air exchange between the coastal strip and the ocean, and with the presence of breezes of humid air over the entire year (ONERN, 1971a, 1971b).

Temperature

In all of the area studied, conditions of moderate subtropical climate prevail, moderated by the marine influence, but with an evident thermal stability. The highest temperatures are reached between December and March (summer), whereas the lowest temperatures occur in from June to August (winter). Average minimum temperatures rarely drop to 8º C on the coastal fringe and there is no occurrence of frost. Nevertheless, temperature range increases toward the interior of the continent and in response to increasing elevation, reaches a diurnal range of temperature of 30º C, such as occurs at the Esmeralda Station in Chile, at 1,180 m elevation, located 110 km from the ocean.

Table 2 indicates that the desert coastal strip of Chile and Peru experiences both in summer and in winter, a large number of hours with temperatures above 8ºC. This is caused by the absence of winter cold, and stimulates the biological activity of *P. dactylifera* without interruption over the entire year. The total number of hours per year with temperatures above 8ºC reaches a maximum of 8,760 hours in Paracas, Peru, and at Azapa in Arica, Chile. This figure exceeds 8,000 hours in practically all of the coastal valleys. It should be noted that in the Azapa Valley there are date palms which continually flower and fruit over the entire year. This climatic indicator (i.e. number of hours with temperatures above 8ºC), creates a special need to carry out relevant studies regarding the future adaptation of *P. dactylifera*.

With respect to the heat units necessary for successful fruit production in *P. dactylifera* (above 18ºC), the areas near the coast (Paracas and Azapa) experience a lower number of degree/days than is recommended for commercial date fruit production (Tables 3 and 4). By contrast, the interior valleys in southern Peru (e.g. Río Grande de Nazca) and the Tamarugal Pampa in Chile (e.g. Esmeralda) can be considered the best endowed as far as heat is concerned in the world, for these locations record more than 5,000 degree/days per year.

Nevertheless, date fruit characteristics vary. At locations close to the coast and with lower heat accumulation (e.g. Azapa), date fruits are soft and juicy, while date fruits grown in interior desert locations (e.g. Esmeralda) tend to be hard and dry.

Precipitation

The strip of coastal desert of Peru and Chile is considered to be the driest place on earth, where rainfall very rarely occurs. There is only sporadic rainfall of short duration, on one or two days in infrequent years, which is followed by dry, sunny periods.

However, in northern Peru (e.g. Zaña, Chiclayo), summer rainfall is determined by the presence of the El Niño effect, a periodic and recurrent event which alters the conditions of climatic stability and produces rainfall of considerable magnitude and duration during the months of date fruit growth, between December and April (Table 5).

Relative Humidity

Compensating for the lack of precipitation, the coastal desert strip of Peru and Chile experiences, up to an elevation of 1,200 m, relative humidity that is relatively high over the entire year, with small monthly differences (Tables 6-A and 6-B). This is especially noticeable during the evening and morning hours, when relative humidity frequently reaches saturation point (100%). Later in the day, solar radiation dissipates the fog cover as temperatures increase, resulting in decreased relative humidity.

By contrast, the desert areas in the interior of the continent, which are at higher elevations, are characterized by humidity conditions comparable to the deserts of the Northern Hemisphere and are subject to dry, warm descending air from the Andes. This relates directly to the greater diurnal and seasonal temperature oscillations, the occurrence of frost and high solar radiation over the year.

The elevated ambient humidity in the valleys of the Pacific Coast of South America considerably minimizes evapotranspiration from cultivated crops in desert agriculture. Based on field observation, it is possible that plants like *P. dactylifera* are able to capture a certain amount of humidity (the volume to be determined) directly from the evening and early morning fogs. To date, there have been no studies carried out to determine the water requirements of *P. dactylifera* under irrigation in Chile or Peru.

The elevated ambient humidity and high degree of cloud cover mostly restrict quality commercial date fruit production to the coastal fringe of Central Peru, comprising the area between Chiclayo to the north and Chincha to the south, including the Peruvian capital of Lima. Therefore, in Peru, optimal conditions of relative humidity for *P. dactylifera* can be found in the dry, sunny valleys of the Ica, Arequipa, Moquegua and Tacna, and also in the interior valleys located to the north of Lima, but at higher elevations and at some distance from the ocean.

Hours of Sunshine

The coasts of Peru and northern Chile are located geographically in the torrid zone of the earth, between the latitudes of 7 and 20º S, close to the equator. For this reason, the area receives higher solar radiation than the traditional zones of date palm cultivation in the Northern Hemisphere, which are located in a subtropical belt between the latitudes of 18 and 39º N.

This particular equatorial zone is distinctive because of the local climatic conditions that have been described (high atmospheric pressure, air subsidence and the cold Peru Current). In this coastal zone of Peru and northern Chile, there is a high number of hours of sunshine over the year, which reach a maximum in the interior desert locations at higher elevations (e.g. Esmeralda, Chile) (Table 7). The months with the highest number of hours of sunshine correspond precisely to the months of date fruit production and ripening (October to May). This condition, combined with the absence of precipitation during the harvest period, provides the potential for the production of date fruits of high quality.

Although no detailed studies for *P. dactylifera* have been carried out regarding photosynthetically active radiation (PAR), it must reach exceptionally high levels in the interior coastal valleys of southern Peru and northern Chile. Combined with the extraordinarily high levels of degrees/days, the absence of a cold period and frost, and the lack of precipitation, a scenario is created of unsurpassable natural conditions for the development of date palm cultivation.

SOIL AND WATER CONDITIONS

Agriculture in the deserts of northern Chile and southern Peru have in common limited water resources, resulting in high levels of salt in both the water and the soil. For this reason, land areas dedicated to agriculture are those where both surface water and underground water are available, characterizing specific localities that in general occur in the valleys located between the ocean and the Andes Mountains.

The date palm adapts well to saline soils and high levels of boron, a circumstance which favors its cultivation in a wide range of soils of varying quality. In Peru, the major concentrations of date palms occur on the plains of Pisco and in the Ica Valley, situated on desert soils containing high levels of salts, but endowed with ground water of good quality at a depth of 2-10 m, the aquifer fed by streams descending from the Andes (Soldi, 1982).

Table 8 illustrates the soil conditions in a series of valleys that represent the agriculture found in the northern part of Chile. These areas lie between approximately

1,500 km of latitude between the cities of Arica and Copiapó, from 18º to 29 ° S. Over this large area, there exist various locations favorable to date palm cultivation such as the Lluta, Azapa, Chiza, Camarones Suca, Liga, Miñi-Miñe, Quillagua, Taltal and Copiapó valleys, some of which are characterized by soils with high levels of salts and boron (Figs. 2 and 3).

Each area in Table 8 represents a variety of agroecological conditions, which are present throughout the Atacama Desert. When assessing the electrical conductivity of the soil (CE), one encounters extreme salinity in the Copiapó, Taltal, Liga and Suca valleys. The Taltal, Suca and Lluta valleys are characterized by extreme levels of boron. The Lluta and Camarones valleys are typically saline with serious limitations regarding their use. In these valleys local varieties of alfalfa and maize, both of which are tolerant to high levels of salinity and boron, are cultivated.

The Azapa, Chaca and Miñi-Miñe valleys offer the best soil and water conditions with scarcely any limitations to the cultivation of a number of fruits and vegetables. These include olives, citrus fruits, guavas, mangoes, tomatoes and green beans. Despite its elevation of 2,000 m, the Miñi-Miñe Valley experiences ideal microclimatic conditions for all types of subtropical agriculture including mangoes, bananas and citrus fruits. Date palms which bear fruit year round are grown in Azapa (Figs. 4 and 5).

Given that electrical conductivity is an indication of salinity, the Azapa and Miñi-Miñe valleys suffer no limitations in this regard. However, other valleys show medium to high levels that indicate certain limitations, especially in the Lluta and Taltal valleys.

However, although there are limitations because of the concentration of mineral salts and boron in the northern region, the development of an important agricultural industry is still possible. Some examples of crops exotic to northern Chile are maize, mangoes and olives, which have been cultivated for over 300 years and can grow under conditions of high salinity and boron. Table 9 refers to a study regarding the quality of irrigation water used in the same valleys.

The Tamarugal Pampa, Chile

The Tamarugal Pampa is a large, flat plateau with an area of 1.5 million ha, extending 300 km from Tiliviche, north of Iquique, to the Loa River, within the Atacama Desert. It is located at a latitude of approximately 20-22° S, an elevation of 1,000-1,200 m and about 50 km from the coast. In this area there are various oases that support the cultivation of tropical and subtropical fruits such as mangoes, citrus fruits, guavas and cherimoyas. Underlying the Tamarugal Pampa extends one of the most important aquifers discovered in northern Chile since 1995, containing an estimated 26,000 million m^3 of fresh water which is near the surface, at a depth of 5-30 m (JICA, 1995).

This area is characterized mainly by the presence of dry lakes with salt deposits at the surface and by the presence of a large forest of *Prosopis tamarugo*, which covers over 26,000 ha of land. of which 3,000 ha is natural forest in the middle of the desert (Figs. 6 and 7). The rest of the land has been forested to create new areas of vegetation. The natural forest contains ideal soil conditions for agriculture over the dry lakes, and its characteristics differ from the artificial forest (Escobar et al., 1998). Table 10 shows the soil quality in both areas.

CURRENT STATUS OF DATE PALM CULTIVATION

Peru has been the only producer of date fruits in South America for over four centuries, and it is estimated that in the 18th and 19th centuries date cultivation reached its widest distribution. The major production zone is located in the Department of Ica (300 km south of Lima), where the traditional date growing areas are found in the districts of Paracas in Pisco; Villacurí, Cachiche and Ocucaje in Ica; and Río Grande in Nazca (INIA, 1998). Decades ago, Gray (1929) briefly described and provided photographs of date production in the Lanchas and Chunchanga Pampas, which are located between Pisco and Ica.

According to Letts and Pavez (2000), in the area including Pisco and Ica in Peru,

there exist 50,000 female seedling dates of productive age, but about 90% of them are long abandoned and exist in a feral state. Currently, only about 4,000 palms are harvested under disorganized conditions, and about 1,000 palms under commercial plantation conditions where they receive care and irrigation. Likewise, there are some 300,000 offshoots of female palms of adequate size for transplanting, but they are of little value because they are from seedling dates where no varietal selection has been done.

Another report (INIA, 1998) indicates the existence of 60,000-80,000 productive date palms in all of Peru. In terms of numbers, the Peruvian date palm populations represent the most important in all of South America. In Peru, palm groves also exist in the Zaña, Chilca, Palpa-Río Grande, Nazca, Acarí, Yauca, Camaná, Tambo, Ilo and La Yarada/Tacna valleys.

Date fruit production in Peru has shown a tendency to decline in the past ten years, fluctuating between 128 mt in 1998 and 260 mt in 2004. The variation is assumed to result from the varying number of palms harvested each year. Assumed yields are very low, with an average of no more than 3,000 kg/ha and 20-30 kg per palm. It must be emphasized that these production figures derive from the harvest of palms that, typically, do not receive any care, irrigation, pollination or pest and insect control. Nevertheless, these nearly feral palms, once pruned, pollinated and given normal care, can achieve high production from individual palms. For example, there are palms that bear as many as 48 fruit stalks and yield more than 500 kg of ripe fruit (Pavez, 2001).

Nearly all of the dates produced are consumed within the production area. There is only one organized company in Peru (Huerto Alamein S.A.) that packages and markets dates commercially. It is located in Lima. There are a few artisanal processors of local importance making preserved dates, e.g. Zaña dates and Camaná dates in Arequipa. On the domestic Peruvian fruit market, date fruits are almost unknown, as is their potential for processing and incorporation into the food industry.

In Chile, according to the agricultural census (INE, 1997), there are three small date plantations with a total area of 35.8 ha, and 26 home gardens containing 377 date palms. Mature date palms can be found in home gardens in the Lluta, Azapa, Chaca, Codpa and Camarones valleys, in the vicinity of Arica and in the Suca, Miñi-miñe, Camiña and Tarapacá gorges, inland from Iquique at elevations of 1,200-1,500 m. The principal small date plantations are located in Pica, on the Tamarugal Pampa at 1,200 m elevation and about 110 km from Iquique.

Chilean date fruit production has surpassed 20 mt in some years, however, the chief plantation and experiment center (Fundo Esmeralda in Pica) is currently not in production (Fig. 8). As in Peru, date fruits are almost all consumed within the production area of Tarapacá; while the domestic Chilean market ignores this fruit and its potential for processing and incorporation into the food industry. This is despite the fact that Chile is the principal fruit exporter of the Southern Hemisphere and is developing a powerful export food industry on a world scale. The current annual consumption of dates in Chile is only 2 gr. per person. Decades ago it was much higher, but was based on imported dates.

In Chile, there is an increasingly important market for date palms for ornamental use in the coastal cities of Arica (Figs. 9 and 10), Iquique and Antofagasta. Nurseries exist for both *P. dactylifera* and *P. canariensis.* In the city of Arica alone it is estimated that 10,000 date palms are grown for ornamental purposes in urban areas, parks, camp grounds and in nurseries for propagation. All of these palms come from seed.

FUTURE PROSPECTS FOR DEVELOPMENT

The primary importance of the seed-derived cultivars found in Chile and Peru is that they constitute a natural germplasm bank of date palms that have adapted to local conditions over 400 years of geographic isolation. They have developed under conditions of lower summer temperatures, lower heat units and higher relative humidity compared to the deserts of North Africa and the Middle East. By contrast, they possess the advantage of optimal conditions of luminosity and radiation for photosynthesis and an absence of

rainfall and frost. This provides specific locations such as Azapa in Arica, Chile, and Paracas in Pisco, Peru, with an uninterrupted period of vegetative growth over the entire year, without winter dormancy, resulting in successive phenological stages in adult palms.

As a result of these environmental conditions numerous date palm cultivars have developed, some with fruit of superior quality and size. Among them are cultivars with fruits weighing 42-45 g, growing under semi-wild conditions, such as in Azapa in Arica. It is of major importance to collect these local cultivars in both countries as they are under threat of depredation and loss of biodiversity. We need to protect them in germplasm collections, with the aim of implementing a program of rehabilitation and development of local varieties that can compete, even in world markets.

Certain locations in both Peru and Chile have strong potential for the cultivation of selected established varieties such as 'Deglet Noor', 'Medjool' and 'Zahidi.' There is an enormous untapped domestic market for dates in the two countries, as well as elsewhere in South America, that could be developed by Peruvian and Chilean date growers and exporters.

There is no reason why both the promising local varieties and imported varieties cannot be developed on parallel tracks.

CONCLUSIONS

The natural conditions found on the Pacific Coast of South America in Peru and northern Chile constitute ideal conditions for the development of date palm cultivation on a large scale.

This coastal zone offers conditions suited to the growth of varieties of *P. dactylifera* and development of commercial date fruit production on a strip extending for 2,300 km from north to south, and from sea level to 1,500 m elevation. The presence of snow-capped mountains, glaciers and lakes along the higher western slopes of the Andes, assure a reliable source of surface and underground irrigation water, in considerable quantities and in most cases of good quality.

In Peru, the climatic conditions of the Central Coast between Chimbote and Pisco, is characterized by a high incidence of cloud cover, which limits commercial date production to interior valleys in the southern departments of Ica, (south of the port of Pisco), Arequipa, Moquegua and Tacna, on the western slopes of the Andes, up to 1,500 m elevation.

In the case of northern Chile, favorable conditions are found in the Tamarugal Pampa. Similar conditions are found on plateaus, slopes and valleys located inland from Arica, from the Peruvian border southward to the Tamarugal Pampa, at elevations of 200-1,400 m.

Because of geographic location and climatic characteristics, this area presents conditions of high temperatures throughout the year, resulting in intense photosynthesis, which translates into high production potential of the best quality fruits containing a high accumulation of sugars and high nutritional value.

Date fruit harvest normally occurs during a long seasonal window extending from March to August, a period when there is no production from the large date growers of the Northern Hemisphere. These circumstances are important for giving direction to the future production of dates destined for export, which should include varieties of high value and superior quality, chiefly of the soft date varieties.

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Tables

Table 1. Reported locations of *Phoenix dactylifera* growth in Peru and Chile.

Country	Region/Location	Latitude (º S)	Elevation (m)	Distance from the ocean (km)
Peru	Zaña /Chiclayo	6º 55´	120	16
Peru	Paracas /Pisco	13º 51´	5	0
Peru	Cachiche/Ica	14º 10´	400	45
Peru	Ocucaje/Ica	14º 22´	330	60
Peru	Río Grande/Nazca	14º 32´	265	55
Peru	Camaná/Arequipa	16º 40´	5	5
Peru	Ilo/Moquegua	17º 42´	35	8
Chile	Azapa/Arica	18º 32´	250	15
Chile	Tarapacá /Iquique	19º 55´	1,500	70
Chile	Esmeralda/Pica	20º 30´	1,180	110

Table 2. Average monthly temperatures (º C) at locations where date palms are grown.

Location/Country	Maximum (warmest month)	Minimum (coolest month)	Annual Average
Paracas/Peru	29.4	9.8	18.7
Ica/Peru	33.3	6.6	21.2
Ocucaje/Peru	35.6	5.9	20.6
Azapa/Chile	28.5	11.5	19.0
Esmeralda/Chile	34.1	1.0	18.8

Table 3. Average maximum daily temperatures (ºC).

Month	Paracas/Peru	Ica /Peru	Ocucaje/Peru	Azapa/Chile	Esmeralda/Chile
January	28.9	31.7	33.1	28.0	34.0
February	28.8	33.3	35.4	28.5	34.0
March	29.4	32.6	35.6	27.7	33.3
April	26.9	30.9	34.8	25.1	31.7
May	26.1	28.2	33.7	22.7	29.9
June	23.5	25.0	27.0	20.3	28.5
July	22.7	23.6	25.1	19.3	29.5
August	21.9	24.1	26.3	19.5	31.1
September	22.4	25.5	29.2	20.7	32.3
October	22.4	27.1	29.1	22.1	34.0
November	23.5	27.8	31.3	23.9	34.1
December	25.9	30.4	32.4	26.0	33.9
Annual average	25.2	28.3	31.1	23.7	32.2

Table 4. Accumulation of degree/days of heat (base 18ºC).

Month	Paracas/Peru	Ica /Peru	Ocucaje/Peru	Azapa/Chile	Esmeralda/Chile
January	337.9	424.7	468.1	310.0	496.0
February	302.4	428.4	487.2	294.0	449.2
March	353.4	452.6	545.6	300.7	474.3
April	267.0	387.0	504.0	213.0	411.0
May	251.1	316.2	486.7	145.7	368.9
June	165.0	210.0	270.0	69.0	315.0
July	145.7	173.6	220.1	40.3	356.5
August	120.9	189.1	257.3	46.5	406.1
September	132.0	225.0	336.0	81.0	429.0
October	136.4	282.1	344.1	127.1	496.0
November	165.0	294.0	399.0	177.0	483.0
December	244.9	384.4	446.4	248.0	492.9
Annual total	2,621.7	3,767.1	4,764.5	2,052.3	5,177.9

Table 5. Precipitacion (mm) at selected locations.

Location/Country	Total Annual Precipitation	Monthly Precipitation (rainiest month)	Rainiest Month
Paracas/Peru	1.6	0.5	August
Ica/Peru	4.4	1.2	January
Ocucaje/Peru	0.3	0.2	December
Azapa/Chile	0.5	0.3	January
Esmeralda/Chile	0.6	0.3	February

Table 6-A. Average daily relative humidity (%) at selected locations.

Location/Country	Maximum Relative Humidty	Minimum Relative Humidity	Annual Average
Paracas/Peru	87.0	77.0	81.5
Ica /Peru	99.0	39.0	69.3
Azapa/Chile	97.0	41.2	72.0
Esmeralda/Chile	85.0	20.0	31.4

Table 6-B. Average monthly telative humidity (%) at selected locations.

Month	Paracas/Peru	Ica /Peru	Azapa/Chile	Esmeralda/Chile
January	82.0	67.5	68.9	40.8
February	80.5	67.0	70.1	42.6
March	80.5	68.0	72.1	43.5
April	80.5	68.0	72.9	36.9
May	82.5	69.5	74.1	28.1
June	83.0	73.0	75.8	28.0
July	81.0	74.5	75.2	23.7
August	81.0	71.5	74.2	25.0
September	82.5	69.5	72.3	23.7
October	81.5	67.0	70.4	23.3
November	81.5	68.5	69.4	28.6
December	81.0	68.0	68.4	32.7
Annual average	81.5	69.3	72.0	31.4

Table 7. Average hours of sunshine in selected locations.

Month	Paracas/Peru	Ica /Peru	Azapa/Chile	Esmeralda/Chile
January	217	223	261.8	350.3
February	199	177	252.8	299.6
March	213	217	265.4	322.4
April	211	223	247.8	312.0
May	177	235	215.4	257.3
June	136	196	177.0	243.0
July	119	184	198.8	313.1
August	153	205	193.7	313.1
September	168	221	217.5	333.0
October	198	260	246.7	334.8
November	197	235	249.5	348.0
December	201	218	269.4	356.5
Annual average	2,189	2,594	2,795.8	3,783.1

Table 8. Chemical analysis of the soil in selected valleys of northern Chile (in saturation extracts).

	Lluta	Azapa	Chiza	Suca	Liga	Miñi-Miñe	Taltal	Copiapó
pH	7.6	7.6	8.5	7.4	6.9	8.1	7.1	8.2
C.E. (mS/cm)	3.07	5.25	2.38	40.6	16.19	0.84	27.1	62.1
Ca (meq/l)	10.1	30.4	6.3	70.2	75.3	3.7	59.0	41.6
Mg (meq/l)	4.8	4.6	2.0	26.2	19.2	1.0	39.8	135.0
Na (meq/l)	14.1	14.63	14.5	315.2	66.4	3.4	157.0	265.2
K (meq/l)	1.5	1.05	0.8	4.2	1.4	0.3	13.0	7.0
HCO_3 (meq/l)	2.8	2.7	9.4	7.5	4.2	2.8	3.8	10.8
Cl (meq/l)	18.3	29.63	11.0	317.2	140.3	5.2	228.8	388.9
SO_4 (meq/l)	6.9	27.5	3.7	52.4	14.8	0	0	165.0
B (mg/l)	13.2	7.82	3.1	17.6	8.8	1.8	24.5	6.4

Source: Escobar et al., 1995.

Table 9. Chemical analysis of irrigation water in the different valleys in northern Chile.

	Lluta	Azapa	Chiza	Suca	Miñi-Miñe	Taltal	Copiapó
pH	7.0	8.6	7.6	6.8	8.2	7.0	7.0
C.E. (mS/cm)	3.15	0.66	1.83	2.01	0.94	5.43	2.06
Ca (meq/l)	5.4	0.9	1.3	16.6	3.2	24.0	9.0
Mg (meq/l)	5.3	0.5	1.5	2.2	1.1	10.7	5.5
Na (meq/l)	20.4	3.1	13.0	4.1	5.3	18.2	6.1
K (meq/l)	1.1	0.3	0.2	0.3	0.2	1.0	0.4
HCO_3 (meq/l)	2.3	3.38	7.7	3.2	4.9	3.5	4.5
Cl (meq/l)	22.0	1.62	8.5	6.7	0.94	45.4	3.7
SO_4 (meq/l)	6.5	3.7	2.8	11.8	0.8	5.2	11.2
B (mg/l)	16.6	0.8	2.2	1.9	1.3	1.2	1.4
R.A.S.	8.8	2.9	11.1	1.3	3.6	4.4	2.3

Source: Figueroa et al., 1993.

Table 10. Chemical characteristics of the soil in forests of *Prosopis tamarugo* Phil.

	Natural forest	Planted forest
pH	8.5	7.4 – 8.02
C.E. (mS/cm)	2.1	2.8 – 16.89
Ca (meq/l)	4.4	4.0 – 1000.0
Mg (meq/l)	2.8	0.6 – 154.6
Na (meq/l)	311.0	463.2 – 2697.0
K (meq/l)	47.7	19.0 – 231.5
HCO_3 (meq/l)	298.9	74.7 – 149.4
SO_4 (meq/l)	88.8	453.6 – 4500.0
B (mg/l)	9.2	2.3 – 47.6
R.A.S.	45.2	7.3 – 89.5

Table 11. Date palm phenology at the Esmeralda Experiment Station.

Variety	Flowering period	Harvest period
'Deglet Noor'	Aug – Sep	October – November
'Medjool'	Jul – Aug	April – June
'Zahidi'	Aug - Sep	September – October

Source: Olave, 1985.

Table 12. Commercial date production in Peru.

Year	Area (ha)	Production (mt)	Yield (kg/ ha)
1966	150	808	5,386
1986	270	777	2,877
2004	97	260	2,680

Source: FAOSTAT - Agriculture, 2005.

Table 13. Importation of dates into Chile.

Year	Imports quantity (mt)	Imports value (US$1,000)
1972	1,369	133
1977	362	128
2004	27	18

Figures

Fig. 1. Location map of western South America showing where the date palm is adapted.

Fig. 2 and 3. Specimens of *P. dactylifera* in Camarones, Chile, under conditions of extremely high salinity and boron.

Fig. 4 and 5. Exceptional fruiting of *P. dactylifera* in the Azapa Valley, Arica, Chile where mature palms produce fruit continuously throughout the year under conditions of adequate soils and water. Photographs taken during the month of July. Source: Escobar et al., 1998.

Fig. 6 and 7. The contrast in natural conditions of the Tamarugal Pampa between areas of salt crust and *Prosopis tamarugo* forest.

Fig. 8. Date palms in the Esmeralda oases, Pica, Iquique, Chile.

Fig. 9 and 10. Ornamental use of *Phoenix canariensis* and *P. dactylifera* in parks and as street trees in Arica, Chile.

Artificial Ripening of Khuneizi Date using Physical and Chemical Methods

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Abstract

Khuneizi date is one of the high quality date cultivars grown in Minab area. However, if grown along the coastal strip where humidity is high, its fruits become sour and fall before ripening. Because of the damage caused to date tissue by unfavorable climatic conditions, it is necessary that this fruit be ripened using artificial methods to prevent the destruction and waste of the date. For this reason a factorial experiment was conducted in Minab in 2000. This cultivar was ripened with both physical and chemical treatments including controlled heat and humidity. In this investigation the laboratory conditions for artificial ripening of dates to prevent date rancidity were evaluated. Khlalas were treated at two TSS levels and pre-treatment properties of the dates were evaluated. These properties included qualification of TSS, total sugar and reduced sugar percentages. The treatments included salt solution (5 and 10 percent), acetic acid solution (2 and 5 percent) and freezing time (24, 48 and 72 hours at -8 degree centigrade). Dates were then`placed in four different temperatures, including 40, 50, 60 or 70 degrees centigrade. The best treatment was freezing followed by placement of the ripened dates at 50 degree centigrade for 48 hours, for khalals containing up to 36 percent TSS at picking time. This treatment resulted in the highest rate of TSS and percentage of rutab from khalal.

INTRODUCTION

Date palm in the growing area of Hormozgan province covers 33998 hectares. A variety of cultivars is grown. Khuneizi is considered to be one of the better cultivars. Artificial ripening is a good option for dates after part of the maturing process has occurred on the tree. This process is done in rooms with controlled temperature, humidity and ventilation. The temperature should not be more than 50°C, and the relative humidity depends on the cultivar. Ripening is directly dependent on the temperature and relative humidity of the environment. When the temperature and relative humidity increase, the dates ripen more quickly. Under natural conditions on the tree, the period from the ripening of the first date until the last is a month. This can be a hazardous time for the crop. In this period some ripe dates fall and are attacked by pests. Artificial ripening is a way of avoiding this situation. There are several different methods for artificial ripening including:

1. Hanging of date clusters with hooks on wooden scaffolding or windows or putting them in front of the sun (Benjamin, Mahdi et al., 1975).
2. In some countries like Egypt and Spain, the fruit is put into a salty solution, then laid in the sun for rapid maturing and marketing (Ashmawi et al., 1955).
3. In the USA, fruit is firstly put at -27°C, and then placed in higher temperatures for 32 hours. However, ripe fruit produced using this method should be kept in the refrigerator (Asif et al., 1983).

In Tunisia the artificial ripening process starts with freezing, before the fruits are placed in 38 - 48°C for 24 hours. In North Africa and Spain, fruit are sprayed with light vinegar and kept for one day wrapped in cloth. Khadrawi dates that are picked after khalal stage are yellow, hard and acrid. They are processed to soft, sweet and edible fruit by using an acetaldehyde solution (1 and 6 percentage). The processing time for Hallawi

when freeze ripened, is less than the time required for chemical and temporal processing. In Bahrain the freezing process is used for ripening of the Khuneizi cultivar. Since humidity at khalal stage is 50 - 60 percent, fruit tissue is soft and transportation is difficult, therefore we can decrease the humidity of this stage in 50°C (Ashmawi et al., 1955).

When dates have about 30 percent humidity, they cannot be preserved using regular methods. The best way of improving the shelflife and transportation of these dates is to use cold storage. If dates are stored at 5°C and 0°C they can be preserved for 4 - 6 months (Barreveld, 1993).

MATERIALS AND METHODS

This research was carried out on two days during date ripening (July 10 and July 19) using a randomized factorial design with 3 treatments. In this investigation, 5 trees growing under the same conditions were selected at Minab Agricultural Research Station. After sampling, the clusters were carried to the laboratory for quality analyzing (TSS) in Hormozgan Agricultural and Natural Resources Research Center. We collected fruit at different stages of maturity to determine precisely when they contain 36-40 percent TSS. We used two methods for processing including physical and chemical methods.

1. Physical Method:

Fruit were frozen at -8ºC for three lengths of times (24, 48 or 72 hours) before being placed in an oven at 40, 50, 60 or 70ºC with 50-70 percent relative humidity for 20 hours.

2. Chemical Methods:

(i) Fruit were floated in a salty solution of two concentrations (5 or 10 percent) before being placed in an oven at 40, 50, 60 or 70ºC with 50-70 percent relative humidity for 72 hours.

(ii) Fruit were floated in acetic acid of two concentrations (2 or 5 percent), before being placed in an oven at 40, 50, 60 or 70 ºC with 50-70 % relative humidity for 72 hours.

After treatment fruit quality factors such as TSS and reduced and total sugar percentages were measured (Benjamin et al., 1975.)

Quality Analysis Methods

1. TSS. The solid materials were measured from the date extract (25gr date) and then mixed with 100ml of distilled water. TSS was calculated using a refractometer.

2. Reduced Sugar Percentage. Percentage of reduced sugar was measured using Lan-Eynons method in the presence of Methylen blue.

3. Percentage of Total Sugar. Percentage of reduced sugar was measured using Lan-Eynons method in the presence of Methylen blue.

No control treatment was conducted in the khalal stage. Dates were placed in 40, 50, 60 or 70ºC and were then stored in the fridge to extend their shelflife (Ranganna, 2000; Saraei, 1996).

RESULTS

The results showed that the interaction effect of salt and heat treatment for both TSS levels (29 and 36 percent) was significantly different at 5 percent level with Duncan's multi-range test. Both levels of salt treatment (5 and 10 percent) were effective for ripening of khalal stage dates but since the treatment had unfavourable effects on date taste, we do not advise use of the higher concentration (10 percent) (Tables 1, 2 and 3).

Also for the acid treatment the results showed that the interaction effect of acid and heat treatment for both TSS levels (29 and 36 percent) was significantly different at 5 percent level with Duncan's multi-range test. Even though both levels of acetic acid treatment (2 and 5 percent) were effective for the ripening of dates at the khalal stage, they had unfavourable effects on date taste and colour. We do not recommend the higher concentration of this treatment (Tables 4, 5 and 6).

Results showed that the interaction effect of freezing and heat treatment for only one TSS level (36 percent) was significantly different at 5 percent level with Duncan's

multi-range test. This treatment increased the reduced and total sugar percentages. Using this treatment we were able to successfully store the khalal stage dates for long periods. Colour and taste of the fruit in this method were favourable (Tables 7, 8 and 9).

When khalal dates with TSS less than 36% at picking were ripened at 50 and 60°C with 70 percent relative humidity, the fruit became undesireable with many cracks and wrinkles on the fruit skin caused by the treatments. When TSS was higher than 36-40 percent in the khalal stage, artificial ripening resulted in high quality fruit including colour, taste and sweetness.

The freezing method combined with heating and humidity resulted in increased quality of dates and reduction of damage caused by pests. High temperatures (60ºC) were effective for artificial ripening and for reduction of humidity of khalals, however, it changed the fruit taste when cooked, so is unsuitable for this purpose.

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Tables

Table 1. Effect of salty solution and heat treatments on TSS percentage.

	July 10				July 19			
Year	10%	5%	Control treatment	Salt Heat	10%	5%	Control treatment	Salt Heat
	33.09c	37abc	32c	40	34.6b	42ab	36.4b	**40**
2000	42.2abc	38.8abc	33.8bc	50	40.2ab	36.3b	48.4ab	50
	44ab	46.8a	42.2abc	60	44.4ab	40.6ab	52a	60
	34.4bc	38.09abc	37.80abc	70	38.4ab	39ab	44.2ab	70
	32abc	32abc	31.50abc	40	38b	39.5b	37b	40
2001	32.40abc	30.8	28c	50	40b	38.50b	39b	50
	32.40abc	38a	33.50bc	60	46a	43.9a	40b	60
	32abc	36ab	30.20bc	70	36.5b	45.50	37	70

Table 2. Effect of salty solution and heat treatments on reduced sugar percentage.

	July 10				July19			
Year	10%	5%	Control treatment	Salt Heat	10%	5%	Control treatment	Salt Heat
	29.81cd	32.10bcd	26.24d	40	27.64e	41.02bcd	33.04dc	40
2000	33.10abcd	33.92abcd	38.1abc	50	43.46abc	36.27cde	36.27cde	50
	40.11ab	41.11a	37.63abc	60	46.44ab	38bcd	50.66a	60
	32.35bcd	36.25abc	35.36abc	70	40bcd	46.44ab	44.84abc	70
	27.64bc	24.13cd	26.20bcd	40	33.1b	26.67c	31.72bc	40
2001	23.52cd	20.83d	26.67bcd	50	30.22bc	26.33c	31.72bc	50
	31.03ab	35.36a	27.38bc	60	26.74c	31.03bc	30.72bc	60
	25.83bcd	31.14ab	30.40ab	70	27.28c	39a	31.34bc	70

Table 3. Effect of salty solution and heat treatments on total sugar percentage.

	July 10				July 19			
Year	10%	5%	Control treatment	Salt Heat	10%	5%	Control treatment	Salt Heat
	26.85b	39.12a	27.64b	40	23.65c	38.86ab	36.19ab	40
2000	27.27b	33.92ab	37.10ab	50	36.19ab	33.04b	32.57b	50
	41.11a	40.11a	35.52ab	60	44.86a	38ab	39ab	60
	33.04ab	41a	32.10ab	70	39ab	40.11ab	43.46a	70
	28.77c	29.40bc	26.74c	40	38.39ab	33.93ab	39.93ab	40
2001	30.45bc	28.68c	27.14c	50	31.72b	34.61ab	37.10ab	50
	28.94c	35.36ab	33.04abc	60	39.21ab	39.21ab	37.10ab	60
	28.30c	37.64a	35.36ab	70	34.20ab	42.35a	35 ab	70

Table 4. Effect of acid solution and heat treatments on TSS percentage.

Year	July 10				July 19			
	5%	2%	Control treatment	Acid Heat	5%	2%	Control treatment	Acid Heat
	29.6d	33.4bcd	32cd	40	39.2cd	32.3e	36.4dc	40
2000	38abcd	33.2bcd	33.8bcd	50	43.2bc	46ab	38.40	50
	30.60abc	41.2bcd	42.2ab	60	46.2ab	46.2ab	52a	60
	43.8a	37abcd	37.8abcd	70	42bcd	40.8cd	44.2bc	70
2001	25.5b	27b	31.5ab	40	38.4bcd	36de	37cde	40
	27b	32ab	28b	50	38.6bcd	33.8e	39bcd	50
	31.5	34ab	33.5ab	60	43.40a	41ab	40abc	60
	33ab	37a	30.2ab	70	41.6ab	43a	37cde	70

Table 5. Effect of acid solution and heat treatments on reduced sugar percentage.

	July 10				July 19			
Year	5%	2%	Control treatment	Acid Heat	5%	2%	Control treatment	Acid Heat
2000	28.08cd	33.61bcd	26.36d	40	31.66dc	26.9c	33.04cde	40
	31.57bcd	31.14bcd	38.1ab	50	42.22abc	42.22abc	36.27bcde	50
	33.90bc	37.27ab	37.63ab	60	43.75ab	50.34a	50.66a	60
	44.7a	36.27ab	35.36bc	70	39bcd	38bcde	44.86ab	70
2001	19.53e	22.53dc	26.2abcd	40	34.61ab	35.45ab	34.2ab	40
	24.19bcde	27.64ab	26.67abcd	50	39a	28.40b	32.17ab	50
	23.39cde	27.72abcd	27.38abcd	60	36.27ab	30.47b	33.79ab	60
	29.23abc	32.35a	30.22ab	70	28c	35.52ab	30.4b	70

Table 6. Effect of acid solution and heat treatments on total sugar percentage.

Year	July 10				July 19			
	5%	2%	Control treatment	Acid Heat	5%	2%	Control treatment	Acid Heat
	29.33a	27.64a	27.64a	40	34.54bcd	28.18d	36.19bcd	40
2000	35.36a	29.23a	37.1a	50	34.68bcd	47.5a	32.57cd	50
	35.92a	39a	35.52a	60	40.11abc	40abc	39abc	60
	36.97a	33/1a	32.10	70	40.11abc	33.1cd	43.46ab	70
2001	22.44bc	21.17c	26.74abc	40	41.72a	40.44a	45.70a	40
	22.67bc	33.07ab	27.14abc	50	42.99a	37.53a	42.24a	50
	29.53abc	30.45abc	33.04ab	60	39.95a	33.77a	43.45a	60
	29.81abc	31.34abc	35.36a	70	44.42a	33.87a	46.45a	70

Table 7. Effect of freezing and heat treatments on TSS percentage.

	July 10					July19				
Year	72 hour	48 hour	24 hour	Control treatment	Freezing Heat	72 hour	48 hour	24 hour	Control treatment	Freezing Heat
	31.4b	30.6b	30.80b	32b	40	39.6bcd	30 g	30.2fg	36.4cdef	40
2000	32.40b	32.55b	32.60b	33.8ab	50	40.35bc	34.40defg	32.4efg	38cde	50
	33.60	34.95ab	37ab	42.20a	60	33.60defg	32.4efg	35.60cdefg	52a	60
	32.4b	35.20ab	33.60ab	37.80ab	70	30.75fg	36.2cdefg	34.30cdef	44b	70
2001	30.5a	29a	27a	31.50a	40	35.60f	35.4f	36ef	37cdef	40
	30a	28a	29a	28a	50	35.60f	36.8cdef	36ef	39bcd	50
	30a	32a	26a	33.50a	60	36.4def	38.7bcde	39ab	40ab	60
	31a	33a	26a	35.20a	70	39.4abc	41.80a	38.40bcde	37cdef	70

Table 8. Effect of freezing and heat treatments on reduced sugar percentage

	July 10					July 19				
Year	72 hour	48 hour	24hour	Control treatment	Freezing Heat	72 hour	48 hour	24hour	Control treatment	Freezing Heat
	31.14abc	31.14abc	30.59abc	26.36c	40	33.90cd	27.17d	33.92cd	33.04cd	**40**
2000	34.61abc	34.61abc	30.40abc	38.45a	50	32.35cd	36.52c	31.89cd	36.27c	50
	36.27ab	36.27ab	36.52ab	37.63ab	60	36.52c	38c	35.52c	50.66a	60
	33.61abc	33.61abc	33.79abc	35.36ab	70	36.40c	39.21bc	34.02cd	44.86ab	70
2001	20.59b	20.59b	24.11ab	26.20ab	40	33.04bcd	33.04bcd	33.79abcd	31.72bcd	40
	20.59b	20.56b	24.51ab	26.67ab	50	33.04bcd	34.54abcd	35.11abcd	31.72bcd	50
	22.60ab	22.6ab	20.35b	27.38ab	60	35.36abc	36.27ab	36.27ab	30.59d	60
	25.76ab	25.76ab	22.53ab	30.40a	70	36.19ab	38a	36.27ab	31.34cd	70

Table 9. Effect of freezing and heat treatments on total sugar percentage.

	July 10					July19				
Year	72 hour	48 hour	24hour	Control treatment	Freezing Heat	72 hour	48 hour	24hour	Control treatment	Freezing Heat
	29.23a	31.66a	33.92a	27.64a	40	37.39abc	30.82c	34.94bc	36.54abc	40
2000	28.08a	34.61a	29.86a	37/1a	50	35.99bc	39.68abc	33.77c	37.07abc	50
	30.59a	36.52a	34.61a	35.52a	60	38.98abc	39.12abc	36.79abc	46.83a	60
	31.66a	32.71a	32.35a	32.1a	70	39.43abc	40.69abc	34.98bc	45.01ab	70
2001	27.72bcd	22.85d	25.76cd	27.17bcd	40	34.54bcd	33.04c	35.36abc	33.93bc	40
	23.52d	21.03d	24.92cd	27.14bcd	50	33.04c	35.36abc	36.19abc	37.1ab	50
	25.77cd	23.37d	23.43d	33.04ab	60	35.36abc	37.1ab	36.27abc	37.1ab	60
	30.84abc	26.24cd	24.42cd	35.36a	70	38a	38a	38a	35.52abc	70

Date Palm Economies and Cultivation Circumstances in Palestine

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Keywords: marketing infrastructure, Medjoul

Abstract

The study aimed to highlight the de facto situation of the date palm industry in Palestine and to improve the circumstances. Change is required from providing good quality and variety of seedlings to ensuring a suitable marketing infrastructure to promote date production both locally and abroad. In this context, the study dealt with the high economic feasibility of date palm cultivation and its impact on the Palestinian economy with reference to the experience of the Palestinian farmers.

The study indicated that the two geographic locations with ideal climate and environmental conditions are the Jordan Valley and Gaza Strip. The advantages and characteristics of the date palm variety Medjoul are described, as well as the experience of PARC and other organizations in resuming the cultivation of date palm and caring for its various issues. The study exposes the problems and constraints that face this cultivation, such as the continued Israeli control of fundamental resources (land, water, market and borders), which is causing high production, transportation and marketing costs. Marketing infrastructure and services are fragile and weak, and there are limited official support policies for date palm cultivation and for the agriculture sector generally.

This study revealed good potential for cultivating Medjoul in the Jordan Valley, due to its suitable climate and environmental conditions and the existence of the necessary production inputs. Furthermore, date palm is able to withstand high temperatures and does not need huge amounts of water.

The study concluded with a number of practical recommendations to overcome the aforementioned obstacles, especially those relating to farmer's performance, who must abide by quality criteria and standards.

INTRODUCTION

The Palestinian farmer is recognized as one of the best farmers in history, known for his/her diligence in transforming the arid rocky lands into arable lands with high productivity of fruits, vegetables and field crops. The Jordan Valley, particularly Jericho, the oldest city in the world, is the earliest cultivated land worked by Palestinian peasants five thousand years ago. The Palestinian peasant utilized the existing springs to irrigate and produce vegetables in this area, which had become the Palestinian food basket, especially in winter when production ceased in the hilly and coastal areas.

Date palm cultivation has been in existence for centuries in the Jordan Valley, Gaza Strip and Sinai. One of the Arab travellers wrote that the Arab conquerors faced a major problem due to the date palm forests in Jericho when they conquered the city.

Throughout history date palm cultivation has deteriorated and almost disappeared, leaving behind small areas of land cultivated with date palm. This has been a result of the hostile Israeli policy of pumping large amounts of fresh water to the Israeli settlements, which were constructed on the Palestinian Occupied Territories. As a result, the water became too salty to grow a variety of vegetable crops, so Palestinian farmers, knowing the feasibility of date palm, started to grow it in very limited areas. Upon Israel's refusal to sell seedlings to the Palestinians during the Intifadah, some Palestinian farmers and investors resolved to establish a pilot project to accumulate expertise and produce seedlings needed for the expansion of date palm cultivation.

This study aimed to highlight the date palm industry in Palestine, and pave the way for improved date palm cultivation, starting with the provision of seedlings, and

assistance with the costs of cultivation and marketing.

The success of date palm cultivation will have a positive impact on the Palestinian agricultural economy, particularly in the Jordan Valley and Gaza Strip. It will generate employment opportunities for farmers and needy families. Furthermore, it will create an alternative to, and a replacement for, production from the Israeli settlements. More importantly, it will provide food security to poor families, especially in times of political crisis and during closures and curfews imposed by the Israeli occupation power. Dates are a part of a balanced diet, easily stored under difficult conditions, and an important food product in the life of Palestinians who use them in their foods and as a traditional desert.

LOCATIONS OF DATE PALM CULTIVATION

Date palm cultivation is concentrated in Gaza Strip where palm trees depend on rainfall (approximately 300- 400 ml annually) and shallow underground water (1-3 meters below the surface in some areas). Date palms, particularly Hayyani variety, are mainly grown in house gardens and along the sides of farms and roads. One thousand dunums of the 300 square km of the area of Gaza Strip are cultivated with commercial date palm. New model farms are also spreading using new cultivars.

In the Jordan Valley (400 square km), date palm cultivation is concentrated in Jericho or 'the date palm city', as people call it. Commercial date palm plantations covering 2000 dunums have recently been started. The local baladi varieties (around 35 kinds) are cultivated around houses, farms and roads. Most of the production is sold fresh while the remainder is dried and sold as pastry.

During the 1980s, the Agricultural Development Association (PARC) focused attention on cultivating date palm, realizing the suitability of the soil and water salinity. However, only a few farmers responded positively to PARC's attempts after they realized the likelihood of success and economic return.

After some years, PARC established a plan to encourage farmers to grow date palm making use of the local varieties, particularly Hayyani, by providing farmers with technical support and extension services. PARC also initiated a pilot project to increase Medjoul variety in the Jordan Valley. The total number of date palms which PARC and the Ministry of Agriculture cultivated was estimated at 25000, most of which were of Medjoul variety.

DATES MARKETING

The share of dates in the Palestinian market in terms of consumption and spending has been relatively stable since the last decade. The annual average of local consumption has reached the global level of 0.9 kg per capita. The growth in local demand for dates is attributed to the natural population growth. Date consumption increased by 28.4% during the years 1994-2000. An increase of 190% in total date consumption is expected by 2020 compared with 1994. Date production in 2004 was 175 tons, which constituted 7.1% of local market needs. The rest of the market demand was met by imports from Israel and other Arab countries.

Date imports constitute a very modest percentage of total food imports. Dates make up only 0.17% of Palestinian food consumption. This is due to the local food consumption pattern, rise in the price of dates and the existence of numerous alternatives. To improve and develop the local date market, consumption patterns must be changed by promotion of this product, highlighting its values and nutrients. A suitable marketing infrastructure must also be provided.

Significant problems face date production and marketing in Palestine.

High Cost of Production

Date production requires intensive labor and expertise, most of which are acquired from workers who have worked or are still working in the Israeli settlements. Since they receive higher wages in the Israeli settlements, employing them incurs a higher cost. Mechanical lifts used in the production process are expensive. They are estimated to cost

15000-20000 US$. Other problems that increase production costs include; the inability to obtain seedlings of good quality resulting from Israel's imposed obstacles to prevent imports, the high cost of the local offsets which reaches 50-60 US$, and the long time that it takes to produce local offsets. These factors restrain the expansion of date palm cultivation.

Transport and Shipping High Costs

Transport and shipping costs form 30% of the total cost of Palestinian exports and imports. This percentage is four times greater and double the cost of Jordanian and Israeli products, respectively. The high cost of transport and shipping is due to the imposition of costly conditions and restrictions such as inspections, as well as extra customs imposed by Israel on Palestinian goods, e.g. the total shipping cost of one container carrying Palestinian products is 50% more than the total shipping costs of Israeli goods.

Weak Marketing Facilities

Palestinian agricultural products, particularly dates, suffer from poor marketing facilities such as grading, filling, transport and storage. This results from an internal failure in the export sector, which is made up of weak technical, management and financial infrastructure of small companies and projects. It is worth noting that unilateral efforts to employ technology and to penetrate the markets, as well as develop marketing infrastructure will not help. A collective effort is required to reinforce bonds between production and marketing in order to create a high competitive capacity. Responding to the local and external market quality standards is a catalyst for further investments in marketing infrastructures and facilities.

The cultivation of Medjoul date palm in the Jordan Valley helped to solve the problems facing agricultural production, however local and external marketing processes still face the following problems:

1. Unequal Competition in the Local Market. This is the result of constant dependence on Israeli dates, which are unsuitable for export. These products are displayed in a manner that increases their price in the Palestinian market. Also there is a large problem of Israeli dates being dumped through smuggling in the Palestinian markets. There is an absence of national regulations to control such activities. Thus, Israel maintains its control over the Palestinian market, continuing to destroy the Palestinian agricultural products and deprive local producers of benefiting from the local sale of their dates. Local date production constitutes only 5% of the local consumption, and should easily be absorbed in the local market. This situation has negative impacts on the future of date palm cultivation and production.

2. Competition in the European Export Markets. The Palestinian production of Medjoul is of no lesser quality than the Israeli product, and yet it faces the following obstacles:

(i) weak modern marketing mechanisms and the use of the manual methods in washing, grading, filling and drying processes;
(ii) poor knowledge by farmers and exporters of global markets in terms of requirements and patterns of consumption;
(iii) lack of commitment by farmers and exporters towards quality standards, particularly in postharvest processes;
(iv) insufficient support for farmers from official and unofficial authorities all through the production processes;
(v) poor agricultural extension services throughout the production processes.

We suggest that the best approach to solve date production marketing is to concentrate and expand the use of modern technologies.

Limited Support for Date Palm Cultivation

The low priority given to the agricultural sector by the government in their official politics, combined with the general lack of official support to this sector, have been

significant in undermining the competitiveness of Palestinian products. Dates are the most marginalized crop, therefore the lack of subsidy to this crop is reflected in the high production costs and the low state of dates in the local market, as well as in the failure to penetrate external markets.

MEDJOUL DATE PALM

The Medjoul variety is one of the newly introduced varieties into Palestine. Its cultivation has succeeded in the Jordan Valley in the past 15 years. Date palms are resistant to difficult environmental circumstances, including high water and soil salinity. They grow in nearly all kinds of soil, but grow particularly well in sandy soil. Date palms endure high temperatures, strong winds and hurricanes, despite the fact that some varieties require dry weather for the drying process and long term storage, such as Dajlat El-Nour and Zhaidi. Others varieties need high humidity such as Birhi and Hayyani. For this reason, certain varieties will flourish in a given location, at the expense of other varieties, e.g. Hayyani in Egypt, Zhaidi in Iraq, and Dajalt El-Nour in Tunis and Algeria. These characteristics are an advantage to date palms.

Morocco and Tunis are the habitat of the Medjoul variety, which was taken to California by the Americans during the First World War. The Americans noted the large size of the dates, the mild percent of sugar, as well as the humidity inside the medium sized dates. They realized that this variety could be sold both fresh, if kept in cold storages, and dried. By keeping 25% of the date's humidity, dry dates could be stored for two years without decay or rot.

Medjoul derives from the Arabic word 'majhoul', which means 'unknown'. It is so called because the origin of this variety is not accurately known. Because it is difficult for the Americans to pronounce the letter "H", the name Medjoul was used. The Americans took good care of the proliferation of this variety. When Israel asked the United States to provide them with varieties of date palms, the Americans sent a shipment of different varieties that included 50 trees of Medjoul. Though the Israelis did not welcome these Medjoul palms, as they preferred to receive Iraqi varieties like Zhaidi, El-Khalas and El-Amri, they cultivated them in Bisan (Beit Sha'an) station where they bore very good results.

Consequently, Israel intensified efforts to obtain and cultivate this variety, so that Medjoul is now the main variety in Israel. By 2003, there were more than one million date palms in Israel, most of them of the Medjoul variety. Israel cultivates only small quantities of other varieties of date palm and plans to increase the number of commercially grown date palms by using refined sewage water for irrigation. The cultivation of date palm has spread from Aqaba gulf in the south up to Bisan plains. However, it is preferred to cultivate date palm in Wadi Araba between the Dead Sea in the north and the Red Sea in the south because this area enjoys a dry climate suitable for high productivity and quality that meets the market need. Israel presently exports large amounts of date fruit, particularly to Europe, at a price of 9 US$ per kilo.

KEY ARGUMENTS PERTAINING TO DATE PALM CULTIVATION

(i) Date palms take only five years to establish and produce. Productivity lasts for 20 years. At 25 years of age, date palm trees are sold to public gardens for beautification projects at a price of 200 USD.
(ii) One offset costs 50- 60 USD, and seedlings proliferated by tissue culture cost 50 USD each.
(iii) The cost of establishing one seedling reaches 100 USD. This includes service, fertilizers, labor, water and price of the offset itself.
(iv) Technical and management supervision is deemed very important for the success of date palms. It is worth noting that in Palestine, experienced and qualified agricultural workers and technicians do exist. They have worked for more than 25 years in this field on established farms.
(v) A seedling requires an average of 50 cubic meters of water annually in the first five

years. After the fifth year it takes 100 cubic meters.
(vi) Date palms are planted in a space measuring 8x9, which means an average of 14 seedlings are planted per dunum. They can also be planted in a space of 8x8 or 7x8 or 7x7, allowing up to 20 seedlings to be planted per dunum.
(vii) It is preferred to cultivate 80% Medjoul variety and to divide the rest of the land equally between Birhi and Zhaidi varieties.
(viii) Two cooperatives for date palm farmers are in existence in the Jordan Valley and Gaza Strip. Both cooperatives encourage and sponsor cooperation between farmers in cultivation, date processing and marketing, purchasing of seedlings and providing extension.
(ix) There is abundant water in the artisan wells, which is relatively saline but suitable for date palm cultivation. There are also other water sources such as springs and streams that can be used in the future for irrigating .
(x) The local price for one kilo sold to merchants is 3 USD and when sold directly to consumers it is 4-6 USD. The global price for this variety is 6-9 USD after it has been packed.
(xi) The cultivation of offsets is quicker than the cultivation of seedlings that are proliferated by tissue culture; they take nearly two years. Although the chances of success with offsets are less than with seedlings, offsets are preferred because of their resistance to disease and insects. Offsets produce quicker than seedlings.
(xii) According to the results reported by Palestinian farmers, a date palm tree produces up to 150 kg per year, however, production drops to 80 kg after the tenth year.
(xiii) The average percentage of success with offsets after cultivation is 90%. It is preferred to cultivate them in early summer i.e. in April and May.
(xiv) The middle and southern parts of the Jordan Valley are well suited to the cultivation of Medjoul because temperatures are hot but the humidity is not too high.
(xv) Tissue culture cultivation has been negatively affected by the presence of seedlings from Ra's An-Naquora farm (north of Israel). These seedlings, which were imported from France, were sick and did not grow normally. Furthermore, they did not possess the same characteristics of the original Medjoul date palm tree. The estimated number of affected seedlings was 12000.

ECONOMIC CALCULATIONS IN TERMS OF THE COST AND PRODUCTIVITY OF DATE PALM

a) Establishment costs per one seedling in US dollar:
- Entrenched offset price per seedling: 55
- Uprooting transport and planting per seedling: 5
- Major and minor pipes per seedling: 10

Total: 70 USD

b) Annual running costs:
- Water cost per seedling: 15
- Labor per seedling: 3
- Fertilizers and medicines per seedling: 2
- Admin. cost per seedling: 5

Total: 20 USD

Total cost during the first 3 years per seedling: 20x 3= 60 USD

Total cost from year one to the 4^{th} year: 70+ 60= 130 USD per seedling

c) Productivity:
(i) Production of dates starts in the third year in small amounts. It is preferred to discard these dates in order to strengthen the tree and allow more offsets to be produced.
(ii) Productivity in the fourth year is estimated at 20 kg and this increases by an annual average of 10 kg until the tree reaches up to 80 kg after ten years. Productivity might reach up to 100 kg annually.
(iii) Productivity depends on the strength of the tree, how much care it is given including fertilization, and the age of the tree.

(iv) Offset production starts in the forth year and continues up to the tenth year with an average of 7 offsets per one tree; i.e. one offset per tree after cultivation in the third year up to year 10.
(v) A date palm seedling gives back all money spent on cultivation with the sale of half of the offsets it produces.
- Offset selling: 40 USD x 7 offsets= 280 USD. The date production is a net profit.
- Cultivation cost until production: 130 USD.
Note: the cost of one offset is 10 USD including entrenching, preparation and uprooting. Therefore, the selling of the offset is estimated at 50 USD from which 10 USD are deducted for entrenching. As such, the net profit becomes 40 USD per offset (Table 1).

Profits after Year 10

Taking into consideration the weather conditions and technical pollination mistakes, it is assumed that 20% of the income will be deducted i.e. $40. As such, the minimum income of one tree is estimated at $180.

Working on a cultivation figure of 14 trees per dunum (8x9 meters), then we can obtain 2520 USD as a net income of one dunum cultivated with Medjoul. This is the highest income that can be achieved compared to the income that other crops can achieve.
Note: the price of one kg of unprocessed dates is estimated at $3, whereas the price of one offset after deducting the cost of uprooting and entrenching is estimated at $40.

RESULTS

(i) We conclude that potential for Medjoul date palm cultivation in the Jordan Valley is good, due to the suitable climate and environmental conditions besides the abundance of necessary input for cultivation. Furthermore, date palms endure high temperatures and do not need large amounts of water, and the land between the palms can be cultivated with with other cash crops.
(ii) According to our production estimation the area of land needed for cultivating Medjoul date palms could reach up to 3000 dunums. This area is within reach in Palestine.
(iii) In the short term, the local marketing potential for Palestinian Medjoul dates is limited because of Israeli competition and the lack of laws and regulations to protect and control the local production. However, in the long term the potential is improved especially if consumer interest and demand is increased. Moreover, promotion by the producers themselves, care for the appearance and quality of the dates, and publicity concerning the nutritional value of dates, shouldl increase the marketing potential of dates, especially if part of the production is processed locally and is used in other food industries.
(iv) It is clear that the Palestinian Medjoul dates have a big advantage in penetrating directly into European markets, especially if we can add value to them by modernizing the production and marketing infrastructure, and abide by quality standards and criteria.

RECOMMENDATIONS

Although the introduction of Medjoul date palm to the Jordan Valley is only recent, it is a promising crop. Continued success requires support from the Ministry of Agriculture, PARC and agricultural societies, and other relevant stakeholders in the following ways:
(i) Provision of loans to farmers as the investment in date palm cultivation takes 5 years before there is a harvest.
(ii) Provision of extension services pertaining to the selection of offsets, how to plant, care for and protect them from insects, how to apply pollination, fertilization and sprinkling processes, etc.
(iii) Providing offsets at suitable prices as the price of the offset currently constitutes 50% of production costs.
(iv) Providing farmers with lifts at low prices or providing loans so that they can purchase

them.
(v) Capacity building of farmers' cooperatives by participating professionals. This will help to overcome the problem of scarcity of qualified and experienced laborers.
(vi) Construction of a factory in the Jordan Valley for grading, filling and storing dates.
(vii) Encouraging the private sector and investors to invest in the industry.

CONCLUSION

The difficulties facing the local marketing of dates should inspire a search for global markets. At a time when the demand for Medjoul dates is increasing, production is still limited. Although the profit average of Medjoul dates is high compared to other date varieties, the shift to Medjoul cultivation is not tangible or quick enough. Unless we overcome some of the obstacles facing date cultivation and invest large funds in this sector, there will not be a quick conversion to date palm cultivation.

The following prerequisites would ensure the foundation of an economically feasible date palm industry in Palestine. As a consequence, thousands of employment opportunities would be provided, land would be protected, and a profitable income would be provided to families and companies working in the Medjoul date sector:
(i) abiding by quality standards and criteria;
(ii) investment in marketing facilities by the private sector;
(iii) installation of cold storages;
(iv) provision of transport and shipping services;
(v) establishment of communication links between local exporters and importers in the global markets, particularly in the European Union states;
(vi) the adoption of measures to prevent smuggling of Israeli dates onto local markets by issuing laws to protect local production, producers and consumers against dumping;
(vii) publicity and promotion campaigns to introduce the nutritional values of dates to consumers.

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Tables

Table 1. Expected costs and production of dates and offsets per one seedling in the first ten years.

Year	Costs in USD	Production: Kg + 1 offset	Difference in income per one seedling
1st	10	-	-
2nd	20	-	-
3rd	30	-	-
4th	30	22 kg (60$) + 40$	70
5th	50	30 kg (90$) + 40$	80
6th	60	40 kg (80$) + 40$	60
7th	60	50 kg (150$) + 40$	130
8th	60	60 kg (180$) + 40$	160
9th	60	70 kg (210$) + 40$	190
10th	60	80 kg (240$) + 40$	220

Early Detection of Genetic Variation in Date Palms Propagated from Tissue Culture and Offshoots by DNA Fingerprinting

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Abstract

In vitro propagation of date palm (*Phoenix dactylifera* L.) through different methods of tissue culture has resulted in large-scale multiplication and distribution to different regions of the world of some elite cultivars which was otherwise difficult to achieve using traditional propagation from offshoots. Propagation by either offshoots or tissue culture generally results in true-to-type plants but some off-types with abnormal phenotypes have developed in tissue cultured plants which may be due to somaclonal variation. In the present study random amplified polymorphic DNA (RAPD) analysis was performed with the aim to detect genetic variation at plantlet stage in female plants of cultivars, 'Khalas', 'Barhy' and 'Sukkary', along with the males of 'Barhy' and 'Sukkary', propagated from offshoots, seeds and through tissue culture. The cultivars showed unique RAPD banding patterns. To facilitate RAPD analysis the 13 DNA samples were divided into two sets of populations. In the first population amplification profiles of 6 DNA samples belonging to two offshoot-derived and four TC-derived plants of cv. Khalas were compared with each other. Out of 18 primers screened, 13 detected polymorphism. The cluster analysis by unweighted paired group method of arithmetic mean (UPGMA) showed two clusters. The genetic distances ranged from 0.634 to 0.825 in the similarity matrix based on Nei and Li's similarity coefficients. In the second population 7 DNA samples belonging to male and female 'Barhy' and 'Sukkary' derived from seedlings and offshoots and a tissue cultured 'Barhy' were compared. All 7 genotypes revealed a unique banding profile with 14 primers used. The genetic distances ranged from 0.232 to 0.810. In this population two clusters were also detected. Offshoots and tissue cultured plants of 'Khalas' when compared showed low level of polymorphism. However tissue cultured 'Barhy' showed a high level of genetic variation when compared with offshoot-derived and seedling-derived male and female 'Barhy'. A significant level of genetic variation was also observed among the cultivars of 'Sukkary' and 'Barhy'. RAPD appears to be an efficient technique for the early detection of genetic variation in plants propagated by tissue culture and from offshoots.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) (2n=2x=36) is of great socio-economic importance in the Kingdom of Saudi Arabia (KSA). It is believed to have originated in Mesopotamia (Wrigley, 1995). The number of known date palm cultivars distributed all over the world is approximately 5000, out of which about 450 are found in the Kingdom of Saudi Arabia (Bashah, 1996). Saudi Arabia is one of the largest date producing countries in the world.

Date palm is a dioecious plant and its heterozygous form makes its progeny strongly heterogeneous (Munier, 1981). Thus the propagation of date palm from offshoots is preferred to seedlings. Since propagation through offshoots is slow with a low survival rate, tissue culture of female plants has been preferred for mass production of true-to-type plants of elite varieties in demand. Propagation by both offshoots and tissue culture generally results in true-to-type plants, but some off-types with abnormal phenotypes

have developed in tissue cultured plants. These abnormalities may be due to somaclonal variation.

Problems concerning fruit set and fruit quality of many date palm cultivars propagated through tissue culture are also thought to be associated with incompatibility of pollen from unidentified male trees (El-Ghayati, 1983). Farmers have been pollinating for years with limited background knowledge of the genotype of the males. The male gamete is derived from pollen and the growing pollen tube secretes chemicals responsible for the development of fruit. The identification, selection and determination of genetic diversity among male date palms through DNA-based RAPD markers, could help in selecting male plants of good quality that match with female cultivars. This window for further research could assist in designing crossing programs that will maximize the yield and improve the quality and quantity of the fruits.

The cultivars of date palm are currently identified on the basis of their morphological features such as leaf shape, pinnae, fruit stalk and fruit characteristics (Nixon, 1950). In recent years, several other methods have been used to identify date palm cultivars. Morphology of pollens was used to identify four male date palm cultivars and other *Phoenix* species (Tisserat and Demason, 1982). Identification and evaluation of genetic diversity between cultivars on the basis of morphological markers is difficult. Identification of trees is not usually possible until the onset of fruit, which takes at least five years. Further, to characterize a variety a large set of phenotypic data is required that is difficult to assess statistically and is variable due to environmental effects (Sedra et al., 1996). Biochemical markers (isozymes and proteins) have proven effective in varietal identification of date palm (Bendiab et al., 1993; Bennaceur et al., 1991). However, they give limited information and are an indirect approach for detecting genomic variation. Among the different techniques used in generating molecular markers, for direct detection of genomic variation at the DNA level, RFLP markers (Restriction Fragment Length Polymorphism) have been evaluated for date palm clone identification (Corniquel and Mercier, 1994), but the technique is laborious and requires radio-labelling of the probes. A rapid technique called Random Amplified Polymorphic DNA (RAPD) may prove to be one of the best molecular techniques to generate markers for the study of a large number of DNA samples and is both time- and cost-effective (Williams et al., 1990; Welsh and McClelland, 1990).

Random Amplified Polymorphic DNA (RAPD) markers have been successfully used for cultivar analysis and species identification in most plants, due to the technical simplicity and speed of the methodology (Gepts, 1993; Askari et al., 2003; Al-Khalifah and Askari, 2003; Sedra et al., 1998; Mokhtar et al., 1998).

The objectives of the present study were (1) early detection of genetic variation in offshoot and TC-derived plants and (2) to determine the genetic relationship among diverse date palm cultivars.

MATERIALS AND METHODS

Plant Materials

A total of 13 samples of young leaves were collected from different male and female plants of the date palm cultivars ‘Khalas’, ‘Barhy’ and ‘Sukkary’, grown both from offshoots and tissue culture. Young leaves of male and female plants grown from seedlings were also included (Table 1).

Total Genomic DNA Extraction and PCR-Amplification

Total genomic DNA was extracted from each of the leaf samples of cv. ‘Khalas’, ‘Barhy’ and ‘Sukkary’, following the protocol described by Dellaporta et al. (1983). The quantity and quality of the DNA were then measured by Hoefer DyNA Quant 200 (Pharmacia Biotech).

PCR-amplification reactions were performed as described by Al-Khalifah and Askari (2003). A total of 46 RAPD primers were used belonging to A, B, C, D and F

series, purchased from Operon Technologies Inc., Alameda, California, USA. The amplified RAPD products were separated by electrophoresis according to their molecular weight in 1.4 % agarose gels submerged in 1 X TBE buffer. The DNA profiles were visualized on UV transilluminator and documented by using Gel Documentation System (Bio Rad). To facilitate the analysis 13 samples were divided into two sets of populations. In the first population amplification profiles of 6 DNA samples i.e. two offshoot-derived and four TC-derived 'Khalas' were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0). In the second population 7 DNA samples of male and female 'Barhy' and 'Sukkary' grown from seedlings and offshoots and a tissue cultured 'Barhy' were compared. The data of all the primers that showed polymorphism (13 primers of population I and 14 primers of population II) was applied to estimate the similarity on the basis of the number of shared amplification products (Nei and Li, 1979). A software "Diversity Data Base" (Bio Rad) was used. Dendrograms were also constructed on the basis of similarity coefficients by using unweighted-paired group of arithmetic means (UPGMA).

RESULTS AND DISCUSSION

The average yields from 300 to 500 mg of young sprouting leaves ranged from 10 to 30 μg ml^{-1} DNA. To facilitate RAPD analysis the 13 different DNA samples were split into two sets of populations.

Population I

In the first population 6 DNA samples extracted from leaves of two offshoot and four TC-derived 'Khalas' were amplified with 18 different RAPD primers. Unique banding patterns were revealed by the 6 samples with 13 primers. Different primers produced different levels of polymorphism among the samples (Fig. 1a). A total of 138 DNA fragments were amplified, with an average of 2.9 RAPD markers per primer. Out of 138 amplified fragments, 98 (71.01%) were polymorphic.

The pair-wise genetic distance estimates of the 6 samples were analyzed and are given in Table 2a. The similarity matrix is based on Nei and Li's similarity coefficient. The genetic distances ranged from 0.634 to 0.825. Cluster analysis using UPGMA revealed cluster groups as shown in Fig. 2a. Offshoots and tissue cultured plants of 'Khalas' when compared showed low level of polymorphism.

Population II

In the second population a set of 7 DNA samples of male and female 'Sukkary' grown from seedlings, male and female 'Barhy' from seedlings, male and female 'Barhy' from offshoot, and a tissue cultured 'Barhy' were amplified. All 7 genotypes revealed a unique banding profile with 14 primers and thus can be used for identification and selection of good performing genotypes. Each primer indicated a different level of polymorphism among the genotypes (Fig. 1b). A total of 187 bands were generated, with an average of 4.6 RAPD markers per primer. Out of a total 187 amplified fragments of DNA, 123 (65.7%) were polymophic.

The pair-wise genetic distance estimates of the 7 genotypes were analyzed and the similarity matrix based on Nei and Li's similarity coefficients are given in Table 2b. The genetic distances ranged from 0.232 to 0.810. Maximum similarity was observed between male and female 'Barhy' from offshoot (0.81). Tissue cultured 'Barhy' in general showed a minimum degree of similarity with all other genotypes ranging between 0.238 to 0.321.

Cluster analysis using RAPD resulted in two cluster groups as shown in Fig. 2b. In cluster B, out of 4 genotypes, male and female 'Barhy' from offshoot were more closely related compared to the rest of the 7 genotypes, with a highest value in the similarity matrix for Nei and Li's coefficient (0.81). Female 'Sukkary' from seedling was 61% similar genomically to male and female 'Barhy' while male 'Barhy' from seedling was 46% genomically related to the three genotypes of the cluster. In cluster A male Sukkary was 60.2% genomically similar to female 'Barhy'. Tissue cultured 'Barhy' was 38%

genomically related to male 'Sukkary' and female 'Barhy', and that was the lowest percentage of similarity among the 7 genotypes.

RAPD appears to be an effective technique for the identification of date palm genotypes. Polymorphism among the date palm genotypes is low (Askari et al., 2003; Al-Khalifah and Askari, 2003) in comparison to other cultivated species like rice (Farooq et al., 1994a), wheat (Farooq et al., 1994b), and cotton (Khan et al., 2000). RAPD markers should be of high value for date palm germplasm characterization and genetic maintenance. A low polymorphism and the lack of evident organization observed among the genotypes taken into consideration could be due to the nature of introduction of the date palm trees into the country and also due to the unorganized maintenance of the germplasm. Development of new recombinants by seedling selection and sexual reproduction and also the exchange of the genotypes between the different plantation areas may have been the main cause for the low diversity among the germplasms. The data generated so far suggests a narrow genetic diversity. In the present communication the average genetic similarity among the genotypes of date palm was more than 50% which makes it difficult to differentiate among the male genotypes compared to more stable females. Nevertheless the results suggest that RAPD markers can be successfully used for the DNA fingerprinting of male date palm if a considerable number of genotypes are included in the experiments and also the number of RAPD primers is increased to generate more data. Only then can a decisive conclusion be made.

ACKNOWLEDGEMENTS

We thank various plant breeders for providing leaf samples from different date palm cultivars. This research was fully supported by King Abdulaziz City for Science and Technology.

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Tables

Table 1. List of 13 DNA samples of leaves, rachis and pollens of male and female date palm from four different cultivars used for determining genetic phylogeny.

No.	Sample name	Cultivar	Source
1.	Offshoot Leaves	Khalas	Research Station, KACST
2.	Offshoot Leaves	Khalas	
3.	Leaves TC1	Khalas	Tissue Culture Lab. NRERI, KACST
4.	Leaves TC2	Khalas	"
5.	Leaves TC3	Khalas	"
6.	Leaves TC4	Khalas	"
7.	Female leaves	Barhy	Riyadh date palm Farm
8.	Male leaves	Barhy	"
9.	Female leaves (seedling)	Sukkary	"
10.	Male leaves (seedling)	Sukkary	"
11.	Female leaves (seedling)	Barhy	"
12.	Male leaves (seedling)	Barhy	"
13.	Tissue Cultured leaves	Barhy	Tissue Culture Lab. NRERI, KACST

Table 2 a. Similarity matrix for Nei and Li's coefficients of 6 DNA samples of two offshoot and four TC-derived plants of date palm cv. Khalas obtained from RAPD markers.

		1	2	3	4	5	6
'Khalas' OD	1	100.0					
'Khalas' OD	2	76.5	100.0				
T.C. 1	3	67.2	69.4	100.0			
T.C 2	4	65.2	69.0	82.5	100.0		
T.C 3	5	70.9	64.9	71.1	72.0	100.0	
T.C 4	6	69.6	63.4	71.3	69.4	81.3	100.0

Table 2 b. Similarity matrix for Nei and Li's coefficients of 7 DNA samples of male and female 'Barhy' and 'Sukkary' grown from seedlings and normal plants and a tissue cultured 'Barhy' of date palm obtained from RAPD markers.

		1	2	3	4	5	6	7
'Barhy' Female SD	1	100.0						
'Sukkary' Male SD	2	60.2	100.0					
'Barhy' T.C.	3	47.1	28.3	100.0				
'Sukkary' Female SD	4	37.4	25.3	26.2	100.0			
'Barhy' Male SD	5	35.5	29.1	28.2	45.0	100.0		
'Barhy' Female OD	6	34.2	34.9	32.1	63.2	48.0	100.0	
'Barhy' Male OD	7	23.2	34.5	23.8	59.2	44.1	81.0	100.0

Figures

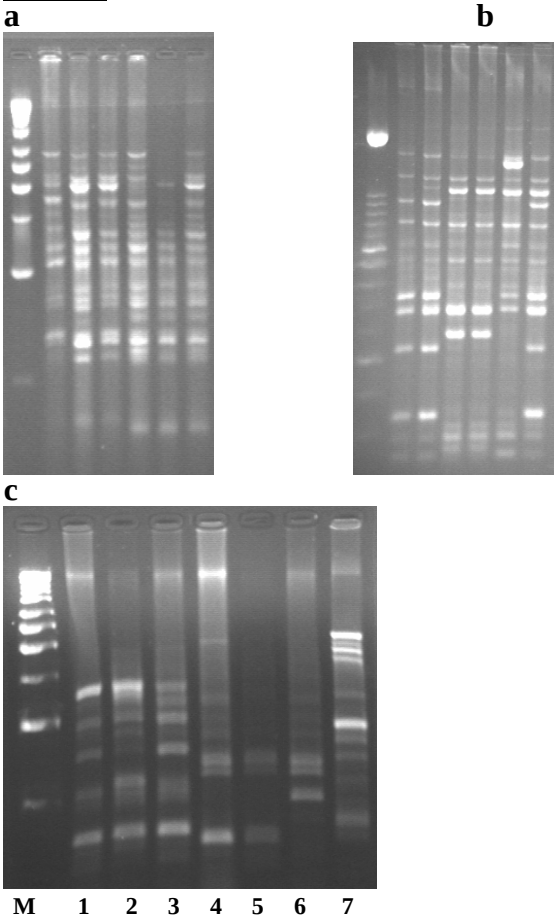

Fig. 1. Banding patterns showing different levels of polymorphism.

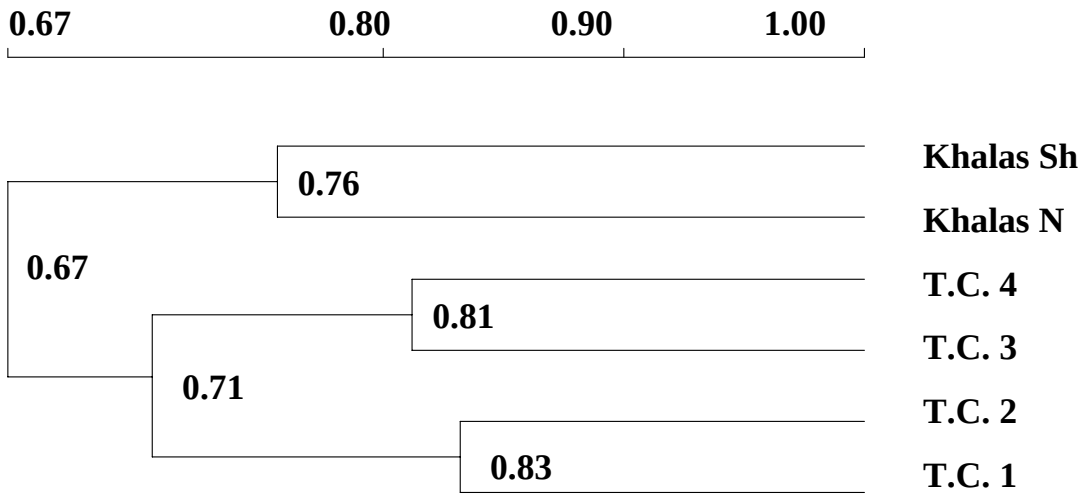

Fig. 2 a. A dendrogram of phylogenetic relationships among 6 DNA samples of two offshoot and four TC-derived plants of cv. 'Khalas', based on Nei and Li's similarity coefficients obtained from 13 RAPD primers.

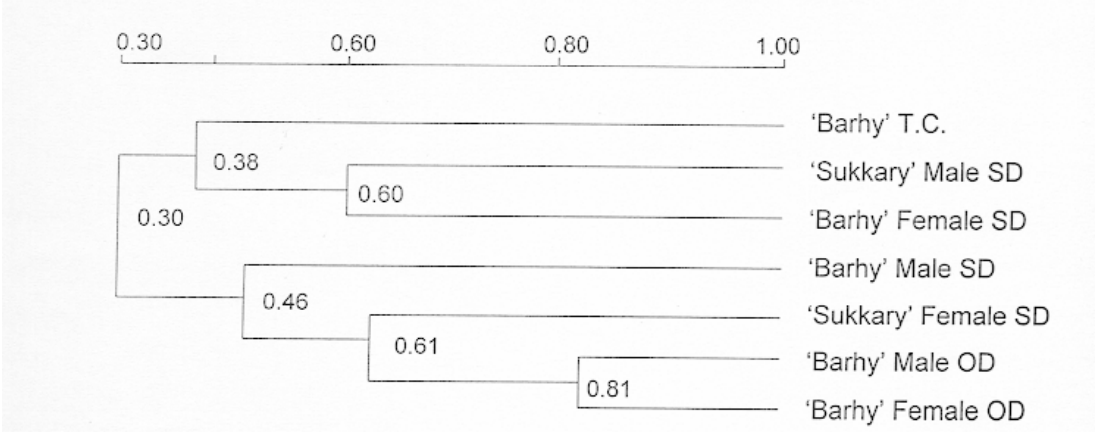

Fig. 2 b. A dendrogram of phylogenetic relationships among 7 DNA samples of male and female 'Barhy' and 'Sukkary' grown from seedlings and offshoot plants and a tissue cultured, based on Nei and Li's similarity coefficients obtained from 14 RAPD primers.

Sequencing of the Date Palm Genome

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Abstract

The date palm represents a major environmental and economic factor in arid climates in many countries around the world. Selection of specific varieties in large date palm groves is decreasing the genetic variation and increasing the susceptibility to a wide variety of diseases and reduced productivity. We are proposing to establish an international consortium to sequence and annotate the entire date palm genome in order to identify those genes responsible for disease resistance and growth characteristics that will allow us to enhance the quality and quantity of the date crop.

INTRODUCTION

Date palm contributes significantly to the economy of arid regions of the world including, but not limited to, the Middle East, Northern Africa and Southwestern USA. As a result it is imperative to continue to develop cultivars that are best suited to specific regions in terms of growth, disease resistance, fruit quality, fruit quantity and drought resistance among other characteristics. In order to take an efficient approach to the modification of the date palm, we are proposing to establish an international consortium of genetic scientists, breeders and growers to sequence the date palm genome. The results of this effort will allow us to improve cultivars in a specific and efficient way based on the genotypes determined from this effort.

Goals of the International Consortium

The central goal of the international consortium will be to determine the sequence of the date palm genome and provide annotation for the genome that will allow identification of genes relevant to specific phenotypes in the plant. Upon annotation of the genome it will be the goal to determine specific polymorphisms that are relevant to development of disease resistance, fruit quality, drought resistance, etc. These polymorphisms will allow determination of the genotypes that lead to specific phenotypes and further identification of genes relevant to the specific phenotypes. We then will be able to identify specific modifications of the genome to produce the phenotypes of interest. This will accelerate the genetic manipulation of the species.

How Will We Accomplish the Goals?

Table 1 shows the sizes of some related genomes. The palm date genome contains 250 megabases in the genome split into 36 chromosomes (Barakat, 1999; Zehdi, 2004) which makes it a relatively small genome. Genomic sequencing will require several routine steps that include DNA purification, cloning of fragmented DNA into bacterial artificial chromosomes (BAC), arraying the clones for sequencing, sequencing, assembly and annotation. The initial steps in the process will be contracted to laboratories that provide these technologies as services. We can purchase arrayed clones from a laboratory in the USA at a cost of $0.30 USD per clone. We will require approximately 100,000 clones to complete the project for a total of $30,000 USD. The latest technology introduced by 454 Life Sciences (Branford, CT, USA) will allow complete sequencing of one cultivar in less than six months at a cost of $2 million USD. This time frame and low cost has not been available until very recently and should be exploited for this project. This includes the assembly of the sequences into large contigs (continuous sequences) that will be ready for annotation and gene identification. We will at this point enlist a central laboratory, likely in United Arab Emirates, to create a web based data collection

and annotation website to allow free access of all consortium members to rapidly evolving data and information. Members will be able to add data from their laboratories and access data from the other member laboratories through this website.

Upon completion of the initial sequencing we will need to recruit laboratories to accomplish the "fill in" sequencing portion of the project, which involves obtaining and sequencing BAC's that contain sequences that were not identified in the initial sequencing. Many laboratories that are medium to high throughput sequencing laboratories around the world will be involved in this stage of the sequencing. During the fill in sequencing stage we will recruit and engage bioinformatics laboratories to identify gene regions throughout the genome utilizing comparative genomics and other plant genomes that are previously annotated. At this point we will incorporate two groups to identify expressed genes (functional genomics) and sequencing laboratories to sequence specific genes in additional cultivars (intraspecies comparative genomics). These groups will be divided into areas of interest such as fruiting genes, disease resistance genes, etc. These groups will focus on areas of interest and contribute information to the consortium through the user interface of the date palm genome website.

Functional genomics laboratories will utilize microarray technologies to identify qualitatively and quantitatively all of the genes expressed under given conditions in specific tissues. These experiments will be carried out on several different cultivars to identify expression patterns that are relevant to specific phenotypes in areas such as fruiting quality, disease resistance, drought resistance, etc. (see Table 2 for categories that will be included in the studies). Comparative genomics laboratories will be similarly divided according to the interest of individual laboratories and data will be compiled on the web site described above.

Genetic engineering laboratories (transgenic facilities) will then be recruited to introduce changes in the desired genomes with the sequences identified by functional and comparative genomics laboratories. Cultivars that are genetically engineered utilizing this advanced information will then be put into production in culturing and expansion laboratories. Those cultivars that respond appropriately will then be placed into "production" in appropriate facilities.

CONCLUSIONS

We are proposing to establish an international consortium to sequence and analyze the genome of the date palm. Due to the significant economic impact of this product in arid regions we plan to focus the effort in those regions that will benefit the most from the genetic modification of this plant. The initial stages of this effort will involve collecting information on those laboratories throughout the world that are interested in participating in the consortium and identifying sources of funding for this project.

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Tables

Table 1. Size of related genomes that have been previously sequenced.

Species	Genome Size
Pine	21,000 Mb
Rice	430 Mb
Date Palm	250 Mb
Arabidopsis	120 Mb
Human	3,000Mb

Table 2. Categories of genes that will be examined for expression and polymorphisms (from www.tigr.org).

Metabolism	**Cell Surface Components**	**Cellular Growth, Organization and Division**	**Cell Motility**	**Biological Niche**
Biosynthesis, catabolism and inteconverison of biomolecules	Objects (membranes, complexes) localized at or near the surface of cells	Morphology, intercellular structures	Chemotaxis, flagella, etc.	Optimal environmental conditions, hosts intercellular structures
Transport	**DNA Handling**	**Virulence**	**Selfish Genetic Elements**	**Quantitative Content**
Movement of molecules across biological membranes	Exchange, repair and modification of DNA	Host range and factors associated with pathogenesis	Stowaways on the ship of life: introns, inteins, phage, etc.	Counts, abundances and other metrics

In Vitro Differentiation of Zygotic Lines of Date Palm: Biochemical and Molecular Approaches to Sex Determination

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Keywords: zygotic embryos, sex type, enzyme activity, RAPD analysis

Abstract

The potential of biochemical and molecular markers in sex identification of in vivo grown and in vitro differentiated cultures of date palm was investigated. In vitro zygotic lines were proliferated from both mature and immature zygotic embryos of date palm. The mature embryo showed more potential in the in vitro differentiation responses compared with immature embryos. Incorporation into tissue culture medium of 3 mg/L 2ip + 5 mg/L 2,4-D gave the highest percentages of embryo germination and growth. Biochemical markers presented as enzyme activities was used for identification of sex type of date palm cultures. High levels of peroxidase activity were observed in adult female and offshoot female samples. Acid phosphatase and glutamate oxaloacetate enzymes gave a strong difference between male and female date palm. Early estimation of sex type of in vitro differentiated lines has been realized using the activity levels of the two enzymes. Random amplified polymorphic DNA (RAPD) technique was used to compare genetic material from male, female and unknown lines of date palm. RAPD analysis showed a relatively close relation between the two females (adult and offshoot) cultures, as they shared a large number of homologous bands. Although there is low relationship between male and female, results of similarity could not confirm link to sex or estimate the sex type of unknown clones.

INTRODUCTION

Date palm is a dioecious, perennial monocot plant that is commercially important in the Middle East and North Africa. The entire tree of date palm is utilized to provide food, shelter, fiber, clothing, furniture and many other products. Moreover, the date palm tree successfully tolerates extremely adverse environmental conditions, including drought, high temperature and salinity, which are the peculiar criteria of desert lands. It makes a significant contribution toward the creation of equable microclimates within the fragile oasis ecosystems, thus enabling sustainable agricultural development in many drought and saline affected regions (Barreveld, 1993). The genetics, morphology, morphogenesis and physiology of date palm is somewhat less understood than other fruit-tree crops. It has been difficult to study because they are native to tropical regions, have long life cycles and have diverse and unique growth habits compared to other fruit-producing trees. Date palm breeding is a long-term endeavor (Carpenter, 1979). Selection of high fruit-producing seedlings must await flowering. Most date palm varieties do not flower until 5-7 years after the germination of the seeds. Further, no viable means exists to identify male and female progeny in date palm. Propagation of date palm through seeds or zygotic embryos is desirable for improvement of the cultivars and for selection of disease resistance, fruit quality and high yield. Also, the early determination of sex type is very important for speeding up breeding programs. Plant tissue culture techniques offer excellent tools for rapid proliferation of sexual clones of date palm and other similar dioecious plants. The culture of mature embryos excised from ripened seeds is used to eliminate seed germination inhibitors or to shorten the breeding cycle. Immature embryo culture can also be used to germinate unique interspecific or intergeneric hybrids that do not survive in nature (embryo rescue) (Hodel, 1977). Embryo culture techniques involve isolating and growing immature or mature zygotic embryos under sterile conditions on an aseptic nutrient medium with the goal of obtaining viable plants (Mark, 1994). Despite its

economic and ecological importance, little research has been undertaken on genetic characterization of germplasm and varieties of date palm. Recently, molecular markers have been introduced in date palm programmes (Shah et al., 1994; Fernandez and Tantaoui, 1994; Coriquel and Mercier, 1994; Saker and Moursy, 1998). No data is available regarding molecular analysis of male and female plants or genetically based sex differentiation of date palm. There have been reports of understanding sex differentiation in *Aspargus officinalis* (Bracale et al., 1990) and the use of RAPD techniques for sex determination of papaya plants (Saker and Rady, 2003). For male sex-associated genetic factors, Y chromosome-specific restriction fragments have been reported from white campione (Dominson et al., 1996).

The objectives of this work were to study the in vitro proliferation of sexual lines of date palm through culturing of immature and mature embryos, analysis of male and female plants using biochemical and molecular markers and early estimation of the sex type of plantlets derived from embryo culture.

MATERIALS AND METHODS

Explants and In Vitro Culturing

Immature (excised from green fruits) and mature (from colored fruits) zygotic embryos of date palm (*Phoenix dactylifera* L.) cv. Zaghlool were used as tissue culture explants. Fruits were surface sterilized with 70 % ethanol for 3 min, 100 % commercial clorox (5.25 % NaOCl) for 20 min and thoroughly washed with sterilized distilled water. Under aseptic conditions, embryos were extracted from the seeds and placed on culture media. To study the role of growth regulators on in vitro differentiation of embryos of date palm, immature embryo explants were cultured on Murashige and Skoog (MS) medium supplemented with different combinations of 2,4-D, BA and 2ip. To investigate the effect of embryo maturation on in vitro morphogenic responses, immature and mature embryos were cultured on three selected combinations of growth regulators dependent on previous experiments. All cultures were incubated in the dark during the first two weeks of culturing and then transferred to light (3000 lux) with a 16/8 photoperiod at 25˚C. Morphogenic responses presented as organogenesis (%), fresh weight (g) and callus frequency (%) were recorded after four weeks of culturing. Experiments were designed in completely randomized design and the data were statistically analyzed using Standard Error (SE) described by Snedecor and Cochran (1967).

Enzymes Extraction and Activities

One gram leaf samples of adult female, offshoot female, adult male and in vitro differentiated zygotic lines (selected randomly) were taken and enzymes were extracted using 500 mM tris buffer, pH 7.0 containing 1 mM EDTA, 300 mM ascerbic acid, 2 % ß-mercaptoethanol, 10 mM EDTA, 5 % Tritonx-100 and 10% (w/v) PVP.

Esterase activity was determined as described by Gottlieb (1974). Assay reaction mixture (1m) contained 60 M mol sodium phosphate buffer, pH 7.0, 2 M mol of p-nitrophenylacetate, suitable dilution of enzyme and complete to one ml by distilled water. The change in absorbance was followed for 5 min with 30 sec intervals at 405 nm at room temperature. One unit of esterase was defined as the amount of enzymes which liberates one M mole of p-nitrophenol under standard assay conditions and specific activity was expressed as unit/mg protein. Peroxidase activity was determined according to Chance and Maehly (1955) by montoring the guaiacol oxidation by measuring the increase in absorbance at 470 nm of reaction mixture containing 0.45 ml of 5 mM hydrogen peroxide and an appropriate amount of crude extract. One unit of enzyme activity was defined as the amount of enzyme which caused on O.D change at 470 nm per min at 25˚C under standard assy conditions. The activity of acid phosphatase was determined according to the method described by Dinan et al. (1983). Glutamate oxaloacetate (GOT) was determined colourimetrically according to the Reitman and Frankel (1957).

Randomly Amplified Polymorphic DNA (RAPD) Analysis

DNA isolation was performed using the Cetyl Trimethyl Ammonium Bromide (CTAB) method of Doyle and Doyle (1990). Half a gram of fresh samples was ground to powder in liquid nitrogen with a pre-chilled pestle and mortar, suspended in 5 ml preheated CTAB buffer, and incubated at 65°C for 1 hour with occasional shaking. The suspension was then mixed with 1/3 volume of chloroform, mixed gently, centrifuged and the upper layer was transferred to a new sterilized tube. Extraction was repeated with an equal volume of chloroform. The aqueous layer was transferred to a new tube, 2/3 volume of isopropanol was added and nucleic acids were either spooled using a Pasteur pipette or sedimentated by centrifugation. The pellet was washed carefully twice with 70% ethanol, dried at room temperature and resuspended in 0.5 ml TE buffer. The enzyme, RNAse A (20µg) was added to the resuspended mixture to digest any contaminating RNA and the tube was incubated at 37°C for 30 min. To remove the enzyme and other contaminating protein, phenol/chloroform extraction was performed.

The polymerase chain reaction (PCR) mixture (25 µl) consisted of 0.8 units of Taq DNA polymerase, 25 pmol dNTPs, and 25 pmol of random primer, and 50 ng of genomic DNA. The reaction mixture was placed on a DNA thermal cycler. The PCR program included an initial denaturation step at 94°C for 2 mins followed by 45 cycles with 94°C for 1 min for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72 °C for 10 minutes were carried out. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. Four 10-mer primers (Operon technologies Inc., Alameda, California) randomly selected were used in RAPD analysis. A 100 bp DNA ladder (Promga) was used as a Marker with molecular size of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The amplified pattern was visualized on a UV transilluminator and photographed. The genetic similarity was calculated using the average linkage between groups according to Lynch (1990).

RESULTS AND DISCUSSION

Effect of Growth Regulators on In Vitro Differentiation

The effect of the supplementation of basal MS-medium with 2,4-D at 5 and 10 mg/L and in combination with 3 mg/L of both BA and 2ip on differentiation of cultured immature zygotic embryos of date palm are summarized in Table (1). It should be noted that the morphogenic responses varied depending on the type of growth regulators. Generally, 2ip showed a superiority over BA in shoot differentiation and organogenesis. However, 2,4-D alone was suitable for callus induction. The highest percentage of immature embryo germination (70%) and the highest value of fresh weight of differentiated cultures (1.30 g) were observed with medium containing 5 mg/L 2,4-D + 3 mg/L 2ip. The lowest percentage of germination (20%) and subsequently the average fresh weight (0.7 g) were registered on medium containing 10 mg/L 2,4-D. However, the highest frequency of non-morphogenic responses was noticed when BA alone was added to culture medium. Addition of 2,4-D alone enhanced callus proliferation and 60 % of the cultures proliferated callus onto MS supplemented with 10 mg/L 2,4-D. The quick and high morphogenic potential recorded here may be due to the meristematic nature of immature embryos as reported by Tisserat (1979 a,b). The results of the present study also revealed that 2ip was more effective in germination and shoot proliferation of in vitro cultured immature embryos of date palm (Fig. 1-A,B). However, relatively high levels of 2,4-D stimulated callus proliferation. Similar observations were reported by Tisserat (1984) and Saker et al. (1998).

Effect of Embryo Maturation on In Vitro Differentiation

Data of morphogenic responses of immature and mature zygotic embryos of date palm cultured in vitro are presented in Table 2. Results indicated that mature embryos showed relatively more potential than immature embryos in their responses to callus

proliferation. Also, the highest percentages of organogenesis and differentiation to complete plantlets were observed with mature zygotic embryos of date palm. This may be due to endogenous hormones and higher storage proteins and carbohydrates contained in mature embryos. Inclusion of 3 mg/L 2ip in the culture medium strongly enhanced the in vitro germination of mature embryos and this treatment produced the highest percentage of organogenesis (85) and resulted in the highest percentage of complete plantlets (75). However, the highest value of fresh weight of differentiated cultures was recorded when mature embryos grew on medium containing 5 mg/L 2,4-D +3 mg/L 2ip. It is important here to mention that the various responses in vitro of both mature and immature zygotic embryos of date were recorded over a relatively short period (3-4 weeks). This illustrates the value of this simple technique in date palm improvement through either genetic manipulation or traditional breeding programs. The present results are in accordance with those of Saker et al., (1998) and Bekheet et al. (2001). However, Mater (1983) used medium contained 2 mg/L IAA + 2 mg/L Kin for plant regeneration from immature embryos of date palm.

Plantlets Development and Acclimatization

Shoots derived from proliferated zygotic embryos were transferred into charcoal containing medium for in vitro elongation and rooting improvement. The plantlets with a healthy root system were successfully transplanted to free-living conditions within a short period of acclimatization (Fig. 1-C). Tisserat (1984) reported that a high survival rate (nearly 100 %) could be obtained when date palm plantlets with 2-3 foliar leaves with a shoot length greater than 10 cm (and a well-developed adventitious root system) were transplanted in pots containing a mixture of peatmoss and vermiculite.

Enzymes Activities

As a step to identify biochemical markers linked to sex in date palm, the activities of esterase, peroxidase, acid phosphatase and glutamate oxaloacetate (GOT) in adult female, offshoot female, adult male and two zygotic lines differentiated in vitro (randomly selected) were measured. Data of esterase activities revealed no clear variation amongst the five clones of date palm cultures. However, high levels of peroxidase activities (1116 and 914 units/g fresh weight) were recorded in adult female and offshoot female, respectively. Concerning acid phosphatase and GOT, there were clearly differences between both female clones and the male clone. It is important here to observe that there was a strong similarity between one zygotic line (1) and the male clone in the activities of the three enzymes. Based on the obtained results, it could be concluded that acid phosphatase and GOT activities can distinguish male from female date palm on a biochemical level and can be used primarily to estimate the sex type of in vitro differentiated zygotic lines. Biochemical markers presented as peroxidase banding patterns have been used for sex identification in papaya by Saker and Rady (2003). Moreover, the search for genes associated with sex chromosomes in asparagus was initiated by screening for enzyme variants encoded by polymorphic loci (Bracale et al., 1990). Their results indicated that gene expression in young male and female flowers is very similar and only later does differential expression take place.

Rapid Analysis

The PCR-based markers for sex prediction in date have the advantage that they are not affected by environmental conditions in which the plants are grown or by epistasis (gene interaction). Thus, sex prediction can be done at any developmental stage of plant growth. In this study, DNA isolated from adult female, offshoot female, adult male and two randomly selected clones of zygotic lines differentiated in vitro were subjected to RAPD analysis. Six random primers (k1-k6) were screened in RAPD analysis for their ability to produce sufficient amplification products (Table 4). The results of DNA fingerprints generated by PCR amplification using the six random primers are presented in Figures 1 and 2 and Table 5. The number of fragments generated per primer varied

between 1 and 5. The total number of bands was 22 and the average percentage of polymorphism was 51.6. The primers k3 and k4 gave the highest number of bands (5) and percentage of polymorphism (80). As shown in Figures 1 and 2 and Table 5, some clones are closely related to each other and have similar patterns of bands but possess one or more different bands that can differentiate between them. RAPD profiles of PCR products using primer k1 indicated that unique DNA fragment (1000 bp) is present in female clones and this band is completely absent in the adult male clone. However, other polymorphic bands were detected among either male or female clones with other primers tested. Data of similarity index (Table 6) revealed that there is low relationship between male and female clones. Also, female clones are relatively closely related to each other and they have a large number of homologous bands. Concerning the in vitro differentiated cultures, there was high similarity with each other. Due to the weak relationship of in vitro plants to either male or female clones, we cannot confirm a clear link to sex or estimate the sex type of these unknown clones. Our results are in line with those of Soliman et al. (2003) on date palm. They added that identification of male date palm needs more advanced molecular studies. In this respect, SCAR has been used successfully to design markers for sex determination in hop (Polley et al., 1997) and asparagus (Jiang and Sink, 1997). Moreover, markers were generated by DNA amplification fingerprinting (DAF) for male, female and hermaphrodite papaya progenies of a cross between Khaeg Dum (Thai cultivar) and Richter Gold (Australian cultivar) (Somsri et al., 1997). In addition, randomly amplified polymorphic DNA (RAPD) markers were also developed for interspecific Carica hybrids (Magdalita et al., 1997).

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Tables

Table 1. Effect of different combinations of growth regulators on the in vitro morphogenic responses of immature embryos of date palm.

Culture media	Organogenesis (%)	Fresh weight (g)	Callus frequency (%)	No responses (%)
MS-basal medium	40	1.10 ± 0.11	20	40
MS+ 5 mg/L 2,4-D	30	0.85 ± 0.09	50	30
MS+ 10 mg/L 2,4-D	20	0.70 ± 0.20	60	20
MS+ 3 mg/L BA	30	0.95 ± 0.12	--	70
MS+ 3 mg/L 2ip	60	1.20 ± 0.30	10	30
MS+ 5 mg/L 2,4-D + 3 mg/L BA	35	0.70 ± 0.15	25	40
MS+ 10mg/L 2,4-D + 3 mg/L BA	20	0.75 ± 0.18	40	30
MS+ 5 mg/L 2,4-D + 3 mg/L 2ip	70	1.30 ± 0.08	10	20
MS+ 10 mg/L 2,4-D +3 mg/l BA	50	0.90 ± 0.25	20	30

Each treatment is the average of 20 replicates. ± SE

Table 2. Effect of embryo maturation in combination with selected culture media on in vitro morphogenic responses of zygotic embryos of date palm.

Culture media	Organogenesis (%)		Callus induction (%)		Fresh weight (g)		Cultures developed to complete plantlet (%)	
	I E	ME	I E	ME	I E	ME	I E	ME
MS basal medium	40	60	20	20	1.05 ±0.10	1.20 ±0.15	20	40
MS + 3 mg/L 2ip	60	85	10	15	1.25 ±0.20	1.40 ±0.30	20	75
MS+5 mg/L 2,4-D + 3mg/L 2ip	65	80	15	20	1.33 ±0.09	1.70 ±0.20	25	70

Each treatment is the average of 20 replicates.
± SE
IE = Immature embryo ME = Mature embryo.

Table 3. The activity levels of esterase, peroxidase, acid phosphatase and glutamate oxaloacetate(GOT) enzymes in male and female plants and in vitro differentiated zygotic lines.

Type of culture	Enzymes			
	Esterase	Peroxidase	Acid phospatase	GOT
Adult female	160	1116	92	249
Offshoot female	137	916	79	214
Adult male	130	350	132	190
Zygotic tissue culture (1)	138	390	125	180
Zygotic tissue culture (2)	153	475	71	206

Table 4. The sequence of the selected random primers, total number of amplification products per primer, number of polymorphic bands and percentage of polymorphism.

Primer	Sequence (5\→3\)	Total number of bands	No. of polymorphic bands	Percentage of polymorphism
K1	TGGCGACCTG	4	3	75
K 2	GAGGCGTCGC	1	4	-
K 3	CCCTACCGAC	5	4	80
K4	TCGTTCCGC	5	4	80
K5	CACCTTTCCC	4	3	75
K6	GAGGGAGAG	3	3	-

Table 5. RAPD products of different date palm clones, generated by PCR amplification using the random primer from K1 to K6.

Primer		M.W. of bands (bp)	Date palm cultures				
			Male (adult)	Female (adult)	Female (offshoots)	Vitroplant (1)	Vitroplant (2)
	1	1000	-	+	-	-	+
K1	2	700	-	+	-	-	-
	3	500	+	+	+	-	+
	1	1000	-	-	-	-	+
K2	2	800	-	+	-	-	+
	3	500	-	+	-	-	-
	4	400	-	-	-	-	+
	1	1000	-	-	-	+	-
K3	2	800	+	+	+	-	+
	3	500	+	-	-	-	+
	4	400	-	+	+	-	-
	1	700	-	+	+	+	+
K4	2	400	-	+	+	-	+
	3	200	+	-	-	-	+
	4	100	+	+	+	+	+
	1	900	+	-	+	-	-
K5	2	450	+	-	-	+	+
	3	200	+	-	-	-	-
	1	850	+	+	+	-	-
K6	2	700	+	-	+	-	-
	3	600	+	-	+	-	-
	4	300	+	+	+	+	-

Table 6. Genetic similarity index of male and female plants and in vitro differentiated zygotic lines of date palm.

Clone	Adult male	Adult female	Offshoot female	Zygotic tissue culture (1)	Zygotic tissue culture (2)
Adult male	100				
Adult female	40	100			
Offshoot female	46	46	100		
Vitroplant (1)	25	27	36	100	
Vitroplant (2)	33	50	50	25	100

Figures

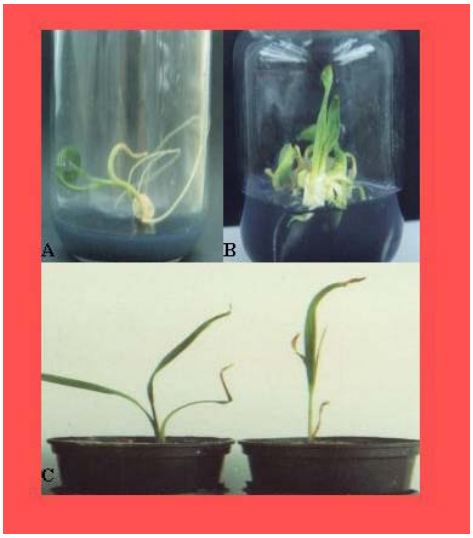

Fig. 1. A) Germination of immature embryos of date palm cultured in vitro. B) Shoot proliferation on MS+ 5 mg/L 2,4-D + 3 mg/L 2ip. C) Date palm plantlets adapted to free living conditions.

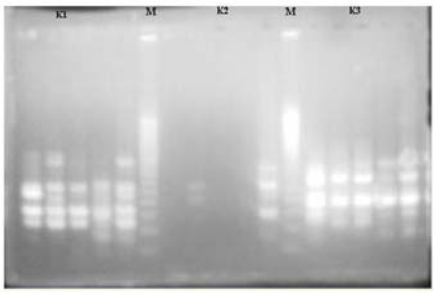

Fig. 2-A. RAPD profile of adult male, adult female, offshoot female, vitro plant 1, vitroplant 2 and the DNA marker (M) from left to right using random primers i.e. K1, K2, and K3.

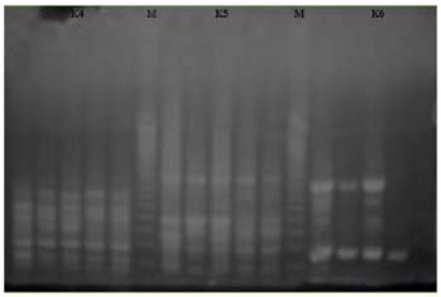

Fig. 2-B. RAPD profile of adult male, adult female, offshoot female, vitro plant 1, vitroplant 2 and the DNA marker (M) from left to right using random primers i.e. K4, K5, and K6.

The Use of RAPDs for the Detection of Genetic Stability of Regenerated Plantlets of Barhee Palm in Iraq

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Abstract

The aim of this study was the use of RAPD-PCR technique to ensure the genetic stability of date palm plantlets produced by tissue culture. To achieve this, shoot tip explants (0.5- 1cm) were excised from date palm offshoots (Barhee) and divided into four equal parts. These parts were cultured on MS media containing NAA (25mg/l), 2-ip (2 mg/1) and activated charcoal (3 gm/l). Nodular callus was produced from shoot tip explants after four subcultures. Somatic embryos developed and formed shoots and roots. At the end of the propagation process young date palm plantlets were produced. RAPD-PCR analysis using universal primers was performed on DNA extracted from the samples taken randomly from the stalk at the process stages: somatic embryos and plantlets, and also from the fresh healthy leaves of the mother offshoot (Barhee). PCR conditions were optimized. Reproducible RAPD patterns were obtained using 30 primers. Three primers (OPC.16, OPG.O8 and OPN.16) of the 30 produced polymorphic bands in some of the samples tested when compared to the DNA fingerprint of the mother offshoot. After mass propagation of date palm via somatic embryogenesis, genetic variation has been reported for several cultivars and has been affected by the plant genotype, number of in vitro subcultures (age of cultures), the nature of the explants and the composition of culture media. Here we report for the first time in Iraq, the efficiency of RAPD fingerprints as a control technique and a simple fast molecular marker for the detection of genetic variations in regenerated plantlets so that tissue culture can be successfully applied for large scale propagation of elite varieties.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) was one of the earliest cultivated tree crops in Mesopotamia. It is considered to be an essential and important plant crop in the Arabian region. Date palms are usually propagated by offshoots, which are mainly produced during the early life of the palm in limited numbers, depending on variety and other factors. Propagation by seeds is undesirable and impractical (Jassim, 1999; Zaid and De Wet, 2002). Prior to 1991, Iraq was the largest producer of dates in the world (Food and Agriculture Organization, 2004). However, as a result of the war, date palm populations have been severely decreased, e.g. the 16 million date palm trees around Basrah was reduced to 3 million in 2003 (MacFarquhar, 2003). Therefore, increasing efforts are being made to propagate high quality varieties.

Plants tissue culture techniques have been used to propagate date palms by somatic embryogenesis, and hundreds of somatic embryos were produced using shoot tips as explants (Omar, 1988; Tisserat, 1991; Jasim and Saad, 2001). Most of the commercial laboratories focus on micropropagation of date palm. Although successful results were reported, no evaluation strategies have been used at an early stage to assess the uniformity of tissue-cultured trees. Long lived plants may have mutants even in apical meristem (Klekowski, 1985). In the last few years, variations have been noticed in in vitro date palm plants (Barhee, Medjool and khalas), e.g. delay in fruiting, fruit set failure and dwarf trees (Al- Wasel, 2000; Zaid and De Wet, 2002). These cases greatly affect the utilization of tissue culture for micropropagation. Various techniques are used to certify the trueness-to-type of the plantlets produced, and recently developed DNA molecular markers for this crop may allow researchers to evaluate tissue culture techniques (Corniquel and Mercier, 1994). In this study Random Amplified Polymorphic DNA (RAPD) markers were used as

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

a reliable, fast and simple fingerprinting tool to confirm if the palms derived from tissue culture are true-to-type to the mother plant.

MATERIALS AND METHODS

Plant Materials and Tissue Culture

Young healthy offshoots were separated from selected date palm mother plants (Barhee) growing at Basrah Date Station, Iraq, and were used for in vitro propagation and DNA extraction. Offshoots were dissected acropetally until the shoot tips were reached. Shoot tips consisting of the apical meristem and soft inner leaves (5 mm in length) were removed from offshoots and placed in an anti–oxidant solution (100 mg ascorbic acid and 150 mg citric acid per liter of water). After surface sterilization, the shoot tips were divided longitudinally into four equal segments as described by Tisserat (1979). Shoot tip pieces were cultured on agar media for callus initiation and growth in conical flasks containing 50 ml of the medium which was composed of MS salts and growth regulators such as: NAA (25mg/L), 2-ip (2mg/L) and activated charcoal (3000mg /L). The pH of all media was adjusted to 5.7 before addition of agar as described by Jasim and Saad (2001). Embryogenic nodular callus initiated from shoot tip segments after 6-8 months. Micropropagation was achieved by transferring small masses of embryogenic nodular callus from high auxin cultures to media containing 0.1mg/L NAA, 200mg/L glutamine and 40g/L sucrose. Uniform sized somatic embryos about 1.5-2 cm were cultured separately on the same media to complete their germination and produce whole plantlets.

DNA Extraction

Total cellular DNA was extracted from healthy leaves of offshoots and from samples taken randomly from the stalk at the process stages: somatic embryos and from the leaves of plantlets. Around 1.5-2 g of leaf tissue was ground to a fine powder in liquid nitrogen. Ten milliliters of hot (60°c) 1x CTAB extraction buffer (1% CTAB, 0.7M NaCL, 0.1M Tris-HCL pH 8, 20 mM EDTA and 1% β- mercaptoethanol) were added, mixed well, and incubated at 60°C in a water bath. After 30 min of incubation with gentle swirling, the resulting cell lyses were extracted with an equal volume of chloroform / isoamyl alcohol (24:1, v/v). The cell lysate was then centrifuged at 8000g at 20°c for 20 min. The aqueous phase was transferred into another tube and precipitation occurred with the addition of 0.66 volume of isopropanol. The precipitate was then collected by centrifugation at 8000 g at 20°c for 20 min. Pellets were washed with 70% ethanol, dried and dissolved overnight at 4°C in 1ml of TE buffer (10mM Tris – HCL pH 8.0, 1mM EDTA). After purification, the resultant DNA (marked as stalk DNA) was quantified and its integrity was determined after agarose gel electrophoresis as described by Sambrook et al. (1989).

RAPD Analysis

A total of 30 random decamer primers (Operon Technologies, Alameda, USA) were used for RAPD amplification based on the protocol of Williams et al. (1990). PCR reactions were carried out in 25 µℓ volumes containing 25 ng of total genomic DNA, 0.2µM of a single primer, 100 mM of each dNTP, 10 mM Tris – HCL pH 8.3, 50 mM KCL, 2 mM $MgCL_2$ and 0.5 unit *Taq* DNA polymerase (perkin – Elmer/Cetus). The mixture was covered with 25 µl of mineral oil. Amplification was performed in Thermal Reactor-Hybaid using program for RAPD: I cycle at 94°c for 4 min, 40 cycles as follows: - 94°c for 1 min, 36°c for 1 min, 72°c for 2 min, the last cycle at 72 °c for 7 min, and an optional soak period at 4 °c. Amplification products were loaded on 1% agarose gels and stained with ethidium bromide (0.5 mg/ml). Amplifications for each primer were performed at least twice and only reproducible products were taken. DNA was visualized on a UV transilluminator and photographed using Polaroid black and white film (667-type). Fragment length was estimated by comparison with standard size markers (λ DNA digested with *hin*d III).

RESULTS AND DISCUSSION

Initiation of in Vitro Cultures

Shoot tip explants cultured on a callus induction medium containing MS salts, supplemented with a high level of auxin NAA (25mg/l), organic nitrogen (200mg/l glutamine) with an enriched sugar concentration (40g/l sucrose) proved to be effective for initiation of good callus growth and somatic embryogenesis in date palm tissues. Our results indicated that the addition of both glutamine at 200mg/l and sucrose at 40g/l increased callus quantity and numbers of somatic embryos produced, compared to the control treatment (0 mg/l glutamine and 30g/l sucrose). This might be because the amino acid nitrogen was more available for absorption than inorganic nitrogen. Sucrose as a source of energy was needed by somatic embryos for growth. This result was consistent with previous studies (Letouze et al., 1998; Jasim and Saad, 2001).

Following repeated subculturing, the aggregated yellowish callus was initiated from the first shoot tip cultures after 6-8 months. Subculturing of embryogenic callus to media containing a low level of auxin, NAA (0.1mg/l), enhanced separation and enlargement of free single nodules and their subsequent germination into small normal embryos (Fig. 1a,b). At this stage of in vitro culture, embryos were transferred to a semi-sold medium containing half strength MS salts, 30g/l sucrose and 3g/l activated charcoal. The cultures were placed under light of 50μmoles m^{-2} s^{-1} for 16 hours light and 8 hours dark.

The resulting plantlets (10-12 cm long) via somatic embryogenesis survived acclimatisation to soil inside a glasshouse (Fig. 2).

Genetic Stability of Somatic-Embryo-Derived Plants

Initially, the RAPD technique was applied as a preliminary genetic comparison between amplified DNA patterns of plants derived from offshoot and plants derived from tissue culture at two stages: somatic embryos (2 samples) and plantlets (4 samples). Total cellular DNA was extracted from the samples tested.

A preliminary experiment was conducted to generate RAPD patterns with a large number of primers to identify those that would be suitable in the present study. To ensure reproducibility of RAPD marker data, the primers generating no or faint (non reproducible) bands were discarded (OPE.08, OPE.20, OPM.20, OPP.10, OPS.13 and OPT.16). Thirty primers showed clear and good amplification results. Most of them, about 27, generated monomorphic banding patterns for all the samples tested (Table 1).

Similar results were reported in previous molecular marker studies (Letouze et al., 1998; El-Hammady et al., 1999). An example of a RAPD monomorphic banding pattern obtained by the primer (OPC.14) is shown in Fig. 3(a).

Only three of the thirty primers showed polymorphic banding patterns in one sample (somatic embryo). These primers were OPG.08, OPC.16 and OPN.16, as shown in Fig. 3(b,c,d). The number of amplified fragments generated by these primers varied from 5 (OPG.08) to 7 (OPN.16) with an average of 6 bands for each sample. Molecular weight of the scored bands ranged from 0.2 to 2.3 kb. Polymorphisms were detected by the presence or absence and molecular weight of amplified fragments for each primer used. For example, it appears from Fig. 3(b,c,d) that there was genetic changes in the DNA amplificaion pattern for sample 3 (somatic embryo) when compared to the offshoots and other plants derived from somatic-embryos. Table 2 summarized the genetic variations revealed by RAPD markers.

Some genetic variation is expected between plants derived from somatic embryo and mother offshoots, although plant tissue culture has been regarded as a rapid means of vegetative propagation in which identical (phenotypically and genetically) clones are produced. Somaclonal variation in some species may result from changes in nuclear, mitochondrial or chloroplast genomes. Previous studies were reported on somaclonal variation in some species resulting in inferior off-types with little commercial value, for example, sugarcane and banana (Taylor et al., 1995; Henry, 1997). Dwarfism and

abnormal floral development were reported as somaclonal variation in tissue culture-derived date palm trees (Al- Wasel, 2000).

Frequency of variations depends on many factors: plant genotype, type of technique used, type of explants, type and concentration of growth regulators, age of callus culture or number of sub-cultures. In our case, at least two reasons could be put forward to explain the results: firstly, long lived plants (date palm and fruit trees) may have mutant tissue in their apical meristems (Klekowski, 1985), and secondly, the technique used for propagation (somatic embryogenesis) is based on callus production, multiplication, and long culture maintenance periods (Al-Wasel 2000).

Several different DNA molecular methods have been used to screen date plants derived from tissue culture for genetic stability e.g. RFLP, RAPD, AFLP and more advanced ISSR (Corniquel and Mercier, 1994). Each technique has its own requirements, sensitivity and reliability. Molecular marker techniques are now relatively routine. RAPD techniques have been reported to be useful for studying genetic variation in date palm (Letouze et al., 1998; El-Hammady et al., 1999). This technique has many advantages such as: experimental simplicity, universal primers, small amount of DNA required, no prior DNA sequence information is required and no radioactive detection. Consequently, more samples can be analysed in one day. We have demonstrated that RAPD markers are an efficient, easier and faster way to evaluate the genetic stability of plants derived from somatic embryo by comparison of their amplified DNA patterns to those of the mother offshoots.

In conclusion, date palm is a much neglected plant crop in Iraq, especially in the last ten years. Therefore increasing attention is being given to micropropagation of high quality varieties. The RAPD technique is useful to confirm genetic stability at an early stage of plants derived from tissue culture. These control techniques (PCR-RAPD) must be updated as new scientific advances in DNA markers are developed, so that tissue culture can be successfully applied to large-scale propagation of elite varieties.

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Tables

Table 1. The set of Operon primers that showed monomorphic bands in all samples tested.

1	**OPA.01**	**7**	**OPB.03**	**13**	**OPC.05**	**16**	**OPD.01**	**21**	**OPE.01**
2	**OPA.02**	**8**	**OPB.05**	**14**	**OPC.07**	**17**	**OPD.02**	**22**	**OPE.02**
3	**OPA.03**	**9**	**OPB.06**	**15**	**OPC.14**	**18**	**OPD.05**	**23**	**OPE.03**
4	**OPA.05**	**10**	**OPB.07**			**19**	**OPD.08**	**24**	**OPE.05**
5	**OPA.08**	**11**	**OPB.09**			**20**	**OPD.09**	**25**	**OPE.07**
6	**OPA.20**	**12**	**OPB.10**					**26**	**OPE.08**
								27	**OPE.20**

Table 2. Polymorphisms revealed by RAPD markers using the primers (OPG.08, OPC.16 and OPN.16). (+) presence of the amplified band. (-) absence of the amplified band.

				Samples tested					
	Primers	M W bp of polymorphic bands	1 offshoot	2	3	4	5	6	7
1	OPG.08 OPG.08	2000 1800	+ +	+ +	- -	+ +	+ +	+ +	+ +
2	OPC,16 OPC.16	2300 2000	+ +	+ +	- -	+ +	+ +	+ +	+ +
3	OPN.16 OPN.16	1900 400	+ +	+ +	- -	+ +	+ +	+ +	+ +

Figures

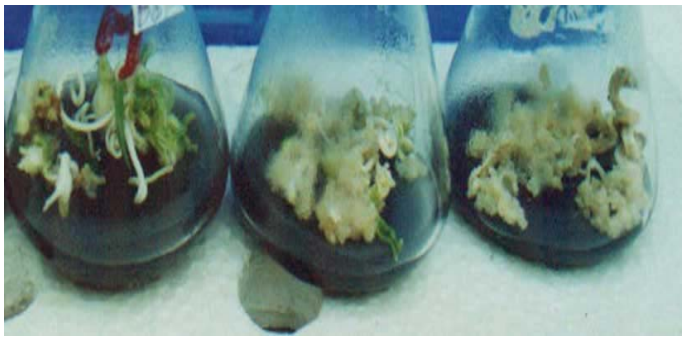

Fig. 1a. Formation of somatic embryos from dividing cells derived from the shoot tip explants.

Fig. 1b. The development of somatic embryos into plants in a growth room in which light and temperature were carefully controlled.

Fig. 2. Healthy date plantlet with good root system after transfer to the soil.

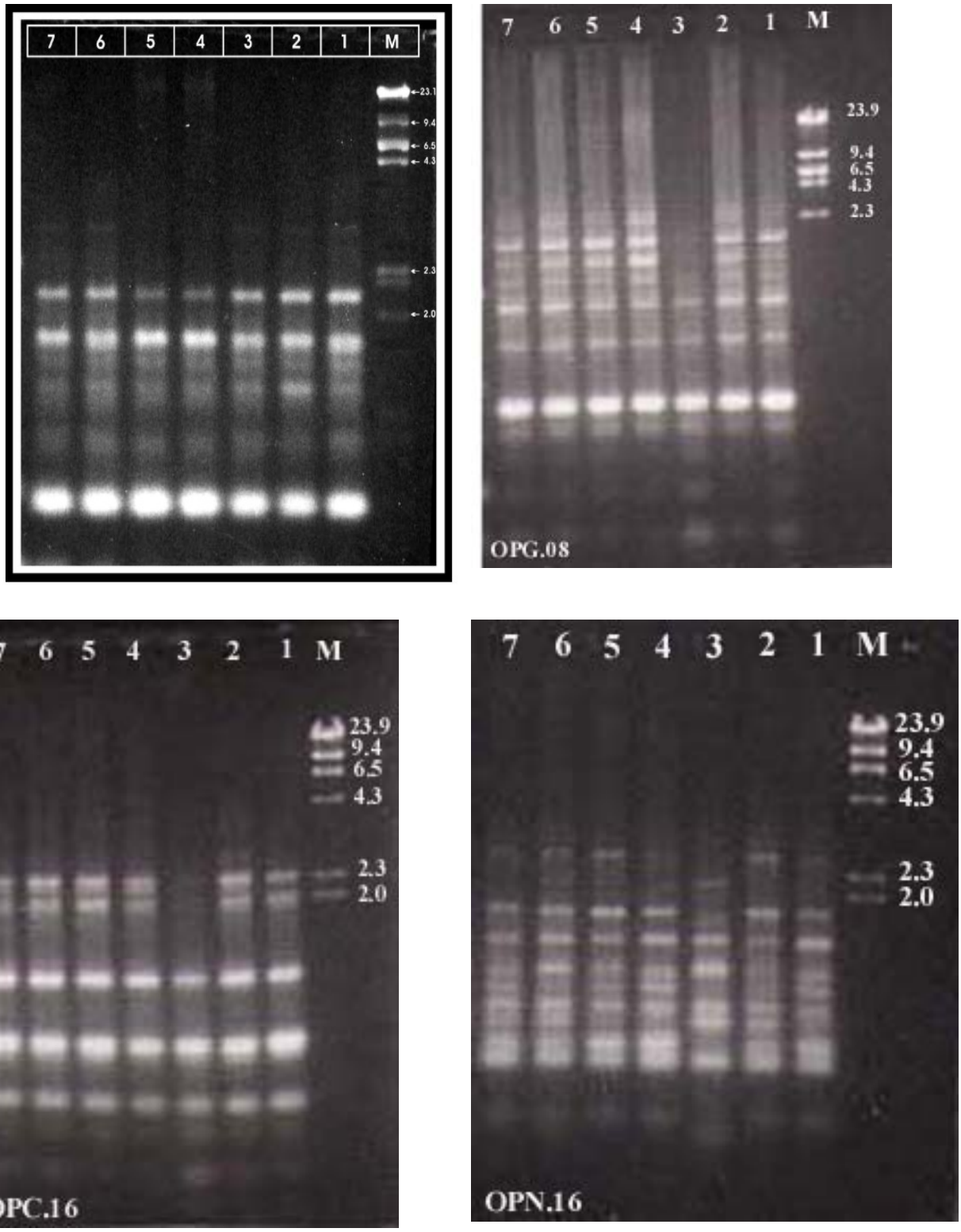

Fig. 3. (a) Monomorphic patterns of the samples tested [(1) Barhee offshoot (2,3) somatic embryos,(4,5,6,7) date plantlet using the primerOPC.14], (b,c,d):Polymorphic banding patterns using the primers (OPG.08,OPC.16,OPN.16), M:Standard molecular weight marker (λ DNA digested with *Hin*d III).

AFLP Variation in Tissue Culture-Derived Date Palm (*Phoenix dactylifera* L.) Plants

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Abstract

Two tissue culture techniques, namely organogenesis and embryogenesis, were compared using ten United Arab Emirates date palm varieties (*Phoenix dactylifera* L.). The frequency of somaclonal variation in the resultant plants was compared and related to the types of variation at the DNA level, estimated by AFLP analysis (amplified fragment length polymorphism). The experimental conditions for the generation of reproducible AFLP markers were optimized using *Eco*RI and *Mse*I primers. All ten date palm varieties were distinguished by AFLP fingerprinting.

The incidence of somaclonal variation in plants regenerated through organogenesis tissue culture was low in contrast to the plants derived from asexual embryogenesis. Furthermore, organogenesis derived plants were genetically closer to the mother plant.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the most important fruit crop trees in a number of Middle East countries. In the United Arab Emirates (UAE), the date palm plays economic, environmental and social roles. The tree was the major source of staple food, shelter, fiber and furniture for earlier societies, as well as being a source of many other by-products and raw materials which are essential to traditional lifestyles in the UAE. Demand for this tree has increased tremendously during the last three decades. Sexual propagation using seeds is not appropriate for commercial production because of the dioecious nature of date palm and high genetic heterozygosity of the species, and results in a lack of uniformity in male and female seedlings. Vegetative propagation using offshoots ensures clonal varieties with economically important traits and true-to-type. However, this traditional method is not ideal because it is slow and inefficient for the rapidly growing demands of the date industry. Therefore, biotechnology has provided a promising asexual alternative for the production of plantlets of elite date palm varieties. Plant tissue culture techniques have been employed to clone a wide range of economically important palms, e.g. coconut (Eeuwens, 1976), oil palm (Rabechault and Martin, 1976) and date palm (Reuveni et al., 1972; Tisserat, 1979a, b; Zaid and Tisserat, 1983a, b).

Organogenesis and asexual/somatic embryogenesis are the two techniques currently used in various laboratories in the world for in vitro mass propagation of date palm. However, these techniques are not without problems. One of the major weaknesses of mass propagation using plant tissue culture techniques is the appearance of undesired plant off-types (somaclonal variants) which are clearly different from the mother plant. Variation phenomena have been described in about 150 different plant species derived from tissue culture techniques (Pierik, 1987), including date palm. In a commercial laboratory where thousands of plantlets are generated from a single explant, genetic uniformity is critical. Unfortunately, in date palm, should an off-type plant be produced with modified fruiting characteristics, such a plant will only be detected four to five years

after purchase from the laboratory, by which time the plant has incurred substantial development costs for the farmer.

Correct identification of date palm cultivars using morphological markers is usually not possible until fruits are produced and frequently requires a large set of phenotypic data that is often difficult to assess and sometimes variable due to environmental influences (Sedra, 2001; Sedra et al., 1998; Kunert et al., 2003). Performance characteristics (pollination, yield, and fruit quality or disease susceptibility) are expressed only in the mature date palm after several years of development. Vegetative characteristics such as leaf length, leaflet zone, leaflet angle, thorn area and thorn length and angle were not reliable traits to assess the uniformity of tissue culture-derived plants against conventionally propagated plants, since there were significant differences not only between the tree types (either tissue culture-derived or conventionally propagated) but also among trees of the same type (Al-Wasel, 2000a, 2000b; 2001).

Due to developments in the field of molecular genetics, various techniques to verify the trueness-to-type, and/or to analyze genetic variation have emerged during the last decade. These include histocytological examination, iso-enzyme analysis and, more recently, DNA fingerprinting techniques such as RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), microsatellites, RDA (representational difference analysis) and microchip technologies.

DNA fingerprinting techniques have an advantage in that the DNA content of a cell is independent of environmental conditions, organ specificity and growth stage (Ainsworth et al., 1996; Kunert et al., 2003). Differences in DNA sequences between genotypes and the somatic stability of DNA sequences within each individual provide the basis for using DNA as a molecular marker (Weising et al., 1995).

The PCR (Saiki et al., 1985; 1988a, b) has been the basis of a growing range of newer techniques such as RAPD method (Williams et al., 1990), arbitrarily primed PCR (AP-PCR; Welsh and McClelland, 1990), DNA amplification fingerprinting (DAF; Caetano-Anolllés et al., 1991), simple (short) sequence repeat (SSR) or microsatellites (Taylor et al., 1992; Wu and Tanksley, 1993), short tandem repeat (STR), sequence tagged site (STS; Chee et al., 1993), sequence characterized amplified region (SCAR; McDermott et al., 1994), cleaved amplified polymorphic sequence (CAPS; Chaturvedi and Al Mssallem, 2001) and amplified fragment length polymorphism (AFLP; Vos et al., 1995).

In most cases, no one fingerprinting technique is ideal for all applications. None of the diagnostic marker techniques so far applied to date palm has fulfilled all of the requirements in terms of cost, ease of use, cultivar identification, applicability to in vitro material and mature plants, ability to detect both genetic and epigenetic variations and the detection of off-types. However, AFLPs satisfy more conditions than any other technique and are becoming the tool of choice for many applications and organisms. A fundamental advantage of AFLP is that its utility can be assessed with a small number of primer pairs that can be extended for more in-depth studies.

AFLP is a recently developed powerful DNA fingerprinting technique that uses PCR to amplify a limited set of DNA fragments from a specific DNA sample (Blears et al., 1998; Vos et al., 1995). The reliability of the RFLP technique is combined with the power of PCR. The execution of AFLP is technically demanding and requires competent users with experience in molecular biology techniques (Karp et al., 1997). AFLPs have been used in a wide variety of fields such as identification of molecular markers for species evaluation. Because of the highly informative fingerprinting profiles generally obtained, AFLPs can be applied in studies involving genetic identity and breeding (Bai et al., 1999; Ma and Lapitan, 1998), population studies and genetic diversity analysis (Winfield et al., 1998; Zhu et al., 1998), linkage mapping (Virk et al., 1998) and sex identification (Griffiths and Orr, 1999). AFLPs allow delineation of complex genetic structures (Powell et al., 1996), and provide reliable and informative multilocus probes (Winfield et al., 1998). The differences in fragment lengths generated by this technique

can be attributed to base changes in the restriction/adapter site, or to insertions or deletions in the DNA fragment. Dependence on sequence knowledge of the target genome is eliminated by the use of adapters of known sequence that are ligated to the restriction fragments. The overall power of AFLP is based upon the molecular genetic variations that exist among closely related species, varieties or cultivars. These variations in DNA sequence are ultimately exploited by the AFLP technology to routinely generate fingerprints of particular genotypes. The AFLP technique has proved to be useful for generating molecular markers in oil palm (Singh et al., 1998). AFLP has also proved to be useful in date palms (Lacaze et al., 2000).

The aim of this study was to develop a molecular fingerprinting method to identify date palm varieties and to assess the variability at the DNA level in plants regenerated from tissue culture; i.e. to verify the trueness-to-type of tissue culture-derived plants. The focus of this study was to optimise experimental conditions for the generation of reproducible AFLP markers. Implementation of a diagnostic marker system as a quality assurance tool in the date palm production process would have the following benefits: to identify closely-related cultivars and to quantify the proportion of specific plant off-types in commercial tissue culture propagation.

MATERIALS AND METHODS

DNA Isolation

1. Plant Material. The plant material used in the DNA isolation study was collected from both in vivo and in vitro samples of date palm (*Phoenix dactylifera* L.). In vivo leaflets, harvested from fruiting adult trees, were taken from the upper third section of the leaflets. In vitro samples were taken from the same date palm varieties under tissue culture propagation (bud generative tissue, leaves of differentiated buds, embryo, and callus stages) derived by two different techniques (i.e. organogenesis and asexual embryogenesis).

2. DNA Isolation Procedure. Various studies conducted to develop an appropriate protocol for date palm DNA isolation resulted in the following method: samples stored at -70˚C were transferred to ice, cut into small pieces (100 mg), wrapped in aluminium foil and immediately placed in liquid nitrogen. 100 mg pieces of leaves from buds in the multiplication stage derived through the organogenesis method and leaves from embryos derived from embryogenesis were similarly wrapped and frozen in liquid nitrogen.

Precooled mortars and pestles were used to grind plant tissue to a fine powder (Fig. 4). To facilitate the grinding process and also to keep the extraction as cool as possible, liquid nitrogen was added during grinding. Because date palm leaves have a high proportion of fibre (Fig. 3), 50 mg of a crushed glass Pasteur pipette was added to mature leaf tissues of adult trees (this procedure was not necessary for tissue culture-derived samples). Using a small precooled spatula, the powder was transferred to a precooled 2 ml microcentrifuge tube which was then immediately returned to the liquid nitrogen until all samples had been ground. Once the grinding process was accomplished, 840 µl of pre-heated (65˚C) extraction buffer [2 % w/v CTAB, 1.42 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 2 % w/v polyvinylpyrrolidone (PVP-40000), 5.0 mM ascorbic acid, 4.0 mM diethyldithiocarbamic acid (Doyle and Doyle, 1990)] was added. This was mixed and incubated at 65˚C for 5 minutes. 14 µl RNase A (20 mg/ml) was added and the mixture was incubated at 65˚C for 15 minutes. 684 µl of a chloroform: isoamyl alcohol mixture (24:1, v/v) was then added and the mixture was shaken by hand before being centrifuged at full speed (13,200 rpm) for 10 minutes (Eppendorf 5415 D) at room temperature. 600 µl of each supernatant was transferred to a new microcentrifuge tube and the DNA was precipitated by adding 0.7 vol. of isopropanol (420 µl). The samples were mixed and immediately centrifuged at full speed (13,200 rpm) for 10 minutes. The DNA pellet was washed with 150 µl of 70 % ethanol, and was then centrifuged at 13,200 rpm for 5 minutes. The ethanol was discarded and the pellets were air-dried for 5-10 minutes and dissolved in 15 µl of TE, pH 8.0. The genomic DNA

samples were stored at 4˚C. Nucleic acid concentration and purity were determined using a spectrophotometer (SP 8-100, UV/VIS, PYE Unicam).

AFLP Study

The genomic DNA was digested with two restriction enzymes (*Apo*I and *Mse*I), with *Mse*I (T/TAA) as the frequent cutter, and *Apo*I (Pu/AATT Py) as the rare cutter. For restriction digestions with *Apo*I, 0.75 µg of genomic DNA was digested in a total reaction volume of 40 µl, containing 5U *Apo*I (4 U/µl), 4 µg BSA and 1x NEB Buffer 3. Reactions were incubated at 50˚C for 3 hours. For restriction digestions with *Mse*I; the *Apo*I-digested DNA was digested in a total volume of 50 µl, with 5U of *Mse*I (10 U/µl), 1 µg BSA, and 1x NEB Buffer 3. Reactions were incubated at 37˚C for 3 hours.

Adaptor Preparation

In screw-capped tubes, stock solutions (200 µM) of adaptors (WD20 + WD50, WD47 + WD48; Table 1) were prepared at a concentration of 50 µM. The adaptors were boiled for 5 minutes, and allowed to cool slowly to room temperature (tubes were placed in a water bath at 37˚C, then transferred to a fridge at 4˚C and then moved to –20˚C).

Ligation of Adaptors

The fragments resulting from the *Apo*I plus *Mse*I digests were ligated to double-stranded adaptors (Table 1). *Mse*I and *Eco*RI adaptors were adjusted to 50 pmoles/µl and 5 pmoles/µl, respectively. To the 50 µl of restricted DNA, 9 µl of ligation mixture comprising the following components was added:

Ligation of adaptors:	Final con.	/reaction
10x OFA Buffer 3	1X	1 µl
5 mM ATP	1.2 mM	2.4 µl
*Apo*I adaptor (5 pmols/µl)	5 pmols	1 µl
*Mse*I adaptor (50 pmols/µl)	50 pmols	1 µl
d.H_2O	-	3.60 µl
		9 µl

1 µl of T4 DNA ligase (5 U/µl) was then added, the tubes were flicked to mix the contents, spun briefly and incubated in a water bath at 37˚C for 3 hours. The tubes were then kept at –20˚C or at 4˚C overnight.

Preamplification Reactions (Non-selective Amplification)

PCR reactions were performed in a GeneAmp® PCR system 9700 machine (Perkin Elmer Life Sciences). Non-selective preamplification reactions used standard *Eco*RI and *Mse*I primers (Table 1).

Primer Mix	Final conc. / 50 µl reaction	/reaction
10x NH_4 PCR buffer	1X	4 µl
50 mM $MgSO_4$	2 mM	2 µl
10 mM dNTPs	0.25 mM	1.25µl
WD16 (100 ng/µl)	100 ng	1 µl
WD19 (100 ng/µl)	100 ng	1 µl
dd.H_2O	-	10.75 µl
		20 µl

Taq Mix	Final conc. / reaction	/reaction
10x NH_4 PCR buffer	1X	1.0 µl
Taq DNA polymerase (5 U/µl)	2.5 U	0.5 µl
d.H_2O	-	8.5 µl
		10 µl

To each thin-walled PCR tube, 20 µl of adaptored DNA, 20 µl primer mix, and 10 µl Taq mix were added. Tubes were gently mixed and briefly spun. The genomic DNA fragments were preamplified using the following AFLP PCR programme:

94˚C : 1 min.

Step 1 (Denaturation)	94˚C : 30 sec.	x 20 cycles
Step 2 (Annealing)	52˚C : 30 sec.	
Step 3 (Extension)	72˚C : 60 sec.	
	4˚C : ∞	

Amplification Reactions (Selective Amplification)

Stock solutions of adaptors used in AFLP studies were prepared in 200 µl aliquots (50 ng/µl *Eco*RI stock and 15 ng/µl *Mse*I stock) and stored at -20ºC. Kinase labelling of the *Eco*RI primer was carried out in a volume of 30 µl (for 48 reactions: 6 µl *Eco*RI primer (50 ng/µl), 3 µl 10x forward buffer, 15 µl d.H_2O, 5 µl - ^{33}P-ATP, 1 µl T4 Polynucleotide kinase (10 U/µl)). The final solution was mixed, briefly spun, and incubated at 37˚C for 1 hour, then was placed on ice. For PCR amplification, to each well of the microtitre plate, the following solutions were added: 1 µl preamplified template (1:10 dilution), 2 µl *Mse*I primer, and 7 µl of AFLP PCR Mix. The AFLP PCR Mix for 48 reactions (420 µl) contained 60 µl 10x NH_4 PCR Buffer, 24 µl 50 mM $MgSO_4$, 12 µl 10 mM dNTPs, 291.6 µl d.H_2O, 30 µl labelled *Eco*RI primer, 2.4 µl 5 U/µl Taq DNA polymerase. To prevent evaporation, and to protect the reactions from any contamination, the PCR plate was sealed with an autoclaveable adhesive sealing sheet.

The amplification conditions were as follows: 13 cycles: denaturation at 94˚C for 30 seconds; annealing (annealing temperature lowered by 0.7˚C each cycle i.e. 65˚C, 64.3˚C, 63.6˚C, 62.9˚C, 62.2˚C, 61.5˚C, 60.8˚C, 60.1˚C, 59.4˚C, 58.7˚C, 58˚C, 57.3˚C) for 30 seconds; extension at 72˚C for 60 seconds. The final temperature reached was 56.6˚C. 23 cycles: denaturation at 94˚C for 30 seconds; annealing at 56˚C for 30 seconds, extension at 72˚C for 60 seconds. After the selective amplification process was accomplished, the samples were placed on ice. The annealing temperature for each primer used in all AFLP experiments was adjusted according to the formula [(total number of Cs + total number of Gs) x 4] + [(total number of As + total number of Ts) x 2].

Following PCR amplification and prior to electrophoresis, an equal volume of formamide loading dye (98 % deionised formamide, 10 mM EDTA (pH 8.0), 0.05 % xylene cyanol, 0.05 % bromophenol blue) was added to each PCR sample to give a total volume of 20 µl. Samples were denatured in a PCR machine at 94˚C for 2-3 minutes and stored in a freezer at -20˚C. The AFLP gel electrophoresis covered acrylamide gel preparation, polyacrylamide gel electrophoresis (PAGE), gel drying and autoradiography, and developing AFLP autoradiographs.

Data Analysis

Cluster analysis using UPGMA clustering analysis (unweighted pair-group method using arithmatic averages) (Sokal and Michener, 1958) was used to develop dendograms revealing the genetic similarities relationship among the ten different date palm varieties and also among the different sources of plant material based on the similarity matrices derived from each type of primer combinations. Based on the presence and absence of the amplified fragments, similarity matrices were generated according to the Dice Coefficient (Sneath and Sokal, 1973).

Three different studies were run in order to improve the AFLP protocol and render it easier and more efficient, as well as to fingerprint the ten date palm varieties with their three production sources (offshoots, organogenesis and embryogenesis techniques).

RESULTS

DNA Isolation of UAE Date Palm Varieties

At the time of conducting this research, few published methods for the isolation of date palm DNA were available. The first aim was therefore to develop a reliable method of DNA isolation suitable for both the fibrous leaf tissues and softer tissue culture samples of date palm. It is important that the same method is used for these two types of samples since using two different methods might affect AFLP patterns.

Plant Material Preparation

To achieve successful DNA isolation in terms of purity and amount, attention was paid to the harvesting process and the selection of healthy date palm leaf samples. The plant material used in the present study was collected from both in vivo and in vitro samples of date palm and was harvested from ten of the most important UAE varieties (Aboumaan, Barhee, Hilali, Jech Ramli, Khlass, Maktoumi, Rziz, Sakii, Sukkari, and Sultana). Fresh leaflets were harvested from fruiting adult trees propagated from offshoots and located at a well-maintained private farm in Al Ain city/UAE (Kuwaitat). Samples from the two tissue culture techniques (organogenesis and asexual embryogenesis) at different stages were also collected.

DNA Preparation

PCR-based methods are widely used in plants for marker-assisted breeding and high-resolution mapping. Because these studies require analysis of large populations, a DNA extraction method that is fast, inexpensive and yielding high quality DNA is needed. Good quality DNA samples are required for obtaining reliable and reproducible results in AFLP analysis (Chen and Ronald, 1999). Therefore, various studies were conducted to develop an appropriate protocol for date palm DNA isolation. To achieve this aim, several published protocols were tested, first on the varieties Medjool and Zaglool (derived from tissue culture and grown in Imperial College glasshouses) and then, on three UAE varieties (Aboumaan, Barhee, and Khlass) derived from tissue culture material. The protocols included those of Aitchitt et al. (1993), Ouenzar et al. (1998) and Chen and Ronald (1999). Final tests involved isolating DNA from 30 samples derived from ten UAE date palm varieties. The results obtained from the protocol published by Chen and Ronald (1999) were promising, although the DNA was slightly degraded in some cases. The DNA samples isolated from leaves harvested from the softer and less fibrous greenhouse plants (Medjool and Zaglool) showed slightly less degradation than those harvested from adult trees. This protocol was originally used for isolating DNA from a variety of plant species (rice, grape, maize, squash, tomato, peppermint and walnut). The date palm is characterized by hard and fibrous tissues (Fig. 3) and several modifications, included the grinding process, tissue/buffer ratio and the concentrations of extraction buffer and RNase were made for date palm.

The grinding process is a key step which significantly affects DNA extraction. Grinding should be executed as quickly as possible to yield a fine powder (Fig. 4) and the plant material should not be allowed to thaw during its disruption. Grinding as stated in the original protocol which used soft leaf crops was found impractical and inefficient for disruption of date palm leaf tissues. The use of a mortar and pestle proved to be more effective, causing less degradation. Using a mortar and pestle, a fine powder of tissue culture leaf samples and callus was achieved within 1-2 minutes, while 3-4 minutes were required for adult tree leaf samples. Addition of glass powder (50 mg) to tissue derived from adult trees facilitated the grinding process, but this step was not necessary for samples derived from tissue culture which are softer.

The DNA yields were still low (2.25 - 3.75 μg/100 mg tissue). Three different plant sources (adult trees, organogenic and embryogenic cultures) were used in an experiment with different types of leaves. Leaflet sections of about 20 cm, taken from the upper third part of the leaves from the middle of the crown (Fig. 1a, b) were found to be more suitable than those from other regions (leaflets from the bottom of the leaf or from all parts of the outer leaves). The more fibrous nature of the other tissues including leaflets taken from lower parts resulted in more degradation and low DNA yield (data not shown). In vitro plantlets derived from the two different tissue culture techniques (organogenesis and asexual embryogenesis) and derived buds and embryos were also found to be the most suitable of the tissue culture samples. Bud generative tissue and callus were found to be impractical due to hardness of the liquid nitrogen frozen tissue which makes the grinding process difficult. Different amounts of leaf tissue (25, 50, 100,

150, and 200 mg) were used to determine the optimum amount of tissue to be used in DNA extraction, while maintaining the amount of extraction buffer. Low amounts of leaf tissue (25 and 50 mg) resulted in low DNA yields, while high amounts resulted in an increase in DNA yield but a decrease in DNA quality. The amount of tissue which gave the best compromise was 100 mg. DNA isolated from tissue culture samples showed less degradation than DNA isolated from greenhouse tissues.

To improve the DNA quality, the protocol was modified in terms of extraction buffer and chloroform: isoamyl alcohol volumes. The volume of these was increased by factors of 1.2, 1.25 and 1.3 (Table 2). These modifications significantly reduced DNA degradation, the best DNA being obtained when the extraction buffer and chloroform: isoamyl were used at 1.2x. Some of the DNA bands are of a much higher molecular weight, which might be due to DNA-polysaccharides complex formation.

The DNA isolated from some tissue samples was contaminated by RNA. Therefore, the concentration of RNase used in the original protocol required modification. In addition to the original amount (140 µg), four additional RNase amounts (20 mg/ml) (70, 140, 280, 560 and1120 µg) were used. Lower amounts of RNase (70 and 140 µg) did not remove the RNA, whereas 280, 560 and 1120 µg RNase (20 mg/ml) completely removed the RNA from the samples. 280 µg of RNase was adopted as the standard.

Assessment of the Modified DNA Isolation Protocol

The modified protocol was found effective for isolating DNA from date palm samples regardless of the source of the tissue (adult tree, organogenesis and embryogenesis tissue cultures). The isolated DNA was pure and intact for the majority of samples. DNA purity, estimated at A_{260}/A_{280} values, ranged from 1.5 to 2.0. Concentrations ranged between 300 and 1000 ng/µl (4.5 µg – 15 µg / 100 mg tissue). To conclude, the modified protocol used for isolating the DNA from ten UAE date palm varieties was tested several times on a large number of date palm tissue samples from different sources. High quality non-degraded and non-contaminated DNA was obtained (Figs. 5a, b, and c).

AFLP Development

Genomic DNA of over 300 different samples extracted from ten date palm varieties from adult trees and tissue culture-derived plants (organogenesis, and embryogenesis) was digested with *Apo*I and *Mse*I. *Mse*I and *Eco*RI adaptors were ligated (50 pmoles/µl and 5 pmoles/µl, respectively) to the digested DNA (0.125µg) and the products were PCR amplified. Three separate experiments were conducted to optimise the AFLP protocol adopted for the fingerprinting of the ten date palm varieties from their three different sources (offshoots (adult), organogenesis and embryogenesis tissue culture-derived plants).

Optimization of the AFLP Technique for Date Palm

The aim of this experiment was to assess the effectiveness of different primers on a trial basis for 24 samples derived from 6 varieties (Aboummaan, Barhee, Zaghlool, Khlass, Hilali, and Jech Ramli) of adult trees and tissue culture samples. Using ApoI +1 (C as selective nucleotide) and MseI +2 primers (CC, CG, CT, GA, GG, GT, TA and TC selective nucleotides). The separation of the AFLP fragments by electrophoresis in denaturing polyacrylamide gels resulted in a large number of bands (about 600) which could not be scored with accuracy. Most of these bands were diffuse and difficult to identify.

To reduce the high number of fragments, reactions with additional selective nucleotides were carried out. The following primers were used with *Eco*RI = E+G, E+GG, E+GGA, E+GGAT, E+GGATC and *Mse*I = M+AA, M+AAG, M+AAGT, M+AAGTA (Table 3). Adult tree samples of Aboumaan and Barhee were used to provide the DNA. Using these primers, fewer bands were generated which were clearer and it was possible with some primers to differentiate between the two varieties (Fig. 6). The

number of bands obtained with the first primer combination varied from 151 to 227 (Table 3). With each of the four *Mse*I primers, the total number of bands decreased with selective nucleotide addition to the *Eco*RI primer. For example, from 227 with *Eco*RI+1 to 151 with *Eco*RI+5. Adding selective nucleotides to the *Mse*I primers also decreased fragment numbers. For example, for the *Eco*RI+1 primers, band numbers were 227, 170, 151 and 159, respectively. Several primer combinations produced fragments which were too diffuse to score. The level of bands which were polymorphic varied considerably with primer combination; from less than 1 % to over 16 %. From (Table 3), it is clear that the best primer combination was *Mse*I+AAG/*Eco*RI+GGA which produced the smallest band number (110) and the highest level of polymorphic bands (16.4 %).

Assessment of AFLP Reproducibility in Date Palm

Having found primer combinations that produce clear AFLP patterns, it was very important to show that the AFLP technique was reproducible. Twelve leaf samples (100 mg leaf material) were harvested from each of two individual Khlass plantlets derived from organogenesis tissue culture. DNA was extracted from the 24 leaf samples and the best 10 (based on DNA amount and quality) from each plantlet were used in AFLP reactions. AFLP fingerprints were generated exactly as before using the primer combination *Eco*RI+GGA/*Mse*I+AAG. The number of bands obtained (about 110) was identical in all samples and there was complete uniformity in the banding patterns (Fig. 7).

AFLP Fingerprinting of UAE Date Palm Varieties

The aim of this study was to assess the usefulness of the AFLP technique for fingerprinting date palm varieties. DNA samples were isolated from adult trees, organogenesis and embryogenesis tissues from ten date palm varieties (Aboumaan, Barhee, Hilali, Jech Ramli, Khlass, Maktoumi, Rziz, Sakii, Sukkari, and Sultana). An AFLP fingerprinting study was carried out using three primer combinations of *Eco*RI+3 and *Mse*I+3 (E+GGA / M+AAG, E+GGA / M+ACT and E+TAT / M+AAG).

Variation between Varieties

The primer combination of EcoRI+3 (E+GGA) and MseI+3 (M+AAG) was tested and showed partial discrimination between the ten date varieties at the adult stage (Fig. 8). In total, 23 differential bands were evident (Table 4). A number of bands (either as a novel or a missing band) were specific to only a single variety: Barhee was the only one (out of ten varieties) that lacked band 20 and 49; Maktoumi lacked band 2; Sultana had band 29; Sakii had band 38a; Rziz had band 68a (Table 4). Thus, five of these varieties could be distinguished by the presence or absence of a single AFLP fragment. Furthermore, 6 different bands were absent or present with just two out of ten varieties: band 4 was absent in Sukkari and Sultana only; band 18 was absent in Maktoumi and Rziz; band 41 was absent in Barhee and Sultana; band 42 was absent in Hilali and Jech Ramli; band 45 was absent in Khlass and Sakii and band 46 was present in Barhee and Sukkari (Table 4). Consequently, taking all these specific-variety bands into account, discrimination between all ten date varieties was possible with a single primer combination.

Based on the presence or absence of the amplified fragments, similarity matrices (Table 5) were generated according to the Dice Coefficient. These similarity tables were used to produce dendograms by Unweighted Pair Group Method with Arithmetic Average (UPGMA) (Sokal and Michener, 1958). These dendograms (Figs. 10 and 11) revealed the genetic similarities between the ten date palm varieties and also between the different sources of plant material (adult tree, organogenesis and embryogenesis). The higher the number, the greater the genetic closeness between the varieties.

The dendogram showed that the ten date palm varieties were composed of five clusters, (I, II, III, IV, and V) (Fig. 10). Sultana (cluster V) did not belong to any of the cluster groups and stands by itself from all remaining varieties. It was 0.60 genetically similar to the other nine varieties. The genetic distance between varieties within the

clusters (I, II, III, and IV) was 0.87, 0.79, 0.82 and 0.75, respectively. The average similarity among the ten varieties was more than 0.74. The average genetic distance between the varieties within four clusters was about 0.8. This high genetic distance reflected the relatively high genetic similarity between varieties. Indeed, the highest genetic distance (0.87) was found between Khlass and Sakii (cluster I), followed by Hilali and Jech Ramli (0.82) (cluster III), and Maktoumi and Rziz (0.81) (part of the cluster IV), and Barhee and Sukkari (0.79) (cluster II). The remaining variety (Aboumaan) was related to both clusters III (Hilali / Jech Ramli) and IV (Maktoumi / Rziz), but fell into the former one. These relationships may be related to the origins of the date palm varieties. For example, Khlass and Sakii, which had the highest genetic similarity (0.87), are originally from the Kingdom of Saudi Arabia. The second highest genetic similarity (0.8) among the remaining varieties was between Hilali and Jech Ramli. Again, these two varieties were originally from the UAE. However, these two groups were relatively closely related (0.65 and 0.72, respectively) to the other varieties which were mostly from Iraq.

Variation between Tissue Sources

AFLP fingerprints from the tissue culture plants were compared with those from the adult tree leaf samples (Fig. 8, Table 6). Surprisingly, in some cases, the fragment banding patterns within a variety were different. Six varieties, however, showed no differences in banding patterns between the three sources of plant material. These varieties were Aboumaan, Barhee, Hilali, Maktoumi, Sakii and Sultana (Table 6). Three varieties (Jech Ramli, Khlass and Rziz) gave identical banding patterns between the two tissue culture sources (organogenesis and embryogenesis) but these differed from the adult tree patterns. Jech Ramli had 5 differential bands (1, 2, 3, 40 and 74), Khlass had 10 differential bands (4, 22, 29, 40, 41, 48, 50, 51, 74 and 92) and Rziz had 5 bands (1, 2, 18, 42 and 68a). The differences between the adult tree patterns and the tissue culture patterns could be due to the intra-varietal polymorphism among date palm varieties. It is known that there is intra-varietal polymorphism among some date palm varieties (Devanand and Chao, 2003). For example, there are three well-known types of the variety Khlass that come from three different geographical regions (KSA, Oman, UAE). Each one has specific characters although they originate from the same genotype (Shabana, personal communication). Thus, the original explants used may have derived from offshoots that were different from the ones used as the sources of leaf samples. Alternatively, the offshoots used in tissue culture may actually be of a completely different variety.

Sukkari showed similar banding patterns between the adult and organogenesis sources but were different from the asexual embryogenesis pattern. Here, there were 9 differential bands (bands 1, 4, 18, 40, 42, 48, 50, 51 and 74). It is proposed that these differential bands may be a consequence of the callus phase in the embryogenesis method. The number of differential bands observed when the 40 embryogenesis samples of Khlass were analysed ranged from 1 to 15. Therefore, it is possible that the observed variation in this instance is a consequence of somaclonal variation.

The tissue culture samples from Jech Ramli and Rziz both differed from their adult tree samples but were clearly identical to each other. They must, therefore, have come from a mis-classified offshoot that was neither Jech Ramli nor Rziz. The patterns are closer to Rziz than they are to Jech Ramli. However, they both lack band 68a which is characteristic (its absence) of Maktoumi.

DISCUSSION

DNA-based molecular markers have emerged as powerful tools for determining genetic relationships (Negi et al., 2004). The availability of a suitable DNA isolation procedure is a prerequisite for performing DNA-based marker studies on plant species (Diaz et al., 2003) and AFLP analysis requires high quality DNA for obtaining reliable and reproducible results (Chen and Ronald 1999; Lacaze et al., 2000). In this work, the testing of several genomic DNA isolation procedures was an essential prerequisite in

order to select the most suitable method for date palm. The procedure must be suited to the hard and fibrous nature of date palm tissues and it must also yield reproducible results to enable the DNA analysis of large sample sizes of different date palm cultivars. Three different genomic DNA isolation procedures were tested for their potential use with date palm tissues: (1) A CTAB extraction method (Aitchitt et al., 1993), (2) A Tris-based isolation procedure, followed by phenol extraction (Ouenzar et al., 1998) and (3) the DNA isolation protocol of Chen and Ronald (1999). A modified Chen and Ronald (1999) method was found to be the only isolation procedure of the three tested to yield good quality DNA. The method was suitable for the isolation of DNA from all the date palm tissue samples used in this work, regardless of the source of the tissue (adult trees or tissue culture samples) or of variety. The DNA yields obtained from highly fibrous date palm tissues were 4.5 to 15 μg per 100 mg tissue (300-1000 ng/μl), which were similar yields obtained previously from other plant species with less fibrous tissues (tomato, peppermint, rice, grape and maize; Chen and Ronald, 1999). The two other methods tested in this study either yielded DNA contaminated with polysaccharides or DNA that was badly degraded. Genomic DNA yields obtained by Aitchitt et al. (1993) and Ouenzar et al. (1998) ranged from 3 - 3.5 μg per 100 mg fresh leaves. This was three to four times lower than those obtained by Chen and Roland (1999), although they reported that the DNA produced by both methods showed little degradation.

Similar results to those of this study were reported by Diaz et al. (2003) when they tested several genomic DNA isolation protocols, including the method of Aitchitt et al. (1993) on date palm samples derived from in vivo and in vivo production. The purest, most intact genomic DNA samples resulted from a CTAB extraction method according to Dellaporta et al. (1983), which yielded 400 ng/μl of DNA from 200 mg leaf tissue. However, they also reported that there were important differences between varieties in the quality and quantity of the DNA extracted. These findings contradict the results obtained in this study, in which the quality and quantity of the DNA extracted from the ten date varieties were not variety-dependent.

The integrity of the genomic DNA extracted from fresh leaves harvested from the ten adult date palm varieties was not affected during the shipping of the tissues from the UAE to the UK on dry ice. In addition, the plant material was stored for several months at -70°C, during which time no significant differences were found in the quality of the DNA isolated. The maintenance of genomic DNA stability in date palm during cold storage was also reported by Diaz et al. (2003).

AFLP Fingerprinting Study

In the past, it has been difficult to identify date palm cultivars based on morphological characteristics, and even more difficult to identify genetic strains of commercial cultivars (Devanand and Chao, 2003). In recent years, several molecular methods have been used for the identification of date palm cultivars. Differences between cultivars were detected by isoenzymes (Saker et al., 2000), RFLP (Corniquel and Mercier, 1994), RAPD (Corniquel and Mercier, 1994; Saker and Moursy, 1998; Sedra et al., 1998, Al-Khalifah and Askari, 2003) and RDA (Vorster et al., 2002) and AFLP (Lacaze et al., 2000; Cao and Chao, 2002; Devanand and Chao, 2003; Diaz et al., 2003).

In this study, the AFLP technique was used to identify ten commercial UAE date palm varieties and to assess the genetic variation among plants originating from both offshoots and tissue culture regeneration systems. A variety of primer combinations with different selective bases (+1 to +5) tested on Aboumaan and Barhee tree varieties revealed different polymorphisms between the samples. A primer combination of E+3 /M+3 which revealed the highest level of polymorphism (16.4 %) was chosen for fingerprinting the ten date palm varieties (Aboumaan, Barhee, Hilali, Jech Ramli, Maktoumi, Rziz, Sakii, Sukkari and Sultana). Diaz et al. (2003) also tested sixty-four primer combinations on three date palm varieties, and concluded that not all the primer combinations revealed polymorphisms in each variety. The best results were obtained with five primer combinations of *Eco*RI and *Mse*I, all with three selective nucleotides.

Other combinations amplified too many or too few fragments. In this work the three primer combinations, E+GGA/M+AAG, E+GGA/M+ACT and E+TAT/M+AAG generated a total of 330 AFLP fragments and a sufficient number of polymorphic bands (23, 19 and 26, respectively) with percentage polymorphisms of 21%, 17% and 23%, respectively. Surprisingly, half of these varieties were easily identified from the others by the presence of one or two variety-specific bands. Furthermore, all ten varieties could be identified with only one AFLP primer combination when all differential bands were taken into account. Similarly, Kjaer et al. (2004) tested 18 primer combinations on Sago palm (*Metroxylon sagu*; Arcaceae) and concluded that the most useful polymorphic bands were generated with E+ACT/M+CAT and E+ACC/M+CTT. Although a relatively low number of polymorphic bands were obtained from the two primer combinations, they were sufficient to enable the identification of 76 accessions of Sago palm in Papua New Guinea.

In the AFLP work of Diaz et al. (2003), five primer combinations displayed between 8 and 25 polymorphic bands in the date palm variety Medjool, whereas varieties Bou-Fegous and E-528 yielded between 10 and 28. These primer combinations yielded significant differences, allowing genetic characterization. An AFLP study carried out on 23 Medjool and 33 Deglet Noor date accessions in the USA revealed that four AFLP primer sets of (E+2 and M+3) were sufficient to identify intra-varietal variation within Medjool and almost none in Deglet Noor (Devanand and Chao, 2003). In previous work, Cao and Chao (2002) stated that AFLP markers could efficiently identify individual date cultivars and they were able to detect polymorphisms in 21 date cultivars by using four primer sets.

Lacaze et al. (2000) tested 20 primer combinations (*Eco*RI+3 / *Mse*I+3) to identify those combinations giving the maximum discrimination between two date varieties of diverse geographical origin (Khlass and Medjool). Two primer combinations, giving a total of 45 polymorphic bands, gave a clear discrimination between all varieties tested.

In wild palm (*Euterpe edulis* Mart) an AFLP study was performed to detect genetic variation in 150 plants from eleven populations. Five pairs of primers (+3) provided a total of 429 markers, 395 (92 %) of which were polymorphic (Cardoso et al., 2000). In a different study, Adin et al. (2004) used the AFLP technique as a tool to compare genetic diversity in peach palm (*Bactris gasipaes* Kunth) populations managed by either indigenous or colonist farming communities in Peru. The genetic variation was detected using two primer combinations of E+3 / M+3 which generated 144 polymorphic bands. In oil palm, eight primer combinations of *Eco*RI+3 and *Mse*I+3 selected randomly were able to screen 10 progenies from two crosses using the AFLP technique (Singh et al., 1998).

The AFLP technique is a useful tool for cultivar identification and assessment of genetic variation in different palm genomes. Apart from Cao and Chao (2002) who used *Eco*RI+2, the majority of investigators including the present study used *Eco*RI+3 and *Mse*I+3 for generating polymorphisms among plants. This primer combination appears to be adequate for fingerprinting varieties and species within the palm family. Date palm shows levels of AFLP polymorphism that are similar to those in other palm species.

The AFLP technology has also proved to be a useful tool in other plant species for several applications. The AFLP technique is clearly a valuable fingerprinting technique for many plants, for example *Brassica napus* (Sobotka et al., 2004), wheat (Tyrka, 2002), tomato (Suliman-Pollatschek et al., 2002), garlic (Ipek and Simon, 2002; Ipek and Simon, 2001); apricot (Hagen et al., 2002), sunflower (Gedil et al., 2001) and *Pinus sylvestris* (Lerceteau and Szmidt, 1999). AFLPs are also useful in estimating genetic diversity, such as in Argentinean garlic (*Allium sativum* L.) (Lampasona et al., 2003) and *Brassica vigra* (Negi et al., 2004).

Cluster analysis of the AFLP data showed that the ten date palm varieties are composed of five clusters based on their genetic similarity. Khlass and Sakii were the two most closely related cultivars. Hilali was closely related to Jech Ramli. These relationships may be attributed to the geographical origin of the date palm varieties.

Khlass and Sakii are originally from Saudi Arabia while Hilali and Jech Ramli are originally from the UAE. This hypothesis is supported by results obtained from AFLP analysis on Sago palm accessions, which showed a significant correlation between genetic and geographical distances (Kjaer et al., 2004) and also with date palms from Saudi Arabia (Al-Khalifah and Askari, 2003).

AFLP analysis confirmed the trueness-to-type of tissue culture plantlets derived from both tissue culture techniques with adult trees propagated vegetatively by offshoots. However, this was true for only six of the ten varieties tested. Lacaze et al. (2000) also used an AFLP technique to confirm varietal status of tissue culture-derived date palms.

In four varieties, AFLP variation was also detected between adult trees and tissue culture samples. This genetic variation could be due to the use of different genetic strains of the same variety that can only be differentiated by molecular markers. In the UAE, there are three well-known accessions of the variety Khlass that come from three different geographical regions (KSA, Oman and UAE) and which cannot be distinguished by morphology. However, it has been recognized for some time that there is genetic variation within commercial date varieties. Mason (1927) reported large and small-fruited strains of Hayany which were indistinguishable in vegetative characteristics. Different strains of Deglet Noor were later reported by Fawcett (1931) and Nixon (1950) with unique fruit colour or late ripening. Differences in band patterns within trees of the same variety have been detected in Medjool, Deglet Noor and Barhee accessions propagated from offshoots using RAPD, RFLP and AFLP markers (Corniquel and Mercier, 1994; Devanand and Chao, 2003). This variability appears to be common in clonally propagated crops, such as almond, cherry, and olive (Devanand and Chao, 2003).

AFLP variation was also observed between plants of the same variety that were propagated by tissue culture. Sukkari samples derived from embryogenesis showed band variation when compared to those from adult trees and organogenesis-derived plants of the same variety. It is likely that this is a consequence of tissue culture and could have been induced in the callus production stage due to the use of high concentrations of 2,4-D. In vitro production of date palm via somatic embryogenesis requires the application of a relatively high concentration of 2,4-D or NAA for the initiation process (Tisserat, 1979; Bhaskaran and Smith, 1995). However, these auxins are known to be associated with genetic instability in plants (Karp, 1989; Phillips et al., 1994; Cullis, 1999). Callus obtained from tissue culture is highly heterogeneous (Reynolds and Murashige, 1979; Tisserat and Demason, 1980) and it is also highly susceptible to mutational changes during somatic embryogenesis (Tisserat and Torres, 1979; Torres and Tisserat, 1980). More than 500 published scientific articles have described the existence of genetic variability in plant tissue culture (Orton, 1983).

The results presented here showed that AFLP can be successfully used for molecular marker testing of different date palm varieties with high resolution and reproducibility and allows the identification of in vivo and in vitro-plants. The present research confirmed the reliability and reproducibility of the AFLP technique. The use of AFLP potentially allows not only differentiation between varieties but also between samples derived from one variety which show somaclonal variation. The AFLP technique appears to be a powerful tool to generate large numbers of markers that are useful in cultivar identification and estimating genetic diversity between different palm genomes. It is sensitive enough to detect low levels of variation, allowing discrimination between highly related varieties. Thus, it is possible to look for linkages between molecular markers and agronomically important traits, and also to identify genetic variation at different stages of the breeding process.

FUTURE WORK

Our future aim is to fingerprint elite UAE date palm varieties in order to compare their genetic relationship with each other, and also to distinguish between them and international (imported) varieties. The identification of genetic variation of date varieties using AFLP techniques will change the direction of future date palm germplasm

collection and preservation efforts. Furthermore, more light is to be shed on intravarietal polymorphism. This could mainly be applied to renown date varieties, such as Aboumaan, Barhee, Khlass, and Medjool, in order to assess potential variations within each. Another future aim, that could be considered a challenge for the date palm industry, is to render the developed AFLP techniques cost-effective and develop an easily applicable DNA-based diagnostic tool that can be standardized for both variety identification and detection of tissue culture off-types. Furthermore, there is a need to convince authorities and decision makers to accept "DNA fingerprints" as "signatures" in legal cases.

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Tables

Table 1. Oligonucleotide adaptors and primers used in preamplification reactions (non-selective amplification).

Name	Sequence
Adaptors used for AFLP analysis	
*Eco*RI adaptor for *Apo*I digests	
WD20	5'-CTCGTAGACTGCGTACC-3'
WD50	3'-CATCTGACGCATGGTTAA-5'
*Mse*I adaptor	
WD47	5'-GACGATGAGTCCTGAG-3'
WD48	3'-TACTCAGGACTCAT-5'
Primers used in preamplification	
*Eco*RI + 0 for *Apo*I digest	
WD16	5' CTCGTAGACTGCGTACCAATT 3'
***Mse*I + 0**	
WD19	5' GACGATGAGTCCTGAGTAA 3'

Table 2. Amounts of extraction buffer and chloroform: isoamyl (24:1) used in DNA extractions.

Concentration (x) (µl)	Original	X 1.2	X 1.25	X1.3
Extraction Buffer	700	840	875	910
Chloroform: isoamyl (24:1)	570	684	712.5	741
Total	1270	1524	1587.5	1651

Table 3. Polymorphic bands obtained with primer combinations with additional selective nucleotides.

Primer combination		Total amplified bands scored	No. of polymorphic bands	% polymorphic bands
*Mse*I	*Eco*RI			
M+AA	E+G	227	1	0.44
	E+GG	173	5	2.9
	E+GGA	162	9	5.5
	E+GGAT	NS	-	-
	E+GGATC	151	1	0.66
Total		713	16	2.24
M+AAG	E+G	170	1	0.6
	E+GG	138	8	5.8
	E+GGA	110	18	16.4
	E+GGAT	NS	-	-
	E+GGATC	120	6	5.0
Total		538	33	6.13
M+AAGT	E+G	151	5	3.3
	E+GG	NS	-	-
	E+GGA	144	10	6.9
	E+GGAT	NS	-	-
	E+GGATC	117	6	5.1
Total		412	21	5.0
M+AAGTA	E+G	159	4	2.5
	E+GG	127	16	12.6
	E+GGA	116	18	15.5
	E+GGAT	fuzzy	-	-
	E+GGATC	104	6	5.5
Total		506	44	8.69 %

NS = not scoreable

Table 4. Differential AFLP fragments in the fingerprints of ten UAE date palm varieties. The primer combination was E+GGA / M+AAG. Samples were collected in 2002 from adult trees.

Lane no.*	1	4	7	10	13	16	19	22	25	28
Band no.	Aboumaan	Barhee	Hilali	Jech Ramli	Khlass	Maktoumi	Rziz	Sakii	Sukkari	Sultana
1	0	1	0	0	1	1	0	1	1	0
2	1	1	1	1	1	**0**	1	1	1	1
3	1	1	1	0	0	1	1	1	0	0
4	1	1	1	1	1	1	1	1	**0**	**0**
18	1	1	1	1	1	**0**	**0**	1	1	1
20	1	**0**	1	1	1	1	1	1	1	1
22	0	0	0	0	1	0	0	1	0	1
29	0	0	0	0	0	0	0	0	0	**1**
37	1	0	0	0	0	0	0	1	1	0
38a	0	0	0	0	0	0	0	**1**	0	0
40	1	1	0	0	0	1	1	1	1	1
41	1	**0**	1	1	1	1	1	1	1	**0**
42	1	1	**0**	**0**	1	1	1	1	1	1
45	1	1	1	1	**0**	1	1	**0**	1	1
46	0	**1**	0	0	0	0	0	0	**1**	0
48	0	1	1	1	0	0	1	0	1	1
49	1	**0**	1	1	1	1	1	1	1	1
50	1	0	0	0	1	0	0	1	0	0
51	0	1	0	1	1	1	1	0	1	1
53	1	0	0	1	0	0	1	0	1	0
68a	0	0	0	0	0	0	**1**	0	0	0
74	0	1	1	0	1	1	1	1	1	0
92	0	0	0	0	1	0	0	1	0	0

(0) Absent band, (1) Present band, * from Figure 8, Variety-specific bands are shown in red and green.

Table 5. Similarity matrix for ten UAE date palm varieties with primer combination of E+GGA / M+AAG. Samples were collected in 2002 from adult trees of A, Aboumaan; B, Barhee; H, Hilali; J, Jech Ramli; K, Khlass; M, Maktoumi; R, Rziz; Sak, Sakii; Sul, Sukkari and Sul, Sultana.

	A	R	Sak	J	H	M	Suk	K	B	Sul
A	100.0	78.6	75.9	75.0	75.0	72.0	71.4	66.7	61.5	58.3
R	78.6	100.0	64.5	76.9	76.9	81.5	80.0	62.1	71.4	69.2
Sak	75.9	64.5	100.0	51.9	66.7	71.4	64.5	86.7	62.1	51.9
J	75.0	76.9	51.9	100.0	81.8	60.9	76.9	64.0	58.3	63.6
H	75.0	76.9	66.7	81.8	100.0	69.6	69.2	64.0	66.7	54.5
M	72.0	81.5	71.4	60.9	69.6	100.0	74.1	69.2	72.0	60.9
Suk	71.4	80.0	64.5	76.9	69.2	74.1	100.0	69.0	78.6	69.2
K	66.7	62.1	86.7	64.0	64.0	69.2	69.0	100.0	59.3	56.0
B	61.5	71.4	62.1	58.3	66.7	72.0	78.6	59.3	100.0	58.3
Sul	58.3	69.2	51.9	63.6	54.5	60.9	69.2	56.0	58.3	100.0

Table 6. Comparison of AFLP banding patterns in ten UAE date palm varieties. Samples were collected in 2002 from adult trees (t), organogenesis (o) and embryogenesis tissue culture samples (e). The primer combination used was E+GGA / M+AAG.

	Varieties showing identical bands from the three tissue sources																		Varieties showing differential bands											
Lane no. *	1	2	3	4	5	6	7	8	9	16	17	18	22	23	24	28	29	30	10	11	12	13	14	15	19	20	21	25	26	27
Band	Aboumaan			Barhee			Hilali			Maktoumi			Sakii			Sultana			Jech			Ramli			Khlass			Rziz			Sukkari		
no.	t	o	e	t	o	e	t	o	e	t	o	e	t	o	e	t	o	e	t	o	e	t	o	e	t	o	e	t	o	e
1	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	0	1	1	1	1	0
2	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	1	1	1	0	0	1
18	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	1	1	1	0
20	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0
37	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
38a	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	0
41	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	1	1	1	1	1	1
42	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	1	1	0
45	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1
46	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
48	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0
49	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
50	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
51	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0
53	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1
68a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
74	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	0	0	1	1	1	1	1	0
92	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Variant	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	10	-	-	5	-	-	-	-	9

(0) Absent band, (1) Present band, * from Figure 8, Diagnostic bands are shown in bold.

Figures

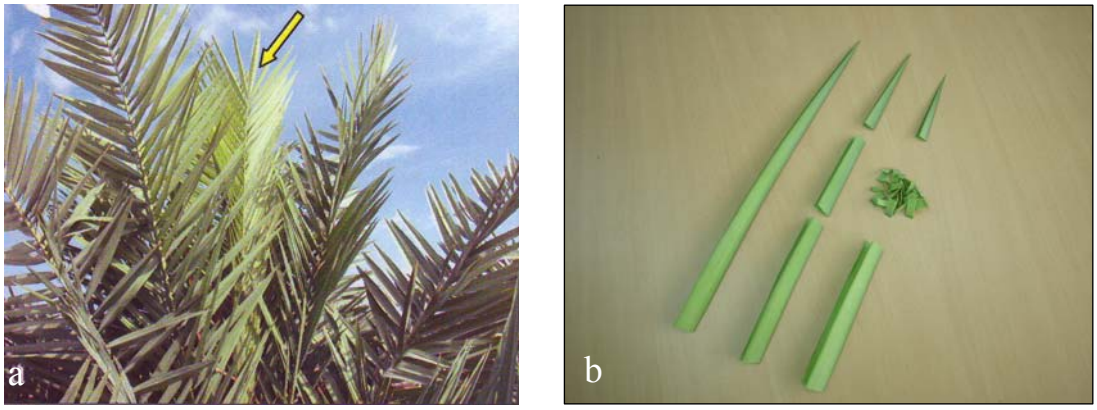


Fig. 1. In vivo leaflet samples used for DNA isolation. a. In vivo samples made of young leaves at the middle of the crown. b. Small pieces were cut from the selected leaflet sections.

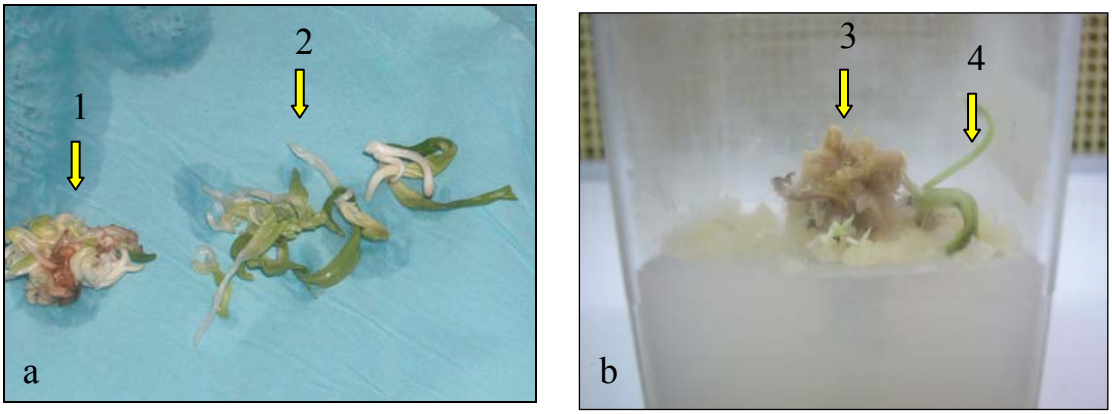


Fig. 2. In vitro samples from organogenesis and embryogenesis used for DNA isolation: a. Organogenesis: 1. Bud generative tissue; 2. Leaves produced by buds. b. Embryogenesis: 3. Callus; 4. Leaves produced by embryos.

Fig. 3. Mature leaf tissues of adult trees before grinding.

Fig. 4. The final fine powder obtained after the grinding procedure.

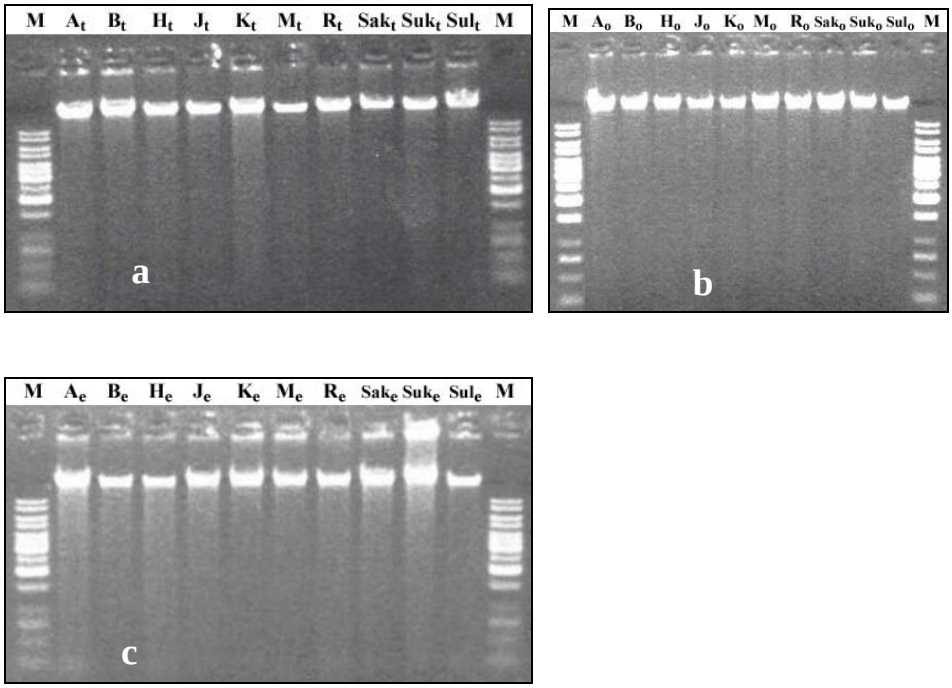


Fig. 5. DNA samples isolated from adult trees (t), organogenesis (o) and embryogenesis (e) tissues of ten UAE date varieties. A, Aboumaan; B, Barhee; H, Hilali; J, Jech Ramli; K, Khlass; M, Maktoumi; R, Rziz; Sak, Sakii; Suk, Sukkari; and Sul, Sultana. Season 2002. a. Adult tree DNA samples, b. Organogenesis DNA samples, c. Embryogenesis DNA samples.

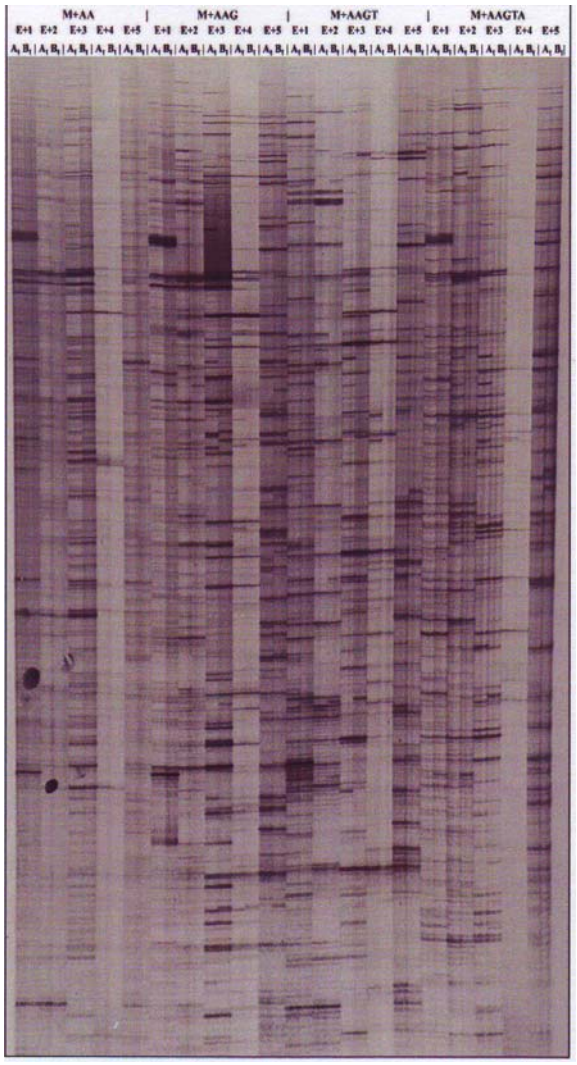


Fig. 6. Autoradiograph showing AFLP fingerprints of genomic DNA from leaves of adult trees. Four primer combinations and two varieties were compared, A_t, Aboumaan; B_t, Barhee. The profiles were generated using the primer combination of *Eco*RI= (1) E+G, (2) E+GG, (3) E+GGA, (4) E+GGAT, (5) E+GGATC and *Mse*I = M+AA, M+AAG, M+AAGT, M+AAGTA.

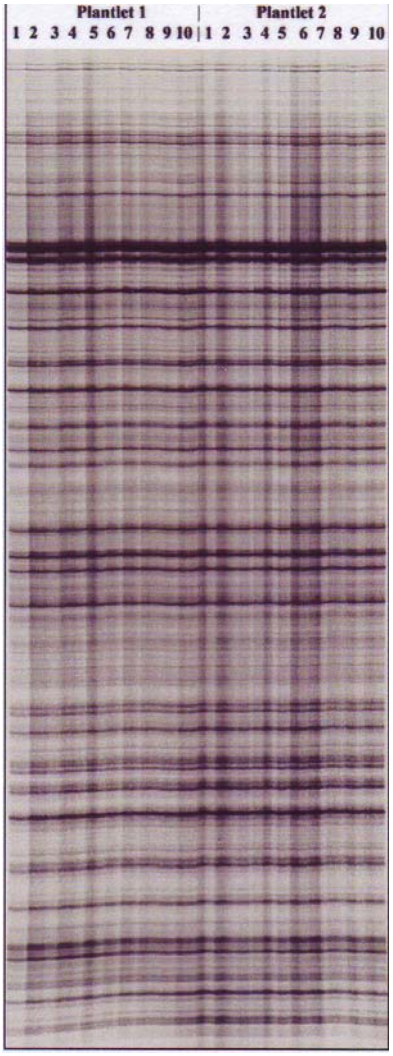
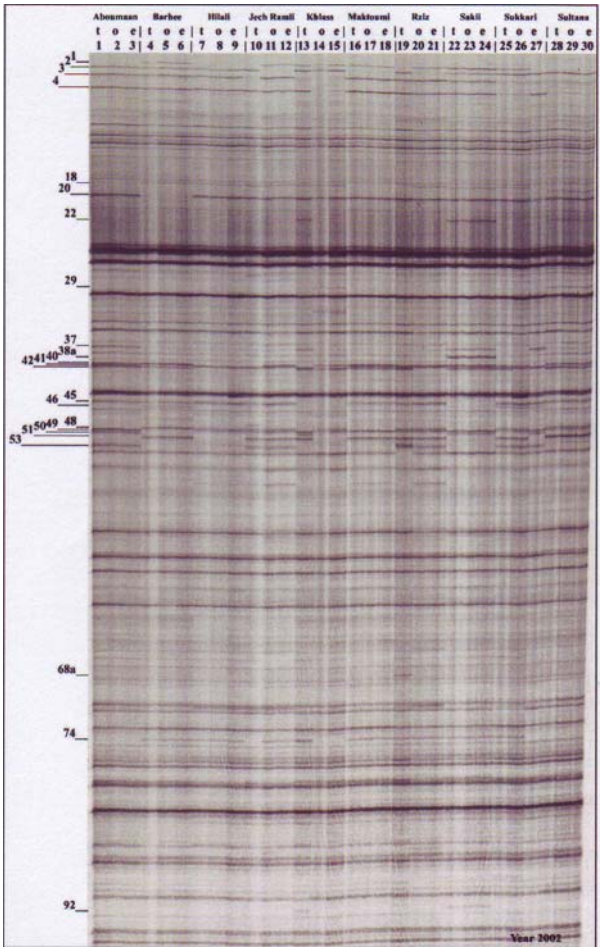
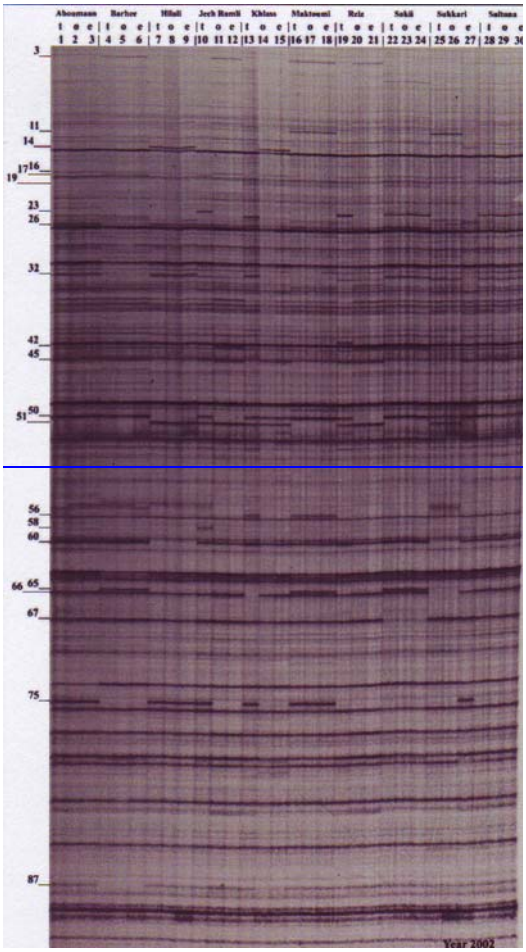


Fig. 7. AFLP reproducibility. Autoradiograph showing AFLP fingerprints of 20 samples derived from two individual Khlass plantlets produced by organogenesis. The profiles were generated using the primer combination of E+GGA/ M+AAG.


Aboumaan
Barhee
Hilali
Jech Ramli
Khlass
Maktoumi
Rziz
Sakli
Sukkari
Sultana
Year 2002


Year 2002

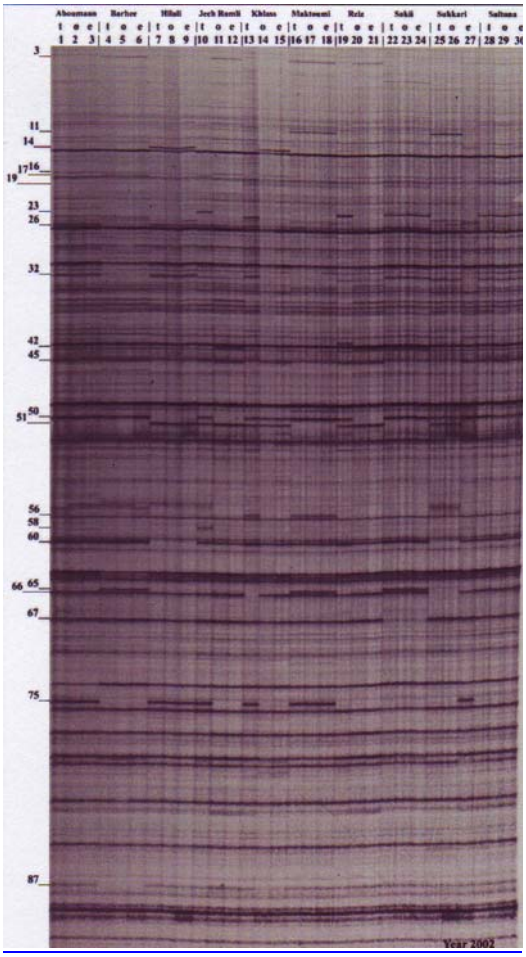


Fig. 8. AFLP fingerprints of ten UAE date palm varieties: A, Aboumaan; B, Barhee; H, Hilali; J, Jech Ramli; K, Khlass; M, Maktoumi; R, Rziz; Sak, Sakii; Sul, Sukkari and Sul, Sultana. The profiles were generated using the primer combination of *E*+GGA/*M*+AAG. Samples were collected in 2002 from adult tree leaves (t), organogenesis (o) and embryogenesis tissue culture samples (e).

Fig. 9. AFLP fingerprints of ten UAE date palm varieties: A, Aboumaan; B, Barhee; H, Hilali; J, Jech Ramli; K, Khlass; M, Maktoumi; R, Rziz; Sak, Sakii; Sul, Sukkari and Sul, Sultana. The profiles were generated using the primer combination of *E*+GGA / *M*+ACT. Samples were collected in 2002 from adult tree leaves (t), organogenesis (o) and embryogenesis tissue culture samples (e).

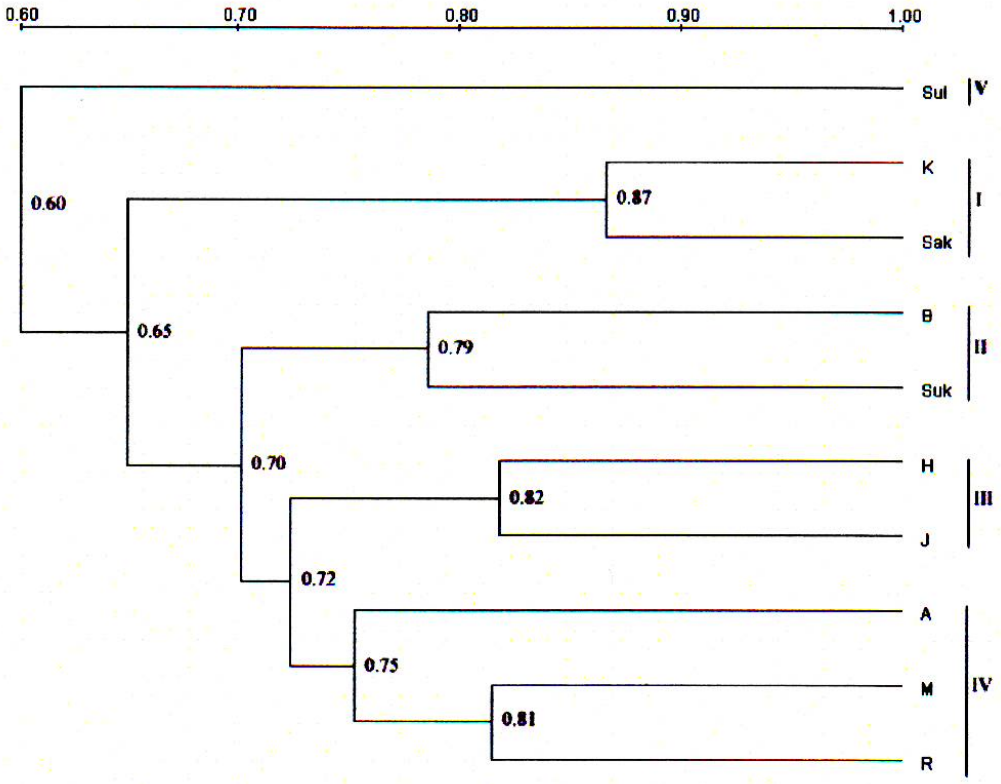


Fig. 10. Dendogram of UPGMA cluster analysis representing the genetic similarity among 10 date palm varieties revealed by AFLP data using the primer combination E+GGA/M+AAG. Samples were collected in 2002 from adult trees of A, Aboumaan; B, Barhee; H, Hilali; J, Jech Ramli; K, Khlass; M, Maktoumi; R, Rziz; Sak, Sakii; Sul, Sukkari and Sul, Sultana.

The Growth and Mineral Composition of Hatamy Date Palm Seedlings as Affected by Sea Water and Growth Regulators

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Abstract

Salinity stress is known to retard plant growth through its influence on vital facets of plant metabolism, including disturbing the concentration of endogenous plant hormones. This disturbance can affect the growth and development of plants. It may be possible to improve the endogenous hormone balance by applying exogenous hormone. The application of NAA in combination with salts reduced accumulation of Na, Cl in leaves. An experiment was conducted at the Research Station of Qatar University to study the effect of IAA, NAA, and a mixture of IAA and NAA and different concentrations of sea water alone or in combination with growth regulators on properties and mineral contents of date palm (*Phoenix dactylifera* L., cv. Hatamy) seedlings.

Salinity in irrigation water reduced leaf and root Mn, Zu, Fe and leaf K, Ca, Mg concentrations and the ratio of K/Na and Mg/Ca, but increased leaf N and leaf and root P, Cu, Na and Cl concentrations, compared with untreated seedlings. Compared to sea water alone, the irrigation of Hatamy seedlings with naphthalene acetic acid in combination with sea water, reduced the adverse effects of salt by reducing Na accumulation in the leaves and roots and Cl in the leaves and the ratio of Mg/Ca in leaves and roots and increasing the N, P and Fe concentrations, the ratio of K/Na in the leaves and roots, and Ca, Mg levels in the roots, number of roots and shoot dry matter percentage of seedlings over the using sea water alone.

INTRODUCTION

Salinity induces growth inhibition and in many cases shoots are affected more than roots (Marber and Lerner, 1994).

It is generally believed that plant resistance to salinity rests with the ability to restrict or prevent the entry of Na^+ to the shoots. This restriction is necessary for vital processes such as photosynthesis to take place without disruption. Salinity stress is known to retard plant growth through its influence on several vital facets of plant metabolism, including osmotic adjustment (Crammer et al., 1990), nutrient uptake (Francios and Kleimon, 1990), protein and nucleic acid synthesis, photosynthesis, organic solute accumulation, enzyme activity, hormonal balance, injury to tissue, alteration in respiration rates, and reduced water availability to crop plants (Alam, 1994). The mechanisms of salt tolerance in cultivated crop species that differ considerably in tolerance, range from restricted ion uptake and translocation into the shoots to structural metabolic changes that decrease salt injury. Salt tolerance under such conditions is generally related to the ability to regulate Na and Cl uptake by plant roots and subsequent translocation to the shoots (Francois and Kleimon, 1990; Johnson, 1991). Salinity stress under certain conditions may promote nutrient uptake by plant species. The simultaneous presence of salts and nutrient elements in the root zones can influence nutrient uptake, thereby affecting their chemical composition. Synergistic and antagonistic effects can increase or decrease the intensity of these processes (Alam, 1994). However, in a saline environment plants take up excessive amounts of Na at the cost of K and Ca. High Na/Ca and Na/K ratios in a saline growth medium may impair the selectivity of the root

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

membrane and result in the passive accumulation of Na in plant roots. The greater accumulation of Na in plant roots (Boursier and Lauchi, 1990) may be due to a regulatory mechanism located within the roots that prevents translocation of excessive amounts of chemicals such as Na, to aerial parts, resulting in Na retention. This Na in plant roots could also be due to the high mobility of Na in the phloem. Roots have a finite capacity to act as storage reservoirs for Na and other ions taken up from the soil. The acquisition of mineral nutrients and water and the tolerance of the plant to the presence of potentially toxic levels of elements such as Na in the soil solution may therefore depend upon the continued growth of the adventitious roots. Adventitious roots in salt-stressed plants represent a potential reservoir for the storage of Na and Cl (Boursier and Lauchi, 1990). The presence of Na and Cl in the rooting environment have been shown to affect plant metabolism by affecting ion uptake (Alam, 1994).

Chloride is a more sensitive indicator of salt damage than Na, since it is stored by plants, whereas Na is absorbed in smaller quantities despite high Na concentrations in the soil (Alam, 1994). Lessani and Marschner (1978) demonstrated that the Cl content varied in various salt stressed crops, but it was always higher than Na.

Salinity reduced the ratio of K/Na, compared to non-saline treated plants (Jacoby, 1994). However the deleterious effect of salt included excess Na^+ accumulation as well as K^+ leakage. Both are prevented by Ca^{2+}. Thus presence of Ca^{2+} seems to be necessary for K^+/Na^+ selectivity and for maintenance of an appropriate K^+ concentration in plant cells. The capability of plants to maintain an adequate K^+ content under saline conditions is also enhanced by ample K^+ supply (Jacoby, 1994).

The response of N, P and K contents in different plants to external Na^+ increments are not uniform, may be increased or decreased or had no effect (Winter and Kirst, 1991). (Champagnol, 1979; Sharpley et al., 1992). Others (Okusanya and Ungar, 1984; Subbarao et al., 1990) found that the K^+ concentration in plant tissue is reduced as the Na^+ salinity or Na^+/Ca^{2+} ratio in the root media is increased.

High Na levels in the root medium markedly reduced the activity of Ca^{2+} available for uptake by plants. It may also displace Ca^{2+} from the plasmalemma of root cells. Root growth and function may be inhibited. The ability of root cells to function in selective uptake of Na from the transpiration stream and the function of membranes as barriers to ion loss from the cells under saline stress may become severely inhibited by depressed tissue Ca^{2+} concentrations. However the presence of sufficient Ca^{2+} in the saline solution is essential to prevent the accumulation of toxic levels of Na, because the Na uptake is strongly regulated by Ca^{2+} in the growth medium (Alam, 1994). Calcium and Mg imbalances in the saline growth medium caused severe leaf chlorosis. In corn blades, increased Ca in concert with decreased Mg reduced photosynthetic activity by deterioration of chlorophyll (Peaslee and Moss, 1966).

It is possible that nutrient disorders could develop in plants that are irrigated with dilute seawater because of a high Mg^{2+}/Ca^{2+} ratio. It has been reported that irrigation water with a Mg^{2+}/Ca^{2+} ratio greater than one reduced growth of plants (Key et al., 1962).

Micronutrient concentration in plant shoots may increase, decrease or have no effect, depending upon the type of plant, tissue, salinity, micronutrient concentration, and environmental conditions (Hassan et al., 1970).

Salinity in irrigation water affected the concentration of endogenous plant hormones, disturbing the hormonal balance of the plant. This disturbance could affect growth and development of the plant. However, it was suggested that the endogenous hormonal balance has an important regulating role in the response of plants to salinity, and it may be possible to improve the endogenous balance by application of exogenous hormones (Mayber and Lerner, 1994).

Irrigation of date palm seedlings with saline water significantly increased leaf Cl^-, Na^+ and K^+ concentrations (Hassan and El-Samnoudi, 1993). Leaf Na^+, Cl^- and Fe^+ concentrations of date palm seedlings were increased, when irrigated with salt application alone or in combination with GA_3 (Aljuburi, 1996) or IAA (Aljuburi and Al-Masry, 2000). Other researchers reported that the application of saline water to citrus plants

decreased K, N, P, Ca, Mg, Fe, Mn, Zn and Cu concentrations in leaves and roots (Banuls et al., 1990). According to other results (Zid and Grignon, 1987) the application of saline water had no effect on N, P and K concentrations but led to large Cl^- and Na^+ accumulation in *Citrus aurantium* leaves. The accumulation of high Na^+ and Cl^- concentrations in date palm leaves may provide a beneficial physiological activity through osmotic adjustment (Hassan and El-Samnoudi, 1993).

Application of GA_3 alone or in combination with salt in irrigation water on date palm seedlings increased the shoot and root Na^+ and Cl^- concentrations (Aljuburi, 1996; Aljuburi and Al-Masry, 2000).

The main objectives of this study were to investigate the effect of diluted sea water when irrigated on date palm seedlings and how to improve the growth and nutrient concentrations in these seedlings by application of exogenous IAA, NAA or a mixture of IAA and NAA alone or in combination with different seawater concentrations.

MATERIALS AND METHODS

This research was carried out in a shade house at the experimental station of University of Qatar at Rawdat Alfaras region in Qatar state, during the growing season of 2001-2002.

Two year old uniform date palm (*Phoenix dactylifera* L.) seedlings of Hatamy cultivar were transplanted into 40 cm tall by 25 cm wide polyethylene bags containing yellow sand and peatmoss (1:1). The seedlings were irrigated with water containing 1337.6 mg/L NaCL every other day in addition to 300 ml/month/seedling with half strength Hoaglands nutrient solution (Hoagland and Arnon, 1950).

The seedlings were arranged in randomized complete block design equally split among four levels of sea water (SW) 0.0%, 20%, 40% and 60%. Salinity treatments were imposed by irrigating each seedling once every 20 days with 300 ml of different concentrations of sea water, in addition to 300 ml of various concentrations of growth regulator solutions (300 mg/L indole-3-acetic acid (IAA), 400 mg/l naphthalene acetic acid or a mixture of the two growth regulators (MGR) (300 mg IAA/L +400 mg NAA/L) added each 20 days). Plants were exposed to salinity in increasing increments of 20% sea water (SW) over a 10 day period to avoid osmotic shock. The experiment consisted of 16 treatments replicated 3 times with seedlings as the experimental unit.

Seedling shoot lengths were measured every 20 days. Other plant growth characteristics (shoot and root dry matter percentages, leaf and root number per seedling, and root lengths) were recorded at the harvest date (120 days after the first treatment) following the procedure described previously (Aljuburi and Al-Masry, 2000). One hundred and twenty days after beginning the treatments, the seedlings were removed from their containers. The roots were washed free of soil and were separated from the shoots. Roots and shoots were oven dried for at least 7d at 65ºC, weighed, ground and stored for mineral analysis.

The nitrogen content of leaves and roots was determined by the micro-Kjeldahl method. Measurement of Ca, Fe, K, Mn, Mg, Na, Zn and Cu concentrations was conducted after wet digestion with a mixture of nitric/perchloric acid (4:1) by atomic absorption spectrophotometer (GBC Avanta). Phosphorus concentration was determined by colorimeter method “spectrophotometer” (UNICAM 8620 UV/VlS spectrometer). Chloride concentration was measured using Ion-chromatograph (DIONEX IC 25) technique.

The data were subjected to analysis of variance and L.S.D. was used for mean comparison with $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Salinity on Plant Growth Characteristics

When Hatamy seedlings were irrigated with 300 mg/L IAA alone or with a mixture of IAA and NAA alone plant length significantly increased compared to control seedlings. The irrigation of Hatamy seedlings with water containing 40% sea water significantly increased plant length relative to untreated seedlings (Table 1).

Number of leaves per seedling significantly increased with application of 40 or 60% sea water alone compared to untreated control plants. Number of roots per seedling significantly increased with application of 40% or 60% sea water alone or in combination with a mixture of IAA and NAA compared to the control. However, the combination of IAA with 20% or 60% sea water, NAA in combination with 60% sea water or a mixture of growth regulators with 20% sea water significantly increased number of roots per plant.

Shoot dry weight matter percentages significantly increased with application of growth regulators, sea water alone or in combination in irrigation water, compared to untreated seedlings, except for the application of a mixture of IAA and NAA in combination with 60% sea water which did not affect the leaf dry matter (%) relative to the control. Irrigation of Hatamy seedlings with growth regulators, sea water alone or in combination, did not affect root dry matter percentage compared with the control, except for 20 and 40% sea water combined with NAA, which significantly reduced root dry matter percentage compared with the control (Table 1).

Effect of Salinity and Growth Regulators on Leaf Mineral Concentrations

Irrigation of Hatamy seedlings with IAA, NAA or salinity alone or in combination significantly increased N concentrations in the leaves compared with untreated seedlings (Table 1). This result is in agreement with those of Zekri (1993) and Aljuburi (1996). They showed that salinity increased leaf N concentrations of citrus leaves or date palm seedling shoots of Khalas cultivar. The results of growth regulators are in agreement with those of Aljuburi (1996), who showed the GA_3 increased N concentration in Khalas seedlings.

Irrigation of date palm seedlings with different concentrations of sea water alone or in combination with IAA or NAA significantly increased leaf P concentrations, with the exception of the 20% sea water in combination with a mixture of IAA and NAA, which significantly decreased leaf P concentration of Hatamy seedlings relative to the control. These results are in agreement with those of Aljuburi and Al-Masry (1996) who demonstrated that leaf P concentrations in date palm seedlings increased with addition of salinity in combination with IAA in irrigation water, and with Zekri (1993) who found the addition of NaCl increased P concentrations in the shoots of citrus seedlings.

Leaf K^+ concentrations of Hatamy seedlings significantly decreased when irrigated with 60% sea water alone or in combination with NAA or 40% sea water in combination with a mixture of IAA and NAA compared to the control. These findings are also in agreement with those of Aljuburi (1996), Banuls et al. (1990) and Grattan and Grieve (1994), who showed that salinity decreased K^+ concentration in date palm seedlings, and citrus leaves, respectively. Other treatments, either growth regulators alone or in combination with different sea water, significantly increased leaf K^+ concentrations compared to the control. These results are in agreement with those of Mayber and Lerner (1994) who hypothesized, that the application of exogenous hormones on stressed plants may ameliorate the endogenous balance of hormones.

Application of NAA alone on date palm seedlings significantly increased leaf Ca^{2+} concentrations over the control (Table 1). Leaf Ca^{2+} significantly decreased with application of a mixture of IAA and NAA or different sea water concentrations alone compared with the control. Leaf Ca^{2+} also significantly reduced with irrigation of Hatamy date palm seedlings with 20 or 40% sea water in combination with a mixture of IAA and NAA, furthermore, Hatamy seedlings irrigated with 60% sea water in a combination with

either IAA or NAA decreased leaf Ca^{2+} relative to untreated seedlings (Table 1). Irrigation of Hatamy seedlings with IAA alone or in combination with 20 or 40% sea water had no effect on leaf Ca^{2+} concentrations. The addition of 40 or 60% sea water in combination with NAA or a mixture of IAA and NAA to irrigation water had no effect on leaf Ca^{2+} concentrations of Hatamy seedlings compared with the control.

Compared to the control, IAA, NAA, a mixture of IAA and NAA, and different concentrations of sea water alone or in combination treatments significantly decreased leaf Mg^{2+} concentrations in Hatamy seedlings except for the treatments containing 40% sea water and 20% sea water in combination with IAA which had no significant effect on leaf Mg^{2+} concentrations. The results of leaf Ca and Mg concentrations of Hatamy seedlings agree with those of Zekri and Persons (1990), Zekri (1993) and Banuls et al. (1990) who showed that salinity alone or in combination with growth regulators in irrigation water decreased leaf Ca^{2+} or Mg^{2+} concentrations in citrus.

Leaf Na concentrations of Hatamy seedlings were increased when irrigated with NAA application alone or in combination with 40% sea water, while it was not affected by NAA in combination with 20 or 60% sea water compared to the control. IAA alone or in combination with 20, 40 or 60% sea water had no significant effect on leaf Na concentrations relative to the control. A mixture of IAA and NAA reduced leaf Na levels, whereas in combination with 40 or 60% sea water leaf Na concentrations were significantly increased. However, the mixture in combination with 20% sea water had no significant effect on leaf Na concentrations compared to the control.

The addition of 40 or 60% sea water to irrigation water each 20 days for 120 days significantly increased leaf Na concentrations, where as 20% sea water reduced leaf Na concentrations compared to untreated seedlings (Table 2).

The indole acetic acid treatment alone or in combination with 20, 40 or 60% sea water significantly increased leaf Cl^- concentrations relative to untreated seedlings. Application of NAA alone significantly increased leaf Cl^- concentrations, but NAA in combination with 40% sea water significantly reduced Cl^- concentrations in Hatamy leaves. A combination of NAA with 20 or 60% sea water had no significant effect on leaf Cl^- levels relative to the control.

Mixture of IAA and NAA alone or in combination with 20 or 40% sea water, did not affect leaf Cl^- concentrations compared to the control, except the mixture in combination with 60% sea water which increased the leaf Cl^- concentrations relative to the untreated seedlings. Date palm seedlings irrigated with saline water (20, 40 or 60% sea water) significantly increased leaf Cl^- concentrations compared to the control.

The results of leaf Na^+ and Cl^- concentrations in Hatamy seedlings are in agreement with those of Zekri (1993), Aljuburi (1996) and Aljuburi and Al-Masry (2000), who showed that salinity in irrigation water increased leaf Na^+ and Cl^- concentrations in citrus and date palm seedlings. Other workers (Aljuburi, 1996; Aljuburi and Al-Masry, 2000) have shown that the application of plant growth regulators alone or in combination with sea water increased, decreased or had no effect on leaf Na and Cl of date palm seedlings.

Application of IAA alone or in combination with 20, 40 or 60% sea water significantly increased leaf Cu concentrations of Hatamy seedlings compared to the control. Date palm seedlings irrigated with water containing NAA alone or in combination with 60% sea water did not affect leaf Cu concentrations, but, NAA in combination with 20 or 40% sea water significantly increased leaf Cu concentrations compared with the control.

A mixture of IAA and NAA alone or in combination with 40% sea water significantly increased leaf Cu concentrations, but when combined with 20% sea water leaf Cu concentrations were decreased compared with the control. Irrigatiing Hatamy with 60% sea water in combination with a mixture of growth regulators did not change leaf Cu concentrations of treated seedlings compared with the results obtained with control seedlings. Leaf Cu concenrations of Hatamy seedlings were reduced with 20% sea water applied alone in irrigation water. Application of 40 or 60% sea water in irrigation

water alone significantly increased Leaf Cu concentrations of Hatamy seedling compared with the control. These results on leaf Cu are consistent with Grattan and Grieve (1994) who reported that the micronutrient concentrations in plant shoots may increase, decrease or have no effect.

Application of IAA alone significantly increased leaf Mn concentrations, but these concentrations decreased when IAA was applied in combination with 20 or 40% sea water relative to the control. The leaf Mn concentrations of Hatamy seedlings irrigated for 120 days with 60% sea water in combination with IAA were not affected, compared to the control seedlings. When irrigated for 120 days with NAA solution alone or in combination with 20% sea water leaf Mn concentrations of date palm seedlings were significantly increased. Application of NAA in combination with 40 or 60% sea water reduced the seedling leaf Mn concentrations compared to the control. Leaf Mn concentrations of Hatamy seedlings were increased when irrigated with a mixture of IAA and NAA solution for 120 days, while they were not affected by the mixture with 20% sea water compared to the control. Application of a mixture of IAA and NAA in combination with 40 or 60% sea water significantly reduced seedling leaf Mn concentrations compared to the control. The addition of 20 or 40% sea water to irrigation water significantly increased leaf Mn concentrations of Hatamy seedlings, but 60% sea water significantly decreased leaf Mn concentrations compared to the control.

Application of IAA alone significantly increased leaf Zn concentration, but the combination of IAA and salinity (20, 40 or 60% sea water) reduced leaf Zn concentrations. Compared with control seedlings, leaf Zn concentrations of Hatamy seedlings significantly increased when irrigated with NAA solution alone or in combination with 20% sea water. The combination of NAA with 40 or 60% sea water significantly decreased leaf Zn concentrations compared to the control. The mixture of IAA and NAA alone or in combination with 20% sea water significantly increased leaf Zn concentration, whereas, application of the growth regulators in combination with 40 or 60% sea water had no significant effect on the concentration of Zn in leaves of Hatamy seedlings relative to the control. Irrigating Hatamy seedlings with water containing 20, 40 or 60% sea water significantly decreased leaf Zn concentration compared with untreated control seedlings.

Application of IAA, NAA or different concentrations of sea water in irrigation water, alone or in combination, reduced leaf Fe concentrations of Hatamy seedlings compared to the control, except the combination of NAA with 40 or 60% sea water, which increased the leaf Fe concentration over the control (Table 2). These results of micro nutrients (Cu, Mn, Zn and Fe) are in agreement with those of Aljuburi (1996) and Grattan and Grieve (1994), who demonstrated that salinity in irrigation water increased or decreased or had no effect on leaf mineral contents.

Application of IAA alone or in combination with different concentrations of sea water increased the leaf K/Na ratio compared to the control. Leaf K/Na ratio significantly increased with irrigation of Hatamy seedlings with NAA solution alone or in combination with 40 or 60% sea water relative to untreated conrol seedlings. Date palm seedlings irrigated with water containing a mixture of NAA and IAA alone significantly increased leaf K/Na ratio over the control, but a mixture of IAA and NAA in combination with 20% sea water did not significantly increase leaf K/Na ratio compared with untreated seedlings.

Compared with the control irrigation, Hatamy seedlings with a mixture in combination with 40 or 60% sea water significantly reduced leaf K/Na ratio. Irrigating date palm seedlings of Hatamy cultivar with 20 or 40% sea water significantly increased leaf K/Na ratio, whereas irrigation of seedlings with 60% sea water alone for 120 days significantly reduced K/Na ratio relative to the control.

These results of leaf K/Na ratio of Hatamy seedlings agree with those of Jacoby (1994) who showed that salinity in irrigation water decreased leaf K/Na ratio and this could contribute to excess Na accumulation as well as K leakage. The results also concur with those of Mayber and Lerner (1994), who showed that the application of salinity in

irrigation water affected the level of endogenous plant hormones, and the hormonal balance of the plant was disturbed. This disturbance could impact on the growth and development of plants, therefore it was suggested that the application of exogenous growth regulators could possibly ameliorate the endogenous balance of plant hormones.

Leaf Mg^{2+}/Ca^{2+} ratio of Hatamy seedlings decreased when irrigated with IAA alone or in combination with 40 or 60% sea water, while it increased with the application of IAA in combination with 20% sea water compared to the control. When date palm seedlings were irrigated for 120 days with NAA alone or in combination with 60% sea water, the leaf Mg^{2+}/Ca^{2+} ratio significantly reduced. Nevertheless, the irrigation of CV. Hatamy seedlings with NAA in combination with 40% sea water did not affect the leaf Mg^{2+}/Ca^{2+} ratio compared to the untreated control (Table 2). Leaf Mg^{2+}/Ca^{2+} ratio of Hatamy seedlings increased when irrigated with NAA in combination with 20% sea water compared to the control.

The addition to irrigation water of a mixture of 300 mg/l IAA and 400 mg/l NAA for 120 days, alone or in combination with 20% sea water, significantly decreased leaf Mg^{2+}/Ca^{2+}ratio, while it was not affected by a combination of the mixture with 60% sea water over the control, but 40% sea water in combination with a mixture of IAA and NAA increased leaf Mg^{2+}/Ca^{2+} ratio compared to the control.

Leaf Mg^{2+}/Ca^{2+} ratio in Hatamy seedlings significantly increased with application of 20 or 40% sea water alone in irrigation water. However, application of 60% sea water alone significantly reduced leaf Mg^{2+}/Ca^{2+} ratio compared to the control (Table 2).

Effect of Salinity and Growth Regulators on Root Mineral Concentration

Cultivar Hatamy seedlings irrigated for 120 days with 300 mg/L or 400 mg/L NAA or a mixture of 300 mg/L IAA and 400 mg/L NAA alone or in combination with different concentrations of sea water significantly increased root N concentrations compared to the the control seedlings, except for the combination of NAA with 20% sea water or the combination of a mixture of IAA and NAA with 20% sea water. These treatments did not show significant effects (Table 3). These effects of salinity on root N concentrations are in agreement with those of Aljuburi (1996) and Aljuburi and Al-Masry (2000), who showed that salinity in irrigation water had no effect root N concentrations of date palm seedlings, however, application of IAA alone or in combination with saline water increased root N concentration of Lulu seedlings (Aljuburi and Al-Masry, 2000).

Application of IAA, NAA, a mixture (IAA + NAA) and different concentrations of sea water, alone or in combination, significantly increased root P concentrations of Hatamy seedlings compared to the control, with the exception of the 20% sea water alone or in combination with IAA, or a mixture of IAA and NAA. These treatments had no significant effect on Hatamy root P concentration compared to the control. These results concur with those of Aljuburi et al. (2005) and Aljuburi and Al-Masry (2000), who demonstrated that the application of NAA or IAA on date palm seedlings increased root N and P concentrations.

Root K concentrations of Hatamy seedlings were decreased when irrigated with IAA alone. Indole acetic acid or NAA in combination with 60% sea water or a mixture of IAA and NAA alone or in combination with 20 or 60% sea water had no effect on root K^+ concentrations compared to the control. Application of IAA in combination with 20 or 40% sea water or NAA alone or in combination with 20 or 40% sea water or a mixture of IAA and NAA in combination with 40% sea water or different concentrations of sea water alone, significantly increased root K concentrations, compared to the control (Table 3). The results are in agreement with those of Winter and Kirst (1991), Champagonal (1979) and Sharpley et al. (1992), who demonstrated that the response of K, N, P contents in different plants to external Na^+ was not uniform, may increase or decrease or have no effect, whereas others (Okusanya and Ungar, 1984; Sabbarao et al., 1990), found that the K^+ concentration in plant tissue is reduced as the Na^+ salinity or Na/Ca ratio in the root media is increased.

Root Ca^{2+} concentrations of date palm seedlings increased when irrigated with

40% sea water alone or IAA alone or in combination with 20% sea water, or NAA or a mixture of IAA and NAA alone or in combination with different concentrations of sea water. However, root Ca^{2+} concentrations were not affected by addition to irrigation water of 300 mg/L IAA in combination with 40 or 60% sea water or 20 or 60% sea water compared to untreated seedlings.

The increase of Ca^{2+} in date palm seedlings may explain why date palms tolerate high concentrations of salinity. Calcium is an essential mineral nutrient that helps maintain membrane integrity and is important in senescence processes. It is known to counteract the harmful effects of Na on crops. Calcium protects membranes from the adverse effect of Na and minimizes the leakage of cytosolic K. Ca plays a vital role in regulating ionic relations in plants and improving the physical condition of the soil (Alam, 1994).

Irrigation of Hatamy seedlings with 20 or 40% sea water, IAA, NAA and a mixture of IAA, NAA alone or in combination with different concentrations of sea water, significantly increased root Mg^{2+} concentration compared to the control, while it was not affected by 60% sea water compared to the control. Root Na^{+} concentrations in Hatamy seedlings significantly increased with application of IAA, NAA, a mixture of IAA and NAA alone or in combination with various concentrations of sea water, or sea water alone, over the control. However, NAA applied in combination with 60% sea water had no effect on root Na concentrations compared to the control.

Application of IAA, NAA or a mixture of IAA and NAA, alone or in combination with 60% sea water or 60% sea water alone, significantly increased root Cl^{-} concentration compared to the control. However, 20 or 40% sea water alone or in combination with IAA, NAA or a mixture of IAA and NAA had no significant effect. These results on Na and Cl concentrations in roots are consistent with those of Alam (1994) and Lessani and Marschner (1978), who reported that chloride is a more sensitive indicator of salt damage than Na, since it is stored by the plants, whereas Na is absorbed in smaller quantities despite high Na concentrations in the soil. They also demonstrated that Cl content varied in different salt stressed crops, but it was always higher than Na.

Irrigation of Hatamy seedlings with different concentrations of sea water alone significantly increased root Cu concentrations compared to the control. The combination of IAA with 20 or 60% sea water or NAA with 20 or 40% sea water or a mixture of IAA and NAA with 20% sea water, significantly increased root Cu concentrations compared with the control. Irrigation of date palm seedlings with IAA, NAA, or a mixture of IAA and NAA alone or in combination with 40 or 60% and 40 or 60% sea water, respectively, had no significant effect on root Cu concentration compared with the control.

Root Mn concentrations of Hatamy seedlings were increased when irrigated with IAA, NAA alone or in combination with 40 or 60% sea water and 20 or 60% sea water, respectively, or the combination of IAA and NAA with 20 or 60% sea water. A reduction in root Mn concentrations occurred when seedlings were irrigated with different concentrations of sea water compared to the control.

Root Zn concentrations of Hatamy seedlings decreased when irrigated with different concentrations of sea water, IAA, a mixture of IAA and NAA, alone or with the combination of IAA, NAA, or a mixture of NAA and IAA and 60% sea water, compared to the control. Application of NAA alone or in combination with 20% sea water, IAA in combination with 20 or 40% sea water or a mixture with 40% sea water, increased root Zn concentration over the control, while it was not affected by the combination of NAA with 40% sea water or a mixture of IAA and NAA with 20% sea water, relative to untreated seedlings.

When irrigated with IAA, NAA, a mixture of IAA and NAA or 20% sea water alone or the combination of IAA, with 40 sea water, NAA in combination with 40 and 60% sea water or a mixture with 20 and 40% sea water, seedling root Fe concentration was higher than that of the control. Salinity (40 or 60% sea water) alone in irrigation water or IAA in combination with 20 or 60% sea water significantly increased root Fe concentration of Hatamy seedlings compared with the control. Nevertheless, the irrigation

of Hatamy seedlings with water containing NAA in combination with 20% sea water, or a mixture of IAA and NAA in combination with 60% sea water did not affect the root Fe concentration compared with the untreated control.

Irrigation of Hatamy seedlings with NAA alone or in combination with 20 or 60% sea water significantly reduced root K^+/Na^+ ratio, but addition of 40% sea water to irrigation water increased root K^+/Na^+ ratio compared with the control (Table 3). Addition to irrigation water of 400 mg/L NAA alone or in combination with 40% sea water significantly decreased root K^+/Na^+ ratio of Hatamy seedlings compared with untreated seedlings. Root K^+/Na^+ ratio of seedlings increased when irrigated with NAA in combination with 20% sea water, while the ratio was not affected by 60% sea water compared to the control.

Irrigation of Hatamy seedlings with a mixture of 300 mg/L IAA and 400 mg/L NAA alone or in combination with 40% sea water, decreased root K^+/Na^+ ratio compared with the control. Irrigation with a combination of IAA and NAA with 20% sea water significantly increased seedling root K^+/Na^+ ratio compared to the control. High salinity concentration (60% sea water) in irrigation water in combination with a mixture of IAA and NAA had no significant effect on Hatamy root K^+/Na^+ ratio compared with the control.

Low or high salinity (20 or 60% sea water) alone in irrigation water significantly increased the root K^+/Na^+ ratio in seedlings compared to the control, whereas the addition of 40% sea water to irrigation water significantly decreased seedling root K^+/Na^+ ratio compared with the control. The results showed that the ratio of K^+/Na^+ in leaves was higher than in the roots. A possible explanation is that leaves appear more sensitive than roots to Na, and the enzymes involved in photosynthesis and other metabolic enzymes are also susceptible to Na. Leaves are considered the source of energy and other compounds, therefore the plant may have restricted Na to the roots and replaced it in the leaves with K^+. It appears that roots are more tolerant to high concentration of Na than leaves.

Root Mg^{2+}/Ca^{2+} ratio increased with application of IAA alone or in combination with 40 or 60% sea water compared with the control, while it was not affected by the combination of IAA and 20% sea water compare to the control.

Root Mg^{2+}/Ca^{2+} ratio increased with application of NAA alone or in combination with 40% sea water compared with the control, whereas the combination of NAA with 20 or 60% sea water significantly decreased the ratio of root Mg^{2+}/Ca^{2+} relative to the control. The mixture of 300 mg/L IAA and 400 mg/L NAA alone or in combination with 40 or 60% sea water significantly increased the seedling root Mg^{2+}/Ca^{2+} ratio over the control. Irrigation of Hatamy seedlings with 20 or 40% sea water alone significantly increased the seedling root Mg^{2+}/Ca^{2+} ratio relative to untreated control seedlings. The addition of high salt (60% sea water) to irrigation water had no effect on root Mg^{2+}/Ca^{2+} ratio compared to the control.

The results of Mg^{2+}/Ca^{2+} ratio indicate that date palm trees are tolerant to high concentrations of salinity in irrigation water despite the fact that they are not halophyte trees. It was found that the ratio of Mg^{2+}/Ca^{2+} in most sea water was 5/1 on a molar basis, and, if the ratio of Mg^{2+}/Ca^{2+} increased to greater than one, reduction in growth would occur (Grattan and Grieve, 1994). Therefore this ratio in the experimental date palm seedlings was maintained under one so that growth in all treatments continued, even in 60% sea water for 120 days. These results might support the hypothesis that date palm seedlings are able to absorb water only, without the salt, or they absorb more Ca^{2+} to replace Mg^{2+} ions to protect the membranes, enzymes and protein from degredation or deformity. However, this might be more evidence for the high ability of date palm to tolarate high salinity in irrigation water. It is clear that the ratio in roots was greater than in shoots for most treatments.

DISCUSSION

The results revealed that the spraying of IAA alone on Hatamy seedlings significantly increased leaf N, P, K, Cl, Cu, Mn, Zn concentrations and also increased the

ratio of K/Na, seedling length and dry matter percentage of shoots. It decreased leaf Mg, and Fe concentration, and the ratio of Mg/Ca compared with untreated seedlings. Application of IAA on seedlings significantly increased root N, P, Ca, Mg, Na, Cl, Mn and Fe concentrations and the ratio of Mg/Ca, but root K and Zn concentrations and K/Na ratios significantly decreased compared with the control. The application of a combination of IAA and 60% sea water on date palm seedlings increased leaf N, P, K, Cl and Cu concentrations, the ratio of K/Na, the number of roots and leaves per seedling and shoot dry matter percentage, but significantly reduced leaf Ca, Mg, Zn and Fe concentrations and the ratio of Mg/Ca compared to the control. Root N, P, Mg, Na, Cl, Cu and Mn concentrations and the ratio of Mg/Ca significantly increased, but only root Zn and Fe concentrations and the ratio of K/Na significantly decreased with application of IAA in combination with 60% sea water on Hatamy seedlings compared with the control.

Leaf N, P, K, Ca, Na, Cl, Mn and Zn concentrations, the ratio of K/Na and shoot dry matter percentage were significantly increased when irrigated with NAA alone, but leaf Mg and Fe concentrations and the ratio of Mg/Ca were significantly reduced compared with untreated seedlings. However, all root elements were significantly increased with application of NAA on Hatamy seedlings, while NAA had no significant effect on root Cu concentration and significantly reduced the ratio of K/Na compared with the control seedlings.

Cultivar Hatamy seedlings irrigated for 120 days with 60% sea water in combination with NAA, significantly increased leaf N, P and Fe concentrations, root numbers and shoot dry matter percentage, while leaf K, Ca, Mg, Mn and Zn concentrations and the ratio of Mg/Ca were significantly reduced compared with untreated seedlings. Root N, P, Ca, Mg, Cl and Fe concentrations were significantly increased, while root Zn concentrations and the ratio of Mg/Ca significantly decreased. Nevertheless, the irrigation of Hatamy seedlings with water containing NAA in combination with 60% sea water did not effect the leaf K, Na, Cu, Mn concentrations and the ratio of K/Na compared to the control.

Application of a mixture of IAA and NAA in combination with 60% sea water on Hatamy seedlings increased leaf N, P, K, Na and Cl concentrations and root numbers/plant, and decreased leaf Mg, Mn, Zn and Fe concentrations and the ratio of K/Na compared with the control. Root N, P, Ca, Mg, Na, Cl and Mn concentrations and the ratio of Mg/Ca were significantly increased with application of 60% sea water and a mixture of IAA and NAA, compared to the control. However, the application of the combination of a mixture and 60% of sea water either did not affect the root K, Cu and Fe concentrations and the ratio of K/Na or reduced the root Zn concentrations relative to the control.

Application of high salt concentration (60% sea water) alone on Hatamy seedlings significantly increased leaf N, P, Na, Cl and Cu concentrations and leaf and root number and shoot dry matter percentage compared with untreated seedlings. However, the irrigation of seedlings with 60% sea water significantly reduced leaf K, Ca, Mg, Mn, Zn and Fe concentrations and the ratio of K/Na and Mg/Ca compared to the control.

Root P, K, Na, Cl and Cu concentrations and the ratio of K/Na were significantly increased with application of 60% sea water on seedlings compared to the control, whereas other root elements were significantly decreased, such as Mn, Zn and Fe concentrations relative to the untreated seedlings. Irrigation of Hatamy seedlings for 120 days did not significantly affect root N, Ca and Mg concentrations and the ratio of Mg/Ca compared with the control. Also the results showed that the concentrations of Na and Cl in the roots was greater than in the shoots by about 8-10 times, and in general, the concentrations of Na in both the leaves and roots was less than the concentrations of Cl. Similar results were obtained in previous studies (Francois and Kleiman, 1990; Johson, 1991 and Boursier and Lauchi, 1990). However, increased Na and Cl concentrations in leaves and roots of date palm seedlings, accompanied by significant increases in leaf and root numbers and dry matter percentage of shoots, without any symptoms of salinity stress, may indicate that high accumulation of Na and Cl could play an important role in

osmotic adjustment in vacuoles that improves water balance. Similar results by Aljuburi et al. (2005), Hassan and El-Samnoudi (1993) were obtained.

The results showed that the application of 400 mg/L NAA per seedling every 20 days for 120 days was more effective in reducing salt stress injury than IAA and a mixture of IAA and NAA. It increased leaf and root N, P and Fe and root Ca and Mg concentrations, as well as root number, shoot dry matter percentage and reduced leaf and root Mg/Ca ratio, compared with the control. Furthermore it decreased leaf and root Na and leaf Cl concentrations and increased K/Na ratio in leaves and roots compared to the application of 60% sea water alone in irrigation water on Hatamy seedlings.

These results are in agreement with Aljuburi et al. (2005) and Mayber and Lerner (1994), who reported that the application of growth regulators could assist the accumulation of mineral elements in leaves and roots of treated plants, thereby increasing plant growth and its resistance to salt stress. These findings are also in agreement with other researchers (Hale and Orcutt, 1987; Aljuburi et al., 2005; Alam, 1994), who showed that plant growth regulators can improve nutrient uptake and the efficiency of their use by altering the rate of membrane selectivity to element uptake. This leads to improved growth of shoots and roots and increased volume of soil penetrated by roots.

CONCLUSION

The irrigation of Hatamy date palm seedlings with NAA in combination with sea water reduced the adverse effect of salinity by reducing the accumulation of Na and Cl in the leaves, and reducing Na and the ratio of Mg/Ca in both leaves and roots. It also resulted in increased N, P and Fe concentrations and the ratio of K/Na in both leaves and roots, and increased Ca and Mg concentrations in the roots, increased number of roots per plant and increased shoot dry matter percentage of seedlings compared to using 60% sea water alone.

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Tables

Table 1. Effect of sea water (SW), IAA, NAA and a mixture of IAA and NAA on length of shoots, and roots, mumber of leaves and roots and dry matter percentage of shoots and roots of Hatamy cultivar date palm seedlings.*

Treatments			Seedling length (cm)	Number of leaves/ seedling	Number of roots/ seedling	Dry matter (%)	
						Shoot	Root
Control			96.97	13.00	12.67	33.63	35.27
300 mg/L IAA alone			105.70	12.67	13.33	37.65	35.91
+ 20% SW.			99.73	13.67	17.00	36.37	33.47
+ 40% SW.			107.07	15.00	15.33	35.04	34.75
+ 60% SW.			101.63	15.67	16.33	36.05	36.93
400 mg/L NAA alone			102.33	12.33	13.00	35.45	35.41
+ 20% SW.			99.00	11.67	13.33	35.42	32.47
+ 40% SW.			105.30	14.00	15.00	38.02	31.07
+ 60% SW.			104.50	14.33	16.00	38.71	36.97
Mixture of IAA and NAA			112.67	13.67	13.67	38.34	33.53
+ 20% SW.			90.13	16.33	16.33	35.56	35.69
+ 40% SW.			97.73	15.00	16.33	35.91	34.31
+ 60% SW.			89.63	14.67	16.33	34.21	35.08
20% SW alone			104.60	13.33	13.67	35.18	35.17
40% SW alone			106.77	15.00	16.00	35.44	34.83
60% SW alone			95.76	15.33	15.00	36.09	34.64
L.S.Δ.	0.05	G.R.	8.153	1.023	1.533	0.636	0.927
		S.W.	8.153	1.023	1.533	0.636	0.927
		G.R.X S.W.	16.31	2.046	3.067	1.272	1.914
	0.01	G.R.	10.98	1.378	2.065	0.856	1.289
		S.W.	10.98	1.378	2.065	0.856	1.289
		G.R.X S.W.	21.96	2.755	4.130	1.713	2.577

*Values are mean of analysis of three seedlings: Three replications each with one seedlings.

Table 2. Effect of sea water (SW), IAA, NAA and a mixture of IAA and NAA on leaf mineral concentration of Hatamy cultivar date palm seedlings.*

N	P	K	Cu	Mg	Na	Cl	Cu	Mn	Zn	Fe

nts		Dry weight (%)							Dry weight (ppm)				Mg/Ca
		0.724	0.176	0.956	0.467	0.305	0.089	0.203	2.621	18.200	32.740	133.185	0.65
IAA alone		1.041	0.204	1.183	0.462	0.226	0.086	0.258	5.005	20.211	39.118	128.944	0.49
W		0.873	0.252	1.149	0.422	0.304	0.086	0.228	7.008	16.258	28.012	124.602	0.72
W		0.967	0.255	1.722	0.480	0.279	0.095	0.226	4.505	16.188	26.386	100.842	0.59
W		0.995	0.243	1.026	0.369	0.210	0.088	0.237	4.010	18.168	28.366	97.475	0.57
NAA alone		1.009	0.223	1.594	0.523	0.290	0.093	0.268	2.712	19.996	41.509	124.704	0.55
W		0.836	0.232	1.749	0.354	0.246	0.088	0.218	3.720	20.020	31.101	106.300	0.69
W		0.886	0.189	1.157	0.421	0.67	0.097	0.178	3.539	16.301	18.765	157.478	0.63
W		0.993	0.208	0.908	0.407	0.236	0.088	0.215	2.334	13.456	31.082	144.886	0.58
of IAA and NAA		1.190	0.219	0.990	0.392	0.224	0.077	0.188	5.227	19.650	50.690	126.430	0.57
W		1.019	0.152	0.938	0.402	0.233	0.089	0.187	1.935	17.837	43.770	110.417	0.58
W		1.094	0.198	0.918	0.356	0.251	0.110	0.219	3.250	12.150	19.800	115.450	0.71
W		0.885	0.223	0.989	0.422	0.267	0.117	0.235	2.619	12.549	26.334	84.881	0.63
alone		0.898	0.257	1.690	0.342	0.243	0.077	0.242	2.090	18.682	31.692	97.811	0.71
alone		1.086	0.272	1.615	0.383	0.310	0.097	0.250	5.208	21.925	19.145	87.202	0.81
alone		0.983	0.193	0.859	0.419	0.264	0.107	0.303	3.247	17.043	20.929	107.243	0.63
	G.R.	0.049	0.009	0.010	0.025	0.010	0.004	0.009	0.173	0.317	0.687	2.746	0.013
0.05	S.W.	0.049	0.009	0.010	0.025	0.010	0.004	0.009	0.173	0.317	0.687	2.746	0.013
	G.R.X.S.W	0.099	0.018	0.021	0.050	0.021	0.008	0.019	0.346	0.635	1.373	5.491	0.026
	G.R.	0.066	0.012	0.014	0.034	0.014	0.005	0.013	0.233	0.428	0.925	3.698	0.017
0.01	S.W.	0.066	0.012	0.014	0.034	0.014	0.005	0.013	0.233	0.428	0.925	3.698	0.017
	G.R.X.S.W	0.133	0.024	0.232	0.067	0.028	0.011	0.026	0.466	0.855	1.850	7.395	0.033

* Values are mean of analysis of three seedlings: three replications each with one seedlings.

Table 3. Effect of sea water (SW), IAA and a mixture of IAA and NAA on root mineral concentration of Hatamy cultivar date palm seedlings.*

nts	N	P	K	Cu	Mg	Na	Cl	Cu	Mn	Zn	Fe	Mg/Ca
	Dry weight (%)							Dry weight (ppm)				
	0.380	0.104	0.910	0.324	0.347	0.513	0.268	2.587	5.970	9.751	134.627	1.07
L IAA alone	0.521	0.116	0.855	0.397	0.499	0.763	0.384	2.478	7.334	7.879	158.375	1.26
SW	0.443	0.098	1.046	0.371	0.400	0.644	0.278	5.815	5.169	10.911	99.105	1.08
SW	0.471	0.129	1.268	0.317	0.443	0.609	0.259	2.390	6.823	11.703	137.072	1.40
SW	0.459	0.118	0.886	0.347	0.586	0.969	0.371	4.000	8.000	5.650	120.500	1.69
L NAA alone	0.509	0.113	1.058	0.373	0.439	0.648	0.348	2.600	6.750	11.800	168.000	1.18
SW	0.401	0.113	1.778	0.443	0.452	0.625	0.275	6.350	5.650	10.700	135.050	1.02
SW	0.541	0.125	1.212	0.376	0.415	1.062	0.280	4.446	6.639	9.641	174.761	1.10
SW	0.486	0.129	0.908	0.424	0.408	0.476	0.359	2.393	6.082	8.830	142.522	0.96
of IAA and NAA	0.581	0.112	0.974	0.446	0.545	0.829	0.355	2.886	5.821	7.910	145.771	1.22
SW	0.400	0.099	0.901	0.436	0.404	0.449	0.259	4.113	6.492	10.358	168.731	0.93
SW	0.483	0.117	1.347	0.429	0.516	1.031	0.276	2.350	6.100	8.450	142.450	1.20
SW	0.467	0.119	0.873	0.389	0.583	0.672	0.373	3.197	7.835	5.862	131.511	1.50
alone	0.379	0.101	0.959	0.331	0.394	0.645	0.262	2.986	5.263	5.164	158.540	1.19
alone	0.365	0.129	1.074	0.413	0.490	1.038	0.277	4.980	4.183	4.781	83.068	1.19
alone	0.360	0.120	1.041	0.337	0.357	0.690	0.362	4.270	4.965	5.362	102.781	1.06
G.R.	0.021	0.004	0.034	0.017	0.016	0.023	0.014	0.165	0.201	0.271	1.650	0.014
0.05 S.W.	0.021	0.004	0.034	0.017	0.016	0.023	0.014	0.165	0.201	0.271	1.650	0.014
G.R.X.S.W	0.042	0.008	0.069	0.034	0.032	0.045	0.027	0.331	0.401	0.542	3.301	0.027
G.R.	0.029	0.006	0.046	0.023	0.022	0.030	0.018	0.223	0.270	0.365	2.222	0.018
0.01 S.W.	0.029	0.006	0.046	0.023	0.022	0.030	0.018	0.223	0.270	0.365	2.222	0.018
G.R.X.S.W	0.057	0.011	0.093	0.045	0.043	0.061	0.037	0.445	0.540	0.730	4.445	0.037

* Values are mean of analysis of three seedlings: three replications each with one seedlings.

Nutritional Dynamics of Date Palm (*Phoenix dactylifera* L.)

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Keywords: nutrient, mineral, status, concentration

Abstract

Leaves and fruit of date palms (*Phoenix dactylifera* L.) cv. 'Deglet Noor' grown under commercial conditions were assessed during the course of an entire growing season for nutrient levels of most macro- and micro-nutrients. The different nutrients showed various patterns in the different organs throughout the year. Implications of these patterns for nutritional analysis are discussed. Since nutritional studies on date palms in the USA have shown varied results over the decades, a comparison is made with results from other countries, particularly Egypt.

INTRODUCTION

Nutritional analysis of date palms (*Phoenix dactylifera* L.) has not received attention in the USA for nearly 50 years. Diagnostic values for nutrient concentrations have not been established and values published in guides have generally been those utilized for oil palm (*Elaeis guineensis* Jacq.) or those simply reported but not correlated with horticultural performance. Most nutritional work with date palms in the USA is several decades old and focused more on responses to fertilization rather than nutrient concentrations in organs of date palms. However, there have been a few reports dealing with nutrient concentrations in date palms grown in the Coachella Valley of California, the major date-producing region of the USA.

Reuther (1948) sampled leaf pinnae from leaves of different phyllotactic age and from different positions on the rachis from 'Deglet Noor'. There was a distinct gradient from the basal to the distal portions of the leaf for most elements, and from younger to older leaves on the tree. Reuther (1948) thus considered that pinnae from the middle portion of the rachis represented the average concentrations for the various elements. No obvious mineral deficiencies were observed in surveying a number of date gardens, and neither were differences in nutrient concentrations noted between low and high vigor palms. The work of Reuther (1948) was performed in the spring; however, he recommended that sampling be done in the fall from leaves near the bunches.

Furr and Cook (1952) sampled 'Deglet Noor' and 'Khadrawy' utilizing the technique of Reuther (1948). N concentrations were higher in leaves of fertilized as compared to non-fertilized trees. However, there was no consistent correlation between yield and either fertilization or leaf N levels. There was an apparent weak response from 'Deglet Noor' but none observed with 'Khadrawy'. Embleton and Cook (1947) analyzed macro-elements in pruned leaves and fruit stalks in an attempt to estimate the amount of these elements removed from the tree. This was found to be about the same amount as was returned to the soil by cover crops. Labanauskas and Nixon (1962) sampled healthy and declining palms but noted no significant differences in nutrient concentrations. The values reported for most elements were lower than those established for citrus.

Unfortunately, there has been little nutritional work on date palms in California since the early 1960s despite changes in fertilization and other cultural practices. However, Abdul-Baki et al. (2002a) surveyed concentrations of certain elements in 'Deglet Noor' and 'Medjool' trees in the Coachella and Bard Valleys in California. They observed no differences between the two varieties. However, there were wide variations between and within orchards, which were attributed to differences in soil conditions. Concentrations of individual macro-elements were similar to those reported 50 years earlier by Reuther (1948) and Embleton and Cook (1947) and those of individual micro-

elements were similar to those reported by Labanauskas and Nixon (1962). Abdul-Baki et al. (2002b) also reported higher concentrations of the elements in fruit strands of 'Medjool' than those reported by El-Shurafa (1984). Abdul-Baki et al. (2002b) observed very high concentrations of most elements (especially Ca, Mg, and B) except K in the distal portion of the strands as compared with the other portion and associated this accumulation with a browning, dieback, and eventual senescence of distal portions of the fruit strands.

The related species *P. canariensis* L. is often grown for ornamental purposes in certain regions of the USA. Broschat (1997) found that nutrient concentrations in the pinnae had a different pattern of variation than that reported by Reuther (1948). A gradient in most nutrients between younger and older leaves was observed by Broschat (1997) and seasonal variations were reported for some elements. For instance, N was lowest in the fall, and this would suggest fall as the best time for determining nutrient status. These results are interesting but cannot be directly compared to *P. dactylifera* due to fewer leaves (based upon pruning style) and a smaller fruit load in *P. canariensis*.

Various reports from other countries are available. In Egypt, Shawky and Mougheith (1974) reported macro-element concentrations somewhat higher than those reported by the USA workers. Minessy et al. (1976) reported seasonal trends in micro-elements in Egypt, with values somewhat higher than those reported in the USA. Conversely, El-Shurafa (1984) reported values from Libya that were similar to those reported in the USA but noted that his concentrations were lower than those generally reported from the Middle East. Al-Ghamdi and Hussain (1988), Ba-Angood and Ahmad (1983), and Azab (1994, 1995) reported values of various elements from several countries on the Arabian Peninsula. These values are rather variable. In some instances, they are comparable to values reported in the USA but in many cases they are higher.

The fact that most reports from other countries report higher nutrient concentrations than do reports from the USA, and are more recent than most reports from the USA raises some questions about the nutrient status of date palms currently growing in the USA. Furthermore, the older reports were largely based upon studies of trees produced using organic fertilizers, whereas many producers now use inorganic fertilizers. Finally, there are no established standards for nutrient concentrations for date palms in the USA. The current study was intended to begin work towards establishing nutritional standards in date palms by providing a better understanding of date palm nutrient dynamics. The objectives of the study were to estimate concentrations of most major and minor elements in date palm leaves and fruits, and to determine whether seasonal variations in these concentrations exist.

MATERIALS AND METHODS

The date palms studied were cv. 'Deglet Noor' growing in a commercial date garden in Thermal, California (33.6 deg. N., 116.2 deg. W.). 'Deglet Noor' is the most widely grown cv. in the Coachella Valley, and thus particularly appropriate for study. The trees were approximately 10 years old at the time of the study. Spacing of the trees was 9 M X 9 M. Fertilizer in the form of UAN32 was applied twice per season (February and May) at a rate of approximately 1.36 kg N (actual) per tree per year. No cover crops were present. Irrigation was by flood.

Ten trees were selected for uniformity in one area of the block. Selecting trees from the same area of the block was done to avoid influences from any soil variability that might be present in the block. Due to selection of the trees for uniformity, the experimental design became a completely random design. Sampling was done during the 2001 season on dates corresponding to fruit developmental stages: March 23, 2001 (bloom; leaves only); June 04, 2001 (kimri); August 22, 2001 (khalal); November 15, 2001 (rutab); and the following season in the spring (May 05, 2002; leaves only). The trees were sampled following Reuther (1948) along the phyllotactic 'spiral of 13' described by Mathez and Bliss (1942). The trees had been pruned to an average of 84 leaves in the canopy, and the lowest leaves were an average of 6 leaves within the 'spiral

of 13' from the base. Samples were designated as low, medium, and high. The low samples were taken from 4 positions around the tree from within the lowest 2 ranks within the 'spiral of 13', the medium samples from within the next 2 ranks, and the high from within the next 2 ranks. These were all fully expanded and hardened leaves. Due to the structure of the tree canopy the highest leaves and immature leaves were not accessible. On the first sampling date, three pinnae were taken from the basal, medial, and distal portions of the leaf. Thereafter, only middle pinnae were sampled. The trees averaged 16 fruit clusters each. Fruit samples were taken from four bunches around the tree.

The samples were dried to a constant weight at 40 deg. C. Analysis of total N, K, P, S, Mg, Ca, B, Na, Cl, Mn, Zn, Fe, Cu, and Mo at the University of California Division of Agriculture and Natural Resources Analytical Laboratory at Davis, California. Protocols used can be accessed at:

http://groups.ucanr.org/danranlab/Methods_of_Analyses545/. Statistical analysis was done using SAS 9.1 (SAS Institute, Cary, NC, USA) using the General Linear Models and other appropriate procedures.

RESULTS AND DISCUSSION

Previous work in our laboratory (data not shown) had suggested that the observations of Reuther (1948) regarding gradients in nutrient concentrations from the basal to the distal pinnae were not valid. However, those observations were made in September rather than in the spring as were those of Reuther (1948). Pinnae from leaves sampled in March did indeed show a gradient from lower concentrations in the basal pinnae to higher concentrations in the distal pinnae for most elements surveyed (Tables 1a and 1b). Exceptions were Na and Cu, for which no significant differences based upon position were observed. For N, Zn, and Mo, the basal pinnae showed significantly lower concentrations than the middle and distal pinnae, but the middle and distal pinnae did not have significantly different concentrations from each other. For K and Cl, the gradient was reversed, with the distal portions having the highest concentrations. These results for K are consistent with Reuther (1948) and fairly consistent with Shawky and Mougheith (1974). The concentration of B in the distal pinnae was very high as compared to the basal and middle pinnae. This figure was consistent across all 10 replicates. It is possible that this concentration of B in the distal portion of the rachis is related to the observations of Abdul-Baki et al. (2002b) of high B concentrations in the distal portions strands of the bunches. The concentrations in the middle pinnae were in line with those of Labanuaskas and Nixon (1962) (who sampled along the entire rachis), while the higher concentrations in the distal portion are close to those reported by Haas (1944) (who did not state which portions of the rachis were sampled). The significance of this high concentration of B in the distal portion of the leaves is not known at this time.

Based upon these observations, the middle portion of the leaf was chosen for further sampling. This represents an average or intermediate value for the concentrations of the elements in the leaves, as per Reuther (1948). A disadvantage is that unusually high or low concentrations, which are possibly physiologically significant, would be missed. It should be noted that for most elements, the differences in concentration, while statistically significant, would probably not be of physiological import. Exceptions are B, as noted, and possibly Fe and Mn. The following discussion concerns pinnae sampled from the middle portion of low (older), medium, and high (younger) leaves.

The effects of leaf position and sampling date on nutrient concentrations were analyzed using the Repeated Measures Analysis within the General Linear Model Procedure of SAS. The position of the leaf in the canopy significantly ($P<0.05$) influenced the nutrient concentrations of N, K, P, Ca, Cl, and Mo. Sampling date significantly ($P<0.05$) influenced nutrient concentrations for all elements surveyed except for Zn and Cu. The interaction of leaf position and sampling date was significant ($P<0.05$) only for K, B, Na, Cl and Mn.

For the most part, the trend was for the high leaves to have higher concentrations

of nutrients than the medium and low leaves (Tables 2a and 2b). In many instances, the medium and low leaves did not differ significantly in nutrient concentration. For a few elements (Ca, S, and Mo), the opposite trend was observed: lower leaves showed higher concentrations. The results for N, P, and K are consistent with the results of Reuther (1948). However, Shawky and Mougheith (1974) did not find consistent trends in this respect across several cultivars studied. Furthermore, Mg showed this trend only on the first sampling date, which is consistent with Reuther's (1948) observation of a lack of a definite trend for concentrations of Mg in leaves of different ages. These observations are consistent with the concept that older leaves export nutrients as they age. The date palms studied were pruned annually, and there were no leaves that could be considered senescent present. This is reflected in the fact that actual differences in concentrations were generally small even when they were statistically significant. The age effect of the leaves on nutrient concentrations was consistent across the entire year only for N, P, K, and Cl. Other nutrients showed these differences only in the first and second samplings (S, Ca, Mg) or sporadically during the season (B, Zn, Mn, Mo). Na and Fe showed significant differences only on the second sampling date and Cu never showed significant differences.

The seasonal changes in nutrient concentrations in leaves from the middle portion of the canopy are shown in Figures 1a and 1b. The middle leaves were selected as they would represent a sort of average concentration of the leaf mineral concentrations when the above-mentioned trends exist. Since the differences in concentrations between leaves from different heights in the canopy were generally small, the trends for these middle leaves are representative of general trends for most elements. In the following discussion, 'significance' refers to $P<0.05$.

Although the seasonal patterns varied somewhat, for most macro-elements except for Ca the lowest concentrations were found on the June and August sampling dates. N and Ca concentrations dropped between the first and second sampling dates, whereas those of K and Cl rose. Ca was at its lowest during June and rose between June and August. Even though P and S had a few statistically significant changes in concentration, their concentrations were low throughout the entire year. The patterns in the micro-nutrients were different. Zn and Cu remained at low levels during the entire period of study, and Mn showed only a moderate increase over time. Although the levels of Fe were high as compared to those of Na, both showed a trend towards increasing during the season but returning to lower levels the following year. Both showed rather large fluctuations. B also showed large fluctuations but did not return to a low level in the 2nd year. Rather, it increased dramatically. This, in combination with the high levels of B in the distal pinnae (see above), suggests that there is something unusual about B either in date palms in general or in this particular block. This is possibly related to water quality as B is sometimes known to be present in high concentrations in the irrigation water used in this area.

Fruit was sampled at the kimri, khalal, and rutab stages rather than five times as for the leaves. All elements showed significant decreases in concentration within the date fruit between the kimri and rutab stages, although the patterns of decline varied slightly (Figs 2a and 2b). The decreases in concentration were probably related to fruit growth. The figures for fruit N are slightly lower than those reported by Embleton and Cook (1952).

Consistent with the earlier reports, the concentrations of elements in the leaves is generally lower than those reported from different geographic areas. For instance, N concentration in the current report are in the range of 1.3 – 1.6 %, whereas in Egypt the rates range from 1.8 – 2.7 (Shawky and Mougheith, 1974; El-Assar et al., unpublished data). Perhaps the most striking difference is in P, where the values here of less than 0.1 % are dramatically different than in Egypt (0.2 % and up; Shawky and Mougheith, 1974; El-Assar et al., unpublished data). However, values from other areas (El-Shurafa, 1974; Azab, 1995) are somewhat closer to the valued in the current report. N appears to be the most consistently low element in comparison with reports from other countries. Date

palm nutrient concentrations are lower than in some other subtropical crops, such as citrus. It is possible that the date palm, evolving in sandy soils low in nutrients, developed into a relatively parsimonious user of nutrients. It should be noted that the date palms studied were well-fertilized, vigorous, and did not exhibit any signs of nutritional stress.

In the Coachella Valley, generally only N fertilizer is applied, thus it is of interest to calculate the amount of N removed from fruit harvest and leaf removal. An average of 32 leaves was removed from each palm, each of which had an average dry weight of 1.3 kg. Using an estimate of the pinnae N content at the time of pruning, leaf removal resulted in approximately 0.5 kg of N being removed. This over-estimates the amount of N in the leaves as the petiole and rachis have lower N concentrations than the pinnae (data not shown). This figure is slightly lower than that reported by Embleton and Cook (1952), but their pruning weights were much larger than those estimated here. This is probably related to changes in cultural practices over the 50-year period. The figures in the current report are slightly higher per palm than those reported by El-Shurafa (1984). The leaves were of the palms in the current study were left as a mulch and thus some of the N was returned to the soil. Fruit yield from this block averaged 143 kg per tree during the season under study. Estimating N removed by the fruit utilizing the N concentration at the rutab harvest, approximately 0.6 kg of N was removed from each tree by the fruit harvest. This is slightly higher than the figure reported by El-Shurafa (1984). Thus, 1 kg of N or slightly more was removed from the trees. Therefore, the 1.36 kg of N applied each year by the producer should be adequate to replace the N removed by the fruit and leaves, although the soil effects and other potential N losses are not known. Some attention is needed on the efficiency of N application under Coachella Valley conditions.

Regarding the data presented, several things are apparent that may influence sampling of leaves for nutrient analysis. Although most reports have suggested sampling in the spring (April), the data presented above suggests that sampling later in the year would be more useful in assessing nutrient status. For most macro-elements, sampling between kimri (June in this report) and khalal (August in this report), preferably towards the end of this period, would generate values when they are at their lowest status. The exception is possibly Ca, but this element is generally not used as fertilizer for date palms and so is of less importance than the other macro-elements. This would also be a suitable time for assessing Zn, Cu, and Mn status. This period finds a transient peak in Na. However, this (and Cl) are elements that are of more concern when found in excess and thus this period would also be suitable for sampling if an accumulation of Na was a concern. Fe and B exhibited unusual patterns of seasonal variation in concentration in this study and no real conclusions about the best time for assessing them can be made, although the pattern of Fe suggests that the period around kimri may be the best time to sample. These results are consistent with the suggestion of Reuther (1948) as to the appropriate time for nutrient analysis of date palms, and of Broschat (1997) for ornamental *P. canariensis*. Fruit at the khalal stage have the greatest sink strength and thus the demand for nutrients during this stage of fruit development is greatest.

Broschat (1997), working with ornamental *Phoenix* spp., stated that most nutritional sampling of palms is done with the youngest fully expanded leaves, but that for N, P, K, and Mn older leaves would be more appropriate. This statement was made on the basis of the nutrient re-mobilization patterns of these elements. It should be noted that the palms with which Broschat (1997) worked were pruned as per ornamental usage and carried an average of 63 leaves, which is only about 75 % of the number of leaves on the date palms in the current study. The ratio of leaves to fruit is important in date production, whereas it is not important for ornamental useage. The range (age) of leaves suggested for analysis by Broschat (1997) would more or less correspond to the middle leaves utilized in this study. Since pruning practices vary, it is somewhat unrealistic to designate leaves from the bottom up for use in sampling as these may represent a variable age leaf. Therefore, ‘counting down’ from the younger (higher) portion of the tree may be more useful. With this in mind, the fact that generally the elemental concentrations were lower in the middle leaves than in the high leaves, suggests that what in the current study are

referred to as middle leaves should be utilized for sampling purposes. This would be 2 – 3 ranks in the 'spiral of 13' from the top of the tree. As a practical matter, this could be estimated by eye rather than necessarily counting spirals. It should be noted that sampling from the highest portions of the tree is also difficult due to the dense structure of the canopy. This would represent a good compromise for the bulk of the elements since sampling more than once would be onerous.

This study has provided a first step towards the development of nutritional standards for date palms under California conditions. The results suggest that sampling from intermediate age leaves in the late summer or early fall would provide the most reliable indication of nutrient status for most elements. Some elements, particularly Fe and B, had unusual seasonal patterns in this study and need more attention. It remains to determine whether or not the nutritional status of any particular element is correlated with yield or other economic parameters and to determine the efficiency of N fertilization as affected by soil conditions and other factors.

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Tables

Table 1a. Effect of rachis portion on concentrations (% d.w.) of nutrients present in high concentrations in date palms[1].

Portion	N	K	P	Ca	Mg	S	Cl
Basal	1.23a	0.63a	0.07a	0.69a	0.16a	0.15a	0.64a
Middle	1.48b	0.43b	0.08b	0.83b	0.22b	0.19b	0.45b
Distal	1.47b	0.35c	0.08c	0.97c	0.25c	0.19c	0.31c

[1] Means (N=10) followed by different letters are different at the 5 % level using Tukey's adjustment. Note that some figures may not appear different due to truncation of significant digits.

Table 1b. Effect of rachis position on concentrations (ppm d.w.) of elements present in low concentrations in date palms[1].

Portion	B	Na	Zn	Mn	Fe	Cu	Mo
Basal	11a	45	16a	38a	184a	7	0.92a
Middle	19a	33	19b	55b	244b	10	1.06b
Distal	183b	37	18b	111c	305c	7	1.08b

[1] Means (N=10) followed by different letters are different at the 5 % level using Tukey's adjustment. Note that some figures may not appear different due to truncation of significant digits.

Table 2a. Effect of position of leaf within canopy on concentrations (% d.w.) of macro-nutrients in leaves of date palm on 5 sampling dates[1].

DATE	POSITION	N	K	P	S	Ca	Mg
3/23/01	H	1.53a	0.558a	0.084a	0.178a	0.693a	0.227a
3/23/01	M	1.48ab	0.388b	0.073b	0.188b	0.882b	0.227a
3/23/01	L	1.41b	0.339b	0.073b	0.189b	0.927b	0.196b
6/4/01	H	1.38a	0.937a	0.086a	0.167	0.538a	0.221
6/4/01	M	1.26b	0.541b	0.073b	0.160	0.729b	0.215
6/4/01	L	1.16c	0.373c	0.069b	0.167	0.942c	0.219
8/22/01	H	1.39a	0.584a	0.080a	0.170	0.750	0.230
8/22/01	M	1.26b	0.350b	0.077a	0.170	0.870	0.227
8/22/01	L	1.22b	0.363b	0.070b	0.171	0.827	0.215
11/15/01	H	1.51a	0.579a	0.086a	0.168	0.665	0.233
11/15/01	M	1.32b	0.355b	0.076b	0.167	0.864	0.230
11/15/01	L						
5/10/02	H	1.32	0.820a	0.076a	0.169	0.497	0.197
5/10/02	M	1.31	0.494b	0.068b	0.155	0.684	0.193
5/10/02	L	1.23	0.319c	0.063b	0.166	0.862	0.202

[1] Means (N=10) followed by different levels are different at the 5 % level using Tukey's adjustment.

Table 2b. Effect of position of leaf within canopy on concentrations (d.w.) of micro-nutrients in leaves of date palm on 5 sampling dates[1].

DATE	POSITION	B (ppm)	Na (ppm)	Cl (%)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Mo (ppm)
3/23/01	H	18a	39	0.587a	22.1a	68.8a	219	11.7	0.95
3/23/01	M	20b	27	0.436b	18.2b	57.2a	255	8.7	1.07
3/23/01	L	22b	34	0.321c	17.0b	39.4b	259	8.9	1.17
6/4/01	H	92	62a	0.781a	16.7a	64.0	217a	8.8	0.71a
6/4/01	M	92	69a	0.643b	16.0a	69.1	249b	10.3	1.02b
6/4/01	L	92	83b	0.553b	13.5b	58.3	270b	9.0	1.08b
8/22/01	H	115	111	0.730a	17.7a	76.3a	346	11.8	0.78a
8/22/01	M	102	123	0.463b	14.8b	73.0ab	330	10.6	0.94b
8/22/01	L	76	253	0.526b	13.5b	59.4bb	329	10.5	1.04b
11/15/01	H	183a	101	0.653a	18.9	86.8	417	15.2	0.69a
11/15/01	M	135b	124	0.499b	17.3	77.1	393	13.5	0.88b
11/15/01	L								
5/10/02	H	174	73	0.739a	66.3	68.4	176	120.8	0.73a
5/10/02	M	190	60	0.603b	14.2	79.2	163	10.5	0.86ab
5/10/02	L	149	66	0.464c	14.3	75.5	267	11.7	1.01b

[1] Means (N=10) followed by different levels are different at the 5 % level using Tukey's adjustment.

Figures

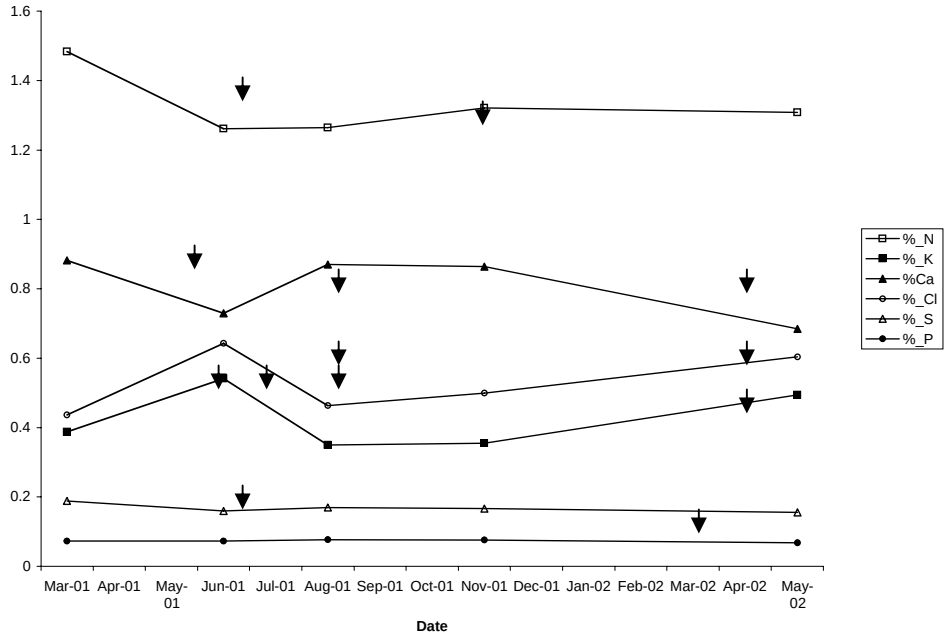

Fig. 1a. Seasonal changes in % d.w. of macro-elements and Cl in leaves from the middle portion of the canopy of date palms (N=10). Segments marked with an arrow change significantly (P<0.05).

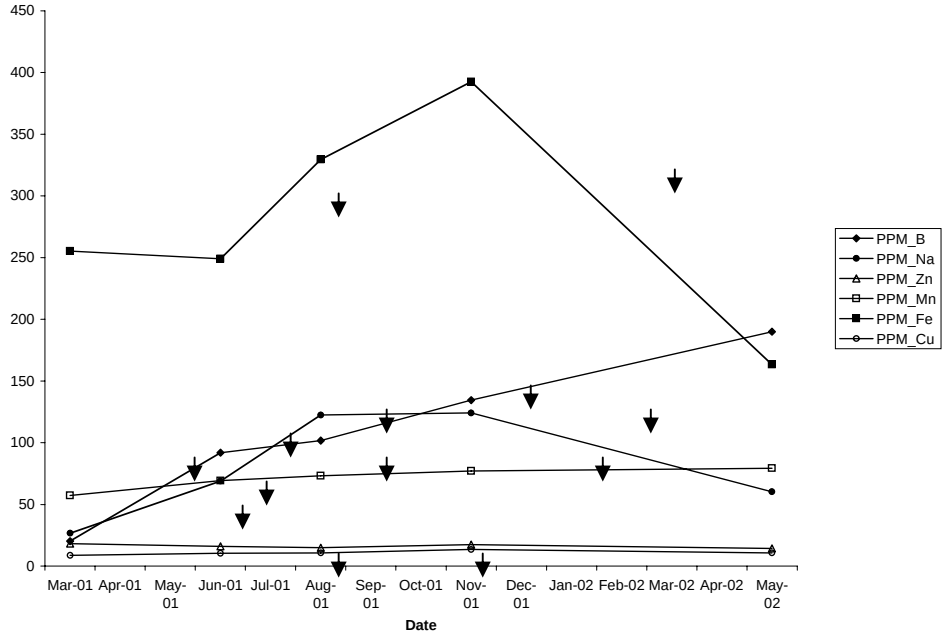

Fig. 1b. Seasonal changes in ppm d.w. of micro-elements in leaves from the middle portion of the canopy of date palms (N=10). Segments marked with an arrow change significantly (P<0.05).

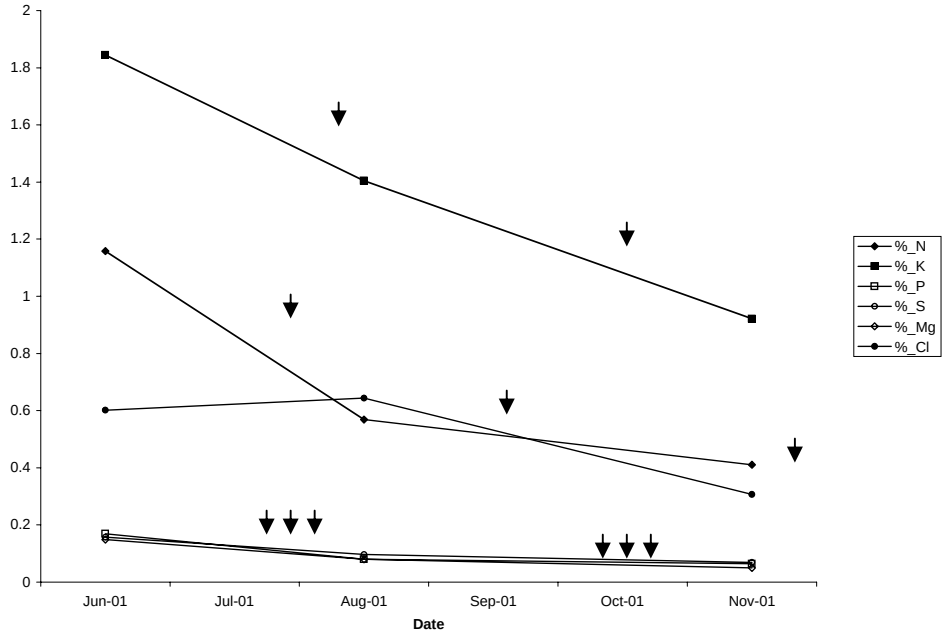

Fig. 2a. Seasonal changes in % d.w. of macro-elements and Cl in fruit of date palms (N=10). Segments marked with an arrow change significantly (P<0.05).

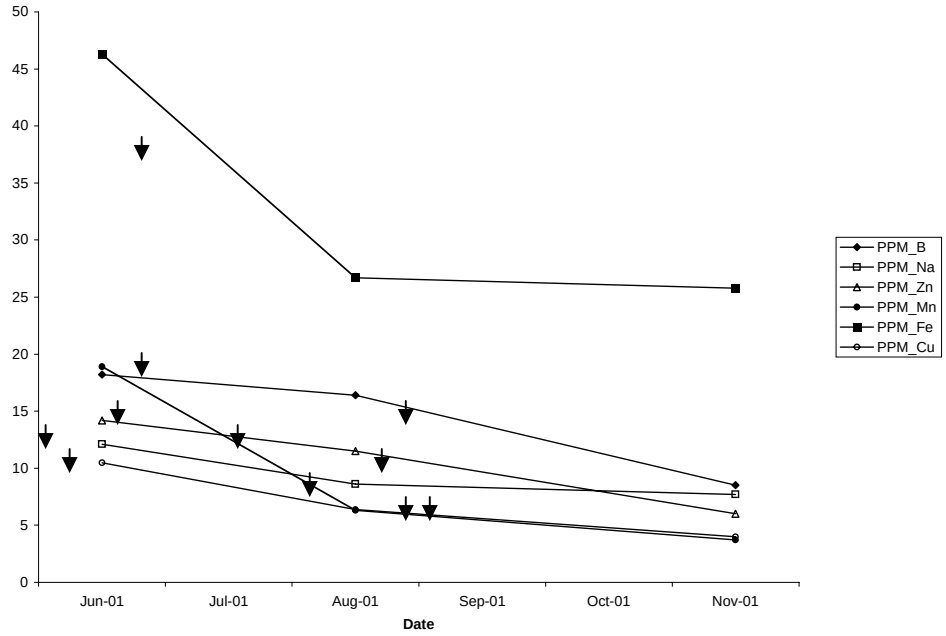

Fig. 2b. Seasonal changes in ppm d.w. of micro-elements in fruit of date palms (N=10). Segments marked with an arrow change significantly ($P<0.05$).

Effects of Different Methods and Degrees of Fruit Thinning on Yield and Fruit Characteristics of Barhee Date Cultivar

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Keywords: fruit drop, fruit quality, fruit ripening, kimri, khalal and rotab stages

Abstract

Barhee is one of the most desirable date cultivars of Iran grown in Khouzestan province. It is mainly consumed at khalal stage. To study the effects of different methods and degrees of fruit thinning on yield and fruit characteristics of this cultivar an experiment was conducted in a randomized complete block design with 7 treatments and 4 blocks in Ahwaz region in 2004. At first, the 8 leaves to 1 bunch ratio was applied for uniformity and then thinning treatments were done at the beginning of kimri stage (6 weeks after pollination). The treatments were: removal of one third of the strand length; removal of one fourth of the strand length; removal of one third of the entire central strands; removal of one fourth of the entire central strands; removal of one fourth of the bunches; removal of half the bunches; and control (without thinning). At the beginning of rotab stage, the percentage of fruits at khalal and rotab stages were calculated in each palm. Fruit characteristics and yield at khalal stage were measured. The results showed that the thinning treatments had no significant effect on fruit weight, volume, length and diameter, seed weight, length and diameter, pulp to seed ratio, percentages of fruit water content, TSS, total sugar and fruit ripening. Furthermore, removal of one third and one fourth of the entire central strands and removal of one fourth and half the bunches resulted in significant decrease in palm fruit yield. According to the results, fruit thinning at the beginning of kimri stage is not recommended for improving Barhee fruit quality.

INTRODUCTION

In 2003, fruit-bearing date groves in Khouzestan province covered about 13 percent of the land and the crop represented 11.5 percent of date production in Iran (Ministry of Jihad-e-Agriculture of Iran, 2004). Most of the desirable commercial date cultivars of Iran are grown in this region, including Barhee. It is usually consumed at khalal stage and each palm yields up to 350kg fruit.

Fruit thinning is one of the main principles of commercial date production. It increases fruit size and quality, reduces alternate bearing and fruit drop, enhances fruit ripening and has other advantages (Zaid, 1999). The effects of different methods or degrees of fruit thinning on yield and fruit characteristics of some date cultivars have been studied for several years. The methods of thinning used in these experiments included removal of part of the length of the strand (El-fawal, 1962; Tavakkoli et al., 1994; Samavi, 1998; Khademi, 1999; Sayyahpoor, 2002); removal of some of the entire central strands (Schroeder and Nixon, 1958; El-fawal, 1962; Tavakkoli et al., 1994; Khademi, 1999); removal of some of the fruits on the strand (Tavakkoli et al., 1994); and removal of some of the bunches on a palm (El-fawal, 1962; Davoodian, 1999). However, there are no studies on the effects of different methods and degrees of fruit thinning on yield and fruit characteristics of Barhee date cultivar in Iran, so this study was undertaken.

MATERIALS AND METHODS

This experiment was conducted in a randomized complete block design with 7 treatments and 4 blocks in Ahwaz region in 2004. At first, the 8 leaves to 1 bunch ratio

was applied for uniformity by keeping 8 bunches on each palm with about 64 leaves. Thinning treatments were done at the beginning of kimri stage (6 weeks after pollination). The treatments were: T1) removal of one third of the strand length; T2) removal of one fourth of the strand length; T3) removal of one third of the entire central strands; T4) removal of one fourth of the entire central strands; T5) removal of one fourth of the bunches; T6) removal of half the bunches; T7) control (no thinning).

The experiment consisted of 28 uniform palms (experimental units) for which all horticultural practices were done similarly according to the last scientific findings. At the beginning of rotab stage, 5 strands of each of the bunches on each palm were selected and the number of fruits at kimri, khalal and rotab stages were counted. Average number of fruits per strand and the percentages of fruits at khalal and rotab stages were calculated for each palm.

Furthermore, by selecting 4 bunches from different directions on each palm and by counting the number of strands, the average number of strands per bunch was calculated for each palm. Then 50 fruits at khalal stage were randomly picked from each palm for measuring fruit characteristics. Fruit yield for each palm at khalal stage was calculated by multiplying together the number of bunches on the palm, average number of strands per bunch, total number of fruits per strand and mean fruit weight. Data were analyzed by MSTATC using Duncan's Multiple Range Test for comparison of means (at P=0.05).

RESULTS AND DISCUSSION

Effect of Thinning Treatments on Fruit Characteristics and Ripening

The thinning treatments had no significant effect on fruit characteristics, i.e. on fruit weight, volume, length and diameter, on seed weight, length and diameter, on pulp to seed ratio, on percentages of fruit water content, total soluble solids and total sugar and on fruit ripening (Tables 1 and 2). It has been reported that the removal of bunches did not increase fruit size, weight and quality or enhance fruit ripening (El-fawal, 1962).

On the other hand, the results of other studies showed that bunch thinning by removing the tips of strands or removing entire central strands, may result in different effects on fruit characteristics and ripening, depending on the amount of thinning, its application time and also the cultivar used. Schroeder and Nixon (1958) found that removing one fourth of the strand length or one third of central strands at pollination time did not affect fruit weight and length of Deglet Noor dates, although removing half of the strand length slightly increased cell length which resulted in increased fruit size.

Sayyahpoor (2002) showed that cutting 5, 10 or 15 cm off the strand 1 week after pollination had no effect on fruit volume, but treatments 10 and 15 cm resulted in significant increases in fruit length and in fruit weight and diameter of Sayer dates, respectively. Samavi (1998) also found that in Mordasang cultivar, removing the tip of strands at 10 or 15 cm levels 3 weeks after pollination improved fruit size and weight but had no effect on qualitative fruit characteristics such as the percentage of fruit water content, TSS and total sugar and also on seed weight and volume.

Furthermore, it has been indicated that in Shahani cultivar, cutting back one third of the tip or central strands at the beginning of kimri stage (identical to the treatments used in our study) increased fruit weight and length, pulp to seed ratio, fruit water content and TSS but did not affect fruit diameter (Tavakkoli et al., 1994). It was also found that in Samani cultivar, removing one third of the tips or central strands 8 weeks after pollination resulted in significant increases in fruit weight, length and diameter, pulp weight and fruit TSS percentage. However, considering the results of other studies, thinning at pollination time has been recognized as more effective to increase fruit size and quality than later thinning (El-fawal, 1962). Khademi (1998) showed that cutting back one third of the central strands at pollination time was much more effective in increasing fruit size and quality and accelerating ripening of Kabkaab dates than the same treatment at the time of bunch lowering (mid-kimri).

Inability to remove the strand tips or entire central strands to improve Barhee fruit

characteristics and ripening in the present study may be attributed to insufficient degree and/or late time of thinning. Furthermore, based on the results of both present and previous studies, it seems that cultivars with longer-shaped fruits (e.g. Shahani cultivar) are more likely to improve their fruit characteristics, ripening and possibly other advantages after thinning than those with fruits of shorter, round-like shape (such as Barhee and Mordasang cultivars, respectively).

Effect of Thinning Treatments on Yield and Fruit Drop

1. Fruit Yield in Palm. There were no significant differences in fruit yield per palm between removal of one third or one fourth of the strand length (T1 and T2 treatments) and the control; but removing one third and one fourth of the entire central strands (T3 and T4) and cutting back one fourth and half of the bunches (T5 and T6) significantly decreased yield, with decreases increasing with thinning amount (Fig. 1). Fruit thinning by removing each of the strand tips, entire central strands or bunches has been previously reported to reduce fruit yield in palm. However, it has also been shown that bunch removal always results in lower yield than other methods of thinning at the same degree, as was found in the present study (El-fawal, 1962; Samavi, 1998; Sayyahpoor, 2002).

2. Fruit Drop. Total number of fruits remaining on strands at the beginning of rotab stage decreased more after cutting back one third or one fourth of the strand length compared to the control and other treatments (Fig. 2). The frequency of fruits at the tip of the strand is usually higher than on other parts of the strand, possibly due to the tendency for better fruit set to occur at the tips (El-fawal, 1962). The removal of one third and one fourth of the strand length should have reduced the fruits per strand by at least 33% and 25%, respectively, compared to the control. However these treatments were insignificant and resulted in only 17% lower number of fruits per strand at the beginning of rotab stage than the control (Fig. 2), suggesting that these treatments were effective in decreasing fruit drop, thereby compensating the yield reduction related to reduced fruit number per palm after their application.

Removal of half the bunches on a palm (T6) led to a significant increase in number of fruits per strand (Fig. 2), indicating a reduction in fruit drop. This result agrees with that obtained by Davoodian (1999). However, fruit drop reduction resulting from this treatment did not prevent the high decrease in fruit yield related to reduced number of fruits per palm.

CONCLUSIONS

In the present study, removing one third or one fourth of the strand length were the only treatments effective in reducing fruit drop and maintaining yield (Fig. 1 and Fig. 2). There are several studies in which cutting back the tips of strands showed greater increase in fruit size, weight and quality than removing entire central strands at the same levels (Nixon and Crawford, 1942; El-fawal, 1962; Khademi, 1998).

Nixon and Crawford (1942) found that a transverse notch cut made in the fruit stalk while the fruits are quite small (such cuts can be made by the larvae of rhinoceros beetle, *Oryctes* ×*rhinoceros* Linné, on the base of the fruit stalk at the time of pollination), reduces the amount of fruit growth and consequently the final fruit size and weight on the strands growing in the same direction. In other words, there is a limitation in cross-translocation of water and carbohydrates between the vascular elements leading to different strands within a fruit stalk (Nixon and Crawford, 1942).

This limitation probably resulted from the separation of vascular elements found between and within vascular bundles of date palm, as in other monocots. Therefore, it seems that the translocation of excess water, carbohydrates and probably some other materials to the remaining fruits on the bunch is made easier after removing tips, reducing fruit drop and preventing yield reduction, in comparison to removing entire strands.

The results showed that the thinning treatments had no significant effect on fruit size, weight and quality and on fruit ripening. Furthermore, removal of one third and one fourth of entire central strands and removal of one fourth and half the bunches resulted in

significant decrease in palm fruit yield. According to the results, fruit thinning at the beginning of kimri stage is not recommended for improving Barhee fruit quality.

ACKNOWLEDGEMENT

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Tables

Table 1. Effect of thinning treatments on quantitative fruit characteristics.

Thinning treatments (removal of)	Quantitative fruit characteristics							
	Fruit weight (gr)	Fruit volume (cm^3)	Fruit length (mm)	Fruit diameter (mm)	Seed weight (gr)	Seed length (mm)	Seed diameter (mm)	Pulp/ seed ratio
1/3 of the strand length	11.9 a[i]	11.5 a	33.2 a	25.1 a	0.78 a	18.7 a	8.9 a	14.2 a
1/4 of the strand length	12.0 a	11.7 a	33.6 a	25.1 a	0.75 a	18.6 a	8.8 a	15.2 a
1/3 of central strands	11.3 a	11.0 a	32.3 a	24.8 a	0.70 a	17.9 a	8.6 a	15.2 a
1/4 of central strands	12.0 a	11.8 a	33.0 a	25.2 a	0.78 a	18.5 a	9.0 a	14.5 a
1/4 of the bunches	10.9 a	10.5 a	32.2 a	24.4 a	0.72 a	18.2 a	8.6 a	14.2 a
1/2 of the bunches	11.0 a	10.7 a	32.2 a	24.6 a	0.75 a	18.3 a	8.8 a	13.8 a
Control	11.0 a	10.6 a	31.9 a	24.4 a	0.72 a	18.0 a	8.8 a	14.2 a

[1]Within columns values followed by different letters differ significantly at P=0.05

Table 2. Effect of thinning treatments on qualitative fruit characteristics and fruit ripening.

Thinning treatments (removal of)	Qualitative fruit characteristics			Fruit ripening		
	Water content (%)	Total sugar (%)	TSS (%)	Khalal fruit (%)	Rotab fruit (%)	Kh.+Ro. fruit (%)
1/3 of the strand length	61.1 a[ii]	18.6 a	29.2 a	18.1 a	0.9 a	19.0 a
1/4 of the strand length	61.6 a	20.3 a	27.3 ab	15.7 a	0.5 a	16.2 a
1/3 of central strands	59.8 a	19.5 a	28.1 ab	12.7 a	0.8 a	13.5 a
1/4 of central strands	61.6 a	20.4 a	28.1 ab	14.6 a	0.5 a	15.1 a
1/4 of the bunches	59.4 a	20.4 a	28.9 a	15.6 a	0.1 a	15.7 a
1/2 of the bunches	60.8 a	18.4 a	26.2 b	19.0 a	0.5 a	19.6 a
Control	60.9 a	17.5 a	27.9 ab	11.1 a	0.2 a	11.3 a

[2]Within columns values followed by different letters differ significantly at P=0.05

Figures

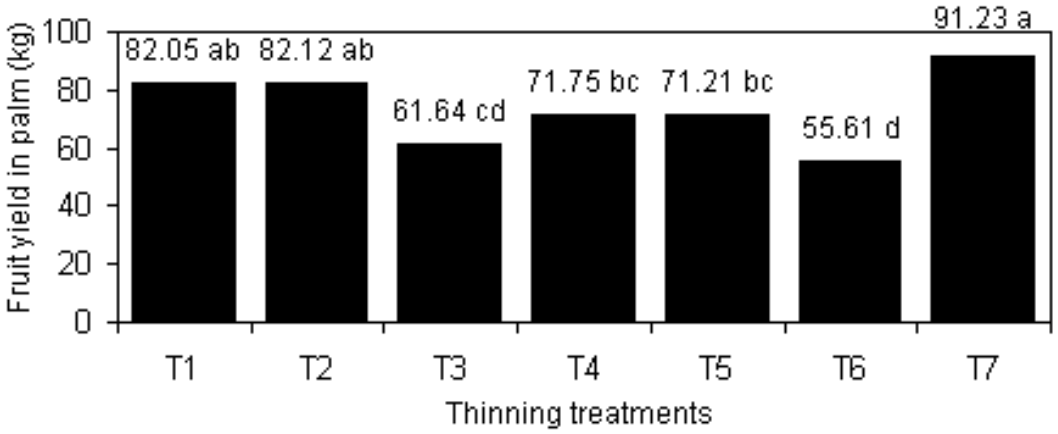

Fig. 1. Effect of thinning treatments on fruit yield in palm.

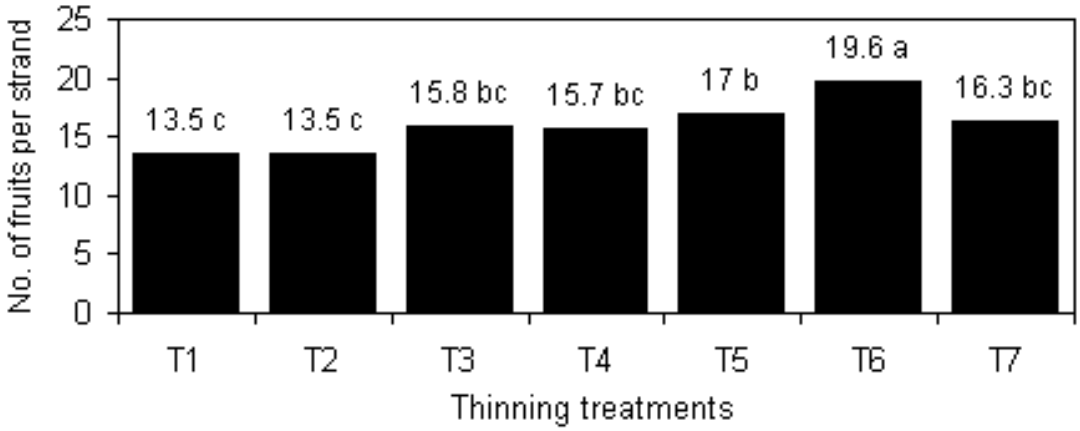

Fig. 2. Effect of thinning treatments on number of fruits per strand.

Date Palm Flowering and Fruit Setting as Affected by Low Temperatures Preceding the Flowering Season

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Abstract

This study was conducted during two consecutive seasons, 2003 and 2004, on flowering and fruit setting in date palm trees at Tuhama region in Yemen. The study included three local and imported groups of cultivars trees: date palm trees grown from offshoots; date palm trees grown from tissue culture using embryogenesis; and date palm trees grown from tissue culture using organogenesis. Under the conditions of this study the flowering and fruit setting of date palm trees were affected by insufficient coldness through the winter season. It was concluded that if temperatures in the winter months are not low enough, flowering will not be normal. The warm winter temperatures of Tuhama region in Yemen, which are higher on average than those in other world regions where date palm is cultivated, may be the main reason for the failure of flowering and fruit set.

INTRODUCTION

Date palm trees are distributed widely throughout the world, from Pakistan in the east to North and South America in the West, and are concentrated in the near East (GCC countries, Iraq, Iran) and North Africa. Recently date palm plantations have been established in new regions such as South African countries, India and Australia (Zaid and Arias, 2002). Date palm trees in these areas are producing a good quality of date fruits, while in some regions the trees face problems of flowering and fruiting (Al Baker, 1972).

A lot of research has been done on the effect of climate factors on date palm growth and production, such as: tolerance of date palm trees to low temperatures and freezing waves, determination of the endurance of different cultivars to high humidity, and thermal accumulation requirements of cultivars (Zaid and Arias, 2002; Albaker, 1972; Shabana et al., 2006). The effect of low temperatures during the period preceding flowering on fruiting has not been studied.

The goal of this study was to determine the effect of temperature through the cold season on flowering and fruit setting of date palm trees.

MATERIALS AND METHODS

This study was conducted in the Tuhama region of Yemen. The period of study was 2003 and 2004. Cultivars included old Yemen local cultivars of Menasaf, Aljahri, Hifris Alkhathari Aljoosh and Alanebari; and recently imported cultivars of Barhi, Nabtat saif, shishi, Khlass khnaizi lulu and abu maan. This group was planted from 1992 to 2001. Two propagation methods were used. Firstly, offshoots, were taken from all local cvs. and from some imported cvs. Secondly, tissue cultured plants, derived from both embryogenesis and organogenesis were used. About 30,000 date palm trees were planted and are still being cultivated.

The following data were recorded: vegetative growth of the trees, including number and length of leaves, color of leaves and trunk diameter; health status of trees, including flowering and fruit setting percent; and, minimum temperatures in different date palm cultivation regions of the world and in Tuhama region in Yemen.

This study was a completely randomized design with 10 replications (percentage of fruit setting).

RESULTS AND DISCUSSION

Vegetative growth of date palm trees in this study was measured by number of

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

leaves and leaf length, and trunk diameter of trees,of both groups of cultivars. The results showed that plants propagated from both offshoots and tissue culture exhibited normal growth (Table 1). No deficiency symptoms of nutritional elements were noted (Fig. 1).

Date palm trees planted between 1992 and 2001 in Tuhama valley, derived from both offshoots and tissue culture (embryogenesis or organogenesis origin), all faced problems with flowering and fruit setting, rangeing from partial to complete failure (Figs. 2, 3, 4).

The results showed that the propagation method (offshoots, tissue culture from embryogenesis or organogenesis) had no effect. The trees of local cvs flowered and set fruit normally (Table 1 and Figs. 2, 3, 4).

As a result of a large number of studies, which included most fruit trees and even evergreens such as olive and citrus (Al Jondia, 2003), it is known that low temperatures are an effective factor in flower initiation (Weaver, 1972). Studies have also shown that normal bud growth and development may be prevented by unfavorable environmental factors such as high temperatures. It is concluded that date palm trees may also need a number of cold hours during the period preceding the flowering season, to induce the biological reactions needed for normal growth and development of flower buds.

This conclusion was supported by the following facts. Firstly, floral initiation like other physiological processes is determined by genotype and its interaction with environmental factors. Secondly, it is well known that the origin of date palm is Iraq and GCC countries, which are characterized by hot (high temperatures) in summer and cold (low temperatures) in winter. Thirdly, because of low winter temperatures at Hadramout province (Al Hubaishi and Holnshtine, 1984), imported date palm cvs both flowered and set fruit normally. Fourthly, date palm trees require high temperatures. Therefore flowering and fruit setting of date palm must occur in warm regions. However date palms flourish only in regions with cold winters and hot summers.

These factors affected environmental adaptation of trees, so that low temperatures followed by relatively high temperatures, as in regions famous for the cultivation of date palm trees and date production, are essential for inducing flowering of the trees (Table 2 and 3).

CONCLUSION

Date palms did not face any problems with flowering or fruit setting in most regions, nor with flourishing growth and productivity. Problems with failure to flower and set fruit appeared in regions with relatively warm winters, such as Tuhama. Flowering buds require cold for development before the temperature rises. Due to the importance of lower winter temperatures for flowering and fruit setting of date palm trees, we advise the selection of the best local cvs and propagation by tissue culture to fulfill the demands of farmers. It is hoped to continue these studies to determine the total cold requirement and optimum winter temperatures.

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Tables

Table 1. Vegetative growth, flowering and fruit setting characteristic for local and imported cultivars planted at Tumaha region.

Cvs	Propagation method	No. of leaves	Leaf length(cm)	Trunk diameter(cm)	Flowering status(Spathe no.)	Fruit setting %
Barhi	T.C	120	355	75	No flowering	o
Saaqi	T.C	98	275	60	Partial flowering(2)	20
Saaqi	Offshoot	91	260	58	Partial flowering(2)	17
Nabtat saif	T.C	110	280	70	Partial flowering(3)	15
Nabtat saif	Offshoot	108	265	68	Partial flowering(4)	11
Shishi	T.C	85	210	65	Partial flowering(3)	7
Shishi	Offshoot	82	207	61	Partial flowering(2)	9
Mjhool	T.C	75	205	60	No flowering	0
Local Cvs.	Offshoot	-	-	-	Full flowering	80

LSD 0.05= 31.62
LSD 0.01= 39.43

Table 2. Minimum and maximum temperatures in regions where date palms are grown and harvested.

Location	Recording period (years)	Minimum temp. °C	Minimum temp.°F	Maximum temp.°C	mum temp °F
Toghert - Algeria	15	3.38	38.1	35.8	96.6
Colmb bakar - Algeria	8	1.2	34.2	34.4	94
Tozer Tunisia	40	5.27	41.5	35.5	96.1
Arfod Morroco	12	1.27	34.3	36.4	97.5
California - andio	25	3.66	38.6	37.6	99.7
Basra-Iraq	19	6.44	43.6	37.4	99.4
Halfa- Sudan	30	1.11	34	40.2	104.4
Alain- U.A.E	30	10.4	76.3	34.3	101.3
Mean	23.4	4.19	43.21	36.54	98.91

1-6 Items took from Albaker (1972), 7 from Shabana and Al Shuraiki (2000)
Minimum temperature represents mean of January and maximum temperature was recorded 1-31 October

Table 3. Average minimum winter temperatures in Tuhama valley.

Year	November	December	January	Mean
1990	16.4	15	16.4	15.9
1991	13	16	16.4	15.1
1992	17	17.6	16	16.68
1993	17.6	16.6	18.5	17.56
1994	18	17	14.2	16.4
1995	16.8	18	15.8	16.86
Mean	16.46	16.7	16.21	16.44

Tuhama Region climate station

Figures

Fig. 1. Normal vegetative growth of date palm trees.

Fig. 2. Flowering failure in Barhi cv.

Fig. 3. Date palm plantation (5000 date palm trees) in Tuhama Valley where flowering has failed.

Fig. 4. Partial failure of fruit setting in Nabtat saif cv..

Development and Testing of a Shaker-System for the Selective Harvest of Date Fruit

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Abstract

A shaker-system for the selective removal of mature dates from the bunch has been designed and manufactured. This tractor-mounted system is powered by the tractor's power take off shaft. The shaker produces a vertical stroke of 50mm. It has been successfully subjected to field tests, in which several date bunches were shaken at frequencies of 300, 450 and 600 cycle/min. The experimental results indicated that a shaking frequency of 450 cycle/min. provided the best results in terms of selective mature date fruit drop and the time required for this to occur (3.5-5 sec.). A lower frequency prolonged the time for fruit removal, while a higher frequency resulted in the removal of immature dates as well. The use of this shaker-system significantly reduced both the time required for date removal from the bunch and fruit damage, in comparison with the conventional hand removal of dates from the bunch.

INTRODUCTION

Harvesting of dates represents 45% of the total production cost (Brown et al., 1984), which emphasizes the need for mechanization of date harvesting.

In the USA during the 1960s, the mechanical harvesting of dates was investigated on Deglet Noor, a semi-dry variety which represented 92% of total date production in the USA (Brown and Perkins, 1967). Several systems for elevating workers to the fruit, removing the fruit from the bunch and handling the harvested fruit between the field and the packinghouse were investigated (Perkins et al., 1972). As a result of these investigations, the harvesting system adopted throughout the date industry in the USA uses a truck-mounted crane, similar to a sign installation crane, to position a man in a basket near the bunches. Mature bunches are cut from the palm and dropped into the basket. The basket is lowered to a truck-drawn shaker-trailer, where the fruit are mechanically shaken from the bunches and fall into bulk bins (Rygg, 1977). The hydraulically-powered shaker produces a vertical stroke of 82.5 mm at a frequency of 600-700 cycles per min. (Perkins and Brown, 1964).

By 1966 almost 80% of the USA date crop was being harvested mechanically. This reduced harvesting labor by 75% and harvesting costs by 50%. Harvesting the varieties Deglet Noor, Zahdi and similar dry or semi-dry dates is practical with this system (Brown et al., 1984).

However, the design of the shaker in the USA was based on field tests and not on a correlation between detachment force requirement and fruit maturity or any other criteria for the selective removal of mature fruit. This resulted in the removal of mature dates as well as immature ones or culls, thus creating sorting problems in date processing and packinghouse. Vis et al. (1969) investigated several methods to overcome this deficiency by modifying the shaker to make it more selective and suggesting methods for fruit separation after harvest.

In shaker-harvester systems, the removal of fruit depends on the inertia force produced by the shaker, which is a function of shaker stroke and frequency. Parchomchuk and Cooke (1972) in their experimental analysis of fruit-stem dynamics, demonstrated that the manner of fruit detachment is frequency dependent. An unnecessarily long shaking duration and unnecessarily larger stroke length must be used for fruit detachment if the frequency is not considered.

In Iraq, during the 1980s, interest in the mechanization of date palm production has led to the development of a tractor-mounted hydraulic-lift, manufactured locally by

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

the Mechanical Industries Company in Alexandria. This machine resembles the truck-mounted crane adopted in the USA and is used in a similar manner for positioning the worker at the tree top to perform different cultural operations. One of these operations is cutting the mature date bunches during harvest and lowering these bunches to the ground. However, to accomplish date harvesting operation, a bunch-shaker system is required to remove the dates from the bunch.

The design of this shaker system must take into account the fact that dates do not ripen at the same time, which means there will be mature and immature dates or culls on the same bunch. This implies that the shaker system must be characterized by selectivity in dropping the mature dates only and leaving the immature ones or culls on the bunch.

An investigation was carried out in Iraq in 1986 to determine the detachment force required to remove Zahdi date fruit from the bunch during different stages of fruit maturity, with the aim of establishing a criterion for the selective harvest of dates based on detachment force differentials with maturity (Ibrahim et al., 1986). The experimental results indicated that fruit detachment force decreased significantly in the final stages of fruit development (tamar), thus establishing the possibility of selective shake-harvesting of Zahdi date fruit.

The aim of this investigation was to develop and test a selective bunch-shaker system which has the capability of dropping the mature dates only and leaving the immature dates or culls on the bunch. To achieve this goal, a shorter shaking stroke and a range of shaking frequencies were examined.

EXPERIMENTAL WORK

The experimental work in this investigation involved the design, manufacture and field testing of a tractor-mounted bunch-shaker system.

The shaker system (Fig. 1) consisted of a slider crank mechanism which produced a vertical stroke of 50mm. A clamp for holding the bunch in position during shaking trials was bolted to the slider mechanism. The shaker was welded to a frame readily adaptable for mounting on the tractor's three point hitch system. Power to drive the shaker was supplied from the tractor's power take off (PTO) shaft, via a propeller shaft.

The experimental procedure involved subjecting several date bunches to shaking trials at three different shaking frequencies: 300, 450, and 600 cycles per min. All date bunches subjected to shaking trials were of the Zahdi variety, which represents 72% of the total Iraqi date production (Ibrahim et al., 1986). The date bunches were tested within three hours of being cut from the palms. A tachometer was used to measure the rotational speed of the PTO shaft. The date bunches were weighed before and after shaking using a scale. A digital stop-watch was used to keep a record of the shaking time required for the removal of dates. A digital video camera was also used to keep a record of the sequence of events and time during shaking trials.

RESULTS AND DISCUSSION

The experimental results of bunch shaking trials are given in Table 1. For each shaking frequency, the time required for mature date removal and the total shaking time for fruit drop (time from the start of shaking until fruit drop ceased) are given, in addition to the mass of date bunch before and after shaking.

The effect of shaking frequency on fruit removal is thoroughly discussed in the following paragraphs:

1. Shaking at 300 cycle/min: At low shaking frequency a slow rate of fruit drop was observed as indicated by the long shaking duration, which was in agreement with conclusions of previous work by Parchomchuk and Cooke (1972). The removal of mature dates (tamar) took 15-20 sec., and then a very small amount of semi-mature dates (rutab) dropped, until fruit drop ceased after 30 sec. A relatively heavy after shaking bunch mass was noticed due to the remaining immature and semi-mature dates on the bunch, which indicated the high selectivity of fruit removal at this frequency.

2. Shaking at 450 cycle/min.: A significantly higher rate of fruit drop was obtained with the increase in shaking frequency to 450 cycle/min. Removal of mature dates took 3.5-5 sec. which was approximately 25% of the time required at the lower frequency (300 cycle/min.). Fruit drop ceased after 10 sec. with the removal of an appreciable amount of rutab and very few immature dates (khalal).
3. Shaking at 600 cycle/min.: The time required for mature date removal was reduced to 2.5-3 sec., which was comparable to the shaking time reported in the USA of 3 sec. (Perkins and Brown, 1964). However, as shaking continued a large number of rutab and khalal also dropped until fruit drop ceased after 5 sec. The large amount of fruit drop at this high frequency was indicated by the relatively small after shaking bunch mass of 0.7-1.25 kg.

In view of the above results, the most appropriate shaking frequency seemed to be 450 cycle/min. This is due to the fact that a lower frequency results in prolonged shaking time, while a higher frequency leads to the dropping of immature dates and consequently the loss of selectivity in fruit removal.

CONCLUSIONS

1. Date fruit removal from the bunch by a shaker-system designed and manufactured for this purpose has been proven feasible for the first time in Iraq.
2. This shaker-system is tractor-mounted which facilitates its use in the field by date growers.
3. Selectivity of fruit removal such that only mature date fruits are dropped by the shaker, can be achieved by selecting a suitable shaking frequency. For the shaker-system developed in this work with a 50mm vertical stroke, this frequency was found to be 450 cycle/min.
4. This shaker-system has the capability to remove the mature dates from the bunch within 5 sec., without causing any damage to the fruit. This significantly reduces harvesting labor, time and damage of fruit incurred by hand removal of dates from the bunch.

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Tables

Table 1. Experimental results of bunch shaking trials.

Shaking frequency (Cycle/min.)	Time required for mature fruit removal (sec)	Total shaking time for fruit drop (sec)	Bunch mass before shaking (kg)	Bunch mass after shaking (kg)
300	15-20	30	6-8	1.5-2.5
450	3.5-5	10	7-8	1 -1.5
600	2.5-3	5	7-9	0.7-1.25

Figures

Fig. 1. The tractor mounted date bunch-shaker system during trials.

Recent Advances in Date Palm Tissue Culture and Mutagenesis

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Keywords: somatic embryogenesis, micropropagation, mutation induction, temporary immersion system

Abstract

Micropropagation techniques for rapid shoot proliferation are achieved from any part of the plant such as shoot tip, tiny stem cuttings, roots, and auxiliary buds. It is critical to select proper genotypes and grow mother plants under a controlled environment, and to determine the maximum number of subcultures before initiating new cultures. By failing to do so, in vitro grown plants will show somaclonal variation in the field. Somatic embryogenesis is an ideal technique for clonal propagation of woody and fruit plants and genetic gain can now be captured through it. It has several additional advantages, such as the ability to produce large numbers of plants, potential for automation, the opportunities for synthetic seed, long-term storage (cryopreservation), packaging, direct delivery systems and genetic manipulations. Somatic embryogenesis is highly genotypic dependent, and it would be useful to modify the culture medium accordingly. For mutation induction, fine 'embryogenic cell suspension cultures' are gamma irradiated. Irradiated cells are further cultured onto fresh medium for the development, maturation and germination of mutated somatic embryos. This approach produces mutated somatic seedlings in a short period of time and also prevents chimerism problems which otherwise would require that plants be multiplied up to M_1V_4 generation for chimera dissociation.

INTRODUCTION

Plant tissue culture refers to the growth and multiplication of cells, tissues and organs of plants on defined solid or liquid media in an aseptic and controlled environment. The micropropagation technique that is most suitable for rapid shoot proliferation, is primarily achieved from suckers (offshoots) in date palm. The process of micropropagation is usually divided into several stages: prepropagation, initiation of explants, subculture of explants for proliferation, shooting and rooting, and hardening. The genotype is critical for high performance of in vitro plant multiplication. The determination of maximum number of subcultures before initiating new fresh cultures is critical for preventing somaclonal variation in the field, which has become a major problem for date palm growers and has resulted in severe economic loses. Somatic embryogenesis is an ideal technique for clonal propagation of woody and fruit plants (Jain and Gupta, 2005) and has great potential for large-scale propagation of superior clones, potential for automation, long-term storage and genetic manipulations. It is highly genotypic dependent. For large-scale production of somatic embryos, 'bioreactor' systems work well, e.g. 'temporary immersion system' (RITA bioreactor). This system has not yet been tried in date palm.

Mutation-assisted breeding have been quite successful for the production of new mutant cultivars by changing the plant characteristic for a significant increase in plant production among both seed and vegetatively propagated crops. This has been achieved by gamma irradiation and most recently with heavy ion beam (Jain, 2005). Fine somatic embryogenic cell suspension cultures are well suited for mutation induction and directly mutated somatic embryos can be produced and germinated into mutated somatic

seedlings. This approach produces mutated somatic seedlings in a short period of time and also prevents chimerism problems which otherwise require that plants be multiplied to M1V4 generation for chimera dissociation. Alternatively, shoot tip or bud wood can be irradiated and plants multiplied to M1V4 generation, producing pure mutants by dissociation of chimerism. 'Micro-grafting' would be an ideal system for shoot multiplication of mutant lines and chimera dissociation. This approach is being investigated for several fruit trees, including date palm.

DATE PALM

The date palm (*Phoenix dactylifera* L.) is called 'tree of life' in the bible, belongs to the monocot family Arecacea and is classified as a dioecious tall evergreen. Date palm female trees bear fruits after 3-5 years and are fully matured at 12 years (Jain, 2006). It is distributed throughout the Middle East, North Africa and South Sahel, areas of East and South Africa, and even in certain parts of Europe and USA. The total number of date palm trees worldwide is approximately 105 million, covering an area of 800,000 ha. Date palms also makes a significant contribution towards the creation of equable microclimates within oasis ecosystems, thus enabling agricultural development to be sustained in many drought- and saline-affected areas. The rich fruit plays an important role in the nutrition of human populations in these regions, as well as being used as a livestock feed supplement. Secondary products are generated from fruits such as jams and ice creams, which means that both small- and intermediate –scale industries can be supported for long-term periods in both urban and rural situations. This is reflected in the widely acknowledged sustainability value of date palm in social, economical and ecological terms.

Why Date Palm Tissue Culture

The propagation of date palm has been traditionally achieved using offshoots that are produced from axillary buds situated on the base of the trunk during the juvenile life of the palm. Offshoots develop slowly and their numbers are limited. Offshoot numbers vary from 10 to 30, depending on the genotype, and are produced only within a certain period of time in the mother palm's life. There is no field-based method yet available for increasing the number of offshoots per plant.

Sexual propagation of date palm is unsuitable for commercial propagation of true-to-type elite genotypes due to the heterozygotic characteristics of seedlings and the their dioecious nature. Half of the progeny are generally male, which produce no fruits, and large variations in phenotype can occur in progeny. Furthermore, no method is known at present for sexing date palms at an early stage of tree development for the elimination of non-productive male trees in the nursery before planting on the field scale. Another drawback of seed propagation is that the growth and maturation of seedlings is extremely slow. A date palm seedling may take 8-10 years, or more, before fruiting occurs and that has hampered date palm breeding.

The use of tissue culture is the most suitable approach for large-scale plant multiplication in vegetatively propagated crops including horticulture and forestry (Jain and Ishii, 2003). Both organogenesis and somatic embryogenesis approaches have been successfully applied for plant propagation for a wide range of plants (Jain and Ishii 2003; Jain and Gupta, 2005). Somatic embryogenesis has limitations, e.g. genotype and poor germination rate of somatic embryos, which have hampered greater commercial use in some plant species.

MICROPROPAGATION

Until now, micropropagation of date palm has had limited success. The world market needs about 1-2 million date palms per year. Regardless of the significance of date palms in dry regions, demand may not increase due to water scarcity and urban migration from dry regions. Of the approximately 25 known groups active in date palm research worldwide, private companies are located in Israel, UAE, USA, UK, France and Morocco.

Companies located in Israel, USA, UK, and France multiply date palm plantlets through somatic embryogenesis. The Moroccan company, El Bassatine, has arguably been the most successful in date palm micropropagation by adopting the organogenesis approach, i.e. plant regeneration from a single cell via organ structures, often shoots. The Institute National de Recherche Agronomique (INRA) of the Moroccan government, in close collaboration with El Bassatine, has found protocols to scale up few varieties with organogenesis. Most of the over 3000 date palm varieties grown worldwide require specific protocols for large-scale micropropagation. For example, some varieties need more sugar in the medium, while others require more vitamins, nitrogen or calcium. Basic research to tackle these differences systematically is scarce. However, Smith and Aynsley (1995) reported results on field performance of tissue cultured date palm clonally produced by somatic embryogenesis. They fruited within 4 years from field planting of small plants with leaf length of 100 cm and 1.5 cm diameter at the base. Fruit from the tissue culture derived plants, cultivar Barhi, was indistinguishable from fruit of plants which had originated from suckers (offshoots). These results justify the commercial scale up of micropropagation procedures using somatic embryogenesis to provide a rapid, cost-effective means of obtaining elite date palm planting material. Recently, at the Date Palm Research Centre (DPRC) at Basrah University, Iraq, scientists have developed in vitro cloning systems for Iraqi date palm varieties. This technology will make possible the production of up to 60,000 clones from one parent that can reach fruiting maturity in four years, compared to 8-10 years using traditional methods. Before the war, Iraq was home to 40 million trees of about 624 varieties. Now the number has dwindled to 10 million, and this new clonal propagation system will help to replace destroyed date palm trees in around a decade.

Advantages of Date Palm Micropropagation

a) High quality plants: Tissue culture plants are of a known, selected origin; they are uniform and of superior quality, and are available at any time without any special preparation in the plantation; b) Large quantity planned planting: Date palm tissue culture micropropagation enables the supply of large quantities of plants at a specific, planned date; c) A large and profitable plantation: The use of tissue culture plants permits the rapid attainment of a large scale and economically viable plantation unit; d) Maximum receptivity: Field establishment is close to 100%, and transportation of plants from the nursery to the field is simple and easy; e) Healthy plants: Tissue culture plants leave the laboratory and nursery completely clean of pests and diseases, a fact of special importance when shipping plants from one country to another; f) Early production: Tissue culture plants produce rapidly and give fruit as early as 3-4 years after planting; and g) Plant on demand: With tissue culture propagation it is possible to propagate and supply planting of rare or highly desireable plants.

There is no information available showing profit gain per plant by the companies. Certainly, micropropagated date palm trees have not shown any decline in yield. Prof. Drira Safax, Tunisia, has 8-year-old Deglect Nour date palm variety, derived from tissue culture, in the field, that is showing better yield and 4-year early fruiting. Fki et al. (2003) developed somatic embryogenesis protocols of date palm var Deglect Noor, and the regenerated plants did not show any changes in ploidy level, which opens the way for further scaling up this process for mass clonal propagation. They produced 10,000 somatic embryos per litre per month, and obtained 85% somatic embryo germination by partial desiccation of embryos.

Culture Medium

The most common culture medium being used for micropropagation via somatic embryogenesis is MS (Murashige and Skoog, 1962). Rarely B5 medium has been used. Several groups have modified the medium composition by adding vitamins, adenine sulfate, thiamine, glycine, glutamine, myo-inositol, and activated charcoal (Fki et al., 2003; Al-Khyari, 2005). Among plant growth regulators, 2,4-D (10-100 mg/L) is

commonly added either alone or in combination with cytokinin (kinetin, 2-isopentyl phosphate) in the culture medium. In some cases, 2,4-D is replaced by NAA. The role of vitamins in date palm somatic embryogenesis is not well defined except for the positive influence of thiamine and biotin on callus growth and somatic embryo production. Sucrose, 20-30g/L, is generally used as the major source of carbon and energy in tissue culture media. Often in combination with sucrose, other sugars such as mannitol, maltose, and sorbitol have been added in the culture medium to enhance somatic embryogenesis. In addition to being a major carbon source, sorbitol and mannitol act as osmotic agents to alter osmotic potential of the medium. Sorbitol-induced osmotic stress hastened induction of somatic embryogenesis. The addition of polyethyl glycol (PEG 8000) in the culture medium enhanced somatic embryo maturation and germination. Addition of silver nitrate, ethylene inhibitor, also had a stimulatory influence on somatic embryogenesis, and response to it was genotypic dependent.

Cultivars Used

The most commonly used cultivars for the induction of somatic embryogenesis are: Deglect Noor and Barhee, which did not show somaclonal variation (Fki et al., 2003; Smith and Aynsley, 1995); Bou-Feggous (BFG), Jihel (JHL) and Bou-Skri (BSK), Sair S 16 (S16) and Sair S 35 (S35) clones (4); Medjhool; Thoory and Zahdi; Tagaza and Takerboucht (Algeria) and Zaghloul (Egypt).

Explant Used

Various explants have been used to initiate date palm in vitro cultures, and their response to various plant growth regulators have been studies. The explants used were: mature and immature zygotic embryos, leaf segments excised from seedlings and young offshoots, leaf and merismatic tissues excised from in vitro plants, and inflorescence tissue. The most frequently used explants are apical shoot tips and lateral buds, since they have been the most responsive to in vitro culture.

Major Advantages of Somatic Embryogenesis

The major advantages of using somatic embryogenesis are:

a) It is possible to produce an unlimited number of plantlets under controlled conditions;
b) Cost effective large-scale production of plantlets in liquid medium, e.g. bioreactor that can lead to automation for somatic embryo production;
c) Cryopreservation for long-term storage;
d) Somatic embryos have both shoot and root meristem that develop in the same step of the process, and this enables direct plant regeneration like seed germination;
d) Encapsulation for artificial seed production;
e) Genetic transformation.

Somatic Embryogenesis Has Major Limitations

The limitations of using somatic embryogenesis are:

a) Low numbers of field-plantable clonal plantlets are produced per embryo culture;
b) The process is highly genotypic dependent for high numbers of plantlet production;
c) The risk of inducing mutations in plantlets which may not be detectable at the early stage of plant development and may appear at the later stage, causing severe economic losses to growers; and
d) Gradual fluctuation and eventual decline in embryogenic culture productivity.

Major Limitations of Tissue Culture in Plant Propagation

One of the major limitations of tissue culture in plant propagation is genetic variability or somaclonal variation (SCV). SCV has the characteristic of induced mutagenesis, that may be strongly influenced by oxidative stress at excision of the tissues, and is genotype-dependent. It is influenced by the explant source and tissue culture protocol, callus phase, age of the donor plant, type and concentration of plant growth

regulators in the culture medium, culture conditions applied such as solid and liquid medium; number of in vitro subcultures used for propagation including age of cultures; and genetic stability of mother plant material including chimeras. However, it is necessary to produce true-to-type micropropagated date palm plants for the survival of tissue culture commercial companies and growers. The in vitro production of date palm via somatic embryogenesis requires the application of relatively high concentrations of auxin-type plant growth regulators, such as 2,4-D or NAA, for process initiation. However, these auxins are known to be associated with genetic instability in plants, and have become a known cause of genetic variability in date palm. Furthermore, variation in DNA methylation may be an important factor in initiating genetic variation, and also activation of retrotransposable elements, e.g. *Tnt1A* retrotransposon expression. The question is whether tissue culture processes activate retrotransposons, or is aberration connected to cell division program activation or to stress responses activation or both???? (Jain, 2001). Djerbi (2000) reported the abnormal fruiting of date palms cv Barhee derived from somatic embryogenesis, where more than 100,000 date palms planted at the beginning and middle of the 1990s in Saudi Arabia showed 80-100% parthenocarpic fruits, sometimes with the development of more than 3 carpels. Saker et al. (2000) detected somaclonal variation in tissue culture-derived date palm plants by using isozymes and RAPD fingerprinting. Smith and Aynsley (1995) had no obvious abnormalities in somatic embryogenesis derived Barhee plants. Similary, Al-Ghamdi (1996) also observed no significant differences in flowering and fruit setting when two cultivars, Thoory and Zahdi, were investigated. Therefore, it has become essential to develop diagnostic molecular markers to detect plant off-types at an early stage of plant development, before transfer of date palm plants to the field (Azeqour et al., 2002; Saker et al., 2000).

Date palm somaclones have shown changes in traits such as morphology and structure, excessive vegetative growth, leaf variegation, dwarfism, higher susceptibility to diseases such as black scorch, delay in flowering time, production of bastard offshoots, leaf whitening, pollination failure, abnormal fruiting and seedless fruits.

Plant production via micropropagation is costlier than the conventional methods of plant propagation. It is a capital-intensive industry, and the unit cost per plant becomes unaffordable due to the high cost of production and know-how. The major constraints in reducing costs are chemicals, energy, labour and capital. In the developed world, expensive labour adds to a higher cost per plant. In the developing countries of Africa, Asia and Latin America, labour is relatively cheap, but consumables and electricity raise the cost of plant production. Therefore low-cost alternatives are needed to reduce the cost of tissue-cultured plants (Jain, 2005). Temporary immersion systems, RITA bioreactor, could be tried in date palm in vitro propagation (Jain, 2006).

MUTAGENESIS

Genetic variability, which can be either spontaneous or induced, is an important aspect of any plant improvement program,. The rate of spontaneous mutation is very low and that is why mutations are induced by the use of chemical and physical mutagens. Induced mutations are random changes in the nuclear DNA or cytoplasmic organelles, resulting in chromosomal or genomic mutations that enable plant breeders to select useful mutants such as high yield, disease resistance etc. Induced mutations have been applied for the past 70 years to produce mutant cultivars by changing the plant characteristic for a significant increase in plant production among both seed and vegetatively propagated crops (see http://www-mvd.iaea.org), and since then, over 2300 officially released mutant varieties have been listed (Jain, 2005). So far, there is very little work done on induced mutations in date palm, due to poor plant regeneration systems. However, FAO/IAEA Technical Cooperation project in North Africa (Algeria, Morocco and Tunisia) on date palm, has made substantial progress in date palm improvement to increase tolerance to Bayoud disease. Somatic embryogenic cell suspension cultures were irradiated with gamma rays. The regenerated plants were transferred to the greenhouse and treated with

Bayoud toxin isolated from the causal fungus *Fusarium oxysporum* f.sp. *albedini*. They have isolated several mutants tolerant to Bayoud disease in the greenhouse (Fig. 1), which are being further evaluated in the field (Jain, 2006).

The advantage of radiation treatment to somatic embryogenic cultures is minimising the chances of chimersim, since embryogenic cultures are of a single cell origin. The irradiation of a multicellular structure, e.g. seed, meristem tissue or offshoots, may result in chimerism in regenerated plants. It would require a lot of work for the dissociation of chimerism by multiplying plants up to M1V4 generation.

CONCLUSIONS

A robust date palm micropropagation - organogenesis and somatic embryogenesis-system associated with a cost effective DNA-based detection technique are needed for both cultivar identification and detection of somaclonal variants. Despite the risk of genetic variability in regenerated plantlets, the most common approach to micropropagation has been somatic embryogenesis, which is very much dependent on genotype and culture medium. It would be more appropriate to optimise tissue culture conditions for specific genotype(s) by further investigating features such as the controlled growing conditions of the mother plant; explant age, type and source; culture medium modifications; liquid (temporary immersion system) vs. solid medium; type and concentration of plant growth regulators including ethylene inhibitors; determination of number of subcultures; light quality; and measurement of osmotic potential of the medium. The germination rate of somatic embryos should be improved to the range of 80-85% to reduce the cost of in vitro plants for commercial feasibility.

Induced mutations are effective in generating genetic variability, and have been very effective in both seed and vegetatively propagated crops. Somatic embryogenic cell suspension cultures would be ideal for mutation induction, and useful mutants can be identified, multiplied and field tested. Initially, mutant plants could be tested in the farmer's field before release of the variety.

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Figures

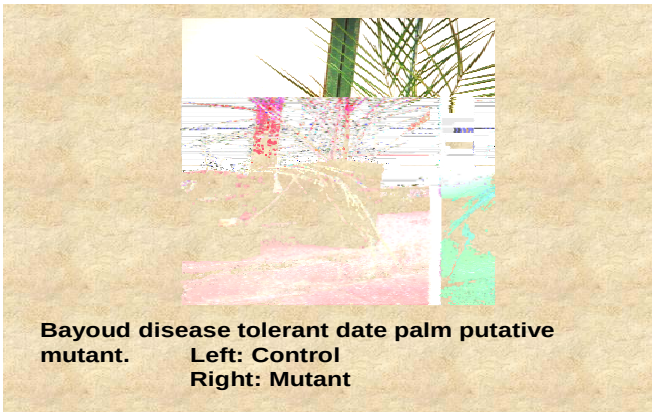

Fig. 1. Date palm 'Deglect Nour' putative mutant showing tolerance to Bayoud toxin in the greenhouse.

Micropropagation of Date Palm (*Phoenix dactylifera* L.) var. Maktoom through Direct Organogenesis

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Abstract

This study aimed to determine the best combinations of plant growth regulators and other conditions in order to achieve organogenesis and multiplication directly from shoot tips of date palm (*Phoenix dactylifera* L.) var. Maktoom without callus formation so as to avoid any possibility of undesirable genetic variability. Results revealed that MS modified medium supplemented with 2.0 mg/L 2ip, 1.0 mg/L BA, 1.0 mg/L NAA and 1.0 mg/L NOA was the best for bud formation from shoot tip after 16 weeks (6.2 bud per explant). Subculturing the formed buds on liquid agitated multiplication medium supplemented with 4.0 mg/L 2ip, 2 mg/L BA, 1.0 mg/L NAA and 1.0 mg/L NOA gave the optimum average of buds number (12.6 buds). In elongation stage MS medium with 0.5 mg/L GA_3 and 0.1 mg/L NAA enhanced plantlet length to 5.3 cm. Optimum rooting percentage 90% was achieved when shoots were transferred to a medium with 1.0 mg/L NAA. The average root number after 8 weeks was 5.4 with 9.0 cm length. Rooted shoots (plantlets) were transplanted in small pots containing a mixture of peatmoss and perlite (2:1) and placed in plastic tunnels or in a greenhouse. The survival percentage was 85% after 3 months when the plants were transferred to bigger pots. These results define a successful protocol for the in vitro propagation of Maktoom cv. date palm.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious tree and its cultivation has extended to Iraq and most Arab countries. It also occupies special significance for its distinguished economic, nutritional, esthetic, historic and social values. It is propagated traditionally by seeds or offshoots, but because of heterozygosity the plantlets produced from seeds are not identical, are lesser in quality than the mother plant, and are approximately 50 % male. Therefore, propagation by offshoots is better, but the numbers produced from the tree are limited, especially from superior and rare cultivars, so it cannot satisfy the need to establishing new groves. The use of plant tissue culture to supplement propagation by offshoots is necessary. Since the first attempts at date palm propagation by tissue culture (Shroeder, 1970; Reuveni, 1972), two methods of propagation have appeared: the first was direct organogenesis and the second was somatic embryogenesis through embryogenic callus produced from explants. Important successes were reported of direct oganogenesis by some researchers in axillary branching of shoot tips (Tisserat, 1984; Hameed, 2001). Al-Maari and Al-Ghamid (1997); Al-Khateeb et al. (2002), were successful in enhancing adventitious bud formation on shoot tips. The second method, the production of somatic embryos from embryogenic callus, has been reported by many researchers (Al–Khalifah, 2000; Al-Musawi, 2001; Al–Khayri, 2003). Despite the fact that the second method is the most commonly used in commercial plant tissue culture labs, it involves the possibility of undesirable genetic variability in the derived plants which is not apparent until the fruiting stage. The first method enables the production of plants that are genetically identical and true-to-type with the mother plant, therefore this method represents an effective means of large scale vegetative propagation of date palm. The aim of this study was to determine the best combinations of plant growth regulators and culture growth conditions to stimulate the initiation and multiplication of adventitious buds directly from shoot tips (without callus formation), to elongate these buds to shoots, to root and acclimatize the plantlets in order to transfer them successfully to soil.

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MATERIALS AND METHODS

Explants Preparation and Sterilization

Young offshoots of Maktoom cultivar (2 – 3 years old) were chosen and detached from the mother palm. Offshoots were dissected acropetaly until the shoot tips appeared. Shoot tips of 3 cm (apical meristem with soft inner leaves) were excised with immature fiber 2 cm in diameter and then applied in autioxidant solution consisting of 150 mg/L cirtic plus 100 mg/L ascorbic acid (Tisserat, 1991). Explants were sterilized in commercial bleach (sodium hypochlorite) 20 % containing eight drops of tweed – 20 as emulsifier for 20 minutes with vacuum, and rinsed three times with sterile distilled water. Then they were transferred to petri dishes and all leaf primordia were removed except two pairs surrounding the apical meristem.

Initiation Stage

The medium used in the initiation stage (Table 1) was composed of MS (Murashise and Skoog, 1962) plus the following (in mg/L): thiamine – HCl, 1.0; pyridoxine – HCl, 1.0; adenine sulfate $2H_2O$, 50; myo-inositol, 100; $NaH_2\ PO_4$. 2 H_2O, 170; glutamine, 200; sucrose, 30000 activated charcoal, 2000 and agar-agar 7000. The pH of the medium was adjusted to 5.7 with 0.1 N NaOH or HCl, before the addition of agar. Media were dispensed into culture jars with 25 ml in each, then covered with polypropylene caps. All vials with media were autoclaved at 121°C and 1.04 kg/cm² for 15 minutes. Apical meristems were cultured into the jars aseptically in a laminar air flow cabinet and cultures were incubated in the dark to reduce phenolic secretions from the explant for one month (Fig. 1). The apical meristems were then removed and divided longitudinally into four equal segments and cultured on media of the same composition supplemented with benzyl adenine (BA), kinetin, isopentenyladenine (2ip), naphthaleneacetic acid (NAA) and naphthoxyacetic acid (NOA) (Table 1). The activated charcoal was changed by 2 g/L of polyvenypyroledone (PVP).

All cultures were incubated in a culture room under low light intensity of 1000 lux for 16 hours daily at 27 $\pm$ 1°C for four weeks. They were subculture four times at four week intervals until the buds had initiated, at which time data was recorded. There were ten replicates of each treatment.

Multiplication Stage

The formed buds were divided into small clumps, each one containing not less than three buds, and cultured on medium of the same composition except for the hormones. Depending on the initiation stage, kinetin was removed and 2ip was added in various concentrations (1.0, 2.0, 4.0, 6.0, mg/L) plus 1.0 mg/L of NAA and NOA. There were ten replicates for each treatment. Cultures were incubated under the same conditions as above. Subculture was carried out every four weeks, and data recorded after eight weeks. The physical status of the medium was evaluated when buds were transferred to liquid medium containing the best combination of plant growth regulators achieved from the multiplication stage. Stationary medium and rotating medium on an orbital shaker (40 rpm) were examined. Results were recorded after eight weeks and compared with those on solid medium.

Elongation Stage

In order to increase the shoot length, shoots were transferred to elongation medium with the same composition except the addition of GA_3 in various concentrations (0.1, 0.3, 0.5, and 1.0 mg/L) in the presence of 1 mg/L NAA with ten replicates for each treatment. Data were recorded after eight weeks, while the subculture was done every four weeks.

Rooting Stage

Resultant shoots were transferred to test tubes (one shoot / test tube) containing 25

cm³ of rooting medium consisting of MS salts and the following (in mg/L) : Thiamine HCl 0.4, myo–inositol 100, sucrose 60000 and agar 7000. The auxin NAA was added separately in different concentrations (0.1, 0.3, 0.5, and 1.0 mg/L). There were ten replicates for each treatment, and cultures were incubated in a culture room at 27 ± 1 ° C and 1000 lux light intensity for 16 hours daily. Rooting percentage, average number of roots and root length were recorded after two months of culture.

Acclimatization Stage

There were two stages of acclimatization. Firstly, the rooted shoots were taken out of the test tube and the root system was washed with tap water to remove the medium. Every washed plantlet was transferred to a new test tube containing 20 cm³ of MS salts and was incubated for two weeks at 27 ± 1°C with 3000 lux light intensity for 16 hour days.. Subsequently,all plastic covers were removed for one week in the culture room. Secondly, the plantlets were washed with distilled water and treated with fungicide (Benlet 2g / L) for 10 minutes, then transplanted into peat moss and perlite alone or into a mixture (1 : 1, 1 : 2 and 2 : 1). Plants were placed in pots with a 10 cm diameter, that were filled with a peat/perlite mixture and placed in a greenhouse or under plastic tunnels. Pots were irrigated with 1/2 strength MS salts and plastic covers were removed gradually for eight weeks. The survival of acclimatized plants was then recorded. The experimental design used in this study was a randomized complete block design (RCBD), and wherever there was a significant effect, less significant difference (LSD) was used to compare means at 5 % level of probability.

RESULTS AND DISCUSSION

Initiation Stage

It was clear that the type and concentration of cytokinin affected the response percentage as well as the formation of buds (Table 2). No response was noticed among explants cultured on media free from cytokinin (medium 2, Table 1) or those supplemented with 0.1 mg/L kinetin (medium 3, Table 1). The medium containing 2 mg/L 2ip plus 1 mg/L BA gave a better result in terms of growth response percentage (80 %) and average bud formation (6.2 bud), (Fig. 2). These results indicated the superiority of BA over other cytokinins (kinetin and 2ip) for the initiation and development of buds (Fig. 3).

Multiplication Stage

The multiplication of buds was slightly more enhanced by the addition of 2ip than BA. The average number of buds formed was 4.5 buds when 6.0 mg/L 2ip was added, which was significantly better with other concentrations, e.g. 4.2 buds with 4.0 mg/L of BA (Table 3). The highest number of the buds was 8.6 when 4.0 mg/L 2ip was combined with 2 mg/L BA and 1 mg/L NAA. This number was significantly higher than all other treatments (Fig. 4). This result shows the optimum combination of plant growth regulators for high multiplication rates. This result is consistent with other results where BA and 2ip have been used in the medium for initiation and multiplication of date palm in vitro (Al–Marri and Al–Ghamdi, 1997; Bekheet and Saker, 1998; Al- Khateet et al., 2002). For the effect of physical status of the medium, results indicated that the agitated liquid medium significantly increased the number of buds which was 12.6 in comparison with 8.6 buds in solid medium and 5.8 bud with stationary liquid medium (Table 4). This effect could be attributed to the increase of nutrient availability and uptake in the liquid medium compared to the solid medium, as well as the movement of explants which lead to gas exchange, and removal of some mineral deficiency symptoms that occur in solid medium (Pierik, 1987).

Elongation Stage

GA_3 had a positive effect on the elongation of shoots produced in the

multiplication stage (Table 5). Shoot length increased with the increasing GA_3 concentration in the medium, but some malformations were noticed at 1.0 mg/L in spite of its superiority over other treatments. The average length of shoots was 7.4 cm, but they were slender and difficult to root and transplant. Therefore, the concentration of 0.5 mg/L was considered a better treatment with an average shoot length of 5.3 cm which was significantly different from other treatments. This result directs our attention to the well-know role of gibberellins in the elongation of the plant cells (1PG SA, 1998).

Rooting Stage

Results of rooting are shown in Table 6. They indicated that the addition of NAA leads to an increase in rooting, with the concentration 0.5 mg/L resulting in the best rooting percentage (90%) and average root length (5.4cm), which were significantly different from other treatments. No reduction in root length was observed with the increasing of auxin concentration. It is known that auxins play an active role in root formation by the induction of root initials (IPGSA, 1998). Our results are not consistent with those by El-Hammady (1999) using NAA. He noticed that the average root length decreased with the increasing auxin concentration. Many researchers have mentioned the importance of NAA in the rooting of date palm shoots in vitro (Al-Maari and Al-Gamdi, 1997; Tisserat, 1984; Mater, 1990), (Fig. 5).

Acclimatization Stage

Acclimatization of plantlets derived from tissue culture confirmed the efficiency of the method used, where the transformation of rooted shoots to the MS salts solution and increasing light intensity enhanced the plantlets for photosynthesis and then changing from heterotrophic to autotrophic status. The gradual lifting of plastic covers both in the culture room and the greenhouse assisted in formation of the cuticle layer (Fig. 6) and regulation of stomatal action. In spite of the significant differences in the percentage of acclimatization successes, the mixture containing 2 peatmoss: 1 perlite was the best and gave 80% survival of acclimitaized plants (Table 7, Fig. 7). According to these results, we can say that all steps in this study were practically successful and could be used as a protocol for micropropagation of date palm, Maktoom cv.

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Tables

Table 1. Plant growth regulators used in initiation stage.

Conc. mg/L					Medium No.
NOA	NAA	2ip	Kinetin	BA	
-	-	-	-	-	1
1.0	1.0	-	-	-	2
1.0	1.0	-	1.0	-	3
1.0	1.0	-	2.0	-	4
1.0	1.0	-	3.0	-	5
1.0	1.0	1.0	-	-	6
1.0	1.0	2.0	-	-	7
1.0	1.0	3.0	-	-	8
1.0	1.0	-	-	1.0	9
1.0	1.0	-	-	2.0	10
1.0	1.0	-	-	3.0	11
1.0	1.0	-	2.0	1.0	12
1.0	1.0	2.0	1.0	-	13
1.0	1.0	2.0	-	1.0	14
1.0	1.0	1.0	1.0	1.0	15

Table 2. Effect of various concentrations of cytokinin and its interaction in the percentage of response and the number of buds formation from the apical meristem of date palm, Maktoom cv. after 16 weeks of culture.

Medium No.	Number of Buds	%Response
2	0.0	0
3	0.0	0
4	0.3	10
5	0.4	10
6	1.8	20
7	2.7	40
8	3.2	50
9	3.3	30
10	4.2	60
11	3.8	50
12	3.5	40
13	3.7	50
14	6.2	80
15	3.4	20
L .S .D	2.62	38

Table 3. Effect of BA and 2ip and its interaction on adventitious bud multiplication for Maktoom cv. after 8 weeks of culture in the presence of 1 mg/L of NAA and NOA.

2ip Mg/L BA	0.0	1.0	2.0	4.0	6.0	AVE BA
0.0	3.0	3.3	3.7	4.2	4.5	3.74
1.0	3.2	4.0	4.6	5.5	4.9	4.44
2.0	3.8	4.3	5.2	8.6	5.4	5.46
4.0	4.2	4.6	5.4	6.3	5.0	5.10
6.0	3.5	3.7	4.3	5.2	4.1	4.16
AVE 2ip	3.54	3.98	4.64	5.96	4.78	
			LSD **:** Interaction, BA = 0.2, 2ip = 0.2 =0.5			

Table 4. Effect of physical status of the medium on adventitious bud formation for Maktoom cv. after 8 weeks.

Physical status	Buds number
semi solid	8.6
Stationary liquid	5.8
Agitated liquid	12.6
LSD	0.78

Table 5. Effect of various concentrations of GA_3 on the elongation of shoots produced from Maktoom cv. after 6 weeks in presence 1 mg/L NAA.

GA_3 Conc	Shoots Length (cm)
0.0	2.23
0.1	3.12
0.3	4.58
0.5	5.30
1.0	7.42
LSD	0.34

Table 6. Effect of different concentrations of NAA in the percentage of rooting, roots number and root length after 8 weeks.

NAA Conc. mg/l	Rooting %	Average of root / shoot	)Roots length cm
0.0	20	0.3	6.2
0.1	50	2.3	8.0
0.3	80	4.4	6.8
0.5	90	5.4	4.7
1.0	70	3.0	4.1
LSD	42	0.49	0.68

Table 7. Effect of culture mixture on the percentage of acclimatization for date palm plant, Mektoom cv.

Culture mixtures	Survival **%**
peatmoss	60
perlite	50
peatmoss : perlite(1:1)	70
peatmoss: perlite(2:1)	70
peatmoss : perlite(1:2)	80
LSD	0.49

Figures

Fig. 1. Quarter of apical meristem (explant) after four weeks in the dark on hormone free medium.

Fig. 2. The development of explant to adventitious buds of Maktoom cv. on MS medium plus (mg/L) BA 1, 2ip 2, NAA, and NOA 1 after 16 weeks .

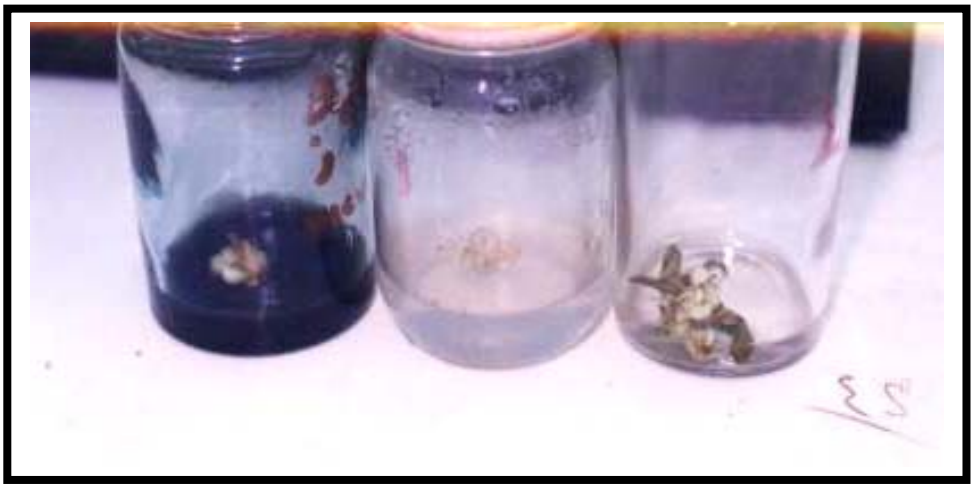

Fig. 3. Apical meristem development stages of Maktoom cv. to adventitious bud after 8, 16 and 24 weeks of culture .

Fig. 4. Bud multiplication of Maktoom cv. on agitated liquid medium supplemented with (mg/L) BA, 2, 2ip, 4, NAA and NOA 1 after 8 weeks.

Fig. 5. Stages of microprpagation of Maktoom date palm culture, from the left (initiation, multiplication, elongation and rooting) .

Fig. 6. Acclimazation of Maktoom date palm under the plastic tunnel in the greenhouse.

Fig. 7. Date palm plants in green house after 8 months after acclimatization.

Fertilization Failure and Abnormal Fruit Set in Tissue Culture-Derived Date Palm *(Phoenix dactylifera* L.)

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Abstract

Tissue culture technologies are commonly used worldwide for the mass propagation of date palms. Productivity and yield of date palm trees are of great importance to date palm growers. However, the occurrence of infertility in plants propagated from tissue culture presents major economic problems. The present study assessed the fertilization failure phenotype in two elite date cultivars, 'Khlass' and 'Barhee', with respect to the different techniques used for their propagation. Propagation by organogenesis was found to be successful, resulting in highly productive trees with a fertilization percentage of 94-98% in both cultivars, whereas somatic embryogenesis resulted in abnormal fruit set. Both direct and indirect embryogenesis generated a high fruit set failure in 'Khlass'. In 'Barhee', only plants propagated by indirect embryogenesis were more susceptible to this abnormality. Most abnormal parthenocarpic fruits showed 2-5 carpels.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious fruit tree grown in arid regions of the Middle East and North Africa. Its importance in the United Arab Emirates (UAE) is well demonstrated at the social, economic, environmental and agricultural level. The conventional method of date palm propagation by offshoots is not commercially practical. This vegetative propagation is limited by the number of offshoots produced during the palm's lifetime, the low planting survival rate and the possibility of spreading diseases and pests (Zaid and De Wet, 1999). The development of tissue culture propagation methods has enabled date palm to be rapidly propagated on a larger scale. Organogenesis and somatic (or asexual) embryogenesis are two major techniques currently used worldwide to mass-propagate date palm. The production of genetically uniform and stable in vitro date palms is of critical importance. Unfortunately, undesirable plant off-types produced by somaclonal variation are quite common among date palms (Zaid and Al-Kaabi, 2003; Cohen et al., 2004; Gurevich et al., 2005). Somaclonal variations can be epigenetic or genetic, resulting from in vitro culture conditions (Pierik, 1987; Zaid et al., 1999; Kaeppler et al., 2000). Plant somaclonal variants are clearly different from the mother plant. These variations have been reported in many plant species including date palm. The abnormalities found include changes in morphology and structure, excessive vegetative growth, leaf variegation structure, dwarfism, leaf whitening, production of bastard offshoots, delayed flowering time, fertilization failure, formation of seedless fruits and higher susceptibility to diseases (McCubbin et al., 2000; Zaid and Al-Kaabi, 2001; 2003). The most economically serious abnormality among these is fertilization failure. This abnormality affects to a great extent the productivity and yield, and consequently the economics of the whole cultivation process.

Low levels of fruit set and supernumerary carpels were detected in many date palm trees produced by tissue culture. In Saudi Arabia, more than 100,000 tissue culture-derived date palm trees were severely affected (Djerbi, 2000). All pollinated bunches showed 80-100% parthenocarpic fruits with the development of more than 3 carpels. This abnormal fruiting was found to be mainly associated with the use of somatic

embryogenesis. A survey in Israel revealed that 1,500 out of 3,500 tissue cultured 'Barhee' trees had abnormal fruit set. Structural abnormalities in flowers were correlated to this poor fruit set phenotype (Cohen et al., 2004).

Molecular characterization of trees showing this abnormal fruiting using AFLP analysis did not reveal any DNA marker specific to this phenotype (Gurevich et al., 2005; Cohen et al., 2005). However, this abnormality was found to be characterized by changes in DNA methylation patterns (Cohen et al., 2005). Varughese (2000) also reported abnormalities in fruiting ability and fruit characteristics, e.g. abnormal fruit size and shape, in tissue culture-derived date palms. Varughese raised the possibilities that abnormalities observed in in vitro plants could be due to the tissue culture procedure or other physiological factors. These abnormalities may be temporary since, in many cases, poor fertility was noticed only in the first few years of fruiting. Alleviation of the low level of fruit set and supernumerary carpels was observed during maturation of the trees. Cohen et al. (2004) reported that about 50% of trees reverted to normal after several years in the field.

The present study aims to assess the fertilization failure abnormality in two important date palm cultivars propagated by tissue culture in the UAE: 'Khlass' and 'Barhee'. Evaluating the importance of this phenomenon and identifying the cultivars most sensitive to this abnormality will provide valuable information for future selection of the most appropriate propagation technique and for improvement of the protocol in order to avoid such shortcomings.

MATERIALS AND METHODS

The assessment of the level of fruit set was done via a survey in several date palm plantations throughout the UAE during 2003-2004. The survey was designed to give a quantitative representative picture of fertilization failure abnormality in tissue culture-derived date palms. Two cultivars 'Khlass' and 'Barhee' propagated by tissue culture techniques (organogenesis; and direct and indirect somatic embryogenesis, AE/B and AE/A, respectively), and offshoots were analyzed. From each propagation source (other than organogenesis), trees of three different ages were randomly selected. From each age group, five trees were randomly selected in each cultivar. For the organogenesis source, only five trees were examined in each cultivar, due to the limited number of palms available of the same age. The total number of palm trees examined was 100 of both 'Khlass' and 'Barhee' cultivars. From each palm tree, three normal representative bunches were selected, the number of spikelets and flowers were counted, and the percentage of fruit set was determined.

RESULTS AND DISCUSSION

A total number of 100 trees from two of the most common date palm cultivars from the UAE, namely 'Khlass' and 'Barhee', were selected to investigate the fertilization abnormality resulting from different propagation techniques. The assessment of the level of fruit set was conducted through a survey in several date palm plantations throughout the country. The survey revealed that failure of fruit set occured in many date palm trees. Fruit set was compared between trees originating from offshoots, organogenesis, and direct and indirect somatic embryogenesis (AE/B and AE/A, respectively).

Fruit Set in Khlass Produced by Offshoots and Tissue Culture

Low levels of fruit set and the formation of multicarpel parthenocarpic fruits were detected in many date palm trees produced by tissue culture (Table 1). This phenotype appears to be mainly associated with propagation by somatic embryogenesis. A high fruit set failure was obtained in both direct (AE/B) and indirect (AE/A) asexual embryogenesis-derived trees. About 20-50% of fruits were parthenocarpic, whereas this abnormality occurred to a lesser extent in trees propagated from offshoots and by organogenesis (0.2% and 6.2%, respectively). A large variation of abnormal fruits with 2-

6 carpels was observed (Fig. 1). In plants that originated from offshoots, all parthenocarpic fruits had 3 carpels, while trees from organogenesis had multicarpel fruits ranging between 2-5 carpels and no fruits with 6 carpels were recorded.

The proportions of abnormal fruits with 2, 3, 4, 5 and 6 carpels recorded in date palm trees from all propagation techniques are illustrated in Fig. 2. Both indirect and direct asexual embryogenesis resulted in similar distribution of multicarpel fruits. The average for 2, 3, 4, 5 and 6-carpels fruits was about 43, 32, 14, 7, and 4.5%, respectively. Parthenocarpic fruits from AE/B consisted mainly of 2 and 3 carpels (Fig. 3). Abnormal multicarpel fruits with 4, 5 and 6 carpels were detected in smaller proportions. Plants propagated through AE/A had a very good fertilization percentage of 80% (Fig. 4). Most parthenocarpic fruits had 2-3 carpels, with fewer 4, 5 and 6-carpels fruits. Trees originating from organogenesis had a very good fertilization percentage of 94%. Parthenocarpic fruits had mainly 2 carpels (Fig. 5), though triple carpel fruits were also detected. Fruits with 4-5 carpels were rare, and no fruits with 6 carpels were recorded.

Fruit Set in Barhee Produced by Offshoots and Tissue Culture

Table 2 illustrates the results obtained in the 'Barhee' cultivar. Unlike 'Khlass', a high percentage of parthenocarpic fruits was recorded only with indirect asexual embryogenesis (about 35%). Of these abnormal fruits, most had 3 carpels (49%), while 31% had 2 carpels, and 20% had 4 carpels. There were almost no cases with 5 and 6 carpels. Date palm trees originating from offshoots, organogenesis and direct asexual embryogenesis displayed almost 99 % fertilization, whereas trees from indirect asexual embryogenesis had only 66% fertilization. Altogether, parthenocarpic fruits were mainly made of 2, 3 and 4 carpels and very few fruits had 5 carpels (Figs. 6 and 7).

Fertilization failure and abnormal fruit set in date palm are two major problems associated with propagation by somatic embryogenesis. 'Khlass' showed more variations in the development of parthenocarpic fruits. Around 20-50% of fruits were parthenocarpic in plants produced by indirect and direct embryogenesis, compared to only 6.2% from organogenesis and 0.2% from offshoots. In 'Barhee', the development of parthenocarpic fruits was restricted mainly to indirect embryogenesis with 66% fertilization compared to 99% in organogenesis. It is clear that the use of somatic embryogenesis generates a higher fruit set failure than organogenesis and offshoots in both cultivars. Both direct and indirect embryogenesis produced this abnormality in 'Khlass', while only indirect embryogenesis causes this problem in 'Barhee'. The date palm 'Barhee' is less susceptible to fertilization failure and abnormal fruiting when compared to 'Khlass'.

Somoclonal variation as aresult of somatic embryogenesis was found in several plants species including palms (D'Amato, 1978). Zaid (1987) detected inter-specific variations for several Arecacae species. Scientists, who are cautious about this technique, often explain that the passage through a callus phase is considered as a possible cause of variation, because callus is constituted of a group of non-organized and non-differentiated cells in which cytological abnormalities are commonly observed. Furthermore, in monocotyledonous plants such as palms, induction of callus requires a relatively high concentration of an auxin-type growth regulator like 2,4-D (2,4 Dichlorophenoxyacetic acid). 2,4-D is a mutagenic and very potent compound with auxin activity. It may cause undesirable features on cultured callus, including loss of differentiation ability with prolonged sub-cultures, chromosomal aberrations, mutations and endoduplication resulting in polyploidy and possibly sister chromatid exchange (Loubser, 1980; Omar and Novak, 1990). Furthermore, unlike various other plant growth regulators, 2,4-D is not broken down and is continuously recycled by the plant. It has also been found that the 2,4-D effect is cumulative and, since it remains in the plant tissues for long periods, the effect is long-lasting (Loubser, 1980). Whereas the organogenesis technique is based on the use of the naturally existing potential at the meristematic regions (meristems, auxillary buds at the base of young leaves) to produce meristemic buds when the nutrient media and incubation conditions are suitable (Zaid and Arias, 1999). The technique avoids passage through the callus phase by using media with very low concentrations of

hormones and excludes the use of 2,4-D.

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Tables

Table 1. Fruit set of offshoots and tissue culture-derived ‘Khlass’ trees.
AE/A: indirect asexual embryogenesis; AE/B: direct asexual embryogenesis.

	Offshoots	Tissue Culture		
		Organogenesis	AE/A	AE/B
Percentage of parthenocarpic fruits	0.2	6.2	19.9	49.25
Parthenocarpic fruits with 2 carpels (%)	0	70	42	43
Parthenocarpic fruits with 3 carpels (%)	100	23.4	35	29
Parthenocarpic fruits with 4 carpels (%)	0	5.5	15.6	12.3
Parthenocarpic fruits with 5 carpels (%)	0	1.2	5	9
Parthenocarpic fruits with 6 carpels (%)	0	0	2.4	6.5

Table 2. Fruit set of offshoots and tissue culture-derived ‘Barhee’ trees.
AE/A: indirect asexual embryogenesis; AE/B: direct asexual embryogenesis.

	Offshoots	Tissue Culture		
		Organogenesis	AE/A	AE/B
Percentage of parthenocarpic fruits	0.8	2	34.2	0.5
Parthenocarpic fruits with 2 carpels (%)	33	25	30.7	95
Parthenocarpic fruits with 3 carpels (%)	67	75	49	4.6
Parthenocarpic fruits with 4 carpels (%)	0	0	20	0
Parthenocarpic fruits with 5 carpels (%)	0	0	0.2	0
Parthenocarpic fruits with 6 carpels (%))	0	0	0	0

Figures

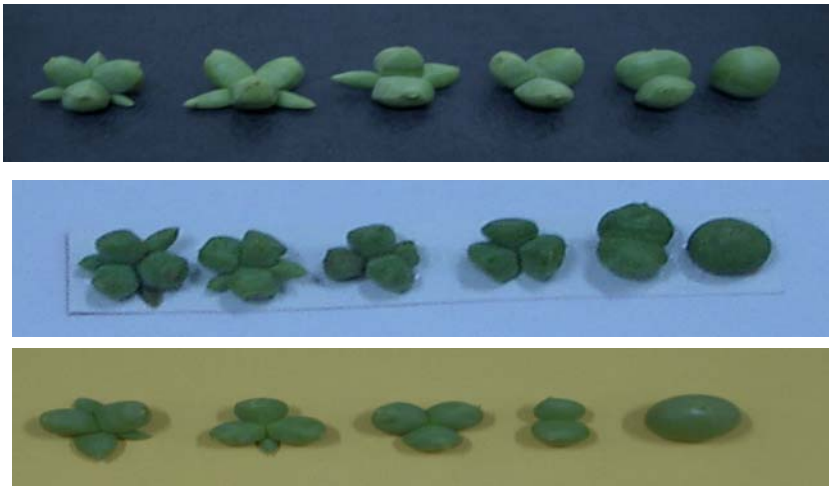

Fig. 1. Multicarpel formation in 'Khlass' propagated by AE/A **(A)**, AE/B **(B)** and organogenesis **(C)**.

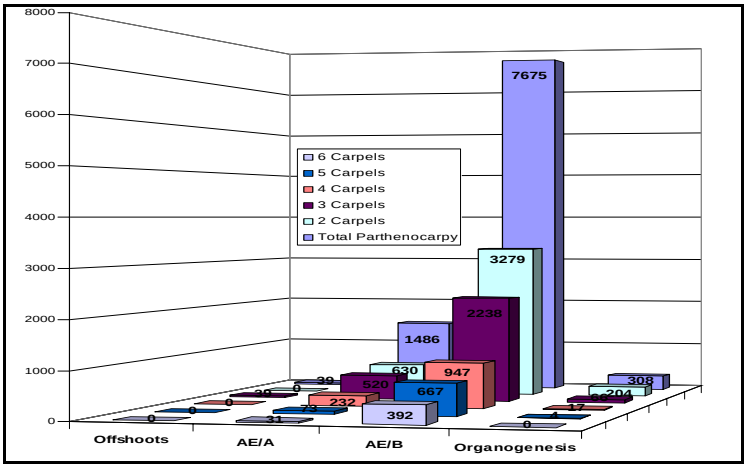

Fig. 2. Multicarpel distribution in 'Khlass' trees. Numbers indicate the number of parthenocarpic fruits. AE/A: indirect asexual embryogenesis; AE/B: direct asexual embryogenesis.

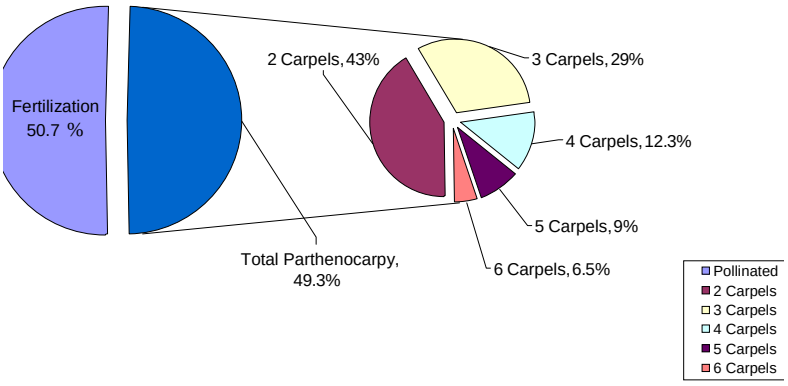

Fig. 3. Multicarpel distribution in 'Khlass' trees propagated by direct asexual embryogenesis (AE/B).

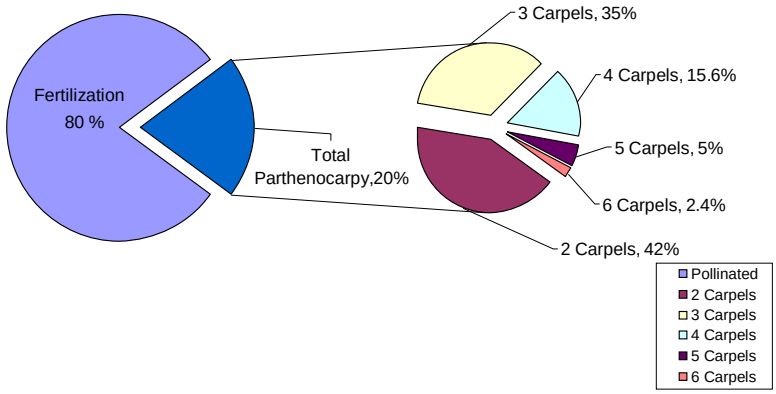

Fig. 4. Multicarpel distribution in "Khlass" trees propagated by indirect asexual embryogenesis (AE/A).

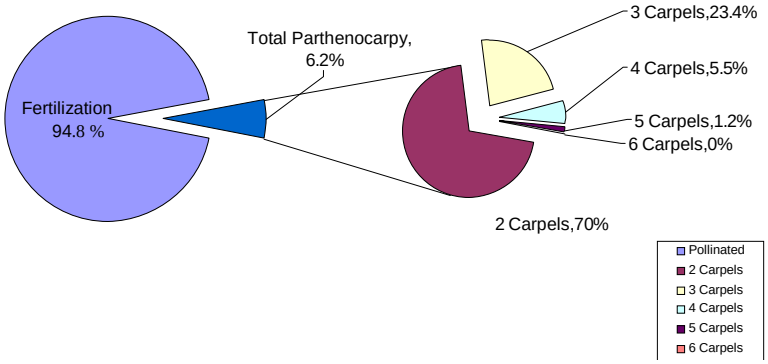

Fig. 5. Multicarpel distribution in 'Khlass' trees propagated by organogenesis.

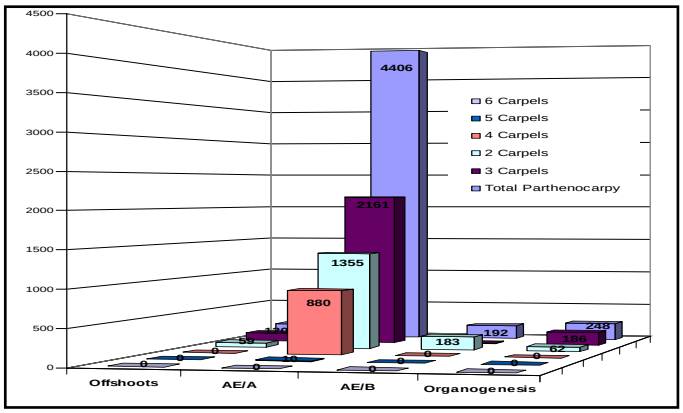

Fig. 6. Multicarpel distribution in 'Barhee' trees. Numbers indicate the number of parthenocarpic fruits.

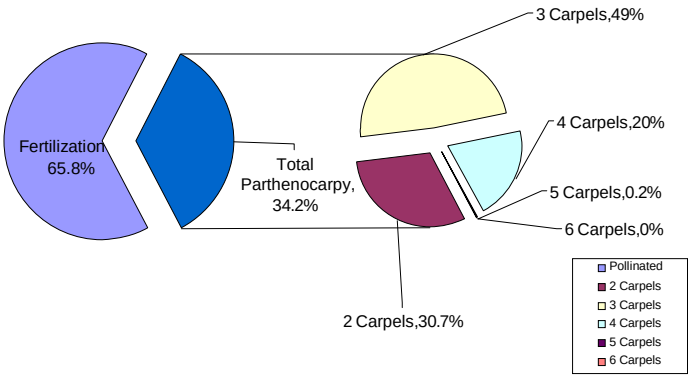

Fig. 7. Multicarpel distribution in 'Barhee' trees propagated by indirect asexual embryogenesis (AE/A).

Micropropagation of Some Date Palm Cultivars: Changes of Some Chemical Constituents Related to Embryogenesis

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Keywords: *Phoenix dactylifera* L., shoot tip, Bartamuda, Gondila, Shamia, SDS-PAGE, isozymes, peroxidase, polyphenol oxidase, tissue culture

Abstract

The present investigation aimed to study growth gradation from explant to plantlet and the biochemical changes at different stages via tissue culture propagation. Shoot tip explants from three dry date palm cultivars (Bartamuda, Gondila and Shamia) were used. Samples were taken from different developmental stages in tissue culture for morphological observations (including callus formation and degree of browning) and biochemical studies (including indole and phenol contents, SDS-PAGE of water soluble protein fraction, isozymes of peroxidase and polyphenol oxidase). The morphological studies indicated that shoot tip explants of Bartamuda cv. produced a higher degree of callus and a lower degree of browning than the other cultivars. The shoot tip explant of Bartamuda cv. had a significant and positive effect on indole and phenol contents compared to other developmental stages. Generally, the increase in indole content was more than phenol content in different developmental stages except phenol content of plantlet. The combination between Bartamuda cv. and shoot tip explant or plantlet stage was more effective on indole and phenol contents in most cases. These combinations markedly increased the previous chemical components. SDS-PAGE of water soluble protein fraction and isozymes of peroxidase and polyphenol oxidase were used to identify and characterize the effect of genotypes and their developmental stages via embryogenesis. SDS-PAGE of water protein fraction was used to determine the molecular weights of protein subunits. The electrophoregrams revealed about 17 protomer bands of different intensities, with molecular weight ranging from 14 to 102 kD. The results revealed that a number of polypeptides increased or decreased in intensity. At the same time, some polypeptides disappeared, or new one synthesized. Most of these modifications appear to be part of the metabolic changes in response to developmental stages. Isozymes analyses by electrophoresis detected the differences, at gene levels, corresponding to genotypes and developmental stages. The peroxidase patterns showed different peroxidase isozyme bands, with five different intensity bands. Meanwhile polyphenol oxidase profiles showed three isozyme bands with different intensities. The increment of isozymes (peroxidase and polyphenol oxidase) was observed in explant and plantlet stages. This may be correlated with the ratio of phenol and phenol-indole (auxin).

INTRODUCTION

Palms belong to the Palmaceae (Arecaceae) family (McCurrach, 1960) which contains several palm members, such as date palm (*Phoenix dactylifera* L.), coconut palm (*Cocos nucifera* L.) and oil palm (*Elaeis guineesis* Jacq), that are widely cultivated for their fruit crop products. In addition they are important as a source of fiber, fuel and furniture. Date palm is the most important fruit and ornamental crop in the Middle East and Arabian lands (FAO, 1984; Moursy and Saker, 1996). Date palm is a monocot and dioecious fruit tree that is vegetatively propagated through offshoots. However, there are many problems associated with this system (Popenoe, 1973).

The genetics, biochemistry, physiology and morphogenesis of tree crops are neglected compared to herbaceous species. Tree crops such as the date palm are difficult to study due to their long life. In vitro techniques appear to be promising tools to study palm growth and development, compared to field and greenhouse experimentation

(Tisserat, 1983).

Lee and Skoog (1965) and Grambow and Langenbeck-Schwich (1983) reported that the substitution pattern of phenols affected the rate of IAA degradation. Some monophenolics increased the rate, while some 3-substituted phenols depressed it. Phenols were found to react with hydrogen peroxide produced during IAA degradation, thereby protecting cellular constituents from its toxic effects. Lee (1980) found that in maize, some phenolic compounds altered the relative proportions of free and bound IAA.

Booij et al. (1993 b) showed that changes in soluble peroxidases correlated well with budding. The modification of peroxidase activities and expression of isoperoxidases always preceded the morphological appearance of buds. It can therefore be considered as an interesting marker of the budding process. The study of the evolution of peroxidases during a subculture can lead us to a better understanding of the physiological processes concerned. It can also allow us to establish optimal conditions for the culture of date palm strains, notably those that concern transfer of dates (in a subculture) and the ideal stage for the induction of rooting. Nevertheless, the peroxidase activity is inversely proportional to the endogenous auxin levels. It would be interesting to verify that the evolution of this endogenous auxin level agrees with the author's observations and make measurements of endogenous (active) auxin. As accumulation of cytokinin in plants is shown by an increase in ethylene synthesis, one should verify that an ethylene peak accompanies each peak of peroxidase activity.

Baaziz et al. (1994) compared the explant, calli and germinated embryos of two cultivars of date palm by denaturing Polyacrylamide gel electrophoresis (SDS-PAGE). They found that embryogenic calli of the two date palm cultivars could be identified by a concentrated polypeptide of molecular weight 27500 and polypeptides of molecular weights 70000 and 11500. Embryogenic calli showed high levels of soluble, ionically and covalently bound peroxidases. The soluble acidic isoperoxidases of R_f 0.6, revealed in these calli and germinated embryos could be a marker of the two tissues. Polyphenoloxidase activities detected in calli and embryos were very low when compared with these of explants.

Booij et al. (1995) found that nine out of 13 date palm cultivars were distinguished on the basis of enzyme polymorphism. Various zymograms were obtained for the five to ten enzymes studied, showing differences in isoenzymes number, band intensities and relative mobility value. These variations were due to genetic differences among cultivars.

Rival et al. (1997) measured peroxidase activity in in vitro rooting induction of oil palm. They demonstrated that, for clones with high rooting rates, changes in peroxidase activity during in vitro rooting followed patterns similar to those already described for many other species. Peroxidase activity was studied just before root induction, to test its value as an early marker for rooting ability.

The aim of this research was to study growth gradation of the explant plantlet (several morphological forms of callus, embryo development and seedling growth) via embryogenesis. For this purpose dry cultivars of date palm were propagated to investigate correlations between biochemical changes and different stages of propagation.

MATERIAL AND METHODS

The present study was performed throughout the period 2000 – 2003 at the central laboratory of development of Date Palm Research at Giza, Egypt.

Culture Technique

1. Plant Material. Healthy offshoots were separated from date palm trees of dry cultivars Bartamuda, Gondila and Shamia grown in Aswan governorate. Shoot tips were obtained from offshoots (2 – 4 years and 5 – 7 kg).

2. Explant Preparation and Sterilization. External leaves and fiber sheaths were carefully removed from offshoots by using saw cutting and a sharp knife until the store core tissue had been exposed. Shoot tips were composed of apical meristem, sub-apical tissue and numerous leaf primordia. Shoot tips were soaked in running tap water for 1 – 2

hours and were left in antioxidant solution containing ascorbic acid (100 mg/L) and citric acid (150 mg/L) until they were surface sterilized.

The shoot tips were surface sterilized in a laminar air flow hood. Sterilization of explants was achieved by soaking the shoot tips and axillary buds in 70 % ethanol for 1 min followed by immersion in 0.1 % mercuric chloride ($HgCl_2$) for 10 min and rinsing in sterilized distilled water. They were then soaked in two percentages of commercial Clorox (5.25 % sodium hypochlorite) solution containing 2 drops of Tween-20: the first was 40 % Clorox for 25 min and the second was 60 % Clorox for 25 min. They were then rinsed with sterilized distilled water three times. After sterilization treatments which were conducted under aseptic conditions, the surrounding leaves were removed, and the apical meristem was divided longitudinally into four equal segments and cultured on nutrient medium.

3. Nutrient Medium. The basic salts and vitamins of Murashige and Skoog (1962) (MS) were used in the medium for in vitro propagation of date palm. The acidity of the final medium was adjusted to pH 5.6 ± 0.1 prior to the addition of agar (6 g/L). Media was dispensed into culture jars (200 ml) containing 30 ml / jar and the jars were capped with polypropylene closures and autoclaved at 121 °C and 1.5 kg/cm^2 for 20 min.

4. Culture Conditions. Initial explant and callus cultures were incubated in the dark at 27 ± 2°C. Cultures were transferred to fresh medium every 4– 6 weeks.

Micropropagation

1. Callus Formation. The experiment was conducted to study the effect of date palm genotype (Bartamuda, Gondila and Shamia) on callus formation. The explants were cultured on basal nutrient MS medium supplemented with 170 mg/L NaH_2PO_4. H_2O, 100 mg/L myo-inositol, 0.04 mg/L adenine sulphate, 0.4 mg/L thiamine HCl, 200 mg/L glutamine, 100 mg/L 2,4-D, 3 mg/L 2iP, 30 g/L sucrose, 6 g/L agar and 3 g/L activated charcoal.

According to procedures of Zaid and Tisserat (1983), cultures were transferred to fresh media every 6 weeks, and incubated in total darkness for 24 weeks.

The following data were recorded: 1. Degree of callus formation, 2. Degree of browning.

Compact callus which formed from the previous culture was placed on medium containing 10 mg/L 2,4-D and 3 mg/L 2iP to produce friable callus. Data were calculated visually using the following scores (Pottino, 1981):

Negative results (-) =1
Below average results (+) =2
Average results (++) =3
Good results (+++) =4

2. Embryogenic Callus Formation. Friable callus was transferred to the following: MS + 0.1 mg/L NAA + 0.05 BA. For embryogenesis 3 replicates, each containing about 1x1 cm friable callus, were performed. Cultures were incubated in total darkness for 3 months. Data were calculated visually as scores (Pottino, 1981).

3. Germination and Growth of Somatic Embryos. Individual embryos were separated from the previous stage and cultured on the following media: MS + 0.1 mg/L NAA + 0.05 mg/L BA.

4. Rooting. Individual shoots were used as explants in this stage and they were cultured in ¼ MS supplemented with NAA at the rate of 2 mg/L. All culture media of each treatment were distributed into culture tubes, and were placed for 16 h illumination of 3000 lux light intensity (white fluorescent lamps) at 27 ± 2°C.

Chemical Analyses

Plant samples were harvested for chemical analyses at different stages (explant, callus, embryogenic callus, shoot, plantlets). Chemical analysis was conducted to determine the biochemical changes at different stages of explant growth and development.

1. Determination of Total Indoles. Total indoles were determined in the methanolic extract using p-dimethyl amino benzaldehyde (PDAB, 1 g was dissolved in 50 ml HCl conc. and 50 ml ethanol 95 %) test according to Selim et al. (1978). The intensity of the resultant color was spectrophotometrically measured at 530 nm. A standard curve was established which indicated the relationship between different concentrations of IAA and their corresponding absorbance values.

2. Determination of Total Phenols. Phenol determination was carried out according to Danial and George (1972). Optical density of these samples was measured by a colorimeter using wavelength 730 nm. Concentrations of total phenols in different samples were calculated as mg phenol/100g f.wt. Amount of total phenolic compounds was calculated according to the standard curve of pyrogalol (99.5 %).

3. Determination of Polypeptide Chains by SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The separation of polypeptide chains and the determination of their molecular weights were achieved by using SDS-polyacrylamide gel electrophoresis technique as described by Laemmli (1970) and as modified by Studier (1973).

4. Electrophoretic Separation of Isozymes.

Peroxidase: PER. Polyacrylamide gel electrophoresis of peroxidase isozyme was carried out according to Graham et al. (1964).

Polyphenol Oxidase. Polyacrylamide gel electrophoresis of polyphenol oxidase isozyme was carried out according to Sato and Hasegawa (1976).

Experiments were conducted in a complete randomized block design with three replicates,and analyzed using L.S.D test at 5% according to Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

Morphological Observations

Data presented in Table 1 showed the effect of genotypes on callus formation. The highest value of callus formation was observed with Bartamuda and Gondila cvs. compared to Shamia cv. Gadalla (2003) likewise reported that Gondila cv. produced the highest significant value of callus formation (81.64%) compared to other genotypes under investigation. This percentage was reduced significantly to 73.94% and 65.75 % with Shamia and Bartamoda cultivars.

In this respect, Murashige (1974) mentioned that explants consisting of shoot tips or isolated meristems, which contain mitotically active cells, were especially successful for callus initiation and subsequent plantlet regeneration.

Hervan et al. (1991) and Bakry (1994) found in date palm tissue culture, a superior feature of shoot tip explants was that they stimulated callus production of the best type (more granular texture). Furthermore, it was found that shoot tips showed pronounced positive responses. This may be because shoot tips consist of mitotically active cells, which are lower in secondary product contents compared to other types of explants.

Table (1) showed that the lowest degree of browning was achieved with Bartamuda and Gondila cvs., compared to Shamia.

Similar results were obtained by Gadalla (2003), who reported the highest significant value of browning (47.24%) occurred with Shamia cv., while the lowest significant value was obtained with Gondila and Bartamuda cvs. (37.33 and 31.04 %).

El Shafey et al. (1999) mentioned that shoot tips showed pronounced positive responses, i.e. less browning, more swelling and callus induction and consequently higher values of callus fresh weight. This may be because shoot tips, consisting of mitotically active cells, are significantly lower in total soluble phenol content.

Chemical Analyses

Tables 2 and 3 and Figures 1 to 3 illustrated data of total indole, phenol, SDS-PAGE water soluble protein fraction, isozymes of peroxidase and polyphenol oxidase.

1. Total Indole Content. Data in Table 2 showed that shoot tip explants showed a significant increase in indole content compared to the other developmental stages of date

palm genotypes. Embryogenic callus produced the lowest value of indole content.

Bartamuda cv. gave the highest value of indole content compared to other cultivars (Gondila and Shamia cvs.).

Regarding the combination between developmental stages and date palm genotypes (Table 2), the highest significant value of indole content was recorded in shoot tip explants of Bartamuda cv. (14.4 mg / g f.wt.), while the lowest significant value of indole content was observed in the callus stage of Gondila and Shamia cvs. (0.33 and 0.35 mg / g f.wt. respectively).

2. Total Phenol Content. Table 3 revealed that the highest significant value of phenol content was observed in the plantlet stage, followed by shoot tip explants, compared to the other developmental stages.

Concerning genotypes, Bartamuda cv. induced a greater increase in phenol content compared to Gondila. In respect to the combination between developmental stages and date palm genotypes, the highest value of phenol content was found with Bartamuda cv. in the plantlet and shoot tip stages (1.51 and 1.28 mg / g f.wt. respectively), followed by Shamia cv. in the plantlet stage (1.27 mg / g f.wt.). On the other hand, the lowest value of phenol content was recorded for Bartamuda and Gondila cultivars in the embryogenic callus stage (0.09 and 0.29 mg / g f.wt.).

Concerning the effect of developmental stage on biochemical components, shoot tip explants showed a significant increase in biochemical-studied constituents. On the other hand, total phenol content was increased in the plantlet stage.

Concerning the effect of different genotypes on biochemical changes, the highest values of biochemical components were recorded for Bartamuda cv. Regarding the effect of interaction between developmental stages and different genotypes biochemical constituents, the obtained results indicated that the biochemical contents were increased especially for Bartamuda cv. combined with shoot tip or plantlet stages.

Similar results were obtained with *Sequoiadendron giganteum* by Monteuuis et al. (1987), who reported that phenolic compounds were found to decrease in concentration when shoots were moved to a root induction medium. The activity of peroxidases in the induction medium increased during 7 – 11 days and then decreased, roots appearing as the phenol levels decreased. Peroxidase activity was reversely correlated with phenol content.

The levels of indole and phenol could be a good parameter for growth in vitro. Bakry (1994) reported the changes of phenols in cultured explants, and he added that elimination of shoot tip explants from offshoots led to an increase in phenol content. Grambow and Langenbeck-Schwich (1983) reported that phenols were found to react with H_2O_2 produced during IAA degradation, thereby protecting cellular constituents from its toxic effects. Phenols were found in large amounts in plantlets to form the basic material that would be used later during secondary wall formation (Thorpe and Gaspar, 1978; Abdallah et al., 2001). Additionally, Bakry (1994) found that axillary buds contained higher levels of phenols than shoot tips.

3. SDS-PAGE Water Soluble Protein Fraction. To determine the molecular weights of water soluble protein subunits, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was used to throw the light on the different developmental stages of three date palm genotypes.

The SDS-PAG electrophoretic patterns of water soluble protein fractions of the three date palm genotypes are illustrated in Fig. 1. A visual inspection of electrophoregrams, showed about 17 protomer bands of different intensities, with molecular weights ranging from 14 to 102 kD.

We could easily distinguish between the three genotypes and the different developmental stages by the number of bands and their intensities in the electrophoretic patterns. The absence of two bands with MW about 50, 18 kD was used to distinguish between Shamia cultivar and the other two cultivars in the shoot tip stage. On the other hand, the increase of intensity with MW about 38, 18 kD in Gondila cultivar may represent quite an acceptable feature for characterizing this cultivar in the shoot tip stage. Also, the absence of bands with MW about 90, 18kD in Shamia cultivar in the callus

stage could be taken as a clear indicator for its differentiation from the other two cultivars. Meanwhile, the presence of bands with MW about 97, 80 kD were considered a characteristic property of Bartamuda cultivar in the callus and embryogenic callus stages. During the embryo stage we distinguished between three cultivars by the presence of a band with MW about 38 kD in Gondila cultivar, a band with MW about 18 kD in Bartamuda cultivar and conversely, the absence of bands with MW about 58, 18 kD in Shamia.

From these results it was concluded that there was a remarkable change in polypeptides during different developmental stages and in different genotypes. It appeared that a number of polypeptides increased or decreased in intensity; others disappeared, or new polypeptides expressed. Most of these modifications appear to be a part of the metabolic changes occurring in response to developmental stages.

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Tables

Table 1. Effect of date palm genotypes on callus formation and degree of browning.

Cultivar	Callus formation degree	Browning degree
Bartamuda	2.63	2.3
Gondila	2.38	2.25
Shamia	1.96	2.78
L.S.D at 5 %	N.S	0.5

Table 2. Effect of developmental stages and date palm genotypes on indole content (mg / g f.wt.).

Developmental stage (A)	Cultivar (B)			Mean
	Bartamuda	Gondila	Shamia	
Shoot tip	14.40	3.00	1.53	6.31a
Callus	6.95	0.33	0.35	2.55b
Embryogenic callus	0.99	1.03	1.02	1.01d
Embryo	3.20	1.13	1.41	1.91c
Plantlet	2.82	1.13	1.51	1.82c
Mean	5.67a	1.32b	1.16b	

L.S.D at 0.05 A = 0.49 B = 0.27 AB = 0.6

Table 3. Effect of developmental stages and date palm genotypes on phenol content (mg / g f.wt.).

Developmental stage (A)	Cultivar (B)			Mean
	Bartamuda	Gondila	Shamia	
Shoot tip	1.28	0.76	0.67	0.90b
Callus	0.93	0.43	0.35	0.57c
Embryogenic callus	0.09	0.29	0.67	0.35c
Embryo	0.71	0.46	0.63	0.60c
Plantlet	1.51	0.81	1.27	1.20a
Mean	0.90a	0.55b	0.72ab	

L.S.D at 0.05 A = 0.25 B = 0.19 AB = 0.75

Figures

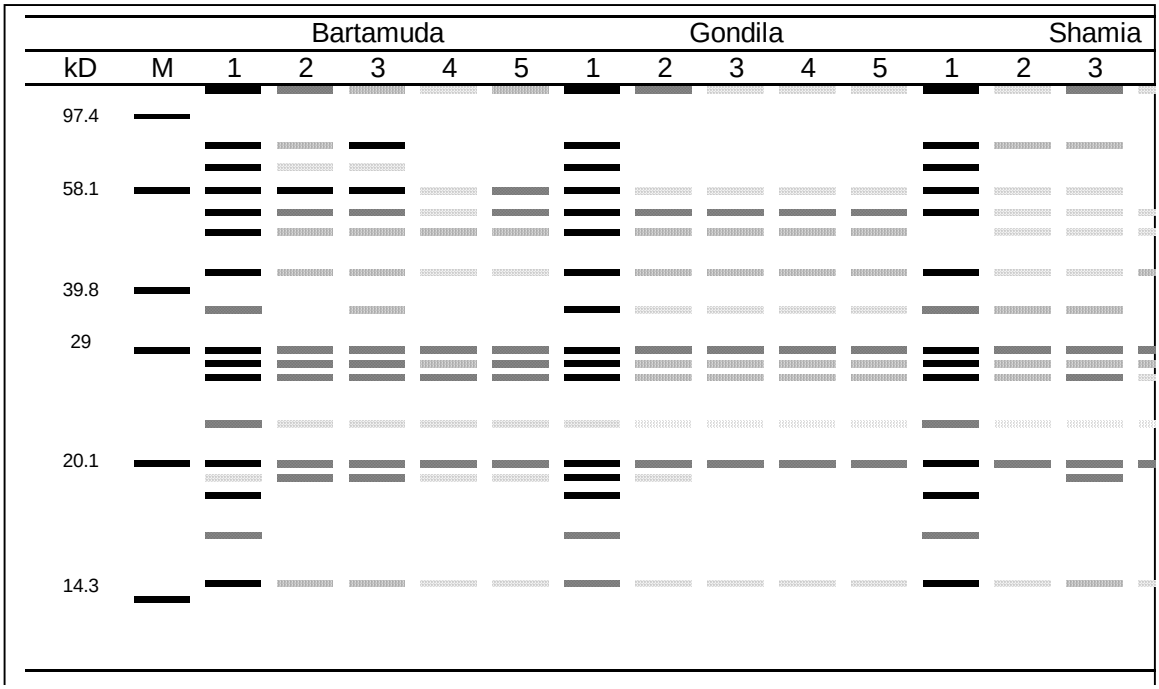

Fig. 1. SDS-PAGE patterns of water soluble protein fractions extracted from date palm cultivars samples were: 1 shoot tip, 2 callus, 3 embryogenic callus, 4 embryo and 5 plantlet.

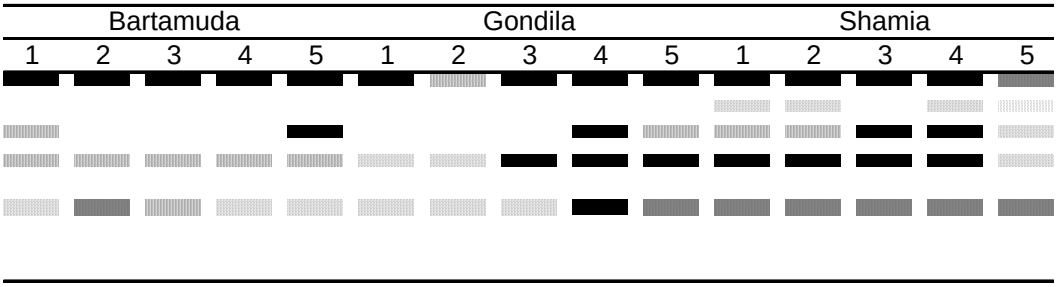

Fig. 2. Electrophoresis patterns of peroxidase extracted from date palm cultivars samples were: 1 shoot tip, 2 callus, 3 embryogenic callus, 4 embryo and 5 plantlet.

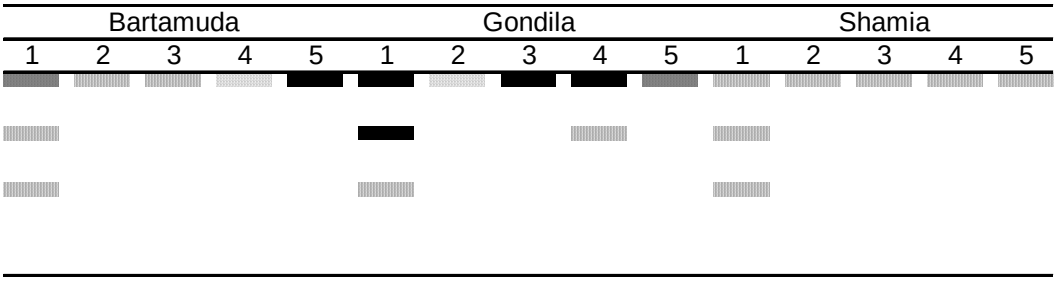

Fig. 3. Electrophoresis patterns of polyphenol oxidase extracted from three date palm genotypes samples were: 1 shoot tip, 2 callus, 3 embryogenic callus, 4 embryo and 5 plantlet.

Large Scale In Vitro Propagation of a Rare and Unique Male Date Palm *(Phoenix dactylifera* L.*)* Using Inflorescences Technique

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Keywords: organogenesis, tissue culture

Abstract

Since tissue culture was developed as a means of large scale propagation of the date palm *(Phoenix dactylifera* L.*)*, the offshoots growing at the base of the mother tree constituted the only source of explants used for the initiation stage. Because of the low success level at this starting stage, a large number of offshoots were often needed. But in some date varieties, this quality of plant material is not always available, and there are even cases where no offshoots are produced at all. No offshoots were available of an Al Ain city male, a unique date palm having many interesting and exceptional metaxenia effects Inflorescences were the only source of primary explants for the large scale propagation of this specimen. Following the steps for organogenesis (initiation, multiplication, elongation and rooting), of vitro plants were produced. By use of this technique, several thousand plantlets were produced, acclimatized and planted in the field, demonstrating that large scale in vitro propagation using an inflorescence technique is possible.

INTRODUCTION

Inflorescences of several species have been cultured in vitro (Nitsh, 1963). Since 1973, several workers have attempted to culture palm inflorescences. Explants of female and male oil palm inflorescences were cultured on a variety of media and usually developed somewhat normally, but callus was not obtained (Smith and Thomas, 1973). It was speculated that a high auxin level was necessary to disrupt normal development. This has subsequently been confirmed in date palm (Eeuwens and Blake, 1977).

Date palm ovules, carpel tissue, parthenogenetic endosperm, and the fruit stalk blackened within 24 hours after culturing on nutrient media, and subsequently died (Reuveni and Kipnis, 1974). Cultures of date palm floral bud reproductive tissues and especially male anthers, usually turned brown and died after a few weeks in culture (Tisserat et al., 1979). De Mason and Tisserat (1980) found that addition of auxins to media increased the frequency of visible expanded carpels that developed from supposedly male date palm flowers.

Vestigial female date carpels on surviving male flowers enlarged and became quite prominent (Tisserat, 1979). White friable callus usually initiated from the floral bud strand (Tisserat et al., 1979). In some cases, roots and embryoids were initiated from explants of Cocos inflorescence rachillae (Eewens, 1978) and from date palm (Tisserat, 1979). Roots have not been initiated on inflorescence rachis explants which lacked leaf or meristem tissue. The culture of date palm inflorescences was thoroughly investigated by Drira (1981) and Lotfi (1989). Morphogenetic responses were found to be dependent on the origin of the physiological stage of the explants.

However, no laboratory has so far succeeded in commercially propagating date palm specimens (males or females) from inflorescences (personal communication, A. Zaid, 2003). This paper reports a case where the inflorescences were the only existing source of the initial explants to mass propagate a rare and unique male date palm: Al ain city Male.

The main characteristics of the Al Ain City Male (short name: MP) include its high metaxenia effect. MP has many important characteristics compared to the other males (Table 1). To take advantage of this male, it was necessary to propagate it on a large scale. All attempts using traditional methods failed because MP did not produce any

offshoots. Consequently plant tissue culture using inflorescences as a source of the initial explants was the only available way to propagate this tree.

MATERIALS AND METHODS

Plant Materials

Two early emerging male spathes S_1 and S_2 (51 and 54 cm in length, respectively) were collected from the mother tree on February 27, 2001, using the following the steps Spathes as young as possible were selected. Sterilized tools were used. Once cut, the injured extremity of the spathe was dipped in heated paraffin wax for a few seconds then exposed to the air. This operation was repeated until a continuous insulator layer of wax was formed to avoid external contamination of the spikelets. After cleaning with 70% ethanol, the spathes were then wrapped in aluminum foil to start the sterilization process in the laboratory.

Sterilization Technique

To disinfect the inflorescences, two distinct steps were followed:

1. Disinfestation of Spathes. This first step of disinfestation took place outside the laminar flow hood and before spathes were opened. The latter were sprayed with 90% ethanol and burned for a few seconds to burn external hairs. Then, the spathes were soaked in a fungicide solution of Aliette (2g/l) for 20 min. using a sterilized "cooking pot" followed by a second dip in a solution of sodium hypochlorite (NaOcl at 5.25 %) for 20 min. (Figs. 1 and 2)

2. Disinfestation of Spikelets. During this second step, which took place in a completely aseptic condition (in a laminar flow hood), the spathes were rinsed three times with autoclaved double distilled water. They were then gently opened with a pre-sterilized knife, and the spikelets were removed and cut into halves that were split into two groups. The first group was soaked in a solution of sodium hypochlorite at the same concentration but for only 10 min. The other half did not undergo a second disinfection treatment. The inflorescences were then rinsed three times with autoclaved double distilled water and dipped in an antioxidant solution.

Nutrient Media

Macro and micro elements of Murushige and Skoog (1962) were combined with 150mg/l $NaH_2PO_4H_2O$, 2 mg/L thiamine-HCl, 200 mg/L L.-glutamine, 100 mg/l myo-inositol, 1.5 g/l polyvinyl pyrolidone (P.V.P.), 40 mg/L iodine sulfate, 3 g/L activated charcoal, 40 g/L sucrose and 7 g/L agar, in addition to different plant growth regulator combinations. The ph was adjusted to 5.7 prior to the addition of agar. The media were then poured into 15 mm x 150 mm pyrex test tubes, closed with Belco covers and aluminum foil and autoclaved at 120°C for 20 min.

Culture and Incubation

The spikelets were cut into small segments containing 2 to 3 flowers and cultivated in eight (8) different media. A total of 1194 explants were cultivated as follows:

Nature of Technique / Spathe Ref	Organogenesis				Embryogenesis				Total
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	
S_1	79	80	79	77	79	75	77	78	**624**
S_2	71	71	71	72	71	72	71	71	**570**
Total									**1,194**

During the initiation phase cultures were kept in a dark, temperature-controlled

room at 27 ± 2°C, while stages two through four (multiplication, elongation and rooting) took place in a temperature-controlled growth chamber, which was maintained at 27 ± 2°C, with a 16/8h photoperiod provided by fluorescent lights.

Inflorescences Follow-up

During the incubation period, the observation of flower explants focused on the following points:

1. Contamination rate
2. Negative reactions, and
3. Positive reaction via both embryogenesis and organogenesis.

RESULTS AND DISCUSSION

Contamination Rate

Since bacterial contamination was one of the constraints encountered when offshoots were used as a source of initial explants, close attention was paid to this problem. Flower explants were assessed every two days during a two-weeks period for their percent contamination. The main findings after the final assessment for contamination are summarized below:

- There was a negligible contamination rate in disinfested as well as non-disinfested spikeletes,
- There was no significant difference in the contamination rates between the disinfested and non-disinfested spikelets, and
- The contaminated explants were of an exogenous nature in both treatments.

Diverse Reactions of the Flower Explants

The growth period during which flower explants were assessed could be divided into three phases;

1. First Phase: Negative Reactions. This phase was mainly characterized by a pronounced browning of the plant materials leading to a total necrosis of the explants. This phenomenon appeared from the third week of initiation (sometimes later), according to the nature of the nutrient media, the place from where the flower was taken along the spikelets (base, middle or summet), and the disinfestation technique applied. The browning was more pronounced among the explants that had undergone disinfestation of the spikelets and among the nutrient media used for organogenesis.

2b. Second Phase or Transitional Phase. This transitional phase came after, and sometimes overlapped, the first phase. It was characterized by:

- Flower hypertrophy in about 10% of the explants in both organogenesis and embyogenesis.
- Rooting of the flower, which affected two to three explants only, forming one large root per explant.
- Some cell division scattered on the surface of the flower without any further divisions, and
- Multiplication of the flower bud leading to the formation of a cluster of flowers (> 10 flowers) shown by one explant only (Fig. 9).

After the two previous phases, around 5% only of the total explants were retained.

3. Third Phase: Positive Reaction. Because the objective was different for the two methods of regeneration, the results of each will be discussed separately.

Somatic Embryogenesis

An assessment of the culture situation before and at the end of the two previous phases is summarized in Table 1.

In order to exploit all possible organogenic and embyogenic potential, half of the cultures that formed callus were transferred to new media in an attempt to regenerate plants by organogenesis rather than embryogenesis, while the remainder were kept on the

same medium under the same conditions until embryogenic callus was obtained.

Embryogenic Callus

After the multiplication phase of the callus in the same medium, the cultures were transferred to new medium without activated charcoal and free of growth regulators from which approximately fifty (50) seedlings were regenerated and acclimatized (Table 2).

Organogenic Callus

The formation of indirect buds was attempted from organogenic callus (Fig. 11). Once produced, the buds were subject to a series of multiplication cycles followed by the same steps used to micropropagate date palm by organogenesis (elongation and rooting stages). Around fifty plantlets were produced and acclimatized through indirect organogenesis. Many more plants could have been produced via somatic embryogenesis or indirect organogenesis.

Direct Organogenesis

Table 3 presents an assessment of cultures at the end of the first two phases. After 7 months of culturing, the first direct bud was obtained. Once initiated, (Fig. 12) the bud was transferred to a caulogenesis medium for multiplication (Fig. 13). The multiplication process was repeated through many cycles (Table 3) until the required number of buds were obtained. These were then separated and individually sub-cultured on an elongation medium. After 4 to 5 weeks of incubation, the elongated shoots were transferred to a rooting medium before their transfer to greenhouses for the acclimatization process.

Based on a (x1.5) monthly multiplication rate, a tentative production plan aimed to produce 1,000 plantlets over 28 months (see Annex 1). Surprisingly, compared to the actual production, the target was reached in about half the planned period (15 months). Approximately 40,000 in vitro plants were produced within two years (see annex 2) because of the high multiplication rate (MR) recorded during the multiplication stage (Table 2). The regenerated in vitro plants were acclimatized and hardened-off using the same technique used for the plants derived from offshoots. Table 4 shows the number of male plants in the greenhouse that were derived from inflorescences.

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Tables

Table 1. Assessment of the culture situation.

At the initiation stage		After the two mentioned phase	
Number of media used	4	Number of media retained	1
Total number of the explants (flower) cultivated	594	Number of the explants positively reacted (callus formation)	22 (3.70%)
Number of the explants cultured in the efficient medium	150	Number of the explants positively reacted from the efficient medium (callus formation)	22 (14.66)

- Only one medium among the four tested has given satisfactory results.
- Regarding the number of explants, the majority has been discarded. 3.70% of the total number of the explants developed callus.

Table 2. Production of MP through somatic embryogenesis technique.

Year	Month	Number
2003	June	15
	September	39
Total		54

Table 3. Assessment of the culture situation.

At the initiation stage		After the first two phases	
Number of media used	4	Number of efficient media	1
Number of flower explants cultivated	600	Number of explants generated buds	1 (0.16%)
Number of the explants cultured in the efficient medium	150	Number of the explants positively reacted from the efficient medium (bud formation)	1 (0.66)

Table 4. Cycle follow-up / multiplication and budding rate of (MP).

Cycle Number	Multiplication Rate (MR)	Budding Rate (BR)	(MR + BR)
C_1	2.44	1.50	3.94
C_2	3.67	1.42	5.09
C_3	3.80	1.43	5.23
C_4	3.21	1.95	5.16
C_5	2.48	2.33	4.81
C_6	2.24	2.35	4.59
C_7	1.84	2.51	4.35
C_8	1.50	2.50	4.00
Average	2.64	1.99	4.64

Annexes

Annex 1. Tentative production planning of the male date palm (MP).

- Date of first introduction 27 February 2001.

07 months

- Date of first reaction and obtention of the mother tissue 03 October 2001.

04 months

- Date of the initial multiplication process (we have now 7 test tubes and 7 boxes) 01 February2002.

We are here now

11 months

- At a monthly multiplication rate of about (x 1.5), the laboratory will produce about 1,000 cultures on the 31 December 2002.

06 months

- Of which half cultures will be channeled for elongation to produced 1,000 plants on the 01 July 2003.

06 months

- The first hardening process in the tunnels will taken an advantage of 6 months 01 January 2004.

06 months

- Then the plants can go to the nurseries for further hardening (+- 6 months) 01 July 2004

Annex 2. Actual Production of the Male Date Palm (MP).

Year	Month	Subtotal	Total
	August	22	
	October	55	
2002	November	22	
	December	25	
			157
	January	270	
	February	69	
	March	347	
	April	321	
	May	561	
	June	1,224	
2003	July	2,696	
	August	2,352	
	September	4,848	
	October	2,207	
	November	6,191	
	December	6,273	
			27,359
	January	5,896	
2004	February	3,881	
	March	4,943	
			14,720
Total			42,236

Annex 3. Greenhouse situation of (MP).

Vitro plant stage	VP_1	VP_2	VP_3
Number of plants	*53,171	5.065	2,923

* (±25%) = ±2,400

Distribution.

Date	No.
March 7, 2003 – March 18, 2004	1,304
September 27, 2004 – December 28,2004	3,000
February 8,2005 – February 1,2006	11,000
General Total of Production / Distribution from March 7, 2003 - February 1,2006	15,304

Follow-up in the field.

Date of first plantation in the field	Number	Date of flowering	Number of flowered plant	Percentage of flowered plant	Duration from plantation to flowering (month)
11-12-03	70	15-1-05	54	77	13
		27-2-05	67	96	14

Main characteristics of MP.

Characteristics	Al Ain City Male (MP)	Other Males (Normal Males)
Pollen's viability (%)	≥ 97	≤ 75
Length of the spathe (cm)	≤ 120	≤ 80
Amount of pollen / spath (g)	72	≤ 30
Number of spathes/ tree	≤ 22	10-30

Figures

A. Different steps of the sterilization technique.

Fig. 1 and 2. Spath sterilization.

Fig. 3. Spath opening under aseptic conditions.

Fig. 4. Spikelets sterilization.

Fig. 5. Spikelets in an antioxidant solution.

Fig. 6. Cut of the spikelets into segments of 2 to 3 flowers each (explants).

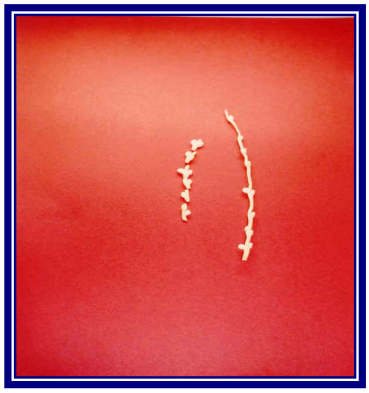

Fig. 7. Full spikelets taken from a surface disinfected spathe (right) and interflorl nodesready to br cultured (left).

Fig. 8. Explants culture in an initiation.

B. Different reactions obtained.

Fig. 9. Cluster of flowers.

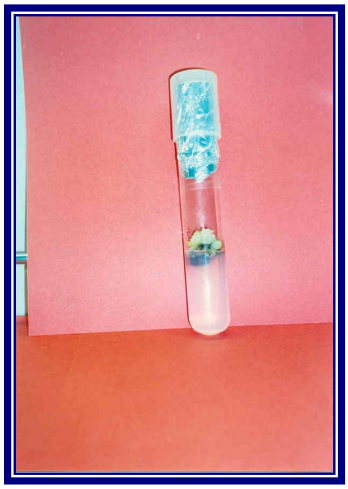

Fig. 10. Callus stage.

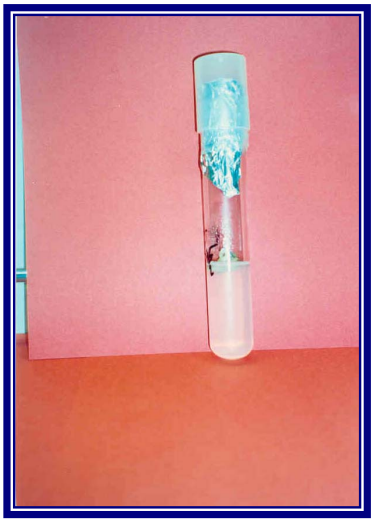

Fig. 11. Bud initiation from an organogenic callus: indirect caulogenesis.

Fig. 12. Direct caulogenesis.

C. Different stages of MP mass propagation through direct organogenesis technique

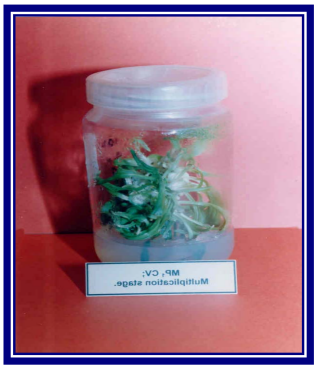

Fig. 13. Multiplication stage (cluster of buds)

Fig. 14. Elongation stage.

Fig. 15. Rooting stage.

Fig. 16. Acclimatization of the vitro plants: VP_1 stage.

Fig. 17. Hardening off of the vitro plant.

Fig. 18. VP_2 stage.

Fig. 19. MP embryo.

Would a Combination of Organogenesis and Embryogenesis Techniques in Date Palm Micropropagation be the Answer?

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Keywords: *Phoenix dactylifera*, off-types, in vitro
Abbreviations: 2,4-Dichlorophenoxyactetic acid (2,4-D), 1-Naphthaleneacetic acid (NAA), 6-(γ,γ Dimethylallyamino) purine (2-iP), Polyvinylpyrrolidone (PVP), Naphthoxyacetic acid (NOA), Sodium hydroxide (NaOH), Hydrochloric acid (HCl).

Abstract

Since first reports of off-types in date palm plants produced using micropropagation techniques, there has been much controversy regarding the process by which tissue cultured plants should be produced. Using high concentrations of plant growth regulators in some embryogenesis protocols have been criticized, while organogenesis protocols have been criticized as being too lengthy and therefore less cost effective. Using plant growth regulators in low concentrations during the initiation process may help to reduce possible variation. Research done on initiation techniques showed that activated charcoal, and plant growth regulator type and concentration played a role in stimulating plant somatic changes. Embryogenesis techniques for the second phase of multiplication are suggested.

INTRODUCTION

Date palms (*Phoenix dactylifera* L.) are grown in many countries in the world, especially in the Middle East and North Africa; they play an important role in the socio-economic development in these countries. Conventionally, date palms have been propagated through seeds and off-shoots until 1970, when reports were made of techniques used to propagate date palms by tissue culture (Schroeder, 1970; Tisserat, 1979). Various explants and methods were used for in vitro propagation.

The tissue culture of date palms provides many advantages over the traditional means of propagation. Where there was a shortage of off-shoots, mass production of date palms of a certain clone enabled the expansion of commercial plantations all over the world. Disease free plants can be widely distributed without fear of spreading diseases such as *Fusarium oxysporum* f.sp. *albedinis* that plague some parts of the world. Tissue culture is also an important tool in multiplying elite cultivars already in existence, or from F1 hybrids of previously select and seed only originated palms as well as from selected strains based on yield or disease resistance. Plants free of pests are able to cross country borders.

The micropropagation of date palms requires that plants are true-to-type. This means that the plants produced from tissue culture are to be identical to the mother plant from which the explant is taken. However, since 2000 there have been reports of off-types in date palms produced from tissue culture (Al-Wasel, 2000; Djerbi, 2000). Some of these off-types may be a result of the methods employed during the in vitro process.

Currently, there are 3 methods used for the micropropagation of date palms. These techniques include organogenesis (where axillary buds and meristematic tissue is multiplied), and direct (embryo multiplication) and indirect (callus and embryo multiplication) somatic embryogenesis. Date palm off-types have been reported from tissue cultured plants derived via both organogenesis (Oihabi and de Wet, 2005) and embryogenesis (McCubbin et al., 2000). High levels of plant growth regulators, light, damage to the meristematic areas and modifications in growth environment are possible causes of somaclonal variation in vitro (Kaeppler et al., 2000).

The objective of this study was to apply the three techniques available in the mass propagation of date palm and to compare these methods. Although much work has been published on each technique individually, very little has been published on comparative analysis of the three methods, detailing the merits and drawbacks of each. This study explored the possible combination of techniques to help reduce the risks associated with somaclonal variation, while at the same time ensuring that the methods remain commercially viable.

MATERIALS AND METHODS

Observations were made on tissue cultured plants over a period of 5 years and variations in vitro were recorded.

For comparative studies on the three methods used, a table of problem areas associated with the tissue culture of date palms was compiled.

For a study on initiation methods for the 3 techniques, 5 Medjool off-shoots were collected from Kakamas South Africa. The exposed cut surfaces were covered in copperoxychloride powder. At the laboratory, the off-shoots were dipped in fungicide (Benomyl). After removal of the leaves, the terminal buds found at the base of each axil, the shoot tip and its surrounding primordial leaves were excised and placed into chilled ascorbic (100 mg/L) and citric (150 mg/L) acid until they were surface disinfected. Inflorescence buds were discarded. The shoot tips and their surrounding leaf bases were cut to an approximate size of 70 mm X 50 mm and surface treated with 2.6 % sodium hypochlorite solution containing 3 drops of Tween 20 for 20 minutes. The explants were rinsed three times in sterile de-ionzed water and then cut further removing the primordial leaves containing auxillary buds surrounding the shoot tip. They were inocultated onto four types of sterile media prepared as shown below, dividing the explants up equally between the media types. Approximately 15 explants were obtained from each off-shoot.

Medium 1: Murashige and Skoog salts and vitamins (Murashige and Skoog, 1962), Adenine (40mg/L), PVP (2g/l), Glutamine (0.2g/l), Inositol (100 mg/L), Ascorbic acid (75 mg/L) and sucrose (30 g/l), NAA (1mg/L), NOA (3 mg/L), IAA (1mg/L) and 2-iP (0.1 mg/L)

Medium 2: Murashige and Skoog salts (Murashige and Skoog, 1965), Myoinositol (100 mg/L), thiamine (20 mg/L), activated charcoal (3g/l), sucrose (30 g/l), 2,4-D (0.1g/l), 2-iP (3 mg/L)

Medium 3: Same as medium 1 with added activated charcoal (3g/l)

Medium 4: Murashige and Skoog salts and vitamins (Murashige and Skoog, 1962), Thiamine (0.2g/l), Myo-inositol (100 mg/L), sucrose (30 g/L), NAA (0.5mg/L) and 2-iP (2.5 mg/L) and activated charcoal (3g/l).

The media was mixed and buffered at pH 5.7 with 1M NaOH and/or 1M HCl, solidified with 8g/L agar-agar and dispensed into test tubes. The test tubes were autoclaved at 121°C for 25 minutes and allowed to cool on a laminar flow bench. After inoculation of explants onto media, the test tubes were placed into a dark growth room at 25°C. Transfers were done every 4 weeks onto fresh medium. As tissue expansion occurred, explants were placed into larger glass jars with polycarbonate screw caps using 40 ml medium per jar. After 3 months, explants from medium 1 were transferred to a medium containing no activated charcoal (media 3). Observations were made on somatic changes and browning of initiations.

RESULTS AND DISCUSSION

In Vitro Variations

The variations observed on in vitro date palm plants were recorded as:

a) Dwarfs (thick plants with short expanded succulent leaves)
b) Disproportionate shoot: root ratio
c) Sheath restriction of leaf emergence (a leaf chokes the shoot tip). Upon dissection, fibrous tissue near the leaf base had expanded around the shoot tip leaving no room

for the next leaf to appear (Fig. 1)

d) "Blind" meristems where the shoot meristem appeared to be blocked or restricted (this phenomenon occurs in all the techniques used)
e) Flowering in vitro
f) Variegation of the leaves

Technique Problem Areas

A table was compiled comparing the three techniques used in date palm culture (Table 1).

Combination Methods

Expansion of tissues occurred on all explants, however, after the third transfer all explants from the medium containing no activated charcoal (medium 1) showed browning. The browning continued on this medium until all tissues were dead. The explants on medium containing activated charcoal however, remained creamy white in colour. The explants on the medium containing 2,4-D (medium 2) started to form callus at 8 months. The callus (Fig. 2) was transferred onto medium containing no 2,4-D and plantlets were formed.

Explants placed on media 3 and 4 started to brown after the fourth month. These explants did not dedifferentiate and were later discarded after 14 months.

Explants placed on organogenesis medium 3 with activated charcoal and then transferred to organogenesis medium (medium 1) differentiated within 6 months and plantlets were formed after 8 months.

This experiment showed that a medium containing low concentrations of plant growth regulators can be used to initiate plants within a short period provided that activated charcoal is used initially, then removed after 3 months in culture. Activated charcoal plays an important role in reducing browning at initiation (Zaid, 1984). Although the medium containing 2,4-D also gave good results, high concentrations of this plant growth regulator may trigger mutagenesis causing somaclonal variation. Callus, being somatic cell clumps, may also be more prone to somaclonal variation than differentiated organs or tissues such as embryos or buds. The organogenesis technique has been criticized for its long period before saleable plants are formed. The culture of embryos may be an option for speedier mass propagation (Fig. 3).

Although plants have been propagated using this method, it is still too early to establish whether these plants are true-to-type. The authors would not recommend the use of any methods until long-term data has been collected to verify trueness-to-type. The use of methods for phenotypic and molecular characterization may assist with earlier identification of off-types (Cohen et al., 2005); while careful selection of mother material, shorter culture period, restricted use of plant growth regulators, changes in culture environment, and limiting the number of plants produced per explant may help to reduce the possibility of off-types.

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Tables

Table 1. Problem areas relating to the tissue culture of *Phoenix dactylifera* L. comparing the three techniques used commercially.

TYPE	Indirect somatic embryogenesis	Direct somatic embryogenesis	Organogenesis
Initiation	*Destruction of off-shoot with the use of shoot tip meristem *Browning of explant leading to death of tissues (+/- 30%) *Length of time taken to produce nodular callus and somatic embryos approx. 6-12 months * Use of 2,4-D in high concentrations increasing risk of somaclonal variation * Contamination losses *Varietal response differences	*Destruction of off-shoot with the use of shoot tip meristem * Browning of explant leading to death of tissues * Length of time taken to produce somatic embryos approx 6-12 months * Use of auxins for dedifferentiation * Contamination losses * Varietal response differences	*Desctruction of off-shoot with the use of shoot tip meristem *Browning of explant leading to death of tissues * Length of time for shoot proliferation is long (up to a year). *Contamination losses *Yield of the technique per off-shoot is low
Multiplication	*Callus has limited life span and more sensitive to environmental changes * Risk of variation * Good multiplication rates (+/- 7) * Vitrification of callus *Unsychronized embryo development (small and large) * Loss of totipotency in some varieties	*Embryo proliferation is plant growth regulator and light sensitive * More stable than callus cultures and has an extended life span * Embryo selection is critical * Loss of totipotency in some varieties * Multiplication rates (+/- 4 embryos) is variety dependent	* Selection of plantlets is critical * Multiplication rates are low * Light sensitive * Loss of totipotency in some varieties * Precocious rooting sometimes occurs-decreasing regeneration capacity
Elongation	Not necessary	Not Necessary	Necessary step
Rooting	Low efficient rooting (2 stage rooting)	Rooting not problematic (2 stage rooting)	Not all plants are successfully rooted
Acclimatization	Hardening-off is generally 80% successful (location dependent)	Hardening-off is generally 60% successful (location dependent)	Hardening-off is generally high (90-98%) Location dependent

Figures

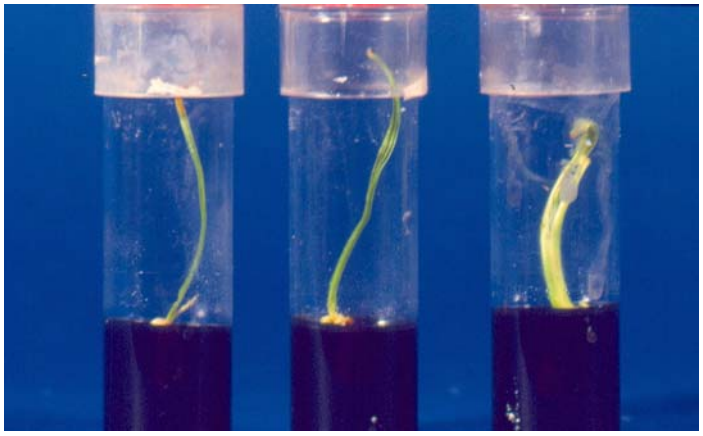

Fig. 1. “Blind”or blocked meristem growth variation of date palm in vitro with restricted leaf emergence.

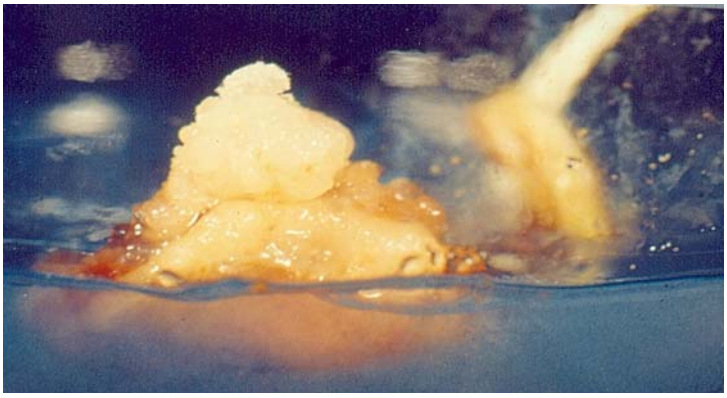

Fig. 2. Date palm callus from “Medjool” initiations using indirect somatic embryogenesis technique.

Fig. 3. Date palm somatic embryo cultures and plantlets using the direct somatic embryogenesis technique.

Volatile Compounds in Date Palm Fruit

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Keywords: volatiles, flavor, aroma, gas chromatography, mass spectrometry

Abstract

Volatile compounds from date palm (*Phoenix dactylifera* L.*)* fruits were extracted using Likens and Nickerson´s apparatus. Several extraction parameters such as weight of the pulp portion, dilution with water, solvent volume and extraction period were standardized to obtain highly characteristic date palm fruit aroma extracts. The extracts were concentrated and analyzed for the identification of volatile compounds using a system of high resolution gas chromatograph coupled with mass spectrometer. Better separation was achieved in a polar capillary column (HP-INNOWax 30 m x 0.25 mm x 0.25 μm). Compounds were positively identified when the mass spectrum and retention index data of the identified compound matched that of the authentic standard run under identical analytical conditions. A total of 78 components were separated, of which 35 compounds were positively identified and 8 tentatively identified. The other components could not be identified. Among the identified components in the fleshy pulp of date palm fruit were 14 esters representing an area of about 23%, 10 alcohols (average area 17%), 4 lactones (average area 8%), 8 aldehydes, (15%) and 3 ketones (average area 3.5%). The principal volatile compounds present were ethyl acetate, acetaldehyde, isopropyl acetate, δ-valerolactone, octanal, furfuryl alcohol, 5-methyl furfural, linalool, δ-valerolactone and γ –undecalactone.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) fruits represent a major production crop in the desert regions of Middle Eastern countries where their cultivation has been known to exist since ancient times. The fruits are practically ready to eat and do not need much processing. They are highly cherished for their organoleptic sweet taste and fleshy feel in the mouth. In several countries, these fruits are still considered a delicacy and are of high commercial value. The fruits are also considered of special ornamental value all over the world and generally form the baskets for religious occasions and other special festivities.

The chemical composition of several date varieties as influenced by stage of ripening was studied by Ismal et al. (1995). They determined proximate chemical composition, sugar, glucose, fructose and some mineral contents. They showed that glucose and fructose contents increase rapidly with maturation while total sugars may represent over 50% of the fresh weight at Tamar (fruits maturing on the tree) stage and these fruits contain potassium as a major mineral source. Quality characteristics and oxidative stability of date seed oil has also been studied in detail and oleic acid is known to be present as a major component of oil (Besbes et al., 2004). These compositional characteristics prove the importance of date fruits as a nutritional supplement, besides enjoying an esthetic sensory appeal.

Date palm fruits are very much cherished for their mouth feel and exotic aroma. Aroma development in fruits accompanies the maturation process which involves many changes such as metabolic reactions of synthesis and degradation of many substances, besides the complex energy transfer involved during various phases of fruit development. In general, aroma and taste are determining factors for selection of date palm fruits. The basic taste of date palm fruit is due to non-volatile components such as high sugar content. However, the characteristic flavor is attributed to a large number of volatile compounds

which are present in extremely small concentrations such as ppm or ppb.

There has not been any detailed research published on the identification of volatile compounds in date palm fruits and if some work has been done, it is quite limited. However, for Zahdi dates, 38 volatile compounds were reported to be identified, which consisted of 2 unsaturated hydrocarbons, 5 aldehydes, 6 ketones, 5 alcohols, 3 phenols and 11 free fatty acids (Jaddou, 1984). It was the purpose of this work to identify the compounds present in the fleshy portion of date palm fruit, which may give help identify the major volatile compounds responsible for the fruit aroma.

MATERIALS AND METHODS

Date palm fruits were bought from the Central Market in the city of São Paulo, Brazil. The variety was not stated on the packaging. The fruits were transported to the laboratory in the city of Aracaju, Brazil. Fruits free from any apparent skin damage were selected for analysis. The solvents and authentic standard flavor compounds used in volatiles identification were of pure grade (purity >97.7%) of Merck and Sigma/Aldrich companies, respectively.

Volatiles Isolation

The volatile compounds were extracted using Likens and Nickerson´s (1964) apparatus, which applies the simultaneous distillation and extraction technique. The fruits, after being washed with distilled water, were cooled to 2°C. The whole fruits were left in distilled water for 12 hours with the proportion of fruit varying with water (1:5; 1:10; 1:15) and then, after withdrawal of the seed, the fleshy fruit portion was macerated, using the same water, complemented with further addition of water. The extraction conditions were initially optimized by varying the parameters such as fleshy fruit weight (50, 100, 150g), volume of water used for dilution (100, 150, 200, 250, 300 ml), solvent (hexane or a mixture of pentane and ethyl ether) and extraction period (40, 50, 60, 70, 80 min), with the objective to separate a large number of compounds on chromatographic analyses. The volatile extracts were finally obtained at optimized conditions by usage of 100 g of fleshy pulp diluted with 150 ml of distilled water and extraction performed with 20 ml of pentane-ethyl ether (2:1) for 60 min. The extracts were concentrated to a final volume of 0.3 ml under the flow of nitrogen gas.

High Resolution Gas Chromatography/Mass Spectrometry

A combined system of Varian gas chromatograph (GC 3800) coupled with mass spectrometer (Saturn 2000R) and the workstation was used. Five micro liters of the concentrated volatile extract were injected in the column in a split (1:50) mode. Capillary GC investigations were carried out on a 30 m (length) x 0.25 mm (internal diameter) innophase bondable polyethylene glycol polar capillary column (HP-INNOWax; 0.25 μm film thickness; Hewlett Packard, Inc., Palo Alto, USA). The carrier gas used was helium and column head pressure was maintained at 11.5 psi having a flow rate of 1 ml/min. The oven temperature was programmed: initiation at 30°C for 5 min, increased at 7°C/min to 100°C, maintained at 100°C for 5 min, increased at 1°C/min to 130°C, increased at 10°C/min to 195°C wherein maintained for 45 min. The temperatures of the injection port and the GC/MS interface were 175°C and 195°C, respectively. The mass spectrometer was operated in the electron ionization mode with an electrical energy of 70 eV and an ion source temperature of 250°C. The mass spectrum was scanned between 33 and 450 atomic mass units at 0.3 sec interval.

Compounds Identification

The linear retention index (RI) values for unknowns were determined based on retention time data obtained by analyzing a series of normal alkanes (C_8-C_{21}). Volatile components were positively identified by matching their RI values and mass spectra with those of standards, also run under identical chromatographic conditions in the laboratory.

RESULTS AND DISCUSSION

Table 1 lists the volatile compounds identified in the date palm fruits. The two chromatograms were selected on variations in the volatile profile. However, the variety of date palm fruits was the same in both chromatographic analyses presented in the table. The data lists the retention indices and peak area percent values for various compounds identified. It is observed that some compounds have the superscript letter[b], which signifies that the compound was tentatively identified since there was no pure standard compound which could be run under the identical analytical conditions. Thus the identification was considered tentative when it was based mainly on matching an unknown mass spectrum with a spectrum available on the NIST (National Institute of Standards and Technology, USA) mass spectral data system or the literature (Jennings and Shibamoto, 1980; Kondjoyan and Berdagué, 1996). The table also presents the characteristic odor of the compounds as described in Aldrich flavor catalogue and by Arctander (1969).

In a typical chromatogram analyzed for the volatile extracts obtained from fleshy pulp of date palm fruit, a total number of 78 components were separated, out of which 35 compounds were positively identified and 8 tentatively identified. The other components could not be identified. Among the identified components in the the fleshy pulp of date palm fruit were 14 esters representing an area of about 23%, 10 alcohols (average area 17%), 4 lactones (average area 8%), 8 aldehydes, (15%) and 3 ketones (average area 3.5%).

The main volatile compounds positively identified in the date palm fruit were ethyl acetate (9%), acetaldehyde (6.5%), isopropyl acetate (6%), δ-valerolactone (6%), octanal (3.7%), furfuryl alcohol (3.7%), 5-methyl furfural (2.4%), while linalool (3.9%) and γ -undecalactone (1.5%) were tentatively identified. The aroma nutty notes are known to be due to the presence of compounds like acetaldehyde, tolualdehyde, δ-valerolactone, δ-decalactone and 2-hexenal. All of these compounds were identified in this work. Although 10 alcohols were identified, their total concentration was low (15.19%) compared to that of the esters (22.5%) present in date palm fruits. A little higher percentage (8.5%) of lactone compounds may be characteristic to the flavor of date palm fruits.

The esters represent a higher number as well as percent area representation of compounds present in date palm fruits. It may hence be concluded that the generation of these compounds is characteristic to date palm aroma. Ethyl acetate is one of the most prominent compounds used in the preparation of flavor chemicals. It constitutes a major part of imitation flavors of many berry-type fruits, banana, grape, pineapple, peach, lemon, tutti-frutti, almond, butterscotch, whisky, butter, mint, pear, melon etc. (Arctander, 1969). The pronounced increase in the concentration of ethyl acetate and isopropyl acetate in date palm fruit shows that these compounds could contribute significantly for the fruit aroma.

Among other important compounds which could be generated due to the high sugar content may be furfural and 5-hydroxymethyl furfural (HMF) which may be formed due to the caramelization reactions initiated at higher temperatures of volatile extraction performed in this study. Since most of these compounds, although belonging to different organic classes such as esters, alcohols, terpenes and aldehydes are known to possess characteristic sweet, floral, nutty, creamy and fruity odors representing different fruits, it could be concluded that to a large extent these prominent compounds contribute to the characteristic aroma and flavor of data palm fruits.

Although in low concentrations, the presence of three lactones - α-angelica lactone (5-methyl-2-furanone), γ-valerolactone, and γ-undecalactone was observed in data palm fruits. The highest percentage being that of δ-valerolactone (6%) this is characteristic of the nutty flavor. These lactones mostly represent peach-like aroma and were also present in mango fruit cultivar Tommy Atkins grown in Vale de São Francisco, in the northeast region of Brazil (Narain & Galvão, 2004).

CONCLUSIONS

This work identifies the presence of the main volatile compounds identified in date palm fruit as being ethyl acetate (9%), acetaldehyde (6.5%), isopropyl acetate (6%), δ-valerolactone (6%), octanal (3.7%), furfuryl alcohol (3.7%), 5-methyl furfural (2.4%), linalool (3.9%) and γ -undecalactone.

ACKNOWLEDGEMENTS

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Tables

Table 1. Volatile compounds in date palm fruit analyzed from two major chromatograms.

Nº	Compounds	RI	Area (%)* Date Palm Sample 1	Area (%)* Date Palm Sample 2	Characteristic Odor
Aldehydes					
1	Acetaldehyde	690	5.80	7.21	Sharp, Nutty
2	Propionaldehyde[a]	780	0.98	0.58	Sharp, pungent
3	(*Z*)-2-hexenal[a]	1218	0.41	0.14	Citrus, melon
4	Octanal[a]	1286	3.55	4.03	Floral, citrus
5	Furfural[a]	1455	1.61	1.22	Sweet, woody
6	(*E*)-2-nonenal[a]	1518	0.22	0.07	Penetrating, fatty
7	5-methyl furfural[a]	1586	2.32	2.49	Caramel like
8	m- tolualdehyde[a]	1630	0.43	0.03	Sweet, mild, almond
Alcohols					
1	Ethyl alcohol[a]	930	2.71	3.67	Sweet, ethereal
2	3-hexanol[a]	1185	1.11	1.44	Alcoholic, ethereal
3	Furfuryl alcohol[b]	1198	3.44	4.06	Low odor, Cooked sugar taste
4	3-methyl-2-pentanol[a]	1202	0.58	0.22	Sweety, floral
5	3-methyl-2-buten-1-ol[b]	1245	2.23	2.83	Fresh, green
6	3-octanol[a]	1383	0.03	0.07	Oily, citrus
7	α- terpineol[a]	1687	1.44	0.78	Fragrant
8	Geraniol[a]	1797	0.46	0.71	Sweety, floral
9	Benzyl alcohol[a]	1821	0.92	1.43	Sharp burning taste, faint aromatic
10	Eugenol[a]	2155	0.12	0.22	Strong, spicy
Esters					
1	Ethyl acetate[a]	819	8.25	9.38	Pineapple, ethereal
2	Isopropyl acetate[a]	882	6.82	5.23	Ethereal, sweet,banana
3	Diethyl sulfide[a]	903	1.17	1.39	Coffee, meaty
4	Propyl acetate[a]	966	0.73	0.90	Powerful, celery odor
5	Ethyl butyrate[a]	1029	2.13	2.44	Fruity, sweet
6	Butyl acetate[a]	1065	0.37	0.26	Fruity,diffusive
7	Dimethyl disulfide[a]	1091	0.59	0.89	Vegetable, cabbage
8	Cyclohexyl formate[b]	1304	0.42	0.16	Fruity, jam-like
9	Hexyl acetate[a]	1308	0.44	0.31	Apple, floral
10	2-hexenyl acetate[b]	1514	0.37	0.42	Fruity, banana
11	(*E*)-5-decenyl acetate[b]	1721	0.73	0.55	Rose petal like
12	Isobutyl cinnamate[b]	2230	0.36	0.25	Fruity, peach
13	Methyl octadecanoate[a]	2429	0.39	0.48	Green, fruity
14	Ethyl octadecanoate[a]	2464	0.31	0.02	Fatty

Nº	Compounds	RI	Area (%)		Characteristic Odor
			85(dias)	112(dias)	
Ketones					
1	1-penten-3-one[a]	1014	0.22	0.21	Pungent, mustard
2	3-hexanone[a]	1049	1.09	1.04	Ethereal, grape
3	Acetophenone[a]	1611	2.17	2.32	Sweet, floral
Terpenes					
1	δ-limonene[a]	1181	3.13	2.73	Citrus, lemon
2	(*E*)-linalool oxide[a]	1424	0.94	1.27	Sweet, woody, penetrating
3	Linalool[b]	1537	4.39	3.38	Creamy floral
4	α- phellandrene[a]	1745	0.18	0.27	Minty, herbaceus
Lactones					
1	5-methyl-2-furanone [a]	1430	0.52	0.94	Oily, nutty
2	γ-valerolactone[a]	1616	0.23	0.40	Sweet, herbaceous
3	δ-valerolactone[a]	1821	5.41	6.62	Sweet
4	γ -undecalactone[b]	2214	1.39	1.64	Fruity

[a] identified positively based on retention index datum and spectrum verification from the NIST mass library or literature (Adams, 1995; Jennings and Shibamoto, 1980) along with the standards run under identical analytical conditions.

[b] identified tentatively based on retention index datum and spectrum verification from the NIST mass library or literature (Adams, 1995; Jennings and Shibamoto, 1980).

Plant-off-Types in Tissue Culture-derived Date Palm (*Phoenix dactylifera* L) Plants

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Keywords: organogenesis, embryogenesis, somaclonal variation, AFLP, true-to-typeness

Abstract

Trueness-to-type and occurrence of abnormalities are the most serious problems associated with date palm tissue culture. The aim of the present investigation was to study the morphological abnormalities in tissue culture-derived date palms. A survey of embryogenesis-derived trees in the field identified various abnormalities including abnormal leaves and inflorescences, dwarfing, leaf bleaching, deformed offshoots, delayed flowering time, pollination failure and abnormal fruiting. Some varietal specificity was noted. AFLP was used as a fingerprinting tool to verify somaclonal variation in plants derived from tissue culture. Variability amongst 40 date plants produced by organogenesis and embryogenesis, in terms of numbers of plants which showed aberrant patterns, was found to be 5 % and 12.8 %, respectively. However, based on the total numbers of variant DNA fragments, plants derived from embryogenesis showed a much higher level of variability (0.6 %) than those regenerated via organogenesis (0.038 %). The identification and early detection of off-type plants will help avoid the occurrence and propagation of these abnormalities in the future.

INTRODUCTION

More than 500 articles have described the existence of genetic variability in plant tissue cultures (Orton, 1983). The terminology used for plant off-types is diverse. The name “phenovariant” was first coined by Sibi (1971). Sibi (1989) also proposed the terms “vitro variants” and “vitro variations” as new general and practical terms for this type of variation. The term “somaclonal variation”, which is commonly used, was proposed by Larkin and Scowcroft (1981).

Somaclonal variation can be an epigenetic or genetic change, sometimes expressed as a new trait, resulting from in vitro culture of higher plants (Pierik, 1987; Zaid et al., 1999). Differentiation between the two types of variation can be determined by a study of their offspring. In comparison to epigenetic variation, genetic variation follows Mendelian segregation ratios.

MATERIAL AND METHODS

A field survey covering most UAE date palm orchards recently planted with tissue culture-derived plants, was conducted during two growing seasons, 2001/02, 2002/03. These plants were obtained from several different commercial laboratories worldwide which used embryogenesis to generate date palm plantlets. The survey targeted all abnormalities and off-types that appeared in these date palm plantations. They included plants with abnormal morphology and structure such as twisted inflorescences and broader leaves, excessive vegetative growth, leaf variegation, dwarfing, leaf bleaching, bastard offshoots, delayed flowering time, pollination failure and abnormal fruiting (Tables 1, 2).

AFLP technique was used as a fingerprinting tool to verify somaclonal variation in plants derived from tissue culture. The research was carried out on plants derived from

both tissue culture regeneration systems (organogenesis and embryogenesis). Eighty individual Khlass plantlets derived from both systems (40 from organogenesis and 40 from somatic embryogenesis) were compared. The DNA from these 80 individual samples was extracted, digested and adaptored using the primer combination of *Eco*RI+GGA / *Mse*I+AAG.

RESULTS AND DISCUSSION

Date Palm Morphology and Structure

Morphological abnormalities were detected at the hardening phase in the nursery and included the following: absence of an onion like base; thin stems with weak, juvenile leaves; abnormal phyllotaxy; very poor growth of the root system with no more than three to four thin roots per plant (about 2 mm in diameter).

The occurrence of abnormalities clearly depended on plant producers and their respective production / selection processes (Table 2). For example, abnormalities at a rate of up to 63 % in a date palm nursery containing 2,000 hardened in vitro Medjool plants was observed in UAE (Fig. 1a). By contrast, a recently established date palm orchard in Namibia contained only 5 % Medjool plants with abnormal characteristics (Zaid and Arias, 1999). A case of abnormal phyllotaxy was observed with Barhee (Fig.1b).

Excessive Vegetative Growth

An abnormality frequently found in the date orchards that were surveyed was an excessive degree of vegetative growth. These plants were found to have broader leaves, compact growth and a different spine structure (Figs. 1c, 1d). Out of 2,000 Barhee palms derived from asexual somatic embryogenesis, only two plants (0.1 %) were observed with this abnormal vegetative growth (Table 2). By contrast, McCubbin et al. (2000b) found a much higher ratio of 1.4 % in a survey carried out in South Africa on Medjool plants that had been produced by three separate tissue culture laboratories using somatic embryogenesis.

Leaf Variegation

Some plants had a creamy-coloured stripe on their leaves that ran parallel to the leaf margin (Fig. 1e, 1f). This abnormality occurred on all leaves but there was some variation in stripe width. Although the plants seemed healthy, they clearly suffered from reduced growth rate. Twelve out of 50,000 greenhouse plants (0.024 %) of several date palm varieties showed variegation (Table 2).

Variegation as an off-type is common among tissue cultured date palm plants and has also been reported in other plant species such as banana (Al Wasel, 2001). Variegation might depend on factors such as virus/microbial contamination, in vitro media nutrient deficiency or genetic variation (McCubbin et al., 2000a). Leaf variegation can occur because of mutations in the photosynthetic apparatus. Furthermore, since many genes are involved in chlorophyll synthesis, many independent mutations can cause the variegated phenotype.

Dwarfism

The dwarfing phenomenon in plants derived from tissue culure manifests as a severe restriction in growth. Dwarf date palms were less than one metre in height after four to five years in the field, compared to an average height of 3 m for a normal date plant of the same age (Fig. 2a). However, most dwarf date palms are identified only in the first or second year after field planting, when stem elongation occurs (Fig. 2b). The older leaves were sometimes normal and dwarfism started by affecting the younger leaves (Fig. 2c). In some cases, dwarfism only affected the outer (older) leaves (Fig. 2d). Dwarfism reduced the leaf length (up to 2/3 shorter than a normal leaf) and also affected the leaflets, which were severely reduced in size (Fig. 2e). This reduction in leaf structure and canopy size affected leaf function resulting in low photosynthesis and consequently in greatly

reduced growth. Dwarfism also severely weakened date palms and reduced offshoot production.

The survey was carried out in the western region of UAE, where date palm varieties, such as Sukkari, Barhee, Sultana, Khlass and Oum Dahn, were found showing dwarfism, with Sukkari being the most affected. In one orchard, 20 dwarf trees were identified among 50 Sukkari plants (40 %), while at another orchard 15 Sukkari plants out of 42 (35 %) were affected. By contrast, Barhee and Sultana plants were less affected and only 17 out of 300 Barhee plants (5.6 %) and 2 out of 200 Sultana plants showed dwarfism (1 %) (Table 1). Khlass was even less affected with only 20 dwarf plants out of 5,000 plants (0.4 %) detected in the survey.

Dwarfism has also been described in the varieties Ajoua (Al Wasel, 2000 a, b), Sultana and Nabt Saif (Abo El Nil, personal communication) with the frequency of dwarfism varying from 0 to 30 %, depending on variety (Al Wasel, 2001). Dwarfism has also been commonly observed with date palm plants derived from embryogenesis (Cohen et al., 2003).

The causes of dwarfism in date palms are not known. A dwarf phenotype is also associated with black scorch disease (Fig. 2g). Black scorch, also called Medjnoon or Fool's disease is caused by the pathogen *Ceratocystis paradoxa* (Amira et al., 2000). Black scorch has been observed on a total of 19 date palm varieties, including Thoory, Hayani, Amhat, Saidy, Halawy, Medjool and Barhee (Djerbi, 1983; Zaid and Al Kaabi, 2001). While genetically dwarf plants will not recover after planting into the soil, and remain dwarf even after 3 consecutive years of chemical treatment, black scorch-affected date palm trees can recover from the disease after repeated chemical treatment. Date palm plants derived from tissue culture are apparently more susceptible to this disease than offshoots and immediately after the attack development of their meristems is restricted. In this study, several genetic dwarf trees were examined and showed normal root systems (Fig. 2f).

Leaf Bleaching / Albinism

The bleaching of leaves of certain date palm varieties, such as Khlass, Sultana, Barhee and Nabt Saif was observed (Fig. 3a) and was due to partial or total loss of chlorophyll. Usually, 2 to 4 leaves were affected per tree. However, this abnormality was found to be rare in orchards (affecting 53 out of 864 plants surveyed) and is therefore of no great economic significance. Normally, the affected tree grows out of this phenotype and the photosynthesis process restarts in the affected leaves which slowly turn back to green (Fig. 3d, e, f). This phenomenon has not been previously described.

Deformed Offshoots

It is well known that date palm plants derived from tissue culture have a better growth habit and produce more uniform date palm orchards than those derived from offshoots (Al Wasel, 2000b). They also produce more primary and secondary offshoots. However, this fast growing habit and the abundance of offshoots is sometimes accompanied by the appearance of abnormal offshoots and twisted inflorescences. Deformed vegetative buds and their conversion to floral buds (Fig. 3b, c) are commonly observed. The frequency of these abnormal offshoots was approximately 1 in 20 trees, but was only observed in trees in their first year of flowering. However, frequency estimates are difficult as abnormal offshoots are removed by the grower and in subsequent years are replaced by normal offshoots. This deformed condition can be caused by an infestation with the date palm bud mite (*Makiella phoenicis* K.) (Cohen et al., 2003), or may be due to reduction in growth caused by an inequilibrium of endogenous growth regulators accumulated during in vitro propagation (Cohen et al., 2003). Better knowledge is required of the cytokinin and auxin levels within the plant and the level of plant growth regulators that are needed to induce a response during in vitro propagation.

Delayed Flowering Time

Although tissue culture plants are known for their faster growth compared to offshoots (Al Wasel, 2000b), there is increasing concern about possible delay in their first fruit production. In one instance, an orchard of 10 hectares of Barhee date palm trees derived from somatic embryogenesis (about 2,420 plants) took more than 7 years for 50 % of the trees to reach the floral stage. Delayed flowering time may be caused by the prolific vegetative growth as a result of juvenile vigour in tissue culture-derived plants (Cohen et al., 2003).

Pollination Failure and Abnormal Fruiting

An observation made during the course of this survey was of pollination failure and very low fruit set for tissue cultured plants of Barhee and Medjool derived from asexual embryogenesis (Table 2). All pollinated bunches showed 80 to 100 % parthenocarpic fruits during the first year of production (Figs. 4a, b, c) and sometimes the development of more than 3 carpels (Figs. 4d, e). This phenomenon has also been found in somatic embryogenesis-derived orchards of Barhee date palm worldwide, including Namibia, South Africa, Kingdom of Saudi Arabia, and the United Arab Emirates (Djerbi, 2000; McCubbin et al., 2000b). Other varieties such as Khlass, Sukkari, Ajoua and Deglet Nour have also been reported to be affected, but to a lesser degree (Djerbi, 2000; Al Wasel, 2001).

Pollination failure of this type appears to be associated with plants produced via somatic embryogenesis (Djerbi, 2000; McCubbin et al., 2000b). The normal parthenocarpic fruits are not suitable for consumption, causing economic loss (Al Wasel, 2000a; Cohen et al., 2003). This phenotype appeared similar to the “Mantled” phenotype of tissue culture-derived oil palms that were associated with epigenetic variations (Corley et al., 1986; Matthes et al., 2001; Jaligot et al., 2002).

More research needs to be carried out to identify the causes of pollination failure in date palm plants derived from tissue culture. It is possible that these palms require heavier pollination than plants derived from offshoots. However, there is evidence that alleviation of abnormalities such as the pollination failure and the subsequent low level of fruit setting may occur after several years (Cohen et al., 2003).

Although genetic abnormalities such as changes in chromosome number have been found in date palm plants derived from somatic embryogenesis, this contrasts with oil palm where plants derived from somatic embryogenesis show very few abnormalities (D’Amato, 1978; Corley et al., 1979; Brackpool et al., 1986)

The key difference between date palm and oil palm somatic embryogenesis may be that meristematic cells from oil palm do not require any exogenous cytokinin which is known to induce genetic variation (Corley et al., 1981).

The morphological differences which were observed included albinism and floral sex differences (Corley et al., 1986; McCubbin et al., 2000b; Sharma et al., 1980; 1984). In addition to date palm and oil palm, somaclonal variation has been repored in about 150 different plant species (Pierik, 1987).

Comparative AFLP Analysis of Variability in Date Palm Organogenesis and Embryogenesis

A major aim of this research was to assess the potential variability induced by two different regeneration systems for tissue culture of date palm, i.e. organogenesis and embryogenesis.

Fragment patterns from 80 individual Khlass plantlets derived from both in vitro regeneration systems (40 from organogenesis and 40 from somatic embryogenesis) were compared. DNA from these 80 individual samples was extracted, digested and adaptored using the primer combination of *Eco*RI+GGA / *Mse*I+AAG.

Thirty eight of the 40 organogenesis samples gave identical fragment patterns. In two of the samples (27 and 34), one band (band 86) was more intense than the others (Fig. 5). It is surprising that the same band should differ in these two samples. The intensity of

this band was much greater in sample 34 than in 27. This could result from carry-over between adjacent tubes during the AFLP procedure. If the additional/more intense band in both is not an artefact, then the overall level of variation, on a per plant basis, was 5 %. If the additional band in sample 27 is considered an artefact, this figure was 2.5 %. On a per band basis, the level of variation was 0.019 % or 0.038 %.

Results from the 40 plants from asexual embryogenesis are given in Figure 6. Five samples out of the 39 (there was one drop-out lane) had different band patterns. Samples 4, 5, 35, 38, and 39 varied in fragment pattern from the standard by 15, 1, 1, 13 and 1 fragments, respectively (Tables 3, 4 and 5). In most cases the differences were due to a novel fragment. In terms of numbers of plants, the level of variation was 12.8 %. In terms of fragments, the embryogenesis samples showed a level of variability of 0.6 %.

Confirmation of Variability

Having shown that the AFLP technique was able to identify variation in plants derived from tissue culture, it was necessary to confirm that there were no artefacts. The variant organogenesis sample (Ko34), and the variant embryogenesis samples (Ke4 and Ke38) were re-extracted and used in a further AFLP analysis (Fig. 7). Samples Ko37 and Ke2 were also re-extracted and used as controls. The AFLP patterns which resulted were identical to those obtained in the previous experiment and it is clear that the observed variation was of genetic origin and was not an artefact.

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Tables

Table 1. Dwarfism in date palms derived from asexual embryogenesis..

Variety	Plants scored	Number of dwarf plants	% Plants affected	Author
Sukkari	50 42	20 15	40 35	Present study
Khlass	5,000	20	0.4	Present study
	218	53	24.3	Al-Wasel (2001)
Barhee	300	17	5.6	Present study
	234	42	17.7	Al-Wasel (2001)
Sultana	200	2	1	Present study
Various varieties	N.S	N.S	4	McCubbin et al. (2000b)

N.S: Not specified.

Table 2. Morphological abnormalities identified in date palm.

Abnormality	Variety	Plants scored	Plants affected	%	Author
Morphology and structure	Medjool	2,000	1,260	63	Present study
		1000	50	5	Zaid and Arias, 1999.
Excessive vegetative growth	Barhee	2000	2	0.1	Present study
	N.S	N.S	N.S	1.4	McCubbin et al., 2000b
Leaf variegation	Several varieties together	50,000	12	0.024	Present study
	Khlass	218	2	0.92	Al Wasel, 2001.
Pollination failure	Barhee & Khlass	100,000	100,000	100	Djerbi, 2000.
	Barhee	N.S	N.S	Up to 100	McCubbin et al., 2000b
	Khlass & Sukkari	1000	786	78.6	Al Wasel, 2001.
	Ajoua	500	430	86	

N.S: Not specified.

Table 3. AFLP fingerprint of plantlets of the date palm variety Khlass derived via organogenesis and embryogenesis..

In vitro technique used	No. of individuals	Total scoreable bands	True-to-typeness	Individuals with differential bands. *
Organogenesis	40	131	38/40	27 (1), 34 (1)
Embryogenesis	39	131	35/39	4 (15), 5 (1), 35 (1), 38 (13), 39 (1)

* No. of differential bands per individual is shown in parentheses

Table 4. Differential banding patterns in samples of Khlass plants derived from embryogenesis compared to the standard pattern.

Band number	standard	Differentials				
		Ke4	Ke5	Ke35	Ke38	Ke39
5	1	1+	1	1	1	1
15	0	0	1	0	0	0
24a	0	1	0	0	1	0
26a	0	1	0	0	0	0
26b	0	0	0	0	0	1
32a	0	0	0	0	1	0
38a	0	1	0	0	1	0
39	1	0	1	0	0	1
44	1	0	1	1	1	1
48	1	0	1	1	0	1
49a	0	0	0	0	1	0
51a	0	0	0	0	1	0
52a	0	1	0	0	0	0
53	1	1	1	1	0	1
81	0	1	0	0	1	0
85a	0	0	0	0	1	0
94	1	0	1	1	0	0
101a	0	1	0	0	0	0
107a	0	1	0	0	1	0
108	1	0	1	1	0	1
115	0	1	0	0	0	0
127a	0	1	0	0	0	0
Total differential bands		15	1	1	13	1

(0) Absent band, (1) Present band, (1+) Intensive band.

Table 5. Khlass samples from plants derived from organogenesis and embryogenesis exhibiting differential bands.

Individuals exhibiting differential bands	Differentials			
	Present (1)	Absent (0)	Intense band (1+)	Total differential bands
Ko27	-	-	1	1
Ko34	-	-	1	1
Ke4	9	5	1	15
Ke5	1	-	-	1
Ke35	-	1	-	1
Ke38	8	5	-	13
Ke39	1	-	-	1

Figures

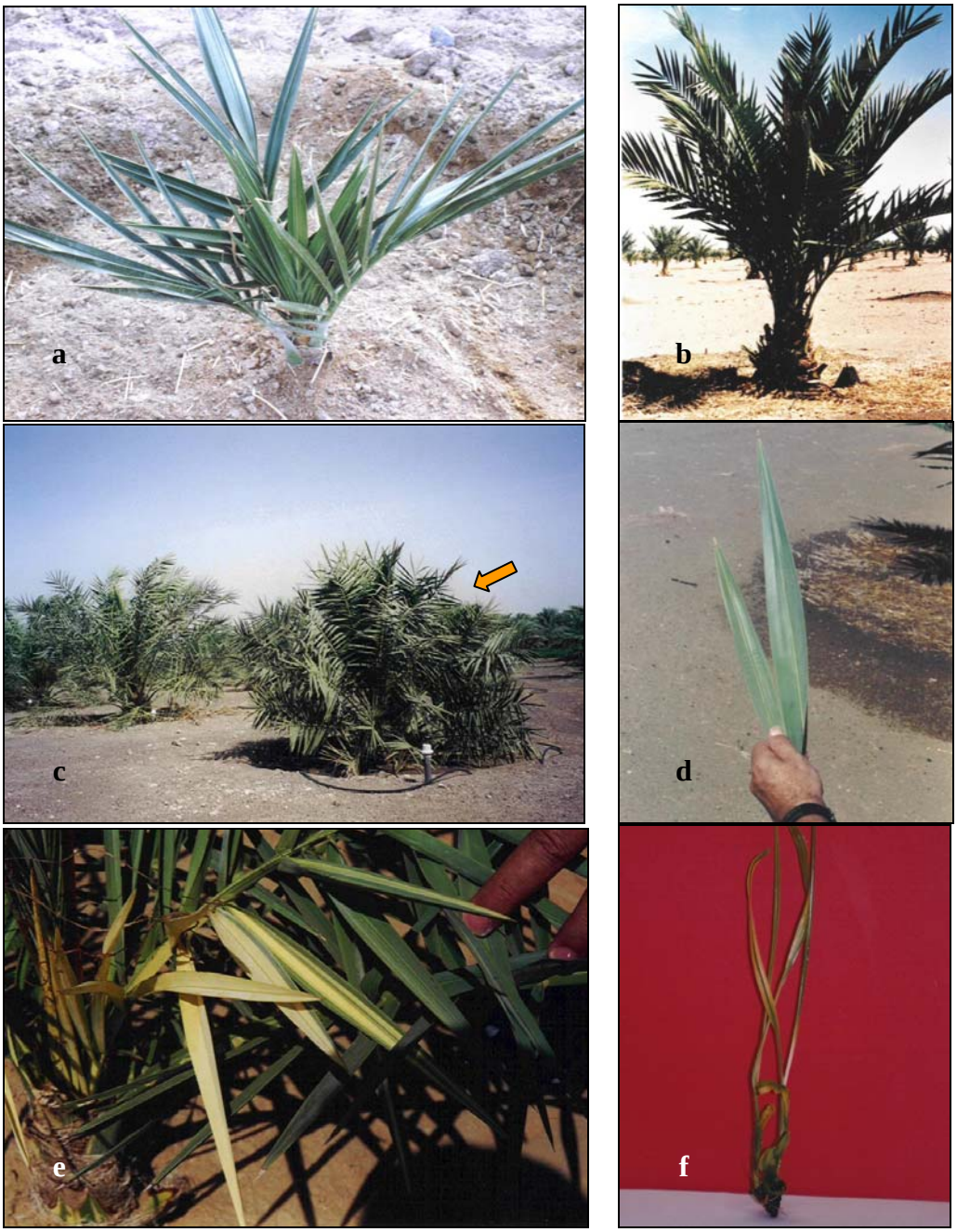

Fig. 1. Abnormal date palm tree morphology:
a. Abnormal morphology and structure of a two year old date palm (Medjool) produced via somatic embryogenesis; b. Seven year old date palm (Barhee) derived from somatic embryogenesis showing abnormal leaf structure and size; c. A Barhee tree showing excessive vegetative growth (arrowed) with a normal tree in the background; d. Abnormally large leaf of a Barhee tree (right: abnormal Barhee; left: normal Barhee leaf); e. Variegation observed on one year old Sukkari at the Liwa area in UAE. f. Leaf variegation on a Barhee plantlet derived by tissue culture, at the elongation stage.

Fig. 2. Dwarfism in date palms derived by asexual embryogenesis:
a. Dwarf Barhee date leaf (arrowed) in comparison to a normal grown leaf; b. Dwarf Oum Dahn date tree showing a variegation abnormality (arrowed); c. Dwarfism on a four year old Barhee plant derived from somatic embryogenesis with older leaves not showing dwarfism; d. Dwarfism on a four-year old Barhee plant derived from somatic embryogenesis. Outer, older leaves affected; e. Dwarfism on young fronds of a one year-old Medjool plant derived from somatic embryogenesis; and f. Uprooted three year-old dwarf Sukkari plant with a normal root system and g. Black scorch disease on a four-year old Medjool plant.

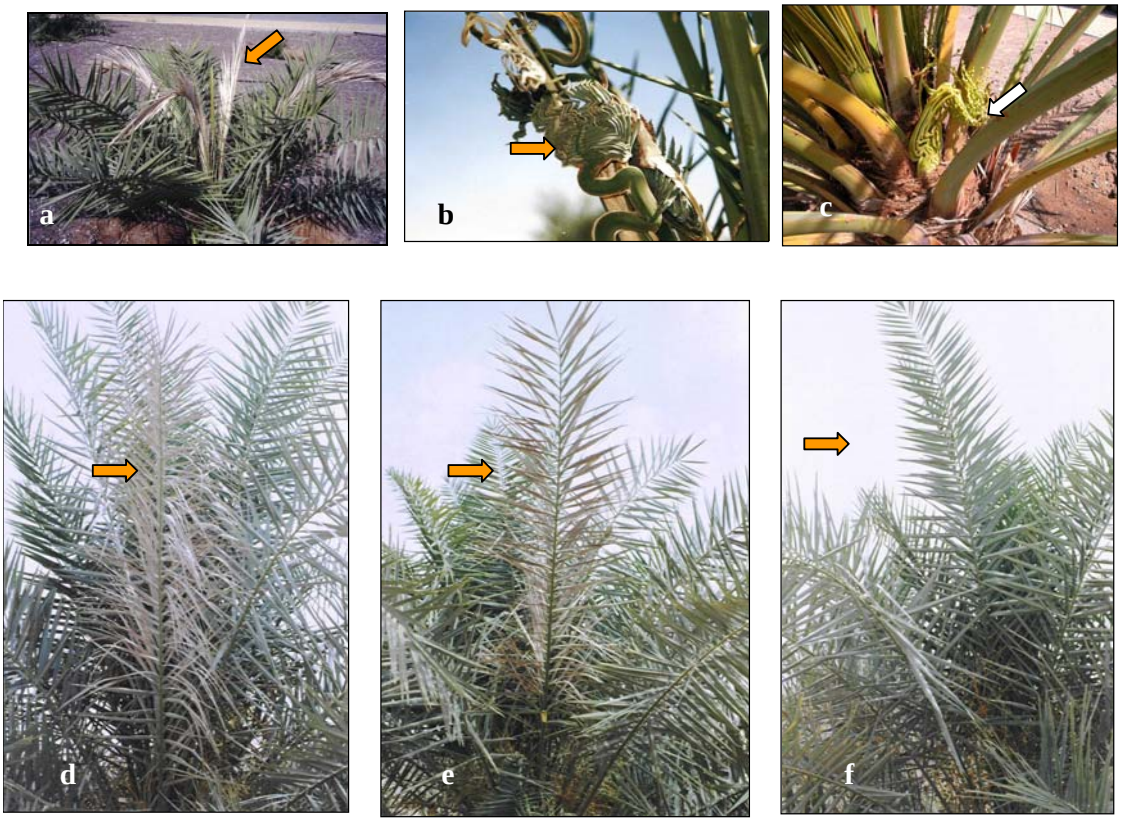

Fig. 3. Date palm morphological abnormalities:
a. Abnormal white leaves on a three year-old Barhee plant; b. Deformed offshoot on a Barhee plant derived from somatic embryogenesis (arrowed); c. Twisted inflorescence on a four year-old Barhee plant from tissue culture (arrowed); d, e, f. Progressive stages of recovery of chlorophyll in the whitened leaves of a six year-old Khlass tree derived from tissue culture. Note: the affected leaves turn back to the normal green colour (in the order d, e and f).

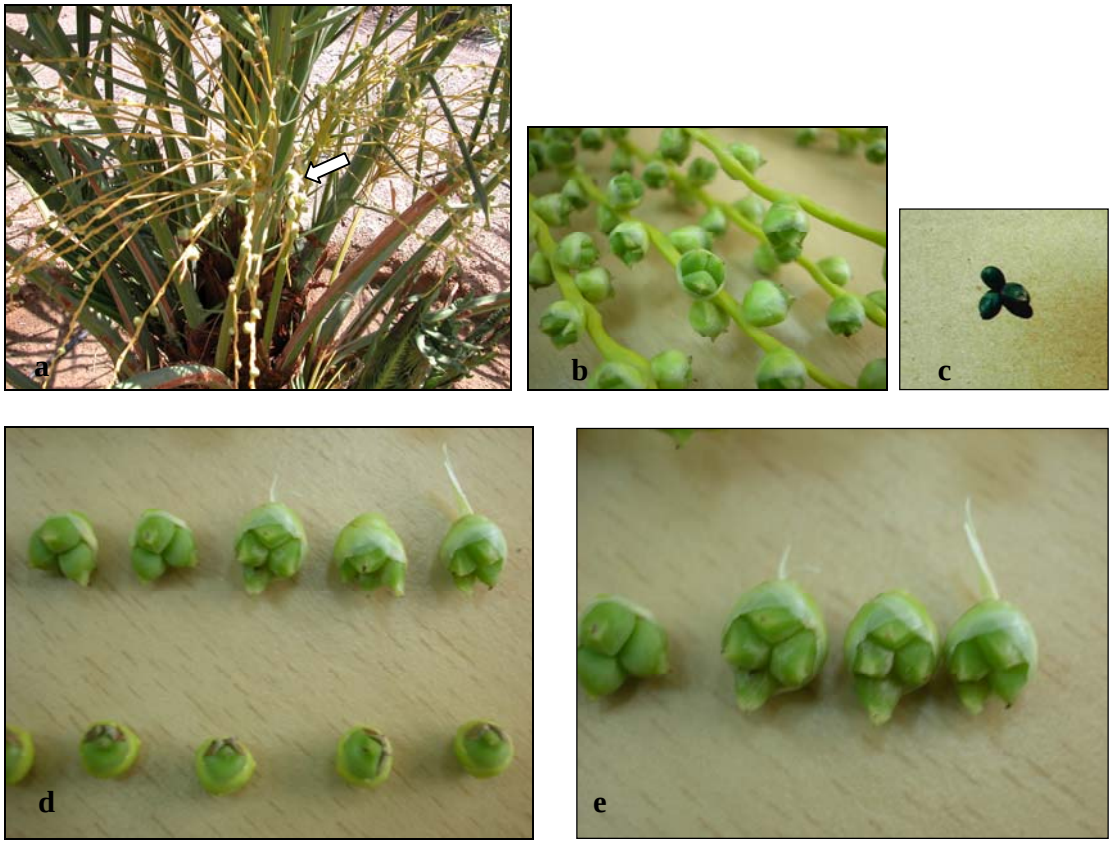

Fig. 4. Fruiting abnormalities in date palms derived from asexual embryogenesis-: a. Pollination failure on a Khlass tree derived from tissue culture, showing more than 90 % loss in fruit set; b. A Khlass tree derived from tissue culture showing more than 80 % parthenocarpic fruits; c. Development of three carpels instead of one; d. Parthenocarpic fruits of Khlass (top) compared to the normal simple carpel development (bottom of figure); and e. Parthenocarpic fruit showing the development of more than three carpels.

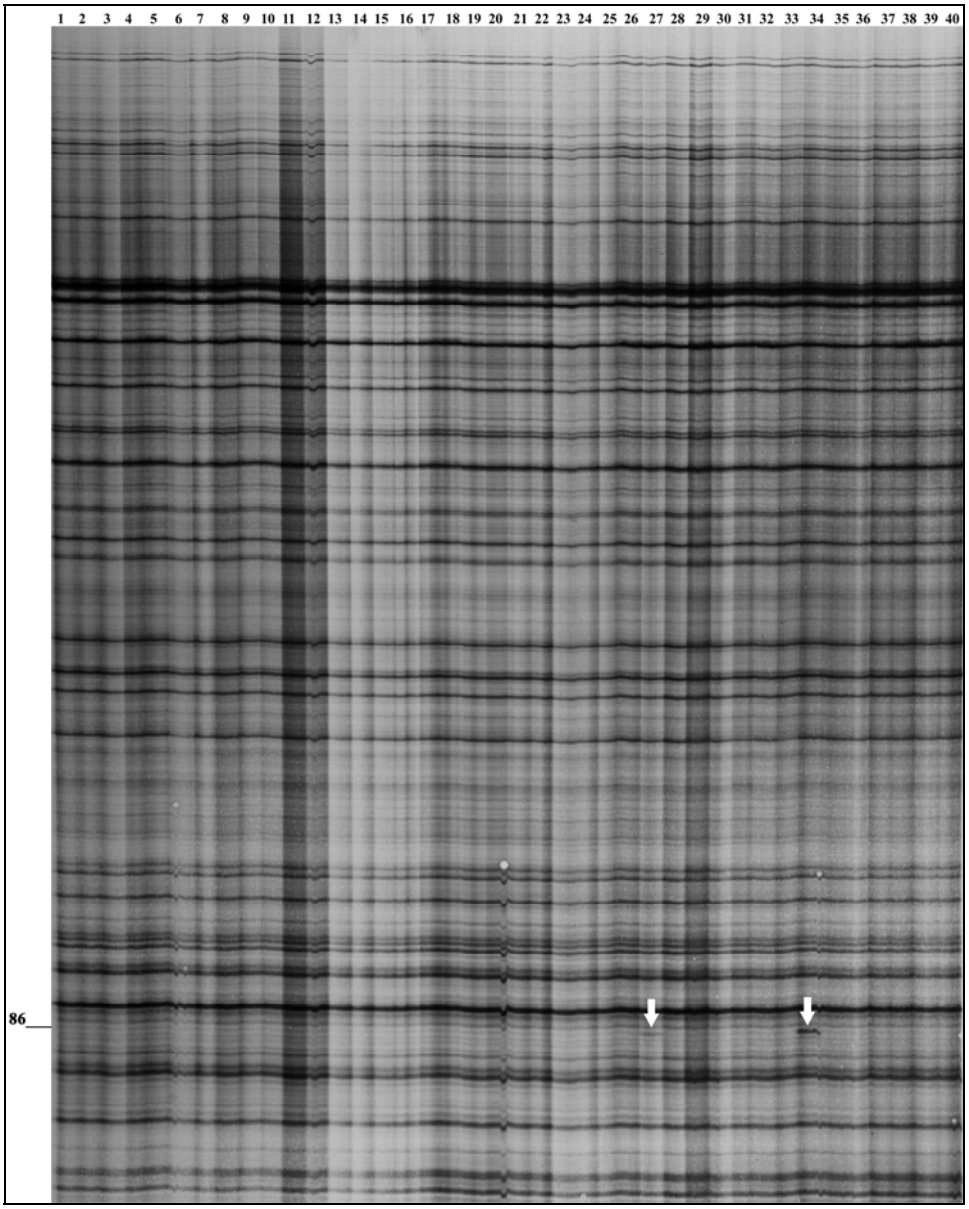

Fig. 5. AFLP variability assessment in plantlets from organogenesis.
Autoradiograph showing AFLP fingerprints of genomic DNA of 40 Khlass plantlets of organogenic origin. The profiles were generated using the selective primer combination of E+GGA / M+AAG.

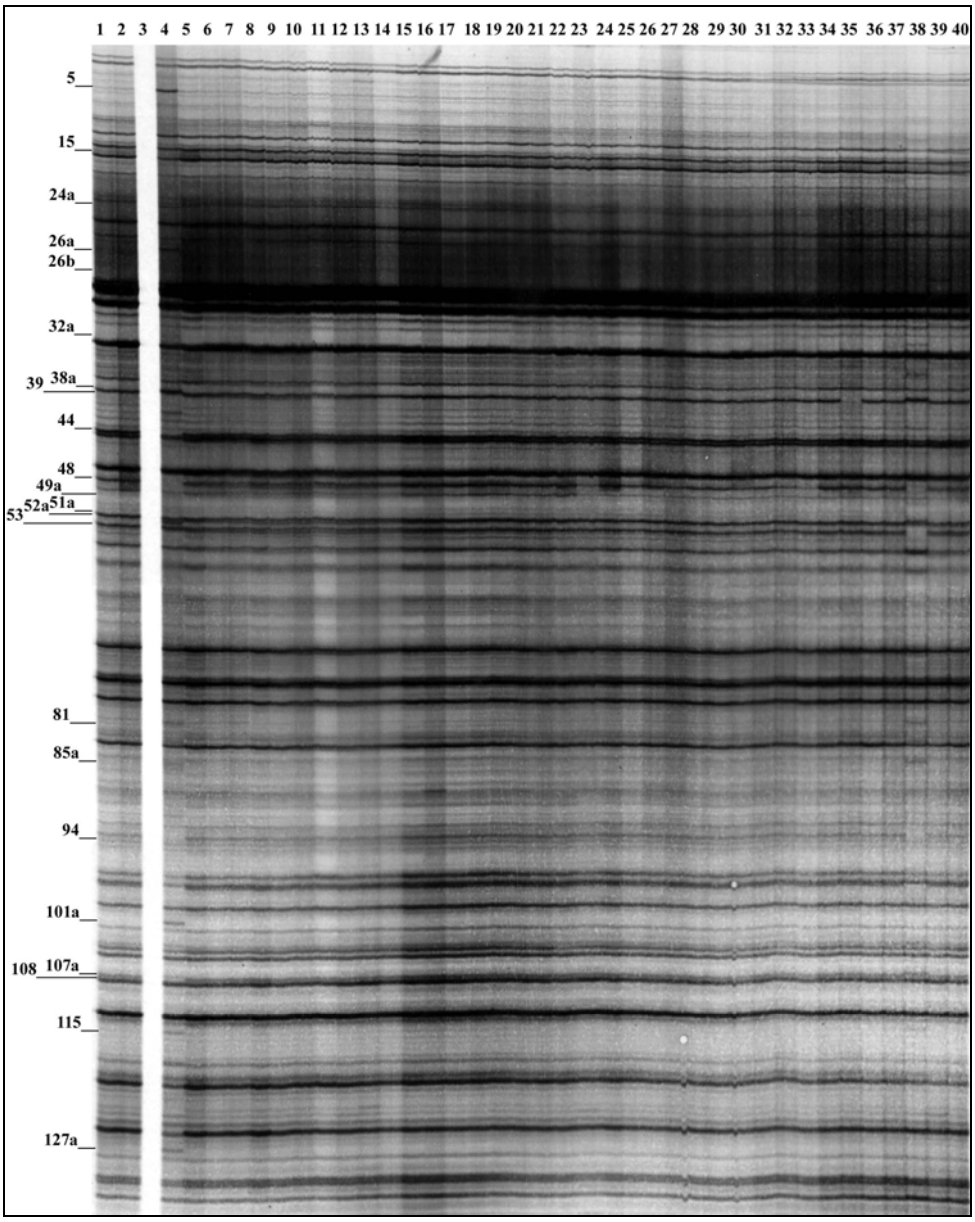

Fig. 6. AFLP variability assessment in plantlets from embryogenesis.
Autoradiograph showing AFLP fingerprints of genomic DNA of 40 Khlass plantlets of embryogenic origin. The profiles were generated using the selective primer combination of E+GGA / M+AAG.

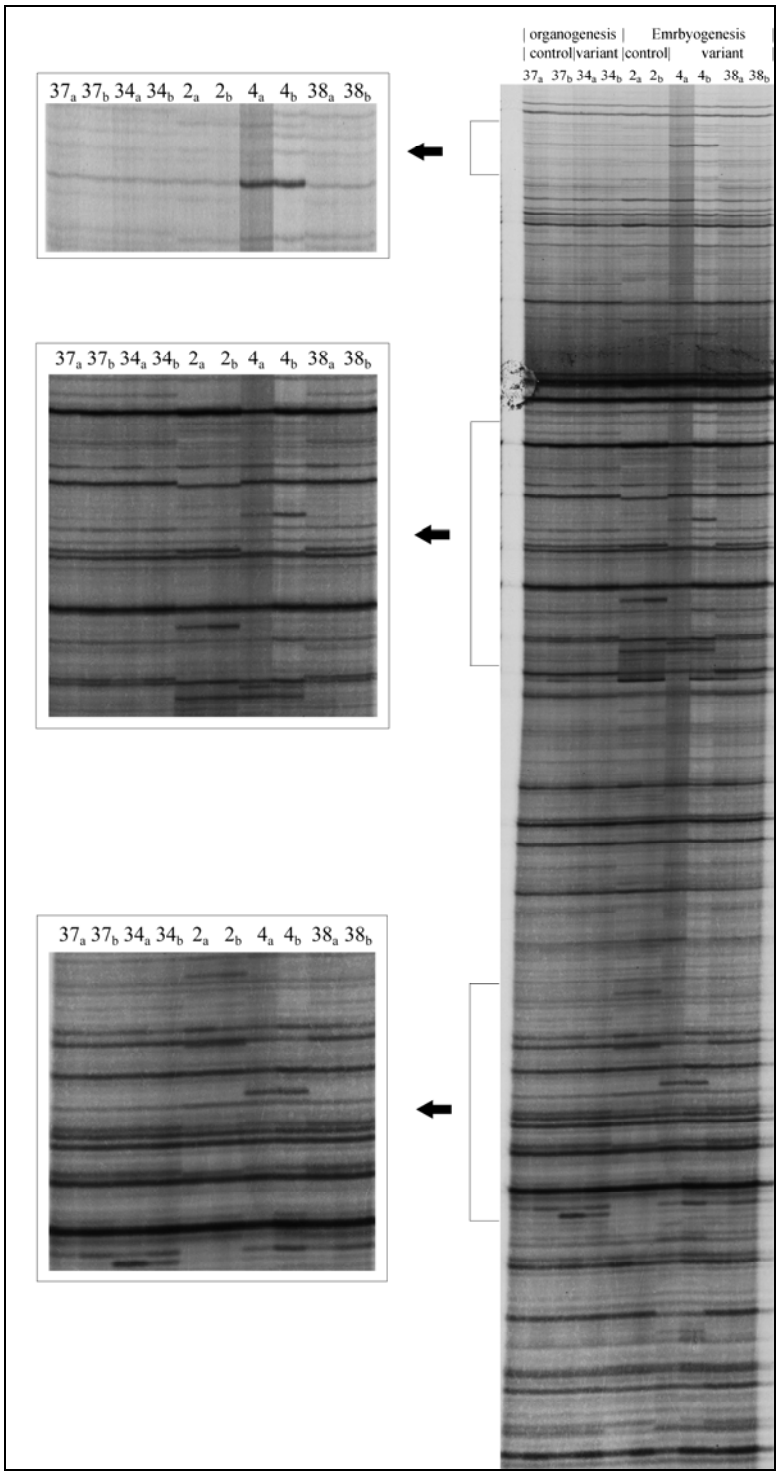

Fig. 7. The variant organogenesis sample (Ko34), and those of the variant embryogenesis samples (Ke4 and Ke38) that were re-extracted and used in further AFLP analysis. Samples Ko37 and Ke2 were also re-extracted and used as controls.

Cryopreservation of Date Palm (*Phoenix dactylifera* L.) Cultured In Vitro

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Keywords: RAPD analysis

Abstract

A very simple cryopreservation method with potential application to a wide range of date palm cultivars is described. Undifferentiated tissue cultures were successfully cryopreserved by freezing methods and plants were subsequently regenerated. Nodular cultures were initiated by culturing shoot tip explants (excised from offshoots) on medium containing 10 mg/L dichlorophenoxyacetic acid (2,4-D) + 3 mg/L dimethylaminopurine (2ip). The effect of dehydration caused by air drying on cryopreservation of date palm tissue cultures through direct immersion in liquid nitrogen was investigated. Cultures with about 65% water content resulted from a 20 min air drying period registered the highest percentage of survival and in vitro conversion to plantlets. Of the different types of sugars used as osmotic agents in a preculture medium, sucrose was the best for the survival of cryopreserved date palm tissue cultures. To determine the effect of vitrification on freezing tolerance, cultures were exposed to a vitrification solution (22% glycerol, 15 % ethylene glycol, 15% propylene glycol, 7% dimethyl sulfoxide) at 25 or 0˚C for 20-100 min. The maximum rate of survival was obtained with cultures exposed for 80 min at 0 ˚C followed by 40 min at 25˚C. Random Amplified Polymorphic DNA (RAPD) technique was used to study the genetic stability of cryopreserved tissue cultures of date palm. According to RAPD analysis, plantlets derived from cryopreserved cultures were identical to those derived from nontreated cultures and both were similar to field grown plants. Finally, complete plantlets retrieved from the cryostored cultures were successfully adapted to free living conditions after acclimatization procedures.

INTRODUCTION

Plant germplasm preservation is an integral part of any plant breeding program. The most efficient and economical method of germplasm storage is in the form of seeds. However, seed storage is not always feasible because: 1) some plants do not produce seeds and thus can only be propagated vegetatively, 2) seeds remain viable only for a limited duration, 3) some seeds are very heterozygous and therefore not suitable for maintaining true-to-type genotypes, and 4) seeds of certain species deteriorate rapidly due to seed-born pathogens.

Because plant cells are inherently totipotent, tissue culture techniques in conjunction with careful manipulation of cryobiological methods could be profitably used for the storage and preservation of important and recalcitrant plant germplasm. Preservation of plant cells, meristems and somatic embryos has become an important tool for the long-term storage of plant species using a minimum of space and maintenance (Shuji et al., 1992). One of the principle long-term in vitro conservation methods is cryostorage. Cryopreservation is generally understood as storage between -79 and -196°C, the lower extreme being the temperature of liquid nitrogen. The major advantage is that both metabolic process and biological deterioration of plant material at such temperatures are considerably slowed or even halted (Kartha, 1981). In addition, it is believed that cryopreserved material remains genetically stable, thus affording an advantage over conventional conservation methods (Withers, 1980, 1983). Successful cryopreservation requires the optimization of numerous variables including the size of specimen, the correct type and concentration of cryoprotectant, sample water content and rate of

freezing and thawing. The survival of plant tissues after cryopreservation can be increased by pretreatments such cold acclimation (Kuo and Linberger, 1985; Reed, 1993), air drying (Uragami et al., 1990) and preculture on a medium supplemented with abscisic acid (Chen and Gusta, 1983) or Me_2SO (Kartha et al., 1980; Kumu et al., 1983). Studies on cryopreservation of asparagus (Uragami et al., 1990), carrot (Dereuddre et al., 1991) and date palm (Bagniol and Engelmann, 1992) tissues have revealed that survival rates after cryopreservation can be increased by preculturing the tissues on media that contained high concentrations of sugar, instead of other cryoprotection procedures. Moreover, vitrification treatment eliminated the need for controlled slow freezing and permitted tissues to be cryopreserved by direct transfer to liquid nitrogen (Kohmura et al., 1992). As a dioecious plant, date palm is generally propagated vegetatively by offshoots. Thus its germplasm cannot be effectively stored long-term using conventional means. In an earlier report (Bekheet et al., 2001), we developed a protocol for in vitro medium-term storage of date palm using a slow growth technique. The present work aimed to develop an effective system for cryopreservation of tissue cultures of date palm by investigating the effects of dehydration, sugar-rich medium and vitrification as cryoprotectant pre-treatments on survival of cryopreserved tissue cultures.

MATERIAL AND METHODS

Induction of Aseptic Nodular Cultures

Date palm (*Phoenix dactylifera* L. cv. Zaghlool) offshoots detached from adult female plants were used as plant material. The outer leaves were removed and the tips were kept in an anti-oxidant solution (citric acid of 150 mg/L concentration). Shoot apices were then disinfested with 70 % ethanol for 1 min followed by immersion for 20 min in a 2.6 % sodium hypochlorite mix and thorough washing with sterile distilled water. External leaves were removed and shoot tips 1 cm in length were excised with a small part of submeristematic tissues and then cultured on Murashige and Skoog (MS) medium supplemented with 100 mg/L myo-inositol, 40 mg/L adenine sulfate, 170 mg/L KH_2PO_4 and different combinations of growth regulators. Cultures were then incubated in darkness at 25°C and subcultured every five weeks. After three subcultures, nodular culture frequency, culture fresh weights and the percentage of differentiated cultures were recorded.

Cryopreservation

Small pieces (1 cm^3 size) of the nodular cultures of date palm were aseptically taken and used for cryopreservation experiments. The potential of dehydration of cultures caused by air drying, preculturing on sugar-rich medium and vitrification were evaluated for the very low temperature storage of date palm tissue cultures. For the air drying treatment, cultures were placed on dry sterile filter paper and exposed to the air flow of a laminar air-flow cabinet for 10-50 minutes. Water content of the cultures (determined by drying samples at 70°C for 48 hr) was expressed as percentage of fresh weight. The cultures were then transferred to cryotubes and prepared for the storage procedures. To determine the role of high concentration of sugars in decreasing freezing injury, fructose, glucose, sorbitol and sucrose at two levels, i.e. 0.5 and 1.0 M, were added separately to preculture medium. After one week of sugar-treatments, the healthy cultures of date palm were taken for cryostorage. In the vitrificatin experiment, segments of nodular cultures of date palm were treated with vitrification solution described by Uragami et al. (1989). This solution contained 22 % (w/v) glycerol, 15 % (w/v) ethylene glycol, 15 % (w/v) propylene glycol and 7 % (w/v) dimethyl sulfoxide (DMSO) in MS-medium containing 0.5 M sorbitol with pH adjusted to 5.8. After 48 hr, the cultures were transferred into cryotubes contained vitrification solution and held at 25 or 0°C for 1 hr before being plunged into liquid nitrogen.

Freezing Procedures and Recovery

The cryotubes (containing date palm cultures) were kept at 0°C for 2 hr and were then plunged directly into liquid nitrogen (-196°C) for 48 hr. For recovery, the cultures were rapidly thawed in a warm water bath (37°C) and then inoculated on regrowth medium. Four weeks after inoculation, survival was evaluated by examining the color of cultures: the green ones (with increase of the volume) had survived; the brown ones had died. Also, the conversion to differentiated cultures was observed.

Randomly Amplified Polymorphic DNA (RAPD) Analysis

DNA isolation was performed using the Cetyl Trimethyl Ammonium Bromide (CTAB) method of Doyle and Doyle (1990). A half gram of fresh sample was ground to powder in liquid nitrogen with a prechilled pestle and mortar, suspended in 5 ml preheated CTAB buffer, and incubated at 65°C for 1 hour with occasional shaking. The suspension was then mixed with 1/3 volume of chloroform, mixed gently, centrifuged and the upper phase was transferred to a new sterilized tube. Extraction was repeated with an equal volume of chloroform. The aqueous layer was transferred to a new tube, 2/3 volume of isopropanol was added and nucleic acids were either spooled using a Pasteur pipette or sedimentated by centrifugation. The pellet was washed carefully twice with 70% ethanol, dried at room temperature and resuspended in 0.5 ml TE buffer. The enzyme RNAse A (20µg) was added to the resuspended mixture to digest any contaminating RNA and the tube was incubated at 37 °C for 30 min. To remove the enzyme and other contaminating protein, phenol/chloroform extraction was performed.

The polymerase chain reaction (PCR) mixture (25 µl) consisted of 0.8 units of Taq DNA polymerase, 25 pmol dNTPs, and 25 pmol of random primer, and 50 ng of genomic DNA. The reaction mixture was placed on a DNA thermal cycler. The PCR program included an initial denaturation step at 94°C for 2 mins followed by 45 cycles at 94°C for 1 min for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72°C for 10 minutes were carried out. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. Four 10-mer primers (Operon technologies Inc., Alameda, California) randomly selected were used in RAPD analysis (Table 1). A 100 bp DNA ladder (Promga) was used as a Marker with molecular size of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The amplified pattern was visualized on a UV transilluminator and photographed.

Culture Conditions and Statistical Analysis

Tissue culture media were solidified with 0.7 % agar and adjusted to pH 5.8 before autoclaving at 121°C and 1.5 Ib/M² for 25 min. Cultures were normally incubated at 25°C with a 16 hr photoperiod. Each experiment was set up as a separate completely randomized design with 20 replicates per treatment. Data were statistically analyzed using standard error (SE) according to the method described by Snedecor and Cochran (1967).

Plantlets Development and Acclimatization

Shoots were proliferated from nodular cultures using phytohormone-free medium. The shoots were elongated and rooted in vitro using a medium containing 1 mg /L indole-3-acetic acid (IAA) and 02% activated charcoal. Healthy plantlets were washed with tap water and disinfected by soaking in benlate solution (1g /L) for 20 min. Then plantlets were transplanted into plastic pots containing peat moss and vermiculite (1:1). The pots were covered with clear polyethylene bags which were sprayed with water to maintain a high relative humidity. Gradually, humidity was reduced and covers were completely removed within four weeks of transplanting.

RESULTS AND DISCUSSION

Morphogenesis of Date Palm Tissue Cultures

The effect of various combinations of phytohormones added to culture medium on differentiation of date palm tissue cultures is summarized in Table 2. The results reveal that morphogenetic responses vary depending on type and concentration of phytohormones used. The auxin (2,4-D) was more effective on in vitro morhogenesis of date palm compared to naphthaleneacetic acid (NAA). Also, the cytokinin (2ip) in combination with auxins strongly enhanced the differentiation and growth of date palm tissue cultures. In this respect, the highest percentage of nodular cultures (80%) as well as the highest value of growth presented as culture fresh weight were observed with medium containing 10 mg/L 2,4-D + 3 mg/L 2ip (Table 2 and Fig. 1-A). However, the highest percentage of differentiation was noticed when 5 mg/L 2,4-D +3 mg/L 2ip was added to culture medium. From these results, it was clear that the combination of 2,4-D and 2ip appeared to be the most suitable compared to other treatments used. These results are similar to those of Taha et al. (2003). They used medium contained 10 mg/L 2,4-D + 3mg/L 2ip for induction of embryogenic cultures and subsequent differentiation of date palm shoot tip and leaf premordia cultures. The results also are in line with those reported by Mater (1986) and Madhuri et al. (1998).

Cryopreservation

1. Effect of Air Drying. Sufficiently dehydrated seeds and small seedlings were directly immersed in liquid nitrogen (LN) from room temperature and stored there without freezing injury (Becwar et al., 1983). Thus desiccation prior to direct immersion in LN seems to be practical for cryopreservation of plant materials. In this experiment we investigated the effect of reducing the water content of in vitro proliferated nodular cultures of date palm caused by air drying on survival and conversion of cryopreserved cultures. The resulted presented in Table 3 show that survival as well as conversion percentages of cryopreseved tissue cultures were strongly affected by their moisture levels. The highest rates of survival and subsequent conversion to plantlets were observed with 65.80% water content which was caused by air drying for 20 min. Results also revealed that a high proportion of date palm cryopreserved cultures were irreversibly damaged when their moisture content dropped below 60%. Our results are in line with those of Uragami et al. (1990) on *Asparagus officinalis*. They reported that the axillary buds tolerant to dehydration remained alive after exposure to liquid nitrogen. In a study on cryopreservation of somatic embryoids of date palm, Mycock et al., (1997) mentioned that drying samples down to the range of 0.7-1.2 $g.g^{-1}$ allowed for a 32% survival rate. On the other hand, a reduction of the moisture content of banana meristem cultures by exposure of the meristematic clumps to sterile air flow did not result in an increased survival rate of cryopreseved cultures (Pains et al., 1996).

2. Effect of Sugar. Although an effect of water content is one possibility, the precise mode of action of sugar preculture in enhancing freeze resistance is not exactly known (Pains et al., 1996). Sugars reduce moisture content slowly due to osmotic action. As a result of its uptake, sugar decreases the freezing point and the amount of freezable water present in the tissues. In the this work, the role of fructose, glucose, sorbitol and sucrose added separately to preculture medium on freezing tolerance of cryopreserved nodular cultures of date palm was investigated. Survival and conversion percentages after freezing, together with their moisture contents resulting from treatments are shown in Table 4. Data presented indicate that most non-precultured cultures were killed by freezing. Generally, the increase of sugar concentration from .05 to 1.0 M decreased the survival rate of cryopreserved cultures of date palm. The addition of various other sugars to the preculture medium effectively promoted survival, however, sucrose was more effective in increasing freezing tolerance. The results concur with those of Pains et al. (1996) on banana. They reported that sucrose preculture improved post-thaw survival of cryopreserved meristem cultures. They added that the concentrations of 0.4 and 0.5 M were especially effective,

resulting in a regrowth of 25 and 42%, respectively. In this connection, Uragami et al. (1990) mentioned that a considerable amount of sucrose uptake and its subsequent dissociation into glucose and fructose during preculture in the presence of high sucrose levels was demonstrated with axillary buds of asparagus. This dissociation inside the cell causes a considerable increase in osmolarity since 1 M sucrose results in 2 M of monosaccharides. An indirect effect of sucrose could be the accumulation of endogenous compounds induced by a mild osmotic stress, that then offer protection against further water stress and cryopreservation.

3. Effect of Vitrification. For successful cryopreservation, it is necessary to avoid lethal intracellular freezing, which occurs during rapid cooling in liquid nitrogen (LN). Thus, cultures have to be sufficiently dehydrated or concentrated to be capable of vitrifying before being immersed into LN. Another possible approach could be extensive concentration using a highly concentrated vitrification solution. The vitrification method is very simple and seems promising as a routine method for cryopreservation of tissue cultures. In this work, to determine the effect of exposure to vitrification solution on freezing tolerance of date palm tissue cultures, samples were treated for 20-100 min at 25 and 0°C before being plunged into LN. (see Table 5 for exposure to vitrification solution produced time dependent survival and conversion percentages). Results revealed that the survival of nontreated cultures was much lower than those exposed for different periods. The highest rates of survival were obtained with cultures treated with vitrification solution for 80 min at 0°C or 40 min at 25 °C, respectively. Moreover, the highest percentages of cultures converted to plantlets were observed when cultures were exposed at 0°C for 60 and 80 min respectively. From the obtained results, it is clear that to reduce the injurious effects of the high osmolarity of the vitrification solution, low temperatures are needed and to decrease the time of exposure, high temperatures are preferable. The present results are in line with those reported by Fahy et al. (1984) and Uragami et al. (1989). In this connection, Kohmura et al. (1992) mentioned that the keys to success of cryopreservation by vitrification are to carefully control the procedures for dehydration and cryoprotectant permeation and to prevent injury by chemical toxicity or excess osmotic stresses during treatment. Thus, successful vitrification requires the use of a highly concentrated yet non toxic solution of cryoprotectants and the optimum exposure time.

Molecular Analysis

Due to its high sensitivity and accuracy in detecting every single base change, DNA-based analysis was applied to study the genetic stability of plant tissue cultures (Williams et al., 1990). RAPD technology has been used successfully for measuring diversity in plants, and the patterns of variation observed have been shown to closely resemble those obtained using more classical characters (Howell et al., 1994). In the present investigation, RAPD-DNA analysis was used to determine the genetic stability of cryopreserved and non-cryopreserved tissue cultures of date palm, and to compare both types of cultures to field grown plants. Four randomly selected primers were used in this investigation. Only one of them (K1) did not give reproducible and sufficient amplification products. As shown in Fig. 2, DNA fragments varied in number and size depending on the primers used. The banding revealed that the three types of cultures were identical to each other with primers K2 and K4. However, with primer K5, a band of 600 bp was not present in the sample of the field grown cultures. It is particularly important to confirm that cryopreserved cultures of date palm produce plantlets genetically similar to both nontreated and plants grown in free-living conditions. From the obtained results, we can conclude that there was no genetic variability of the frozen-thawed nodular cultures of date palm. The present results are consistent with those reported by Saker et al. (2000). They mentioned that no significant variation was observed in plantlets derived from tissue culture. RAPD analysis showed genetic variation in only 4% of analyzed plants (70 regenerants) which were incubated for 6-12 months under 25 °C. In this respect, genetic marker analysis has been used to study the degree of genetic change in plants regenerated

in vitro such as pea (Cecchini et al., 1992), sugarbeet (Sabir et al., 1992) and wheat (Brown et al., 1993).

Plantlets Development and Acclimatization

In this part of the study, shoot cultures were regenerated from nodular cultures on recovery medium (Fig. 1. B). Because the development of a good root system on the in vitro grown plantlets of date palm is considered one of the most important factors affecting acclimatization to the ex vitro environment (El-Bahr et al., 2004), shoots derived from cryopreserved cultures were transferred into charcoal containing medium for in vitro elongation and rooting. The plantlets with healthy root systems were successfully transplanted to free-living conditions within a short period of acclimatization (Fig. 1. C). Similarly, Tisserat (1984) reported that a high survival rate (nearly 100 %) could be obtained when date palm plantlets with 2-3 foliar leaves and of shoot length greater than 10 cm (with a well-developed adventitious root system) were transplanted in pots containing a mixture of peatmoss and vermiculite.

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Tables

Table 1. Primers used and their annealing temperatures.

Primer	Sequence 5'- 3'	Annealing Tm °C / Sec
K1	TGGCGACCTG	36
K 2	GAGGCGTCGC	
K4	TCGTTCCGC	
K 5	CACCTTTCCC	

Table 2. Effect of growth regulators on in vitro morphogenesis of date palm tissue cultures.

Media	Nodular culture frequency(%)	Culture fresh weight (g)	Differentiated cultures(%)
MS +1 mg/L NAA	20	0.70 ± 0.20	-
MS + 2 mg/L NAA	30	0.90 ± 0.19	10
MS +5 mg/L 2,4-D	50	1.11 ± 0.33	20
MS +10 mg/L 2,4-D	60	1.20 ± 0.25	20
MS +1mg/L NAA+3 mg/L 2ip	25	1.00 ± 0.18	-
MS +2mg/L NAA+3 mg/L 2ip	35	1.15 ± 0.22	15
MS +5 mg/L 2,4-D+3 mg/L 2ip	70	1.30 ± 0.40	60
MS +10 mg/L 2,4-D+3 mg/L 2ip	80	1.48 ± 0.33	55

Each treatment is the avearge of 20 replicates. ± SE.

Table 3. Effect of drying on survival and conversion of date palm tissue cultures cryopreserved at -196˚C.

Drying time (min)	Water content (%)	Survival (%)	Conversion (%)
0	90.10	10	-
10	75.90	70	60
20	65.80	80	65
30	60.30	40	30
40	57.80	20	10
50	50.00	10	-

Each treatment is average of 20 replicates.

Table 4. Effects of sugar types and their concentrations in pre-culture medium on survival and conversion of date palm tissue cultures cryopreserved at-196˚C.

Sugar	Concentration (M)	Water content (%)	Survival (%)	Conversion(%)
None		90.20	10	
Fructose	0.5	75.50	70	70
	1.0	70.20	60	50
Glucose	0.5	73.90	70	60
	1.0	69.50	65	55
Sorbitol	0.5	76.00	75	70
	1.0	71.80	65	60
Sucrose	0.5	72.50	70	60
	1.0	65.00	80	75

Each treatment is the average of 20 replicates.

Table 5. Effect of exposure to vitrification solution at 25 and 0 ˚C on survival and conversion of date palm tissue cultures cryopreserved at -196 ˚C.

Exposure time (min)	Survival (%)		Conversion (%)	
	Exposure at 25 ˚C	Exposure at 0 ˚C	Exposure at 25 ˚C	Exposure at 0 ˚C
0	10	20	-	10
20	60	55	40	45
40	80	75	60	65
60	50	85	40	75
80	40	75	30	70
100	20	70	10	60

Each treatment is the average of 20 replicates.

Figures

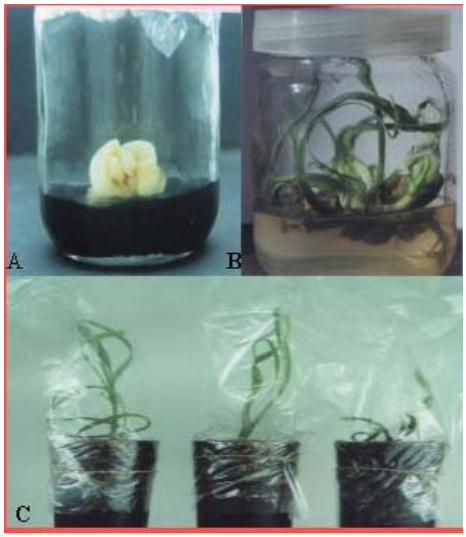

Fig. 1. A- Nodular cultures of date palm proliferated on medium contained 10 mg/L 2,4-D + 3 mg/L 2ip; B- Shoot cultures regenerated form cryopreserved cultures on recovery medium; C- Successful acclimatization for transplanting of plantlets to free-living conditions.

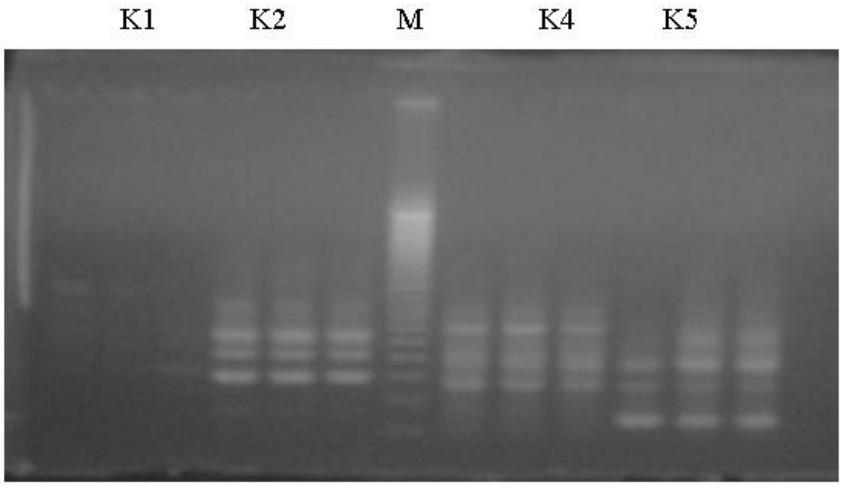

Fig. 2. RAPD profile of field grown plant (lane 1), nontreated tissue cultures (lane 2), cryopreserved tissue cultures of date palm (lane 3) and the DNA marker (M) from left to right using rabdom primers i.e. K1, K2, K4 and K5.

Quantity and Quality Comparison of Offshoot and Tissue Cultured Barhee Date Palm Trees

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Keywords: commercial cultivars, plantations, production, export

Abstract

Barhee is one of the most commercial and popular date cultivars in the world. The province of Khuzestan in the southwest of Iran is one of the main producing regions of this cultivar but many of the plantations were damaged during the Iraq and Iran war (1980-1989). Therefore, in order to renew these plantations and also to restore the old ones, the government prepared and distributed many tissue cultured plantlets of Barhee date cultivar in this region. Many date growers who were worried about the incidence of abnormalities (genetic and epi-genetic changes) in these trees, were not interested in using tissue cultured plantlets. These abnormalities relate to modifications of chromosome structure during the in-vitro propagation process that may affect some properties of the tree and its fruit, such as shape, color, size and sugar content of fruits, and growth pattern of the tree like height, trunk diameter, number of leaves, leaflets and thorns, or their position. Therefore this study was carried out using tissue culture offshoot propagated Barhee date palms from 1999 to 2002 on a date palm germplasm collection of Date Palm & Tropical Fruits Research Institute of Iran at Ahwaz city, using a completely randomized design with 12 replications. The results showed significant differences in only 16 of the 97 evaluated characteristics of the two groups. There were no important morphological changes related to genetic abnormalities in the trees propagated by tissue culture technique.

INTRODUCTION

Date palm is the second most important fruit crop in Iran. According to FAO reports in 2003, Iran is the second largest producer in the world, where more than 184000 hectares were harvested producing about 885000 tons of fruit, of which 120056 tons were exported., Major plantations of date palm are located in the southwest to southeast regions of Iran. The provinces of Hormozgan, Kerman, Fars, Sistan & Baluchestan, Bushehr, and Khuzestan produce more than 99 percent of the total annual production and are the main producing provinces of Iran. About 400 of the 3000 cultivars of date palm that have been reported from Iran are placed first in the world. Although the exact distribution of them are being investigated, the commercial cultivars viz Piarum, Zahidi, Deiri, Sayer, Mordasang, Hallavy, Shahani, Kabkab, Mozafati, Almehtari, Khasoui and Barhee are well known.

Barhee is one of the most commercial and popular date cultivars in the world. The province of Khuzestan in the southwest of Iran is one of the main producing regions of this cultivar but many of the plantations were damaged during the Iraq and Iran war (1980-1989). To renew these plantations and old ones using offshoots was impossible due to their limited number, so in recent years the government produced and distributed many tissue cultured plantlets of cultivar Barhee date to the region.

An investigation of the propagation of date palms by tissue culture has been ongoing for 25 years, starting with Eeuwens and Blake (1977). They believed that propagation of date palm and coconut was not efficient using offshoots. Further studies showed many parts of the date palm tree, such as young leaves, root tips and immature fruits could be used for micropropagation (Reuweni, 1979; Scharma et al., 1980), but meristematic stem tips proved to be the best explant (Shakib et al., 1993; Khoshkam et al.,

1995). The possibility of using this technique for propagation of date palm has been studied in Iran since 1990 (Majidi et al., 1991) and has led to success in both government and private sectors. Early results of tissue cultured production showed that propagated plantlets were true-to-type and uniform in phenotypic appearance, but other cases were characterized by a great deal of variability (Hartmann et al., 1990). Variability can be categorized, although it is not always possible to assign a specific variant to a given category. Because of this, many Iranian date growers worried about the incidence of abnormalities (genetically and epi-genetically changes) in tissue cultured trees and were not interested in using micropropagated plantlets. The changes in tissue cultured plantlets related to modifications of chromosome structure occurring during the in-vitro propagation process and affected some important properties of both tree and fruit, such as shape, color, size and sugar content of fruit, and growth pattern of the trees such as height, trunk diameter and number of leaves, leaflets and thorns or their position. Trueness-to-type as the basic principle must be tested through phenotypic and genotypic selection procedures under field conditions.

MATERIALS AND METHODS

This study was carried out using Barhee date trees propagaed from both tissue culture and offshoots from 1999 to 2002 on a date palm germplasm collection of Date Palm & Tropical Fruits Research Institute of Iran at Ahwaz city using a completely randomized design with 12 replications. Evaluated characteristics were divided into vegetative and reproductive groups. For the first, 3 leaves were selected randomly and removed from each replication. For the second, a sample of 50 fruits was selected randomly from each harvested bunch in Khalal, Rotab and Tamar stages. The means were compared by T-test at 1% level of probability after the analysis of variance tables were computed for all of the data.

RESULTS AND DISCUSION

Vegetative Characteristics

The vegetative characteristics that were evaluated are listed and compared in Table 1. The results showed that there were no significant differences between plants derived from offshoots and tissue culture in the following vegetative qualities: height of tree, trunk diameter of tree, number of leaves, diameter and length of leaf stalk, long and short distances between two consecutive thorns, long and short distances between two consecutive leaflets, tallness of thorns, tallness of leaflets, and greater and lesser angles of leaflets on the leaf.. Although the greater angle of thorns on leaves in the offshoot group was less than the tissue culture group, results for the lesser angle, length of leaf, width of leaf, number of thorns and leaflets, length of thorny and leafy areas on a leaf were the opposite. Therefore it appeared that there were not only significant differences between offshoot and tissue cultured trees, but also the latter (plants derived from tissue culture) had more uniform growth patterns, especially in terms of the angle of thorns on the leaf. Al-Wasel (2000) reported that tissue cultured plantlets of Barhee in Saudi Arabia had more uniform growth than those from offshoots. Diaz et al. (2003) did not observe any changes in vegetative characteristics caused by genetic variation in cultivars such as Medjool and Bou Fegous in Spain. Of course they did not report any priority of tissue cultured than offshoot plantlets in this regard.

Reproductive Characteristics

The evaluation of reproductive characteristics is presented in Table 2. There were no significant differences in yield, number of bunches, length of main axis of the bunch, length of bunch stalk, length of strand, length of fruit, diameter of fruit, volume of fruit, length of seed, volume of seed, total soluble solid and water content. But, the number of strands per bunch, number of fruits per strand, diameter and weight of seed, and pulp to seed ratio were different in offshoot and tissue cultured trees at Khalal, Rotab and Tamar

stages.

1. Number of Strands per Bunch. The number of strands per bunch of offshoot and tissue cultured trees showed no significant difference at Rotab stage (60.52 and 58.28, respectively). However, the bunches of trees propagated by offshoot had 62.11 and 59.81 strands at Khalal and Tamar stages, in comparison to tissue cultured ones that had 59.14 and 56.93, respectively (Fig. 1).

2. Number of Fruits per Strand. Results showed that the number of fruits per strands of offshoot trees was similar to tissue cultured trees at Khalal and Rotab stages, but at Tamar stage the bunches of tissue cultured trees had 15.9 fruits per strands in comparison to offshoot trees with 14.7 fruits (Fig. 2).

3. Seed Diameter. At Rotab stage the diameter of seeds was 9.87 and 8.17 mm in offshoot and tissue culture trees, respectively. This is a significant difference (Fig. 3). Although the diameter of seeds at Khalal and Tamar stages of offshoot trees was more than tissue cultured trees, the difference was not significant.

4. Seed Weight. At Khalal stage seed weight showed no significant difference between the two groups, but at Rotab and Tamar stages, the seeds of tissue cultured trees had 0.92 and 1/02 g, in comparison to offshoot trees with 1.05 and 1.17 g, respectively (Fig. 4).

5. Pulp to Seed Weight Ratio. The results showed pulp to seed weight ratio of the tissue cultured group was higher than the offshoot group at Rotab and Tamar stages (Fig. 5). The ratios were 10.22 and 8.56 at those stages in tissue cultured trees, and 8.93 and 7.41 in offshoot trees, respectively. There was no significant difference at Khalal stage.

In previous studies reproductive characteristics between offshoot and tissue cultured Barhee date palm were not evaluated and analyzed precisely. Al-Wasel (2000) studied the vegetative phase due to non-bearing of the trees. He showed plantlets of the two groups in 1992 when many of them were too young to bear. He reported informally on the incidence of abnormality in the number of fruit carpels but believed that further evaluation of the fruit would have to wait until trees had reached economic bearing age (at least 12 years old). Our results showed no significant differences in yield, fruit size, total soluble solid and fruit water content (Table 4). Although the number of strands per bunch of tissue cultured trees was less than offshoot trees (Fig. 1), there was a greater number of fruits per strand, with a smaller seed diameter and weight in tissue cultured trees (Figs. 2, 3 and 4). Consequently, the total amount of pulp in fruits of tissue cultured trees was more than that from offshoot trees (Fig. 5). Ma'aravi (2003) recommended using tissue culture for mass propagation of Barhee, Medjul and Deglet Noor varieties. He not only reported that there were no genetical abnormalities in the commercial propagation program of these varieties, but also that the quantitative and qualitative characteristics of fruits were better in tissue cultured trees than offshoot trees.

CONCLUSION

The use of tissue culture techniques for commercial or mass propagation of date palm can be recommended. The main advantages of this method are as follows (Pezhman, 2001; Al-Wasel, 2000; Scharma et al, 1980):

- Trueness-to-type
- Ability to mass propagate
- Independance on time and ecological conditions
- Time-saving
- High establishment percent (less mortality of sowed plantlets) due to extended root systems
- Low cost of transplanting of plantlets to the field
- Low risk of the distribution of pests and diseases
- Production of uniform plantations

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Tables

Table 1. Comparison of vegetative characteristics between offshoot and tissue cultured Barhee date palm trees.

Description of Characteristic	Tissue Culture	Off Shoot
Height of tree (m)	2.43 a*	2.72 a
Trunk diameter of tree (m)	1.96 a	1.99 a
Number of leaves	35.5 a	34.7 a
Length of leaf (cm)	383.1 b	447.5 a
Width of leaf (cm)	70.2 b	77.6 a
Diameter of leaf stalk (cm)	22.5 a	19.9 a
Length of leaf stalk (cm)	10.1 a	12.2 a
Number of thorns	30.5 b	39.3 a
Number of leaflets	177.5 b	203.2 a
Length of thorny area on a leaf (cm)	86.8 b	92.7 a
Length of leaflety area on a leaf (cm)	243.7 b	296.8 a
Long distance between two consecutive thorns (cm)	8.9 a	9.9 a
Short distance between two consecutive thorns (cm)	1.8 a	1.5 a
Long distance between two consecutive leaflets (cm)	4.8 a	5.0 a
Short distance between two consecutive leaflets (cm)	1.4 a	1.0 a
Tallness of thorn (cm)	10.4 a	11.0 a
Tallness of leaflet (cm)	42.5 a	45.8 a
Greater angle of thorn on leaf (degree)	36.7 a	30.1 b
Less angle of thorn on leaf (degree)	20.2 b	28.0 a
Greater angle of leaflet on leaf (degree)	46.6 a	45.4 a
Less angle of leaflet on leaf (degree)	32.7 a	32.5 a

*Means with the same letters were not significantly different at 1% level of T- test.

Table 2. Comparison of reproductive characteristics between offshoot and tissue cultured Barhee date palm trees.

Description of Characteristic	Khalal		Rotab		Tamar	
	Tissue Culture	Off shoot	Tissue Culture	Off shoot	Tissue Culture	Off shoot
Yield (Kg/tree)	137.43 *	136.49	117.38	114.09	100.72	94.60
Number of bunches	11.41	10.94	11.41	10.94	11.41	10.94
Length of mail axis of bunch (cm)	136.95	139.66	139.77	141.94	143.51	145.48
Length of bunch stalk (cm)	102.66	100.02	108.25	106.24	111.17	108.06
Length of strand (cm)	38.17	36.75	39.44	38.05	40.89	39.97
Length of fruit (cm)	39.31	40.19	36.91	37.62	32.83	34.05
Diameter of fruit (cm)	30.95	30.45	29.60	28.50	25.85	25.84
Weight of fruit (g)	15.99	16.36	14.73	14.69	12.00	12.42
Volume of fruit (cc)	11.129	11.098	10.323	10.381	9.752	9.835
Length of seed (cm)	16.32	17.00	18.01	18.93	19.97	21.44
Volume of seed (cc)	0.63	0.75	0.75	0.87	0.89	1.00
Total soluble solid (%)	52.93	52.65	63.56	63.29	72.93	72.67
Water content (%)	53.80	54.40	27.80	27.30	19.70	19.50
Fruit shape (length / diameter ratio)	1.27	1.32	1.25	1.31	1.27	1.31

*For each stage of fruit growth, the means were not significantly different at 1% level of T- test.

Figures

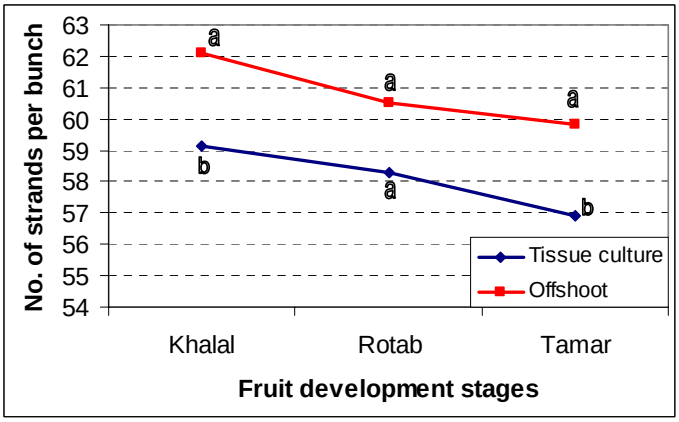

Fig. 1. Comparison of number of strands per bunch between offshoot and tissue cultured Barhee date palm tress.
Means with the same letters were not significantly different at 1% level of T- test.

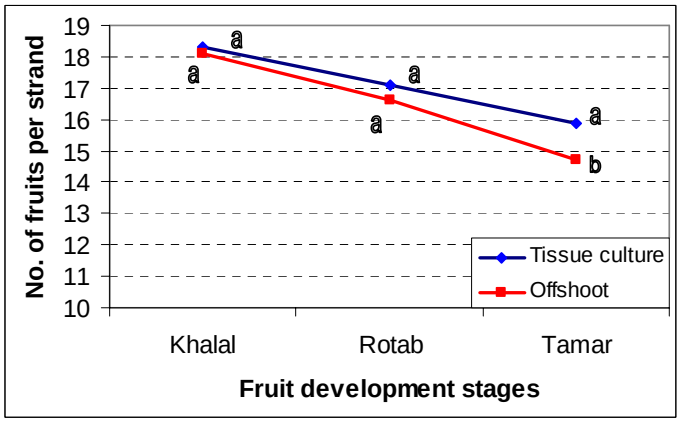

Fig. 2. Comparison of number of fruits per strand between offshoot and tissue cultured Barhee date palm tress.
Means with the same letters were not significantly different at 1% level of T- test.

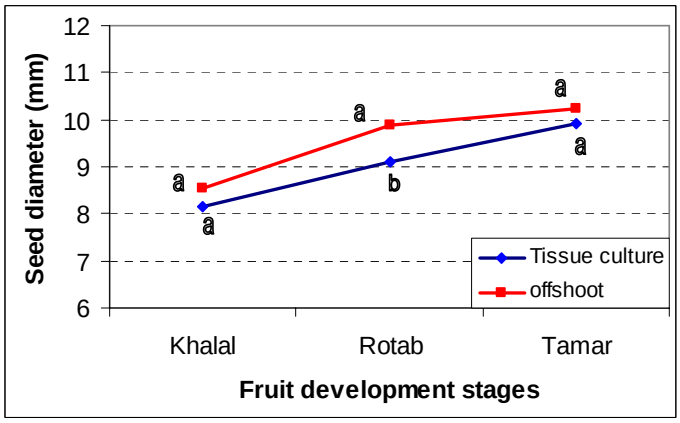

Fig. 3. Comparison of seed diameter between offshoot and tissue cultured Barhee date palm tress.
Means with the same letters were not significantly different at 1% level of T- test.

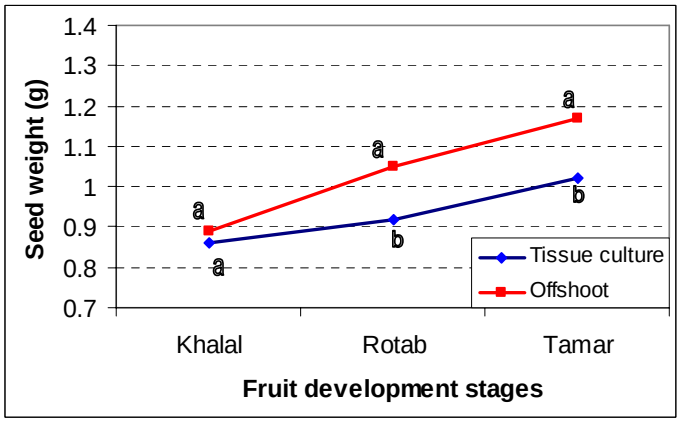

Fig. 4. Comparison of seed weight between offshoot and tissue cultured Barhee date palm tress.
Means with the same letters were not significantly different at 1% level of T- test.

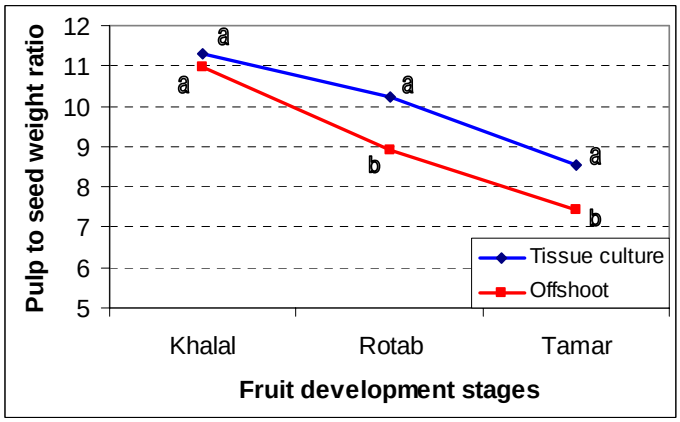

Fig. 5. Comparison of pulp to seed weight ratio between offshoot and tissue cultured Barhee date palm tress.
Means with the same letters were not significantly different at 1% level of T- test.

Evaluation of In Vitro Screening Techniques for Salt Tolerance in Date Palm

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Abstract

The date palm is an important source of income and nutrition in a number of countries. It is considered a salt tolerant plant species. However, very limited information is available concerning variation in salt tolerance among the cultivars currently growing. This investigation aimed to evaluate the potential of employing tissue culture techniques to screen date palm genotypes for salt tolerance. The effect of NaCl on calli derived from immature embryos of four local date palm cultivars was examined at two distinct stages, which were dedifferentiation and fast growing stages. The results suggest that employing callus induction might be more efficient than using the fast growing stage to identify variation in salt tolerance among date palm genotypes. The fast growing callus system was unable to detect significant differential performance, whereas the callus induction system was capable of detecting variation among the genotypes. Salinity affected date palm immature embryos significantly and complete inhibition occurred when 3.0% (w/v) NaCl was incorporated into the induction medium. In both cases, callus growth retardation was clearly evident with increasing salinity. Both types of calli also exhibited tissue dehydration symptoms, which were recorded as a significant increase in callus dry weight ratio. Callus Na^+ content increased dramatically with increasing NaCl level, whereas K^+ content decreased causing a significant reduction in callus K^+/Na^+ status. Proline, which is considered a compatible osmoticum, increased significantly in both types of calli in response to salinity. During the induction stage, the increase in endogenous free proline content was more pronounced in the progeny of the cultivar, which exhibited higher percentage of callus induction. Therefore, the better dedifferentiation process could be related to proline content, which adjusts the intracellular osmotic pressure between the cytoplasm and the vacuole.

INTRODUCTION

Salinity is one of the major environmental stresses that challenge plant growth and crop productivity worldwide (Sen and Mohammed, 1994). Producing sustainable and profitable crops under these conditions needs technological and biological approaches, including selection of new, more salt tolerant cultivars of named plants using conventional breeding programs or tissue culture techniques.

Date palm is an important source of income and nutrition in a number of countries. It is considered a salt tolerant plant species (Ismail et al., 1993). However, limited information is available concerning variation in salt tolerance among the cultivars currently grown (Al Juburi, 1992). Because of the difficulty involved in using conventional screening methods, the current study investigated the use of in vitro techniques to establish rapid, feasible and reliable approaches for screening date palm germplasm for salinity tolerance. The effect of NaCl on calli derived from immature embryos of four local date palm varieties was examined at two distinct stages, which were dedifferentiation and fast growing stages.

MATERIALS AND METHODS

Callus Induction and Growth on Media Containing NaCl

In order to verify the variation in salt tolerance at callus induction stage, immature embryos of four local date palm cultivars: Khlass (KL), Khnazy (KN), Merzaban (M) and

Khasbat Ausfoor (KA), were excised aseptically from surface sterilized, semisolid endosperms. The embryos were inoculated onto callus induction medium composed of modified Murashige and Skoog basal salts (1962) supplemented with 100 mg 2,4-D and 3 mg 2iP (Tisserat, 1979) and different levels of NaCl (0.0, 0.5, 1.0, 1.5 and 2.0% (w/v)). The cultures were incubated in a temperature controlled growth room at a constant temperature of 28±2^{o}C in complete darkness. Twenty-four weeks after inoculation, the percentage of callus induction was evaluated. Numerical scoring was used to indicate callus size. Callus fresh and dry weight was measured.

For the fast growing stage, 200-300 mg pieces of morphologically similar calli were selected and inoculated onto basal induction medium devoid of growth regulators but amended with the above levels of NaCl. Cultures were incubated in a temperature controlled growth chamber with a program of daily exposure to 16 hours of daylight at 28^{o}C and 8 hours of complete darkness at 24^{o}C. After six weeks, tissues were harvested and the final fresh and dry weight was determined.

Estimation of Na^+, K^+ and Proline

In both systems the concentrations of Na^+ and K^+ were measured using a flame photometer after tissue digestion with concentrated nitric acid. Proline accumulation was evaluated in the calli using Ninhydrin reagent as described by Bates et al. (1973).

Data Analysis

The results obtained were assessed by standard analysis of variance for a randomized complete block design. Overall means were compared using LSD test. The interrelationship between the measured characters of the two types of calli was examined by correlation analysis using the genotype treatment means.

RESULTS

Assessment of Salt Tolerance

Salt tolerance assessed on the basis of callus induction percentage (Ci%) and callus numerical scoring (CiNS) showed a strong, significant positive correlation ($r=0.946$; $P<0.001$) to callus fresh weight. However, for the fast growing screening technique, salt tolerance was evaluated on the basis of callus growth rate (CuGR), which was calculated as the difference between the final and the initial fresh weight over the time of incubation:

CuGR (mg/wk) = (Final fresh weight (mg) – Initial fresh weight (mg)) / 6 weeks.

Salinity markedly affected Ci%, CiNS and CuGR ($P<0.001$). The LSD test revealed that as salt was increased, the overall Ci%, CiNS and CuGR decreased (Table 1). Callus induction technique was capable of detecting significant variation among the genotypes ($P<0.001$). All of the genotypes exhibited a significant reduction in Ci% and CiNS. Nevertheless, the intensity of these reductions varied between the genotypes (Fig.1.a and b). In contrast, the fast growing callus technique was ineffectual in detecting significant differential genotype performance under the various salt treatments. However, when absolute means were expressed as relative values (% of the control), variation between the genotypes was noticeable (Fig.1.c).

Callus Dry Weight Ratio

Salinity exhibited a remarkable ($P<0.001$) effect on callus dry weight ratio (DWR) (Table 2). However, a significant ($P<0.001$) variation between the response of the genotypes to the different concentrations of salt was noticed in the callus induction system only ($P<0.001$).

Na^+, K^+ and Proline Content

Callus Na^+ content increased dramatically with increasing NaCl levels, whereas K^+ content decreased causing a significant reduction in tissue K^+/Na^+ status (Table 2). Results of the analysis of variance revealed that the progeny of the four cultivars

exhibited differential performance in terms of Na^+ influx ($P<0.001$) but their ability to exclude K^+ was comparable in the two screening methods (Fig. 2).

Proline increased significantly ($P<0.001$) in both types of calli in response to the added salt (Table 2). Unlike the fast growing stage, calli of the induction stage exhibited differential genotype performance under the distinct salt treatments ($P<0.01$). The relative values (Fig. 2) clearly revealed that during the callus induction stage, the increase in endogenous free proline was more pronounced in the calli of M progeny, whereas the fast growing calli of KN and KA exhibited the higher levels of proline production. Interestingly, the proline content of the induction system did not exhibit significant correlation ($r=0.294$; $P>0.05$) with the proline content of the induction stage (Table 3).

DISCUSSION

This investigation aimed to evaluate the validity of implementing two distinct in vitro techniques to screen date palm genotypes for salt tolerance. The results obtained from this study suggested that employing a callus induction screening method might be more efficient than the fast growing callus technique to identify variation in salt tolerance among date palm genotypes. The callus induction technique was capable of pinpointing significant variation among the genotypes. In contrast, the analysis of variance did not give assurance of the capability of the fast growing calli, which lacked the structural integrity of higher plants, to detect variation in salt tolerance among the various genotypes. However, the relative values (% of the control), which eliminated the underlying differences suggested that the fast growing callus technique has the potential to perceive variation between the genotypes. The inability of the system to pinpoint significant differential performance might be related either to the technique, which was not sensitive enough to detect the variation, or to the degree of the variation in salt tolerance between the genotypes, which was not large enough to be detected. The wide range of genetic diversity found among the progenies of each cultivar could be a crucial elucidation for the inability of the technique to perceive significant variation in salt tolerance.

Salinity inhibited date palm immature embryo dedifferentiation and hampered the growth of the fast growing calli. The weak correlation detected between the growth parameters of the two stages indicated that the effect of salt on callus growth varied according to the level of tissue organization. Consequently, the growth behavior at the callus induction stage cannot be used to predict the growth behavior of the vigorous calli.

The fast growing calli showed tolerance to the lower level of NaCl added to the medium. They continued to grow normally without any sign of cell dehydration or salt induced injuries. This may point to the suitability of the osmotic potential of the medium for date palm cell growth and vigorous proliferation. The results showed that callus Na^+ content increased significantly in relation to this level of salt whereas the K^+ content remained relatively constant. This suggested the possibility of inorganic ion involvement in the internal osmotic adjustment under this level of salinity. The fact that proline did not increase significantly, may indicate that this physiological response did not contribute in the subcellular osmotic adjustment under this level of salt.

Both types of calli exhibited symptoms of tissue dehydration. This could have been a result of direct contact between calli cells and the saline nutrient media that has a lower osmotic potential. Under these conditions water flow can be diverted from the cells into the surrounding medium causing cell water deficit. An alternative interpretation could be the over accumulation of salts in the apoplast rather than the central vacuoles, which creates a water potential gradient and prevents the flow of water into the cells symplast (Gibbs et al., 1989). This physiological imbalance reduces the efficiency of the cells to take up essential nutrients and growth regulators that are required for growth and morphogenesis.

Plants rely on several cellular mechanisms to tolerate salinity including salt accumulation, which have been recorded in many halophytes and salt tolerant glycophytes to maintain cellular osmotic adjustment (Jacoby, 1994). In both growing

systems, callus Na^+ content increased dramatically along with increasing salt level, whereas callus K^+ content decreased under the same conditions. The increase in Na^+ contents is possibly an osmotic adjustment mechanism, which enables the tissues to adapt to the low water potential of the external environment (Sabbah and Tal, 1990). Whereas the reduction in the K^+ content might have resulted from the competition of Na^+ with K^+ uptake or due to changes in the membrane integrity causing a significant reduction in callus K^+/Na^+ status (Lazof and Cheeseman, 1988). The ion contents of the two types of calli showed relatively strong correlations. This suggests that Na^+ influx and K^+ efflux mechanisms may operate similarly under the two distinct levels of tissue organization. Therefore, the variation in salt tolerance could be due to active participation of other mechanisms or be related to the structural variation between the two types of calli. The other consideration could be related to the technique and the time of exposure to salinity.

The high degree of endogenous free proline accumulation in date palm calli in response to salinity stress is a universal phenomenon (Lutts et al., 1996). Usually proline is considered as a compatible osmoticum, which adjusts the intracellular osmotic pressure between the cytoplasm and the vacuole that accumulates salts (Perez-Alfocea, 1994). Proline accumulation was more pronounced in the calli of M progeny, which exhibited higher percentage of callus induction. On the other hand, proline accumulation was more noticeable in the fast growing calli of KN and KA genotypes, which exhibited higher relative growth rate. This may suggest that the better dedifferentiation process and the most vigorous growth could be related to concentration of the endogenous proline. Therefore, proline could be used as an indicator of salinity tolerance in date palm cult ures at the cellular level of organization.

During the process of dedifferentiation, Na^+ accumulation along with maintaining relatively high levels of K^+ were clearly noticed in the calli of the progeny of KN cultivar, which exhibited the lowest percentage of callus induction. On the other hand, proline accumulation was more pronounced in the calli of M progeny, which exhibited higher percentage of callus induction. This suggested that osmotic adjustment, which assures efficient flow of water, nutrients and growth regulators into the plant tissues, could be a crucial factor to achieve salt tolerance during callus establishment. Therefore, the inability to maintain osmotic adjustment could be an explanation for the low salt tolerance recorded for KN progeny during the dedifferentiation process. The high levels of salts in their calli could be accumulated in the apopast instead of the cell vacuoles. This can divert the flow of water, nutrients and growth regulators needed by the explant to undergo dedifferentiation. In contrast, the better performance of the M progeny could be related to their ability to achieve intracellular osmotic adjustment by accumulating high levels of proline in their cytoplasm and sequestering the toxic ions in the central vacuoles.

For centuries emphasis has been directed towards female clonal propagation, which reduced the genetic diversity of date palms and increased the chances of susceptibility to biotic and abiotic stress (Mills, 1989). Therefore, immature embryos, which can easily be dedifferentiated into calli and regenerate plantlets, may offer the potential for selection from the pre-existing date palm gene pool for enhanced salt tolerant genotypes. Furthermore, the possibility of involvement of spontaneous mutation (somaclonal variation), should be considered during implementing somatic embryogenesis, especially with high levels of 2,4-D commonly used for callus induction (Saker et al., 2000). However, it is of great interest to determine if the salt tolerance expressed within the cellular level will be transmitted into the regenerated whole plants and their progeny. If this trait proved to have a genetic basis, then a new avenue is opened for the use of tissue culture techniques to improve date palm salinity tolerance.

The current work opened the opportunity for further intensive research to promote salt tolerance in date palm trees (*Phoenix dactylifera* L.). Including mature plants and a wider range of germplasm is the key for ultimate screening protocols. One of the things that may help in developing new screening protocols, and/or improving the proposed procedures, is the broader understanding of the physiological mechanisms involved in date palm salinity tolerance. More work is needed to elucidate the comparative

interrelationship between the physiological mechanisms and the degree of salinity tolerance in the different cultivars at various levels of tissue organization and at different stages of the plant life cycle before a complete picture of salinity tolerance traits can be applied in screening programs.

ACKNOWLEDGEMENTS

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Tables

Table 1. Overall means of the measured growth parameters of date palm calli in response to different levels of salt.

NaCl%	Callus Induction Stage		Fast Growing Callus Stage
	Ci%	CiNS	CuGR (mg/wk)
0.0	100 [a]	6.4[a]	65.8 [a]
0.5	98.5[a]	5.6[b]	71.7 [a]
1.0	89.2[b]	4.0[c]	38.6 [b]
1.5	76.0[c]	3.0[d]	29.6 [b]
2.0	34.8[d]	1.5[e]	27.6 [b]
$LSD_{0.05}$	6.4	0.29	13.8

Table 2. Overall means of the measured parameters of date palm calli in response to different levels of salt.

NaCl%	DWR		Na^+ content (ppm g^{-1} DW)		K^+ content (ppm g^{-1} DW)		Proline content (µmolg^{-1} FW)	
	(Ci) [s]	Cu	(Ci)[lo]	Cu	(Ci)[lo]	Cu	(Ci)[l]	Cu
0.0	0.29[a]	0.108[a]	3.84[a]	7279.7[a]	4.32[a]	4.41[a]	1.56[a]	63.3 [a]
0.5	0.32[b]	0.111[a]	4.36[b]	20479.7[b]	4.20[b]	4.37[a]	1.82[b]	93.5[ab]
1.0	0.37[c]	0.114[a]	4.41[c]	28869.5[c]	4.17[bc]	4.16[b]	2.38[c]	95.8[ab]
1.5	0.40[d]	0.125[b]	4.48[d]	33815.7[d]	4.09[c]	4.14[b]	2.55[c]	136.3[b]
2.0	0.39[d]	0.135[c]	-	41033.7[e]	-	4.14[b]	2.38[c]	199.7[c]
$LSD_{0.05}$	0.01	0.009	0.045	4174.6	0.09	0.07	0.21	62.2

Means followed by different letters are significantly different using LSD test at p=0.05.
[l]: Data were transformed to the logarithmic scale.
[s]: Data were transformed to the square root scale.
[o]: The 2% (w/v) NaCl was omitted to maintain homogeneous error variances.

Table 3. Correlations between the in vitro measured characters at callus induction and growth level of organization.

Fast Growing Callus Characters	Callus Induction Characters				
	CiNS	$(Na^+)^l$	$(K^+)^l$	$(K^+/Na^+)^t$	$(Proline)^l$
CuRGR	0.383^{***}	-0.426^{**}	0.213^{ns}	0.460^{***}	-0.321^{**}
Na^+	-0.714^{***}	0.825^{***}	-0.654^{***}	-0.835^{***}	0.531^{***}
$(K^+)^l$	0.525^{***}	-0.647^{***}	0.515^{***}	0.652^{***}	-0.484^{***}
$(K^+/Na^+)^t$	0.661^{***}	-0.915^{***}	0.603^{***}	0.932^{***}	-0.600^{***}
Proline	-0.348^{***}	0.162^{ns}	-0.091^{ns}	-0.254^{ns}	0.099^{ns}

***, **, *, ns Correlation is significant at 0.001, 0.01, 0.05 level and not significant respectively (2-tailed).
[t]: Data+1 were transformed to the logarithmic scale.
[l]: Data were transformed to the logarithmic scale.
[s]: Data were transformed to the square root scale.

Figures

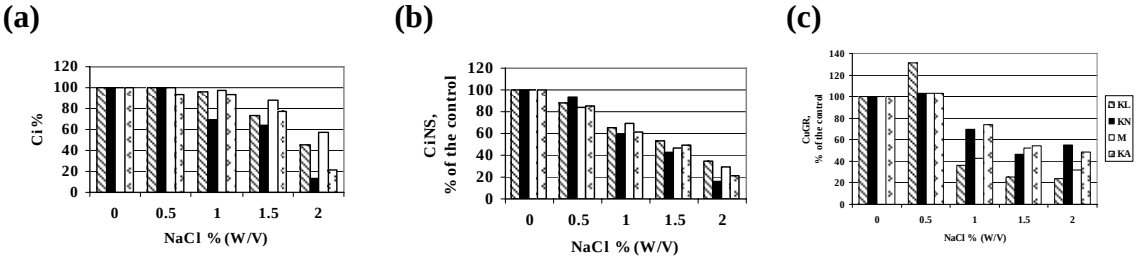

Fig. 1. The relative values (% of the control) of Ci% (a), CiNS (b) and CuGR (c) on different levels of salt.

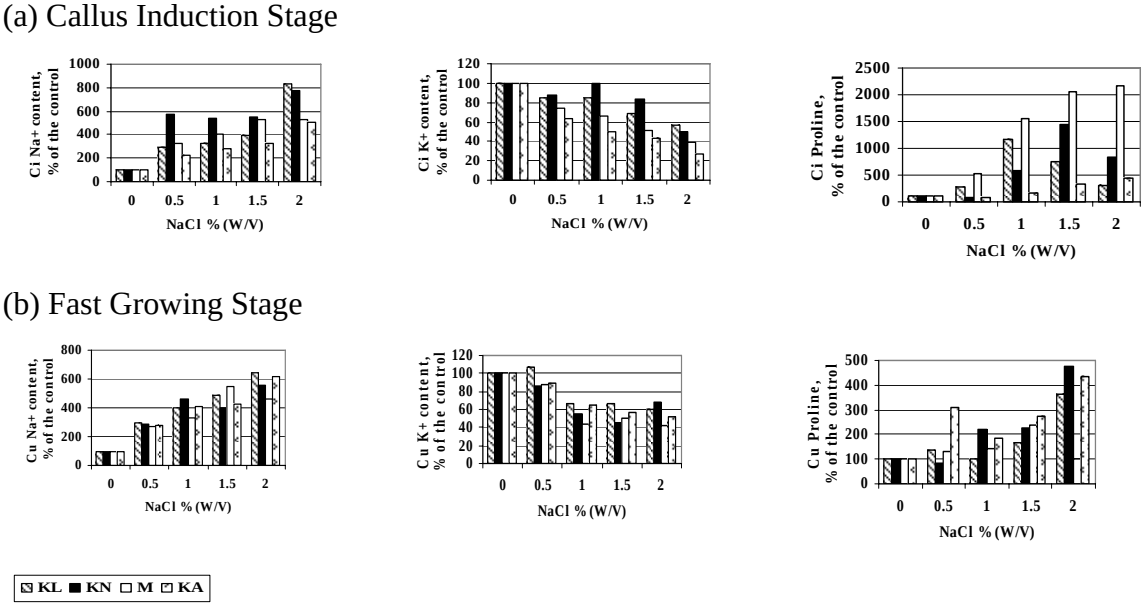

Fig. 2. The relative values of Na^+, K^+ and praline content of date palm calli subjected to different levels of NaCl at callus induction (a) and fast growing (b) stages.

Crop Water and Irrigation Water Requirements of Date Palm (*Phoenix dactylifera*) in the Loamy Sands of Kuwait

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Keywords: arid environment, evapotranspiration, FAO cropwat, irrigation schedule, potential evapotranspiration

Abstract

An investigation was undertaken in Kuwait University to quantify the crop water and irrigation water requirements of date palm grown in the loamy sands of Kuwait. FAO CROPWAT decision support system was used for this purpose. Agro-meteorological data of 43 years were used to estimate the potential evapo transpiration and water requirements of date palm. The annual potential evapotranspiration (ETo) of Kuwait was estimated as 2883mm. The annual crop water requirement (ETc), irrigation requirement (IR) and net irrigation requirement (NIR) of date palm were estimated as 2685mm, 2553mm and 2563.9mm, respectively. The monthly ETc varied from 74mm (January) to 392mm (June). The daily NIR of date palm varied from 97 L tree^{-1} d^{-1} (28th November to 31st December) to 854 L tree^{-1} d^{-1} (19th June to 26th June). An irrigation schedule for date palm grown in the loamy sands of Kuwait was also developed.

INTRODUCTION

Kuwait is one of the smallest countries of the Middle East. Soils are mostly entisols with low water-holding capacity and high susceptibility to wind erosion. The clay and organic matter content is low indicating poor soil fertility. Date palm is one of the most important crops of this country with 1400 hectares under cultivation with this crop (http://www.fao.org/faostat/servelet3?Areas).

In an extremely dry environment with harsh climate and poor soils, agriculture without irrigation is impossible. The efficiency of water use in irrigated agriculture assumes greater significance particularly in semi arid environments (Hatfield et al., 1996). Information on scientific irrigation scheduling is meager for crops in Kuwait. Indirect methods using evapotranspiration measurements are widely used to develop irrigation schedules in many countries.

There are different approaches to developing irrigation schedules. One method is the “water balance” approach, which involves keeping an account of water input into the soil and water output on a daily basis (Doorenbos, 1976). Reference crop evapotranspiration (ETo) is the evapotranspiration rate from a reference surface that is not short of water. It expresses the evaporating power of the atmosphere at a specific location and time of year and does not consider the crop characteristics and soil factors. Reference crop evapotranspiration is also known as potential evapotranspiration (http://www.fao.org/docrep/X0490e/x0490e04.htm). It is different from actual evapotranspiration. Evapotranspiration of a crop (ETc) can be related to the ETo of the area as it is independent of factors other than climate. As such the information on ETo of an area will be a very useful guide for development of irrigation schedules for crops. The important weather data required for the estimation of ETo are air temperature, humidity, wind speed and sunshine hours. A computer program (Hess, 1996) or a spread sheet (Hess and Stephens, 1993) can be used to calculate ETo using the Penman or Penman-Monteith equation. This method has been shown to be reliable in a wide range of environments (Allen et al., 1994). Most water balance irrigation schedule methods are based on a daily estimate of the ETo which is then modified according to the type of crop, stages of growth and soil water content (Hess, 1996). Penman-Monteith equation should

give the best estimate of ETo where daily weather data are available (Hess, 1996). Allen (1998) reports guidelines for computing crop water requirements based on evapotranspiration.

Though several models have been proposed to predict ETo, there is no universal consensus on the suitability of any given model for a given climate, thereby prompting Smith et al. (1996) to conclude that these models require rigorous local calibration before they can be used for the estimation of ETo for irrigation scheduling. The local calibration and validation are more important in semi-arid environments than temperate, because almost all the ETo models were developed, calibrated, and validated for temperate environments using reliable and long term weather data (Jensen et al., 1990; Smith et al., 1996; Allen et al., 1998; Ventura et al., 1999).

For local calibration, the methodology published first in 1974 as bulletin number 24 in the FAO Irrigation and Drainage series and revised in 1977 (Doorenbos and Pruitt, 1977) is widely followed internationally. Penman model frequently overestimated ETo, while the other models showed variable adherence to grass reference. Further, the FAO-24 method assessed for a humid temperate environment in Tottori, Japan (Yano and Hayashi, 1977) using long-term weather data (1952-1974) indicating that Penman and Radiation balance models produced similar ETo estimates. The ETo estimates obtained from six commonly used ETo estimation models indicate that Penman-Monteith method produced the most reliable estimates compared to lysimeter data for the semi-arid Karaj region, in Iran (Hossein et al., 2004).

Trials in Iraq with 20-year-old palms revealed that the total water consumption of the palms was 18000m^3/ha annually (Abou-Khaled et al., 1982). According to Al-Amoud et al. (2000) water use efficiency of date palm was the maximum for trickle-irrigated plots followed by the basin plots.

There are seasonal variations in the water demand of date palm. The irrigation requirements in summer (May to August) are considerably higher compared to the winter period. Irrigation requirement vary between regions of the same country due to differences in soil and climatic conditions. The date growing area of Sahara needs 34190m^3 per hectare per year while Ziran region needs only 15000m^3 per hectare per year. The planting density varies from 120 per hectare (Ziran) to 144 per hectare in Algeria (FAO) (http://www.fao.org.documentry show_cdr.asp?url)

The irrigation requirements of date palm of varying countries as estimated by FAO show wide differences between countries. The values range from 13000 - 20000 m^3 ha^{-1} in Morocco, to 27000 – 36000 m^3 ha^{-1} in California (USA). The irrigation requirement of date palm in Algeria is 15000-35000 m^3 ha^{-1}, in Egypt 22300 m^3 ha^{-1}, in Tunisia 23600 m^3 ha^{-1}, in India 22000-25000 m^3 ha^{-1}, in Iraq 15000- 20000 m^3 ha^{-1}, in Jordan 25000-32000 m^3 ha^{-1}, and in South Africa 25000 m^3 ha^{-1} (http://www.fao.org//docrep/006/y4360e/y4360e0b.htm).

Considering the importance of dates in Kuwait, an attempt was made to quantify the crop water requirement and irrigation water requirement of this crop and to develop an irrigation schedule for the loamy sands of the country.

METHODOLOGY

Collection of Climatological Data

The climatic data such as air temperature, air humidity, rainfall, pan evaporation, wind speed and sunshine hours recorded at the Kuwait International Airport (KIA) were used for the study. The mean data of 1962 to 2004 (43 years) were estimated to calculate potential evapotranspiration and water requirements.

Collection of Soil Data

Kuwait Institute of Scientific Research (KISR) conducted a detailed soil survey of Kuwait and published the results. Soil data such as available water holding capacity, soil depth, bulk density, texture etc. were collected from the soil survey report (KISR, 1999).

Soils of Kuwait vary considerably with respect to their physical and chemical properties. This study focused on the agricultural soils of Wafra where date palm is being grown. No restricting layers were seen up to 1.5 m in depth. The clay content of the soil varied from 4.1 to 10 percent, silt content varied from 2.7 to 21 percent and sand content varied from 87.9 to 93.2 percent. The bulk density of the soil ranged from 1.83 to 1.88 g cm^{-3}. The moisture content of the soil at field capacity was 9 to 10 percent and at permanent wilting point 3 to 4 percent. The available water holding capacity varied from 5 to 7 percent. The pH of the soil varied from 7.8 to 8 and the electrical conductivity (EC) varied from 0.3 to 1.5 dSm^{-1} (KISR, 1999). Textually soils were loamy sands or sand.

Crop Data of Date Palm

Date palm cultivation in Kuwait is concentrated in the Wafra and Abdali areas. Elite varieties like Barhee are being grown at a spacing of 7m x 7m to 8m x 8m. The pit method of planting is adopted. Basins of 1.5m to 2m width are taken around the tree and water is applied in these basins. Certain plantations use tissue culture plants and adopt the drip method of irrigation. Currently irrigation is being done without any scientific basis. Bore well water is mostly used for irrigation and the water is saline.

Estimation of Water Requirements

The reference crop evapotranspiration (ETo), crop water requirement (ETc), irrigation requirement (IR), net irrigation requirement (NIR), the effective rainfall (ER) and the irrigation schedule (IS) of date palm were estimated following the FAO Penman-Monteith method using the CROPWAT decision support system. (Doorenbos and Pruit, 1977). Calculations of crop water requirements and irrigation water requirements were carried out with inputs of climatic, soil and crop data. The development of irrigation schedule was based on a daily soil-water balance. ETc was estimated as a product of ETo and crop coefficient (Kc). The irrigation requirements (IR) were estimated as difference between crop water requirement and effective rainfall. The irrigation water requirement also included additional water for leaching of salts and to compensate for non-uniformity of water application. Effective rainfall was that fraction of the total rainfall that forms part of the consumptive use of the crop. It was estimated by the procedure of Doorenbos and Pruit (1977). The term 'net irrigation requirement' (NIR) refers to the actual quantity of water that is to be applied to the soil as irrigation water. The term irrigation schedule (IS) refers to the quantity and frequency of irrigation during the growing season. The irrigation schedules consider the available water holding capacity of the soil as well.

RESULTS AND DISCUSSION

Climate

Kuwait climate is characterized by extremely high temperatures during summer, short mild winters, high sunshine hours, low humidity and general dry conditions. The average daily maximum temperature varies from 18.7^{o}C during January to 46.1^{o}C in July. The average daily minimum temperature varies from 7.8^{o}C during January to 29.4^{o}C during July. The mean temperature varies from 13.2^{o}C during January to 38.5^{o}C during July.

The total annual rainfall of the area is 138 mm of which 133 was effective rainfall. This rainfall is received mostly from December to April. December and January receive the highest rainfall. There is almost no rain during summer. Rain-fed agriculture is not possible in Kuwait because of the extreme scarcity of rainfall. The mean relative humidity ranges from 17.7 percent during July to 65 percent during January. May to October forms the dry period and November to April forms the cool period.

The wind speed ranges from 252.3 kmd^{-1} during October to 437.2 kmd^{-1} during June. The prevailing winds in Kuwait are from the northwest and the southeast. In the summer months between June and September, the monsoon depression affects the northwesterly winds, which form 59 percent of the total wind (Khalaf, 1989). Dust and

sand storms are typical of Kuwait and occur throughout the year. However, according to Khalaf and Al Ajmi (1993) they are more frequent in spring and mid summer (March to August). The mean sunshine hours range from 6.2 h d^{-1} during December to 11.1h d^{-1} during June. Solar radiation ranges from 11.3 MJ m^2 d^{-1} during December to 26.7 MJ m^2 d^{-1} during June.

Reference Crop Evapotranspiration (ETo)

The ETo values vary from 2.75 mm d^{-1} during January to 14.03 mm d^{-1} during June. The total annual ETo is estimated as 2883 mm. The results indicate that the evapotranspiration demand of Kuwait environment is very high. Being an arid environment with high temperature, wind speed and solar radiation, it is natural that the ETo values are high compared to the other regions. As ETo plays a vital role to decide the ETc of a crop, it is probable that the water requirements of crops in this region could be relatively high. Ullah et al., 2001 reported the potential evapotranspiration in the Indus river basin in Pakistan as 1200 to 1300mm (in the upper and northern part of the basin) and 1700 to 2100mm (in the lowest part of the basin). The high ETo values in Kuwait are mainly due to the harsh weather conditions.

Effective Rainfall (ER)

Of the total annual rainfall of 138 mm, 133 mm formed ER. This fraction contributed to the consumptive use of the crop.

Crop Water Requirement (ETc)

Crop water requirement is the quantity of water needed for normal growth, development and yield and may be supplied by precipitation or by irrigation or by both. The annual ETc of date palm was estimated as 2685mm (Table 1). The ETc was the lowest during the month of January (74mm) and highest during June (392mm). Date palm is a tree crop with a planting density of 156 to 204 trees per hectare per year (spaces: 8m x 8m to 7m x 7m). Spacing depends upon the variety and the soil conditions. The soils being sandy, drip irrigation method is most appropriate.

Irrigation Requirement (IR)

The annual IR of date palm was estimated as 2553 mm (25530 m^3 ha^{-1}) (Table 1.). The monthly IR varied from 41 mm in January to 392 mm during June. The irrigation requirement of date palm is reported as 27000 m^3 ha^{-1} to 36000 m^3 ha^{-1} for California, 15000 m^3 ha^{-1} to 35000 m^3 ha^{-1} for Algeria, 23600 m^3 ha^{-1} for Tunisia, 22000 m^3 ha^{-1} to-25000 m^3 ha^{-1} for India, 25000 m^3 ha^{-1} to 32000 m^3 ha^{-1} for Jordan (http://www.fao.org//docrep/006/y4360e/y4360e0b.htm). The irrigation requirement of date palm estimated for Kuwait is comparable to that of the above countries. The climate, particularly temperature, humidity and wind velocity, decide the potential evapotranspiration and thus the irrigation requirement. Kuwait has a harsh climate with high temperatures during summer (June to August) and very cool period during winter (December to February).

Net Irrigation Requirement (NIR)

The term net irrigation refers to the actual quantity of water that is to be applied to the soil as irrigation water. The annual NIR of date palm is estimated as 25639 m^3 ha^{-1} (Table 2). The NIR was lowest during December and highest during June to August. Several factors like soil salinity, temperature, humidity, wind speed and cloudiness influence the water requirements of date palm. In areas with high temperature the rate of evaporation will be more and the water requirement will also be more. The lower the humidity level, the more water is needed. Similarly high constant wind speed causes high evaporation and thus higher water demands. Water demand will be more during periods of high sunshine. All these factors influence evapotranspiration which strongly determines the water requirement.

Irrigation Scheduling

An irrigation schedule developed for date palm in the loamy sands of Kuwait is shown in Table 2. The NIR of date palm ranges from 97 L tree^{-1} d^{-1} during December to 854 L tree^{-1} d^{-1} during June. The water is to be applied within the boundaries of the root zone. Localized irrigation (drip irrigation) would be more efficient than non-localized (flood irrigation). It would be possible to reduce the irrigation requirement by adopting efficient irrigation methods and also by restricting water application to the active root zone.

SUMMARY AND CONCLUSIONS

The crop water requirement of date palm in Kuwait is estimated as 2685mm and the net irrigation requirement as 2553mm. These values, however, appear to be high. The irrigation schedule developed in this study needs test verification by conducting field experiments, in order to confirm the results.

ACKNOWLEDGEMENTS

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Tables

Table 1. ETo, ETc, TRF, EF and IR of date palm in the loamy sands of Kuwait.

Period	Duration (days)	ETo (mm/period)	Crop Kc	ETc	TRF (mm/period)	EF	IR
1 Jan to 30 Jan	30	83	0.9	74	35	33	41
31 Jan to 1 Mar	30	116	0.9	104	15	14	90
2 Mar to 31 Mar	30	171	0.9	154	15	15	139
1 Apr to 30 Apr	30	236	0.9	212	11	11	201
1 May to 30 May	30	322	0.9	291	3	3	288
31 May to 29 Jun	30	418	0.9	392	0	0	392
30 Jun to 29 Jul	30	409	1.0	388	0	0	388
30 Jul to 28 Aug	30	374	1.0	355	0	0	355
29 Aug to 27 sep	30	304	1.0	288	0	0	289
28 Sep to 27 Oct	30	209	1.0	199	4	4	195
28 Oct to 26 Nov	30	139	1.0	132	16	15	117
27 Nov to 31 Dec	35	104	0.9	96	39	37	58
Total	365	2883		2685	138	133	2553

ETo = Reference crop Evaporation - mm
ETc = Crop Water Requirement- mm
TR = Total Rain fall -mm
ER = Effective Rainfall - mm
IR = Irrigation Requirement- mm

Table 2. Irrigation schedule for date palm in the loamy sands of Kuwait.

Period	Duration days	NIR* (m^3 ha^{-1} $period^{-1}$)	NIR (L $tree^{-1}$ d^{-1})
1/1- 1/2	32	514	103
2/2- 18/2	17	517	195
19/2- 4/3	14	518	237
5/3- 15/3	11	513	299
16/3- 26/3	11	513	299
27/3- 4/4	9	539	384
5/4- 12/4	8	546	438
13/4- 20/4	8	528	423
21/4- 28/4	8	546	438
29/4- 4/5	6	549	587
5/5- 10/5	6	575	614
11/5- 16/5	6	575	614
17/5- 22/5	6	576	615
23/5- 28/5	6	582	622
29/5- 2/6	5	584	749
3/6- 6/6	4	520	833
7/6- 10/6	4	524	840
11/6- 14/6	4	527	845
15/6-18/6	4	532	853
19/6- 22/6	4	533	854
23/6- 26/6	4	533	854
27/6- 30/6	4	529	848
1/7- 4/7	4	517	829
5/7- 8/7	4	517	829
9/7- 12/7	4	517	829
13/7- 16/7	4	517	829
17/7- 20/7	4	517	829
21/7- 24/7	4	517	829
25/7- 28/7	4	517	829
29/7- 2/8	5	611	783
3/8- 7/8	5	588	754
8/8- 12/8	5	588	754
13/8- 17/8	5	588	754
18/8- 22/8	5	588	754
23/8- 27/8	5	588	754
28/8- 1/9	5	541	694
2/9- 7/9	6	564	603
8/9- 13/9	6	564	603
14/9- 19/9	6	564	603
20/9- 25/9	6	564	603
26/9- 2/10	7	558	511
3/10- 11/10	9	553	394
12/10- 20/10	9	553	394
21/10- 29/10	9	560	399
30/10- 12/11	14	539	247
13/11- 27/11	15	525	224
28/11- 31/12	34	512	97
Total	365	25639	

***** NIR = Net Irrigation Requirement**.**

Effect of Pollination Method, Fertilizer and Mulch Treatments on the Physical and Chemical Characteristics of Date Palm (*Phoenix dactylifera*) Fruit I: Physical Characteristics

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Abstract

Khalas and Khasab date cultivars were fertilized by urea, triple superphosphate, potassium sulphate with or without micronutrients and date palm fiber mulch. Half the palms were pollinated by hand and the other half by hand-pollen duster (mechanical) in a CRD design with 3 replications for 3 successive years. Fruit length, diameter, weight, number and weight /strand and total yield were determined. Pollination effect was not significant on Khalas and Khasab fruits number and weight / strand and fruit diameter. Khalas yield was higher after mechanical pollination, but with lower fruit length and weight than hand pollination. This effect was not significant on Khasab. Fertilizer effect was not significant on producing longer Khalas fruits than NPK supported by mulch. Khasab higher yield and longer fruits of larger diameter were produced by NPK with micronutrients and mulch than mulch alone on yield and than all other fertilizer treatments on fruit length and diameter. Interaction effect for pollination method and fertilizer mulch was not significant on all the tested physical parameters. But certain variations indicated an important trend that can furnish guide lines for improving date physical qualities, soil conditioning and reduced cost of production. The general trend with most of fertilizer /mulch treatments indicates that Khalas yield was higher by mechanical whereas, Khasab by hand pollination than mechanical. Mulch increased Khalas yield by mechanical pollination when NPK and micronutrients were applied by 25% over NPK alone. The same treatment combination produced the highest Khasab yield by mechanical pollination. It consistently produced the longest Khasab fruit with largest diameter and heavier single fruit by mechanical pollination than other treatments. Application of NPK with micronutrients and mulch produced comparable Khalas fruit length and diameter and larger than the average Khalas fruit size in Oman. It can be concluded that application of NPK and micronutrients supplemented by date palm fiber combined with mechanical pollination produced the most desirable physical characteristics than most of the treatments. And discrepancies from this could easily be ameliorated or overridden by economical and environmental benefits.

INTRODUCTION

Pollination is considered the most critical operation that directly affects date palm production and date characteristics. This is because the time for its completion in limited, depending on varietal characteristics and environmental conditions (Hussein et al., 1977; Bacha et al., 1988). Likewise, sexual compatibility between the male and female palms influences fruit set percentage and fruit characteristics (Hijazy et al., 1983; Abo Hassan et al., 1983). Pistillate flowers differ in the duration they remain receptive (Al Bert, 1930; Al Bakr, 1982, Al Juburi, 1993; Mekki et al., 1998), and some reports indicated differences among female cultivars in the amount of pollen which produced the best yield (Nixon, 1954; Dowson, 1982; Al Juburi, 1993).

The vast increase in date palm numbers around the world is accompanied by a drastic reduction of experienced hand pollinators. The number of date palms in the

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

Sultanate of Oman in 1979 was 6 million; in 2003 it reached > 8 million. This situation prompted the concerned date palm growers and researchers to look for an alternative method of pollination. Since the dusting device that was developed by Monciero in 1954, several attempts have been made to improve pollen dusters (Furr and Hewitt, 1964; Brown et al., 1970; Al Juburi, 1972; Shabana, 1988). Besides the value of mechanical pollination in solving the problem of scarcity of pollinators, it also saves pollen, time and reduces production costs (Khalil and Al Shaawan, 1983; Shababa, 1988). Moreover, mechanically pollinated palms produced similar yield, fruit size, and weight (Brown et al., 1970; Al Juburi, 1995; Furr and Hewitt, 1964; Shabana et al., 1988).

MATERIALS AND METHODS

Thirty six date palms of each of Khalas and Khasab cultivars were treated in a completely randomized factorial experiment as follows:

a. Fertilizer and Mulch: 1. Control without fertilizer and mulch; 2. 1000g urea with 800g K_2SO_4 (equal doses in March, May, July and October) + 500 g triple super phosphate (200g in March, and 100g in each of May, July and October), (hereafter referred to as NPK); 3. NPK was applied with or without micronutrients and with or without date palm fiber mulch as shown in Table 2 of the results.

b. Pollination: 1. half of the palms were pollinated by applying 6 male strands/ Khasab female spadix (inflorescence) and 3 strands /Khalas spadix. 2. The other half of the date palms were pollinated mechanically using a hand pollen duster designed by the Ministry of Agriculture and Fisheries. Pollen was mixed with grade 1 wheat flour at the ratio of 1 part pollen to 5 parts flour. Both pollination treatments were done after the cracking of the spathe (cover of the inflorescence) using the same season dried pollen. The experiment ran for 3 years. Three strands were collected weekly from each treatment in the Bisir stage and placed in an ice chest containing ice and brought to the Horticulture Laboratory in the College of Agricultural And Marine Sciences, where the physical characteristics of the fruit were immediately measured. The number of fruits / strand was recorded first. Fruit length and diameter of 10 fruits of each treatment were measured by Vanier Caliper. Likewise, fruit weight of the same sample was determined using digital pan balance (Precisa XB 620C). The average length, diameter and weight were then calculated. Total yield was determined by summation of the weights of previously collected samples and the final harvest.

Data was statistically analyzed using SPSS. Software version 12.0.1 with General Linear Model (GLM) and Duncan's range test were employed to separate means.

RESULTS AND DISCUSSION

Effect of Pollination Method on the Physical Characteristics of Fruit from Khalas and Khasab cvs.

Data in Table 1 showed significant reduction in fruit length and weight of Khalas fruits when mechanical pollination was used, but higher yield was produced than with hand pollination. There were no significant differences in the number and weight /strand and diameter of Khalas fruit. In Khasab fruit no significant differences were detected in any of the physical characteristics as a result of the pollination method. These results are in partial agreement with Al Juburi (1995) Shabana et al. (1985).

Effect of Fertilizer and Mulch on the Physical Characteristics of Fruit from Khalas and Khasab cvs.

Fertilizer and mulch treatments did not produce any significant effect on fruit number, weight per strand and fruit weight of Khalas (Table 2) and Khasab (Table 3) fruits. In Khalas fruit there were no significant differences in diameter and yield. However, the control produced the longest fruit and the treatment containing urea, super phosphate and potassium sulfate (NPK) with mulch, produced the shortest fruit, with no significant differences between these treatments and the others on fruit length. In spite of

insignificance in yield, NPK with mulch increased the yield of Khalas by 12.4% over the control and 2.3% over NPK without mulch. These results indicated the importance of mineral fertilizer combined with mulch on increasing yield. The effect of mulch on yield increase can also be observed when NPK and micronutrients were applied with or without mulch; it was higher with mulch by 8.14%.

Fertilizer and mulch produced significant effects on fruit length, diameter and total yield of Khasab. Significantly longer fruits with larger diameter were produced by the treatment containing NPK + micronutrients + mulch. Likewise it resulted in the highest yield, which was higher than when the mulch was excluded by 6.7% and than the mulch treatment by 14.87%.

Results from fertilizer and mulch treatments indicated the importance of mulch and mineral fertilizers (Hussein et al., 1977; Bacha et al., 1983). It appeared that the fertilizer treatments required a longer time to cause pronounced effects, and that the 3 years of this experiment may not have been long enough (Gahgah, 1993). Mulching has been practiced for a long time and proven effective on modifying soil temperature, conserving soil moisture, increasing root activity and nutrient and water uptake. Therefore, it is highly recommended in hot areas like the Sultanate of Oman (Janick, 1986; Hartmann et al., 1988.)

Interaction Effects of Pollination Method and Fertilizer/Mulch Treatments on Physical Characteristics of Fruits

The interaction effects of pollination method and fertilizer treatment were not significant on total yield, number and weight of fruits per strand, fruit length, diameter and weight of fruit from both Khalas and Khasab cultivars. But the following differences were observed and considered for discussion:

1. Khalas Total Yield. The interaction effect of pollination method and fertilizer/mulch treatments on Khalas yield is shown in Figure 1. With the exception of the application of mulch with NPK (t3) and without NPK (t4), all other treatments produced a higher yield by mechanical pollination than hand pollination (traditional). The highest yield of Khalas was obtained when mechanical pollination was used and NPK without mulch (t6 = 48.8 kg /palm) was applied, followed by NPK with micronutrients and mulch (t5 = 45.6kg /palm). Application of mulch increased the yield when NPK with micronutrients (t5) was applied by 25% over the treatment without mulch. But this effect of mulch disappeared when NPK was applied without the micronutrients as indicated above. Consequently treatment 5 (t5) can be recommended with the use of mechanical hand pollen duster. This treatment produced a similar yield to traditional hand pollination.

2. Khasab Total Yield. The interaction effect of pollination method and fertilizer /mulch treatment on Khasab total yield is shown in Figure 2. With the exception of NPK treatment (t6) all others produced a higher yield when hand pollination was used. The treatment of NPK with micronutrients and mulch (t5) produced the highest yields with both mechanical (64.4 kg) and hand pollination (66.5 kg). The lowest yields for the two pollination methods were produced when mulch without fertilizer (t4) was applied. Therefore, mulch alone is not sufficient to increase yield. It must be supplemented by mineral fertilizers.

The general trend showed Khalas yield was higher with mechanical pollination, whereas, Khasab produced higher yields with hand pollination. The increase in yield of Khalas and Khasab by the application of macro and micronutrients supported by mulching was likely due to improved physical condition of the soil and improved water and nutrient uptake (Hartmann et al., 1988). Mulch of date palm leaves was found to save water and reduce irrigation costs (Hussein et al., 1986). Moreover, mulch has been reported to increase yield (Chaudhry and chopra, 1983; Burgers and Nel, 1983); it also improved plant response to nitrogen fertilization (Famaco and Baustista). Fertilizer effect on yield of date palm has been reported by many researchers (Hussein et al., 1993; Bacha and Abo-Hassan, 1983). Moreover, there is accumulating evidence that there was no significant difference in yield of different date palm cultivars in different locations as a

result of pollination method (Brown et al., 1971; Shabana et al., 1988; Al Juburi, 1995). In order to obtain high yield with these two cultivars and to take maximum advantage of mechanical pollination and the application of mulch, it is recommended that NPK and micronutrients also be applied.

3. Khalas Fruit Length. The interaction effects of pollination method and fertilizer /mulch treatments on Khalas fruit length are shown in Figure 3. Except for t4 all others produced longer fruits by hand pollination than mechanical.

The longest fruit was produced by the control using hand pollination. This length was much exaggerated compared to the average normal Khalas fruit length of 3.4 cm as reported by Mekki et al., 1998. In general, Khalas fruits produced by hand pollination were longer than by mechanical pollination. Application of NPK with micronutrients and mulch and mechanical pollination produced the fruits with recommended length of 3.64 cm.

4. Khasab Fruit Length. The interaction effects of pollination method and fertilizer /mulch treatments on Khasab fruit length are shown in Figure 4. Except for t5 and t4 all other treatments produced longer fruits by mechanical pollination. Application of NPK with micronutrients and mulch consistently produced the longest fruits (3.04 cm), and is recommended with mechanical pollination which resulted in the normal fruit length (Mekki et al., 1998).

It was observed that fruits produced by the two cultivars using the two pollination methods and NPK with mulch (t3) and NPK without mulch (t6) were shorter than when they were supplemented by micronutrients. Therefore, mechanical pollination and NPK with micronutrients and mulch can be recommended for Khalas and Khasab.

5. Khalas Fruit Diameter. The interaction effects of pollination method and fertilizer /mulch treatments on the diameter of Khalas fruits are shown in Figure 5. Except for the control, all other treatments produced fruits with a larger diameter when hand pollination was used compared to mechanical. Applying NPK with micronutrients and mulch using mechanical pollination produced fruits that were 2.3 cm. in diameter compared to the average normal of 2.46. This small diameter was compensated for by the advantages of the treatment combination: the mulch improved the soil condition for root growth and function (Hartmann et al., 1988) and mechanical pollination resulted in lower costs and high efficiency (Shabana, 1988)..

6. Khasab Fruit Diameter. The interaction effects of pollination method and fertilizer /mulch treatments on the diameter of Khasab fruits are shown in Figure 6. The same treatment as was recommended above (t5) consistently produced the largest Khasab fruit diameter and it was larger by mechanical (2.38 cm) than hand pollination. This measurement is larger than normal, which is (2.28 cm) as described by Mekki et al., 1998. Other treatments produced similar fruit diameter. Therefore this treatment can be recommended for the two cultivars to produce suitable sized fruits and yield.

7. Khalas Number of Fruits/Strand. The interaction effects of pollination method and fertilizer /mulch treatments on Khalas number of fruits /strand are shown in Figure 7. With the exception of treatment 4, all others produced a larger number of fruits by mechanical pollination. The largest fruit number was obtained when mechanical pollination was used along with the application of NPK and mulch (t3), resulting in 7 fruits/ strand. By hand pollination with the application of mulch alone 7 fruits / strand were also produced. The latter produced the lowest number by mechanical (5 fruits /strand), whereas, t5 produced a little bit higher fruit number /strand. But fruits from t5 with mechanical pollination were heavy with a larger diameter. Thus it can be recommended. The low fruit number could be due to early dropping or low pollen concentration reducing fruit set. Such factors can interrupt the interaction effect of pollination method and fertilizer on fruit set (Furr and Hewilt, 1964; El – Kassas and Mahmoud, 1986; Mekki et al., 1998, El Mardi et al., 2002).

8. Khasab Number of Fruits/Strand. The interaction effects of pollination method and fertilizer /mulch treatments on Khasab number of fruits /strand is shown in Figure 8. Applying mulch with NPK fertilizer (t3) and mechanical pollination produced lower fruit

number (17 fruits) than when applied alone (t4) (21 fruits/ strand). This situation was reversed with hand pollination as t3 produced a larger number than t4. Application of NPK with micronutrients and mulch with mechanical pollination produced 21 fruits; therefore it can be recommended as it combines all favorable conditions for date fruit growth and development.

9. Khalas Fruit Weight. The interaction effects of pollination method and fertilizer /mulch treatments on Khalas fruit weight is shown in Figure 9. Khalas fruits responded differently by the application of NPK plus micronutrients (t2) and NPK alone (t6) from their response when applied with mulch and using the two pollination methods.???? The former with mulch (t5) produced heavier fruits than without mulch (t2), whereas, the latter without mulch (t6) produced heavier fruits than with mulch (t3).

The main difference between t5 and t3 was the addition of micronutrients in t5. The effect of micronutrients on weight can be observed by comparing t5 higher fruit weights to those of t3, indicating that the effect of mulch on increasing weight required micronutrients. Therefore NPK with micronutrients and mulch can be recommended with mechanical pollination even though it produced lower fruit weight (13.33 g) than t4 which contained only mulch (13.86). This was because higher yield was produced by the former (45.56kg/palm) than the latter (40.06 kg/palm), and also a larger number of fruits /strand, 6 and 5 respectively. It also assists in maintaining the nutritional status of the tree by continuous fertilizer supply. Note that Khalas average fruit weight in Oman is 9.6g (Mekki et al., 1998).

10. Khasab Fruit Weight. The interaction effects of pollination method and fertilizer /mulch treatments on Khasab fruit weight are shown in Figure 10. The treatment with NPK with micronutrients and mulch (t5) produced the heavier fruit by hand pollination (10.2 g) and by mechanical than other treatments (9.09g), except (t2) which was slightly heavier (9.1g). Note the average Khasab fruit weight in Oman is 8g (Mekki et al., 1998).

It is observed that the general trend indicated the higher the number of fruits in both cultivars the lighter the average weight of fruit; e.g. t5 produced <6 fruits corresponded to 13.33 g/fruit by mechanical pollination whereas, by hand pollination it produced 5 fruit/strand corresponded to 15.71 g/fruit. Likewise, using the same treatment for Khasab 21 fruits corresponded to 9.01g by mechanical, whereas, by hand pollination 19 fruits/strand corresponded to 10.12g.These results confirm those reported by Al Khateeb et al., 1993 and Nixon, 1940 on the negative relationship between these parameters.

11. Pollination Efficiency. see Table 4.

CONCLUSION

The obtained results indicated that mechanical pollination supported by an appropriate fertilizer program and mulching can improve date palm production with a lower cost and higher efficiency.

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Tables

Table 1. Effect of method of pollination on the physical characteristics of Khalas and Khasab fruits.

Cultivar	Pollination Method	Total yield (kg/palm)	No. fruit+strands	Wt.strand (g)	Fruit wt. (g)	Fruit length (cm)	Fruit diameter (cm)
Khalas	Mech.	43.11 A	6.0 A	154.58 A	13.34 B	3.66 B	2.35 A
	Hand	35.96 B	6 A	172.37 A	14.28 A	3.83 A	2.45 A
Khasab	Mech.	59.98 A	20.0 A	171.36 A	8.8 A	2.94 A	1.92 A
	Hand	62.23 A	19.0 A	180.56 A	9.1 A	2.95 A	1.87 A

Numbers in the same column followed by the same letter are not significantly different at .05 level by Duncun's Multiple Range.
Mech = Mechanical hand pollination duster, Hand = hand pollination.

Table 2. Effect of fertilizer and mulching on the physical characteristics of Khalas fruits.

		Khalas					
	Treatments	yield kg / palm	fruits weight/strand (g)	fruits numbers	fruit length (cm)	fruit diameter (cm)	fruit weight (g)
1	no fert + no mulch	36.95 A	175.01 A	6.63 A	3.94A	2.51 A	14.55 A
2	Mac+Mic + no mulch	36.57 A	161.33 A	6.44 A	3.77 AB	2.44 A	14.04 A
3	Mac+ mulch	42.19 A	141.26 A	6.78 A	3.59 B	2.33 A	12.53 A
4	mulch+ no fert	40.45 A	158.66 A	6.27 A	3.72 AB	2.36 A	13.38 A
5	Mac+Mic + mulch	39.81 A	165.02 A	5.37 A	3.77 AB	2.40 A	14.52 A
6	Mac+ no mulch	41.21 A	179.60 A	5.98 A	3.68 AB	2.38 A	13.84 A

Numbers in the same column followed by the same letter are not significantly different at .05 level by Duncun's Multiple range.
Fert. = fertilizer, Mac = Macronutrients NPK; Mic = Micronutrients.

Table 3. Effect of fertilizer and mulching on the physical characteristics of Khasab fruits.

		Khasab					
	Treatments	Yield palm/kg	Fruits weight/strand (g)	Fruits numbers	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)
1	no fert + no mulch	61.84 AB	190.78 A	20.77 A	2.98AB	1.86 B	9.02 A
2	Mac+Mic + no mulch	61.10 AB	166.56 A	19.04 A	2.89 B	1.88 B	8.99 A
3	Mac+ mulch	62.35 AB	155.94 A	18.83 A	2.89 B	1.82 B	8.62 A
4	mulch+ no fert	55.73 B	167.66 A	19.19 A	2.93 B	1.86 B	8.78 A
5	Mac+Mic + mulch	65.47 A	192.93 A	20.28 A	3.10 A	2.18 A	9.58 A
6	Mac+ no mulch	60.20 AB	185.36 A	17.85 A	2.87 B	1.77 B	8.74 A

Numbers in the same column followed by the same letter are not significantly different at .05 level by Duncun's Multiple range.
Fert. = fertilizer, Mac = Macronutrients NPK; Mic = Micronutrients.

Table 4. Efficiency of pollination method.

Method	No of palms pollinated per hr.	Amount of pollen ml/palm	Time to pollinate one palm	No of pollination per season	No of labors	No of palms that can be pollinated per season
Hand	3---5	5.5-7.0	10-15 min.	2 --3	1	510
Mechanical	52	2----3	51 Sec	3----4	1	2400
Hand duster						
Efficiency mechanized	13x	60%	93%		-	4.88x
	higher	less	lower			

Figures

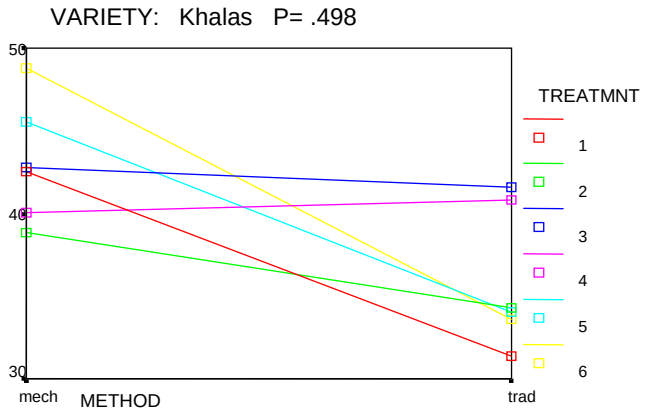

Fig. 1. Interaction effect of pollination method and fertilizer /mulch treatments on Khalas total yield (kg/palm).

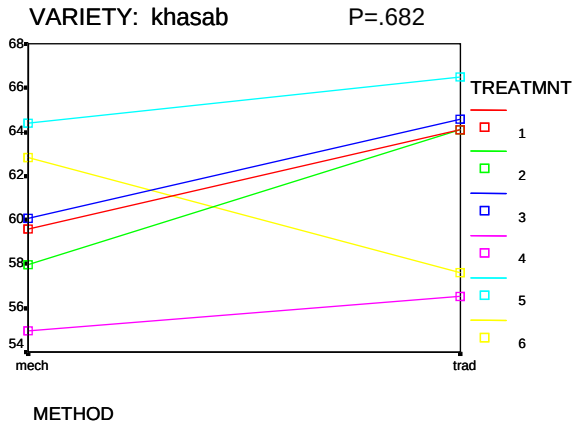

Fig. 2. Interaction effect of pollination method and fertilizer /mulch treatments on Khasab fruit total yield (kg/palm).

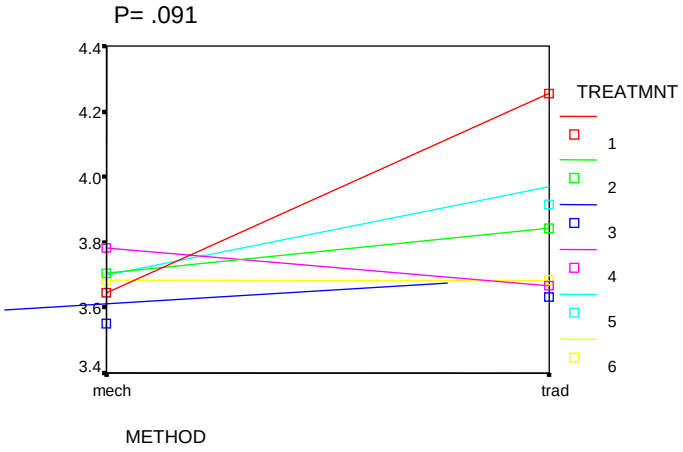

Fig. 3. Interaction effect of pollination method and fertilizer /mulch treatments on Khalas fruit length (cm).

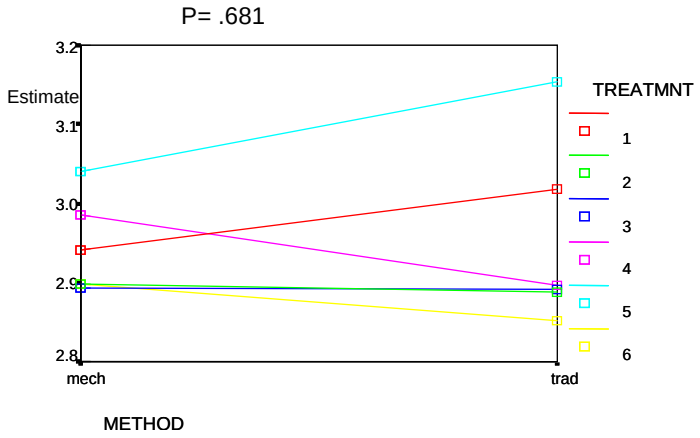

Fig. 4. Interaction effect of pollination method and fertilizer /mulch treatments on Khasab fruit length (cm).

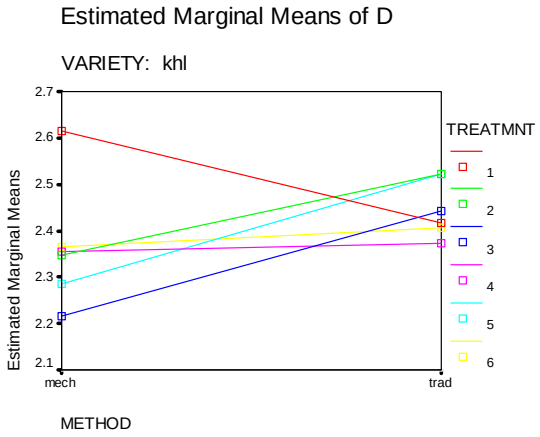

Fig. 5. Interaction effect of pollination method and fertilizer /mulch treatments on Khalas fruit diameter (cm).

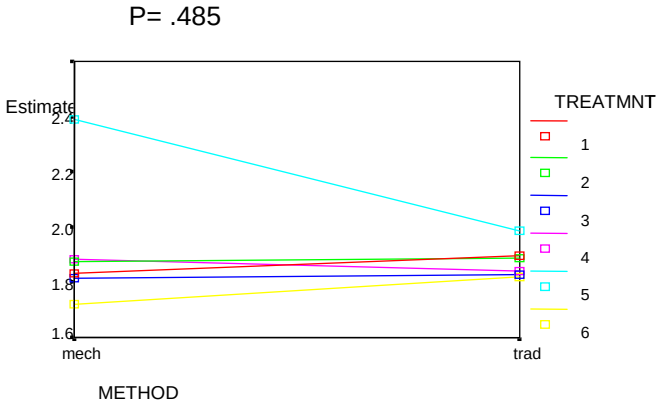

Fig. 6. Interaction effect of pollination method and fertilizer /mulch treatments on Khasab fruit diameter (cm).

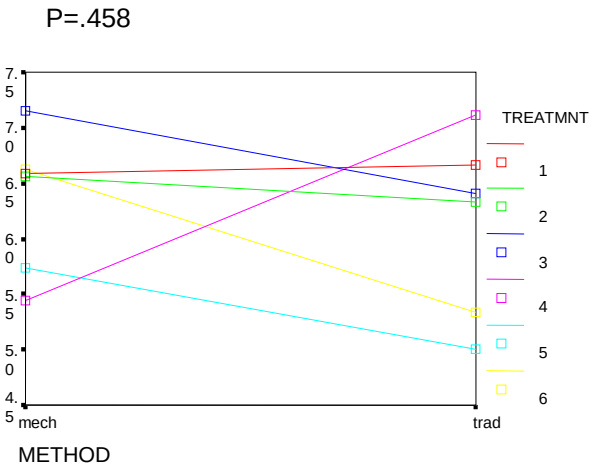

Fig. 7. Interaction effect for pollination method and fertilizer /mulch treatments on Khalas number of fruits/ strand.

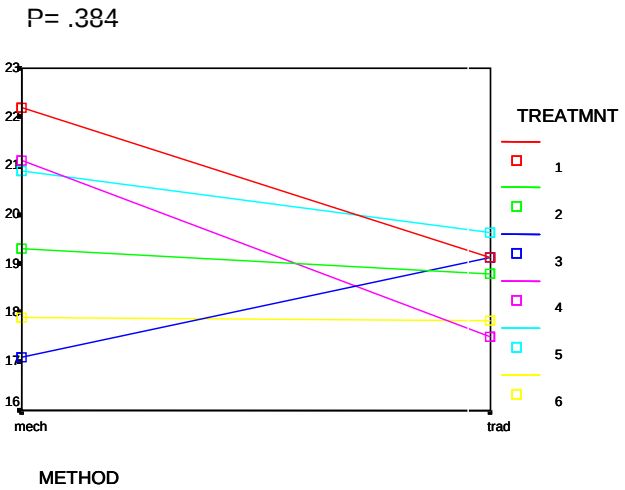

Fig. 8. Interaction effect for pollination method and fertilizer /mulch treatments on Khassab fruits number of fruits/ strand.

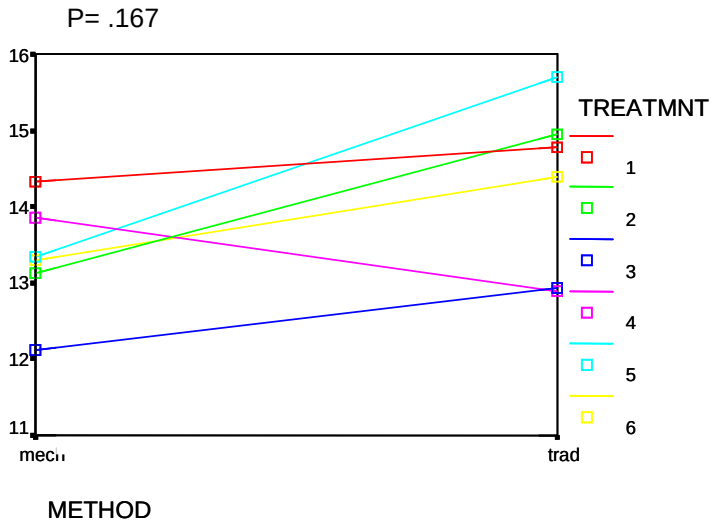

Fig. 9. Interaction effect for pollination method and fertilizer /mulch treatments on Khalas fruit weight.

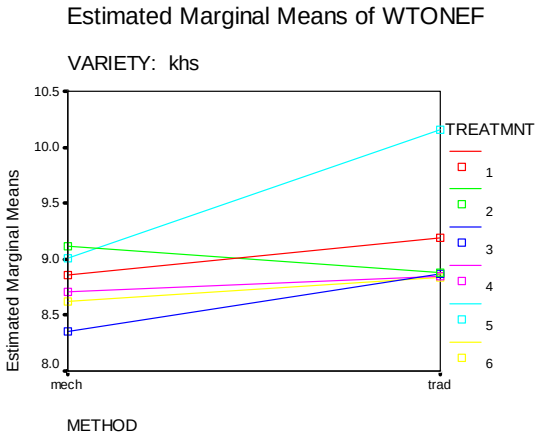

Fig. 10. Interaction effect for pollination method and fertilizer /mulch treatments on Khasab fruit weight.

Morphological Abnormalities in Tissue Culture-Derived Date Palm (*Phoenix dactylifera* L.)

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Keywords: Somaclonal variation, organogenesis, somatic embryogenesis

Abstract

Tissue culture is an important means of mass propagation of date palms. Although this technology presents several advantages, appearance of plant off-types is often associated with tissue culture-derived plants. The present study aimed to assess the occurrence and importance of morphological abnormalities in five important date cultivars propagated by tissue culture techniques. Dwarfism was observed at a high incidence (18%) in 'Sukkari' propagated by indirect embryogenesis. Scarcity in flowering, fertilization failure and bastard offshoots did not exceed 5% in the plants studied. Excessive vegetative growth, uneven ripening, delay in flowering and abnormal morphology and structure occurred with a very low incidence of less than 1%. The results obtained in this investigation provide valuable information to plant tissue culturists and date growers alike as guidance in the selection of the best propagation technique for each cultivar.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the oldest plants cultivated by man, and its origin is said to be either Mesopotamia or the Gulf Region (Sanderson, 2001). Date palm is a major tree crop cultivated in arid regions of the Middle East and North Africa. In the United Arab Emirates (UAE), a significant priority is given to sustainable date palm production. UAE has been able to develop a thriving date production industry and is currently self-sufficient in dates. Date palm plantings include very valuable local germplasm, which is extensive and contains about 120 date cultivars (UAE News Agency, 2000).

The traditional method of date palm propagation is by offshoots. However, this method presents many disadvantages. Offshoots are produced in a limited number, the survival rate of offshoots is low, with high chances of spreading date palm diseases and pests, and the technique is difficult and laborious. The most recent method for date palm propagation is tissue culture which presents many advantages such as the propagation of healthy selected female cultivars, producing males with superior pollen, large scale multiplication, no seasonal effect, production of genetically uniform plants, and finally, economic affordability when large production is needed (Zaid and De Wet, 2002).

Tissue culture can be applied to date palm by two methods: organogenesis and somatic embryogenesis (also called asexual embryogenesis). Date palm organogenesis consists of initiation of meristematic buds, followed by multiplication, then elongation, and finally rooting. Somatic embryogenesis is based on germination and elongation of somatic embryos from the explants. Somatic embryogenesis is induced either directly from the explant, or indirectly through a callus phase. In vitro propagation should normally produce true-to-type plants. However, the appearance of plant off-types has been observed. This process is referred to as somaclonal variation. It occurs in plants as a result of tissue culture conditions, and produces plants different from the original plant (Kaeppler et al., 2000). The existence of genetic variability in plant tissue culture has been reported in many plant species including date palm. The abnormalities found include changes in morphology and structure, excessive vegetative growth, leaf variegation,

dwarfism, leaf whitening, production of bastard offshoots, delayed flowering time, fertilization failure, formation of seedless fruits and higher susceptibility to diseases (McCubbin et al., 2000; Zaid and Al Kaabi, 2001, 2003). A preliminary study was carried out to evaluate the occurrence of plant off-types in tissue culture-derived date palms in the UAE (Zaid and Al Kaabi, 2003). It was found that there were several morphological abnormalities in tissue culture-derived date palms, including abnormal leaves and inflorescences, dwarfing, leaf bleaching, deformed offshoots, delayed flowering time, pollination failure and abnormal fruiting.

The objective of the present study was to investigate in depth the occurrence of morphological abnormalities in five important date cultivars cultivated in the UAE, in order to evaluate the importance of this phenomenon. The identification and early detection of these plant off-types will contribute to avoiding such shortcomings in the future and avoid the occurrence and propagation of such abnormalities.

MATERIALS AND METHODS

A survey aimed at assessing the morphological abnormalities was performed during 2003-2004, and covered 650 date palm farms in the UAE. The survey aimed to record the main abnormalities observed in cultivated date palms obtained using different tissue culture techniques including organogenesis, indirect asexual embryogenesis (AE/A), and direct asexual embryogenesis (AE/B), as well as plants propagated by offshoots. The age and source of plants were recorded, as well as information about the region, year of planting and morphological abnormalities in the planted date palms. Five important date cultivars were investigated in this study: 'Khlass', 'Barhee', 'Nabtet Seif', 'Sultana' and 'Sukkari'.

RESULTS AND DISCUSSION

The survey revealed that tissue culture-derived date palm cultivars have been mass cultured since 1998 and were planted evenly among the various regions of the UAE. The age of these palm trees ranged from one to seven years. The total number of date palm trees investigated was 89,362 with the following distribution: 6,172 originated from offshoots, 375 from organogenesis, 66,443 from AE/A and 16,372 from AE/B. The results obtained are summarized in Table 1.

Abnormal Morphology and Structure

The results indicated that none of the planted offshoots or plants produced by organogenesis analyzed in this study showed any abnormality in morphology and structure. However, all cultivars propagated by asexual embryogenesis exhibited some level of abnormality. 'Khlass' showed a level of 0.31% and 0.36% when propagated by AE/A and AE/B, respectively. Lower levels were observed in 'Barhee' (0.11% and 0.14% by AE/A and AE/B, respectively). The highest level of abnormality occurred in 'Sukkari' (0.42% and 0.36%, by AE/A and AE/B, respectively). In general, the occurrence of abnormal morphology and structure was very low and did not exceed 0.5%. It is therefore not a serious problem.

In general, the features observed in young tissue culture-derived date palms displaying abnormality were the following: 1) Absence of an onion base, 2) Thin stem with weak, juvenile leaves, 3) Abnormal phyllotaxy, 4) Weak root systems with less than three to four thin roots per plant (Zaid and Al Kaabi, 2003). Abnormal leaf shape and leaf bending were reported by Al Ghamdi (1993) in different date cultivars produced using somatic embryogenesis.

Fertilization Failure and Uneven Ripening

Fertilization failure was defined in the present study as none of the female flowers setting, or when a very low fruit set was obtained. All planted offshoots and organogenesis-derived plants surveyed in this study showed normal fruit set. However, propagation by asexual embryogenesis resulted in fertilization failure. In 'Khlass', 0.18%

of plants produced by AE/A showed fruit set abnormality. This level was about 20 times higher in plants produced by direct embryogenesis AE/B (5.52%). In 'Barhee', only plants derived from indirect embryogenesis (AE/A) showed this phenotype at a rate of 2.7% (Fig. 1). In addition to fruit set failure, low yield or uneven ripening was quantified. Only 'Khlass' cultivar showed this uneven ripening of fruits in both indirect and direct embryogenesis techniques with a level of 0.05% and 0.07%, respectively. None of the date palms derived from offshoots, nor from organogenesis showed this abnormality. Thus, in the five studied cultivars, abnormal fruit set does not seem to be a serious problem. This phenomenon was also reported by Al-Wassel (2000) on 'Barhee', 'Khlass', 'Sukkari' and 'Ajoua', with a much more severe fertilization failure between 60-86%. Such abnormality can also vary within the same bunch or within the spikelets of the same bunch, or among bunches of the same tree (Al-Wassel, 2000; 2001). There was no scientific evidence to indicate that fertilization failure was based on genetic variation or was due to a lack of optimal fertilization procedure (Zaid and Al Kaabi, 2003; Gurevich et al., 2005). Epigenetic changes occurring during the tissue culture stage were assumed to be responsible for this abnormal phenotype.

Dwarfism

Dwarf date palm plants are less than one meter high after four to five years in the field, in comparison to a normal date plant of the same age with an average height of 3 m. All date palms derived from offshoots and from organogenesis were normal and showed no sign of dwarfism. However, plants produced by asexual embryogenesis in 'Khlass', 'Barhee' and 'Sukkari' cultivars showed this phenotype (Fig. 2). The highest level was about 18% in 'Sukkari' propagated by indirect embryogenesis (AE/A). Zaid and Al-Kaabi (2001) found that certain date palm cultivars, such as 'Sukkari', 'Barhee', 'Sultana' and 'Oum Dahn' exhibited abnormal dwarfing, with 'Sukkari' being the most sensitive cultivar.

Excessive Vegetative Growth

Excessive vegetative growth appeared as broader leaves, compact growth and different spine structure of the plant. There was no vegetative growth abnormality in the offshoot- and organogenesis-derived date palm trees investigated. However, in 'Khlass' and 'Barhee', both direct and indirect embryogenesis-derived date palms trees showed excessive vegetative growth abnormality (Fig. 3). In 'Khlass', the level of excessive vegetative growth was 0.07% and 0.15%, by AE/A and AE/B, respectively, while in 'Barhee', it was only 0.01% and 0.08%, by AE/A and AE/B, respectively.

Leaf Whitening

Palm trees derived from offshoots did not show any leaf whitening. 'Sukkari' trees derived from AE/A and AE/B did not show any white leaves. However, both 'Barhee' and 'Khlass' cultivars produced from indirect and direct embryogenesis (AE/A and AE/B) had white leaves. There were usually two to four white leaves per affected tree. In 'Khlass', the percentage of leaf whitening was 0.02% by AE/A and 0.07% by AE/B, whereas in "Barhee", it was 4.30% by AE/A and 0.39% by AE/B. Organogenesis-derived plants showed this whitening phenomenon in 'Nabtet Seif' and 'Sultana' cultivars (Fig. 4). Eighty out of the 150 'Nabtet Seif' date palm trees (53.33%) originating from organogenesis had at least one white leaf per tree. In 'Sultana' cultivar, the level was 32%. However, this abnormality was rather rare in a given plantation, and therefore of no great economic significance. Leaf whitening was heavily noticed in regions with gravel soil, which has low water retention, in contrast to regions with clay soil, which retain water better.

Bastard Offshoots

The abundance of offshoot production is accompanied by the appearance of abnormal offshoot/inflorescence specimens. Deformed growth of date palm vegetative buds and their conversion to floral buds, are commonly known as twisted inflorescences. All five cultivars exhibited normal offshoot production with the absence of any twisted inflorescences. However, 'Khlass' and 'Barhee' showed this abnormality in trees derived from both direct and indirect asexual embryogenesis techniques, with a level of 0.62 % and 2.7% by AE/A and AE/B, respectively in 'Khlass', and 0.03% and 0.1% by AE/A and AE/B, respectively in 'Barhee'. Similar results were previously reported by Zaid and Al-Kaabi (2003) on 'Barhee' cultivars. This deformation was attributed to a reduction in growth caused by an inequilibrium of endogenous growth regulators accumulated during in vitro propagation (Varughese, 2000).

In general, the level of abnormality found in this study was very low, and in many cases it did not exceed the limit of one percent. This will indeed strengthen the importance of tissue culture techniques as a reliable means to propagate date palm. Planted offshoots and plants derived from organogenesis did not show any abnormality in the cultivars analyzed. The leaf whitening phenotype appearing in 'Nabtet Seif' and 'Sultana' produced by organogenesis seemed to be a temporary phenomenon since the affected leaves finished by turning back to their original green color. Most of the abnormalities recorded were associated with somatic embryogenesis. The most sensitive cultivars to asexual embryogenesis were 'Khlass' and 'Barhee', which showed 8 abnormalities out of 9. In large-scale multiplication, it is safer to confirm the trueness-to-type of tissue culture-derived plants.

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Tables

Table 1. Morphological abnormalities observed in different date palm cultivars. Data represent the percentage of each abnormality per total number of trees analyzed. AE/A: Indirect Asexual Embryogenesis. AE/B: Direct Asexual Embryogenesis. -: No trees available.

Bastard offshoots	Leaf whitening	Excessive vegetative growth	Dwarfism	Uneven ripening	Fertilization failure	Scarcity in flowering	Delay in flowering	Abnormal morphology and structure	Propagation technique	Cultivar
0	0	0	0	0	0	0	0	0	Offshoots	"Khlass"
-	-	-	-	-	-	-	-	-	Organogenesis	
0.6	0	0.1	0.6	0.1	0.2	1.1	0	0.3	AE/A	
2.7	0.1	0.2	0.3	0.1	5.5	4.4	0	0.4	AE/B	
0	0	0	0	0	0	0	0	0	Offshoots	"Barhee"
-	-	-	-	-	-	-	-	-	Organogenesis	
0	4.3	0	0.1	0	2.7	0.1	0.9	0.1	AE/A	
0.1	0.4	0.1	0.2	0	0	0.1	0	0.1	AE/B	
-	-	-	-	-	-	-	-	-	Offshoots	"Nabtet Seif"
0	53.3	0	0	0	0	0	0	0	Organogenesis	
-	-	-	-	-	-	-	-	-	AE/A	
-	-	-	-	-	-	-	-	-	AE/B	
-	-	-	-	-	-	-	-	-	Offshoots	"Sultana"
0	32	0	0	0	0	0	0	0	Organogenesis	
-	-	-	-	-	-	-	-	-	AE/A	
-	-	-	-	-	-	-	-	-	AE/B	
-	-	-	-	-	-	-	-	-	Offshoots	"Sukkari"
-	-	-	-	-	-	-	-	-	Organogenesis	
0	0	0	17.7	0	0	0	0	0.42	AE/A	
0	0	0	4.7	0	0	0	0	0.4	AE/B	

Figures

Fig. 1. Five-year-old Barhee tree produced by direct asexual embryogenesis (AE/B) showing abnormal fruit setting. (Al Dhaid area / Central region).

Fig. 2. Three-year-old dwarf Khlass plant produced by indirect asexual embryogenesis (AE/A).

Fig. 3. Excessive vegetative growth observed in a five-year-old Khlass tree produced by indirect embryogenesis (AE/A). Top leaf: (Normal), length: 37 cm, width: 4 cm; Bottom: (Abnormal), length: 56 cm, width: 5 cm.

Fig. 4. Leaf whitening in a five-year-old Sultana tree produced by organogenesis, Masafi.

Improvement of Date Palm Production in the Sultanate of Oman

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Abstract

The Sultanate of Oman is located in the south-eastern part of the Arabian Peninsula. The general climate is hot and arid with an average of 100 mm of rainfall per year. High temperatures leads to high rates of evapotranspiration during most of the year. Date palm is the primary crop in the Sultanate of Oman and represents 82% of all fruit trees in the country. Irrigation using scarce water resources is practiced in all date producing regions of Oman. Soil and water salinity, pests and diseases, increased production costs as well as limited market outlets led to a decline in date production in recent years. This paper presents ways to improve date palm production in Oman. Factors that adversely affect date palm production are discussed and solutions are proposed to increase total production of dates in Oman.

INTRODUCTION

Agricultural Production in the Sultanate of Oman

The Sultanate of Oman is located in the south-eastern part of the Arabian Peninsula. This region is classified as arid hence it receives an average of 100 mm of annual rainfall (Fig. 1). Due to high evapotranspiration rates and low rainfall amount and frequency, agriculture is dependent on irrigation using scarce underground water resources. The amount of water reservoirs in Oman is estimated to be 1168 million m^3 (Al-Gheilani and Al-Mulla, 2006). Agriculture uses 92% or 1124 million m^3 of all water resources (Al Sulaiman and Al Wohaibi, 2006). Due to the topographic and climatic diversity of Oman, many crops can be grown from temperate fruit crops in the northern mountains of al Jabal al Akhdar to tropical fruits in the southern province of Dhofar. Data on agricultural area and production of vegetables, fruits, field crops and fodder crops are shown in Fig. 2. Fruit crops occupy the largest area, 48%, with an estimated 41958 ha in 2004 according to the Ministry of National Economy (MNE, 2005) with a total fruit production of 292,720 metric tonnes (MT). Date palm is the primary fruit crop in the country and it constitutes 82% of all fruit crops in Oman.

DISCUSSION AND ANALYSIS

Date Production of the Sultanate of Oman

The Sultanate of Oman contributes a significant amount of dates to the world market. According to the Food and Agriculture Organization of the United Nation (FAO, 2006), in 2005 Oman was ranked the eighth largest date palm producing country in the world with a total production of 238,000 MT. According to a 2004 national agricultural census conducted by the Ministry of Agriculture and Fisheries (MAF), date palm occupied 48% of the total cultivated area of fruits, vegetables, field and fodder crops (MAF, 2006). The total number of date palm trees in 2004 was 7,795,786 (MAF, 2006), which represented 82% of all fruit crops grown in Oman. The largest production area of date palm in Oman is al Batinah, which in 2004 had 41.87% of all date palm trees in the country (Table 1), and 45.85% of the total date production (Table 2) (MAF, 2005, 2006).

There are about 200 cultivated varieties of date palm in Oman. Thirty out of the 200 varieties produce high quality dates that are consumed fresh or processed. Table 2 shows the top ten varieties that contributed to over 70% of the country's date production in 2004.

Economic Value of Dates in the Sultanate of Oman

Although Oman is one of the largest producers of dates in the world, as indicated above, its export share was only 1.4% of date palm exported in 2004 (Table 3) (FAO, 2006). According to MAF (2005), the average amount of exported Omani dates during 2003 and 2004 was 9,000 MT or 4% of all dates produced (Table 4). Within Oman, the majority of dates are used for human consumption (51%), while animal consumption constitutes 22% of all dates produced (Table 4). By comparing these values to the dates produced in Oman, there is an estimated 52,000 MT of excess dates that represent 23% of the total date production. This surplus date production represents an opportunity for enhancing the export market of Omani dates in the world.

Future Prospects for Enhancing Date Production and Export

1. Date Processing and By-product Utilization. Several studies have shown that Omani dates vary in their physical properties and chemical composition including the amounts of sucrose, reducing sugars and fibers (Myhara et al., 1999; Myhara et al., 2000). These physical and chemical properties are affected by several factors related to field operations such as pollination and fertilization (Haffar et al., 1997; El Mardi et al., 2002) and to fruit quality such as moisture content (Rahman and Al-Farsi, 2005). Therefore it is essential to select the appropriate varieties for processing and for manufacturing date by-products. Aspects of certain date processing and by-product utilization of dates need further refinement. These include development of date confectionary, utilization of date palm products in animal feed, and making coffee-substitute drinks from roasted date pits.

2. Marketing. It is essential for government agencies such as the Omani Chamber of Commerce and Industry and date manufacturers to explore new markets and to raise the standard of quality of exported dates in order to be competitive in the world date market. In addition, new date products need further investigation and industrial promotion to utilize the excess dates produced.

3. Factors Limiting Date Palm Production and Utilization. Many factors may have contributed to the reduction of dates from 2001 when the amount of dates produced was 298,000 MT to the 2005 levels of 238,000 MT. Among them is the availability of skilled labor to carry out field operations, pests and diseases, degradation of soil and water quality, and harvest and post-harvest losses.

Labor Costs. Date palm requires several labor-intensive management practices including pollination, pruning and harvesting throughout the year. The current total workforce in agriculture, hunting and forestry is 6.63% of the total workforce (736, 624) (MNE, 2005). This is approximately 1 person per 200 date palm trees. Because of labor shortage, the production cost has increased in recent years. Training of expatriate and local date palm workers is important for maintenance of economically productive trees.

Pests and Diseases. The Dubas bug (*Ommatissus lybicus* Bergevin, Hemiptera: Tropiduchidae) is the most destructive pest in date producing regions of Oman. Despite annual spraying of over 21,000 L of insecticides to combat this pest (MNE, 2005), it has become more difficult to control. An integrated pest management approach might lead to control of this insect pest in areas where pesticide spraying is ineffective. Another pest that threatens date palm production in Oman is the red palm weevil (*Rhynchophorus ferrugineus* Olivier, Coleoptera: Curculionidae). This pest was first detected in 1993 and has been controlled and confined to the north-western boarders of the country. To maintain low infestation levels of this insect pest, appropriate measures such as inter-state quarantine and periodic monitoring should be implemented. In addition, implementing integrated pest management practices is expected to prevent future potential outbreaks of existing insect pests (Murphy and Briscoe, 1999).

Soil and Water Degradation. Agriculture is the largest consumer of water resources in Oman. It is estimated that irrigation of agricultural crops consumes 92% of the country's water resources (Al Sulaiman and Al Wohaibi, 2006). Decreasing water table and increased water salinity were attributed to saline water intrusion (Al Sulaiman and Al Wohaibi, 2006; Al Barwani and Aboulk A'ata, 2006). Salinity due to sea-water intrusion

into ground water in the largest date production region, al Batinah, has contributed to a decline in dates produced in the country. Results of a study that monitored 537 wells in al Batinah region over a period of 12 years, showed a gradual increase in salinity levels to reach 47% of the wells (Al Barwani and Aboulk A'ata, 2006). Modern irrigation systems to replace the dominant basin-flood irrigation systems are needed in date growing regions of Oman to conserve the limited underground water reservoirs. Another constraint facing date palm production is soil desertification. Desertification occurs in regions bordering the coastal line, the Empty Quarters and al Sharqiah Sands. Desertification in these regions is expanding due to abandonment of many date farms and reduced fertility of these soils. Lower soil fertility is possibly due to low use of fertilizers by date growers, especially synthetic fertilizers. However, little has been done on characterization of local practices of irrigation and fertilizer application in date palm growing regions of Oman (Luedeling, 2005). Research aimed at evaluating current soil and water management in date palm production areas is important to provide suitable recommendations for improving management practices.

Harvest and Post-harvest Losses. Harvesting of date palm in Oman is carried out using the traditional methods of cutting fruit bunches and throwing them to the ground where other family members and/or workers collect the fruit. Besides the negative effects on physical appearance, harvested fruits come in contact with the soil that leads to more losses to pests and diseases during storage and transport. Appropriate harvesting and post-harvest handling techniques need to be determined and effectively transferred to date growers to ensure good quality fruits for fresh consumption and for processed date products.

4. Research and Development. Research on date palm production and processing in Oman is conducted by the Ministry of Agriculture and Fisheries (MAF) and the College of Agricultural and Marine Sciences (CAMS) at Sultan Qaboos University (SQU). Date palm facilities at MAF include a live gene bank that was opened in 1988 and is actively growing 166 female and 21 male date palm cultivars (Al-Zidjali, 1996). The MAF tissue culture laboratory in Oman was opened in 1992 to mass propagate selected high-quality date palm trees and then distribute them to growers. There are several MAF agricultural research stations that conduct applied research and provide extension services to date palm growers throughout the country. The Ministry has recently proposed a strategic plan for development of date palm in the country. The plan aims at increasing investments in the date sector. This increase will be achieved through production of high quality dates as well as expansion of the national and international markets for dates throughout the year. Moreover, the plan includes development, extension, and research programs covering all aspects of date field operations, post-harvest management, by-product utilization and marketing. At CAMS, research on date palm is primarily focused on basic research. Application of this research is achieved through collaboration with MAF. Extension activities and training of extension agents are also periodically conducted by CAMS through national and international conferences and periodic workshops such as farmer- and field-days.

CONCLUSION

Omani date growers face numerous challenges in date production and marketing. Determining these problems and proposing feasible solutions will alleviate the extent of these problems and enhance date production in the country. Farmer participation can be effectively achieved through extension and training programs. The role of extension agents is important to increase the production of high quality dates. Monitoring pests and diseases, limiting the degradation of soil fertility and water quality should be given high priority in research and development. These research areas will ultimately provide new avenues for improving date palm cultivation and increasing production of export-quality dates in Oman.

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Tables

Table 1. Regional date palm distribution in the Sultanate of Oman (MAF, 2005 and 2006).

Region	No. of Date Palm Trees	Percentage of total	Production (tonnes)	Percentage of total
al Batinah	3,263,862	41.87	105,929.14	45.85
al Sharqiah	1,502,525	19.27	37,294.65	16.14
al Dhahira	1,333,898	17.11	29,515.50	12.78
al Dakhlia	1,112,959	14.28	44,006.17	19.05
Muscat	322,222	4.13	10,282.98	4.45
Musandam	234,453	3.01	3874.09	1.68
Dhofar	23,679	0.30	132.38	0.06
al Wusta	2,188	0.03	-	-
Total	7,795,786	100	231,034.91	100

Table 2. The ten most important varieties in the Sultanate of Oman in 2004 (MAF, 2005).

Cultivar	Percentage of date production
Um Sella	14.15
Mabsli	13.24
Khasab	11.55
Naghal	10.75
Fardh	7.81
Shahl	4.95
Khunaizi	4.91
Khalas	4.82
Madloki	2.35
Barni	2.15

Table 3. The top 10 date exporting countries in 2004 (FAO, 2006).

Country	Dates exported (MT)	Percentage of total
Iran	94,584	26.9
Pakistan	65,429	18.6
UAE	59,457	16.9
Saudi Arabia	42,453	12.1
Tunisia	40,432	11.5
France	8,386	2.4
Algeria	8,133	2.3
Israel	6,441	1.8
Oman	4,752	1.4
USA	4,202	1.2

Table 4. Market uses of dates in the Sultanate of Oman in 2004 (MAF, 2005).

Consumption Type	Amount (MT)	Percentage of total
Internal consumption:		
Human	120,000	51
Animal	50,000	22
Export (Incl. Processed)	9,000	4
Surplus	52,000	23

Figures

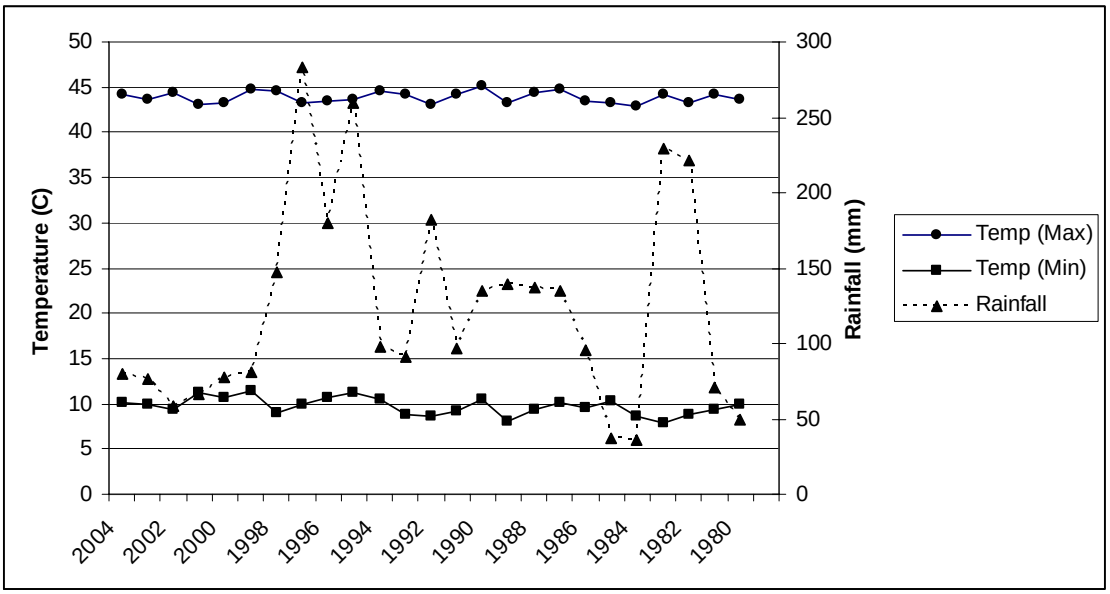

Fig. 1. Average annual maximum and minimum temperatures and rainfall over 25 years from nine weather stations in the Sultanate of Oman (MNE, 2005).

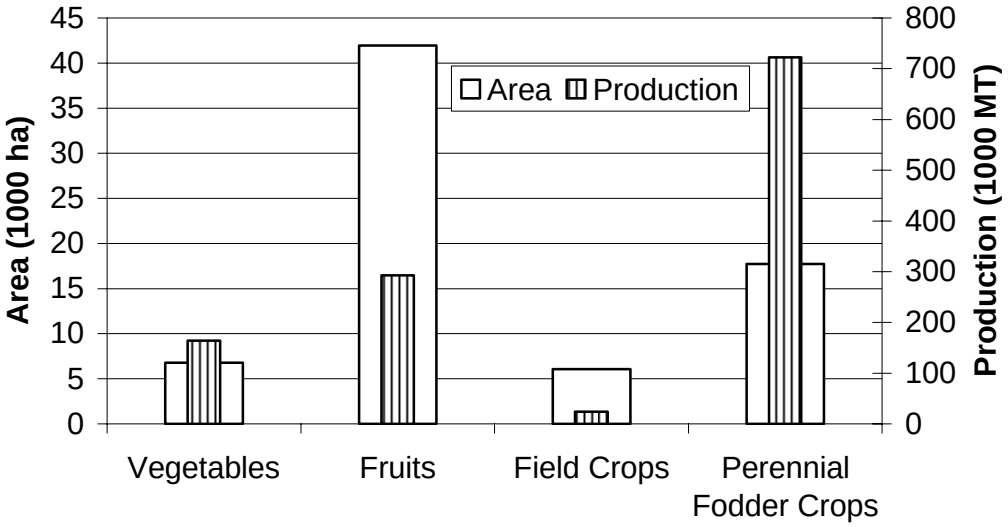

Fig. 2. Area and production of agricultural crops in the Sultanate of Oman in 2004 (MNE, 2005)

Optimizing Trapping of Palm Weevils and Beetles

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Keywords: *Oryctes rhinoceros, Rhynchophorus ferrugineus, Rhynchophorus palmarum*, pheromone, kairomone

Abstract

***Oryctes rhinoceros* is an economically important problem of young oil palm in South East Asia. The optimum trap for *O. rhinoceros* is a pheromone baited, elevated bucket trap containing vanes that protrude into the bucket. Trapping adults using 1 of these traps / 2 ha lowers damage by over 90% within a few weeks and is competitive with insecticide application. *Rhynchophorus palmarum* is most efficiently trapped using a small plastic container strapped to trees and baited with pheromone, ethyl acetate and food. In oil palm trapping *R. palmarum* at 1 trap / 5 ha lowers populations and associated damage by > 80% in one year. In coconut trapping of *R. palmarum* using one trap / ha lowers damage by > 80% over 1 year while in palmito palm the same trap will lower damage by 80% and increase yields by 58%. In the Middle East *R. ferrugineus* infestation of date palm is managed by periodic survey, treatment or removal of infested palms and trapping. There is strong evidence that trapping, in combination with spraying decreases infestation by 64% while smaller scale experiments indicate that trapping alone reduces infestation by 71%. Trapping is most efficient for all palm weevils if aggregation pheromone is combined with, ethyl acetate and moist. Trapping is made difficult by the requirement for replacement of water and food bait in traps. This paper reports that propylene glycol extends the effective life of trap food bait from 2 weeks to 7 weeks. This paper also describes a new natural food bait that is more effective than sugarcane in capture of *R. palmarum* in pheromone traps. Finally, tests of repellants that reduce captures of *R. palmarum* in pheromone traps by over 50% make possible push-pull strategies to improve management of palm weevils.**

This paper summarizes work spanning 10 years on the development and optimization of traps for *Oryctes rhinoceros* and *Rhynchophorus* palm weevils.

TRAPPING OF *ORYCTES RHINOCEROS* IN OIL PALM IN MALAYSIA

Trap Design

Oryctes rhinoceros is a large scarab. Since most scarabs are clumsy flyers it has proven easy to intercept them using pheromone-baited vane traps. In the case of *O. rhinoceros,* buried traps were the least efficient, single vane traps were of intermediate efficiency and double vane traps were most efficient (Fig. 1, 2). A further modification of vaned bucket traps is necessary to prevent escape of captured *Oryctes rhinoceros*. Vanes that protrude within a few centimeters of the bottom of the trap are required.

Trap Location

Oryctes rhinoceros is an important pest of oil palm from month 10 to month 30 after planting. In this period the palms are one meter to two meters high. Work in Malaysia established that efficiency of trapping was increased by 5X if traps were raised above the canopy (Fig. 3, Gait Chung Fee, Sime Darby, unpublished).

Attractants in Trap

The male-produced aggregation pheromone of *O. rhinoceros* is required for traps

to be attractive. The release rate of the pheromone has been varied from 0.3 to 30 mg/day (additional studies to 72mg/day also show increasing captures) and capture rates increased as pheromone release rate increased. When pheromone was released at 0.3 mg/day attraction was statistically equivalent to empty non-baited traps. Between 0.3 mg/day and 3 mg/day captures increased by 700%, which corresponded to ~280% increase in capture / additional mg of pheromone released. When the pheromone release rate was between 3 mg/day and 9 mg/day captures increased by 85% which was a ~14% increase in capture / additional mg of pheromone released. Beyond 9 mg/day the gain was about 2% increase in captures for each additional milligram of pheromone released. Operational trapping uses lures that release approximately 9 mg/day. It has been shown that decaying organic matter added to pheromone-baited bucket traps with crossed vanes increased capture rates by 64% which was statistically significant. The organic matter added much weight to elevated traps making it difficult to maintain the trap in position without extensive bracing. Since the effect of elevating the trap was several times more than the effect of adding organic matter the latter was not used operationally.

Operational Use of Traps

Operational trapping of *O. rhinoceros* employed one elevated trap of the type shown in Figure 3 for each two hectares of young oil palm. If traps were installed soon after planting and felled palm trunk from the last crop was chipped, then *Oryctes* populations were well managed.

TRAPPING *R. PALMARUM*

Weevils of the genus *Rhynchophorus* are a major problem in oil palm. In Central and South America *R. palmarum* is a major economic problem. Larvae often kill trees and adults carry red ring nematode that is lethal to palms. Prior to 1993 management of this pest was by systematic inspection of palms coupled with cutting and spraying of cut palms with insecticide (Chinchilla et al., 1993). This practice did not control red ring infestation carried by the weevil nor did it control direct weevil damage caused by larvae.

Since *R. palmarum* is a large insect and populations are generally low mass trapping was considered a reasonable option. In late 1991 collaborative research between the author's group and Chinchilla's group at the Coto, Costa Rica oil palm plantation showed that the male-produced aggregation pheromone for *R. palmarum* attracted large numbers of male and female *R. palmarum* only when presented with food such as sugarcane (Fig. 4) (Oehlschlager et al., 1992, 1993a, 1993b; Chinchilla et al., 1992, 1993, 1996). Trap and bait optimization studies led to the adoption of a bucket trap containing a pheromone lure and a few pieces of insecticide-treated sugarcane (replaced biweekly) as a useful trap for this insect (Fig. 5).

Trap Design

R. palmarum responded to attractive traps by flying to the location of the trap, landing on the trap or tree to which the trap was attached or on the ground. The final step of capture was walking into the trap (observations reported in Oehlschlager et al., 1993a). Many trap designs have been examined for this weevil but only the most convenient and efficient have been used operationally. Sandwich traps made from palm trunk, quartered palm trunk, vertical sections of palm trunk and baited palm trunk laid on the ground are efficient when baited with pheromone, but labor is intensive and they are not used operationally (Oehlschlager et al., 1993b). Pheromone and food baited plastic bags or plastic buckets (5, 19 liter) were all efficient and buckets were most commonly used as traps because of the ease of servicing (Oehlschlager et al., 1993b). It is important to note that 5 liter containers were as efficient as 19 liter containers and because of their ease of use 5 liter containers are used operationally (Oehlschlager et al., 1993b).

Trap Location

Early tests revealed that when traps are placed between palms, that placement on

the ground led to the greatest captures. Ground level traps captured 55% more weevils than traps on poles 1.7 meters high and 189% more than when traps were on poles 3.1 meters high (Oehlschlager et al., 1993b). When traps were placed attached to palms there was no statistical difference between ground level traps and traps at 1.3, 2.3 and 3.3 meters above ground (Oehlschlager et al., 1993b). For convenience in commercial oil palm plantations, traps are hung on trees at chest height (Oehlschlager et al., 2002).

Attractants in Trap

Prior to the 1990's the most widely employed attractants for *R. palmarum* were palm tissue and sugarcane. Although traps containing these attractants alone are marginally effective they have been used in the Americas to attract *R. palmarum* since the 1970's (for a review see Oehlschlager et al., 1993a). The elucidation of a male-produced aggregation pheromone for *R. palmarum* in 1991 (Rochat et al., 1991) led to the discovery that when traps are baited with pheromone and moist food, attraction to traps is synergized and very large numbers of weevils are captured (Fig. 1, Oehlschlager et al., 1992, 1993a,b).

It is important to view food as a pheromone synergist (Fig. 1). Traps that emit pheromone but have dry or unattractive food will attract weevils but they enter traps in significant numbers only when moist food is present. Moist food is a necessary ingredient to capture weevils in a pheromone trap.

Among the foods that have been found to synergize attraction to pheromone are sugarcane juice, sugarcane, coconut palm heart, coconut palm petiole and oil palm petiole (Oehlschlager et al., 1993a,b). Although sugarcane stalks cracked by hitting with a hammer are marginally more attractive than halved sugarcane (Oehlschlager et al., 1993b) the latter is the most commonly used food additive in *R. palmarum* traps.

In 1993 Jaffee et al. reported that the addition of ethyl acetate to traps containing pheromone and food enhanced captures. Since attraction to sugarcane in traps generally increases for a few days after it is placed in a trap and fermented palm sap (toddy) is a well known attractant of *R. ferrugineus* in India, the author's group (ChemTica) examined (Fig. 6) ethyl acetate, ethanol and the combination of ethyl acetate and ethanol as synergists for pheromone:sugarcane baited traps. We concluded that ethyl acetate alone was as effective as any combination of ethanol and ethyl acetate and more effective than ethanol in synergizing attraction to pheromone. These experiments further confirmed a 50% increase in capture of *R. palmarum* in pheromone:sugarcane baited traps that additionally emit ethyl acetate.

Figure 6 Test conducted May 17-30, 2000. Traps were 19 l white buckets with four 5 cm X 8 cm entry slots near the top. One liter 1% lannate in water was added to each trap at the start of the test and 500 mL was added after the first week. Treatments were sugarcane and ethyl acetate:ethanol lure; fresh sugarcane and ethyl acetate lure; sugarcane and ethanol lure or sugarcane (control). Weevils were counted and removed after the first week at which time trap positions were rerandomized. ANOVA (n = 20) gave no significant differences between treatments.

Even with the addition of ethyl acetate, attractiveness of pheromone:sugarcane bait was significantly decreased after two weeks due to decay and desiccation of the sugarcane. This was remedied by replacement or replenishment of sugarcane after 2 weeks. Several approaches have been reported to avoid servicing traps every 2 weeks. In one approach the author and co-workers (ChemTica) added propylene glycol to pheromone:sugarcane traps. Propylene glycol decreases decay of sugarcane by retarding fungal and bacterial growth. As well propylene glycol is not repellant to weevils. In dry climates propylene glycol also acts to retard the evaporation of water. Experiments using this approach have consistently shown that the life of traps can be extended to at least a month. Thus, in Costa Rica in the dry season, sugarcane bait becomes dry and unattractive after 2 weeks, while in the wet season decomposition renders it unattractive due to decomposition.

Figures 7 and 8 show typical captures of *R. palmarum* in pheromone:sugarcane

traps with propylene glycol. Since the propylene glycol does not evaporate traps containing it do not get dry. Propylene glycol is not toxic to humans and is relatively inexpensive. The addition of propylene glycol prolongs the useful life of pheromone:sugarcane baited traps to at least 7 weeks. In Figure 7, after 4 weeks traps with the propylene glycol are still more attractive than 2 week old traps with water. Traps with propylene glycol were still attractive and contained liquid after 10 weeks.

In Figure 8 traps prepared with propylene glycol on January 14, 2001 remained attractive for 7 weeks. At this point traps containing propylene glycol were still almost as attractive as freshly prepared traps.

In a newer approach the author's group (ChemTica) has found that stabilized natural foods are as effective as sugarcane in synergizing attraction to pheromone. These natural food baits can be stored for over a year and are highly effective as substitutes for sugarcane in *R. palmarum* pheromone traps. In the experiment shown below (Fig. 9) traps with natural food bait were as attractive as sugarcane during the first week and significantly more attractive during the second week of the trial (during which time the sugarcane became dry).

A more sophisticated but less successful approach to development of a long lasting food additive for *R. palmarum* traps has been published by Rochat et al., 2000. In this approach a mixture of chemicals emitted by decaying coconut, sugarcane and other weevil hosts was claimed to be a replacement for sugarcane in *R. palmarum* traps. These claims have been shown to be incorrect by several additional experiments in which the blends functioned poorly as synergists in pheromone baited traps (Rochat, 2005). In Costa Rica the author's group (ChemTica) has repeated the work with much attention to detail and report that the blends reported by Rochat et al., are no more attractive to *R. palmarum* than ethyl acetate alone. In several experiments it was found that traps containing pheromone and the reported synthetic chemical blends were significantly less attractive to *R. palmarum* than traps containing pheromone and sugarcane (Oehlschlager and Gonzalez, unpublished).

The optimal trap for *R. palmarum* is currently a bucket or open plastic gallon baited with pheromone, ethyl acetate and Lannate (0.1%) treated sugarcane.

Mass Trapping *R. palmarum* in Palm

1. Oil Palm. An oil palm plantation of ~ 6,000 ha established near Coto, Costa Rica in the mid 1970's was mature by the late 1980's. Red ring nematode infestation (RRD) was first detected in the Coto oil palm plantation in 1989. In that year 5,171 of ~800,000 palms were diagnosed with RRD. These palms were cut and sprayed with Furadan. During 1990 and 1991 the only measure undertaken to manage RRD was elimination of RRD infected palms. During these years the number of RRD infected palms in the plantation approximately doubled each year. In late 1992, mass trapping of *R. palmarum* in the Coto plantation commenced in sections diagnosed with RRD. Traps were 19 liter plastic containers tied to palms at chest height and were baited with the male-produced aggregation pheromone and insecticide treated sugarcane or palm pieces (Oehlschlager et al., 1993a, 2002). Decomposition and desiccation of food bait decreased attraction to traps after 2 weeks so food bait was replaced every 2-3 weeks. Pheromone lures were replaced at 3-4 month intervals. Since the enhancement by ethyl acetate was not known at this time ethyl acetate lures were not used in this trapping study. While optimum trap densities were not determined, in previous smaller trials it had been found that a trap density as low as 1 trap / 3.5 ha was sufficient to significantly reduce RRD in a ~ 50 ha stand after a few months (Chinchilla et al., 1993). In the Coto plantation surveys determined that stands with palms older than 17 years were more highly infected with RRD than stands with palms younger than 10 years. Throughout the first year of trapping capture rates in the entire Coto plantation declined from 30 weevils / trap / month to 4 weevils / trap / month, or over 80% (Fig. 10). During the period between 1994 and 2001 monthly capture rates were no higher than 2 weevils / trap / month (Oehlschlager et al., 2002).

Between 1989 and 1991 RRD management was limited to surveying and eliminating RRD infected palms. In late 1992, traps were introduced throughout the plantation and thereafter RRD incidence level dropped by >90% (Fig. 11, Oehlschlager et al., 2002).

In another trial plantation-wide mass trapping was conducted on 8,719 hectares of commercial oil palm near Quepos, Costa Rica with similar results (90% decrease in RRD in 2 yrs). Capture rates were initially high but declined to less than 4 weevils / trap / month by 1994. In 2001 the mean capture rate of traps in the Quepos plantation was 1.13 ± 0.16 weevils / trap / month (Oehlschlager et al., 2002).

In a 3,300 ha oil palm plantation in Honduras trapping *R. palmarum* reduced RRD by 50% in 2 years, 80% in 3 years and 94% in 5 years (ASD, 1999).

Through mark-release-recapture experiments in the Coto plantation in 1991 the initial *R. palmarum* population was estimated at 23-57 weevils per hectare (Chinchilla et al., 1993). During the period April 1991-September 1992 an estimated 123,000 weevils were captured in trap optimization and mass trapping experiments (Oehlschlager et al., 1993b; Chinchilla et al., 1993; Oehlschlager et al., 1995). Plantation-wide mass trapping captured another ~80,000 weevils to the end of 1993. The approximately 200,000 weevils removed by trapping during 1991-1993 corresponded to ~ 30 weevils / hectare. During the same period new RRD infection decreased from ~ 22,000 in 1992 to ~ 5,000 palms in 1993.

Trapping, in combination with removal of nematode infected palms, is the principal method by which weevil vectored red ring nematode is managed in oil palm in Central and South America.

2. Mass Trapping *R. palmarum* in Coconut Palm. Trapping of *R. palmarum* in coconut palm is also effective. During 2000-2001 the author's group (ChemTica) in Costa Rica conducted trapping of this weevil in 50 ha of coconut palm using traps similar to those used in the Coto and Quepos trials. In the coconut plantation in which trapping was conducted, direct damage by *R. palmarum* larvae was responsible for palm death. A trap density of 1 trap / ha was used in this trial (Oehlschlager and Gonzalez, unpublished). Capture rates of *R. palmarum* were initially ~11 / trap / week and did not show a significant decline during the 11 month study (Fig. 12). This is in contrast to a > 80% decrease *R. palmarum* capture rates observed in oil palm over 1 year (Oehlschlager et al., 2002).

In the coconut palm study infested palms were allowed to act as *R. palmarum* breeding sites. Thus, in this trial *R. palmarum* capture rates remained high throughout the study. Similarly, rather constant *R. palmarum* capture rates were observed over 26 months of trapping in coconut in Brazil. In the latter case 54 one hundred liter (huge) pheromone:sugarcane baited traps were placed around the perimeter of 54 ha of coconut palm infested with *R. palmarum* carrying red ring nematode. Over 97,000 weevils were captured over a 26 month period with no noticeable decrease in capture rate over the entire period (Moura et al., 2000). Despite this, the number of RRD infested palms decreased from 206 at the initiation of trapping to 3 within a few months after trapping was started. The most reasonable explanation for a rather constant capture rate accompanied by a declining infestation rate is that weevils immigrating into the plantation as well as those escaping from infested palms preferred traps rather than palms. A similar behavior has been noted in mass trapping of *Cosmopolites sordidus*, the banana corm weevil. In 1 ha plots, using 4 traps / ha capture rates remained high for >8 months while infestations due to this weevil decreased significantly after <5 months (Alpizar et al., 1998).

Immediately before commencement of mass trapping in coconut palm we identified and tagged palms with yellowing fronds and verified which of these were infested with *R. palmarum* by a subsequent survey in June 2000. Of the coconut palms initially identified possessing yellowing fronds 49 were eventually confirmed to have died as a result of *R. palmarum* infestation. During the remaining period of the study 4 additional palms were found to be weevil infested (Fig. 13). This is a reduction of >90%

in infestation and compares favorably with the decrease in RRD achieved in oil palm by trapping *R. palmarum* (Oehlschlager et al., 2002).

TRAPPING OF *RHYNCHOPHORUS PALMARUM* AND *METAMASIUS HEMIPTERUS* WITH THE SAME LURE IN PALMITO PALM IN COSTA RICA

The heart of palmito palm (*Bactris gasipaes*, Kunth) is a delicacy in many countries of the world. Increasing demand for dietary fiber continues to fuel demand for palmito heart. Areas dedicated to commercial production in Central and South America in 1996 were about 12,000 ha of which around 4,000 ha were in the Atlantic Region of Costa Rica (Anonymous, Min. Agric. & Gran., 1998 Costa Rica).

Palmito palm propagates from offshoots that grow to a harvestable height of one meter in about 3 months. Harvesting discards parts of the plant except the interior of the stem. In some plantations, competing offshoots are pruned to promote more rapid growth of the remaining offshoots to harvestable size. Harvesting and pruning provide excellent entry points for *Metamasius hemipterus* L. (Vaurie, 1966) and *Rhynchophorus palmarum* L. (Couturier et al., 1996; Vásquez et al., 2000). Female weevils are attracted to and deposit eggs in cut stems. Larvae tunnel the stem and rhizome destroying maturing stems.

The author's group (ChemTica) recently completed and published a study that showed it is efficient to capture both *M. hemipterus* and *R. palmarum* using a pheromone lure that emits the aggregation pheromones of both species (Alpizar et al., 2002). Using traps baited with this combination lure and insecticide treated sugarcane it was shown that damage to palmito by both weevils was reduced over the crop cycle and that the yield increase attributable to trapping was 58% in plots in which pruning was conducted and 70% in plots in which pruning was not conducted (Alpizar et al., 2002).

MASS TRAPPING *R. FERRUGINEUS* IN DATE PALM

Trap Design

Traps should be designed with consideration of weevil behavior. As with *R. palmarum* the red palm weevil *R. ferrugineus* is a strong flyer. It will fly to the area of a trap then land and walk into the trap. Accordingly, the traps of highest efficiency are those that facilitate entry. In the most extensive trap design trials (Fig. 14) the pit trap was the most effective but it was also the most difficult to construct and maintain. Operational use therefore centered on bucket traps. In each of these traps pheromone with food immersed in insecticide laced water was used as bait. The inverted bucket trap was less effective than the upright bucket traps (Fig. 12, Trial A), presumably because it easily lost moisture which is key to trapping *R. ferrugineus*. Additionally, with the inverted trap changing food baits was difficult because the liquid part of the food easily spilled. Upright buckets retain moisture for extended periods in the winter months and for at least a few days in the Middle East summers. Upright buckets are also easy to service. Of the upright bucket traps tested traps containing a mat or rough surface were the most effective (Fig. 12, Trial B).

The preferred trap for *R. ferrugineus* in the Kingdom of Saudi Arabia is the upright bucket trap with mat (Fig. 15) and in the UAE a bucket trap with a molded rough surface is used (Fig. 13).

Trap Location

The attachment of food baited traps for *R. ferrugineus* to coconut trees was common practice in India prior to the 1990's and is still common practice. Initial trials with pheromone and food baits in 1992 and 1993 used bucket traps (Fig. 5) attached to coconut palms in Indonesia and date palms in the UAE (Hallett et al., 1993a). It was subsequently determined that in the desert environment of Egypt placement of traps on the ground or buried in the ground was more effective (Fig. 16).

In Indian coconut gardens (Faleiro, 2005) it was found that traps tied to coconut trees at chest height were more efficient than those placed in other locations including on

the ground.

Overall, ideal trap locations are those where there is a good landing surface near the trap so that it is easy for the attracted weevils to walk into the trap.

In Spain some of the traps tested for monitoring *R. ferrugineus* use moist, insecticide-treated, absorbent plastic cubes and synthetic chemical food bait as well as pheromone (Gomez and Perry, 2002, Fig. 17). These traps are poor for two reasons: a) synthetic chemical food bait for *R. ferrugineus* is not a good attractant for *R. ferrugineus* (Rochat, 2005) and should not be used until it is improved, b) it is difficult for weevils to enter this trap since they must crawl up a very high and smooth surface (30 liter container). Both of these considerations lead to the expectation that in a head to head trial this trap would be considerably less efficient than the Kingdom of Saudi Arabia design or the UAE design or a buried trap of smaller size in which the entry ports were at ground level.

Other traps tested in Spain use insecticide treated date palm petiole as the food synergist to pheromone. Based on studies with *R. palmarum* palm petiole is not a very good synergist (Oehlschlager et al., 1993b).

Attractants in Trap

While the pheromone is reported to be only one component (4-methyl-5-nonanol, Hallett et al.,1993a,b), experiments in the early 1990's in Saudi Arabia showed that addition of a second component (4-methyl-5-nonanone) in a small amount increased capture rates by 65% (Fig. 18, Abozuhairah et al., 1996).

Attractiveness of the male-produced aggregation pheromone of *R. ferrugineus* is dramatically synergized by addition of food to the trap (Fig. 19). Food additives for *R. ferrugineus* traps have been sporadically studied. In India fermented palm sap (toddy) has been recommended as a trap additive for decades. In initial studies of the ability of food to synergize attraction to the pheromone coconut and date palm trunk pieces were found to be good additives (Hallett et al., 1993a) but are difficult to obtain. Molasses and extracted sugarcane stalk have been used in Egypt with some success (Oehlschlager, 1998). The most common additive to pheromone traps for *R. ferruginues* in the Arabian Peninsula is mature date fruit. The key to a good food seems to be relatively high moisture content coupled with the presence of attractive constituents.

In July 1997 ethyl acetate was shown to increase attraction of *R. ferrugineus* to pheromone:date fruit traps by 2.6X in the UAE (Fig. 20, Oehlschlager, 1998). Later the same year it was shown that addition of ethyl acetate to pheromone:molasses,sugarcane stalk traps increased captures by 5X in Egypt (Fig. 18, Oehlschlager, 1998). At the current time the best attractants for *Rhynchophorus* species are the pheromones, moist food and ethyl acetate. Except for ethyl acetate attractiveness to pheromone and food is not increased by addition of synthetic kairomones for either of *R. palmarum* or *R. ferrugineus*.

The author's group (ChemTica) has incorporated both pheromone and ethyl acetate into a single lure that emits these components at a constant ratio over the life of the lure. This combination lure (Ferrolure+WM) functions as well as an individual pheromone lure and individual ethyl acetate lure (Fig. 21). The Ferrolure+WM lures are the most cost effective pheromone:kairomone lure currently available.

The optimized trap for *R. ferrugineus* is a small plastic bucket trap that allows easy entry due to a rough outer surface as is used in the Kingdom of Saudi Arabia or the United Arab Emirates. The optimized lure for *R. ferrugineus* emits 4-methyl-5-nonanol and 4-methyl-5-nonanone (Ferrolure+) as well as ethyl acetate (Ferrolure+WM). Optimal foods are mashed mature date fruit bits in water (Arabian Peninsula) or fermented palm sap (toddy, India).

Insecticide in Traps

Some palm weevil trapping programs have recommended the use of traps without insecticide. In Costa Rica we observed that pheromone:sugarcane traps captured low

numbers of *R. palmarum* unless some killing agent was added (Fig. 22). Experiments in which live *R. palmarum* were placed in traps showed that within 24 hrs traps with no killing agent lost over 90% of their weevils (Oehlschlager et al., 1993b). We determined that several additives to traps would retain weevils, namely detergent, boric acid and insecticide. The latter was the most effective in retaining *R. palmarum*. In the insecticide vs no insecticide experiment shown in Figure 22 we found 10 *R. palmarum* in traps without insecticide. Of these, 7 were alive suggesting that without insecticide *R. palmarum* enter the traps but leave. In Costa Rica we use only 0.1 to 0.25% lannate in traps to retain *R. palmarum*. In the UAE killing agents are not used in R. ferrugineus traps but in Saudi Arabia killing agents are added. The behavior of *R. ferrugineus* in traps appears to be different than that of *R. palmarum,* in that captured *R. ferrugineus* cling to solid material and each other in traps and exhibit little tendency to escape (Author, personal observation).

Mass Trapping *R. ferrugineus*

Mass trapping of *R. ferrugineus* is widely practiced in the Arabian Peninsula where it is a major problem in date palm. Management of *R. ferrugineus* relies on frequent inspection of palms to detect infestation, treatment of infested palms by injection of insecticide or removal, periodic spraying and trapping (Abraham et al., 1998). Although several studies have been undertaken to determine if trapping *R. ferrugineus* lowers infestation, only one in the UAE will be cited here. In the cited study traps were used as shown in Figure 15, baited with Ferrolure+ and contained date fruit bits in water without insecticide. Between 1994 and 1998, 1,466 farms were periodically examined for infestation. In 1994 all farms contained 349,342 palms and had an average infestation rate of 1.9%. In 1995/1996 all farms received chemical treatment and in 1996/1997 45% of the area was treated with chemicals and pheromone traps. while 55% of the area was treated with chemicals only. In trapping areas in 1996/1997 11,711 weevils were captured (Ezaby et al., 1998). A benefit of ~ 30% less infestation appears to be derived from combined use of spray and pheromone traps compared to spray alone (Fig. 23).

It is often argued that traps attract weevils to an area and that not all weevils enter so traps create a situation in which infestation can increase. In the case of *R. palmarum* it has been found that although weevils are attracted to areas where traps are placed that infestation goes down in areas with traps (Oehlschlager et al., 1995). The same situation occurs in the case of *R. ferrugineus.* Thus, in another UAE study in which traps were placed on 6 different farms over one year with no spraying, the highest captures resulted in the greatest reduction of infestation (Fig. 24, Kaakeh et al., 2001). Interestingly, in this study the average reduction in infestation over all 6 farms from one year to the next was 71%. This suggests that trapping *R. ferrugineus* is as effective as trapping *R. palmarum* and that one can expect approximately a 70-80% reduction in infestation over one year if *R. ferrugineus* traps are maintained well and infested palms are treated to prevent further breeding.

When *R. ferrugineus* was first detected in Israel in 1999 a management strategy including regular survey, prophylactic insecticide chemical treatment and mass trapping was installed on 450 ha of date palm plantations in the infested region. In addition, monitoring trapping was conducted outside the infested region. Traps used for both mass trapping and monitoring contained Ferrolure+ lures as well as ethyl acetate lures and fermenting dates and sugarcane molasses (Sorokor et al., 2004). A significant decrease in the number of trapped weevils as well as a significant decrease in infested palms was observed by 2001 and no infested palms have been found since 2002. It is suspected that trapping played a significant role in suppression of *R. ferrugineus* populations (Soroker et al., 2004).

Trapping of *R. ferrugineus* in date and coconut palm has been practiced in the Indian subcontinent for decades. Prior to the discovery of the pheromone for *R. ferrugineus,* traps were usually constructed of hollowed coconut trunk into which fermented palm sap (toddy) laced with insecticide was placed. These traps, while

effective, are much more effective with pheromone added and this strategy is now recommended by several institutions (such as the Coconut Research Institute of Sri Lanka) responsible for vetting techniques for farmers (for example, http://www.dailynews.lk/2002/04/02/fea07.html). In a recent 2 year study in India pheromone:food trapping of *R. ferrugineus* resulted in a 75% decrease in captures suggesting a significant decrease in population (Fig. 25, Muralidharan et al., 1999). No damage assessment was made in this study. The decrease in capture rate observed in this study was very similar to the reduction in capture rate observed in Costa Rica for *R. palmarum* trapping in oil palm (Chinchilla et al., 1993, Oehlschlager et al., 2002). Additionally, (Faliero, 2005) it has been shown that good trapping techniques lead to good management of R. ferrugineus in coconut plantations in India.

Since female *R. ferrugineus* are the primary target of trapping it is often debated as to whether trapping removes young females with high egg laying potential or old females with low egg laying potential. Dr. Falerio of the Goa, Research Station in India conducted an extensive study of female *R. ferrugineus* captured in pheromone traps (Falerio, 2000). In his experiment he removed female *R. ferrugineus* daily from field placed traps and maintained them alone with only other females or in contact with captured males. The captured female *R. ferrugineus* were then allowed to feed and oviposit on sugarcane for the rest of their life. The results indicated that trapped females held separately from males after capture laid an average of 208 eggs during the rest of their life. Since Wattanapongsiri (1966) reported that wild females lay an average of 127-376 eggs during their lifetime these results indicate that young, gravid females are captured by pheromone traps. Thus, trapping is predicted to have a major effect on egg laying potential of the population.

Palm weevils are present in most plantations in relatively small numbers and have a relatively long life (Wattanapongsiri, 1966). These characteristics allow mass trapping to be an efficient management technique since capture of low numbers can significantly impact future populations and a significant proportion of an adult population can be captured over the long period they are susceptible to pheromone and food traps.

Since palm weevils are strong flyers traps can be widely spaced and trapping is more efficient than spraying for weevil management.

Lowering Palm Weevil Attack on Palms

1. Pruning and Off-shoot Removal Practices. It is generally recognized that *R. ferrugineus* can cause infestation of a palm only via oviposition in fresh cut tissue. Since females are (Faliero, 2000) mated upon emergence and must oviposit in fresh palm tissue a recommended practice in the Indian subcontinent and Arabian Peninsula is to leave about 20-30 cm of frond base attached to the tree. This is a preventive action based on the logic that if *R. ferrugineus* eggs are deposited in the cut tissue that drying of the tissue will decrease the egg and larval survival rate. Almost no infestation occurs in the Arabian Peninsula in the crown of date palms. In the Arabian Peninsula the primary entry point of *R. ferrugineus* is at the base (Fig. 26, Khalifa et al., 2001). This is because the common practice of offshoot removal causes trunk damage at ground level. Placement of insecticide on the injured tissue is a very good preventive action.

The highly localized nature of *R. ferrugineus* attack raises the possibility of using repellants to deter attack on that portion of the palm that is most susceptible. Most repellants deter insects only at close range and then can be expected to function only over short ranges. Because of the highly localized nature of *R. ferrugineus* attack the author's group (ChemTica) has been investigating repellants for *R. palmarum* with the assumption that this is a good test species for *R. ferrugineus*. The strategy compares capture efficiency of pheromone:food baited traps with pheromone:food baited traps additionally releasing candidate repellants. This approach allows rapid screening, which is necessary because there are over 9,000 compounds reported to be repellant to different insects. We have narrowed the search by eliminating any compound that has been reported both as a repellant and an attractant and currently have ~ 30 candidates.

In trials conducted to date one potent repellant for *R. palmarum* has been discovered. In Figure 27 release of Repellant A, from highly attractive pheromone:sugarcane:ethyl acetate baited traps decreased capture rates by over 50%. In Figure 28 a similar test of known repellants of other insects is shown. While it could be argued that alpha-pinene would mask the odor of palm trees with that of a non-host pine tree this candidate is not repellant. Likewise, leaf alcohol has been reported to be repellant to many species of insects but is not repellant to *R. palmarum*.

PRESENT STATUS

In Central and South America trapping is well established in well managed oil, coconut and palmito palm plantations to reduce problems associated with *R. palmarum*. Current best practices management of *R. palmarum* in oil and coconut involves removal of infested palms and mass trapping. In the Arabian Peninsula and Indian subcontinent trapping of *R. ferrugineus* is well established in the management of weevil populations. In the Arabian Peninsula monitor and mass trapping are used in combination with regular survey and insecticidal treatment of infested palms. Effective trapping of both *R. palmarum* and *R. ferrugineus* requires the use of pheromone, ethyl acetate and wet food. Although no synthetic chemical combination has been found to be as effective a synergist as natural food for any palm weevil pheromone, the author's group has found stable and storable food bait that is as attractive as sugarcane in *R. palmarum* pheromone traps.

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Figures

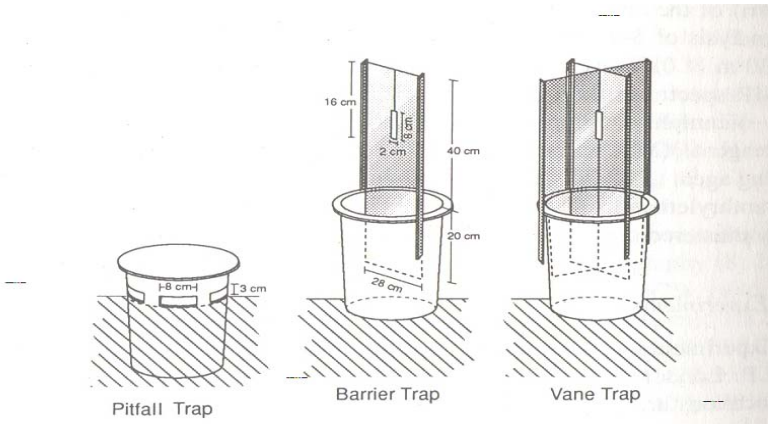

Fig. 1. Traps designed to capture *Oryctes rhinoceros*.

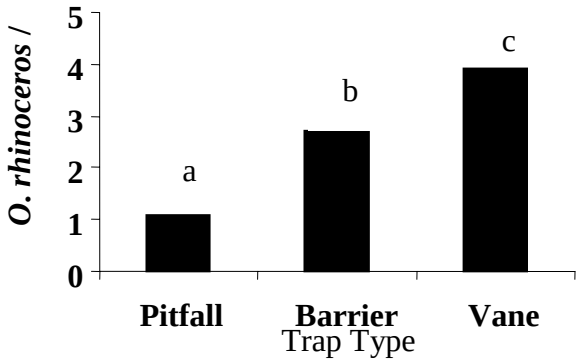

Fig. 2. Capture of *O. rhinoceros* in different trap types (9 repetitions). Male-produced aggregation pheromone released at 30 mg/day.

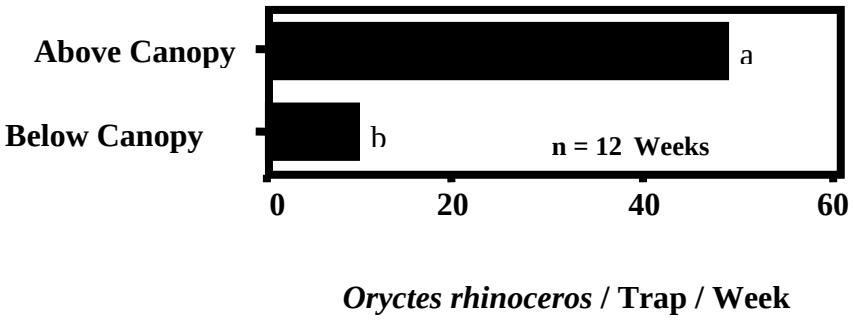

Fig. 3. *Oryctes rhinoceros* vane topped bucket traps elevated above canopy in young oil palm plantation. Capture rates in vane topped bucket traps above and below canopy of young oil palm.

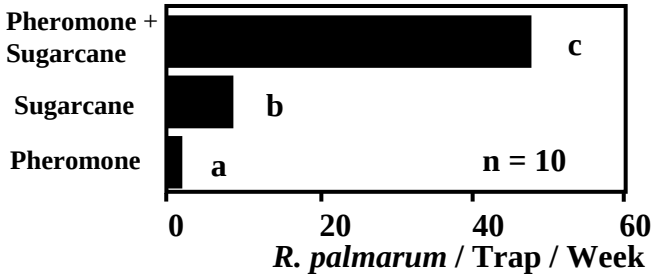

Fig. 4. Attraction of *R. palmarum* to traps.

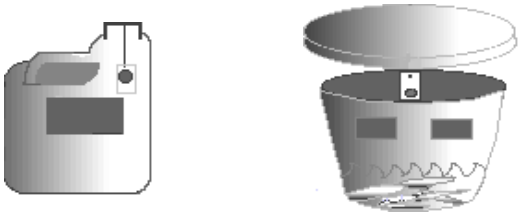

Fig. 5. Traps for *R. palmarum.*

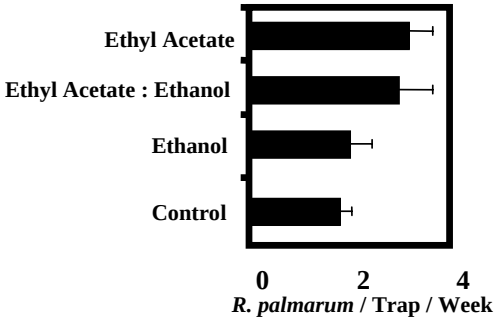

Fig. 6. Effect of ethyl acetate, ethanol and the combination of ethyl acetate and ethanol on attraction of *E. palmarum* to traps.

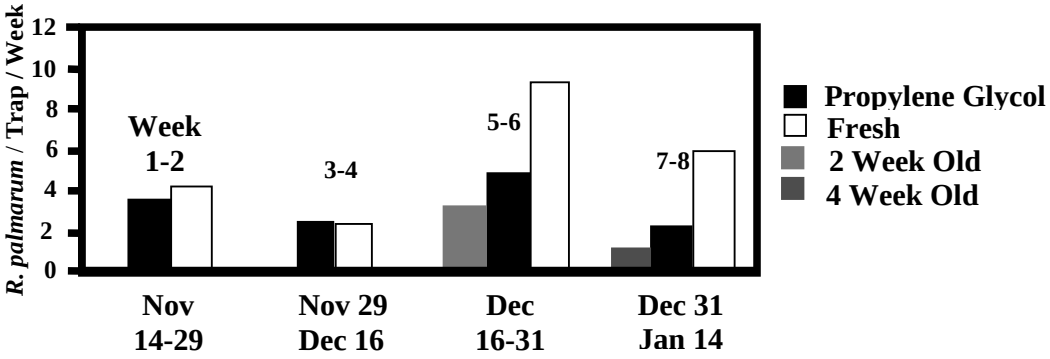

Fig. 7. Experiment set up November 14, 2000. All traps contained commercial pheromone lures (3-6 mg/day, ChemTica). Treatments were traps baited additionally with fresh sugarcane (350-400g) in 750 mL of water containing 0.13% Lannate (New Sugarcane); 2 week old sugarcane in 750 mL of water containing 0.13% Lannate (2 Week Old Sugarcane); 6 week old sugarcane in 750 mL of water containing 0.13% Lannate (6 Week Old Sugarcane) and fresh sugarcane, ethyl acetate (200-400 mg/day) lures in 750 mL of water with 20% Trap Extender and 0.13% Lannate placed November 14, 2000 (Traps with Propylene Glycol). Ten traps of each treatment were placed. Means of capture are presented. ANOVA on data collected November 29 (n = 9-11), December 16 (n = 9-10) and January 14 (n = 9-10) indicated no significant differences between treatments. ANOVA (n = 8-10) on December 31 and Feb 1 (n = 9-10) indicated traps containing new sugarcane were significantly more attractive than other treatments.

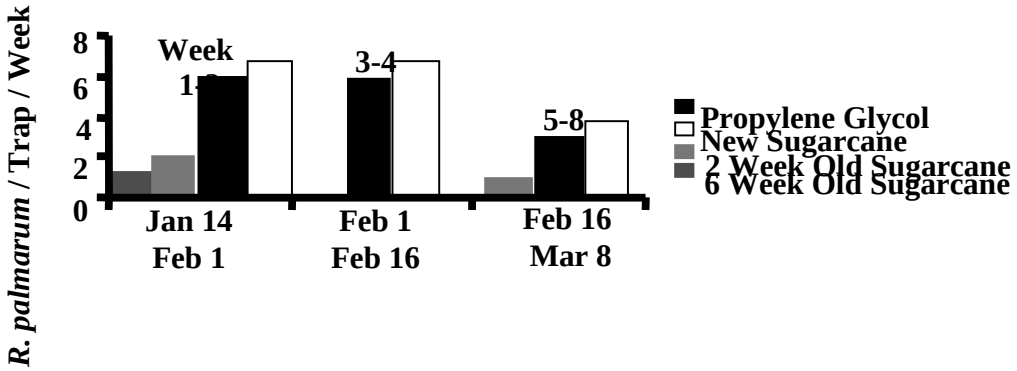

Fig. 8. Experiment set up January 14, 2001 all traps contained commercial pheromone lures (3-6 mg/day, ChemTica). Treatments were traps baited additionally with fresh sugarcane (350-400g) in 500 mL of water containing 0.13% Lannate (New Sugarcane), 2 week old sugarcane in 500 mL of water containing 0.13% Lannate (2 Week Old Sugarcane), 6 week old sugarcane in 500 mL of water containing 0.13% Lannate (6 Week Old Sugarcane) and fresh sugarcane, ethyl acetate (200-400 mg/day) lures in 750 ml of water with 50% propylene glycol and 0.13% Lannate placed January 14, 2001 (Traps with propylene glycol). Ten traps of each treatment were placed. Means of capture are presented.

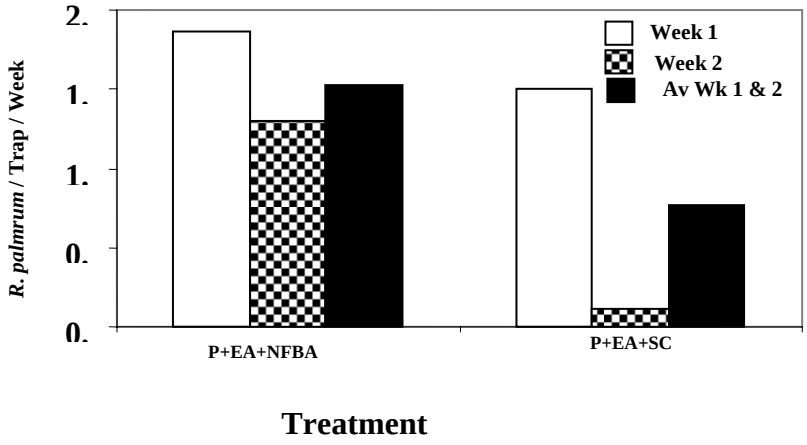

Fig. 9. Ten liter bucket traps containing pheromone lure (P, 3-6 mg/day), ethyl acetate lure (EA, 200-400 mg/day), 500 mL of 0.1% Sevin and either 350-400 g sugarcane (SC) or 440 mL of natural food bait (NFBA). Traps were hung at chest height on coconut trees in a 50 ha coconut plantation in Northeastern Costa Rica 100 meters apart and 50 meters from any border (n = 9).

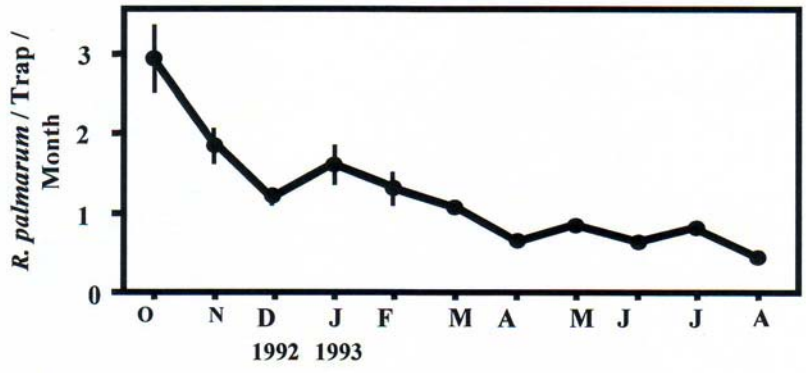

Fig. 10. Mean (SEM) capture rates of *R. palmarum* in all pheromone and sugarcane traps in Coto, Costa Rica oil palm plantation 1992-1993.

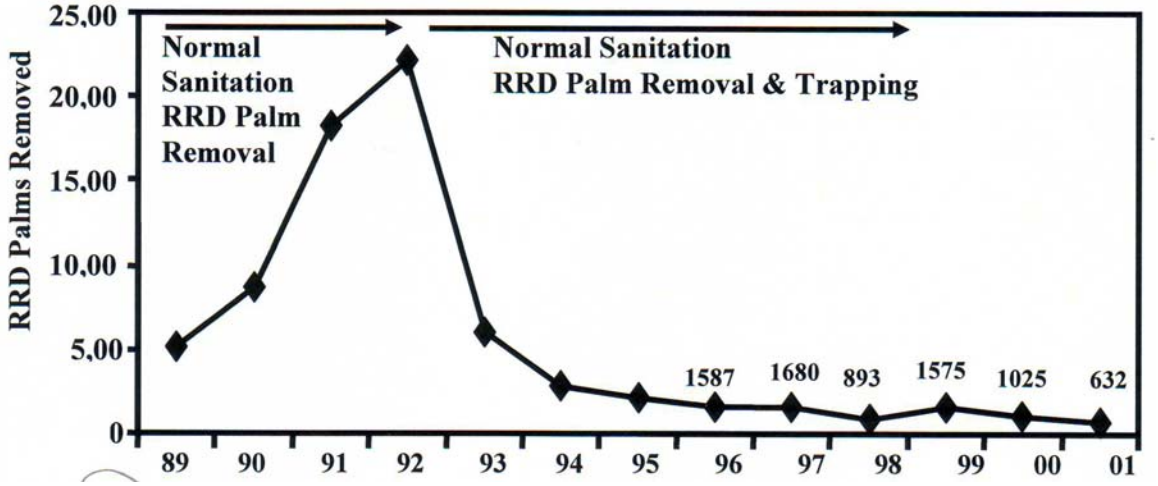

Fig. 11. RRD observed in oil palm plantation in Coto, Costa Rica between 1989 and 2001. Each year of the study all palms were inspected every 2 months and infested palms eliminated. Pheromone and sugarcane trapping was begun late 1992.

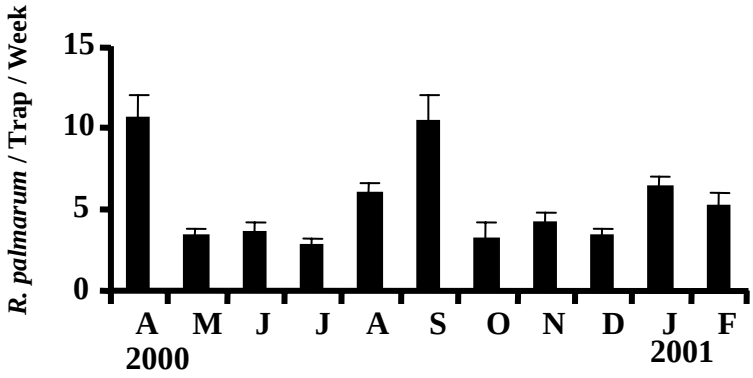

Fig. 12. Average of weekly captures (+SEM) of *R. palmarum* in bucket traps (tied to coconut trees at chest height) baited with pheromone (3-6 mg/day), sugarcane (350-400 g) and ethyl acetate (200-400 mg/day) April 2000 to March 2001 in 50 ha coconut.

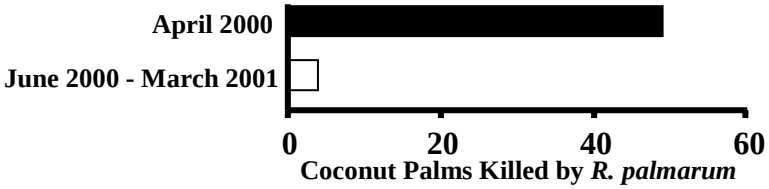

Fig. 13. Coconut palms killed by *R. palmarum* in 50 ha of coconut June 2000 to April 2001 in which trapping was conducted.

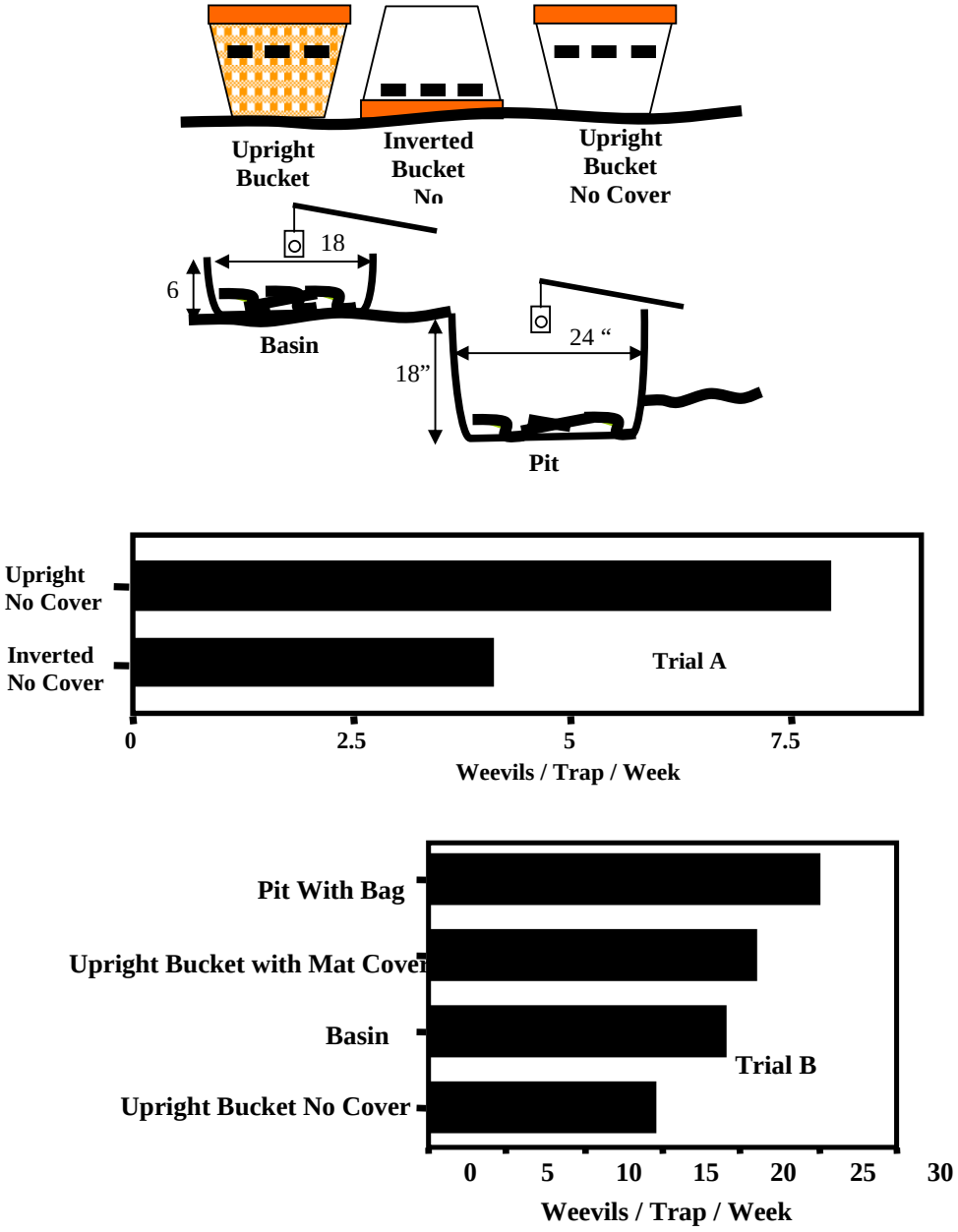

Fig. 14. *R. ferrugineus* traps studied in the Kingdom of Saudi Arabia. Traps baited with Ferrolure+ and insecticide treated palm pieces. Test conducted by Dr. R. A. Abozuhairah, Dr. P. S. P. V. Vidyasagar & Dr. V. A. Abraham, Ministry of Agriculture & Water, Al Hassa, Kingdom of Saudi Arabia. Reported in International Congress of Entomology, Florence, Italy, 1996.

Fig. 15. Rough surface molded plastic *R. ferrugineus* trap used in the UAE. Trap is used buried in ground to level of entry ports.

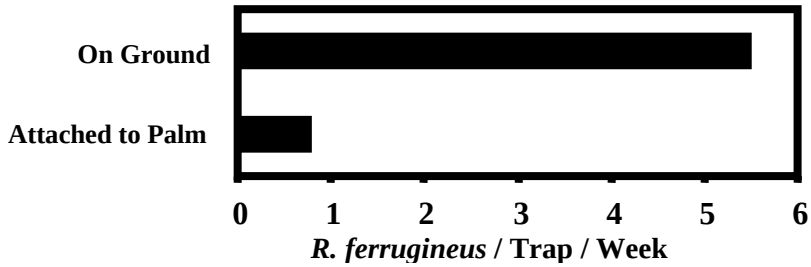

Fig. 16. Test of 20 Bucket Traps on the Ground and 20 Bucket Traps attached to date palms in Northern Egypt, July-November 1996, Traps baited with Ferrolure+ and insecticide laced molasses and sugarcane stalk (extracted). Dr. G. Moawad & Y. El-Sebay, August, 1997 (Published in proceedings of FAO Workshop on Red Date Palm Weevil & its Control, Dec. 1998, Cairo).

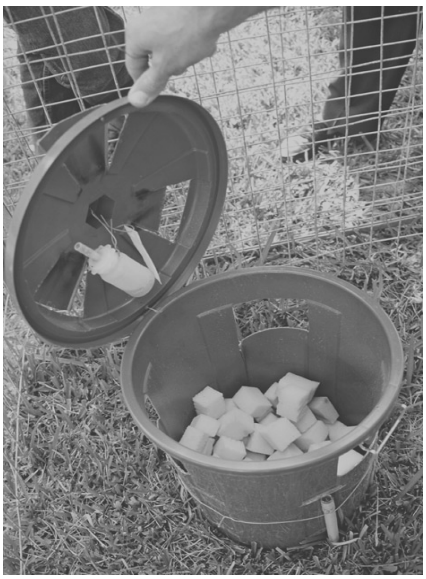

Fig. 17. Spanish trap for *R. ferrugineus* (Gomez and Perry, 2002).

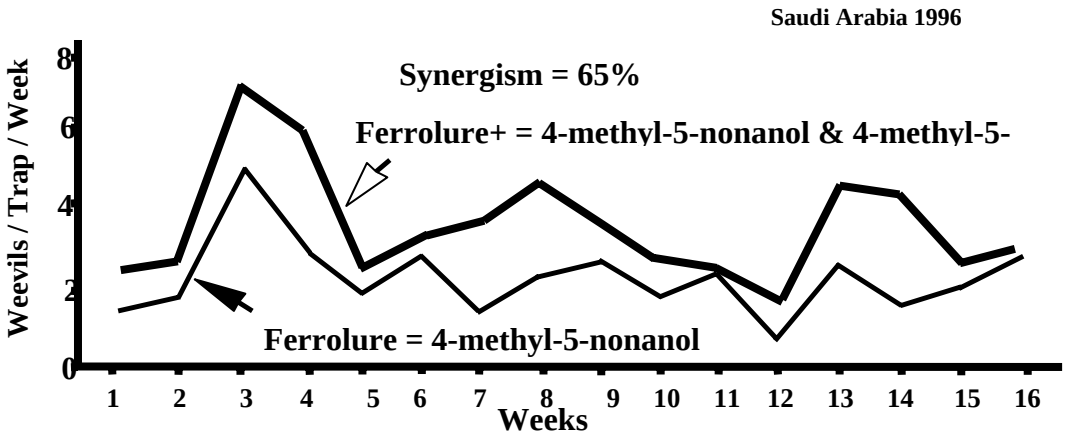

Fig. 18. *R. ferrugineus* captured in upright bucket traps with mat cover when traps are baited with (4-methyl-5-nonanol, Ferrolure), or 4-methyl-5-nonanol and 4-methyl-5-nonanone, Ferrolure+ and insecticide treated palm trunk pieces.

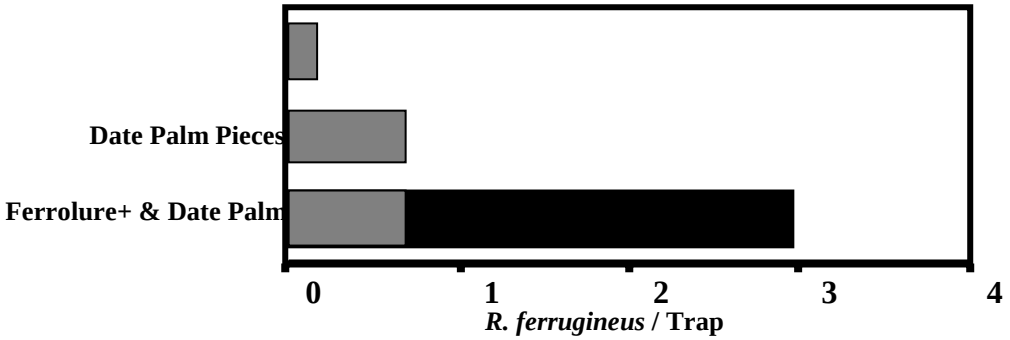

Fig. 19. Capture of *R. ferrugineus* in bucket traps attached to palms Test conducted by Mr. R. K. Al-Shareqi & Dr. S. Gassouma in UAE in 1992 (n = 10). Traps contained insecticide-treated date palm trunk pieces.

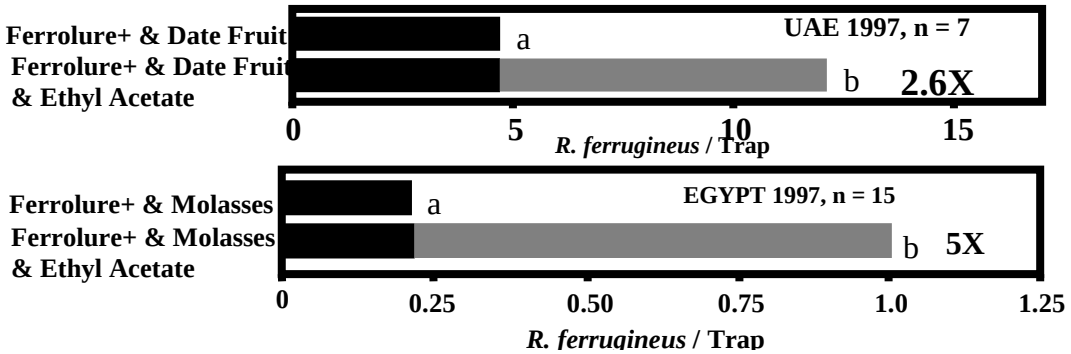

Fig. 20. Increase in capture of *R. ferrugineus* to pheromone:food traps containing lures emitting ethyl acetate. One experiment conducted in UAE (Aswar and Oehlschlager, ChemTica Technical Bulletin) and a second experiment conducted by G. Moawad and Y. El Sebay, PPRI, in Egypt, 1997 (Oehlschlager, 1998). ANOVA on both experiments revealed significant differences between treatments, Bonferonni t-test, P > 0.95.

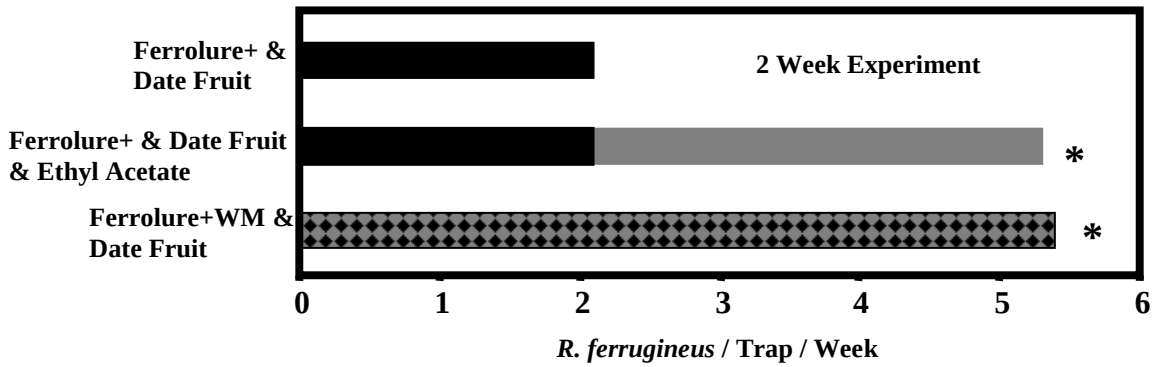

Fig. 21. Experiment conducted using 12 liter buried bucket traps (as in Figure 13) 100 meter intervals. All traps contained 200-300 grams of crushed date fruit and 2 liters of water. Ten traps were baited with Ferrolure+ pheromone lures, ten traps were baited with Ferrolure+ pheromone lures and ethyl acetate bottle lures and ten traps were baited with Ferrolure+ / ethyl acetate combination lures (Ferrolure+WM). Captured weevils were counted and removed each week for 2 weeks. Trap positions were rerandomized at the end of the first week. ANOVA (n = 20) gave a significant difference between Ferrolure+ and Ferrolure+ with kairomone in two or same lure.

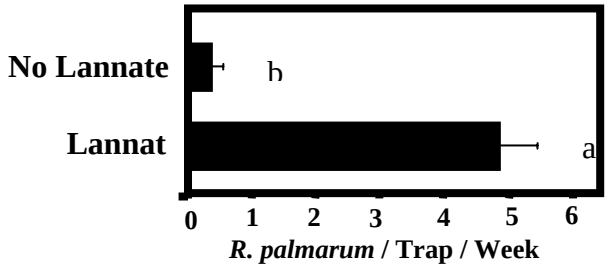

Fig. 22. Experiment conducted July 29-August 12, 2000. Traps contained pheromone lures, fresh sugarcane (350-400 g), ethyl acetate:ethanol (1:1) lures (200-400 mg/day each component) and 1 liter of 0.25% Lannate (Lannate) or 1 liter of water (No Lannate). ANOVA (n = 12-13) gave df = 1, 23 F = 15.07. Means topped by different letter are significantly different by Bonferonni t-test, P>0.95.

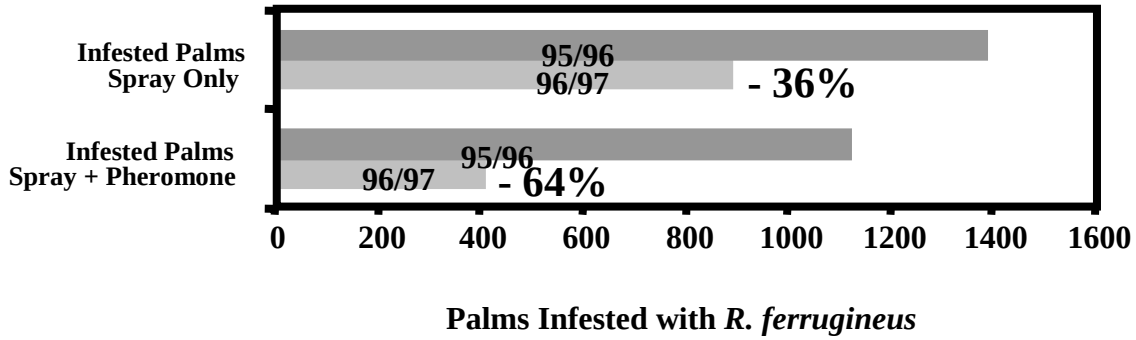

Fig. 23. Survey of 1,466 farms in Al-Ain region of UAE between 1994 and 1998 (Ezaby et al., 1998).

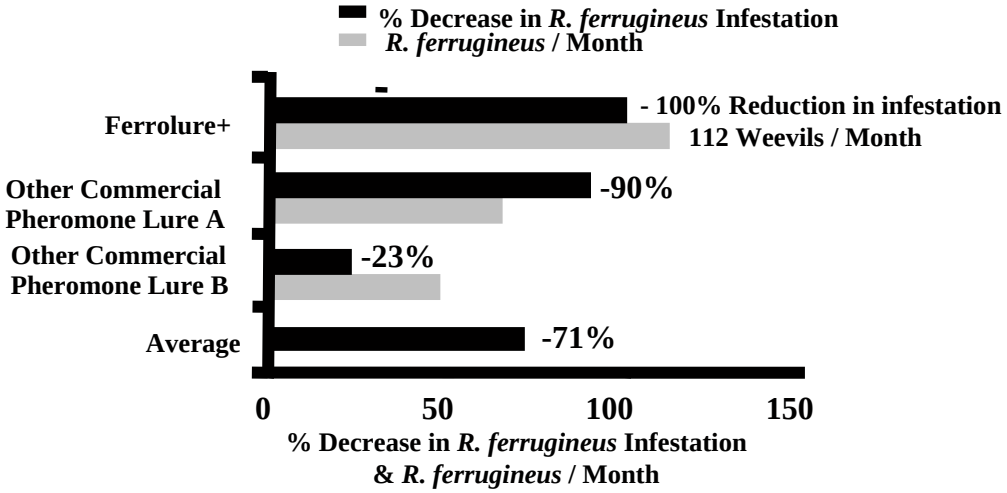

Fig. 24. Trapping *R. ferrugineus* at 6 farms in UAE with 3 different commercial pheromone lures and containing date fruit in water as food synergist. Infestation from first year in which spray only was used to second year in which trapping only was used (Kaakeh et al., 2001).

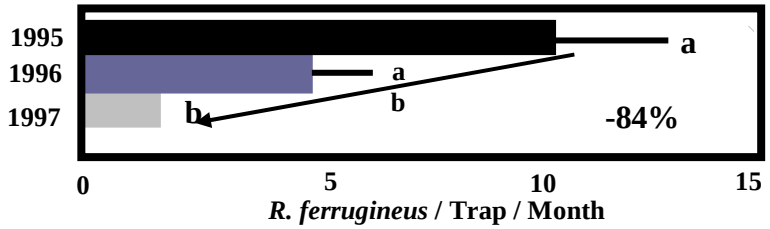

Fig. 25. Average capture of *R. ferrugineus* in 5 date palm gardens in 4 villages in northern India ANOVA (n = 12) gave df = 2, 33, F = 7.0, $p < 0.05$. Means followed by different letter are significantly different, Bonferonnti t-test, $P > 0.95$.

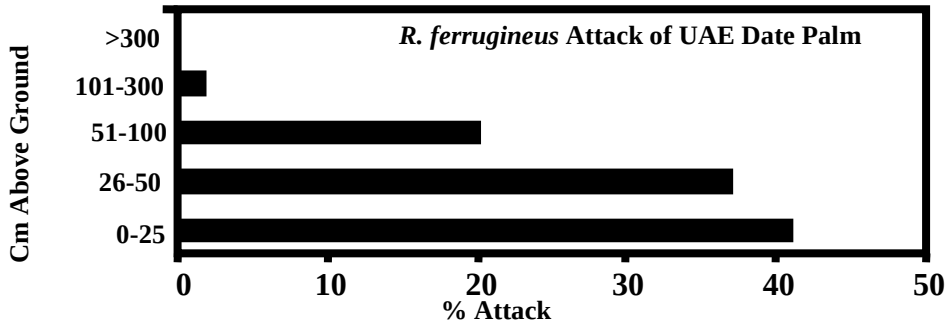

Fig. 26. Survey of 1,325,574 Palms in UAE 1998-2000 of which 2,296 were infested. (Khalifa et al., 2001).

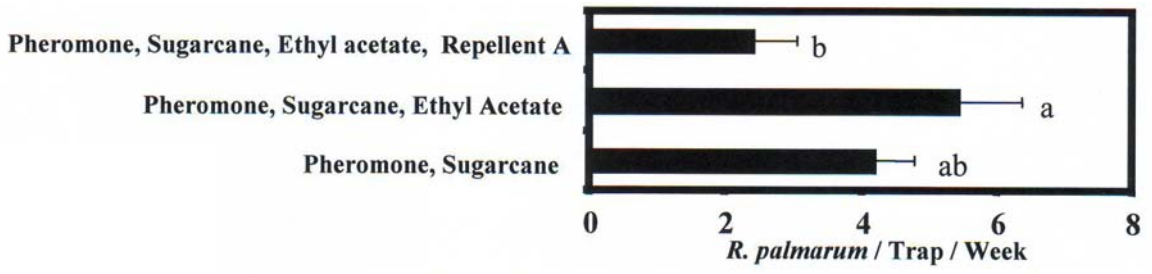

Fig. 27. Experiment conducted November 14-29, 2000 in 50 ha of commercial coconut palm in Costa Rica. Treatments were pheromone and sugarcane in 750 mL 0.13% Lannate; pheromone, sugarcane, ethyl acetate with 750 mL water containing 0.13% Lannate and pheromone, sugarcane, ethyl acetate, and Repellant A with 750 mL water containing 0.13% Lannate. ANOVA (n = 8-10) gave $p < 0.05$. Means followed by different letter are significantly different by Bonferonni t-test, $P > 0.95$.

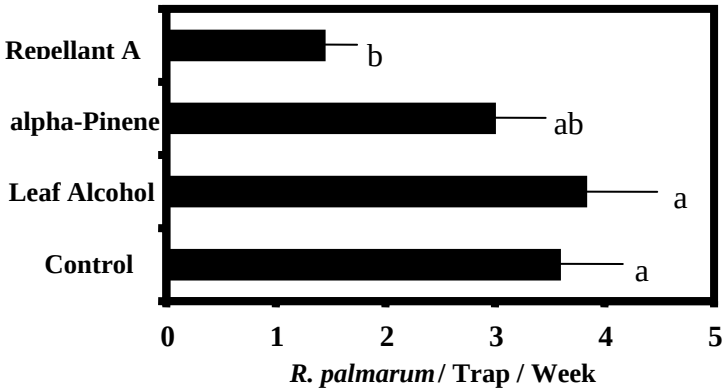

Fig. 28. Experiment conducted May 31-June 13, 2000. All traps contained pheromone lures, ethyl acetate:ethanol (1:1) lures and fresh sugarcane immersed in fresh sugarcane 1 liter of 0.25% Lannate. Repellant candidate traps additionally contained slow release devices containing the indicated candidate repellants. Weevils were counted and removed June 6 at which time trap positions were re-randomized. Analysis determined no differences in capture rates on June 6 and June 13 allowing combination of captures for June 6 and 13. ANOVA (n = 17-20), df = 3, 72, F = 4.62. Means followed by different letter are significantly different by Bonferonni t-test, $P > 0.95$.

The Efficacy of Four Systemic Insecticides Using Two Methods of Application against the Green Date Palm Pit Scale Insect *Asterolecanium phoenicis* Rao.) (*Palmapsis phoenicis)* (Homoptera: Asterolecaniidae) in Northern Sudan

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Sudan

Abstract

A series of small-scale experiments were undertaken in Algaba scheme and Golid area for two seasons, 2002/2003 and 2003/2004, respectively, to evaluate the effectiveness of four systemic insecticides (Actara 25WG, Rinfidor 20%SL, Comodor 20%SL and Confidor 200SL) using two methods of application: soil application and trunk injection. Insecticide doses of Actara 25WG (thiamethoxam) were 9, 12, 15 and 18g /palm and 6, 8 and 10g /palm for soil application and trunk injection, respectively, while doses of Rinfidor 20%SL and Comodor 20%SL of (imidacloprid) were 20, 25 and 35ml/palm and 10, 15 and 20ml/palm for soil application and trunk injection, respectively. Confidor 200SL (imidacloprid) was used as standard (35ml/palm) for soil application and 10, 15 and 20ml /palm for trunk injection. A complete randomized design with six replicates (one palm = replicate) was used. The insects (all developing stages) were counted (cm^2/leaflet). Eight leaflets from each palm were inspected at biweekly intervals. Date yield and quality were determined at harvest. A residue analysis was carried out on dates, soil and intercropping twice. Results indicated that the total number and % mortality of both adult insects and immature stages were significantly higher than the untreated control for the two application methods. The higher doses remained effective throughout the experimental period. Date palms treated with the higher doses of insecticides developed normally and dates reached maturity (ripening) with an increased yield of more than 70% compared to the untreated control. All insecticides checked termites and many other pests, but did not affect mites. The two methods of application are highly economical and safe for the user with minimal environmental impact.

INTRODUCTION

The date palm, *Phoenix dactylifera* L., is cultivated in the Northern Sudan along the banks of the Nile over a distance of about 900 kilometres. The total number of trees is between 5-7 million. According to FAO reports, mean annual production (1990-2000) of dates is 154876 metric tons. This ranked Sudan as the 7th largest producer of dates among Arab countries. Date fruits constitute the most important agricultural crop in the area. They provide food and are a primary source of income to the majority of the inhabitants. As by-products, woods from stems, and fronds are widely used for thatching, buildings, barring and basketry (household utensils).

Although the commonly known and lethal palm insect pests and diseases like Red Weevil and Bayoud have not as yet been reported in Sudan, yield of the date palm is affected by many biotic factors of which insects are the most important. In the Sudan, the green date palm pit scale insect *Asterolecanium phoenicis* (Rao.) is considered the main pest. This genus, a native of central Asia (Iran), (Ezz, 1973, Sherif, 1967) was not known in Sudan before 1986 when it was first reported by Anas (1989) in the Golid area. It is believed to have arrived with the illegal introduction of offshoots from Saudi Arabia in 1974. Later, the pest crossed the natural barrier of the Baja desert to invade Algaba scheme, (150 km south of Dongola, 400 km-north capital Khartoum), probably in 1996, to become a real threat to date palm cultivation in Northern Sudan. The infested area in Golid, Gaba and ancient Dongola is about 5000 hectares, extending over 60 and 40

Proc. IIIrd IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

kilometers along the west and east bank of the river Nile, respectively. The newly reported infestation in Artigasha Island and Orbi in Dongola area and Abuhamad in the River Nile State (13000 infested palm trees) is evidence that the pest continues to spread.

In the past, and due to a lack of indigenous knowledge of appropriate control measures, control efforts were not successful and the level of infestation steadily increased. The insect attacks the leaflets, leaf rachis and fruits. It causes chlorosis, degeneration of the leaves, malformation of fruits before maturity leading to losses in production in a range of 30-50 kg to 5 kg per tree (Ali et al., 1993). The losses may range between 85 and 90% according to rate of infestation, the variety infested and management conditions (Ahmed et al., 2001, Ahmed, 2004).

The Pest and Disease Committee in its 67th meeting (Ahmed et al., 2002) recommended the following package for the control of the pest:

1. Pruning (removal of the dead and highly infested leaves in the lowers rows).
2. Earthing (raising earth around the palm trunk to facilitate irrigation).
3. Pre-watering and frequent irrigation.
4. Application of imidacloprid (Confidor 200SL) with 35 ml/ palm (7g.a.i).

Results of the partial budget analysis indicated the positive profitability for control using the recommended package as shown by a marginal rate of return of 29.8%. A comprehensive program (PPD) has been conducted in Algaba scheme (April-June 2004) where 200,000 date palm trees were treated using the recommended package and very good results were achieved.

This study was conducted to improve the profitability and stability of the package mentioned above to increase farmers' net benefits by introducing new products and techniques which offer a wide range of choices of selection between different methods, beside the recommended product to lower the cost of control as the cost of insecticide alone represents about 50% of the variable cost of the control package.

MATERIALS AND METHODS

A series of small scale experiments were undertaken in Algaba scheme and Golid area for two seasons (2002/2003 and 2003/2004) to evaluate the effectiveness of three systemic insecticides using two application techniques against the green date palm pit scale insect.

Barakawi variety, the most predominant variety was selected. A complete randomized design with 6 replicates (one tree = one replicate) was used.

Soil Application Method, Season 2002/2003

1. Experimental Site. A farm in the middle of Algaba scheme was selected, the infestation of which dated from about 6 years ago and the estimated loss in date yield was more than 60% and 80% for Barakawi and Gondaila varieties, respectively. Palm age was between 15 and 20 years and length between 10 and 15 metres. Flood method of irrigation from the Nile was conducted monthly via the scheme's main canal. Supplementary irrigation was provided using a diesel pump from underground water. Urea was usually added in summer for intercropping fodder crops (maize, durra and legumes). No chemical control had been conducted in the area before.

2. Insecticides in Use. Treatments are shown in (Table 1) for seasons 2002/03 and 2003/04.

3. Insect Count. Samples of eight leaflets (two leaflets from each of the four main directions) were inspected at biweekly intervals and examined under a binocular microscope. The number of living and dead adult females and immature stages were recorded per 3 cm² of each leaflet (tip, top and bottom). An average per cm² was obtained and the following parameters were calculated:

a) Total number of dead insects including adult females and immature stages.
b) Percentage mortality of adult females.
c) Percentage mortality of immature stages.

Pre-spray count was undertaken before insecticide application. Residue analysis

was done at the Gezira Research Station.

4. Yield and Yield Components. At harvest, 50 date fruits were taken from each replicate, and repeated three times to obtain the percentage fruit maturity (ripening).

Samples of ten date fruits were taken to the lab to determine the following parameters:

a) Mean fruit weight (g)
b) Mean fruit length (cm): (L)
c) Mean fruit diameter (cm): (D)
d) The L/D ratio
e) % seed/fruit weight.

Yield in kilogram per palm was determined at harvest. Samples for residual analysis (from date fruits, soil and grasses) were taken to the ARTC laboratory at Wad Medani.

Soil Application Method, Season 2003 /2004

1. Experimental Sites. As a result of the intensive chemical control program in Algaba scheme in April – June 2003, the second season was changed to Golid area. The location selected was a farm about three kilometers from the Nile that was highly infested. The age of trees was between 10 and 15 years and the length ranged between 8 and 10 meters. Intercropping with fodder crops was common. Urea and cattle manure were applied to fodder crops. The farm was irrigated using a diesel pump from underground water.

The estimated loss in yield was more than 60% and 70% for the Barakawi variety in location (1) and (2), respectively. Chemical control was conducted in the two locations using contact insecticides during the extensive program in this area.

Trunk Injection Technique

For the injection process in this experiment holes were bored 15 cm deep into the trunk and an open end snout metallic tube was inserted. The tube was 25 cm in length and 1.5 cm in diameter and was inserted into the hole at an angle of 45°, about one meter above the ground. The tube had to be able to hold at least 25 ml of the diluted insecticide. A calibrated "drench mastic" injection gun (used by Cordova, 1997) was not available, so a 25 ml measuring cylinder was used for this purpose. When the injection was over, the tube was closed with a tight- fitting flap. Apart from gloves the user also wore a mask for additional safety. The following insecticides with given dosage rates were diluted to 25 ml by water before injection. Samples for residual analysis were as described above.

RESULTS AND DISCUSSION

Soil Application Experiments: Algaba Scheme Season 2002 / 2003

Experiments using the soil application technique were conducted in Algaba scheme for the first season using Actara 25 WG (thiamethoxam) at four strengths (9, 12, 15 and 18 g product / tree) and Confidor 200SL (imidacloprid) at the recommended strength of 35ml / tree as a control.

1. Insect Count. Results of biweekly counts (Table 1) indicated that all insecticide doses increased the mortality of adult females and immature stages of the insect. The results were significantly different from the control (untreated) throughout the test period (three weeks after application). The total number of dead insects (adult females and immature stages) / cm² of leaflet after two weeks were 8.5, 7.4, 3.5, 1.9 and 1 for Actara (18 g), Confidor (35ml), Actara (15g), Actara (12g), Actara (9g) and untreated control, respectively. The total dead ranking for the same doses by the end of the count after 12 weeks were 7.9, 7.3, 3.9, 3.0, 1.9 and 1.2, respectively. The percentage mortality of the adult females and immature stages (Table 2) significantly increased in trees treated with insecticides compared with the untreated control. More than 90% of adult female mortality was observed in the higher doses of Actara (18 and 15 g) and Confidor (35ml) after four weeks from application, while similar percentages for mortality of the immature

stage were observed for the same doses only two weeks after application. A high percentage of adult female mortality was observed after twelve weeks (last count) for higher doses of Actara (18g) and Confidor (35ml).

2. Yield and Yield Components. Data presented in Table 3 showed the effect of different doses of Actara and Confidor on the average yield per tree. The percentage of ripe fruits (% maturity) and fruit weight (g), fruit length (L cm), fruit diameter (D cm), L/D ratio and % seeds / fruit weight (as physical characteristics) were shown. Date palms treated with higher doses of Actara (18g) and Confidor (35ml) showed a higher percentage of ripe fruits with no losses. They also showed the highest fruit weight (g) and the lowest percentage of seed/ fruit weight resulting in a higher yield, compared to the untreated control.

Soil Application Experiment: El Golid, Season 2003 / 2004

Based on results obtained from the first season in Algaba scheme, three concentrations of Actara (thiamethoxam) (18g, 15g and 12g/ tree), Confidor 200SL (imidacloprid) (35ml/ tree as standard), as well as two insecticides belonging to imidacloprid group were tested in season 2003 /2004. They were Rinfidor 20%SL and Comodor 20%SL at dosages of 35, 25, and 20 ml / tree for each insecticide. Two locations were selected from different localities.

1. Insect Count. Results in Table 4 indicated that all doses significantly increased total death of adult females and immature stages. The higher dose gave excellent performance for insect suppression. High mortality percentage was recorded at four weeks after application for adult females (Table 5) and after only two weeks for immature stages (Table 6). This confirmed results obtained from the previous season.

2. Yield and Yield Components. All insecticides at higher doses lead to full maturity of date fruits. Physical characteristics of fruits (fruit weight, fruit length (L), fruit diameter (D), L/D ratio and % seed/fruit weight) confirmed the effectiveness of the high insecticide doses for the two locations (Table 7). Physical fruit characteristics and yields in the two locations confirmed results obtained in the first season, where the highest yields were obtained in the treatments using higher doses of insecticides; Actara (18g), Rinfidor (35ml), Comodor (35ml) and Confidor (35ml).

The date palms treated with the higher doses of thiamethoxam and imidacloprid, started re-growing, which was a clear indication that the pest damage had stopped and the palms were no longer under stress. This phenomenon was independent of the pesticide formulation applied to the palm (the two formulations belong to a new pesticide group known as Neonicotinoids). It was observed that the treated trees remained free from termites, white scale insects and ant infestation. However, the two insecticides had no effect against mites. The results were consistence over the years and locations and confirmed the results obtained by Ahmed (2003).

Trunk Injection Method

The three phases of trunk injection process include:

a) Boring the injection channel and inserting a tube.
b) Inserting the semi- diluted insecticide.
c) Closing the injection channel (or tube).

Results of insect mortality, yield and yield components obtained from the two seasons (2003 / 20040) indicated that trunk injection was an effective and reliable method for controlling green pit scale insect.

1. Insect Count. The mean biweekly death (adult female and immature stages)/ cm^2 of leaflet for the first season 2002 / 2003 (Table 8) significantly increased for all insecticides used compared with the untreated control throughout the experimental period. The higher doses of insecticides resulted in the higher number of dead insects. Similar results were obtained in the second season (2003 /2004) in Al Golid (Table 11) when Actara and Confidor were used as well as the new insecticides Rinfidor 20%SL and Comodor 20%SL. The higher doses were superior to other doses and the untreated control in

number of total dead insects, even after 12 weeks (the last count).

In 2002 / 2003 (Table 9) the high effectiveness of insecticides was reflected in the hundred percent mortality of adult female and immature stages from the second week after injection and they remained effective throughout the count intervals. Similar results were obtained in the second season (2003 / 2004) as shown in Table 12 and Table 13. The new imidacloprid commercial compounds Rinfidor and Comodor showed an effective performance similar to Confidor (imidacloprid).

2. Yield and Yield Components. Results in Table 10 indicated that all doses of different insecticides significantly affected yield and physical characters of date fruits compared to the untreated control. The higher dose treatments of Actara (10g) and Confidor (20ml) resulted in the higher fruit weight, fruit length and a lower seed/ fruit weight percentage indicating a higher yield. An increase in yield of about 75% was observed compared to the untreated control.

Results of the second season (2003 / 2004) in Al Golid (Table 14) confirmed the above mentioned results. All treatments were superior to the untreated control in yield and yield components except in fruit diameter and percentage seed / fruit weight. The higher yields (kg / tree) were observed in the higher doses of different insecticides.

As mentioned before, trunk injection requires the use of a systemic insecticide, in this case imidacloprid and thiamethoxam, which is injected into a tube inserted in a hole bored into the lower part of the trunk. From there the agent is translocated in the sap to the leaf tissues. This means that only green pit scale insect is killed, not the natural enemies. Natural control mechanisms are thus activated without any adverse effects on the environment. A wide spray with contact insecticides using airplanes and heavy machinery has been conducted in areas like Al Golid) (Ahmed et al., 1993). Furthermore, the insecticide is not adversely affected by other climatic factors. If we take into consideration that more than 60% of date palm trees in the Sudan are not irrigated, the use of trunk injection is very useful as an alternative to soil application.

This method not only increases user safety, it also allows the work to be carried out in an extremely economical manner. The dose used is decreased by 50% compared to soil application. On the other hand, a 3- man team can do the work: one man boring the hole; the second inserting the tube into the holes; and, the third injecting insecticides and closing tubes. Date palms treated with insecticides by trunk injection have continued to develop normally during the past two seasons. No phytotoxicity has been observed till now in the treated trees. No insecticide residues have been detected from either method in dates, soil or grasses (Abas et al., 2005; El Habib et al., 2005).

CONCLUSION

1- Soil application and trunk injection of imidacloprid (Confidor 200SL, Rinfidor 20%SL and Comodor 20%SL) and thiamethoxam (Actara 25WG) were highly effective in controlling the green pit scale insect.
2- They proved to be very effective as a protective measure against new infestation.
3- The two methods of application do not require any machinery or labour for application. They can be safely applied.
4- Trunk injection is a truly effective and reliable method for controlling the green pit scale insect, with minimal environmental impact.
5- The two methods of application are highly economical and safe for the user and appear to be safe for the beneficial insects.
6- Date palms treated with different insecticides using the two methods, developed normally during the past two seasons. No phytotoxicity has been observed in the treated trees.
7- The tested insecticides checked termites and many other pests, but did not affect mites.

RECOMMENDATIONS

Based on the results, the following insecticides with given dosage rates are recommended using two methods of application:

Soil Application

a) Actara25WG (thiamethoxam) 18g product / tree (4.5 g a.i.)
b) Rinfidor 20%SL (imidacloprid) 35 ml product / tree (7g a.i.)
c) Comodor 20%SL (imidacloprid) 35 ml product / tree (7g a.i.)

The following cultural practices must be conducted before application:

a) Pruning; removal of the dead leaves and highly infested leaves in the lowest rows.
b) Raising earth around the date palm trees to facilitate irrigation.
c) Pre-watering and normal irrigation after application.

Trunk Injection

a) Actara25WG (thiamethoxam) 10g product / tree (2.5 g a.i.)
b) Rinfidor 20%SL (imidacloprid) 20 ml product / tree (4g a.i.)
c) Comodor 20%SL (imidacloprid) 20 ml product / tree (4g a.i.)
d) Confidor 200SL (imidacloprid) 20 ml product / tree (4g a.i.)

Removal of the dead leaves and highly infested leaves in the lowest rows and normal irrigation must be applied.

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Tables

Table 1. Mean biweekly mortality of adult females and immature stages of green pit scale insect treated with different insecticides (soil application method) Algaba scheme, season 2002/2003.

Insecticide	Dosage rate /palm	Weeks after application					
		0	2	4	6	8	12
Actara 25WG	18g (4.5g a.i)	(2)	8 (2.9)^{A}	8.5 (3)^{A}	10.9 (3.4)^{A}	8.1 (2.9)^{A}	7.9 (2.9)^{A}
Actara 25WG	15g (3.75g a.i)	0.7 (1.1)	3 (1.9)^{B}	4.6 (2.3)^{B}	5.6 (2.5)B^{C}	5.8 (2.5)^{B}	3.9 (2.0)^{B}
Actara 25WG	12g (3g a.i)	0.9 (1.2)	1.7 (1.5)^{BC}	3.5 (2.0)^{B}	4.3 (2.2)^{C}	3.6 (2.0)^{C}	3 (1.8)B^{C}
Actara 25WG	9g (2.25g a.i)	0.8 (1.1)	1.1 (1.2)^{C}	1.9 (1.6)^{C}	3.9 (2.1)^{C}	2.5 (1.7)^{D}	1.9 (1.6) ^{CD}
Confidor200SL	35ml (7g a.i)	1.1 (1.2)	6.8 (2.7)^{A}	7.4 (3.8)^{A}	8.1 (2.9)^{AB}	8.5 (3.0)^{A}	7.3 (2.8)^{A}
Untreated control	water only	0.5 (1.0)	0.94 (1.2)^{C}	1.0 (1.1)^{D}	1.2 (1.3)^{D}	1.2 (1.3)^{E}	1.2 (1.3)^{D}
SE±		0.1	0.14	0.1	0.22	0.1	0.14
C.V%		17.4	15.2	6.7	18.6	8.4	13.7

Data in brackets were √x+0.5. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 1 (a): Soil applied treatments (2002/03)
1) Actara 25 WG (thiamethoxam) 18g product/tree (4.5g a.i.)
2) Actara 25 WG (thiamethoxam) 15g product./tree (3.75g a.i.)
3) Actara 25 WG (thiamethoxam) 12g product/tree (3g a.i.)
4) Actara 25 WG (thiamethoxam) 9g product/tree (2.25g.a.i.)
5) Confidor 200SL (imidacloprid) 35 ml/tree (7g a.i.) (Standard).
6) Untreated control (water only).

Table 1 (b): Season 2003/04
1. Actara 25WG (thiamethoxam) 18g product/ tree (4.5g.a.i)
2. Actara 25WG (thiamethoxam) 15g product/ tree (3.75g.a.i)
3. Actara 25WG (thiamethoxam) 12g product/ tree (3g.a.i)
4. Rinfidor 20%SL (imidacloprid) 35ml product/ tree (7g.a.i)
5. Rinfidor 20%SL (imidacloprid) 25ml product/ tree (5g.a.i)
6. Rinfidor 20%SL (imidacloprid) 20ml product/ tree (4g.a.i)
7. Comodor 20%SL (imidacloprid) 35ml product/ tree (7g.a.i)
8. Comodor 20%SL (imidacloprid) 25ml product/ tree (5g.a.i)
9. Comoidor 20%SL (imidacloprid) 20ml product/ tree (4g.a.i)
10. Confidor 200L (imidacloprid) 35ml product/ tree (7g.a.i) (standard)
11. Untreated control, (water only).

Table 2. Mean biweekly percentage mortality of adult females and immature stages of green pit scale insect treated with different insecticides (soil application method) Algaba Scheme, season 2002/2003.

Adult females		Weeks after application					
Insecticide	Dosage rate/palm	0	2	4	6	8	12
25WG	18g (4.5g a.i)	8.5 (17.0)	99.9 (88.3)^{A}	98.6(83.2)^{AB}	99.9 (89.0)^{A}	97 (80.01)^{A}	100 (97.5)^{A}
Actara 25WG	15g (3.75g a.i)	16.2 (23.8)	99.7 (86.9)^{A}	90.0 (71.6)^{BC}	88.0 (69.8)^{B}	89.5 (71.0)^{AB}	99.9 (88.5)^{A}
Actara 25WG	12g (3g a.i)	6.2 (15.45)	66.5(54.6)^{ABC}	81.6 (65.33)^{C}	80 (63.7)^{BC}	80.2 (63.6)^{B}	79.5 (67.6)^{B}
Actara 25WG	9g (2.25g a.i)	14.6 (22.4)	55.1(47.9)^{BC}	78.5 (62.4)^{C}	70.5 (57.0)^{C}	33.8 (35.6)^{C}	79.9 (63.4)^{A}
Confidor200SL	35ml (7g a.i)	9.6 (18.11)	86.2 (68.2)^{AB}	99.9 (88.9)^{A}	100 (99.1)^{A}	96.3 (78.9)^{AB}	100 (96.7)^{B}
Untreated control	water only	14.7 (22.8)	14.0 (22.03)^{C}	20.5 (26.93)^{D}	22.2 (28.1)^{D}	24.8 (29.9)^{C}	20.2 (26.7)^{C}
SE±		4.9	9.5	3.19	3.23	2.24	4.67
C.V%		49.5	31	9.63	9.52	14.2	12.7
Immature stages		Weeks after application					
Insecticide	Dosage rate/palm	0	2	4	6	8	12
Actara25WG	18g (4.5g a.i)	17.5 (24.7)	99.7 (87)^{AB}	100 (97.2)^{A}	100 (97.2)^{A}	100 (93)^{AB}	100 (96.3)^{A}
Actara 25WG	15g (3.75g a.i)	11.2 (19.6)	94.9 (76.9)^{BC}	96.2 (78.8)^{B}	98.1 (82.2)^{AB}	99.4 (85.9)^{B}	99.8 (88.2)^{B}
Actara 25WG	12g (3g a.i)	(12.8)	82.9 (65.7)^{CD}	91.7 (73.3)^{BC}	63.5 (75.2)^{BC}	90.9 (72.4)^{C}	96.2 (78.8)^{C}
Actara 25WG	9g (2.25g a.i)	7.2 (15.5)	80.1 (63.6)^{D}	84.2 (66.6)^{C}	75.9 (60.4)^{C}	76.7 (61.1)^{D}	89.7 (71.3)^{C}
Confidor200SL	35ml (7g a.i)	9.2 (18.1)	100 (93.3)^{A}	100 (99.4)^{A}	100 (91.6)^{AB}	100 (96.2)^{A}	100 (94.8)^{AB}
Untreated control	water only	15.5 (23.2)	17.6 (24.9)^{E}	17.1 (24.4)^{D}	24.5 (29.7)^{D}	22.7 (28.5)^{E}	21.7 (27.8)^{D}
SE±		2.87	3.49	2.24	5.32	2.69	2.13
C.V%		30.3	10.2	6.1	14.6	7.4	5.6

Data in brackets were arcsine transformed. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 2 (a): Algaba scheme, season 2002/203

1) Actara 25 WG (thiamethoxam) 10 g / tree (2.5 g a.i)
2) Actara 25 WG (thiamethoxam) 8 g / tree (2.0 g a.i.)
3) Actara 25 WG (thiamethoxam) 6 g / tree (1.25 g a.i)
4) Confidor 200SL (imidacloprid) 20 ml / tree (4g a.i.)
5) Confidor 200SL (imidacloprid) 15 ml / tree (3g a.i.)
6) Confidor 200SL (imidacloprid) 10 ml / tree (2g a.i.)
7) Untreated control (water only)

Table 2 (b) Golid area, season 2003/04

1) Actara 25 WG (thiamethoxam) 10 g / tree (2.5 g a.i)
2) Actara 25 WG (thiamethoxam) 8 g / tree (2.0 g a.i.)
3) Actara 25 WG (thiamethoxam) 6 g / tree (1.25 g a.i)
4) Confidor 200SL (imidacloprid) 20 ml / tree (4g a.i.)
5) Confidor 200SL (imidacloprid) 15 ml / tree (3g a.i.)
6) Confidor 200SL (imidacloprid) 10 ml / tree (2g a.i.)
7) Untreated control (water only)
8) Rinfidor 20%SL (imidacloprid) 35ml product/ tree (7g.a.i)
9) Rinfidor 20%SL (imidacloprid) 25ml product/ tree (5g.a.i)
10) Rinfidor 20%SL (imidacloprid) 20ml product/ tree (4g.a.i)
11) Comodor 20%SL (imidacloprid) 35ml product/ tree (7g.a.i)
12) Comodor 20%SL (imidacloprid) 25ml product/ tree (5g.a.i)
13) Cooidor 20%SL (imidacloprid) 20ml product/ tree (4g.a.i)

Table 3. Yield and yield components of date fruits (soil application method) Al gaba scheme, Season 2002/2003.

Insecticide	Dosage rate/palm	%ripe fruit	Fruit weight (g)	Fruit length (cm) L	Fruit diameter (cm) D	L/D ratio	%seed/ Fruit wt.	Yield kg/palm
Actara25WG	18g (4.5g a.i)	100^{A}	8.5^{AB}	4.9^{A}	1.6	3.0	9.8^{B}	90.0^{A}
Actara 25WG	15g (3.75g a.i)	90^{B}	7.4^{BC}	4.6^{A}	1.6	2.9	10.5^{B}	59.5^{B}
Actara 25WG	12g (3g a.i)	80^{C}	7.2^{C}	4.5^{A}	1.6	2.8	13.5^{A}	57.7^{B}
Actara 25WG	9g (2.25g a.i)	72.5^{C}	6.7^{CD}	4.4^{A}	1.5	2.8	12.8^{A}	47.0^{B}
Confidor 200SL	35ml (7g a.i)	100^{A}	8.2^{A}	4.7^{A}	1.7	2.9	10.0^{B}	108.3^{A}
Untreated control	water only	48.6^{D}	6^{D}	3.8^{B}	1.6	2.5	14.5^{A}	22.5^{C}
SE±		2.35	0.21	0.12	0.07	0.13	0.52	5.6
C.V%		5.8	5.7	5.3	9	9.1	8.9	17.5

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 4. Mean biweekly mortality of adult females and immature stages of green pit scale insect treated with different insecticides (soil application method) El Golid, season 2003/2004.

Location 1		Weeks after application					
Insecticides	Dosage rate/palm	0	2	4	6	8	12
Actara25WG	18g (4.5g a.i)	0.4 (0.9)	2.9 (1.8)^{A}	2.0 (1.6)^{BC}	1.6 (1.5)^{ABCD}	1.7 (1.5)^{ABC}	1.7 (1.5)^{AB}
Actara 25WG	15g (3.75g a.i)	0.5 (1.0)	1.6 (1.6)^{BC}	1.9 (1.5)^{BC}	1.5 (1.4)^{ABCD}	1.6 (1.4)^{ABC}	1.7 (1.5)^{AB}
Actara 25WG	12g (3 g a.i)	0.5 (1.0)	1.2 (1.3)^{BCD}	1.8 (1.5)^{BC}	1.4(1.4)^{ABCD}	1.2 (1.3)^{ABC}	0.6 (1.1)^{B}
Rinfidor 20%SL	35ml (7g ai)	0.5 (1.0)	0.96(1.4)^{BCD}	3.9 (2.1)^{A}	2.4 (1.7)^{AB}	2.5 (1.7)^{A}	3.0 (1.9)^{A}
Rinfidor 20%SL	25mli (5g ai)	0.4 (0.9)	0.9 (1.2)^{BCD}	2.9 (1.8)^{AB}	1.0 (1.2)^{BCD}	1.5 (1.4)^{ABC}	1.7 (1.5)^{AB}
Rinfidor 20%SL	20ml (4g ai)	0.3 (0.8)	0.6 (1.5)^{CD}	0.4 (1.2)^{C}	0.9 (1.2)^{BCD}	0.8 (1.2)^{BC}	0.5 (1.4)^{B}
Comodor 20%SL	35ml (7g ai)	0.5 (1.0)	2.2 (1.7)^{AB}	2.1 (1.6)^{BC}	2.5 (1.7)^{A}	1.8 (1.5)^{AB}	1.1 (1.7)^{AB}
Comodor 20%SL	25mi (5g ai)	0.2 (0.8)	1.3 (1.3)^{BCD}	1.2 (1.3)^{CD}	1.8 (1.5)^{ABC}	1.4 (1.1)^{ABC}	1.7 (1.5)^{AB}
Comodor 20%SL	20ml (4g ai)	0.3 (0.9)	1.3 (1.3)^{BCD}	1.1 (1.3)^{CD}	1.3 (1.1.3)^{ABCD}	1.4 (1.4)^{ABC}	1.2 (1.3)^{AB}
Confidor 200SL	35ml (7g ai)	1.0 (1.2)	1.2 (1.3)^{AB}	4.3 (2.2)^{A}	1.8 (1.5)^{ABC}	2.1 (1.6)^{A}	1.3 (1.3)^{AB}
Untreated control	water only	0.6 (1.1)	0.5 (0.9)^{D}	0.4 (1.0)^{D}	0.5 (1.0)^{D}	0.6 (1.0)^{C}	0.3 (0.9)^{B}
SE±		0.08	0.13	0.14	0.14	0.13	0.53
C.V%		16.6	19	18	19.5	18.5	29
Location 2		Weeks after application					
Insecticides	Dosage rate/palm	0	2	4	6	8	12
Actara 25WG	18g (4.5g a.i)	0.7 (1.1)	4.2 (2.2)^{A}	5.3 (2.4)^{A}	3.9 (2.1)^{A}	3.9 (2.1)^{A}	5..9 (2.5)^{A}
Rinfidor 20%SL	35ml (7g ai)	0.8 (1.1)	2.3 (1.7)^{A}	4.4 (2.2)^{AB}	3.1 (1.9)^{A}	3.6 (2.0)^{A}	3.6 (2.0)^{B}
Comodor 20%SL	35ml (7g ai)	0.7 (1.1)	4.8 (2.3)^{A}	3.3 (1.9)^{B}	4.3 (2.1)^{A}	3.6 (2.0)^{A}	3.6 (2.0)^{B}
Confidor 200SL	35ml (7g ai)	0.5 (1.06)	3.5 (2.0)^{A}	3.7 (2.0)^{AB}	4.0 (2.1)^{A}	3.9 (2.1)^{A}	4.0 (2.1)^{B}
Untreated control	water only	0.7 (1.0)	1.0 (1.2)^{B}	0.7 (1.1)^{C}	0.9 (1.2)^{B}	0.9 (1.2)^{B}	0.8 (1.2)^{C}
SE±		0.06	0.16	0.11	0.11	0.11	0.07
C.V%		9.2	15.2	9.9	10.3	9.8	6.2

Data in brackets were arcsine transformed. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 5. Mean biweekly percentage mortality of adult females of green pit scale insect treated with different insecticides (soil application method) El Golid, 2003/2004 (Location 1 and 2).

Location 1		Weeks after application					
Insecticides	Dosage rate/palm	0	2	4	6	8	12
Actara 25WG	18g (4.5g a.i)	15.1 (22.9)	72 (58)A^{B}	99.7 (875)^{A}	100 (98.8)^{A}	100 (99.3)^{A}	100 (98.6)^{AB}
Actara 25WG	15g (3.75g a.i)	16.0 (23.6)	65.5 (54.5)^{AB}	83.3 (65.1)^{C}	100 (98.2)^{A}	100 (96.2)^{A}	100 (90.4)^{BCD}
Actara 25WG	12g (3 g a.i)	21.6 (27.7)	59.8 (50.7)^{ABC}	77.1 (61.4)^{C}	100 (91.5)^{A}	99.7 (87.2)^{B}	99.9 (88.5)^{CD}
Rinfidor 20%SL	35ml (7g ai)	23.0 (28.7)	89.3 (71.1)^{A}	99.3 (85.2)^{A}	100 (94.6.3)^{A}	100 (95.3)^{AB}	100 (96.7)^{ABC}
Rinfidor 20%SL	25mi (5g ai)	19.7 (26.4)	73.8 (59.2)^{AB}	81.6 (64.7)^{C}	99.3 (85.3)^{AB}	100 (96.1)^{AB}	100 (91.9)^{ABCD}
Rinfidor 20%SL	20ml (4g ai)	25.6 (30.4)	58.5 (49.9)^{ABC}	77.6 (61.8)^{C}	91.8 (73.4)^{B}	100 (93.5)^{AB}	99.7 (87.1)^{D}
Comodor 20%SL	35ml (7g ai)	20.5 (26.9)	72.2 (58.2)^{AB}	99.6 (86.6)^{A}	100 (98.7)^{A}	100 (94.9)^{AB}	100 (99.1)^{A}
Comodor 20%SL	25mi (5g ai)	10.6 (19.0)	58.1 (49.7)^{ABC}	89.9 (71.5)^{BC}	100 (94.8)^{A}	100 (91.1)^{AB}	99.2 (85.0)^{D}
Comodor 20%SL	20ml (4g ai)	7.1 (15.5)	52.8 (49.0)^{BC}	78.1 (62.1)^{C}	100 (92.8)^{A}	99.9 (89.5)^{AB}	99.2 (85.1)^{D}
Confidor200SL	35ml (7g ai)	11.1 (19.8)	79.0 (62.7)^{AB}	100 (99.4)^{A}	100 (98.8)^{A}	100 (99.2)^{A}	100 (98.6)^{AB}
Untreated control	water only	21.7 (27.8)	20.4 (26.8)^{C}	23.1 (28.7)^{D}	18.9 (25.8)^{C}	3.4 (107)^{C}	7.2 (15.6)^{E}
SE±		3.15	6.97	4.41	3.88	3.15	2.52
C.V%		25.8	26.1	12.6	9.0	7.3	5.9
Location 2		Weeks after application					
Insecticides	Dosage rate/palm	0	2	4	6	8	12
Actara 25WG	18g (4.5g a.i)	20 (26.5)	89.7 (71.3)^{A}	96.9 (76.8)^{B}	95.6 (77.9)^{B}	100 (92.5)^{A}	99.3 (84.7)^{A}
Rinfidor 20%SL	35ml (7g ai)	16.6 (24.1)	91.5 (73)A	100 (90.9)^{AB}	100 (99.5)^{A}	100 (99.5)^{A}	100 (92.5)^{A}
Comodor 20%SL	35ml (7g ai)	18.3 (25.3)	90.8 (72.3)^{A}	100 (99.7)^{A}	100 (99.7)^{A}	100 (99.7)^{A}	100 (99.6)^{A}
Confidor 200SL	35ml (7g ai)	25.6 (3.4)	96.5 (79.2)^{A}	100 (99.7)^{A}	100 (99.6)^{A}	100 (99.7)^{A}	100 (99.7)^{A}
Untreated control	water only	18.6 (25.6)	16.5 (24.^{B}	21.9 (27.6)^{C}	22.4 (28.3)^{C}	24.3 (29.5)^{B}	24.4 (29.6)^{B}
SE±		1.75	3.57	4.27	1.4	3.31	5.19
C.V%		11.5	9.7	9.4	3.0	6.8	11.1

Data in brackets were arcsine transformed. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 6. Mean biweekly percentage mortality of immature stages of green pit scale insect treated with different insecticides (soil application method) El Golid, 2003/2004 (Location 1 and 2).

Location 1		Weeks after application					
Insecticides	Dosage rate/palm	0	2	4	6	8	12
Actara 25WG	18g (4.5g a.i)	13.2 (21.3)	100 (99.5)^{A}	100 (94.2)^{A}	100 (98.7)^{A}	100 (99)^{A}	100 (98.6)^{AB}
Actara 25WG	15g (3.75g a.i)	12.1 (20.4)	100 (99.5)^{A}	100 (93.7)^{A}	100 (98.2)^{A}	100 (96)^{AB}	100 (90)^{BC}
Actara 25WG	12g (3 g a.i)	11.9 (20.2)	89.3 (71)^{B}	100 (94.1)^{A}	100 (91)^{AB}	99.7 (87)^{B}	99.9 (88.6)^{C}
Rinfidor 20%SL	35ml (7g ai)	9.9 (18.3)	100 (99.5)^{A}	100 (98)^{A}	100 (99.9)^{A}	100 (95)^{AB}	100 (96)^{AB}
Rinfidor 20%SL	25mi (5g ai)	11.7 (20)	100 (99.5)^{A}	100 (96.1)^{A}	100 (96)^{AB}	100 (95)^{AB}	100 (91.9)^{ABC}
Rinfidor 20%SL	20ml (4g ai)	9.6 (18)	72.7 (58.5)^{D}	100 (98.1)^{A}	89 (70)^{C}	100 (93)^{AB}	99.1 (84)^{C}
Comodor 20%SL	35ml (7g ai)	14 (21.9	100 (99.5)^{A}	100 (95)^{A}	100 (98)^{A}	100 (94)^{AB}	100 (99)^{A}
Comodor 20%SL	25mi (5g ai)	9.1 (17.5)	100 (99.5)^{A}	100 (95)^{A}	100 (94)^{AB}	100 (91)^{AB}	99.2 (85)^{C}
Comodor 20%SL	20ml (4g ai)	10.5 (18.9)	80 (68.5)^{C}	100 (97)^{A}	100 (92)^{AB}	99.9 (89)^{AB}	99.3 (84)^{C}
Confidor200SL	35ml (7g ai)	18.3 (25.3)	100 (99.5)^{A}	100 (97)^{A}	100 (98)^{A}	100 (97)A^{B}	100 (98)^{AB}
Untreated control	water only	17.8 (24.9)	25.7 (30.5)^{E}	23.5 (29.2)^{B}	18.9 (25.8)^{D}	3.4 (10.7)^{C}	15 (7.2)^{D}
SE±		4.36	1.41	1.53	3.55	3.20	2.41
C.V%		42.3	3.4	3.7	8.2	7.4	5.7
Location 2		Weeks after application					
Insecticides	Dosage rate/palm	0	2	4	6	8	12
Actara 25WG	18g (4.5g a.i)	22.9 (28.7)	93.1 (74.8)^{A}	99.9 (89.6)^{A}	84.2 (66.6)^{AB}	100 (99.5)^{A}	100 (99.2)^{A}
Rinfidor 20%SL	35ml (7g ai)	22.9 (28.6)	99 (84.3)^{A}	100 (99.4)^{A}	100 (99.4)^{A}	100 (99.6)^{A}	100 (99.5)^{A}
Comodor 20%SL	35ml (7g ai)	24.9 (29.9)	99.9 (89)^{A}	100 (99.5)^{A}	100 (99.4)^{A}	100 (99.4)^{A}	100 (99.5)^{A}
Confidor 200SL	35ml (7g ai)	26.6 (31.1)	99.8 (87.7)^{A}	100 (99.6)^{A}	100 (99.5)^{A}	100 (99.5)^{A}	100 (99.6)^{A}
Untreated control	water only	24.9 (29.9)	24.7 (29.8)^{B}	27.6 (31.7)^{B}	22.4 (28.3)^{B}	23.1 (28.7)^{B}	25.6 (30.4)^{B}
SE±		1.4	6.28	2.55	14.54	0.37	0.88
C.V%		8.2	14.9	5.3	32	0.75	1.8

Data in brackets were arcsine transformed. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 7. Yield and yield components of date fruits (soil application method) El Golid, season 2003/2004 (location 1 and 2).

Location 1 Insecticide	Dosage /palm	% Ripe fruit	Fruit wt. (g)	Fruit length, L (cm)	Fruit diameter, D (cm)	L/D ratio	% seed/ fruit wt.	Yield (kg/palm)
Actara 25WG	18g (4.5g a.i)	100^{A}	5.7^{ABCD}	4.5^{A}	1.6	3.0^{ABC}	10.8^{C}	82.5^{A}
Actara 25WG	15g (3.75g a.i)	92.5^{AB}	5.0^{ABCDE}	3.5^{B}	1.6	2.3^{D}	14.8^{BC}	59.5^{CD}
Actara 25WG	12g (3 g a.i)	75^{C}	4.0^{EF}	3.7^{B}	1.6	2.3^{D}	17^{B}	50^{D}
Rinfidor 20%SL	35ml (7g ai)	97.5^{A}	5.7^{ABC}	4.5^{A}	1.5	3.03^{AB}	12.8^{BC}	78.5^{AB}
Rinfidor 20%SL	25mi (5g ai)	$97.^{5A}$	5.1^{ABCDE}	3.8^{B}	1.6	2.5^{BCD}	15^{BC}	59.3^{CD}
Rinfidor 20%SL	20ml (4g ai)	85^{BC}	4.6^{BCDEF}	3.5^{B}	1.5	2.4^{D}	15.5^{BC}	55.8^{CD}
Comodor 20%SL	35ml (7g ai)	100^{A}	6.3^{A}	4.6^{A}	1.5	3.1^{A}	11.5^{BC}	72.5^{B}
Comodor 20%SL	25mi (5g ai)	93^{AB}	4.2^{CDEF}	3.7^{B}	1.4	2.7^{ABC}	13.5^{BC}	61.5^{C}
Comodor 20%SL	20ml (4g ai)	75^{C}	4.1^{DEF}	3.6^{B}	1.5	2.4^{CD}	12.5^{BC}	54.3^{CD}
Confidor200SL	35ml (7g ai)	100^{A}	5.9^{AB}	4.6^{A}	1.5	3.0^{ABC}	12.5^{BC}	83.5^{A}
Untreated control	water only	45^{D}	3.1^{F}	2.8^{C}	1.3	2.1^{D}	22.8^{A}	12.3^{E}
SE±		3.42	0.45	0.19	0.07	0.18	1.68	2.87
C.V%		7.8	18.4	9.7	9.4	13.6	23.3	9.4
Location 2 Insecticide	Dosage /palm	% Ripe	Fruit wt. (g)	Fruit length, L (cm)	Fruit diameter, D (cm)	L/D ratio	% seed/ fruit wt.	Yield (kg/palm)
Actara 25WG	18g (4.5g a.i)	**100^{A}**	8.6^{A}	4.6^{A}	1.6	4.5^{A}	9.0^{B}	103.7^{A}
Rinfidor 20%SL	35ml (7g ai)	100^{A}	6.4^{BC}	4.6^{A}	1.7	4.0^{AB}	10.0^{B}	96.7^{A}
Comodor 20%SL	35ml (7g ai)	100^{A}	6.4^{BC}	4.4^{A}	1.6	2.8^{BC}	11.0^{B}	96.3^{A}
Confidor 200SL	35ml (7g ai)	100^{A}	8.4^{AB}	4.4^{A}	1.7	2.6^{C}	9.0^{B}	103.3^{A}
Untreated control	water only	53.3^{B}	5.1^{C}	3.3^{B}	1.5	2.3^{C}	19.0^{A}	30.0^{B}
SE±		1.49	0.55	0.16	0.06	0.34	0.59	4.13
C.V%		2.9	13.6	6.4	6.8	18.2	8.83	8.3

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 8. Mean biweekly mortality of adult females and immature stages of green pit scale insect using different insecticides (trunk injection) in Al gaba scheme season 2002/2003.

Insecticide	Dosage rate per palm	Weeks after injection					
		0	2	4	6	8	12
Actara 25WG	10g.p (2.5g.a.i)	0.4 (0.9)	6.2 (2.6)^{AB}	9.3 (2.5)^{AB}	9.1 (2.7)^{A}	4.6 92.6)^{A}	4.7 (2.3)^{AB}
Actara 25WG	8g.p (2g.a.i.)	0.94 (1.2)	5.6 (2.5)^{AB}	4.0 (2.1)^{ABC}	5.4 (2.5)^{AB}	3.9 (2.1)^{AB}	3.8 (2.1)^{BC}
Actara 25WG	6g p (1.5g. a.i)	0.97 (1.1)	4.1 (2.1)^{BC}	2.9 (1.8)^{C}	4.4 (2.2)^{AB}	3.1 (1.9)^{AB}	1.9 (1.5)^{CD}
Confidor 200SL	20ml (4g. a.i)	1.6 (1.5)	8.8 (3.1)^{A}	6.6 (2.7)^{A}	5.9 (2.5)^{AB}	4.0 (2.1)^{AB}	6.7 (2.7)^{A}
Confidor 200SL	15ml (3g. a.i)	0.6 (1.0)	2.9 (1.9)^{BCD}	4.1 (2.1)^{ABC}	4.7 (2.3)^{AB}	1.2 (1.3)^{AB}	3.4 (2.1)^{BC}
Confidor 200SL	10ml (2g. a.i)	1.0 (1.2)	1.4 (1.0)^{D}	3.2 (1.9)^{BC}	3.2 (1.9)^{B}	3.2 (1.9)^{BC}	1.7 (1.5)^{CD}
Untreated control	water only	0.9 (1.2)	1.4 (1.3)^{D}	0.95 (1.2)^{D}	0.73 (1.1)^{C}	0.5 (1.0)^{C}	1.3 (1.3)^{D}
SE±		0.16	0.22	0.17	0.19	0.24	0.17
C.V%		23.3	18	14.4	15.04	22.0	15.5

Data in brackets were √x+0.5. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 9. Mean biweekly percentage mortality of adult females and immature stages of green pit scale insect using different insecticides (trunk injection) in El Gaba scheme season 2002/2003.

Adult Females		Weeks after injection					
Insecticide	Dosage rate/ palm	0	2	4	6	8	12
Actara 25WG	10g.p (2.5g.a.i)	9.6 (18.1)	100 (95.6)^{AB}	100 (99.2)^{A}	100 (97.2)^{A}	100 (97.9)^{A}	100 (92.4)^{A}
Actara 25WG	8g.p (2g.a.i.)	13.3 (21.4)	100 (91.2)^{AB}	99.3 (85.2)^{BC}	100 (91.3)^{AB}	99.3 (85.3)^{B}	98.5 (82.8)^{BC}
Actara 25WG	6g p (1.5g. a.i)	14.3 (22.2)	98.3 (82.5)^{BC}	93.6 (75.3)^{CD}	94.6 (76.5)^{C}	86.8 (68.7)^{C}	92.7 (74.3)^{CD}
Confidor 200SL	20ml (4g. a.i)	11.4(19.7)	100 (99.6)^{A}	100 (99.6)^{A}	100 (99.8)^{A}	100 (92.9)^{AB}	100 (99.2)^{A}
Confidor 200SL	15ml (3g. a.i)	13 (21.1)	94.6 (76.4)^{C}	99.9 (89.9)^{AB}	100 (90.1)^{AB}	90.7 (72.3)^{C}	100 (90.0)^{AB}
Confidor 200SL	10ml (2g. a.i)	12 (21)	91.9 (73.5)^{C}	90.4 (72)^{D}	95.9 (78.3)^{BC}	79.8 (63.3)^{C}	88.3 (70)^{D}
Untreated control	water only	11 (20.2)	8.3 (16.7)^{D}	10.7 (19.1)^{E}	14 (22.0)^{D}	23.9 (29.3)^{D}	25.1 (30.1)^{E}
SE±		1.99	3.77	2.89	3.72	3.34	2.62
C.V%		16.8	8.5	6.5	8.1	8	5.9

Immature stages		Weeks after injection					
Insecticide	Dosage rate/ palm	0	2	4	6	8	12
Actara 25WG	10g.p (2.5g.a.i)	14.4 (22)	100 (99.6)^{A}	100 (99.7)^{A}	100 (99.4)^{A}	100 (95.6)^{AB}	100 (98.1)^{AB}
Actara 25WG	8g.p (2g.a.i.)	16.5 (24.1)	100 (96.8)^{A}	100 (92.5)^{AB}	100 (92.4)^{B}	99.8 (88)^{AB}	99.6(86.6)^{ABC}
Actara 25WG	6g p (1.5g. a.i)	15.3 (23)	100)93.8)^{AB}	100 (91.3)^{B}	99.9 (88.7)^{B}	91.2 (72.7)^{CD}	99.2(85)^{C}
Confidor 200SL	20ml (4g. a.i)	13.4 (21.5)	100 (99.2)^{A}	100 (99.6)^{A}	100 (99.7)^{A}	100 (99.7)^{A}	100 (99.1)^{A}
Confidor 200SL	15ml (3g. a.i)	17 (24.4)	99.2 (85.2)^{BC}	100 (92.7)^{AB}	95.4 (77.7)^{C}	98.4 (82.7)^{BC}	99.4 (85.7)^{BC}
Confidor 200SL	10ml (2g. a.i)	15.4 (23.1)	97.9 (81.7)^{C}	96.7 (79.6)^{C}	87.2 (69)^{D}	78.4 (62.3)^{D}	94.4 (76.3)^{C}
Untreated control	water only	9.3 (17.8)	19.9 (26.5)^{D}	21.4 (27.6)^{D}	21.6 (27.7)^{E}	22.8 (28.5)^{E}	25.5 (30.3)^{D}
SE±		1.46	2.57	2.12	1.45	3.93	3.56
C.V%		11.3	5.4	4.4	3.2	9.0	7.7

Data in brackets were arcsine transformed. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 10. Yield and yield components of date fruits (trunk injection) Al gaba scheme. Season 2002/2003.

Insecticide	Dosage rate/palm	% ripe fruit	Fruit weight (g)	Fruit length (cm) L	Fruit diameter (cm) D	L/D ratio	% seed/ Fruit wt.	Yield kg/palm
Actara 25WG	10g.p (2.5g.a.i)	100^{A}	7.2^{A}	4.9^{A}	1.6	3.0	9.3^{D}	109^{A}
Actara 25WG	8g.p (2g.a.i.)	93.3^{AB}	6.8^{AB}	4.9^{A}	1.5	2.9	11.3^{BC}	85.5^{ABC}
Actara 25WG	6g p (1.5g. a.i)	85^{BC}	6.2^{B}	4.3^{BC}	1.7	2.8	12.7^{B}	81.77^{ABC}
Confidor 200SL	20ml (4g. a.i)	100^{A}	7.6^{A}	4.7^{AB}	1.6	3.0	10^{CD}	103.67^{AB}
Confidor 200SL	15ml (3g. a.i)	86.7^{BC}	7.4^{AB}	4.6^{AB}	1.6	2.9	11.7^{BC}	63.67^{BCD}
Confidor 200SL	10ml (2g. a.i)	80^{C}	7.0^{AB}	4.5^{AB}	1.5	2.8	12.3^{B}	57.77^{CD}
Untreated control	water only	48.3^{D}	4.9C	3.9^{C}	1.5	2.6	15.7^{A}	28.3^{D}
SE±		2.47	0.37	0.14	0.06	0.17	0.56	11
C.V%		5.0	9.5	5.6	6.5	9.9	8.1	25.1

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 11. Mean biweekly total mortality of green pit scale insect treated with different insecticides (trunk injection) El Golid, season 2003/2004.

Insecticides	Dosage rate/palm	Weeks after injection					
		0	2	4	6	8	12
Actara 25WG	10g.p (2.5g.a.i)	0.51(1.0)	7.7 (2.8)^{A}	8.5 (3.0)^{A}	5.7 (2.5)^{A}	3.7 (2.0)^{AB}	5.3 (2.4)^{A}
Actara 25WG	8g.p (2g.a.i.)	0.71 (1.1)	3.5 (2.0)^{B}	4.3 (2.2)^{BC}	3.1 (1.9)^{CD}	2.4 (1.7)^{BC}	3.4 (1.9)^{BC}
Actara 25WG	6g p (1.5g. a.i)	0.6 (1.1)	2.7 (1.8)^{BCD}	3.1 (1.9)^{C}	2.4 (1.7)^{DE}	1.3 (1.3)^{CD}	2.3 (1.7)^{CD}
Rinfidor 20%SL	20ml (5g. a.i)	0.7 (1.1)	6.3 (2.6)^{A}	5.8 (2.5)^{B}	4.3 (2.2)^{AB}	3.5 (2.0)^{AB}	3.9 (2.1)^{AB}
Rinfidor 20%SL	15ml (4g.a.i)	0.9 (1.2)	3.3 (1.9)^{BC}	3.1 (1.9)^{C}	2.4 (1.7)^{DE}	2.5 (1.7)^{BC}	3.1 (1.9)^{BC}
Rinfidor 20%SL	10ml (3g.a.i)	0.9 (1.2)	1.8 (1.5)^{D}	1.5 (1.4)^{D}	1.7 (1.3)^{E}	1.2 (1.3)^{CD}	2.3 (1.7)^{CD}
Comodor 20%SL	20ml (5g.a.i)	1.1 (1.3)	6.8 (2.7)^{A}	5.4 (2.4)^{B}	4.8 (2.3)^{AB}	4.3 (2.2)^{A}	4.3 (2.2)^{AB}
Comodor 20%SL	15ml (4g.a.i)	0.9 (1.1)	3.9 (2.1)^{B}	3.5 (2.1)^{C}	2.9 (1.8)^{D}	2.6 (1.8)^{ABC}	3.1 (1.9)^{BC}
Comodor 20%SL	10ml (3g.a.i)	1.1 (1.2)	2.1 (1.6)^{CD}	1.8 (1.5)^{D}	2.4 (1.7)^{DE}	1.9 (1.5)^{CD}	2.4 (1.7)^{CD}
Confidor 200SL	20ml (5g.a.i)	1.1 (1.3)	7.3 (2.8)^{A}	7.9 (2.9)^{A}	4.2 (2.2)^{AB}	3.9 (2.1)^{AB}	3.1 (1.9)^{BC}
Confidor 200SL	15ml (4g.a.i)	0.9 (1.1)	3.5 (2.0)^{B}	4.3 (2.2)^{BC}	1.9 (1.5)^{E}	1.4 (1.4)^{CD}	2.1 (1.6)^{CD}
Confidor 200SL	10ml (3g.a.i)	1.0 (1.2)	2.1 (1.0)^{D}	3.1 (1.9)^{C}	1.7 (1.5)^{E}	1.0 (1.2)^{D}	1.5 (1.4)^{DE}
Untreated control	water only	0.9 (1.1	0.5 (1.0)^{E}	0.7 (1.1)^{E}	0.5 (1.0)^{F}	1.0 (1.2)^{D}	0.5 (1.0)^{E}
SE±		0.14	0.10	0.11	0.1	0.13	0.12
C.V%		24.6	8.7	9.2	8.4	14.1	11.7

Data in brackets were √x+0.5. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 12. Mean biweekly percentage of adult females of green pit scale insect treated with different insecticides (trunk injection) El Golid, season 2003/2004.

Insecticides	Dosage rate/palm	Weeks after injection					
		0	2	4	6	8	12
Actara 25WG	10g.p(2.5g.a.i)	23.1 (28.7)	91 (72.8)^{A}	98.6 (88.4)^{A}	95.8 (76.6)^{ABC}	100 (98.5)^{A}	99.6 (86.5)^{A}
Actara 25WG	8g.p (2g.a.i.)	22.9 (28.5)	89.0 (70.8)^{A}	91.5 (73.4)^{ABCD}	87.2 (69.0)^{ABC}	100 (98.6)^{A}	100 (95.7)^{A}
Actara 25WG	6g p (1.5g. a.i)	27.0 (31.3)	69.4 (56.4)^{BC}	87.7 (69.5)^{ABCD}	65.3 (53.9)^{CD}	100 (97.9)^{AB}	99.8 (87.2)^{A}
Rinfidor 20%SL	20ml (5g. a.i)	13.6 (21.7)	89.2 (70.8)^{A}	80.0 (97.0)^{ABC}	100 (98.0)^{A}	100 (99)^{A}	100 (97.7)^{A}
Rinfidor20%SL	15ml (4g.a.i)	12.3 (20.5)	61.4 (51.6)^{C}	77.2 (61.5)^{BCD}	100 (99.0)^{A}	98.9 (84.4)^{AB}	100 (99.2)^{A}
Rinfidor20%SL	10ml (3g.a.i)	20.7 (27.1)	51.6 (45.9)^{C}	72.4 (58.0)^{CD}	98.7 (83.7)^{ABC}	91.2(80.5)^{AB}	97 (80)^{A}
Comodor20%SL	20ml (5g.a.i)	23.2 (28.8)	88.7 (70.4)^{A}	97.6 (81.2)^{ABC}	100 (94)^{AB}	100 (99.2)^{AB}	100 (99.5)^{A}
Comodor20%SL	15ml (4g.a.i)	14.7 (22.6)	66.8 (54.8)^{C}	85.3 (67.3)^{ABCD}	100 (91)^{AB}	96 (78)^{BC}	100 (98)^{A}
Comodor20%SL	10ml (3g.a.i)	13.7 (21.7)	50.9 (45.5)^{C}	67.0 54.0)^{D}	78 (62.0)^{BC}	92 (74.7)^{AB}	100 (91.9)^{A}
Confidor 200SL	20ml (5g.a.i)	19.9 (26.3)	86.6 (67.7)^{AB}	100 (91.0)^{A}	100 (99)^{A}	100 (99.4)^{A}	100 (99.7)^{A}
Confidor 200SL	15ml (4g.a.i)	27.9 (31.9)	70.4 (57.0)^{BC}	99.5 (86.0)^{AB}	98.7 (83)^{ABC}	100 (99.5)^{A}	100 (98)^{A}
Confidor 200SL	10ml (3g.a.i)	11.9 (20)	70.6 (57.2)^{BC}	84.8 (67.1)^{ABCD}	89.1 (71.9)^{ABC}	89.2 (71)^{B}	100 (91)^{A}
Untreated control	water only	17.3 (24.6)	15.0 (22.0)^{D}	19.0 (24.9)^{E}	25.7 (30.6)^{D}	19.2 (26)^{C}	13.3 (21.4)^{B}
SE±		3.76	1.3.47	7.13	9.75	6.32	6.26
C.V%		25.4	10.5	17.8	21.6	12.8	12.3

Data in brackets were arcsine transformed. {Means with letter(s)} in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 13. Mean biweekly percentage mortality of immature stages of green pit scale insect treated with different insecticides (trunk injection) El Golid, season 2003/2004.

Insecticides	Dosage rate/palm	Weeks after injection					
		0	2	4	6	8	12
Actara 25WG	10g.p (2.5g.a.i)	8.2 (16.6)	100(99.3)^{A}	100(98.4)^{A}	100(99.6)^{A}	100(98.1)^{A}	100(98)^{A}
Actara 25WG	8g.p (2g.a.i.)	20.8 (27.1)	97.8 (81.4)^{ABC}	100(95.2)^{A}	100(99.6)^{A}	100(96.3)^{A}	100(96.2)^{A}
Actara 25WG	6g p (1.5g. a.i)	2.5 (9.1)	96.3(78.9)^{BC}	100..(92.1)^{A}	100(99.1)^{A}	98.6(83.3)^{B}	99.7(86.9)^{A}
Rinfidor 20%SL	20ml (5g. a.i)	23.2 (28.8)	100(99)^{A}	100(95.4)^{A}	100(98.2)^{A}	100(99.2)^{A}	100(97.5)^{A}
Rinfidor 20%SL	15ml (4g.a.i)	9.9 (18.3)	99.9(88.3)^{AB}	100(97.7)^{A}	100(98.9)^{A}	100(97.8)^{A}	100(99.2)^{A}
Rinfidor 20%SL	10ml (3g.a.i)	4.5 (12.3)	85.5(67.6)^{C}	100(90.7)^{A}	98.2(82.3)^{B}	100(99.3)^{A}	100(97.2)^{A}
Comodor 20%SL	20ml (5g.a.i)	25.6 (30.4)	100(99.5)^{A}	100(98.5)^{A}	100(99.3)^{A}	100(99.2)^{A}	100(98.5)^{A}
Comodor 20%SL	15ml (4g.a.i)	1.5 (1.4)	100(99.4)^{A}	100(98.4)^{A}	100(96.2)^{A}	100(98.6)^{A}	100(99.1)^{A}
Comodor 20%SL	10ml (3g.a.i)	0.4 (1.0)	97.9(81.6)^{ABC}	100(95.2)^{A}	100(90.9)^{A}	100(98.7)^{A}	100(99.5)^{A}
Confidor 200SL	20ml (5g.a.i)	25.2 (30.1)	100(99.3)^{A}	100(99.3)^{A}	100(99.6)^{A}	100(99.4)^{A}	100(98.^{A}
Confidor 200SL	15ml (4g.a.i)	11.7 (20.0)	100(98.9)^{A}	100(98.8)^{A}	100(99.4)^{A}	100(99.4)^{A}	100(99.4)^{A}
Confidor 200SL	10ml (3g.a.i)	12.4 (20.6)	100(97.3)^{A}	100(98.1)^{A}	100(95.3)^{A}	100(99.2)^{A}	63.5(52.8)^{B}
Untreated control	water only	17.5 (24.8)	22.3(28.2)^{D}	24.4(29.6)^{B}	24(29.3)^{C}	23.2(28.8)^{C}	24.3(29.5)^{C}
SE±		4.8	5.23	3.47	2.73	2.66	7.22
C.V%		45.3	10.5	6.6	5.2	5.0	14.1

Data in brackets were arcsine transformed. {Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test}.

Table 14. Yield and yield components on date fruits treated with different insecticides (trunk injection) El Golid, season 2003/2004.

Insecticides	Dosage rate/palm	%ripe fruit	Fruit wt. (g)	Fruit length (cm) L	Fruit diameter. (cm) D	L/D Ratio	%seed per fruit wt.	Yield kg/palm
Actara 25WG	10g.p (2.5g.a.i)	100^{A}	7.3^{A}	4.6^{A}	1.6^{A}	3.0^{A}	9.3^{FG}	97.3^{AB}
Actara 25WG	8g.p (2g.a.i.)	86.7^{BCD}	7.1^{AB}	4.1^{ABCD}	1.6^{A}	2.6^{ABCD}	12.7^{BCDEF}	87.3^{BC}
Actara 25WG	6g p (1.5g. a.i)	70^{EF}	6.9^{ABC}	3.4^{EF}	1.6^{A}	2.1^{CD}	8.3^{G}	79^{CDE}
Rinfidor 20%SL	20ml (5g. a.i)	$90A^{BC}$	5.7^{ABCD}	4.2^{ABC}	1.4^{AB}	3.0^{A}	11.3^{DEFG}	96^{AB}
Rinfidor20%SL	15ml (4g.a.i)	83.3^{CD}	5.4^{BCD}	3.7^{BCDE}	1.6^{A}	2.3^{ABCD}	14.7^{ABCD}	86^{BCD}
Rinfidor20%SL	10ml (3g.a.i)	65.1^{F}	5.1^{CD}	3.5^{DEF}	1.4^{AB}	2.6^{ABCD}	16.3^{AB}	72.7^{E}
Comodor20%SL	20ml (5g.a.i)	96.7^{AB}	5.9^{ABCD}	4.1^{ABCD}	1.6^{A}	2.6^{ABCD}	9.7^{EFG}	96.7^{AB}
Comodor20%SL	15ml (4g.a.i)	80^{CDE}	5.1^{CD}	3.7^{CDE}	1.4^{AB}	2.5^{ABCD}	13.3^{BCDE}	75.7^{DE}
Comodor20%SL	10ml (3g.a.i)	60^{F}	4.7^{D}	3.4^{EF}	1.3^{B}	2.8^{ABC}	14.7^{ABCD}	68^{E}
Confidor 200SL	20ml (5g.a.i)	100^{A}	5.9^{ABCD}	4.3^{AB}	1.5^{AB}	3.1^{AB}	10^{EFG}	99.7^{A}
Confidor 200SL	15ml (4g.a.i)	83.3^{CD}	5.3^{BCD}	3.6^{DE}	1.6^{A}	2.2^{BCD}	11.7^{CDEFG}	86.3^{BCD}
Confidor 200SL	10ml (3g.a.i)	78.6^{DE}	4.2^{D}	3.2^{DEF}	1.4^{AB}	2.2^{BCD}	15.3^{ABD}	68.7^{E}
Untreated control	water only	50^{G}	4.3^{D}	3.1^{F}	1.4^{AB}	1.9^{D}	18.3^{A}	24.7^{F}
SE±		3.15	0.51	0.18	0.07	0.21	1.13	3.28
C.V%		6.8	15.8	8.4	8.2	14.5	15.4	7.1

Means with letter(s) in common are not significantly different at 5% level according to Duncan's Multiple Range Test.

Biocontrol of the Lesser Date Moth *Batrachedra amydraula* Meryrick *(*Cosmopteridae = Batrachedridae*)* on Date Palm Trees

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Abstract

Bioassays of some botanical extracts, granulosis virus, paraffined summer oil and bacteria toxin *Bacillus thuringiensis* and plant extracts (matrine) were conducted against the first generation of lesser date moth *Bactrachedra amydaula* at several sites during two campaigns (2004 and 2005) in Saudi Arabia in order to assess their efficacy against this important pest in the region.

Several trials focussing on biopesticides were conducted in date palm areas (Bicha, Riyadh, Haer, Oyayna) using the following bio-products according to the infestation level: *Bacillus thuringiensis* kurstaki (Condor) 300-1000g / hl; Spinosad (tracer 480): 100 cc/hl; Carpovirisin: (CYD-X): 500cc/hl; Sunspray 98.8% (summer oil) 1l/hl; Matrine3: Herbal source: 150 cc/hl.

A trial protocol was adapted to each region according to its specificity. The application was made at the rate of 3 to 7 liters per tree, washing all bunches of the tree according to the size of trees and their bunches. In each bioassay, samples of 400 to 500 fruits were analyzed from each plot weekly during one month after treatment. The results showed that when applied at the time corresponding to the oviposition and hatching periods (a few days after fruit set), the biopesticides controlled the lesser date moth well. The protection period was important in plots treated with Matrine one, Sunspray7E and *Bacillus thuringiensis* Kurstaki, but was less important in the plots treated with Spinosad and Carpovirusin. The efficacy of all tested biopesticides was improved if good sanitation was applied in the orchard by removing over-wintering larvae in fruit stalks and dry fruits.

INTRODUCTION

The lesser date moth is a pest of date palm from the west of the Arabian Peninsula to Pakistan (Al-Haidari, 1980). In Libya its damage in coastal areas is slight (Martin, 1958, 1959 and 1972). Gharib (1968, 1969) reported that in the southern regions of Iran, it is a serious pest and it has been registered as a serious date palm pest in Iraq (Downson and Aten, 1962), in India (Martin, 1972), in Yemen (El Baker, 1952 and 1972), and in Saudi Arabia (Arafat, 1974), where it occurred in different areas (Bicha, Riyadh, El Ihsaa) including the Eastern region. The pest has 3 generations from March to July. Lepesme (1947) stated that the lesser date moth attacked only *Phoenix dactylifera* from April till the end of July. Larvae attacked flowers and bored into young fruit. Martin (1959) reported that many generations may occur. The first spring generation is the most dangerous in many countries (Crossa Raymond, 1960; Calcat 1959, Buxton 1920, Hussein 1974, Hussein et al., 1971). It appears in April, the second generation appears in late May and the third generation in late June to July. The larvae of the last generations over-winter in cocoons and pupate in spring to start the first generation of the new season.

In general, the adults fly in April and oviposition on young fruit during fruit set. The larvae bore into the base of immature date fruits. In Saudi Arabia, imagos fly earlier in southern regions (Bicha), adult activity starts in March, and the larvae of the first generation was found during the beginning of April. It was observed that females laid eggs on the calyx of small fruits. Hatching larvae bore inside fruits and sometimes consumed seeds of the tender varieties (Khothari and Sufry, in Bicha, Nbout Seif in Oyayna and in Ryadh).

The larvae feed inside the fruit but some authors have reported that they feed on flowers also (Gharib, 1968; Hussein, 1974). Larvae spin webs around the attacked flowers

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

and later infest the fruit on the strand. The larvae perforate the fruit throughout the calyx of the young fruit and feed inside the pulp of the fruit or on flowers, and immature seeds. A larva seldom eats more than one third of the fruit before it seeds others and may damage three or four fruits during its lifetime. Carpenter and Elmer (1978) reported that damages in the regions of Iraq are estimated at 75% of the crop. A quarter of a million palms may lose practically all their fruit within 2 months after pollination. The same authors reported that there were no differences in degree of infestation by the lesser date moth amongst approximately 40 date varieties in Iraq.

In Saudi Arabia, we found that damage caused by the lesser date moth varied from one locality to another and there were certain varieties that appeared more vulnerable to attack than others. In southern areas of Saudi Arabia (Bicha), the most infested variety during 2004 was Elkhothari and Sufry. In Jouf area, Elhilwa was more prone to attack, but in Riyadh areas, Nbout-Sif was more infested than other varieties.

In Bicha areas, all surveyed orchards were infested (all bunches of the palm tree were infested) but on each bunch, infested fruit numbers varied from 10 to 35%. The lesser date moth is a pest of immature dates in bunches and in storage (Carpenter and Elmer, 1978). Infested fruits do not develop and need 4 weeks to darken, dry and fall or stay on the bunch.

In severe infestation, most of the fruits and sometimes the whole bunch darkens. Infested fruit will usually fall, causing bunches to cease growing and to dry. Larvae leave attacked fruit before ripening, leaving frasses and other debris. Attacked fruit must be removed.

The natural enemy of the lesser date moth are parasitaoids. They are from the families Braconidae and Trichogrammatidae and control the caterpillars and eggs of date moths in many date palm areas (Dhouibi, 1995; Dhouibi and Jemmazi, 1995). The most important species are: *Habrobracon hebetor*: larval parasitoid; *Bracon brevicornis*: larval parasitoid; *Phanerotoma ocularis* or *Phanerotoma flavitestacea*: egg larval parasitoid; and *Trichogramma* sp.: egg parasitoid.

Until now date palm growers in Yemen have used predatory ants of the *Crematogaster* sp. to help control insect pests. Farmers collect these predators in mountains and sell them to growers to be released in date palm orchards. This method is still used in certain oases.

Control Means: Sanitation

Maintaining good sanitation by removing the over-wintering larvae in fruit stalks, dry fruits and fibers decreases the insect population level next campaign.

In several date palm areas, farmers cover date bunches with craft bags to enhance pollination of certain varieties, mainly those derived from tissue culture. The bagging operation was effective in excluding the lesser date moth. These bags are removed 50 to 60 days after pollination, otherwise the craft bags would be split by the size of the bunch. These bags may also have an effect on the color of fruits (Dhouibi, 2000).

Several authors (Michael, 1970; Michael and Habib, 1971; Hussein, 1974; Hussein et al., 1971; Gharib, 1968) have recommended the application of insecticides (Fenthion, Thionazin, Malathion, Trichlorfon, Diazinon) with high volume one week after fruit set and again 15 days after the first application.

The pollination period covers a large range of time because female inflorescences to not become receptive to grains of pollen at the same time. This causes difficulties in applying chemical treatments by spray because they wash and remove pollen grains. Therefore we recommend that chemicals should be applied by dusting during the pollination period. Deltamethrine, Carbaryl (sevin 10%, malathion 5%) controlled the lesser date moth well during the pollination period. The effectiveness of new biopesticides (Spinosad, *Bacillus thuringiensis* Kurstaki and Rotenone) were also tested as dust treatments during this period.

MATERIALS AND METHODS

Several bioassays focused on biopesticides in different areas (Bicha, Riyadh, Hayer, Oyayna…).

The biopesticides tested on the lesser date moth *Batrachedra amydraula* during 2004 included:

+ *Bacillus thuringiensis* Kurstaki 32 000 UI/mg (condor WP) 43% Lepidopteron active toxins: 500 -1000 g/100L,
+Spinosad (tracer) 480: 100cc/100l
+Baculovirus CYD-X 3x1013 virus units: 0.5 – 1 liter/100L
+Sunspray: Ultra paraffin oil 7E 98,8%: 1l/100L

During 2005 the following biopesticide treatments were investigated:

+*Bacillus thuringiensis* Kurstaki 32 000 UI/mg (condor WP) 43% Lepidopteron active toxins: 300g/100l,
+Baculovirus CYD-X 3x1013 virus units: 0.5 – 1 liter/100L
+Matrine 3: Baico 3: (0.45% matrine + Emamectin benzoate compound slight emulsion: containing extract from *Sophora flavescens*, Neem, *Melia azedarach* and Emamectin benzoate. Target pests included Beet Armyworm (*Spodoptera exigua*), Tobacco Cut Worm (*Spodoptera litura*), Cabbage Armyworm, and Cotton Boll Worm (*Helicoverpa armigera*). It was used at a dilution rate between 2000 and 3000. Its effect is touch killing firstly and stomach poison secondary. It is used at 250cc/hl.

Treatments were applied with sprayers of 600L capacity to cover bunches of treated trees in all localities. The quantity of water used per tree varied from 3 to 7 liters according to the size of bunches on each date palm tree.

RESULTS AND DISCUSSION

In all experiments conducted at different sites on lesser date moth, the results presented in Figures 1 to 5 showed that the biopesticides used had an effect on the pest but the efficacy varied according to the pest infestation level, the application time and the dose of the bio-agent. Most of the biopesticides were satisfactory and performed when they were applied at the beginning of the pest cycle when larvae crawled outside the fruits and had direct contact with bio-agents. Therefore treatment should be applied while larvae are still outside the fruit and before they penetrate into the fruit. These biopesticides act by contact or ingestion, thus to control lesser date moth using them, it is recommended the behavior of the pest be followed using different techniques (sexual traps, sampling…) in order to apply the biopesticide at the right time to achieve a satisfactory result. Once larvae have penetrated inside fruits, the treatment does not cause a reduction of the pest population, even by chemical products.

The results suggested that the following doses of bio-agents would be effective: Spinosad 480E: 100cc/100L of water; *Bacillus thuringiensis* Kurstaki 32: 1kg/100L of water; Matrine3: 150cc/100L of water; Sunspray 7E: 1l/100L of water; Virus CYD-X : 500g/100L of water.

The *Bacillus thuringiensis* dose was high, and could be reduced to 500g/100L of water and remain effective against the lesser date moth. The dose of Spinosad may be increased to 150 cc/100L of water and applied each 10 to 15 days. The same recommendation was made for Sunspray7E and the virus CYD-X, particularly when the weather is relatively hot.

It is concluded, that to control a pest well, recommended treatments must be applied at the right time when the pest level is still low. Therefore it is crucial to understand the biology and behavior of the pest (emergence, pullulating and over-wintering….) and to know the sensitive period for application of bio-agents. It is imperative to emphasis the importance of sanitation and agronomic techniques as a further control method, ensuring the removal of all residual dates on the ground and on date palm trees. The bagging technique is recommend to protect bunches against the lesser date moth. In some areas this technique is used for the entire oasis against several pests and birds, snails and bat attack.

The use of biopesticides does not have a great impact on the natural enemies of the lesser date moth. Such predators are very active on dates and act against several pests. They help to maintain a positive balance in an oasis by limiting the population level of certain pests. In some places these techniques are used instead of chemical control, especially in organic date palm farming.

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Figures

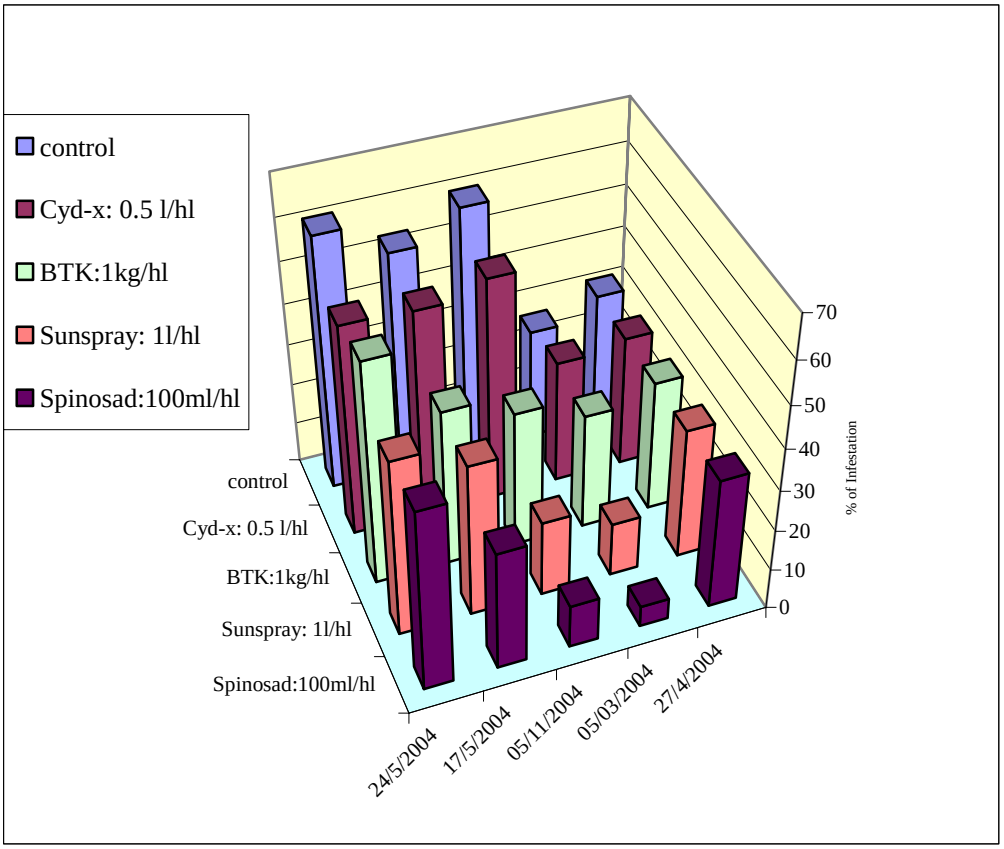

Fig. 1. Evolution of date infestation in Trad Mohamed in Bicha.

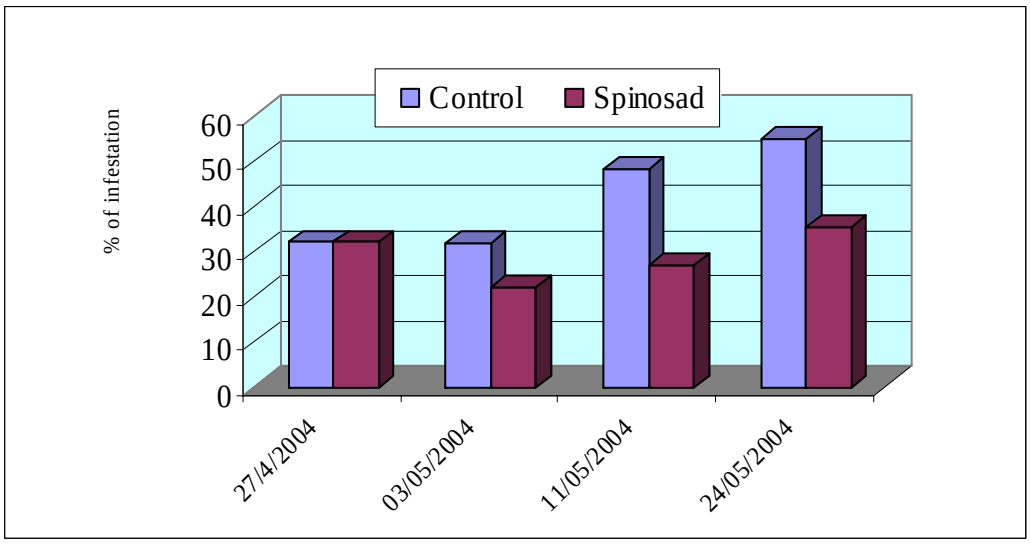

Fig. 2. Lesser date moth infestation in Ali Sallouli orchard 2004.

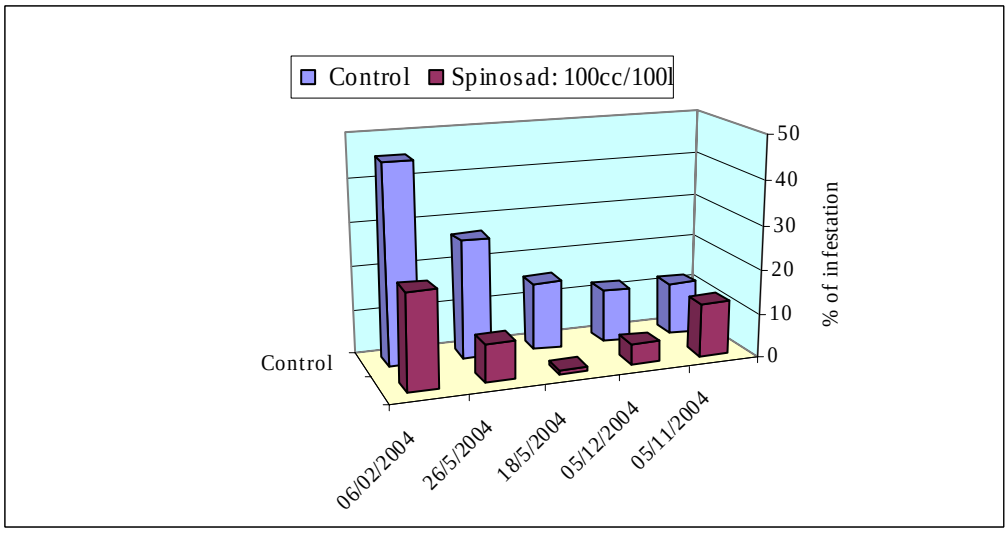

Fig. 3. Evolution of lesser date moth infestation in Oyayna 2004.

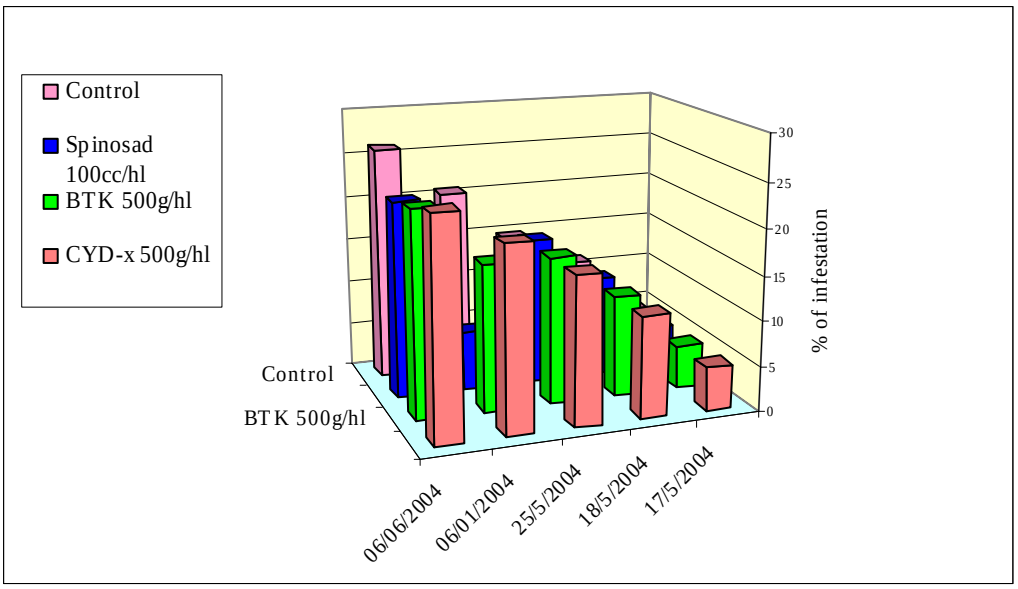

Fig. 4. Evolution of infestation of lesser date moth in Emir Fahd ben Turki 2004.

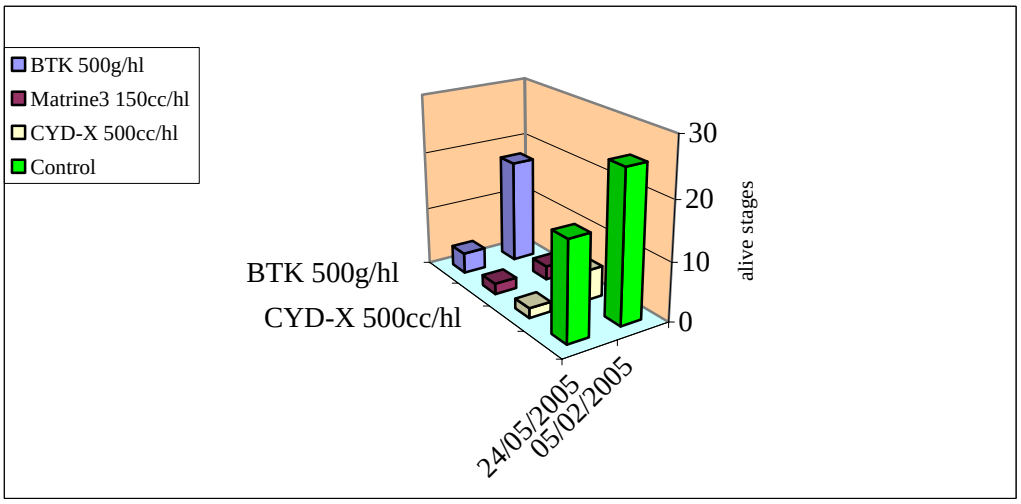

Fig. 5. Bioefficacy of biopesticides against lesser date moth in Bicha 2005.

Biological Control of Red Palm Weevil, *Rhynchophorus ferrugineus* (Col.: Curculionidae) by the Entomopathogenic Fungus *Beauveria bassiana* in United Arab Emirates

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Keywords: Conidia, mass production, storage, virulence, spray, dusting

Abstract

Biological control of the red palm weevil, *Rhynchophorus ferrugineus* was studied using the local strain, UAE-B2 of the entomopathogenic fungus *Beauveria bassiana.* For mass production of dry conidia, a new economic simple medium containing granulated rice was evaluated and used. The culture medium yielded 5.2 mg conidia/cm^2 with a potentiality of 91.7% on adult weevils. The conidia were stored at – 10 ^{o}C for 13 months without decrease in its virulence. Preliminary field investigations were carried out in date palm plantations at Ras Al Khaimah to evaluate the efficacy of the fungus. Spraying date palm trees with an oil formulation containing 5x10^7 con. / ml at a rate of 5 L / tree caused a mortality of 13.7-19.2% in the adult population during the three weeks after application with a monthly delayed mortality of 2.3-12.5% in the following four months. Dusting a date palm tree with 40 g of a powder formulation containing 5% conidia killed 8.9% of adult population during the three weeks after application and caused monthly delayed mortality of 4-5.9% in the following three months.

INTRODUCTION

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Oliv.) (Col.: Curculionidae) is now the principle insect pest of the date palm, *Phoenix dactylifera* in the Arabian Gulf Region, particular in United Arab Emirates (UAE) (El Ezaby et al., 1998). Since its occurrence in UAE in 1985, the insect has spread throughout date palm plantations causing considerable damage and death of the trees. In UAE, India and other countries, control efforts were mostly directed against the larval stage through the injection of chemical insecticides into the tree stem (Muthuraman, 1984; Abraham et al., 1975; El Ezaby, 1997). However it was difficult for chemical insecticides to reach the larvae in their deep branched tunnels. In addition, use of chemical insecticides is no longer desireable because of the destruction of parasitoids and predators, and because of environmental pollution and their impact on non-target organisms including humans.

The Biological Control Project (BCP) of RPW (3rd phase) conducted by Arab Organization for Agricultural Development (AOAD) in cooperation with Ministry of Agriculture and Fisheries, UAE, was begun in September 2004 with the objective of developing integrated biological control technology against RPW using local microbial control agents. The entomogenous fungi, Deuteromycetes were considered of great importance in the integrated pest control programs because of their broad host ranges and ability to grow and sporulate on more generalized media (Burge, 1988; Boucias and Pendland, 1998). The Deuteromycetes *Beauveria bassiana* (Balsamo) Vuillemin is widely distributed in nature and has the potential to control over 70 insect pests including the Colorado beetle, *Leptinotarsa decemlineata*, the Japanese beetle, *Popillia japonica* and the Egyptian alfalfa weevil, *Hypera prunneipennis* (Mueller-Koegler, 1965; El-Sufty and Boraei, 1987; Lacey et al., 1994; Sewify, 1998; Bextine and Thorvilson, 2002).

The present paper reports on a new method for mass production of the fungus *B. bassiana* and storage of the conidiospores, as well as preliminary field experiments to

control RPW using the local strain UAE-B2.

MATERIAL AND METHODS

Experimental Insects

Adults of *R. ferrugineus* were collected weekly from date palm plantations using insecticide free pheromone-kairomone traps developed by BCP and maintained for a week in plastic containers and provided with food (moistened pieces of palm wood). Dead and injured insects were discarded and healthy ones were used in the experiments.

The Fungus

A naturally occurring entomopathogenic fungus has been found infecting adults and pupae of RPW in several date palm plantations in UAE. The fungus was isolated for the first time by BCP and found to be potentially important for RPW control. The fungus was identified by CABI Bioscience, UK, and samples were also dispatched to Brook University, Canada, for fingerprinting. The fungus was designated as a local strain of *Beauveria bassiana* (UAE-B2).

Mass Production of Conidia

The fungus was maintained on Sabouraud's dextrose agar supplemented with yeast extract (SDAY), as described by Goettel and Inglis (1997), with regular passages through RPW-adults. For mass production of conidia, a surface culture on an economic simple medium was developed and evaluated. The new medium (GR) composed of 40 g granulated rice grains, 8 g peptone, 10 g agar and 2 g yeast extract per 1L water. The components were mixed, autoclaved at 121^{o}C and 15 Ib pressure for 20 min. After cooling, the medium was poured into autoclaved aluminium trays (29 x 21 x 3 cm) to a depth of 5 mm. Twenty four h later, the medium were inoculated with germinated hyphae and blastospores grown for 2-3 days in liquid medium composed of 20 g D-glucose, 5 g peptone, 1 g yeast extract and 500 ml water containing 0.5% Tween 80. The trays were covered, incubated at room temperature for 2 weeks and left partly covered for a week to dry. Conidia were harvested by scraping from the surface using paint brush.

To evaluate conidia production, 5 Petri dishes of GR-medium and of SDAY-medium were inoculated with 1 ml/dish of the liquid medium. The dishes were incubated at 25 ± 1^{o}C for 21 days. Nine cm^{2} of the Petri dish center were cut out and transferred to a sterilized plate. The conidia were scraped from the surface medium using a fine brush and weighed. To evaluate virulence, conidia produced on GR-medium were used to contaminate 20 RPW adults in 4 replicates (5 insects each) by individual dipping in 50 ml of a suspension containing 10^{8} conidia /ml and 0.1 Tween 80 for 15 seconds. For comparison, 20 adults were treated with conidia produced on SDAY- medium. Another 20 untreated adults served as the control. Each replicate was maintained inside plastic containers provided with palm wood pieces. The containers were kept at room temperature. Insects were daily examined and dead individuals were removed. Recording continued for 12 days.

Conidia Storage

Samples of conidia produced on GR-medium were stored inside a deep freezer (-10^{o}C), in a refrigerator (3-5^{o}C) and at room temperature. Monthly sub-samples were taken to evaluate their viability and virulence. Viability was assessed using culture technique on microscope slides as described by Fuehrer and El-Sufty (1979). One mm thick medium film was inoculated with 0.1 ml of a suspension containing 10^{5} con/ml on a cm^{2} of the center, incubated at 25 ± 1^{o}C for 24 h and microscopically examined. For each sub-sample, 4 medium films were used. For each film, 10 groups of conidia were randomly selected and germination percentage determined. Virulence was assessed monthly as previously described using 20 weevils in 4 replicates, each of 5 adults, for the sub-sample.

Field Experiments

To evaluate the fungus efficacy, preliminary field experiments were carried out in 5-10 year old date palm plantations at Ras Al Hkaimah, UAE. Two methods of applications were applied. In the first method, spray treatments were applied in two plantations using 5 L per tree of an oil formulation containing $5x10^7$conidia/ml. In the first plantation (Omran: 416 trees), 56 infested trees were treated on 30. 3. 2005 while in the second one (Darwish: 500 trees), 51 infested trees were treated on 11. 5. 2005. The spray was directed at leaf axils and continued to cover about 1 m of the trunk above the soil surface and the soil area around the trunk in a diameter of 2 m. In the second method, dusting treatment was applied in a plantation (Ebed: 510 trees) using 40 g per tree of a powder formulation containing 5% conidia. Fifty-one Infested palm trees were dusted on 11 .5. 2005 using a hand-held plastic inoculator to disperse the powder to leaf axils and the soil around the trunk.

Efficacy of treatments was assessed based on the monthly mortalities caused by the fungus in the RPW-adult population in treated plantations. Therefore, adults were collected weekly by pheromone-kairomone traps a month before application and continued till July 2005. Dead weevils were subjected to mycosis test as described by Lacey and Brooks (1997). The cadavers were individually placed on moistened filter papers inside Petri dishes. Plates were maintained at room temperature for 10 days and fungus growth was observed. Living weevils were maintained inside plastic boxes provided with moistened pieces of palm wood for 7 days and any that died were tested for mycosis. Cadavers showing external growth of *B. bassiana* were considered killed by the fungus.

Statistical Analysis

Mortalities were corrected according to Abbot' formula (1925) and analysis of variance was used to evaluate the impact in bioassays.

RESULTS AND DISCUSSION

Surface culture of the fungus on GR-medium produced sufficient quantities of conidia. The medium yielded 5.2 mg dry conidia per cm^2 of the medium surface compared with 4.3 mg for SDAY-medium. Differences were significant (P=0.01). Studies on virulence indicated that conidia of GR-medium showed nearly equal power of infection as that of conidia grown on SDAY-medium (Fig. 1). Accumulated mortalities for conidia cultured on GR-medium and those cultured on SDAY-medium were 91.7 and 94.4%, respectively. Differences were not significant.

The entomopathogenic fungi are easily produced on artificial media in large quantities. Fermentation techniques have allowed easy production of species such as *B. bassiana* and *Metarhizium anisopliae* (Ferron, 1978; Goettel and Inglis, 1997). However, high-technology is not available at laboratory level. Therefore, several investigators have developed simple techniques using cheaper nutritive substrates such as rice, bran, barley and rice husk for large-scale production (York, 1958; Arreger, 1992; Mendonca, 1992; Mazumder et al., 1995). The present type of mass production seems to be economic and suitable for a sufficient supply of *B. bassiana* conidia. The GR-medium supported better yield of conidia. It enabled aerial growth and sporulation of the fungus as well as complete separation of conidia from the nutritive medium.

Fig. 2 and 3 reveal that *B. bassiana* conidia remained viable and virulent for 13 months at -10^oC. This storage method seemed to be technologically simple and inexpensive for conidia maintenance. Refrigeration storage at $3\text{-}5^oC$ preserved the conidia in a viable and virulent state for 5 months. During the 6^{th} month, germination percentages and mortalities significantly decreased to reach 60.6% and 20%, respectively. Conidia kept at room temperature survived for 2 months. During the 3^{rd} month, germination percentages and mortalities significantly decreased to reach 79.1% and 57.8%, respectively.

Burge (1988) stated that Deuteromycetes conidia were sensitive to environmental

extremes and their viability must be sustained, not only during harvest and formulation but also during some period of storage. Goettel and Inglis (1997) mentioned that Hyphomecetes conidia could be stored at 4^{o}C for up to several weeks or even months. Conidia of *B. bassiana, M. anisopliae* and *Nomuraea rileyi* were stored in a frozen state for different lengthy periods without loss of viability (Balardin and Loch, 1988; Boucias and Pendland, 1998). Conidia of *Fusarium* have been stored for more than 10 years on silica gel at – 20 ^{o}C (Windels et al., 1993).

Fig. 4 demonstrates that the spray method of application caused 13.7-19.2% mortality in the population of RPW adults during 3-4 weeks after treatment. In both experimental plantations, the fungus induced monthly delayed mortality ranging from 2.3-12.5% in the following 3-4 months.

The dusting method of application was less effective with 8.9% mortality during the three weeks after application. The monthly delayed mortality values ranged from 4 - 5.9% in the following 3 months. A natural mortality of RPW-adults caused by the fungus was 1.5% and was recorded only in Omran plantation in March 2005 before spray treatments were commenced.

These preliminary field experiments indicate the potential of *B. bassiana* as a biological control agent for RPW. A single spray application yielded considerable mortalities of adult weevils over a relatively long period. The higher mortality in Omran plantation may be due to the application date, as the population density of RPW adults was higher in April than in May. Mueller-Koegler (1965) stated that the high population density of host insect enhances the development of the fungus disease in the population. The delayed effect of the fungus may be attributed to the development and sporulation of the fungus on cadavers of infected weevils, thereby spreading the fungus inoculum in the population. Ferron (1978) mentioned that under favorable conditions the auto-multiplication of the fungus inoculum resulted in a heavier contamination of healthy insects.

The results represent the first report of the use of the local strain UAE-B2 of *B. bassiana* against RPW in date palm plantations. Additional work is still needed to improve the effectiveness of the fungus and increase the mortality rates. It is also important to clarify the possible manipulation of the fungus in an integrated biological control program for RPW in United Arab Emirates.

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Figures

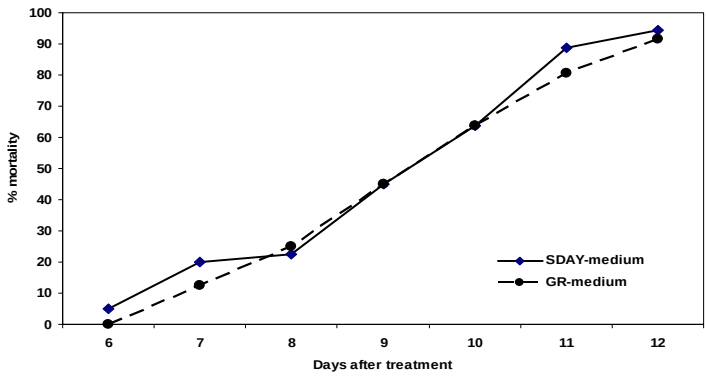

Fig. 1. Mortality curves of *Rhynchophorus ferrugineus* adults treated with *Beauveria bassiana* cultured on GR-medium and SDAY-medium.

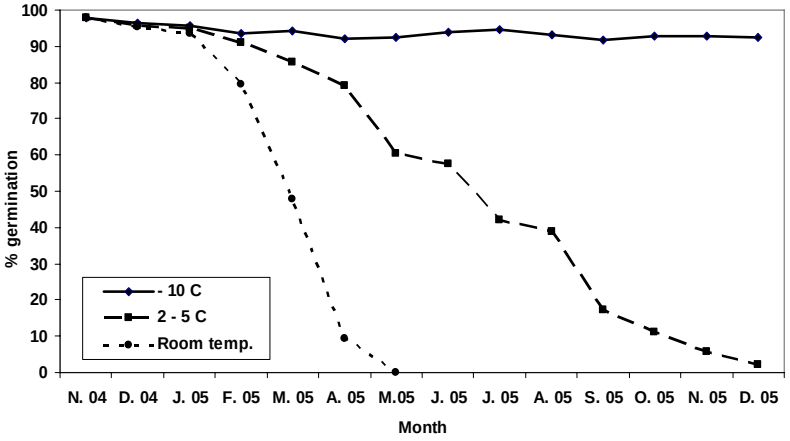

Fig. 2. Monthly values of germination percentages for *Beauveria bassiana* conidia.

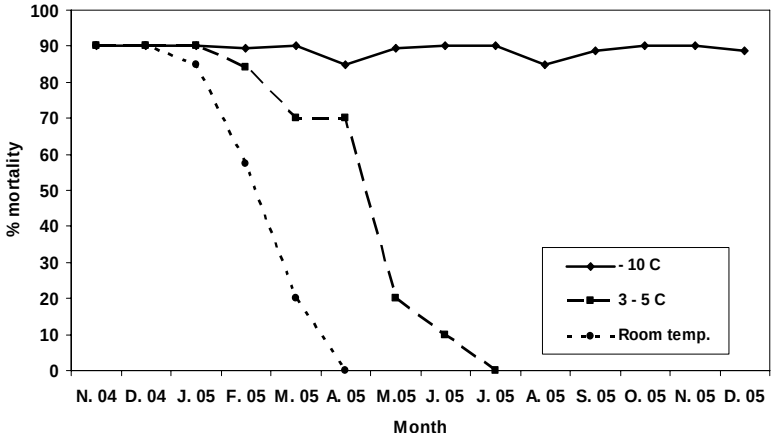

Fig. 3. Monthly mortality of *Rhynchophorus ferrugineus* adults treated with *Beauveria bassiana* conidia stored at three temperatures.

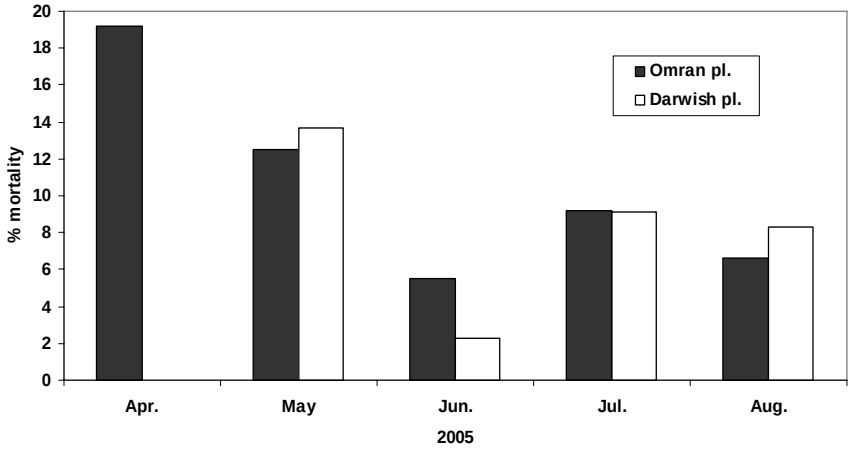

Fig. 4. Mortality of *Rhynchophorus ferrugineus* adults caused by *Beauveria bassiana* UAE-B2 after spray applications in two date palm plantations.

Importance of Date Fruit in Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) Aggregation Pheromone Traps

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Keywords: date palm, aggregation pheromone traps

Abstract

Experiments were conducted in four date palm plantations at Al-Rahbba from May 2004 to April 2005. Twelve pheromone traps were set in each plantation, to investigate the effect of adding dates as a bait, on the capture of the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Curculionidae: Coleoptera). The traps contained the aggregation pheromone 4-Methyl-5-Nonanol 90% + 4- Methyl-5-Nonanon 10% and 350g of dates. The catch was compared to the catch in traps containing either the pheromone alone or dates alone. The pheromone lure in each trap was changed every three weeks, while the water and dates were changed every 2 weeks. The number of RPW captured in the 16 traps was 1752, 181 and 54 insects for the three treatments, respectively. There were significant differences between the three treatments. The first treatment catch value was 10 and 32 times the second and third treatment value, respectively. The second treatment catch value was more than three times the third treatment catch value. The experiment also showed that adult RPW were present throughout the year, and the number of females was higher than the number of males. The numbers of captured insects were 420, 452, 542 and 573 adults at the four plantations, respectively. The reason behind the variation in numbers could be explained by a variety of factors such as farm practices, differences in tree age and source, and farmer knowledge.

INTRODUCTION

Palm trees are infested by several insect pests, Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) is one of the most important and dangerous. It constitutes a major threat to palm trees all over the world (Frohlich and Rodewald, 1970; Sharif and Wajih, 1983; Al-Saadani, 1993; Faleiro et al., 1998; Al- Ajlan, 1999; Al-Saoud, 2004).

There are several features of RPW that make it dangerous. Firstly the early stage of infestation are difficult to detect. This delays the instigation of control measures. It has been reported (Lever, 1969; Al-Asoud, 2004 b; Asoud, 2004 c) that infestations of insects is often discovered only after complete destruction of the tree: trees fall over or the trunk collapses or bad odors are emitted with a gel material from the trunk, destroying the crown of the tree. Secondly, weevil captures have been reported to be female dominated during various times of the year (Abraham et al., 1999; Faleiro and Rangnekar, 2000; Vidhyasagar et al., 2000; Faleiro et al., 2000a; Al-Saoud, unpublished), and thirdly, the presence of RPW has been reported throughout the year (Ghosh, 1912; Al-Ahmadi, 2002; Al-Saoud, unpublished). The activity period of this insect differs with the location. Pheromone trap captures have indicated that under coastal humid conditions of Western India, RPW is most active between October and November, and least active between June and July (Faleiro and Rangnekar, 2001a). Under arid conditions in the Middle East, weevil captures in pheromone traps were highest during May and November, with low activity being recorded during February and August, the peak of winter and summer, respectively. Also in the Middle East infestation of RPW in date palm increased with the severity of the summer, indicating that the peak weevil activity observed during May was crucial in determining the high infestation level in the field during the subsequent summer

months. The severe winter months from December to February in the region probably inhibited the hatching of eggs laid during the second activity peak of November, leading to a decline in the infestation level during winter (anonymous, 1998). The worst activity of the RPW in Kingdom of Saudi Arabia was from April to November in 1995, from May- Jun to October in 1996, and from May to September in 1997 (Abraham et al., 1999)

Large numbers of RPW were attracted by aggregation pheromone traps from February to August in Al-Ain in UAE. The numbers decreased after April in the south of the city, while the numbers increased in the western part of the city during November (Khalifa et al., 2004).

There are several methods to control this pest. To follow one of the methods is insufficient to control the pest effectively. Different methods should be followed to get an acceptable result in minimizing injury and preventing the spread of this pest. Abraham et al. (1998) stated that the Integrated Pest Management Programme (IPM) succeeded in controlling RPW injuries in AL-Qatif area from 1994 to 1997. Similarly, infestation levels were brought down from 6.6 % in 1993 to 2.5 % in 1997, due to the use of pheromone traps, as well as other control measures, in the areas of palm tree cultivation (Vidyasagar et al., 2000). This is similar to reports by Faleiro et al. (1998).

It is well known that this insect is a hidden enemy, and infestations are often discovered late even by trained and practiced specialists. The percentage of severely infested palm trees that were eradicated at the beginning of 1994 was 31.53%, and this figure decreased to 19.53 in 1997.

Several scientists have confirmed the importance of aggregation pheromone traps in RPW control (Abraham et al., 1998; Abraham et al., 2000; Abraham et al., 2001; Faleiro et al., 2002a; Faleiro et al., 2003; Al-Saoud, 2004b). The intensive and continuous efforts to control this insect have led to a significant decrease in their numbers and has prevented their increase in the field. Pheromone traps are considered to be the major key in the IPM programmes to reduce the insect population in the field.

Initial attempts to control RPW in the Kingdom of Saudi Arabia with insecticides were not successful (Bokhari and Abozahairrah, 1992). The Integrated Pest Management (IPM) strategy has successfully suppressed the pest in date plantations of Saudi Arabia, and mass trapping of the pest occurs widely in the Arabian Peninsula where it is a major problem in date palm (Abraham et al., `1998). Various ways should be followed to achieve this goal. The aggregation pheromone traps have played a vital role in this regard by collecting and killing the adults to interrupt the life cycle of the insect and its reproduction. During the period April 1991- September 1992 an estimated 123000 weevils of *R. palmarum* were captured in trap optimization and mass trapping experiments (Chinchilla et al., 1993; Oehlschlager et al., 1995).

In 1998, it was found (Anonymous) that the percentage of trees infested with RPW was reduced from 30.53% in 1994 at the beginning of use of pheromone traps to 19.53% in 1997 at Al-Hassa region. In India it was reported that the use of aggregation pheromone traps for two successive years on palm trees farms to control RPW led to a 75% reduction in numbers of captured insects (Muralidharan et al., 1999). Abraham et al. (2000) stated that the average number of captured insects in heavily infested areas was 2.55 weevils/ trap / month during 1999, and decreased to 1.41 weevils / trap / month during 1997, indicating that RPW pheromone traps substantially curtailed the building of the population in the field. Oehlschlager (2000) showed that throughout the first year of trapping, capture rate in the entire Coto plantation declined from 30 weevils / trap / month in 1994 to 4 weevils / trap / month, a decrease of over 80%. During the period between 1994 and 2001 monthly capture rates were no higher than 2 weevils / trap / month.

The trap components are important, especially the nutritional material added to the traps and its renewal. Kurian et al. (1979, 1984) and Abraham (1998) found that adding coconut juice and cetic cid to the pheromone traps helped to attract RPW to the traps. Faleiro and Satankar (2002 a) reported that replacement of the food bait in pheromone traps was required every 10 days. Large numbers of the insect were caught when dates were used as food bait, compared with coconut (Nair et al., 2000). Trials conducted in

India showed that the use of banana and sugarcane as food baits in pheromone traps gave positive results in increasing the efficacy of the traps. Faleiro and Satarkar (2003) said that water and food should be changed every 15 days maximum. Faleiro's results (2003) showed that the use of oil palm fruits as food bait had a repelling effect on RPW, preventing them from approaching the pheromone traps, instead of attracting them. Oehlschlager et al. (1993, 2003) found that adding sugarcane pieces or palm tree parts to the aggregation pheromone traps of *R. palmarum* led to the increase in captured weevils in the traps.

The efficacy of aggregation pheromone traps used in RPW control is affected by several factors, such as (a) the addition of nutritional material, (b) regular servicing of the food and water in the traps (c) the manner in which the traps are used and other factors.

This study aimed to determine the role of dates as food in fortifying the efficacy of aggregation pheromone traps of RPW, by counting numbers of the insect collected from the traps that contain date fruits comparing with those that contain pheromone or date fruits only.

MATERIALS AND METHODS

A trial was conducted to test the effect of adding palm date fruits as food bait to Red Palm Weevil aggregation pheromone traps, on the number of insects collected from these traps.

The trial consisted of 48 traps (buckets). They were used for 3 different treatments,with 16 traps per treatment. The first group (t1) contained palm date fruits, the second group (t2) contained pheromone only and the third group (t3) contained both pheromone and 350g of date fruits.

The date fruits used were forage fruits or those that had dropped around palm trees in the farms. These date fruits are not consumable and incur no cost, according to the farmers. The aggregation pheromone that is designed to attract RPW adults was used. It contains 400 mg of the active ingredient 4-Methyl-Nonanol-5 (9 parts) + 4-Methyl-Nonanol-5 (1 part), purity 95%.

These treatments were distributed at 4 farms in Al-Rahba region, Abu Dhabi. Each farm contained nearly 140 palm trees, some of which had been transplanted as seedlings from the mother tree, while others had been grown as tissue seedlings. The age of the trees was between 3 and 20 years.

The agricultural practices varied from farm to farm (i.e. pruning, irrigation system, fertilizing, grass removal, separation of seedlings from their mother, covering the stem biases with sand, covering the roots with sand, pesticide use, cleaning the head and the trunk of the palm tree etc.)

Twelve traps were placed at each farm. They were set about 50 m apart. Every trap was provided with 4-5 liters of water, with a water level inside of 4-5 cm, which was lower on the side of the opening of the bucket. Serial numbers were assigned to each trap and locations were numbered from 1 to12 on every farm. The study period began on 28/4/2004 till 4/5/2005. The traps were placed 3-4 m from palm trees. They were fixed in a hole of 12-15 cm depth in the sand, and part of the trap was covered by sand to fix it securely in place, and to avoid the trap being over-turned by wind, animals or any other external factors. The traps were put in position so that insects could reach through the trap side-openings. Trap maintenance and food change were done every 2 weeks, and the pheromones were replaced every 3 weeks. Number of weevils captured (female, male and total) were recorded weekly. Every trap was moved to a new location after weekly results had been taken, to avoid a location effect, and to know the insect numbers in every location and in every treatment on each farm during the study period. The data was processed and subjected to ANOVA test.

The trap consisted of a plastic bucket treated with ULV, with a 8- 10 liter capacity, a cover, density polyethylene (HDPE), with four windows (3 x 8 cm) cut equidistantly 4 cm below the upper rim of the bucket, for the entrance of the attracted adults.. The bucket was rough on the outside to allow the weevils to crawl up easily to the opening. The

pheromone hung from the inner side of the lid.

RESULTS AND DISCUSSION

Seasonal Activity of Red Palm Weevil in Al-Rahba

From the results presented in Fig. 1, it can be seen that RPM dose not enter diapause and it is found throughout the whole year in date palm areas. Similar results were recorded by Ghosh(1912), Abraham et al. (1999), Vidhyasagar et al. (2000), Al-Ahmadi (2002) and Al-Saoud (unpublished). Consequently, reproduction of the insect occurs all year, damage from RPM is increased, and control is difficult to achieve especially using chemicals, because application of pesticides must cease during mid February until the end of March (pollination period), and from the beginning until the end of the maturing and harvest of the crop (June- October).

Fig. 1 showed that weevil captures were female dominated during different months of the year. This has been found by several workers (Abraham et al., 1999; Vidhyasagar et al., 2000; Al-Saoud, 2004; Al-Saoud, unpublished). The adult female lays eggs on the date palms, spreading the infestation, so mass trapping of RPW helped suppress the build up of the pest. Fig. 1 indicated that the capture of adults increased during March- May and October- November, and decreased during December- February in the 2004/ 2005 season. Al-Saoud (unpublished) found similar trends in Al-Khatim region, with some differences due to environmental conditions, especially temperature and relative humidity, in the two regions. Similar results were recorded in the date palm plantations of Al-Ain in United Arab Emirates (Khalifa et al., 2004) with some differences. They found that the number of weevils trapped increased in February and March reaching maximum catch in April. It then decreased to the least number in October. The period of red palm weevil was from February to August in most of Al-Ain districts. The minimum activity period was after April in the southern Al- Ain areas, and the activity increased in the western areas during November. Studies in Arab Saudi Kingdom showed that the maximum activity of the weevil was during April and November in 1996 and in May and September in 1997 (Abraham et al., 1999).

Effect of Date Fruits in Aggregation Baited Pheromone Traps on the Collected Number of RPW

The data presented in Table 1 showed that significant differences occurred between treatments. The greatest number of RPW caught was 1752 weevils for the treatment using pheromone + 350 g dates (t3), compared to 181 weevils for pheromone alone treatment (t2) and 54 weevils when dates alone (t1) were used in the trap. The third treatment (t3) was better than the other two treatments, and the 2nd treatment (t2) was better than the first. The results indicated that the addition of date fruits to the pheromone traps was effective in improving the performance of the trap.

The main role of pheromone traps in control programs is the intensive and continuous collection of adult RPW and killing them to prevent their reproduction and spread of infestation. The benefit is higher when greater numbers of the females are collected, for they are considered the main factor in spreading the infestation which occurs as a result of laying large numbers of the eggs. If males are collected preventing them from mating with females, this leads to limited spread of this dangerous pest. Numbers of collected adults from the pheromone traps that contained these treatments showed that the use of pheromone and 350 g date (t3) resulted in the collection of 88.2% of the adults collected, t2 resulted in the collection of 9.1%, and t1 just 2.7%. These results were similar to what Abraham et al. (2001), Faleiro et al. (2002a) and Al-Saoud (2004a, unpublished) found, that a massive controlling program of RPW helped to prevent the increase of the pest in the field. These results were also similar to findings by Nair et al., (2000) and Faleiro and Satarkar (2002a), who showed the importance of using date as a food bait in aggregation pheromone traps of RPW. These results were not in full agreement with above researchers regarding the frequency of changing the food and

water, or adding water to the traps. Dates were changed every 2 weeks with good result during the trial period. Fungi and algas were usually growing on the water surface of the traps if water was not replaced for more than 2 weeks. These results agreed with Oehlschlager et al. (1993a, 2002) who recommended food be replaced every 2-3 weeks. Nair et al. (2000) suggested using banana and sugarcane as food in pheromone traps, because they were both easy and cheap to acquire in India, however, using these materials in UAE, would lead to an increase in costs due to the high price of these materials. Consequently, in UAE, the use of fallen dates is recommended.

Effect of Different Treatments on the Number of RPW captured in Aggregation Pheromone Traps in Al-Rahba, between May 2004 to April 2005

Results presented in Table 2 indicated that there were significant differences between the numbers of RPW caught on the four date palm farms. The number of RPW weevils caught were 420, 573, 452 and 542 weevils on the four farms, respectively. The maximum was on farm 2 (573 weevils) which was significantly higher than the number on the other 3 farms. The number collected on farm 4 was significantly higher than the numbers on farms 1and 3, and, more were caught on farm 3 than on 1. The differences in numbers of RPW caught on the 4 farms may have been affected by many factors, including age of trees, agricultural practices, source of trees, farm sites, and the expertise of farmers and agricultural workers, especially concerning weevil management.

These results showed the role of additional date fruits in aggregation pheromone traps on increasing the numbers of RPW adults caught in these traps compared with these numbers of RPW catches on aggregation pheromone bait traps contain pheromone only or date fruits only.

CONCLUSION

The number of weevils caught in t3 (pheromone + 350g date fruit) was 10 and 32 times greater than t2 and t1, respectively. The question arises, why was t3 (pheromone + date fruits) so superior to the other treatments? The reason may be that the date fruit acted as a food, and the pheromone acted as an attractant to adult RPW. As more adults were attracted perhaps the attraction of other insects combined with these smells created a new type of smell to attract the adults of this insect in comparison to food alone or pheromone alone. Further investigation is required to study these behaviors

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Tables

Table 1. Number of red palm weevil adults collected in aggregation pheromone traps with three different treatments on four date palm farms at Al- Rahba during 28/4/2004- 4/5/2005.

Treatments	Number of insects caught			% for	
	♂	♀	∑	♂	♀
350 g Dates (t1)	18	36	54	33.3	66.7
Pheromone (t2)	68	113	181	37.6	62.4
Pheromone + 350 g Dates (t3)	689	1063	1752	39.3	60.7
Total	775	1212	1987	39	61
Mean	285.3	404	662.3		
LSD 5%			12.63		
LSD 1%			17.52		
F			16.50**		

Table 2. Number of red palm weevils caught in aggregation pheromone traps in four date palm farms in Al- Rahba during 28/4/2004- 4/5/2005.

Farms	No. of weevils caught			Total	Mean
	350 g dates	pheromone	Pheromone + 350 g dates		
First	9	41	370	420	140
Second	20	61	492	573	191
Third	14	44	394	452	150.7
Fourth	11	35	496	542	180.7
Total	54	54	1752	1987	
Mean	13.5	45.3	438	496.8	
LSD 5%			7.96		
LSD 1%			10.67		
F			4.31**		

Figures

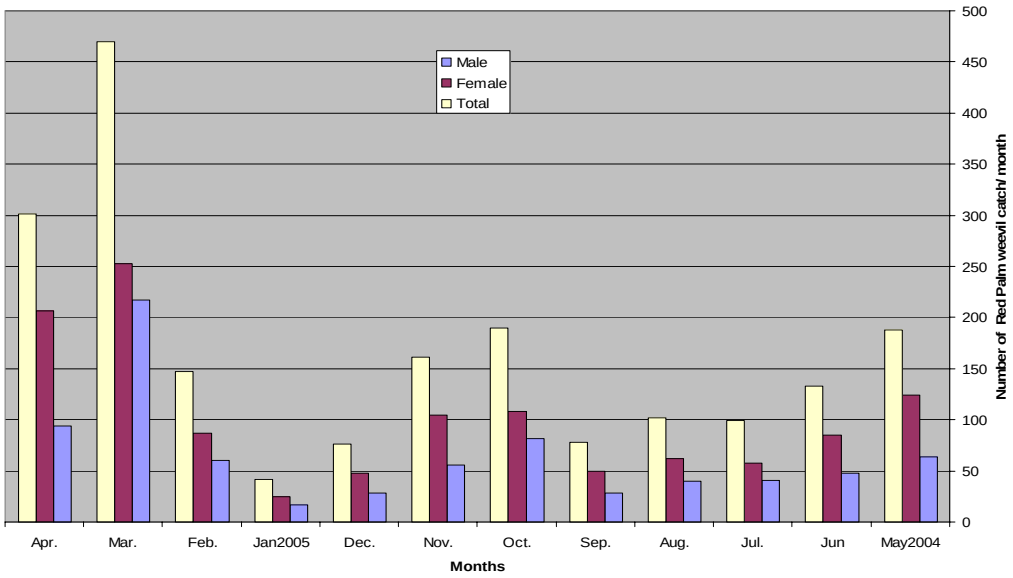

Fig. 1. Monthly RPW *Rhynchophorus ferrugineus* Oliv., captures with aggregation pheromone trapsin Al-Rahba during may 2004 to April 2005.

Efficacy of Entomopathogenic Nematodes against Red Palm Weevil in UAE

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Abstract

The Red Palm Weevil *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae) was introduced to the Middle East Region in the early 1980's. The insect is considered one of the serious pests which threaten date plantations in the region. As part of the research activities of the Biological Control Project of the Red Palm Weevil in the Middle East, investigations were conducted to assess the infectivity, the pathogenicity and the control potential of several isolates of entomopathogenic nematode against the adults of Red Palm Weevil, both under laboratory and field conditions. Results obtained from the laboratory assessments showned that a local UAE isolate of the entomopathogenic nematode *Heterorhabditis indicus* (Poinar et al., 1992), was the most efficient nematode isolate tested against this pest. Field assessments using a novel procedure of targeted application of highly concentrated suspensions of local UAE isolate *H. indicus* resulted in a substantial decline in the population of Red Palm Weevil after two successive applications within a period of two months. We concluded that there is great control potential when *H. indicus* is applied in date plantation fields against adults of the Red Palm.

INTRODUCTION

The Red Palm weevil (RPW) *R. ferrugineus* (Oliver) was recorded for the first time in the Arabian Peninsula in the early 1980s as a serious pest attacking and causing considerable damage to date plantations. (Gush, 1997; Abraham et al., 1998). The infestation begins with females using their rostrum to bore holes inside succulent date tissues, followed by ovipositing eggs. Hatching neonates burrow and feed extensively inside the internal parts of the palm tree. The larval stage, which lasts for up to 120 days, is responsible for the majority of the damage. Larval infestation remains concealed except in the late stages when sticky sap oozes outside the attacked regions of the trunk. Due to extensive larval feeding inside the date trunk and also due to secondary bacterial and fungal infections, death of the tissues of the main trunk occurs, and this is followed by eventual collapse of the infested tree. Due to the concealed nature of the attack and damage, control measures have relied mainly on the irrational use of chemical insecticides but this treatment has met with only limited success. However due to the undesirable effect of chemicals on human health and the environment, there is a need for effective and safe alternative approaches, including biological control agents such as entomopathogenic nematodes. The Biological Control Project of the Red Palm Weevil executed by the Arab Organisation for Agricultural Development aims to adopt the use of a successful and safer control strategy which involves the integration of several biological agents such as entomopathogenic nematodes, fungi and pheromone/kairomone traps.

The entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are among the most promising biocontrol agents of insects and, in particular, for those which live in cryptic habitat (Poinar, 1990). The third infective juveniles of these nematodes (IJs) actively search for their hosts, penetrate and kill them via a highly specialised mechanism. The death of infected insects is caused by the effect of the symbiotic bacteria released in the haemocoel of these insects a few hours after penetration.

This paper reports the findings of studies performed with the objective of assessing the potential and efficacy of UAE local isolates of entomopathogenic nematodes *H. indicus* (Elawad et al., 2006) against the adults of Red Palm Weevil.

Proc. III[rd] IC on Date Palm
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MATERIALS AND METHODS

Laboratory Assessments

The following experiments were designed and conducted with the objective of assessing the infectivity of several entomopathogenic nematode isolates against adults of RPW in the laboratory.

1. Dose Response Experiments. Dose response assessment is robust and very efficient in the determination of the pathogenic ability of nematode strains against specific hosts. The purpose of this experiment was to obtain a mathematical correlation between the different doses of IJs and the mean number of IJs penetrated and established inside the infected insect hosts (Fan and Hominick, 1991; Westerman and Stapel, 1992). The experiment was performed on small plastic plates of 4cm diameter. Two local UAE isolates of *Heterorhabditis indicus,* Deba isolate and Dedna isolate (Elawad et al., 2006), were compared with other known species of entomopathogenic nematodes, *Steinernema riobrave* and *Steinernema abbasi*. The infection arena used was fine sand with 14% moisture content (W/V). For each nematode isolate, five treatments of different doses of infective juveniles (IJs) (50, 100, 200, 400 and 800) were used. Adults of the Red Palm Weevil were introduced individually to each plastic plate after addition of the nematode suspensions containing specific doses. Each treatment was replicated four times. All plates were kept in the dark inside an incubator fixed at 30ºC for 60h. Assessment of the results was performed by counting the number of infective juveniles which penetrated the weevils at each dose. Regression lines resulted from the relationship between doses of IJs and the mean number of IJs established was analyzed and compared using GLM in the SAS computer program.

2. Reproduction Capability inside the Red Palm Weevil Adults. The reproductive capability of an entomopathogenic nematode strain refers to its ability to reproduce inside specific insect hosts. This assay was found to be efficient in assessing the infectivity of nematode strains against insect pests (Elawad et al., 2001). Infection of adult weevils was similar to the previous experiment except that a single dose of 400 IJs was used to infect each weevil. Two local UAE isolates of *H. indicus*, Deba isolate and Dedna isolate (Elawad et al., 2006), were compared with other known species of entomopathogenic nematodes, *S. riobrave* and *S. abbasi*. Each treatment was replicated five times. Six days after infection with nematode strains, dead weevils were transferred individually to White Traps (White, 1927). Emerging juveniles were collected and counted over a period of one week. Assessments and comparisons of the mean number of IJs that emerged for each strain were performed using ANOVA in MINITAB computer program.

3. **LT_{50} Assay.** LT_{50} test is an important test to assess how rapidly different strains of entomopathogenic nematodes can kill insect hosts. In this experiment, forty adult Red Palm Weevils were infected individually with one local UAE isolate of *H. indicus* (Deba isolate) (Elawad et al., 2006). Two other known species of entomopathogenic nematodes, *S. riobrave* and *S. abbasi* were used for comparison purposes. Infection was performed in small plastic plates as described previously and a single dose of 400 IJs from each nematode strain was used. The number of dead insects was counted and recorded every two hours for each nematode strain till all weevils had died. Reason for weevil deaths was confirmed by dissecting the dead weevils after an incubation period of three days. LT_{50} values were computed using GENSTAT computer program.

Field Assessments

A preliminary investigation was performed to assess the effect of applying UAE local isolate of *H. indicus* in date fields against adults of Red Palm Weevil. The trial was performed in two neighboring date fields in UAE. The trial involved applications of two successive spray applications of nematode suspensions, one month apart, to target the adult stages of the weevil. A novel application procedure, using targeted spraying of nematode suspensions onto date trunks and the soil around the main trunk, was used (Elawad et al., unpublished data). Four litres of nematode suspension was applied to each

infected date tree. Each dose of the applied nematode suspension contained 4 million IJs. The application was synchronized with the infestation peak of the weevil. The first application was in mid March while the second treatment was applied in mid April. Assessments and monitoring of the number of weevils before and after application of nematode suspension in both the treated and control fields were recorded by counting the number of weevils captured by Pheromones/kairomone traps installed at the farms. Decline in the population of Red Palm Weevil in the treated field was assessed using Henderson's formula (Henderson and Tilton, 1955).

RESULTS

Dose Response Experiments

Nematode isolates assessed by dose response have shown different infection rates to the Red Palm Weevil. A significant difference in the regression lines was obtained for each nematode isolate ($P \leq 0.001\%$). The nematode isolate which showed high virulence against the Red Palm Weevils was *H. indicus* (Deba isolate) followed by *H. indicus* (Dedna isolate) then *S. riobrave* and *S. abbasi* (Figure 1).

Reproduction Capability inside the Red Palm Weevil Adults

Statistical analysis showed a significant effect in the number of IJs produced from each nematode strain ($P \leq 0.05\%$). The highest number of nematode infective juveniles was recorded by the nematode *H. indicus* (Deba isolate) which indicates why it had a powerful virulence against the adults of the Red Palm Weevil, while the least number of infective juveniles was produced by *S. abbasi* (Fig. 2).

LT_{50} Assay

The shortest time needed to kill adults of Red Palm Weevil was recorded for the nematode *H. indicus* (Deba isolate) followed by *S. riobrave* then *S. abbasi* (Table 1).

Field Assessment Results

Decline in the Red Palm Weevil percentage in the treated field was assessed using Henderson's formula (Henderson and Tilton, 1955) as follows:

$$\% \text{ Decline} = 1 - \frac{C1xT2}{C2xT1} x100$$

Where:
C1 = Mean no. of weevils collected from control before application of nematodes
C2 = Mean no. of weevils collected from control after application of nematodes
T1 = Mean no. of weevils collected from the treated field before application
T2 = Mean no of weevils collected from the treated field after application

Percentage decline of weevils in the treated field one month after the first application of the nematode suspension: % Decline = 12.76%

Percentage decline of weevils in the treated field one month after the second application of the nematode suspension: % Decline = 42.40%

Figures 3 and 4 below show the mean number of weevils captured in the treated field after the first and second applications.

DISCUSSION

All results of this research have clearly indicated that UAE (Deba isolate) of the entomopathogenic nematode *H. indicus* possess great pathogenic capability to infect and kill adults of the Red Palm Weevil. The application of nematode suspensions in the treated field was conducted by a novel method of targeted application. (Elawad, unpublished data). The development of this method of application has relied mainly on the behavior of the Red Palm Weevil at the peak period of infestation. The strategy developed in this method involves application of enough of the nematode suspension to

target Red Palm Weevil in hiding zones either under the bases of old leaves or in soils surrounding the base of the main trunk. During sampling of nematode infected weevils we recovered several pupae which had been infected by *H. indicus* nematodes. This observation indicates that IJs of *H. indicus* (Deba isolate) nematode were able to move and penetrate the pupae inside the cocoon, located at sites very close to the peripherals of the main trunk. The decline in insect population recorded after the first application was relatively low. However a greater decline in insect population was recorded after the second application. The greater decline of weevils after the second application could be attributed to the cumulative number of IJs from the two successive applications. Further investigations may be needed to confirm this hypothesis. We strongly recommend the adoption of two successive applications of targeted sprays of nematode suspensions during the peak period of infestation of RPWs as an effective strategy in combating the Red Palm Weevil in date fields.

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Tables

Table 1. LT_{50} values for three nematode isolates against the red palm weevil.

Nematode Isolate	Value LT_{50}
Heterorhabditis indicus (Deba isolate)	19.8 hr
Steinernema riobrave	28.0 hr
Steinernema abbasi	35.2 hr

Figures

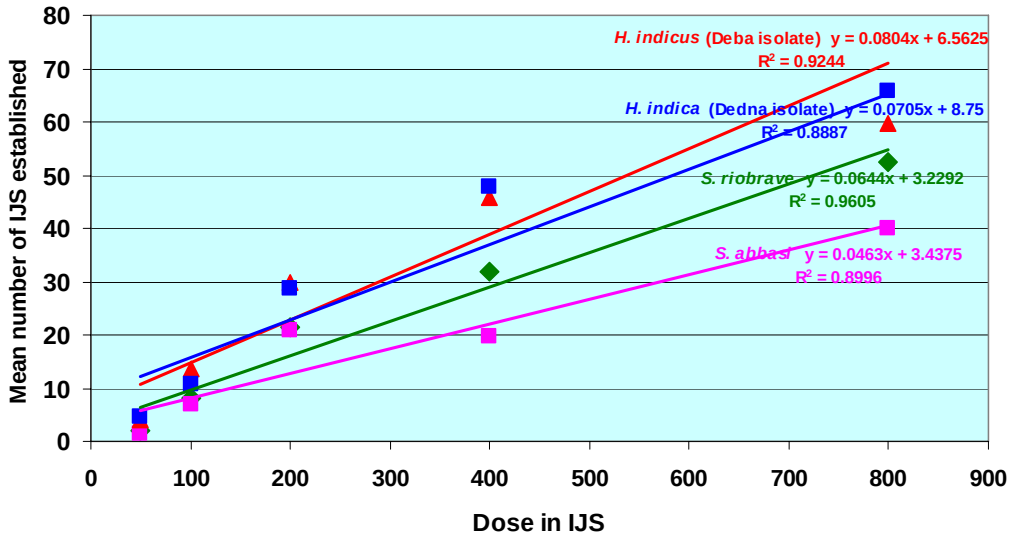

Fig. 1. The establishment of 4 isolates of EPNS inside adults of the RPM.

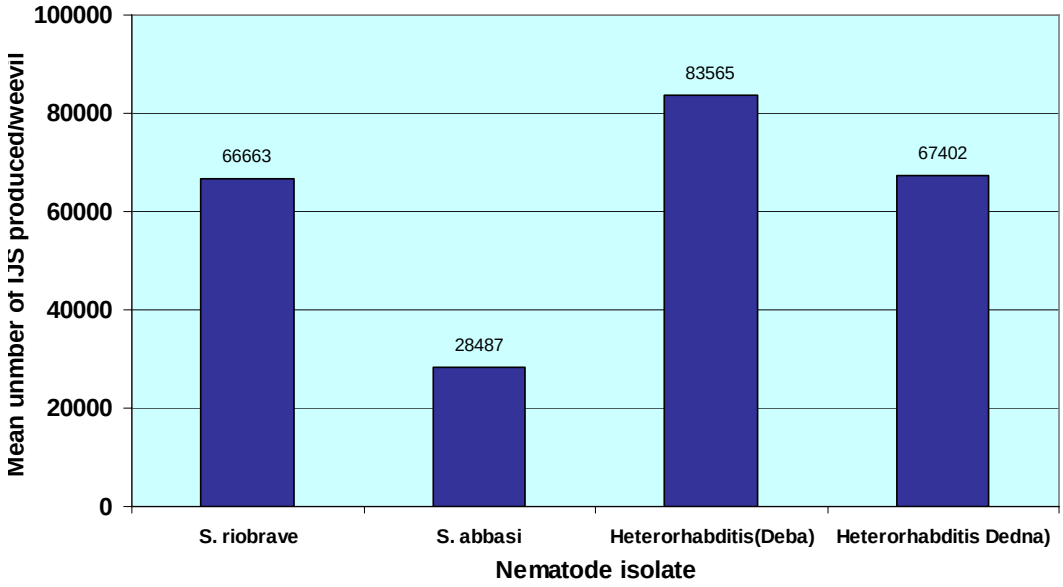

Fig. 2. Reproduction of 4 isolates of EPNS inside adults of RPW.

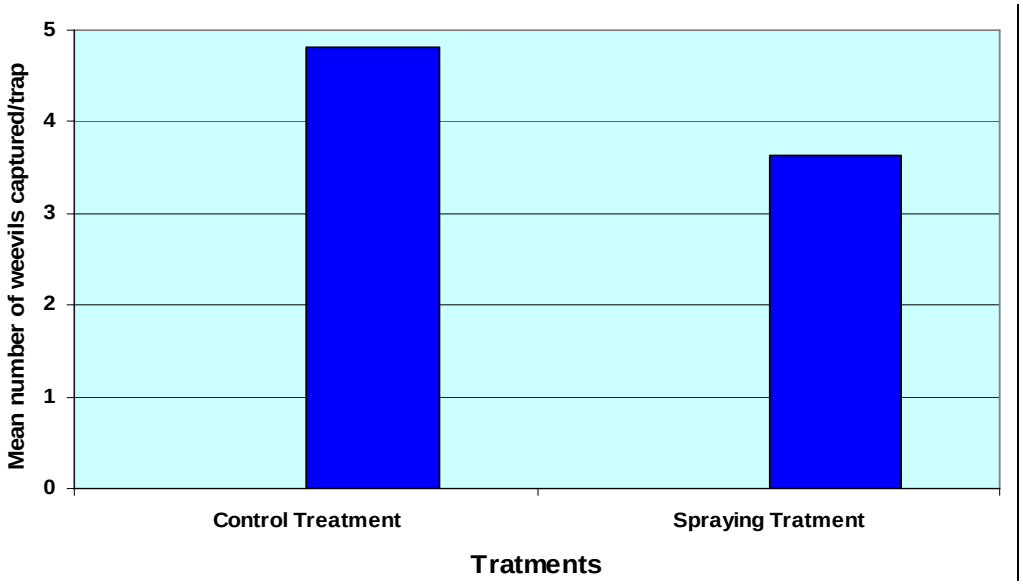

Fig. 3. Mean number of weevils captured/trap one month after the first spraying treatment of nematode suspension.

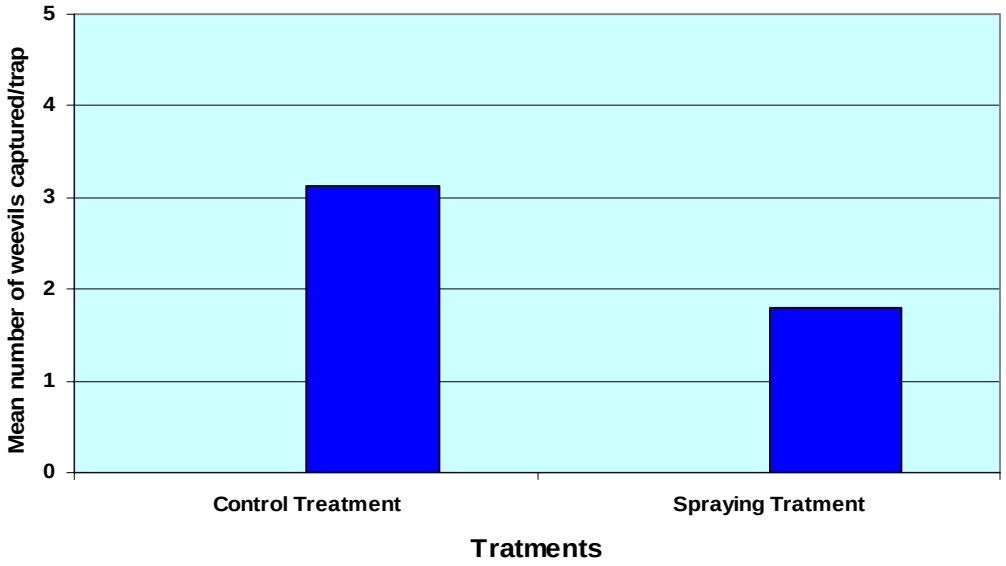

Fig. 4. Mean number of weevils captured/trap one month after the second spraying treatment of nematode suspension.

Distribution and Pathogenesis of Date Palm Fungi in Egypt

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Keywords: geographic spreading, wounding, *Thielaviopsis*, *Diplodia*, *Botryodiplodia*, *Fusarium*

Abstract

Twenty one fungal species belonging to fifteen genera were isolated from diseased date palm samples collected from different Egyptian localities. *Thielaviopsis paradoxa* was the most prevalent fungus followed by *G. phoenicis, D. phoenicum, B. theobromae* and *F. oxysporum*. *Alternaria alternata, F. solani* and *F. moniliforme* had moderate frequency percentages. *Fusarium equiseti, Phomopsis* sp., *Phoma* sp., *Mycosphaerella* sp., *Chaetomium* sp., *Gliocladium* sp., *Omphalia* sp., *Chaetosphaeropsis* sp., *Mauginiella* sp. and *F. semitectum* had low frequency percentages. The lowest frequency was recorded for *Paecilomyces* sp. Some date palm pathogens were previously recorded in Egypt, such as *G. phoenicis, Diplodia* sp., *T. paradoxa, Phomopsis* sp., *M. scaettae, Chaetosphaeropsis* sp., *Botryodiplodia* sp. and *Chaetomium* sp., while the isolated species of *Fusarium, Alternaria, Mycosphaerella, Omphalia* and *Phoma* were recorded for the first time in Egypt as fungal pathogens of date palm trees. The fungal genera isolated from diseased date palms were attributed to the isolation sites on date palm tree, i.e. root, trunk, leaf or inflorescence. *Diplodia, Fusarium* and *Thielaviopsis* were isolated from all diseased roots, trunks and leaves of date palm. On the other hand, *Phomopsis, Omphalia, Chaetomium* and *Paecilomyces* were also isolated from the roots. *Diplodia, Fusarium, Gliocladium* and *Thielaviopsis* was isolated from diseased trunks of date palm. Diseased leaf samples of date palm exhibited 11 fungal genera, i.e. *Alternaria, Botryodiplodia, Chaetosphaeropsis, Diplodia, Fusarium, Graphiola, Gliocladium, Mycosphaerella, Phoma, Phomopsis* and *Thielaviopsis*. Rotted-root samples of date palm exhibited 8 fungal genera, i.e. *Chaetomium, Diplodia, Fusarium, Gliocladium, Omphalia, Paecilomyces, Phomopsis* and *Thielaviopsis*. However, rotted inflorescences yielded only *Mauginiella scaettae*. A study of the geographical distribution of date palm fungi in Egypt proved that the prevalence of each one was affected by environmental conditions. *Thielaviopsis* isolates were present in all examined localities, except the hot areas at New Valley. *Botryodiplodia* isolates were associated with Sharkia, Ismailia and Behaira. The causal agent of false smut disease and Gliocladium leaf spot were found at Damietta and North Sinai which had experienced rainy weather. *Diplodia* isolates were obtained only from samples from Behaira. The other leaf spot fungi, i.e. *Altrenaria, Mycosphaerella, Chaetosphaeropsis, Phoma* and *Phomopsis,* were associated with the conditions of the Sahara climate. The tested fungi varied in their dependence on wounds for entry to host tissues. *Phomopsis* sp., *Chaetosphaeropsis* sp., *Mycosphaerella* sp., *Gliocladium* sp. and *Phoma* sp. formed some limited brown lesions on the midrib of date palm leaflets at puncture sites. It was suggested that these fungi were wound parasites. In the case of *Alternaria* sp., *Thielaviopsis* sp., *Diplodia* sp. and *Botryodiplodia* sp., wounds were not always necessary as a host entrance for these fungi. The second bioassay of detached roots was designed to measure peroxidase enzyme activity as an indicator of successful root infection. Date palm root tissues infected with *Omphalia* sp., *Chaetomium* sp. and *Paecilomyces* sp. exhibited high levels of peroxidase enzyme activity in punctured tissues only. This means that these fungal isolates are wound parasites. By contrast, roots infected with *Fusarium* spp. exhibited high levels of peroxidase activity in punctured and unpunctured tissues. This means that wounds are not necessary for *Fusarium* infection.

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INTRODUCTION

A review of the recording of date palm pathogenic fungi in Egyptian, reveals that *Graphiola phoenicis* (false smut disease) was recorded as early as 1925 by Briton-Jones and Fawcett (1931). Further studies on false smut disease in Egypt were carried out by Gafar et al. (1979) and Soleman et al. (1985).

Thielaviopsis paradoxa (black scorch disease) was recorded by Klotz (1931). Brown and Bahgat (1938) observed the perfect stage of *T. paradoxa* (*Ceratocystis paradoxa*), however Gafar et al. (1978) reported that this fungus was normally present on Egyptian palms only in the imperfect stage.

Anthostomella molleriana (brown striate discoloration) and *Gloeosporium* sp. (anthracnose disease) were recorded by Fawcett in 1931.

Mauginiella scaettae (Khamedj disease) was recorded on Sinai's palms and studied by Sabet and Michael (1965), Michael (1969), Michael and Sabet (1970) and Badawy and Abd El-Al (1982).

The occurrence of *Chaetosphaeropsis* sp. (black leaf spot disease) was recorded by Mostafa et al. in 1971.

In addition, Melchers (1931) recorded four rare fungal isolates, i.e. *Coniothecium heterosporium, Clasterosporium lindavianum, Phyllosticta palmarum* and *Ustilago phoenicis.*

From available world review, date palm leaves that wilt and die-back are infected with *Fusarium oxysporum* f. sp. *albedinis* (Brochard and Dubost, 1969; Feather et al., 1989) and *Gliocladium vermoeseni* (Hahn and Nussboum, 1988). Decayed date palm leaf-stalks are infected with *Diplodia phoenicum* (Fawcett, 1930). Rooted date palm spathes are infected with *F. solani* and *F. moniliforme* (Al-Roubaie et al., 1987) and necrotic date palm tops are infected with *Botryodiplodia theobromae* (Brun and Laville, 1965). Declined date palm roots are infected with *Fusarium oxysporum* (Djerbi et al., 1986), *F. solani*, *F. moniliforme* (El-Arosi et al., 1983) and with *Thielaviopsis paradoxa* (Abbas et al., 1989). The attack on date palm leaf bases of old trees by Thielaviopsis, Omphalia, Fomes or *Phomopsis* is preliminary to rotted heart and root (Streets, 1933; Bliss, 1944; Sharif and Wajih, 1982; Abbas et al., 1989).

The thickness and toughness of the outer wall of epidermal cells of palms are apparently important factors in its resistance to certain pathogens (Cochrane, 1958). Fawcett and Klotz (1932) reported that wounding is the usual means of introduction for many date palm fungal infections. The wounds made in pruning of leaf-stalks (Fawcett, 1930), feeding of insects (Lindgren et al., 1948), invasion of nematodes to roots (Al-Khoury, 1986), cracking of ripening fruits (Rieuf, 1963) and natural wounding of roots (Matheron and Ben-Badis, 1985) provide easy entry for fungi.

MATERIALS AND METHODS

Isolation and Identification

Diseased samples of date palm trees were collected during the active growth period of two seasons. The samples included new and old rachis, terminal buds and roots from different date palm areas in Egypt, i.e. Damietta (Senania), Sharkia (Zagazig), Ismailia (Abo Souir), Monofia (Shibin El-Koum), Fayoum (Sannors), Behaira (Nobaria), New Valley (Dakla and Kharga oases) and North Sinai (Pair El-Abd). The samples were put in plastic bags and kept in a refrigerator until laboratory examination. They were then washed thoroughly with tap water to remove any adhering particles, cut into small pieces, sterilized with 0.1% mercuric solution for 2 mins, washed in sterilized water and dried between two sterilized filter papers.

The surface sterilized pieces were transferred individually to Petri dishes, each containing 20 ml of PDA medium. After 3 days incubation at 30˚C, fungi developed from the cut surfaces, then hyphal tips or single spores were transferred to test tubes containing slant PDA medium. The purified fungi were verified and identified by the author according to Barnett (1960), Subramanian (1971) and Tousson and Nelson (1976).

In order to study the geographical spread of date palm fungi in Egypt, fungal genera isolated from diseased date palm samples from each Egyptian Governorate were documented.

Frequency of fungi isolated from date palm diseased samples was made according to the following equation:

% Frequency = (No. of isolates / each fungus) / Total No. of fungal isolates X100

In order to determine the susceptibility of different date palm organs to infection with the isolated fungi, all identified fungi were attributed to their isolation site on the date palm tree, i.e. root, trunk or terminal bud.

Inoculation of Detached Date Palm Leaflets with Fungal Isolates after Wounding

In order to explain the importance of wounding for successful fungal infection of date palm leaves, excised leaflet bioassay according to El-Sherbeeny and Mohamed (1980) was applied. Freshly excised leaflets of date palm were used. Four small needle punctures were made in each leaflet, and then a droplet of prepared inoculum suspension was applied on each puncture. Other leaflets without mechanical injury had droplets of the same inoculum applied. In each case, control leaflets were treated with distilled water droplets. All leaflets were put in petri dishes in a growth chamber at 25˚C under continuous light from two cool-white fluorescent lamps. Lesion formation within 4-6 days was an indicator of a positive reaction. Induced spotting was determined as follows: - = Not infected, + = Limited spotting, ++ = Extended spotting.

For reaction in date palm roots, the method described by Shrara (1967) was applied. Sterilized-surface root segments (4 cm length) were put on layers of moist paper tissues in sterilized Petri dishes. Four small needle punctures were made in each segment and then a droplet of prepared inoculum suspension was applied on each puncture. Root segments without mechanical injury had the same inoculum applied. In each case, control root segments were treated with distilled water droplets. The root segments were incubated at 25˚C for 72 hrs. The increase of peroxidase enzyme activity/min in the treated leaflet tissues was an indicator of a positive reaction.

RESULTS AND DISCUSSION

Data presented in Table 1 show that twenty-one fungal species belonging to fifteen Genera were isolated from diseased date palm samples collected from different Egyptian localities. *Thielaviopsis paradoxa* was the most prevalent fungus (12.5%) followed by *Graphiola phoenicis, Diplodia phoenicum* and *Botryodiplodia theobromae* and *Fusarium oxysporum* (11.25, 10.00, 8.75 and 7.50%, respectively). *Fusarium solani, F. moniliforme* and *Alternaria alternata* had moderate frequency percentages (6.25, 5.0 and 5.0%, respectively). *Fusarium equiseti, Phomopsis* sp., *Phoma* sp., *Mycosphaerella* sp., *Chaetomium* sp., *Gliocladium* sp., *Omphalia* sp., *Chaetosphaeropsis* sp., *Mauginiella scaettae* and *F. semitectum* had low frequency percentages (3.75-2.50%). The lowest frequency was recorded with *Paecilomyces* sp. (1.25%).

Some date palm pathogens were previously recorded in Egypt, such as *Graphiola phoenicis* (Briton-Jones, 1925), *Diplodia* sp. (Fawcett, 1930), *T. paradoxa* (Fawcett, 1931), *Phomopsis* sp. (Melchers, 1931), *Mauginiella scaettae* (Sabet and Michael, 1965), *Chaetosphaeropsis* sp. (Mostafa et al., 1971), *Botryodiplodia* sp. and *Chaetomium* sp. (Rashed, 1991).

Species of *Fusarium* (*oxysporum, solani, semitectum, equiseti* and *moniliforme*), *Alternaria* sp., *Mycosphaerella* sp., *Omphalia* sp. and *Phoma* sp. were recorded for the first time in Egypt as fungal pathogens of date palm trees.

The fungal genera isolated from diseased date palm were attributed to the isolation sites on date palm tree, i.e. root, trunk, leaf or inflorescence as shown in Table 2. *Fusarium, Diplodia* and *Thielaviopsis* were isolated from all diseased roots, trunks and leaves of date palms. *Phomopsis, Omphalia, Chaetomium* and *Paecilomyces* were also isolated from the roots. This agreed with the findings of Bliss (1944), Lambe and Wills (1975), Abbas et al. (1989) and Rashed (1991). *Alternaria, Botryodiplodia,*

Chaetosphaeropsis, Graphiola, Gliocladium, Mycosphaerella, Phoma and *Phomopsis* were also isolated from the leaves. This agreed with Mostafa et al. (1971), Soleman et al. (1985) and Hahn and Nussboum (1988).

On the other hand, *Gliocladium* was also isolated from diseased trunk of date palm. This concurred with the findings of Feather et al. (1989). Only *Mauginiella scaettae* was isolated from rotted inflorescences. This agreed with the findings of Sabet and Michael (1965) who studied the biology of kamedj disease in Sinai.

Studying the geographical spread of date palm fungi in eight Egyptian Governorates during two seasons showed that the prevalence of each one was affected by environmental conditions. Data presented in Table 3 show that *Thielaviopsis* isolates were present in all examined localities, except the hot areas at New Valley. This agreed with Klotz and Fawcett (1932) and Nambiar et al. (1986) who stated that enhanced decay due to *T. paradoxa* may be linked to the conditions of high humidity and comparatively moderate temperatures. *Botryodiplodia* isolates were associated with Sharkia, Ismailia and Behaira. This agreed with Brun and Laville (1965) who found that *B. theobromae* leaf spot was encouraged under drought conditions. The causal agent of false smut disease (*G. phoenicis*) and Gliocladium leaf spot (*G. vermoeseni*) were found at the locations of Damietta and North Sinai, which are rainy. This somewhat fits with Fawcett (1931) who reported that Graphiola leaf spot was the most common disease on date palm trees in Egypt, especially in the Delta and at Fayoum. *Diplodia* isolates were obtained from Behaira only. This agreed with Waked (1973) who recorded *Diplodia* spp. at Rashied and Aswan but not at Egyptian oases. The other leaf spot fungi, i.e. *Altrenaria, Mycosphaerella, Chaetosphaeropsis, Phoma* and *Phomopsis* were associated with the conditions of the Sahara climate. This result was confirmed by Munier (1955). Fusarium isolates were obtained from leaf samples collected from North Sinai only.

Rotted-root samples of date palm exhibited 8 fungal genera, i.e. *Chaetomium, Diplodia, Fusarium, Gliocladium, Omphalia, Paecilomyces, Phomopsis* and *Thielaviopsis*. The Agaric fungus, *Omphalia* sp. was isolated from Behaira (Nobaria) only for the first time in Egypt. Bliss (1944) reported that rapid Omphalia infection occurred in soil at 24-28˚C in a pH range of 5.1 to 9.7. However, decayed inflorescences yielded only *Mauginiella scaettae*.

Data presented in Table 4 indicate that the tested fungi varied in their dependence on wounds as an entry site to host tissues. *Phomopsis* sp., *Chaetosphaeropsis* sp., *Mycosphaerella* sp., *Gliocladium* sp. and *Phoma* sp. formed some limited brown lesions on the midrib of date palm leaflets at puncture sites. It was suggested that these fungi were wound parasites. This result was confirmed by results of Klotz (1931), Mostafa et al. (1971), Feather et al. (1979) and Vessey (1981).

In the case of *Alternaria alternata, Thielaviopsis paradoxa, Diplodia phoenicum* and *Botryodiplodia theobromae*, there were some unlimited brown lesions on punched and unpunched midribs of date palm. It was suggested that wounds contributed to infection with these fungi but were not always necessary for entrance. This result was evident in the investigations of Fawcett (1930), Streets (1933), Sheir et al. (1982) and Matheron and Ben-Badis (1985).

The second bioassay of detached roots was designed to measure the peroxidase enzyme activity as an indicator of successful root infection as shown in Table 5. Date palm root tissues infected with *Omphalia* sp., *Chaetomium* sp. and *Paecilomyces* sp. exhibited high levels of peroxidase enzyme activity in punched tissues only. This means that these fungal isolates are wound parisites. This is in agreement with the findings of Bliss (1943), Lambe and Wills (1975) and Rashed (1991). By contrast, roots infected with Fusarium spp. exhibited high levels of peroxidase activity in both punched and unpunched tissues. This means that wounds were not necessary for infection by *Fusarium*.

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Tables

Table 1. Frequency of fungi isolated from diseased date palms in Egypt during two seasons.

Isolated Fungus	No. of Isolates	% Frequency
Alternaria alternata	4	5.00
Alternaria sp.	2	2.50
Botryodiplodia theobromae	7	8.75
Chaetomium sp.	2	2.50
Chaetosphaeropsis sp.	2	2.50
Diplodia phoenicum	8	10.00
Fusarium equiseti	3	3.75
Fusarium moniliforme	4	5.00
Fusarium semitectum	2	2.50
Fusarium solani	5	6.25
Fusarium oxysporum	6	7.50
Fusarium sp.	2	2.50
Gliocladium sp.	2	2.50
Graphiola phoenicis	9	11.25
Mauginiella scaettae	2	2.50
Mycosphaerella sp.	2	2.50
Omphalia sp.	2	2.50
Paecilomyces sp.	1	1.25
Phoma sp.	2	2.50
Phomopsis sp.	3	3.75
Thielaviopsis paradoxa	10	12.50
Total	80	100

Table 2. The fungal isolates attributed to different sites of diseased date palms during two seasons.

Isolated fungus	Diseased date palm sample			
	Root	Trunk	Leaf	Inflorescence
Alternaria alternata	-	-	+	-
Altenaria sp.	-	-	+	+
Botryodiplodia theobromae	-	-	+	-
Chaetomium sp.	+	-	-	-
Chaetosphaeropsis sp.	-	-	+	-
Diplodia phoenicum	+	+	+	-
Fusarium equiseti	+	+	+	-
Fusarium moniliforme	+	+	+	-
Fusarium semitectum	+	+	+	-
Fusarium solani	+	+	+	-
Fusarium oxysporum	+	+	+	-
Fusarium sp.	+	+	+	+
Gliocladium sp.	-	+	+	-
Graphiola phoenicis	-	-	+	-
Mauginiella scaettae	-	-	-	+
Mycosphaerella sp.	-	-	+	-
Omphalia sp.	+	-	-	-
Paecilomyces sp.	+	-	-	-
Phoma sp.	-	-	+	-
Phomopsis sp.	+	-	+	-
Thielaviopsis paradoxa	+	+	+	-

+ = Present, - = Absent.

Table 3. Fungal genera isolated from date palm samples collected from some Egyptian Governorates during two seasons.

Isolated fungus	**Egyptian Governorate**							
	Damietta	Sharkia	Ismailia	Monofia	Fayoum	Behaira	New Valley	North Sinai
Alternaria alternata	-	-	+	-	+	-	+	-
Alternaria sp.	-	-	+	-	+	-	+	-
Botryodiplodia theobromae	-	+	+	-	-	+	-	-
Chaetomium sp.	-	-	-	+	-	-	-	-
Chaetosphaeropsis sp.	-	-	-	-	-	+	-	-
Diplodia phoenicum	-	-	-	-	-	-	-	-
Fusarium equiseti	-	+	-	-	-	-	-	-
Fusarium moniliforme	-	-	-	-	-	-	-	+
Fusarium semitectum	-	-	-	-	-	+	-	-
Fusarium solani	-	-	+	-	-	-	-	-
Fusarium oxysporum	+	-	-	-	-	-	-	-
Fusarium sp.	-	-	-	-	-	-	-	+
Gliocladium sp.	+	-	-	-	-	-	-	+
Graphiola phoenicis	+	-	-	-	-	-	-	+
Mauginiella scaettae	+	-	+	-	-	+	+	-
Mycosphaerella sp.	-	-	-	-	-	-	+	-
Omphalia sp.	-	-	-	-	-	+	-	-
Paecilomyces sp.	-	+	-	-	-	-	-	-
Phoma sp.	-	-	-	-	-	-	+	-
Phomopsis sp.	-	-	-	-	-	-	+	-
Thielaviopsis paradoxa	+	+	+	+	+	+	-	-

Table 4. Reaction (as a streaking size) on detached date palm leaves to infection with the isolated fungi after puncture or no puncture of the tissues.

Fungal inoculum	Punched tissue	Punched Tissue
Alternaria alternata	++	++
Alternaria sp.	-	-
Botryodiplodia theobromae	++	++
Chaetosphaeropsis sp.	+	-
Diplodia phoenicum	++	++
Gliocladium sp.	+	-
Mycosphaerella sp.	+	-
Phoma sp.	+	-
Phomopsis sp.	+	-
Thielaviopsis paradoxa	++	++
Control	-	-

-=Not infected, += Infected with limited streak, ++= Infected with not limited streak.

Table 5. Reaction (as a peroxidase enzyme activity/min.) in detached date palm roots to infection with the isolated fungi after puncture or no puncture of the tissues.

Fungal inoculum	Punched tissue	Not punched tissue
Fusarium oxysporum	2.65	2.61
Fusarium solani	2.84	2.81
Fusarium semitectum	2.62	2.58
Fusarium equiseti	2.58	2.53
Fusarium moniliforme	2.54	2.50
Omphalia sp.	1.82	0.42
Chaetomium sp.	1.73	1.44
Paecilomyces sp.	1.41	0.44
Control	0.43	0.40

Study on Possible Influence of Pathogenic Fungi on Date Bunch Fading Disorder in Iran

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Keywords: Date palm, fruit wilt, toxigenic fungi, environmental stresses

Abstract

Date palm (*Phoenix dactylifera* L.) is one of the most important commercial fruit trees growing in about 220,000 hectares of Iran, especially in six southern provinces. In accordance with recent investigations, there are more than 400 cultivars in date palm plantations of Iran (Karampour, 2002; Mirzaei et al., 2001). Date bunch fading disorder (DBF) is the most harmful phenomenon that damages both the quality and quantity of date yield. During the last 5 years this disorder has caused wilting and drying of bunches and finally severe defoliation of date fruits in southern Iran, especially on commercial cultivars in khelal-rutab stages. This investigation was carried out during 2002–2004, starting with the collection of more than 150 samples of vegetative and generative tissues of affected trees from Hormozgan and from several areas of south Kerman Province. Samples were collected, cut into 3–5 mm pieces, surface-sterilized in 1% hypochlorite for 2–3 minutes, then placed on potato dextrose agar (PDA) containing 1 ml/L lactic acid (25% concentration) and incubated at 26 ±1ºC for one week. The fungi *Aspergillus niger, A. flavus, A.* sp., *Alternaria* sp., *Trichoderma* sp., *Penicillium* spp. *Thielaviopsis paradoxa, Fusarium* sp. and *Rhizoctonia* sp. were isolated. All fungi obtained from infected generative tissues such as pedicels, strands and peduncles exclusively, were purified and identified as associated fungi based on morphological and growth characters. The asexual stage of *Ceratocystis paradoxa* was isolated from more than 50% of infected vegetative samples. This fungus was identified by the production of two types of spores; the dark cylindrical microconidia which formed endogenously in uniseriate chains, and ovate macroconidia measuring 11–16 × 8–15 µm. Pathogenicity tests were carried out under laboratory conditions (T: 35 ±5°C, Rh: 25 ±5%) on the cut bunches (in vitro) and under natural conditions in the orchard in a RCB statistical design on susceptible cultivar 'Mordaseng', during August – September of two years. Among nine species of associated fungi, the thermophillic fungus *Thielaviopsis paradoxa* produced symptoms similar to DBF disorder, both in vitro and in vivo. Also two species of *Aspergillus* spp. produced necrotic longitudinal lesions similar to DBF disorder on the cut peduncles under in vitro conditions only. It is likely that with date palm, *T. paradoxa* is an opportunistic secondary pathogen attacking stressed trees (in this case, the affected trees were growing under conditions of severe drought, hot winds and salinity. *T. paradoxa* which also causes terminal bud rot, black scorch and bending head diseases on date palms in southern Iran (Karampour et al., 2002), is a widespread fungus and its pathogenicity to date palm is well documented, especially in areas where drought and salinity are prevailing (Abbas et al., 2003; Carpenter et al., 1966; Elmer et al., 1968; Karampour et al., 2002; Ploetz et al., 2003). Date bunch fading disorder (DBF) has not been observed and reported in the world until now. This is the first report of DBF disorder from Iran and the world.

INTRODUCTION

The date bunch fading disorder (DBF), which is also known as date palm bunch fading, was first reported in 1997 in the south of Kerman province (Iran) on the 'Mozafti' cultivar. In the last 5 to 6 years, this disorder has been the most harmful phenomenon on

date yields in the plantations of southern Iran. Reports of the amount of damage at different regions and in different years have varied from 0 to 80 percent loss of yield, but the mean has been 30–50% (Karampour, 1999, 2002; Karimipour et al., 2002; Mirzaei et al., 2001). The worst DBF damage happened during Khelal to Rutab stages, which occur in the limited period of mid-July until early September (Karampour, 1999, 2002, 2005; Poozesh et al., 2004; Rahkhodaei, 2004). The incidence of DBF in mid-ripened cultivars, which are important commercial dates, is usually high, but its severity in the early-ripening and late-ripening cultivars is very low (Karampour, 1999, 2002; Poozesh et al., 2004), (Figs. 1–5). The disorder does not show any symptoms and/or infectious appearance in vegetative growth of date palm trees such as leaflets, leaves, trunks, root systems, crown, buds, shoots and also in young inflorescences and spathes (Karampour, 1999, 2002; Karimipour et al., 2002; Poozesh et al., 2004), (Figs. 7 and 8). This disorder occurs only in the generative maturing tissues, starting with light yellow lesions on peduncles (the main stalk of bunch), and gradually developing to longitudinal pale brown strips on the whole peduncle (Figs. 2, 4, 5). Date fruits wilt, usually from the bottom of the strand up, and then the pedicels, peduncles and whole bunch will dry (Figs. 1–3). At the very least the fruits will dry and severely defoliate (Karampour, 1999, 2002; Mirzaei et al., 2001; Pejman et al., 2003; Poozesh et al., 2004). In accordance with past studies and rapid distribution of DBF, the climatic and environmental agents such as hot dry winds and very low relative humidity (high drought) are effective in its occurrence and severity (Karampour, 1999, 2002; Poozesh et al., 2004; Rahkhodaei, 2004; Roosta, 2003; Sarhadi et al., 2004). Although a lot of studies have been carried out in Iran over the last 5 years to found the animate pathogens, there are no any confirmed cases. The Plant Pests and Diseases Research Institute of Iran (PPDRI), started investigations into DBF in 1997(Alavi and Najafinia, 2004). In 1999, the disorder was reported as "wilt and defoliation of date fruits" on ‘Kabkaab’ cultivar from Boushehr province (Karampour, 1999, 2002). The studies carried out in 2000–2001 on damaged tissues of ‘Mozafti’ cultivar in Jiroft and Bam, reported the presence of *Aspergillus* spp., *Bipolaris australiensis* and *Nattrassia mangifera* as associated fungi to DBF. Many researchers in Iran (2001–2004) have reported the relative high suppressive effects of bunch covers on the susceptible date cultivars. They recommended an integrated management program for reducing commonly occurring damage caused by DBF in date palm plantations consisting of different cultural, chemical and nutritional measures such as regular irrigation especially at nights, regular nutrition especially with Ca^{++} and K^{+} compounds, intercropping of annual forage crops especially alfalfa and sorghum, and date protection against common pests and diseases in date palm orchards (Karampour, 2002; Karimipour et al., 2002; Mirzaei et al., 2001; Pejman and Roosta, 2003; Sarhadi and Ghalebi, 2004). Over the past five years (2000–2004), the influence of viruses, viroids, bacteria and phytoplasma agents have been studied by PPDRI researchers in Iran (Alavi and Najafinia, 2004; Azadvar et al., 2003; Farzdfar et al., 2003; Karampour, 2002). The results showed no direct influence or pathogenicity of these group of pathogens on DBF, but the investigation of the indirect effects of their metabolites and toxicities merits more study in the future (Alavi and Najafinia, 2004; Karampour, 2002). with a review of literature, internet searches and communications with honorable African and Arabian date scientists, confirmed that there are no reported diseases or disorders that exhibit symptoms like DBF disorder as it occurs on date palm in Iran. Although many studies have been conducted to find the living, infectious agents of DBF disorder in Iran, there is no confirmed pathogen. Only associated fungi have been isolated from infected tissues. (Alavi and Najafinia, 2004; Karampour, 2002; Karimipour et al., 2002).

This research was conducted to identify the probable fungal influences on DBF disorder and to investigate their pathogenicity.

MATERIALS AND METHODS

Sampling

The samples were obtained from both generative tissues such as infected strands, fruits, pedicels and peduncles and vegetative tissues like root systems, leaves and leaflets. They were put into plastic bags and taken in an ice container to the laboratory. The samples were collected over a period of early summer to early autumn from date palm plantations in Hormozgan regions and in Kerman province. They were collected from cultivated date palms (e.g. Mordaseng, Mozafti, Kariteh, Shahani, Kabkaab, Khassouei, Khanizi and Halili).

Isolation and Purification

1) Preparing infected tissues: 3-5mm pieces were cut from between infected and healthy tissues.
2) Surface sterilization: the external surfaces of prepared pieces were sterilized and cleaned in 1% sodium hypochlorite by flooding the pieces for 2–3 minutes under laminar flow conditions, before being washed in sd water.
3) Culturing: 5 sterilized pieces were placed on each Petri dish containing potato dextrose agar (PDA) media for different times. The experiment was replicated in 3 Petri dishes and incubated at 26 ±1ºC. The isolated fungi grew well under incubator conditions and observations were documented daily.
4) Reculturing and purification; the rings of isolated fungal colonies were separated by cork borer and placed on APDA media (PDA + 25% lactic acid for suppression of growth of bacterial saprophytes). All isolates were then purified by single spore and/or hyphal tip methods.
5) Growth in warm conditions: all new pure cultures of isolated fungi were incubated and grown in a temperature of 30–34ºC, which was close to the climatic conditions of date palm plantations. Cultures were observed and their macroscopic and microscopic characters such as color and diameter of colony, colony shape, sporulation, spore type and abundance were documented everyday.

Pathogenicity Tests

The pathogenicity tests were carried out under two different conditions,
1) Laboratory conditions: a suspension of spores and vegetative propagules of dominant and frequently occurring fungi were prepared from one week old cultures of the same (1×10^6 propagules/ml) in a shaker-incubator with 200 rpm. They were then sprayed onto wounded peduncles and strands of the susceptible date cultivar Mordaseng. The material was taken from healthy palm trees and were wounded with sterilized needles. There were two bunches for each fungus. In addition, there was a control treatment of one bunch of each cultivar, which was sprayed with sterile water only. The bunches were incubated in fluorescent light at 30–34˚C. The treatments were observed two times everyday (morning and evening) and all symptoms and changes were documented. When the external symptoms had been observed, the fungal agents were isolated from infected tissues on APDA media in sterile conditions.
2) Natural conditions: a healthy date palm tree of cv. ‘Mordaseng’ was selected from a date palm plantation in the summer of 2002. There were 6 bunches on the tree, all of which appeared healthy. The experimental tree and surrounding trees had been sprayed with Omite (0.5 ml/L) against main pests, especially *Olygonychus afrasiaticus*, seven days before the pathogenicity test of fungi. At the same time a suspension of spores and vegetative propagules of two dominant fungi (*Thielaviopsis* and *Aspergillus*) were prepared in liquid media (1.5×10^6 propagules/ml), which was obtained from 10 day pure cultures of the same. The suspension of each fungus was sprayed onto the entire external surfaces of peduncles, strands and fruits of two bunches for each fungus. Only sterile water was sprayed in the control treatment without any wounding. The amount of sprayed suspension per treatment was 1 liter/bunch. Documentation was made of daily

observations. Another experiment was carried out in a complete randomized block design (CRBD) with 3 treatments in 4 replications (each tree as a replication) in the summer of 2003 under the same conditions. The treatments were as follows; T_1: fungi suspensions sprayed on entire bunch, T_2: water sprayed on entire bunch, and T_3: control not sprayed with either water or fungi. The experiment was carried out on four date palm trees of cv. 'Mordaseng' at each side of an imaginary amygdale?? shape orchard (north, south, east and west). Each replication was a bunch. At fruit coloring stage (Khelal), 3 bunches were selected on angles of an imaginary triangle, separate from each other, then the bunches were sprayed. The observations were taken at two different times. First observations occurred 7 days after spraying and the second set was taken 7 days later at the change from khelal to rutab stage. All symptoms were documented and infected samples were collected randomly. In each infected bunch, 20 strands were cut (12 bunch × 20 strands = 240 total strands), put in clean plastic bags separately, taken to the laboratory and the following data collected:

1. Total number of fruits on each strand and all fruits in plastic bag (NF),
2. Number of infected fruits per strand that had on wilted, shrivelled or dried (NIF),
3. Percentage of infected fruits per strand (PIF) which was calculated using this formula:

$$pif = \left(\frac{nif}{nf}\right) 100 \text{ and}$$

4. Weight of each strand including all healthy, wilted and dried fruits (GF).

Analysis of variance and comparison of treatment means by Duncan's multiple–ranged test were done. The fungi reisolated from infected tissues of peduncles, strands and pedicels were cultured again on APDA media.

RESULTS

Vegetative Studies

In all cases of cultured vegetative tissues selected from date palms infected with DBF, no living pathogenic agents were isolated. No symptoms or malformations such as chlorosis, necrosis, scorching, yellowing and other discoloration in vegetative tissues were observed. Also was no rotting or other disorders on the root system. Only a few isolated saprophytic soil born fungi such as *Rhizoctonia* sp., *Fusarium* sp., *Trichoderma* sp., *Aspergillus* sp., *Alternaria* sp. and *Penicillium* sp. were obtained from rhizosphere very rarely. ??

Internal Tissues Studies

In the longitudinal section from the trunk and from neck cross sections of an infected 12 years old date palm 'Mordaseng', no abnormalities or symptoms were observed. Also there were no signs or symptoms like rotting, discoloration and necrosis in meristemic tissues (in folk "paneer"), central cylinder tissues and veinal systems. The young spathes and meristemic tissues were entirely white, healthy and normal (Fig. 8). The necrotic brown strips on infected and cracked peduncles of wilted and dried bunches continued longitudinally until they reached 3–5 cm from the end of the peduncle where it attaches to the veinal system (Fig. 7). The fungus *Thielaviopsis* sp. was isolated on APDA from the necrotic end tissue between healthly tissue and the brown infected strip of the peduncle.

Fungi Identification

In accordance with microscopic, morphologic and morphometric studies, the following fungi were isolated on APDA media from different parts of the cracked necrotic strips on the peduncle, from dry pedicels and strands and from internal infected brown tissues of the peduncle: *Thielaviopsis* sp. (more than 53%), *Aspergillus* spp. (17%) and *Alternaria* sp. (about 6% abundance). Also *Aspergillus niger*, *Aspergillus* sp. and a species of Alternaria were isolated with 6%, 14% and 1% abundance, respectively, from infected bunches of Jiroft date palm plantations.

Results of the Pathogenicity Tests

1) Laboratory conditions (in vitro): 6 days after inoculation with Thielaviopsis, the first symptoms appeared as pale brown spots, then a dark brown strip appeared on peduncles. Finally, after 15 days, both the peduncle and strand were dried in 'Mordaseng'. Cv. 'Halili' also displayed these symptoms when Thieleviopsis was applied.?? (but not completely similar to natural conditions). In Aspergillus treatments, only a small number of pale brown spots were observed 7 days after inoculation on the wounded spots of the peduncle. The damage was not severe. In the control treatments, no malformation or discoloration was observed. The original fungi *Thielaviopsis* and *Aspergillus* were reisolated on APDA.

2) Natural conditions (in vivo); In the summer of 2002, 9–12 days after Thielaviopsis inoculation, both bunches showed symptoms of necrosis on the upper surface of peduncles, which then developed into a long brown strip. Peduncles began to dry from 15–17 days after Thielaviopsis inoculation. The fungus Thielaviopsis was reisolated from infected tissues and identified again. The treatments sprayed with Aspergillus suspension showed only mild symptoms, such as a little wilting in fruits, but symptoms were observed on peduncles, strands, pedicels and other tissues.

In none of the cases was soft rot observed. In addition, neither *Thielaviopsis* nor the other fungi and/or pathogens were isolated from vegetative tissues. The results of another pathogenicity test of *Thielavioosis paradoxa* on 'Mordaseng' which was carried out in the summer of 2003 are presented.

In the first documentation (10 Aug. 2003), a necrotic strip on peduncles had begun and a few fruits were shrivelled and wilted. The bunches in treatment 2 (water spray) did not show any symptoms in this stage. In the second documentation (17 Aug. 2003) severe fruit wilting and drying of strands had occurred in treatments 1 and 3, that seemed to develop from the base to the top of strands. A deep brown cracking was observed on peduncles and strands. Bunches in T2 (water spray) showed wilting, drying of strands and fruits and defoliation. There were no symptoms on vegetative tissues. The symptoms and infection occurred on peduncles, pedicels, strands and fruits exclusively. The fungus *Thielaviopsis paradoxa* with diagnostic characteristics were reisolated and identified from necrotic tissues of peduncles. In T3 (fungal suspension sprays) the severity of symptoms was much higher than for other treatments. From T3 *Thielaviopsis paradoxa* alone was obtained, but in T2 and T1, in addition to *Thielaviopsis*, the saprophytic fungi *Aspergillus* were isolated. There were no fungi isolated from fruit tissues in these treatments. The results of analysis of variance and comparison of treatment means showed that there was no statistical difference between all treatments in total fruits on each strand, thus they were placed in same statistical group.

The highest number of fruits/strand was in T3 (23.535) and the lowest was in T2 (19.650). There was a statistical difference between all 3 treatments in number of infected fruits.

In accordance with Duncan's multiple-range test, T1 (control) with mean 5.593 was in statistical class B, T2 (water spray) with mean 3.162 in class C and T3 (fungal suspension spray) with mean 8.030 was in class A. Thus applying a fungal suspension spray of *Thielaviopsis paradoxa* was effective on the number of infected fruits in 'Mordaseng' dates. The relationship among treatments, based on the percentage of infected fruits, was as follows: T2=15.62%>T1=27.58% >T3=34.22%. This relationship showed that water spraying on bunches in date palm plantation conditions from 10th-20th August decreased bunch wilting and fruit drying in 'Mordaseng' dates. This method increased yield weight (36 g/strand) and decreased infection (12%) in comparison to natural conditions. The relationship based on 'weight of strands with their fruits' was as follows: T2=204>T1=168>T3=148. These results showed a statistical difference between T1 and T3.

The final result of these experiments showed that *Thielaviopsis paradoxa* as a pathogen, had an effective role in DBF incidence and an effect on date palm yield quality and quantity.

DISCUSSION

Fungal pathogens are the most important agents of plant diseases in the world, according to their host range, variability and geographical distribution. There are about 25 diseases and disorders affecting date palm worldwide. Among them, 14 are caused by fungi which 4 important cases of them (e.g. inflorescence rot, black scorch, bending head and terminal bud rot) happened by anamorphs of *Ceratocystis* spp. which they are opportunistic anamorphs with a closely association to date palm trees. With the exception of some postharvest fruit rots caused by yeasts, bacteria, and actinomycetes, there are no confirmed bacterial and/or viral diseases on date palm (*Phoenix dactylifera*) in the world (Azadvar et al., 2003; Carpenter, 1966; Karampour, 2002; Mirzaei et al., 2001). Before the occurrence of DBF, the Khamedj disease (*Mauginiella scaetea*), terminal bud rot or TBR (*Thielaviopsis paradoxa*), leaf spots (i.e. *Massariella palmarum*) and the false smut (*Graphiola phoenisis*) were reported as the most important diseases on date palms in Iran (Karampour, 2002). Recent investigations have revealed no investigations into the effective role of viruses, viroids, MLOs and other bacteria in DBF disorder occurrence. *Fusarium eqiseti* and *Ceratocystis radicicola* were isolated from roots of affected date palms in Iran, but the pathogenicity tests of these fungi were not successful (Alavi, 2000). He believes that the causal agents of DBF are both animate and inanimate, especially climatic agents, with different effects and severity. Also *Bipolaris australiensis*, *Aspergillus flavus, A. niger* and *Nattrassia mangifera* were isolated from date bunches as associated fungi to DBF in Kerman province but the pathogenicity tests of them were not proved (Najafi et al., 2004). *Bipolaris australiensis* were reported as agent of peduncle necrosis of date palms in Jiroft, Iran (Ershad et al., 2004). In this investigation, although many fungal species were isolated from affected date palm trees, such as *Alternaria* sp., *Aspergillus* sp., *A. flavus, Penicillum* sp., *Aspergillus niger, Fusarium* sp., *Trichoderma* sp. and lastly *Thielaviopsis paradoxa*, only a few of them caused necrosis strips, brown spots, dry rot and some discoloration on peduncles, pedicels and strands in vitro which were not entirely similar to DBF symptoms in vivo conditions. Among the isolated fungi, *Thielaviopsis paradoxa* had the ability to increase the incidence of DBF disorder on date trees 'Mordaseng' under drought and hot winds stresses in the natural climatic hard conditions of date palm plantations in Hormozgan province exclusively. The results of this investigation and other similar recent studies in Iran, showed that the associated fungal agents had no direct and/or primary role in occurrence of DBF disorder, but the indirect and secondary effects of them, especially the opportunistic anamorphs of *Ceratocystis* spp. like *Thielaviopsis paradoxa* on DBF severity in unfavorable conditions (hot winds, drought stress) require further investigation in the future, as toxigenic agents.

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Tables

Table 1. The results of pathogenicity test of *Thielaviopsis paradoxa* on 'Mordaseng'.

Treatment	Replicate	Number of Fruits/strand (NF)	Number of Infected fruits (NIF)	Percentage of Infected fruits (PIF)%	Strand and fruits weight (GF)g
T_1: Control without fungi	1	21.25	3.37	16.23	147.15
	2	26.70	7.90	28.34	187.35
	3	14.40	3.55	24.36	127.60
	4	18.80	7.55	41.13	185.25
T_2: Water spray	1	17.55	2.70	15.77	196.85
	2	21.50	4.25	19.08	243.00
	3	16.85	2.00	11.66	177.10
	4	22.70	3.70	15.96	198.55
T_3: Fungi suspension spray	1	27.20	8.65	23.94	162.00
	2	24.45	7.95	30.32	137.05
	3	21.44	6.67	32.02	146.83
	4	21.05	8.85	41.61	130.30

Table 2. Analysis of variance of fruits/strand (NF).

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	3	69.243	23.081	2.328	0.147
Treatment	2	34.728	17.346	1.751	0.252
Error	6	59.488	9.915		
Total	11	163.458			

Coefficient of variation: 14.88%

Table 3. Analysis of variance of infected fruits/strand (NIF).

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	3	15.694	5.231	3.683	0.082
Treatment	2	47.358	23.693	16.678	0.004
Error	6	08.523	1.421		
Total	11	71.603			

Coefficient of variation: 21.30%

Table 4. Analysis of variance of total weight of strand and fruits.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Replication	3	1664.577	554.859	0.851	
Treatment	2	6277.758	3138.879	4.813	0.057
Error	6	3912.635	652.106		
Total	11	11854.971			

Coefficient of variation: 14.70%

Figures

1. Beginning of wilting on fruits and strands.
2. Necrosis strip on peduncle of ‘Mozafti’.
3. Wilting on ‘Mordaseng’ bunch.
4. Necrotic brown lesion and deep cracking of end of strands in ‘Kabkaab’.
5. Symptoms of DBF on ‘Kariteh’ bunch.
6. ‘Thielaviopsis’ spores.
7. Limited brown strip on peduncle.
8. No infection on young bunches and spathes of ‘Kabkaab’.

Fruit Set Failure in Tissue Culture-Derived Date Palm Trees (*Phoenix dactylifera* L.) cv. 'Nabt Saif' as Affected by Pollinator Type and Pollination Density

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Keywords: somaclonal variation, pollination, fertilization, sugar palm, fruit quality

Abstract

There is a special interest in date palm plants regenerated by tissue culture. However, one of the major weaknesses of mass tissue culture propagation is the appearance of undesired off-type plants, such as poor fertility and delayed flowering. During the 2004 and 2005 seasons, the effect of five date palm pollinators, namely 'Ahmar', 'Akhdar', 'Fard', 'Khenizy' and 'Maghool' as well as sugar palm (*Phoenix sylvestris*), each with five different pollination densities (10, 20, 30, 40 or 50 strands/female spathe), was studied on fruit set, multi-carpel fruit formation, fruit drop, and fruit quality of eight-year tissue culture derived 'Nabt Saif' palm trees growing at Al-Ain oasis, United Arab Emirates, The results showed different degrees of compatibility among the pollinators on 'Nabt Saif'. The pollinator 'Khenizy' resulted in the highest (34.3% and 28.7%, for 2004 and 2005, respectively) and 'Fard' the lowest (11. 7% and 20 % for 2004 and 2005, respectively) fruit set level. The pollinator 'Sugar palm' (*Phoenix sylvestris*) was as effective as or better than the other pollinators that belong to the species *Phoenix dactlifera*. There were no significant differences between the different pollination densities on fruit set and fruit quality during both seasons. Generally, neither the pollinators nor the pollination density resulted in a satisfactory level of fruit set. These results may suggest that other endogenous factors are responsible for such abnormality in the tissue culture derived 'Nabt Saif' palms. Some metaxenia effects of the different pollinators on fruit quality characteristics were recorded both at Bisir and Tamr stages. In this respect, the 'Sugar palm' pollinator significantly increased fruit and flesh weight, fruit length and diameter and seed weight percentages at Bisir stage, but decreased the concentration of total soluble solids, acidity and vitamin C at both Bisir and Tamr stages compared to other pollinators. More research work is needed on tissue culture derived date palms to understand more clearly the nature of abnormalities that appear in some cultivars.

INTRODUCTION

Date palm is the most successful (and extremely important) subsistence crop in most of the hot arid desert regions (Botes and Zaid, 1999). There is a high demand for offshoots for new plantations but the number of offshoots which are naturally produced by fruiting palms is not sufficient. There is a special interest in date palm plants regenerated by tissue culture since this technique can provide homogenous plants that are true-to-type, free of diseases and can be produced on a large scale (Zaid and de Wet, 1999).

Organogenesis and embryogenesis are the two major techniques currently used worldwide to mass-propagate date palms. However, one of the major weaknesses of mass tissue culture propagation techniques is the appearance of undesired off-type plants caused by somaclonal variation (Zaid and Al-Kaabi, 2003). Such variations can be epigenetic (non-hereditary variation) or genetic (hereditary variation). Thus the production of genetically stable and uniform plants is critically important in determining the efficient use and success of the tissue culture technique. The occurrence of variability

in tissue culture derived plants has been described in several plant species including date palms (Larkin and Scowcroft, 1981; Orton, 1983; Pierik, 1987; Zaid, 1987; Zaid and Al-Kaabi, 2003). Sensitivity differences in the occurrence of abnormalities among date palm cultivars have been observed (Zaid and Al-Kaabi, 2003). They reported that 'Barhee' cultivar was more susceptible to pollination failure and abnormal fruiting than 'Medjool', 'Khalas' and 'Deglet Nour'. Also, 'Sukkari' cultivar was more susceptible to dwarfism (restricted vegetative growth) than 'Khalas', 'Barhee', 'Sultana', 'Nabt Saif' and 'Oum Dahn'. They concluded that the frequency of abnormalities was generally about 5%, with the exception of pollination failure in 'Barhee' cultivar. Such incidence of abnormalities was acceptable to date palm growers. Most of these abnormalities occurred mainly in embryogenesis-derived palms and, to a lesser extent, in organogenesis-derived palms (Zaid and Al-Kaabi, 2003). However, in a survey of abnormalities occurring in tissue culture-derived date palms in United Arab Emirates, it was found that leaf-whitening abnormality occurred at a high rate in 'Nabt Saif' (53%) and 'Sultana' (32%) cultivars regenerated by organogenesis technique (Al-Mazroui, 2005). Fruit set failure (formation of 80 to 100% parthenocarpic fruits in the pollinated bunches) occurred in 'Khalas' cultivar at a rate of 6.2%, 20% and 49% for organogenesis, indirect asexual embryogenesis and direct asexual embryogenesis techniques, respectively. For 'Barhee' cultivar the rates were 2%, 34% and 0.5% for organogenesis, indirect asexual embryogenesis and direct asexual embryogenesis techniques, respectively (Al-Mazroui, 2005). Such abnormalities were also recorded in many orchards around the world, including Saudi Arabia, South Africa, Namibia and UAE (Al-Ghamdi, 1993; McCubbin et al., 2000; Djerbi, 2000; Cohen et al., 2004). Fruit set failure was found in more than 100,000 date palms planted in the early and mid-1990's (Djerbi, 2000). However, there was no evidence to prove that such fruit set failure was based on either change in the DNA or on the pollination process (pollinator type or pollination density) and/or due to environmental factors. It is well known that fruit set percentage as well as fruit quality characteristics are greatly influenced by type of pollinator and pollination density (El-Amer et al., 1993; Krueger, 1998; Moustafa, 2001).

Thus, the aim of this study was to evaluate the effect of pollinator type and pollination density on fruit set, multi-carpel fruit formation, fruit drop and fruit quality of eight-year-old tissue culture derived 'Nabt Saif' palms that were showing severe fruit set failure, in an attempt to understand the nature of such abnormality.

MATERIALS AND METHODS

Plant Materials and Experimental Procedure

During the 2004 and 2005 seasons, thirty palms of tissue culture-derived 'Nabt Saif' (regenerated via an organogenesis technique) cultivated in the 1997 season at the Experimental Farm of the College of Food and Agriculture, UAE University, Al-Fooa, UAE, were selected for this experiment. These palms were similar, growing in a sandy soil 10.0 m apart, drip irrigated, and receiving normal cultural practices. These palms, as well as an additional 25 palms of the same cultivar, has a normal vegetative growth pattern, but they showed severe fruit set failure.

Spathes were selected from five date palm pollinators, namely 'Ahmar', 'Akhdar', 'Fard', 'Khenizy' and 'Maghool', as well as sugar palm (*Phoenix sylvestris*), growing at Al-Kuwaytate Research Center, Department of Agriculture and Livestock, Al-Ain, UAE. Mature spathes were collected from the male palms and transported to the Horticulture Laboratory of the College of Food and Agriculture, UAE University, for air drying of the strands at ambient temperature. On each selected female palm, 4-5 spathes of equal size were selected and labeled before cracking. Each of the selected spathes was hand pollinated with one of the six pollinators at one of the following densities: 10, 20, 30, 40 or 50 strands/female spathe, in a completely randomized design according to procedures outlined by Steel and Torrie (1980). Subsequently, the experiment consisted of 30 treatments, where each treatment was replicated 4 times. The entire experiment contained

a total 120 spathes. The selected spathes were pollinated on the same day of spathe cracking or on the next day. The pollination processes for all spathes were generally completed within 10-days during both seasons (started on 25th of February and 2nd of March for the first and the second season, respectively). After pollination, all spathes were bagged with paper bags, each bag with 8 small holes, to prevent contamination from air or other surrounding pollinating treatments. The spathes remained covered for five weeks after pollination with frequent hand shaking during the first week for effective pollen dispersal.

Fruit Set, Multi-carpel Fruit and Fruit Drop Percentage

Fiftly days after pollination, 20-strands were randomly selected from each bunch. The percentage of fruit set, multi-carpel fruit and fruit drop per bunch were determined by counting the number of normal fruit, multi-carpel fruit and dropped fruit, dividing it by the total number of the twigs on the respective strands per bunch and multiplying by one hundred.

Fruit Quality Measurements

In the 2004 season, at both Bisir and Tamr stages (mature stage), 20 sound fruits per bunch (replicate) for each treatment were picked and immediately transported to the Horticulture Laboratory for quality measurements. Fruit weight, flesh and seed weight were recorded. Total soluble solids (TSS) were measured in fruit juice with a hand refractometer. Titratable acidity was determined in juice by titrating with 0.1N sodium hydroxide in the presence of phenolephthalene as indicator (Ranganna, 1979) and the results were expressed as a percentage of malic acid. Ascorbic acid (vitamin C) was measured according to Ranganna (1979) by the oxidation of ascorbic acid with 2,6-dichlorophenol endophenol dye and the results were expressed as mg/100 ml juice.

Statistical Analysis of Data

All data were analyzed by analysis of variance (ANOVA) using the MSTAT Computer Software (Michigan State University, East Lansing, MI). Comparisons between means were made by *F*-test and the least significant differences (LSD) at 5% level.

RESULTS

Fruit Set, Multi-carpel Fruit and Drop Percentages as Affected by Pollinator Type and Pollination Density

Fifty days after pollination fruit set percentage of the 'Nabt Saif' cultivar was generally lower than the classical off-shoot derived trees (Tables 1 and 2). Fruit set percentage was generally lower in 2004 than in 2005, except for the 'Fard' pollinator which gave the opposite results. Maximum fruit set percentages (34.3% and 28.7%, for 2004 and 2005, respectively) were achieved by the pollinator 'Khenizy' during both seasons. However, the pollinator 'Fard' produced the lowest fruit set percentages (11.7% and 20% for 2004 and 2005, respectively) during both seasons. In this respect, the pollinator 'Sugar palm' (*Phoenix sylvestris*) was as effective as, or higher than, the other pollinators belonging to *dactlifera*. In the 2004 season, the pollinators 'Khenizy' and 'Sugar palm' produced significantly higher percentages of fruit set than all other pollinators (Table 1). In the 2005 season, the pollinators 'Khenizy', 'Maghool' and 'Sugar palm' produced significantly higher percentages of fruit set than all other pollinators (Table 2).

Regarding the percentage of multi-carpel fruit results showed that in the 2004 season, 'Khenizy' produced the highest and 'Akhdar' produced the lowest level. The pollinators 'Khenizy' and 'Sugar palm' produced significantly higher percentages of multi-carpel fruits than 'Maghool' and 'Akhdar' (Table 1). In the season 2005, the pollinators 'Khenizy', 'Maghool' and 'Sugar palm' resulted in significantly higher levels of multi-carpel fruit than all other cultivars (Table 2). The pollinator 'Fard' produced the

lowest level of multi-carpel fruits (Table 2). During both seasons of the study, the pollinator 'Fard' produced a higher level of fruit drop percentage than all the other pollinators during both seasons (Tables 1 and 2). The pollinators 'Ahmar' and 'Akhdar' produced higher percentages of fruit drop than 'Khenizy' and 'Sugar palm'. The pollinator 'Khenizy' resulted in the lowest level of fruit drop that was significantly different than all the other pollinators (Tables 1 and 2).

Considering the effect of pollination density, the application of 20 strands/bunch resulted in the highest level of fruit set, followed by 30 strands/bunch in the 2004 season (Table 1). In the 2005 season, the highest density (50 strands/bunch) resulted in a significantly higher level of fruit set than other treatments (Table 2). There were no significant differences between the first, third, fourth, and fifth pollination densities during both seasons (Tables 1 and 2). The percentage of multi-carpel fruits was not affected by pollination density during both seasons (Tables 1 and 2). In the 2004 season, the second pollination density (20 strands/bunch) resulted in a significantly lower level of fruit drop than other treatments (Table 1). However, in the 2005 season, the highest density (50 strands/bunch) resulted in a significantly lower level of fruit drop than other treatments (Table 2). There were significant interaction effects between pollinator type and pollination density during both seasons (Tables 1 and 2).

Fruit Quality and Metaxenia Effects

The fruit quality of 'Nabt Saif' cultivar at the Bisir stage was significantly affected by the pollinator type in the 2004 season (Table 3). In this context, the 'Sugar palm' pollinator significantly increased fruit and flesh weight, fruit length and diameter, and seed weight percentages compared to other pollinators in the 2004 season. The pollinators 'Ahmar' and 'Fard' significantly increased fruit and flesh weight, and fruit diameter compared to other pollinators, except for 'Sugar palm'. The pollinator 'Khenizy' significantly decreased fruit length compared to other pollinators. The pollinator 'Ahmar' significantly increased seed weight compared to other pollinators, except for 'Sugar plam'. The pollinator 'Fard' significantly decreased seed weight compared to other cultivars. The male cultivar 'Khenizy' significantly increased dry matter percentage compared to other pollinators, except for 'Maghool'. The pollinators 'Sugar palm' and 'Ahmar' significantly decreased dry matter percentage compared to other pollinators. There were no significant effects for the pollination density or the interaction between pollinator type and density on physical fruit qualities during the 2004 season.

At the Bisir stage, the pollinator 'Sugar palm' showed the lowest concentration of TSS, acidity and vitamin C compared to other pollinators (Table 4). There were no significant differences in TSS concentration between the pollinators 'Ahmar', 'Khenizy', 'Akhdar' and 'Maghool'. The pollinator 'Fard' significantly increased acidity concentration compared to other pollinators, except for 'Maghool'. Vitamin C concentration was significantly higher in fruits pollinated with 'Maghool' than other pollinators. At the Tamr stage, the pollinator 'Maghool' produced the highest concentrations of TSS, acidity and vitamin C. The 'Sugar palm' pollinator resulted in the lowest concentration of TSS, acidity, and vitamin C. There were no significant effects for the pollination density or the interaction between pollinator type and density on fruit chemical qualities at the Bisir and Tamr stages during the 2004 season.

DISCUSSION

One of the major weaknesses of mass tissue culture propagation is the appearance of undesirable off-type plants caused by somaclonal variation, such as poor fertility and delayed flowering time. During the 2004 and 2005 seasons, the results of this experiment showed different degrees of compatibility among the used pollinators with the tissue culture derived 'Nabt Saif' cultivar. In this context, the pollinators 'Khenizy' and 'Fard' resulted in the highest and the lowest fruit set percentage, respectively. However, neither of the used pollinators nor the pollination density resulted in a satisfactory level of fruit set (Tables 1 and 2). Such fruit set abnormality has been reported in different date palm

cultivars (Al-Ghamdi, 1993; McCubbin et al., 2000; Djerbi, 2000 and Cohen et al., 2004). Fruit set failure might be due to environmental factors and/or endogenous factors. In general, the tissue culture derived palms bear economic yield in the fifth or sixth growing season from transplanting. However, some cultivars might show fruit set failure only during the first to the third bearing season, but thereafter bear normally (Zaid, A; personal communication). Interestingly, in the same orchard as this experiment, tissue culture derived date palms of the cultivar 'Sultana' were growing that showed a heavy bearing level already in the sixth growing season. Also, these palms were produced in the same laboratory as the 'Nabt Saif' cultivar, cultivated on the same date, received the same cultural practices and were pollinated with similar pollinators as the 'Nabt Saif' cultivar. Accordingly, the effect of environmental factors could be eliminated. Sensitivity differences in the occurrence of abnormalities among date palm cultivars have been previously recorded (Zaid and Al-Kaabi, 2003). Also, the results of this experiment indicated that the pollination process (pollinator type and pollination density) was not a major factor involved in fruit set failure. Therefore these results suggest that other endogenous factors are responsible for such abnormality in the tissue culture derived 'Nabt Saif' palms. However, a number of offshoots, which were naturally produced by some of the 'Nabt Saif' trees used in the current experiment, produced bunches with an acceptable fruit set level. This may indicate that this fruit set failure is a non-hereditary variation and is not based on any changes in the DNA of these palms.

There is a common practice among date palm growers to apply larger amounts of pollen grains to the tissue culture derived palms than to palms derived from classical offshoots. However, in this experiment there were no significant effects for pollination density either on fruit set level or on fruit quality (Tables 1 and 2). These results confirm those of Haffar et al. (1997) who found that neither pollen concentration nor the frequency of application on 'Khalas' date palm cultivar by mechanical dusting affected fruit set and final yield. They concluded that in general, increased pollen application resulting from either higher mixture concentrations or more frequent applications resulted in a high fruit drop and consequently a significant drop in yield. El-Kassas and Mahmoud (1986) reported that bunch weight was not affected by pollen concentration in the range of 20, 40, 60, 80 and 100%.

Some metaxenia effects for the different pollinators on fruit quality characteristics were recorded both at the Bisir and Tamr stages (Tables 3 and 4). In this context, the pollinator 'Sugar palm' significantly increased fruit and flesh weight, fruit length and diameter, and seed weight, but decreased the concentration of TSS, acidity and vitamin C compared to other pollinators in the 2004 season (Tables 3 and 4). These results partly confirm those of El-Amer et al. (1993), Krueger (1998) and Moustafa (2001).

In conclusion, the production of genetically stable and uniform plants is critically important in determining the efficient use and success of tissue culture techniques applied to the date palm industry. Generally tissue culture derived date palms grow and yield similarly to those of classical offshoots. However, more research work is needed on tissue culture derived date palms to understand more clearly the nature of abnormalities that appear in some cultivars.

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Tables

Table 1. Fruit set, multi-carpel fruit and fruit drop percentage in 'Nabt Saif' date palm trees as affected by pollinator type and pollination density, season 2004.

	Fruit set (%)	Multi-carpel fruit (%)	Fruit drop (%)
Pollinator type			
Ahmar	31.14	5.76	63.10
Fard	11.66	6.23	82.12
Akhdar	32.45	5.25	62.31
Maghool	32.18	5.54	62.28
Khenizy	34.31	7.23	58.45
Sugar palm	34.10	6.62	59.29
F-test	***	***	***
LSD 5%	*1.98*	*1.05*	*2.35*
Pollination density			
10 strands/bunch	28.67	6.25	65.10
20 strands/bunch	31.00	6.53	62.47
30 strands/bunch	29.79	5.34	64.87
40 strands/bunch	28.22	6.17	65.61
50 strands/bunch	28.84	6.23	64.93
F-test	*	*NS*	*
LSD 5%	1.82	-	2.15
Pollinator X density	***	***	***

(*) and (***), significant at P = 0.05 and 0.001, respectively; (ns), not significant; (-), not calculated.

Table 2. Fruit set, multi-carpel fruit and fruit drop percentage in 'Nabt Saif' date palm trees as affected by pollinator type and pollination density, season 2005.

	Fruit set (%)	Multi-carpel fruit (%)	Fruit drop (%)
Pollinator type			
Ahmar	25.37	4.63	69.99
Fard	19.98	3.63	76.39
Akhdar	25.02	4.14	70.84
Maghool	27.64	6.51	65.85
Khenizy	28.73	6.71	64.56
Sugar palm	27.65	6.01	67.01
F-test	***	***	***
LSD 5%	*1.94*	*0.74*	*1.95*
Pollination density			
10 strands/bunch	23.81	5.29	70.90
20 strands/bunch	24.33	5.55	70.11
30 strands/bunch	24.11	5.34	70.54
40 strands/bunch	25.23	5.42	69.36
50 strands/bunch	30.62	4.75	64.63
F-test	***	*NS*	***
LSD 5%	1.77	-	1.78
Pollinator X density	***	***	***

(***), significant at P = 0.001; (ns), not significant; (-), not calculated.

Table 3. Physical quality characteristics of 'Nabt Saif' date palm fruit at the Bisir stage as affected by pollinator type and pollination density, season 2004.

	Fruit weight (g)	Fruit length (cm)	Diameter (cm)	Flesh weight (g)	Seed weight (g)	Dry matter (%)
Pollinator type						
Ahmar	15.65	3.50	2.74	14.52	1.14	32.3
Fard	15.67	3.50	2.71	14.74	0.92	37.0
Akhdar	14.15	3.45	2.60	13.10	1.05	36.2
Maghool	14.64	3.50	2.59	13.63	1.00	38.8
Khenizy	14.26	3.35	2.54	13.19	1.10	40.6
Sugar palm	18.99	3.91	2.95	17.79	1.20	32.7
F-test	***	***	***	***	***	***
LSD 5%	*0.92*	*0.12*	*0.11*	*0.91*	*0.06*	*2.25*
Pollination density						
10 strands/bunch	15.23	3.52	2.65	14.15	1.10	35.93
20 strands/bunch	15.80	3.51	2.68	14.75	1.05	37.10
30 strands/bunch	15.55	3.53	2.72	14.48	1.10	36.30
40 strands/bunch	16.04	3.61	2.74	14.96	1.05	36.49
50 strands/bunch	15.17	3.52	2.65	14.10	1.07	35.60
F-test	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
LSD 5%	-	-	-	-	-	-
Pollinator X density	NS	NS	NS	NS	NS	*NS*

(***), significant at $P = 0.001$; (ns), not significant; (-), not calculated.

Table 4. Chemical quality characteristics of 'Nabt Saif' date palm fruit at both the Bisir and the Tamr stages as affected by pollinator type and pollination density, season 2004.

	Bisir stage			Tamr stage		
	TSS (%)	Acidity (%)	Vit. C (mg/100ml juice)	TSS (%)	Acidity (%)	Vit. C (mg/100ml juice)
Pollinator type						
Ahmar	35.5	1.26	5.88	81.8	5.21	3.47
Fard	32.6	1.50	5.96	83.0	5.23	3.49
Akhdar	34.9	1.44	6.45	82.9	5.54	3.50
Maghool	34.5	1.57	8.13	83.8	5.67	3.83
Khenizy	35.1	1.44	6.16	82.7	5.34	3.47
Sugar palm	30.3	1.15	5.74	81.0	5.00	3.38
F-test	***	***	***	***	***	***
LSD 5%	*2.24*	*0.08*	*0.30*	*1.08*	*0.26*	*0.16*
Pollination density						
10 strands/bunch	34.5	1.37	6.43	82.5	5.39	3.47
20 strands/bunch	34.4	1.39	6.29	82.1	5.21	3.55
30 strands/bunch	33.9	1.42	6.29	82.8	5.31	3.55
40 strands/bunch	33.2	1.43	6.41	82.5	5.26	3.52
50 strands/bunch	33.2	1.35	6.51	82.8	5.49	3.53
F-test	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
LSD 5%	-	-	-	-	-	-
Pollinator X density	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

(***), significant at $P = 0.001$; (ns), not significant; (-), not calculated.

Distribution of Phythopathogenic Fungi on the Coastal Region of Libya and Their Relationships with Date Cultivars

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Abstract

A field study was conducted to determine the relationship between date cultivars growing in the coastal region of Libya, and the distribution of fungal genera associated with the trees. The cultivars studied were Helawi, Hura, Bayodi, Brulsi, Tabuni, Bekrari, Ammi, Om-Hnash, Najma, Om-Ftity, Tagyat, Fazani, Tabla, Om-Edam, Akfa, Rahat, Zaglool, Semani, Hamuri and Deglet. There were big differences between date cultivars and it was found that *Graphiola phoenicis* was most often associated with Tabuni, Helawi, Bekrari, Ammi and Fazani, although it was associated with all cultivars at a lower rate. Black Scorch fungus, *Thielaviopsis paradoxa,* was found in association with Helawi, Bekrari, Ammi and Fazani while it was found infrequently on Tabuni. It was recorded that the highest infection rate was on leaves and fruits with 90 % and 80 %, respectively. The frequency of *Diplodia phoencium* was higher in all cultivars studied ranging from 6-61 % while the highest distribution on Bekrari, Tabuni, Ammi, Helawi, Hura and Brulsi. *Mauginiella scaettae* was found only on Bekrari.

INTRODUCTION

Libyan date fruit production of approximately 350,000 ton makes it the largest date fruit producer in the world. Accordingly, date palm *Phoenix dactylifera* is considered an important fruit tree in Libya, and a large variety of cultivars are grown. There are 400 cultivars, which are distributed between three regions, namely coastal, middle and southern regions (Edongali, 2005, unpublished data). The expansion of agriculture cultivation, especially date palm, heightens the awareness of all problems that are a hindrance to this expansion, such as different kinds of pests including fungal diseases (Anon, 2004; Zaid et al., 2005). Many researches in this area have verified that date palm trees are infected with fungal diseases and are also colonized by a large number of fungal genera in Libya, but no indication has been given to the relationship between there fungi and date palm cultivars planted (Abd-Alkader et al., 1997; Alnuesray et al., 1986; Be-Saad et al., 1981).

MATERIALS AND METHODS

This study was carried out during the 2004 and 2005 seasons on common date palm cultivars *P. dactylifera*. Three farms were selected from the coastal area and samples were taken from ten plants on each farm. Samples were taken from five places on each tree. The samples of infected parts were collected randomly from twenty-two cultivars such as, Helawi, Hura, Bayodi, Brulsi, Tabuni, Bekrari, Ammi, Om-Hnash, Njma, Om-Ftity, Tagyat, Fazani, Tabla, Om-Edam, Akfa, Gerjim, Raht, Zaglool, Semani, Hamuri and Deglet. All samples were collected in plastic bags separately with all information, and then stored at 4°C in the laboratory until examined to identify the pests. A piece from each infected plant tissue was surface sterilized with 70 % ethanol followed by three washes in sterile distilled water. These samples were then placed on a petri dish containing PDA or CMA media. All plates were incubated at room temperature (22 ± 2°C) for a few days, and then examined for identification.

RESULTS AND DISCUSSIONS

There were 17 genera of fungi associated with coastal date cultivars, namely *Graphiola phoenicis, Thielaviopsis paradoxa, Diplodia* spp*, Mauginiella scaettae, Alternaria* spp*, Cladosporium* spp*, Drecheslera* spp*, Helminthosporium* spp*, Stemphylium*

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

spp, *Colletotrichum* spp, *Pestalotia* spp, *Penicillium* spp, *Aspergillus* spp, *Nigrospra* spp, *Fusarium* spp, *Rhizopous* spp, *Botrydiplodia* spp. (Table 1). All these genera were associated with the following cultivars: Helawi, Hura, Bayodi, Brulsi, Tabuni, Bekrari and Ammi. The frequency of distribution of these fungi was variable, but the most frequently occurring were, *Alternaria, Penicillium, Aspergillus, Nigrospra, Fusarium, Rhizopous, Graphiola, Thielaviopsis* and *Diplodia* (Table 1). These fungi are considered to be the primary disease-causing fungi in these cultivars.

*Graphiola phoenici*e (Fig. 1) was found mostly frequently on Tabuni, Helawi, Bekrari, Ammi and Fazani as follows: 95, 98, 83, 87 and 77 %, respectively. It was found on Hura, Om-Hnash, Om-Ftity, Om-rwanni 69 % of the time, while it ranged from 33 – 43 % on other cultivars.

Black scorch fungus (*Thielaviopsis paradoxa*) (Fig. 2) was found most frequently on Hellawi (90 %), while it was varied from 44 -65 % on other cultivars.

Diplodia phoencium fungus (Fig. 3) was found on almost all cultivars with a range of 6 – 61%. This fungus causes leaf front spots. The most susceptible cultivar was Bekrari (61 %), followed by Tabuni (57 %), Ammi (55 %), Helawi (50 %), Brulsi (59 %), Hura (33 %), while other cultivars were less susceptible (6 – 20 %). These rates of infection were linked to weather conditions (relative humidity, temperature), farming practices and inter-cropping systems that led to an increase in relative humidity.

Mauginiella saettae fungus (Fig. 4) was associated with Bekrari cultivars where it was found on 75 % of the samples collected. However, it was not recorded on any other cultivars.

Other fungi are known to be associated with leaf spotting (Fig. 5), such as *Alternaria, Cladosporium, Drecheslera, Helminthosporium, Stemphylium, Colletotrichum, Pestalotia* and *Diplodia*. The highest incidence of these fungi were found on Bekrari, Ammi, Tabuni, Brulsi, Hura and Helawi, while lower rates were found on other cultivars.

Fungi found on date palm fruits (Fig. 6) included *Alternaria, Penicillium, Aspergillus, Nigrospra, Fusarium, Rhizopous* and *Botrydiplodia*. They were found on most cultivars, however with high frequency on Bekrari (45 %), Hura (41 %), Byodi (41 %), Helawi (33 %), Brulsi (18 %), Tabuni (27 %) and with frequency on Om-Edam (9 %) and Najma (10 %) cultivars.

These results were in agreement with Abd-Alkader et al. (1997), Alnuesray et al. (1986) and Zaid et al. (2005), who have documented fungal genera associated with date palm trees in Libya and elsewhere in the world. Alnuesray et al. (1986) reported the incidence of most of these fungal genera in association with palm trees and different diseases around Libya, but did not study the relationship between these fungi and date palm cultivars. The common fungal genera they reported were *Graphiola, Thielaviopsis, Diplodia* and *Mauginiella*. Our results indicated a higher incidence of these fungi in the cultivars Tabuni, Helawe, Ammi, Bekrari, Brulsi and Hura and the lowest incidence in association with Tabela, Om-Edam, Akfa, Jerjim, Raht, Zaglool, Alsemai, Hamuri and Deglet.

In general, the variation among the cultivars in the incidence of different fungi may be related to factors other than genetic factors, such as the dominant environmental conditions, tree age, irrigation method and other agricultural practices. These factors need to be investigated in order to better understand the rate of tolerance or susceptibility of the different cultivars to any specific fungal genera.

Most of these fungi have a harmful effect on date palm plantations. The damage occurs at different rates, either on foliage or on fruits, and is dependent on region and dominant abiotic factors. All these need to be studied in depth.

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Table

Table 1. Frequency of fungal genera associated with date palm cultivars.

Associated fungi	% Frequencies distribution						
	Ammi	Bekrari	Tabuni	Brulsi	Bayodi	Hura	Helawi
Graphiola phoenicis	87	83	95	0	76	69	98
Thielaviopsis paradoxa	65	44	90	0	0	0	50
Diplodia phoenicum	55	61	57	59	20	33	50
Mauginiella scaettae	0	75	0	0	0	0	0
Alternaria spp	90	90	90	90	90	90	90
Cladosporium spp	0	20	40	0	30	0	90
Drecheslera spp	21	32	30	55	0	0	0
Helminthosporium spp	44	45	60	82	90	88	90
Stemphylium spp	0	0	0	0	40	75	0
Colletotrichum spp	12	31	0	0	85	0	80
Pestalotia spp	10	20	0	0	50	0	85
Penicellium spp	61	76	80	80	80	80	80
Aspergillus spp	80	72	70	80	79	80	75
Nigrospora spp	0	0	0	80	77	71	37
Fusarium spp	61	11	35	44	9	13	16
Rhizopous spp	80	77	78	80	61	70	80
Botrydiplodia spp	0	0	0	0	8	8	78

Table 1. continued

Associated fungi	% Frequencies distribution						
	Omedam	Tabla	Fazani	Tagyat	Omftity	Najma	Omhnash
Graphiola phoenicis	0	0	77	0	62	56	60
Thielaviopsis paradoxa	0	0	49	0	0	0	0
Diplodia phoenicum	6	0	6	0	6	6	20
Mauginiella scaettae	0	0	0	0	0	0	0
Alternaria spp	0	0	73	77	90	87	89
Cladosporium spp	40	0	0	0	0	0	0
Drecheslera spp	22	0	0	0	0	0	0
Helminthosporium spp	90	0	70	0	0	0	00
Stemphylium spp	0	0	0	0	0	0	0
Colletotrichum spp	0	0	0	0	0	0	0
Pestalotia spp	0	0	0	0	0	0	0
Penicellium spp	8	35	30	0	80	10	0
Aspergillus spp	0	40	33	0	70	9	0
Nigrospora spp	0	0	0	0	13	0	0
Fusarium spp	0	0	0	0	0	0	0
Rhizopous spp	15	13	13	40	75	15	0
Botrydiplodia spp	0	0	0	0	0	0	0

Table 1. continued.

Associated fungi	% Frequencies distribution						
	Deglet	Hamuri	Semani	Zaglool	Raht	Gerjim	Akfa
Graphiola phoenicis	0	0	33	43	0	0	0
Thielaviopsis paradoxa	0	0	0	0	0	0	0
Diplodia phoenicum	0	0	6	6	6	6	6
Mauginiella scaettae	0	0	0	0	0	90	0
Alternaria spp	0	0	0	80	0	0	0
Cladosporium spp	0	0	0	0	0	0	0
Drecheslera spp	0	0	0	71	0	50	0
Helminthosporium spp	0	0	0	63	0	65	0
Stemphylium spp	0	0	0	0	0	0	0
Colletotrichum spp	0	0	0	0	0	0	0
Pestalotia spp	0	0	0	0	0	0	0
Penicellium spp	80	0	0	0	0	0	80
Aspergillus spp	70	0	0	0	0	0	70
Nigrospora spp	36	0	0	0	0	0	0
Fusarium spp	0	0	0	0	0	0	0
Rhizopous spp	79	0	0	0	0	0	80
Botrydiplodia spp	0	0	0	0	0	0	0

Figures

Fig. 1. *Graphiola phoenicis* fungus associated with date palm leaves.

Fig. 2. Disease symptoms caused by *Thielaviopsis paradoxa* fungus on date palm tree.

Fig. 3. *Diplodia phoencium* fungus associated with date palm leaves.

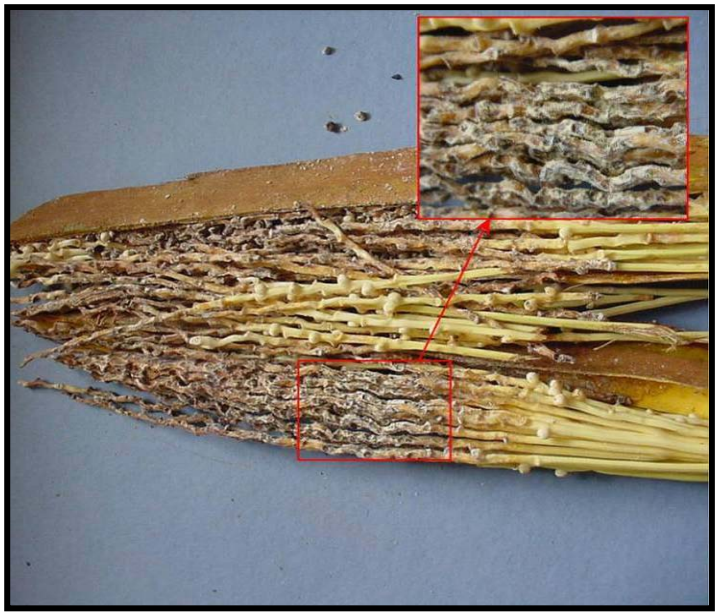

Fig. 4. Inflorescense rot caused by *Mauginiella scaettae* on date palm.

Fig. 5. Symptoms of fungal diseases on date palm leaves.

Fig. 6. Symptoms of fungal diseases on date palm fruits.

The Effects of Relative Humidity and Temperature on Disease Development in Stored Date Fruits

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Abstract

Date fruit rot is widely distributed in Nigeria and causes great losses for farmers and marketers. The role of moisture and temperature in disease development was investigated at 7 relative humidity levels using saturated salt solutions and water at 6 temperature levels with the ultimate aim of controlling rot. Temperature and moisture were found to be important in disease development. Up to 15°C, no rot was observed after 21 days of incubation in all relative humidity levels tested. Similarly, in up to 63% RH, no rot was observed at the temperatures tested. Though the fruits were shriveled and brittle the spores proved viable when transferred to growth media and incubated at 30°C. This indicated that low relative humidity and temperature are not conducive for disease development. At 25-30°C all fungi tested caused rot in 91-100% RH in 2-5 days. All except *Cladosporium* spp. caused rot in 84.5% RH in 2-26 days. Only *Aspergillus vesicolor* and *Malbrachiae* sp. caused rot at 75% RH in 11-15days. Thus date fruits with low moisture content may be preserved at room temperature while those with high moisture content will require lower temperatures.

INTRODUCTION

Date fruit decay is widely distributed in the date growing belt of Northern Nigeria, (Omamor, 1987). It becomes a very serious problem during the rainy season when the atmosphere is humid causing high fruit losses to farmers and marketers.

Economic losses result primarily from the rotten appearance and offensive odor emitted by the infected fruits. Reduction in both quality and quantity of production is caused and fruit is rendered unsuitable for human or livestock consumption. Of the 29 fungal spp belonging to 14 genera associated with stored date fruit, only 20 belonging to 8 genera were pathogenic (Omamor, 1991). Temperature and moisture, two of the most important environmental conditions regulating fungal growth, govern the occurrence and rate of fruit decay. This paper seeks to study the effects of relative humidity and temperature on disease development in stored date fruit with the aim of controlling post harvest fruit rot.

MATERIAL AND METHODS

Source of Pathogen

Infected stored date fruits were obtained from the date palm research substation, Dutse, and other date palm growing areas. Among these were observed black rot, grey rot, and white rot. Green rot, yellow rot, pinkish purple rot and orange rot, were each exhibiting the colour of the colonizing fungus.

Representatives of each colour group were washed in sterile distilled water and surface sterilized in 0.35% sodium hydrochloride for 2 minutes. They were then rinsed thoroughly in sterile distilled water. Some other segments were merely washed in sterile distilled water. Fungal segments of fruits were then plated on PDA (potato dextrose agar), MEA (malt extract agar), MSA (malt salt agar), CZDA (czepe-Dox agar) or CZDSA (czape-Dox sucrose agar) and incubated for 3-21 days at room temperature (27 °C -28 °C). At the end of the incubation period the different organisms associated with the fruit were isolated, purified, identified and maintained at room temperature.

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

Relative Humidity

The effect of relative humidity and temperature on disease development was investigated using saturated salt solutions (Winston and Bates, 1960) to give approximately 32.5, 47, 63, 75, 84.5 and 91% RH at 10, 15, 20, 25, 30 and 35^{o}C.

At all temperatures sterile distilled water was used to give approximately 100% RH. About 30ml of the saturated salt solutions was dispensed separately into 50ml glass jars each containing two (2) virtual glass slides and the mouth greased with Vaseline before the lid was placed on tightly. These jars were left in the relevant temperatures for 3 days in order to attain equilibrium.

The Fruit

Healthy date fruits from fruit rot susceptible plant lines were surface sterilized in absolute alcohol for 30 seconds, rinsed with sterile distilled water, blotted dry, immersed in 1% mercuric chloride for 1 min. and then rinsed and blotted dry. These fruits were then dried in the oven at 78°C for 2 – 3hrs during which time approximately constant weight was obtained. Some fruits were injured while others were left intact.

Inoculation

These fruits were inoculated separately with different fungal species isolated earlier, placed on the two vertical slides in the glass jar of different relative humidity levels with the inoculated portion facing the saturated salt solution and incubated for 2 – 31 days at each of the temperature values. There were 5 replicates for each RH level. At the end of the incubation period all fruits that did not show disease development were transferred to PDA and incubated for 5 days at 30°C.

RESULT AND DISCUSSION

The superficial nature of fungal colonies on the surface of date fruit makes them particularly sensitive to their microclimate conditions, especially temperature and relative humidity.

Temperature and relative humidity were found to be very important in disease development. At 10°C and 15°C, rot was not observed even after 21 days of incubation at all levels of relative humidity tested (32.5, 47, 63. 84.5, 91 and 100% RH). Similarly, at low relative humidity (32.5-63% RH) no rot was observed at any of the temperatures tested (10, 15, 20, 25, 30 and 35°C). The fruits became very dry, slightly shriveled and brittle. However, the spores from both low humidity and temperatures proved viable when transferred to growth media and incubated at 30°C for 5 days. This indicates that low relative humidity and temperature are not conducive to disease development and that the shelf life of fruits will therefore be prolonged under these conditions, but when transferred to a more favorable environment, disease will develop.

High relative humidity (75 – 100% RH) made the fruits succulent but predisposed them to infection. At 20–35°C and 75 – 100% RH, deceased development was observed, but the time taken to initiate disease varied with fungal species, humidity levels and temperature. Of the 6 *Aspergillus spp* only *Aspergillus* versicolor infected fruits at 75% RH after 11 days of incubation (Table 1). All six *Aspergillus* spp. took 4 – 26 days in 84.5% RH to initiate infection and 2 – 5 days in 91 – 100% RH. The 3 *Cladosporium* spp. infected fruits in 91-100% RH only, taking 5 days. *Malbranchae spp* infection occurred in 75 – 84.5% RH in 15 days and in 91 – 100% RH in 5 days. The three (3) *Monascus spp* infected date fruits in 84.5% RH in 10 – 11 days while those in 91 – 100% took 4 days. *Rhizopus oryzae*, *Penicillium spp* and *Paecilomyces varriotii* took 7 – 15 days to infect date fruits in 84.5% RH and 4 – 5 days in 91 – 100% RH.

All fungal species took longer to infect fruits at 20°C in 75 – 100% RH and grew scantily, whereas fungal growth was faster and more luxuriant in 84.5 – 100% RH at 30°C. Similar results on effects of temperature and relative humidity on disease development in fruits and vegetables in storage have been reported by other investigators (Burr et al.,1989; Ross and Bramlage, 1990; Schultheis, 1998).

The most important aspects of these findings are the identification of the temperatures and relative humidity below which rot-causing fungi are not active. There is an interaction between temperature and relative humidity in disease development. Similarly, relative humidity is an important factor over the range of temperatures most conducive for growth of fungi.

CONCLUSION

If date fruits are dried in to a moisture content equivalent to 32.5 – 63% RH, they may be preserved at room temperature. If, however, they have a moisture content equivalent to 75-100% RH, they may be preserved at temperatures below 15°C.

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Tables

Table 1. Time taken for initiation of infection at different relative humidity levels at 30^{o}C.

		75% RH Time in Days	84.5% RH Time in Days	91 – 100% RH Time in Days
1.	*Aspergillus flavus*	-	7	2
	Aspergillus fumigatus	-	5	5
	Aspergillus nidulans	-	5	5
	Aspergillus niger	-	9	2
	Aspergillus termarri	-	26	2
	Aspergillus versicolor	11	4	2
	Aspergillus sp. (brown)	-	11	4
2.	*Cladosporium cladosporiodes*	-	-	5
	Cladosporium oxysporum	-	-	5
	Cladosporium sphaerospermum	-	-	5
3.	*Eurotium amstelodermi*	-	11	4
4.	*Malbrachae* sp.	15	15	5
5.	*Monascus purpureus*	-	10	4
	Monascus rubber	-	10	4
	Monascus sp. (white)	-	11	4
6.	*Paecilomyces varriotii*	-	7	5
7.	*Penicillium citrinum*	-	11	4
	Penicillium purpuragenum	-	15	5
8.	*Rhozopus oryzae*	-	7	4

Evaluation of Alternative Date Harvesting Methods in Iran

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Keywords: Date palm mechanization, harvesting machines, palm harvesting

Abstract

Date is an important agricultural product in the Middle East including Iran. From 1961 Iran has been producing about 15 to 20 percent of the total world production and is the second largest producer of dates. Most of the dates are still harvested manually. There is no mechanized method available that covers all needs. Simple, general purpose lifting machines of various models are an alternative for date harvesting in this country. Using mechanized methods and harvesting machines in date yards of developed countries like USA reduced harvesting costs by 50%. In this research the characteristics of available lifting machines in Iran were measured and compared to find the acceptable range of size and value of features required for a technically and economically feasible machine. The physical properties of trees and tree spacing were also measured to complete the research. Average values of the main features of existing lifting machines in Iran were calculated as: lifting height: 13 meter; weight: 15650 N; payload: 2000N; lateral expansion: 5.58 m; machine length: 5.23 m; width: 2 m; transport height: 3m; average selling price: 150 million Rials. Results revealed that most of the available machines are not acceptable to date growers. They would require machine features in the following range: lifting height: 10 meter; payload: 1100 – 1300 N; climbing speed: 0.41 m/s; machine width: less than 2m; height: less than 2.5 m; lateral accessibility: of 1 m; average selling price: 30 million Rials.

INTRODUCTION

Date is one of the most important fruits in more than 30 countries. Dates are spread around the desert regions of the world. The annual world production was approximately 5211 thousand tons (FAO, 2000), with Egypt, Iran, Saudi Arabia, Iraq, Pakistan and Algeria producing 75 percent of the total world production (Barreveld, 1993). Yearly production of date in Iran is about 900 thousand tons (Iranian ministry of agricultural, 2002).

A study (Elhampuur, 1993) showed that Iran has the potential for further increases in yield. By using mechanized cultivation the quality of dates can be improved. Most harvesting is currently done manually, so there is major interest in the mechanization of the harvesting operation. The most popular cultural practices carried out in palm orchards are: soil digging that is usually done in the winter, pollination that is carried out in spring, dehorning, pruning, fruit thinning, bending and bagging of bunches and pesticide control that are carried out in summer and harvesting which is the most labor consuming is carried out in autumn. Brown (1983) showed that among cultural operations, harvesting, pollination and pruning are the most labor intensive tasks, accounting for more than 80 percent of total production costs. Most operations continue to be done manually in Iran, because a mechanized method that covers all needs is not available. General purpose lifting machines of various models are alternatives to harvesting manually, but date growers do not use these machines and more than 90 percent of dates are still harvested manually. This research was carried out to investigate why date growers do not use the existing machinery and to propose an optimized lifting machine to service the date palms.

Literature Review

The most difficult part of harvesting dates is reaching to the fruits. There are two methods of harvesting date fruits, traditional and mechanical. In Iran, Iraq and Libya the traditional way of harvesting is to use the leaf base to climb the tree (Nixon, 1969). Some workers use a belt to secure themselves to the tree (Dawson, 1962). In Africa, especially in Algeria, workers dig holes in the tree trunk to make climbing easier (Rohani, 1998). They may hammer pegs into the tree trunk or move to the next tree on the leaves or move on a rope. Date growers in USA use ladders to reach the fruits (Nixon, 1969). Depending on the date variety and climate, dates are picked by one of three ways:

1-Bunch cutting: used when all fruits on a bunch ripen simultaneously.
2-Bunch shaking: used when fruits do not ripen at the same time.
3-Selective hand picking: used for those varieties which have fruits that are sensitive to vibration and impact.

During the past decades, labor shortage and increasing interest in mechanical harvesting of date fruits has led to the development of mechanical date harvesting systems. Perkins and Brown (1964, 1965 and 1967) used a system which involved three men, including a truck driver, a boom operator and a bunch cutter or shaker. Davis (1977) developed a coconut tree climbing bicycle. Shamsi (1985) designed a walking machine to carry one person to the crown of the palm tree. Shamsi (1990) designed and tested a rig climbing machine to harvest date (Fig. 1). Tood (1986) introduced machines that climbed smooth vertical surfaces. Al-Suhaibani et al. (1988 and 1990) reported making and testing a date service machine in Saudi Arabia which was designed at Silso College, a "U" shape platform allowing the worker to reach all bunches of dates without any additional movement of the platform. Al-Suhaibani et al. (1992) reported the field testing of the machine in 1990. He showed that a tree could be harvested in 21 minutes which is faster, safer and easier than hand harvesting. Yoav Saring et al. (1989) in Israel developed an integrated mechanical system that could harvest the fruits by shaking the tree trunk. Nicklin (1993) designed a tree climbing test rig to lift a man up the date palm trees. Lawitzky et al. (1997) designed a pipe climbing machine in Germany. Hughes in 1997 developed a portable security surveillance system that was equipped with a CCTV (Closed Circuit Television) to provide aerial images of the surrounding locality. Shamsi et al. (1998) designed a date harvesting machine in Silso College. Abounagmi (2000) designed and developed an experimental shaker for investigating the effect of shaking mode, frequency and amplitude on date fruit detachment. Seirei Kogyo (1996) is currently producing a pruning tree climber. Meier (1996) reports producing a similar machine in Switzerland. Roux et al. (1994) developed a mechatronic tree pruning machine (SELA).

MATERIALS AND METHODS

To find out why date growers do not use the existing lifting machines and to propose an optimized lifting machine to service the date palms, the characteristics of available lifting machines in Iran were measured (Table 1). The physical and mechanical properties of trees and their spacing were calculated and compared to investigate the acceptable range of size and value of features for a technically and economically acceptable machine for date harvesting. Climbing the tree and reaching the fruits are the most difficult part of the harvesting action, therefore attempts to develop machinery have focused on mechanization of tree climbing.

To obtain characteristics of available lifting machines the authors contacted three manufactures of lifters in Iran. Important machine features were found through catalogues and by consulting technicians. Important features are: working height, machine weight, payload capacity, lateral accessibility, power and transmission type, machine size and price (Table 1).

To evaluate the feasibility of available lifting machines, information concerning the physical properties of trees and palm plantings were required: tree trunk height, tree and row spacing, number of bunches, yield, distance to nearest tree and the worker's

climbing speed were measured (Tables 2, 3, 4, 5, 6). Twenty-five random trees in different orchards were selected for these measurements.

RESULTS AND DISCUSSION

Tables 2 to 6 showed that about 72 % of the trees were smaller than 10 m. Table 1 showed that the average lifting height of machines was about 11 m. When the height of the worker was added to this value, the worker's hand could reach to a height of 13 m. Growers do not use this height but they must pay for it if they want to use available machines. Seventy percent of machines weighed more that 10000 N and 60 % of them more than 15000N. These heavy machines need a big truck or tractor to pull them. Extra machine weight would compact the soil in the field and damage the plantings under the shadow of palm trees in dry and hot areas. Table 6 showed that the maximum tree yield was about 1280 N which is harvested in 4 stages. When worker weight is added to it the machine deals with a maximum payload of 1100 to 1300 N. Ninety percent of the existing machines are designed for higher payloads. Tree spacing between and across rows were 3.5 and 4.1 m, respectively. Machines dimensions are in the accepted range but they cause problems when turning. Traditionally,fruit trees such as oranges are cultivated in the cool shadow of palm trees, limiting the height of harvesting machines to less than 2.5 meter, and 70% of machines are out of this range. The lifting speed of machines must be faster than the worker's climbing speed which is about 0.41 m/s. Investigations showed that all machines move slower than skilled workers. Eighty percent of machines need more than 40 hp power. A skilled worker consumes just 0.3 hp power to climb the tree. This variation shows that using available machines was a huge waste of power and it was a major reason for date growers not using machines. It is difficult to bring machines close to the tree and therefore machines need a lateral movement mechanism. Two models of existing machines which are more suitable from other points of view do not have this facility. Table 1 showed that 100% of powered machines are more expensive than 30 million Iranian Rials and some of them cost 4 times this value. An investigation (Shamsi et al., 1998) showed that most date growers can afford to buy machines to the value of 30 million Iranian Rials. They cannot afford to buy existing machines.

CONCLUSIONS

It can be concluded that available lifting machines are not suitable for date harvesting and they must be modified and optimized for this job. A lifting machine must be able to reach to a height of 10 meters, carry a maximum pay load of around 1100-1300N and move laterally by 1 m. The width of the machine must be less than 2m and the height less than 2.5m. It must lift the worker faster than 0.41 m/s and be cheaper than 30 million Iranian Rials.

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Tables

Table 1. Characteristics of available lifting machines in Iran.

Manufactory	Model	Height – Working (m)	Weight (kg)	Payload (kg)	Lateral access (m)	Length (cm)	Wide (cm)	High (cm)	Price (million rial)
Balan sanat	DML 12	12	610	160	------	410	180	250	27
Ahrom vazin	S.T.S SIMON B-9	11	601	170	5.49	510	200	225	97
Ahrom vazin	S.T.S-ZOOM B-14	14	1100	500	9	336	170	210	181
Balan sanat	EHs 1000	10	340	130	------	141	75	215	38
Lajvar	AL 1200	14	1700	200	6	660	230	330	180
Lajvar	TL 1600	18	3200	200	10.5	700	220	320	291
Lajvar	TML 900	11	1600	150	5.4	540	200	315	100
Lajvar	AL 900	11	1400	150	5.4	590	170	310	90
Lajvar	AL 1050	12.5	2400	200	7.5	670	210	310	250
Lajvar	AL 1400	16	2700	200	6.5	660	230	370	330
Max		18	3200	500	9	700	230	370	330
Min		11	340	80	5.4	141	75	215	27
Ave		12.95	1560	200	5.58	522	205	288	150

Table 2. Physical properties of date palm trees and palm plantings.

Tree No.	1	2	3	4	5	6
City	Bam	Bam	Bam	Bam	Bam	Bam
Grove name	Dehghan	Dehghan	Dehghan	Dehghan	Istghah	Istghah
Variety	Mazafati	Mazafati	Mazafati	Mazafati	Mazafati	Mazafati
Tree trunk height, m	8.20	7.60	9.40	7.70	10	7
Row spacing, m	3.5	5	4.2	4.5	6.1	5.2
Across row spacing, m	4.2	7.1	5.3	5.5	6.2	7.2
Bunch & stalk weight, kg	14.5	12.5	13	14.5	12	12.5
Number of bunches	11	10	12	8	9	9
Yield, kg	127.6	100	124.8	92.8	86.4	90
Distance to nearest tree, m	4.1	3.2	4.5	3.5	5	5.2
Climbing speed, m/sec	0.4.	0.35	0.36	0.41	0.25	0.3

Table 3. Physical properties of date palm trees and palm plantings..

Tree No.	7	8	9	10	11	12
City	Bam	Bam	Bam	Bam	Bam	Shahdad
Grove name	Sabzevary	Shahrdadi	Shahrdadi	Shahrdadi	Shahrdadi	Bagherig
Variety	Karoot	Karoot	Karoot	Karoot	Karoot	Ghasb
Tree trunk height, m	6.5	14	16.4	17	15.2	14.1
Row spacing, m	3.8	3.5	4.3	4.1	5.7	4.9
Across row spacing, m	4.1	5.4	6.3	6.7	6.9	5.2
Bunch & stalk weight, kg	13.5	10.1	7.2	6.9	10.1	7.5
Number of bunches	10	8	6	8	5	8
Yield, kg	108	64.5	34.6	44.1	64.6	48
Distance to nearest tree, m	4	2.5	3.1	3.9	3.6	4.9
Climbing speed, m/sec	0.28	0.2	0.28	0.21	0.18	0.31

Table 4. Physical properties of date palm trees and palm plantings.

Tree No.	13	14	15	16	17	18
City	Shahdad	Shahdad	Shahdad	Shahdad	Shahdad	Shahdad
Grove name	Kohestani	Kohestani	Rahmani	Rahmani	Baghe rig	Baghe rig
Variety	Mazafati	Mazafati	Mazafati	Mazafati	Porkoo	Porkoo
Tree trunk height, m	15.3	12.1	16.2	11.2	6.5	7.2
Row spacing, m	4.8	5.7	5.1	4.8	5.1	3.9
Across row spacing, m	6.1	7.1	6.3	5.9	5.5	4.5
Bunch & stalk weight, kg	8.1	7.1	7	8.5	6	10.2
Number of bunches	7	6	7	8	8	6
Yield, kg	45.4	34.1	39.2	54.4	38.4	49
Distance to nearest tree, m	3	3.7	3.1	2.8	3.2	3.5
Climbing speed, m/sec	0.21	0.18	0.31	0.27	0.2	0.22

Table 5. Physical properties of date palm trees and palm plantings..

Tree No.	19	20	21	22	23	24
City	Shahdad	Shahdad	Shahdad	Shahdad	Shahdad	Shahdad
Grove name	Bagherig	Baghe rig	Bagherig	Char farsahk	Char farsahk	Saheb dadi
Variety	Porkoo	Porkoo	Porkoo	Abdolahi	Abdolahi	Bazmani
Tree trunk height, m	6.5	7.5	8	10.1	9.5	7.3
Row spacing, m	4.4	5.1	4.8	3.8	4.5	4
Across row spacing, m	7.3	6.7	6	5.8	5.9	4.5
Bunch & stalk weight, kg	9.8	6.5	8.5	8	6.7	10
Number of bunches	7	8	9	9	8	10
Yield, kg	38.4	49	54.9	41.6	61.2	80
Distance to nearest tree, m	3.2	3.5	2.9	3.6	2.5	4.3
Climbing speed, m/sec	0.38	0.41	0.29	0.37	0.31	0.41

Table 6. Physical properties of date palm trees and palm plantings. .

Tree No.	25	Ave.	Std.	Max.	Min.
City	Shahdad				
Grove name	Saheb dadi				
Variety	Bazmani				
Tree trunk height, m	6.9	10.3	3.6	17	6.5
Row spacing, m	4.9	4.6	0.69	5.7	3.5
Across row spacing, m	5.1	5.8	0.96	7.3	4.1
Bunch & stalk weight, kg	11.1	9.67	2.66	14.5	6
Number of bunches	10	8	2	11	7
Yield, kg	88.8	66.9	28.2	127.6	34.1
Distance to nearest tree, m	3.8	3.66	0.74	5.2	2.5
Climbing speed, m/sec	0.33	0.31	0.08	0.41	0.18

Figures

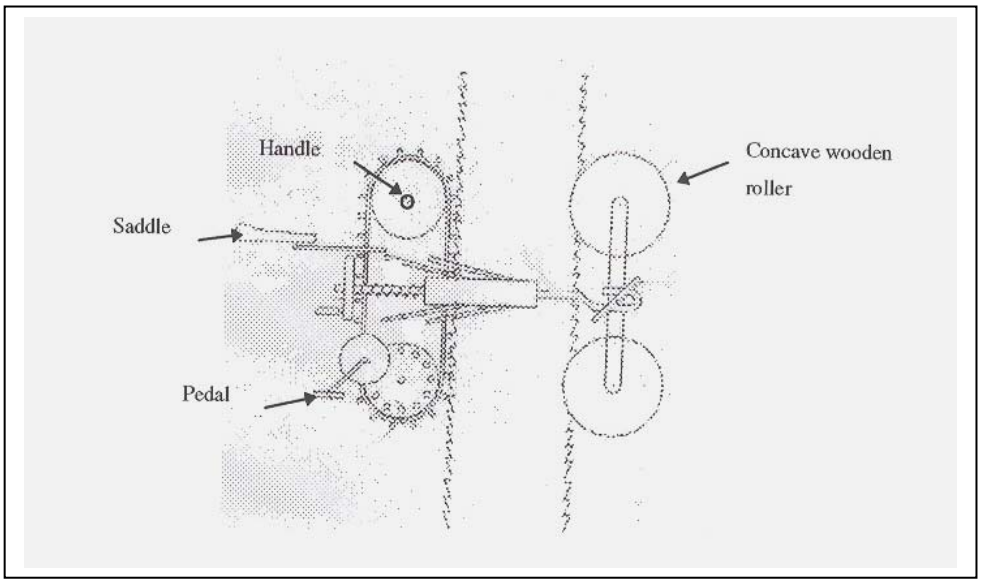

Fig. 1. Tree climbing bicycle.

Effect of Vacuum and Modified Atmosphere Packaging on the Postharvest Quality and Shelf Life of Date Fruits in Khalal Stage

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Keywords: Date palm, (*Phoenix dactylifera* L), storage, quality, cv. Barhee

Abstract

Date (*Phoenix dactylifera* L.) is a berry fruit that can be consumed at three stages of its growth and development, including Khalal, Rutab and Tamar. At the Khalal stage, fruits are physiologically mature, hard and crisp, and bright yellow or red in color. Date cultivars with a low amount of tannin and low astringency are suitable for consumption at Khalal stage but due to the high moisture content, fruits are very perishable with low storage life. In this experiment, the physicochemical properties of Barhee dates under two storage temperatures (4 and 25ºC) in response to vacuum and modified atmosphere packaging (MAP) were studied. Fruits were analyzed at three time intervals after packing (0, 10 and 20 days) and evaluated for quality characteristics: weight loss, flesh firmness, total soluble solids (TSS), water activity, acidity and appearance. Results showed that fruits in MAP treatment had less than 1% weight loss, lowest percentage of Rutab fruits (14.7%), highest water activity (0.957) and low changes in other parameters tested. However, in the vacuum packaging, weight loss and the amount of crumbled fruits were least but a large portion of the fruit had changed to Rutab (22.4%) and fruit firmness was significantly reduced (4.9 kg).

INTRODUCTION

Annual date fruit (*Phoenix dactylifera* L.) production in Iran is estimated at over 900,000 tons, and has an important economic and social role in the south of Iran (Anonymous, 2004). Date fruits are nutritious, being high in carbohydrates, fiber, potassium, certain vitamins and minerals, but are low in fat, and virtually free from cholesterol and sodium. Dates are berry fruits distinguished from most other fruits in that they have a botanical maturity in at least 3 distinct maturation stages including Khalal, Rutab and Tamar. At the Khalal stage, dates are physiologically mature, hard and crisp with over 50 % moisture content, bright yellow or red in color and very perishable. In general, when dates reach the Khalal stage, they are ready for trading as "fresh" fruit but this applies only to those varieties which are sweet, with a low amount of tannin and low astringency (Barreveld, 1993). The most important date cultivars suitable for marketing at Khalal stage are ‘Barhee’, ‘Bereim’, ‘Hayany’ and ‘Khalas’ (Kashani, 1992; Glasner, 2002).

Of these cultivars, ‘Barhee’ is the most important variety suitable for marketing at Khalal stage both worldwide and in Iran and neighboring countries. Harvesting dates takes place from the end of July with the harvesting of the Khalal varieties, especially Barhee. This cultivar provides the first fruit for local markets (Kashani, 1992; Pezhman, 2001). Due to its high moisture content, Khalal fruits are very perishable so they must be transported as soon as possible to ensure that the fruit reaches customers in an unripe state (Glasner, 2002). Any delay in transport or improper storage conditions quickly results in fruits at the Rutab stage, or crumbled fruits. These fruits have a low quality and price and give the grower a low rate of return. Most Barhee fruit is harvested at Khalal stage and information is required on perishability of fruits and packaging methods in order to prolong the shelf life of fruit (Mortazavi, 2005).

Vacuum packaging (VP) and modified atmosphere packaging (MAP) are now

being used to extend shelf-life and reduce the wastage of a wide range of fruits and vegetables. In the VP and MAP packaging systems, the gas mixture surrounding fruits in the package is changed. Elevated concentrations of CO_2 and reduced levels of O_2 inside the package have benefits including reduced respiration, ethylene production and sensitivity to ethylene, retarded softening and reduced decay (Kader et al., 1989). Depletion of O_2 and elevation of CO_2 within the package can be achieved passively by respiration or actively by flushing a desired mixture of gases into the package (Zagory and Kader, 1988; Ballantyne et al., 1988). Passive MAP relies on the respiration of the commodity to consume the O_2 in a sealed bag and replace it with CO_2, a byproduct of normal aerobic respiration. The bag itself restricts the movement of gases in and out of the sealed package due to its selective permeability to O_2 and CO_2. Over time, the system achieves equilibrium with the O_2 lower than that found in the air (20.9%) and the CO_2 concentration higher than that in the air (0.03%). Active MAP introduces a desired gas mixture into the bag prior to sealing, thereby accelerating the process of achieving an equilibrium atmosphere. Vacuum packaging draws a slight vacuum prior to sealing the bag, thereby reducing the headspace in the bag, and accelerating the process of achieving an equilibrium atmosphere (Zagory and Kader, 1988). However, there are no reports about use of vacuum and modified atmosphere packaging for date fruits at Khalal stage. Al-Redhaiman (2004) exposed full mature ´Barhee´ date fruits to modified atmosphere storage conditions with three carbon dioxide concentrations (5, 10 or 20%) during cold storage (0°C). His results showed that fruits stored at 0°C under 20% CO_2 had a significantly longer storage period (lasted for 26 weeks) than all other treatments (i.e. 5 and 10% CO_2 which lasted for 17 weeks) and the control (lasted for 7 weeks). Moreover, fruit stored under MA conditions showed lower decay and weight loss percentage. The influence of vacuum and modified atmosphere packaging with injection of a gas mixture of 20% CO_2 and 80% N_2 on storage ability of 'Deglet Nour' dates at Tamar stage showed that vacuum and modified atmosphere packaging decreased date dehydration during storage (Achour, 2003). The present study was undertaken to ascertain the response of vacuum and passive modified atmospheric packaging on the shelf life and physicochemical properties of Barhee date fruits at Khalal stage at two storage temperatures.

MATERIALS AND METHODS

Fruits of 'Barhee' date cultivar at Khalal stage were purchased from the local market during August, 2005 and after quick precooling, fruits were transported to the laboratory on the same day. Any damaged fruits, crumbled fruits and fruits with Rutab spots were removed and the healthy fruits of uniform size and appearance were washed and randomly distributed into groups of 20 fruits for specific packaging treatments. Soon after harvesting, fruits were analyzed for quality properties.

The experiment was conducted using a completely randomized design (CRD). Fruits were packed using three replications of 20 uniform fruits in low density polyethylene (LDPE) bags (ZIP KIP®, Iran) and were kept in a HENKELMAN vacuum pack instrument (200A) and were sealed to ensure vacuum and passive MAP packages. The control fruits were placed in corrugated cardboard boxes without polyethylene bags. To study the effects of storage temperature on quality and shelf life of date fruits at Khalal stage, packaged and control fruits were subdivided into two sets and stored at two storage temperatures (4ºC and 25ºC). Quality parameters were evaluated on days 0, 10 and 20 after packaging. The data were analyzed with MSTAT-C (version 1.42) statistical package, and means compared by Duncan's Multiple Range Test (DMRT) at 0.01 and 0.05 probability levels. Major quality characteristics were studied including titratable acidity, °Brix, pH, firmness, water activity, weight loss and percentage of Rutab and crumbled fruits. Titratable acidity was calculated as percentage of mailic acid by titrating 25g/200mL of the date extract with a solution of NaOH (0.1N) at pH 8.1. The pH was measured by a Metrohm pH meter, model 744 (Switzerland). The level of sugars was measured as °Brix by a A.Krüss Optronic GmbH refractometer (Germany). Water activity

was measured with a Novasina AW SPRINT (TH 800) instrument and firmness measured with a Wagner pressure tester.

RESULTS AND DISCUSSION

Figures 1 to 6 show the effect of packaging treatments (vacuum, passive MAP and control), storage temperature (4ºC and 25ºC) and storage period (0, 10 and 20 days) on the evaluated quality characters. The physicochemical properties of fruits had different responses to experimental treatments. There was no significant effect of package type and storage temperature on water activity (a_w) of fruits; however, with increase in storage period, a_w was decreased (Fig. 1). Water activity is an important quality factor for dates. It decreases gradually during growth and development of date fruit (author unpublished) and reaches its highest point in Khalal stage (a_w more than 0.95). Water activity is the ratio of the partial vapor pressure of water in equilibrium with a fruit texture to the partial saturation vapor pressure of water vapor in the air at the same temperature. The water activity describes the energy state of water in the food, and hence high water content of fruit texture results in a high a_w (Fontana, 2000). The correlation between fruit water content and its water activity shows that a decrease in a_w resulted in a significant weight loss of fruit. It seems that if water activity is lower than 0.95, fruits undergo a high weight loss and become crumbly.

A gradual increase in pH from 5.8 to 6.4 was seen during the storage period of 20 days (Fig. 2). Samples that were stored at 4ºC temperature and passive MAP had less increase (0.1) in pH during storage. Fruit juice pH is affected by alkaline and acidic compounds of fruit cells and any change in concentration of these compounds will changed the pH quickly (Wills, 1998). Most common pH values for dates range from 5.3 to 6.3. In addition a definite correlation was observed between increasing pH and commercial quality for Deglet Noor dates (Barreveld, 1993).

With prolonged storage period, titratable acidity of fruits increased significantly (102.3 to 141.1 mg/100g), although passive MAP packaging (114.9 mg/100g) and storage at 4ºC (113.8 mg/100g) were the most effective in maintaining the titratable acidity of fruits during the storage period (Fig. 3). Organic acids are a useful index of authenticity in fruit products (Camara et al., 1994). The organic acid composition of fruits is also of interest because of its influence on the sensory properties of fruits, even though they are minor components of fruits, in combination with sugars (Wang et al., 1993). Major organic acids that have been isolated from date flesh are citric-, malic- and oxalic acid, however, generally during growth and maturation of date fruit the acid content tends to go down. Upon storage, and more specifically at the onset of deterioration, second generation organic acids are formed (Barreveld, 1993). This report supports our experimental data that with increasing storage period titratable acidity increased from 102.3 to 141.1 mg/100g of fruit flesh.

Firmness of fruits was significantly affected by type of packaging and storage temperature. Date fruits at Khalal stage had a hard and crisp texture. When they changed to Rutab, fruit firmness decreased. In the present study, treatments that caused minimum change to Rutab had fruits with maximum firmness. Fruits in the passive MAP treatment, when stored at 4ºC, had the highest firmness (5.8 kg and 5.6 respectively), and in all treatments, increased storage duration, gradually decreased firmness (7.1 to 3.3 kg during 20 days). Khalal fruits that lose their hard and crisp texture have reduced quality and attract lower prices. Softening of fruit texture is related to activation of pectin decomposing enzymes such as poly galactronase (PG) which hydrolyses α (1-4) linkage between galactronic acid residues in pectins (Wills, 1998). Changed gas mixture surrounding fruits under modified atmosphere packaging caused slowing enzymatic reactions of fruits such as softening (Kader et al., 1989).

TSS was increased in all treatments except in the passive MAP packaging stored at 4ºC (Fig. 5). TSS is one of the most important maturity and quality indices in many fruits. In general, soluble solid content of date fruits at Khalal stage should be in excess of 30%. With increased fruit development, TSS significantly increases (Behbahani, 2003).

Fully mature Khalal fruits gradually changed to low quality Rutab fruits. The percentage of Rutab fruits at the 3 evaluation times (0, 10 and 20 days) were 0, 18.9 and 44.3%, respectively (Fig. 5). There was a direct correlation between stage of growth and fruit soluble solids content. In all treatments, as storage period increased, higher proportions of fruits changed to Rutab and TSS was increased from 29.1 to 39.9 % by 20 days storage (Fig. 5). Lesser increases in TSS and Rutab% were observed in the passive MAP treatment stored at 4ºC (32.1 and 32.2 %, respectively) suggesting that in these treatments, enzymatic reactions interfering in fruit softening and senescence were retarded. This result was consistent with the report of Kader et al. (1989) for several fruits and vegetables.

In all packaging and at all storage temperatures percentage of crumbled fruits and weight loss significantly increased with increased storage time, (Fig. 6). Fruits at Khalal stage have high water content and active metabolism so unfavorable factors that cause water evaporation from fruits, increase weight loss and percentage crumbled fruits. In the control fruits (without packaging) stored at 25ºC, fruits had highest weight loss (7.52 and 4.1%, respectively) and crumbled fruits (9.5 and 5.1%, respectively). By contrast, in packaged fruits (passive MAP and vacuum), less than 1% crumbed fruits and weight loss were observed.

CONCLUSIONS

The present work is the first study on the shelf life of date fruits at Khalal stage in Iran. The results showed that package type and storage temperature had significant effects on quality characteristics and shelf life of date fruits. Of all the studied treatments, passive MAP packaging resulted in the best maintenance of quality characters that were measured. It is possible that fruit respiration changed the gas mixture inside the MAP package, elevated CO_2 concentration and reduced O_2 levels, thereby retarding fruit metabolism. Vacuum packages had minimum weight loss and percent crumbled fruits but percentage of Rutab and fruit softening was higher, so this package type is not recommended for Khalal dates. Storage of Khalal dates under low temperatures e.g. storage in a refrigerator would retard metabolic reactions, respiration rate and ethylene production and help to prolong shelf life of the fruit.

Some micro-organism contamination was observed in the passive MAP packages stored at 25ºC, however the type of bacteria or fungus that cause contamination was not identified. It seems that high humidity and temperature activate the spores of fungus that are present in the surface of the fruits. This type of microbial contamination was not observed until 15 days after storage.

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Figures

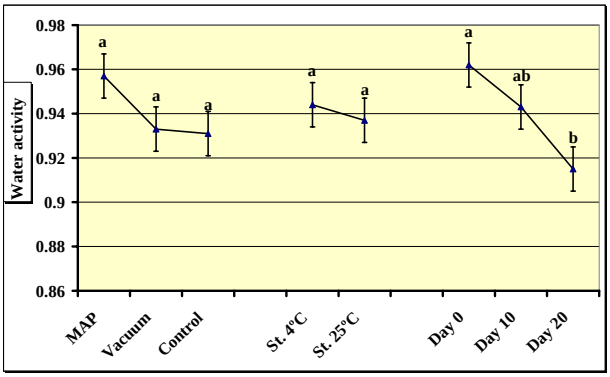

Fig. 1. Effect of package type, storage temperature and storage period on water activity

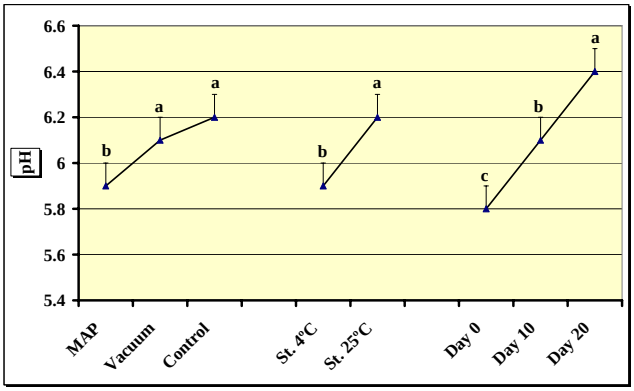

Fig. 2. Effect of package type, storage temperature and storage period on fruit juice pH.

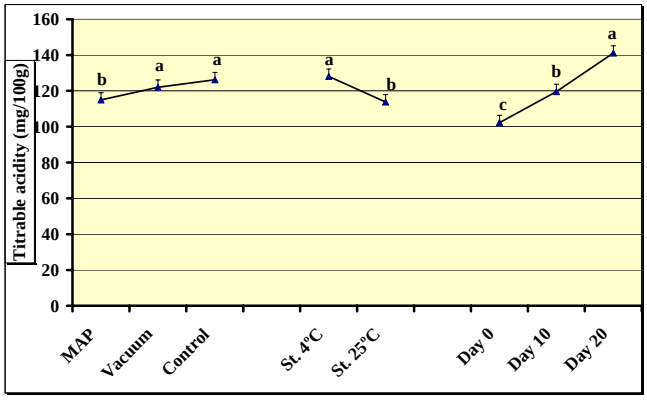

Fig. 3. Effect of package type, storage temperature and storage period on titratable acidity.

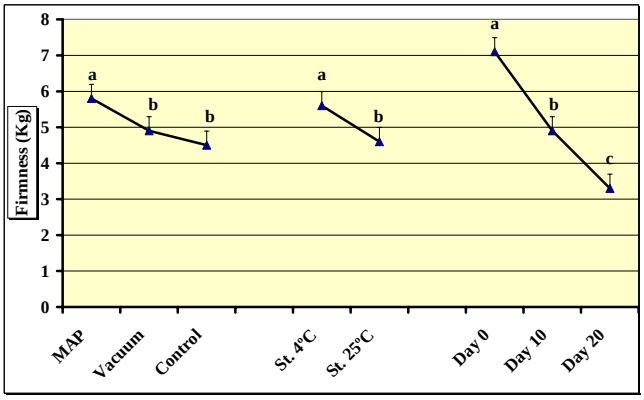

Fig. 4. Effect of package type, storage temperature and storage period on flesh firmness.

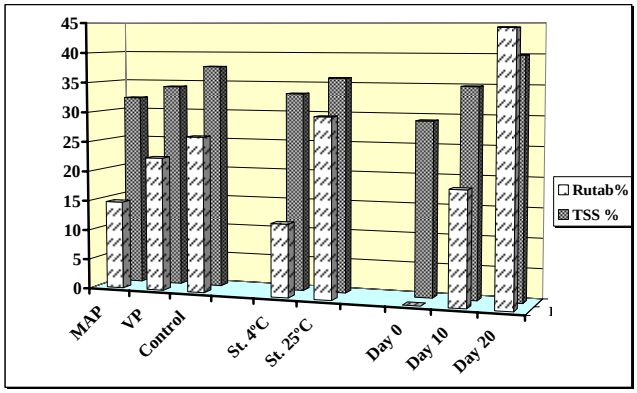

Fig. 5. Effect of package type, storage temperature and storage period on TSS (%) and Rutab fruits (%).

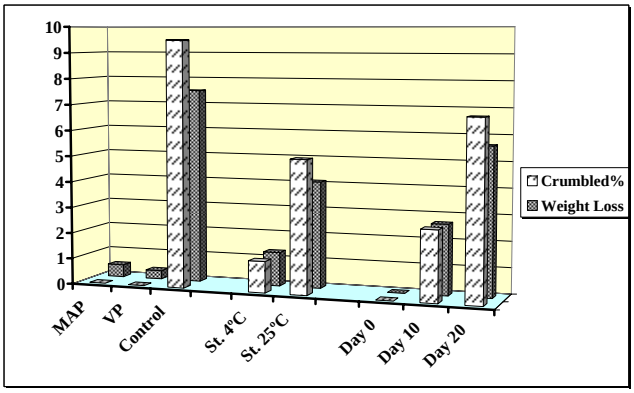

Fig. 6. Effect of package type, storage temperature and storage period on weight loss (%) and crumbled fruits (%).

Functional Properties of Omani Dates (*Phoenix dactylifera* L.)

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Keywords: Proximate composition, dietary fiber, antioxidant, carotenoids, phenolics, phenolic acids

Abstract

Three sun-dried date varieties grown in Oman, namely Fard, Khasab, and Khalas, were examined for their proximate composition and functional constituents; dietary fiber, selenium, antioxidant, carotenoids, phenolics as well as phenolic acids. The study was conducted on dried dates due to their higher consumption compared with fresh dates. All results are expressed as mean value ± standard deviation (*n*= 3) on a fresh weight basis. Date varieties were found to be low in fat and protein, but rich in dietary fiber and antioxidants. They were found to be a high source of energy (278-301 kcal/100 g), due to the high carbohydrate content. Total dietary fiber content of dates varied from 6.26 to 8.44 g/100 g, of which 84-94% was insoluble fiber. Dates were found to be a good source of antioxidant constituents including selenium (0.356 to 0.528 mg/100 g), total antioxidants (8212-12543 µmol of Trolox equiv/g), carotenoids (0.92-2.91 mg/100 g), and phenolics (217-343 mg of ferulic acid equiv/100 g). Date varieties had different levels and patterns of phenolic acids. Nine phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, and *o*-coumaric acid) were tentatively identified. Ferulic acid was the major phenolic acid for all date varieties. The total content of phenolic acids ranged between 20.24 mg/100g for Khasab and 63.41 mg/100g for Khalas. Of the date varieties studied, Khalas, which is considered to be of premium quality, had higher antioxidant activity, total carotenoids and phenolic acids than other varieties. These results suggest that all date varieties serve as a good source of natural antioxidants and could potentially be considered as a functional food or functional food ingredient.

INTRODUCTION

Dates *(Phoenix dactylifera* L.) are produced largely in the desert regions of the world and are marketed globally as a high-value fruit. The world production of dates has increased from 2.8 million tons in 1985 to 6.8 million tons in 2004 (FAO, 2005). The major date producers in the world are situated in the Middle East and North Africa, including Oman. Date production in Oman has greatly increased over the past two decades, and the latest estimate was 231 000 metric tons in 2004 (MAF, 2005), which constituted 3.4% of total global production. According to the Omani Ministry of Agriculture and Fisheries (MAF, 2005), date palm cultivation accounted for 49% of the total cultivated land and date production represented 81% of the total fruit production. The per capita daily consumption of dates in Oman is estimated at 55-164 g (MAF, 2005), and dates are considered to be a vital component of the daily diet. In Oman, dates are consumed either fresh (30-40%) or sun-dried (60-70%). Sun-dried dates are consumed throughout the year, but their use reaches a peak during Ramadan, for breaking the fast before eating. It is, therefore, of prime importance to examine the nutritional and functional characteristics of sun-dried dates.

Dates are rich in certain nutrients and provide a good source of rapid energy due to their high carbohydrate content (70-80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body (Ahmed et al., 1995; Myhara et al., 1999). The high nutritional value of dates is also based on their

dietary fiber content, which makes them suitable for the preparation of fiber-based foods and dietary supplements. Dietary fiber has important therapeutic implications for certain conditions such as diabetes, hyperlipidemia, and obesity and may exhibit a protective effect against hypertension, coronary heart disease (CHD), cholesterol, colorectal and prostate cancers and intestinal disorders (Anderson and Bridges, 1988; Tariq et al., 2000). Dates have also been reported to serve as a good source of essential minerals (Al-Showiman et al., 1994; Mohamed, 2000; Al-Shahib and Marshall, 2003).

High fruit and vegetable consumption is associated with a reduced risk of several chronic diseases such as cancer, cardiovascular disease, coronary heart disease, and artherosclerosis, as well as neurodegenerative disease and inflammation (Anderson and Bridges, 1988). The compounds thought to be responsible for the protective effects of a fruit and vegetable-rich diet include carotenoids and antioxidant vitamins. However, there is growing evidence that other phytochemicals (non-nutritive components) contribute to varying degrees to the antioxidant activity of individual fruits and vegetables. In this regard, attention has been focused on the significance of phenolics such as phenolic acids, flavonoids, and in particular anthocyanins (Surth, 2003; Shahidi and Naczk, 2004; Rice-Evans, 2001). Antioxidants can be classified into two groups according to their solubility; hydrophilic antioxidants (water-soluble), such as the majority of phenolic compounds and ascorbic acid, and lipophilic antioxidants (fat-soluble) such as carotenoids and vitamin E (Namiki, 1990).

Detailed information about the health-promoting components of dates could lead to a better understanding and an increased consumption, including their use as functional foods and ingredients in nutraceuticals, pharmaceuticals, and medicine. The objectives of this research were to examine the functional characteristics of three native Omani date varieties. The health aspects of these components, where possible, are discussed.

MATERIALS AND METHODS

Date Samples

Sun-dried date varieties were procured from a local farm in Fanjh, Oman, at the beginning of the 2003 harvest season. Khalas is regarded as being of premium quality due to its sensory qualities and high price. The sensory quality and price of Khasab are lower than those of Khalas. Fard is known as an industrial quality date and is mainly used for processing purposes. Upon arrival at the laboratory, the samples (100-150 g portions) were packed in polyethylene bags, sealed and stored at –30 °C until analyzed.

Proximate Analysis

Percentages of moisture by vacuum oven (method 934.06), protein by Kjeldahl nitrogen (method 920.152), and ash by direct analysis (method 940.26) were determined according to the Association of Official Analytical Chemists methods (AOAC, 1995). The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25 (AOAC, 1995). The Bligh and Dyer method (Hanson and Olley, 1963) was used to determine the lipid content. Total carbohydrates were calculated by subtracting the total percent values of other measurements from 100. The energy value was calculated according to the method of the Ministry of Agriculture, Food, and Fisheries (MAFF, 1995). Available carbohydrates were calculated by subtracting the total dietary fiber from total carbohydrates.

Dietary Fiber Analysis

Determination of dietary fibers was carried out using the AOAC enzymatic-gravimetric official method 991.43 (AOAC, 1995). Contents of crude protein (nitrogen x 6.25) and ash determined by using the methods described above were used to correct the fiber content. Dietary fiber was expressed as grams per 100 g of fresh weight.

Measurement of Oxygen Radical Absorbance Capacity (ORAC)

An improved ORAC method of Ou et al. (2001) using fluorescein (FL) as the fluorescent probe was used with slight modifications. The $ORAC_{FL}$ assay measures the ability of antioxidative compounds in test materials to inhibit the decline in fluorescence induced by free radical generator (AAPH). The $ORAC_{FL}$ values were calculated according to the method of Wang et al. (1996) and $ORAC_{FL}$ values are expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

Measurement of Total Phenolics

Total phenolics were determined colorimetrically using Folin-Ciocalteau reagent as described by Velioglu et al. (1998) with slight modification using a UV-1601 spectrophotometer (Shimadzu). The concentrations are expressed as milligrams of ferulic acid equivalents (FAE) per 100 g of fresh weight.

Measurement of Total Carotenoids

Total carotenoids were extracted according to the method of Talcott and Howard (1999) with slight modifications using a UV-1601 spectrophotometer (Shimadzu). Total carotenoids were calculated according to the method of Gross (1991) and expressed as milligrams per 100 g of fresh weight.

Extraction, Hydrolysis, Identification, and Quantification of Phenolic Acids

Phenolic acids in date varieties were determined according to the high-performance liquid chromatographic method of Mattila and Kumpulainen (2002) and Alasalvar et al. (2005). Extraction, hydrolysis (alkaline and acid), identification, and quantification of phenolic acids together with HPLC column, pump, diode array detector, and auto-sampler used were the same as those described in a previous study (Alasalvar et al., 2005). Phenolic acids are expressed as milligrams per 100 g of fresh weight.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD) (n = 3) on a fresh weight basis. Statistical significance (t test: two-sample equal variance, using two-tailed distribution) was determined using the Microsoft Excel Statistical Data Analysis. Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Proximate Analysis

The proximate composition and caloric value of three Omani date varieties are summarized in Table 1. Carbohydrate was the predominant component in all varieties, ranging from 77.13 g/100 g in Fard to 83.41 g/100 g in Khalas, followed by moisture, along with small amounts of protein, fat, and ash. Significant ($p < 0.05$) varietal differences existed in proximate composition among varieties, with some exceptions. These values were within the range of results previously published in the literature (Ahmed et al., 1995; Al-Shahib and Marshall, 2003). The energy values determined ranged from 278 kcal/100 g in Fard to 301 kcal/100 g in Khalas, due to their high carbohydrate content (mainly sugar). Similar energy values for different date varieties were reported by other researchers (Ramadan, 1990; Rabie, 2003). The energy requirement of adult men ranges from 2300 to 2900 kcal/day and is 1900-2200 kcal/day for adult women (RDA, 1989). Hence, a typical portion of 100 g of dates supplies approximately 11-15% of the total energy requirement per day for adults.

Dietary Fiber

Table 2 presents the content of dietary fiber (insoluble, soluble, and total) in dates. Total dietary fiber contents in Fard, Khasab, and Khalas were 8.00, 8.44, and 6.26 g/100g, respectively. Significant differences ($p < 0.05$) existed in insoluble, soluble and total

dietary fiber contents among varieties. Insoluble and soluble fibers contributed 84-94 and 6-16% to the total dietary fiber present among varieties, respectively. Recently, Al-Shahib and Marshall (2003) who surveyed the total dietary fiber contents of 14 date varieties from various countries, found that the percentage of total dietary fiber was in the range of 6.4-11.5%, depending on variety and degree of ripeness. Our results were within the range of their findings. The contents of dietary fiber, obtained by using the Prosky method, in dried apricots, prunes, figs, and raisins were 7.7, 8.0, 12.2, and 5.1 g/100 g, respectively (Marlett et al., 1994; Vinson, 1999; Camire and Dougherty, 2003). Thus, dates serve as a good source of fiber compared with other dried fruits.

Although no RDA has been set, most health/nutrition professionals agree on the benefits of increased consumption of dietary fiber to 25-35 g/day (Dreher, 1987). Eating approximately 330-460 g of dates per day is adequate for this requirement. Dietary fiber (indigestible carbohydrate) is not a nutrient, but still plays a very important role in maintaining good health (Anderson and Bridges, 1988). Soluble fiber dissolves in the gut to form a viscous gel that slows the release of some nutrients, particularly glucose, into the bloodstream. Blood cholesterol is a major risk factor for CHD, and increasing consumption of dietary fiber has been recommended as a means to lower cholesterol levels. In addition, many studies have found that high-fiber diets, especially those high in soluble fiber, can reduce the risk of prostate cancer (Tariq et al., 2000). The high content of insoluble fiber is of benefit in weight control and the health of the large intestine. It is present at high levels in dates and has a sponge-like effect in the gut, soaking up water and swelling in size. This effect produces a feeling of fullness and adds bulk to the gut contents, increasing the mass frame waste matter and speeding it through the large intestine, thus reducing the risk of constipation and possibly even cancers of the digestive system (Nicklas et al., 1995).

Selenium

Dates were also found to be a rich source of selenium (0.356-0.528 mg/100 g, table 3). These results are, in general, comparable with those published previously on different date varieties (Al-Showiman et al., 1994; Rabie, 2003). With regard to human nutrition, all date varieties have significant selenium content. Eating approximately 13.4 and 17 g of dates per day supplies 100% of the recommended dietary allowances (RDA) of selenium (RDA, 1989) for adult women and men, respectively. Selenium plays a major antioxidant role, protects cell membranes by preventing free radical generation, thereby decreasing the risk of cancer and diseases of the heart and blood vessels. Medical surveys have shown that increased selenium intake decreases the risk of breast, colon, lung, and prostate cancer and may preserve tissue elasticity (Oldfield, 1991).

Total Antioxidant

The total antioxidant content of dates measured using the $ORAC_{FL}$ assay of dried dates are shown in Table 4. The total antioxidant content ranged from 8212 to 12543 μM/g. Khalas had the highest content of antioxidant followed by Fard and Khasab. The antioxidant content of bilberry and elderberry when determined by the $ORAC_{FL}$ assay were found to be 2646 and 2221 μM/g, respectively (Ou et al., 2001). Thus in comparison to these fruits, dates are a rich source of antioxidants. This finding is supported by Guo et al. (2003). Although these researchers used different assay methods, which make the quantitative comparison invalid, Guo et al. (2003) reported that dates had the second highest antioxidant value of 28 fruits commonly consumed in China.

Total Phenolics

The mean total content of phenolics ranged from 217 to 343 mg of FAE/100 g in sun-dried date varieties (Table 4). Of the varieties studied, Fard had the highest amount of total phenolics. Mansouri et al. (2005) studied the phenolic profiles of seven different varieties of ripe date fruits grown in Algeria. They found that total phenolic content ranged from 2.49 to 8.36 mg/100 g of fresh weight, expressed as gallic acid equivalents.

These levels are much lower than those found in this study. Use of different phenolic acid standards (ferulic acid and gallic acid, which make the quantitative comparison invalid) or various factors such as variety, growing condition and maturity, among others, might be responsible for the observed differences.

However, there are many data available for total phenolics in other fruits including: 18-54 mg/100g yellow nectarines; 28-111 mg/100g white peach; 21-61 mg/100g yellow peach; 42-109 mg/100g plum; 694-3820 mg/100g blackcurrant; 114-178 mg/100g raspberries and 181-458 mg/100g berries (Prior et al., 1998; Gil et al., 2002; Moyer et al., 2002). In comparison to these examples, dates may be considered to be a rich source of total phenolics. The average consumption of dates (164g) provides up to 563 mg of total phenolics to the Omani diet.

Total Carotenoids

The carotenoid content of dried dates ranged from 0.92 to 2.91 mg/100g (Table 4). Khalas dates had the highest carotenoid content followed by Fard and Khasab. The total carotenoid content in dates varied significantly ($P < 0.05$) especially between the yellow and red coloured varieties. The high carotenoid content of Khalas dates was expected, as this is a yellow variety, whereas the other two are red. Fruits that are yellow-red usually contain hydrocarbon carotenoids such as lycopene, neurosporene, gamma-carotene, delta-carotene, alpha-carotene, beta-carotene, phytofluene and phytoene. The yellow-orange coloured fruits contain, in addition to the carotenoids listed above, a complex mixture of carotenol fatty acid esters (Fennema, 1996).

The available published value for carotenoid content of dates was reported by Ben-Amotz and Sishler (1998) who reported a carotenoid concentration of an un-named date variety of 0.22 mg/100g. Their value is much lower than those recorded in this study, probably due to the differences between the two samples in variety, maturation, storage and analysis conditions. Typical carotenoid concentrations in other fruits range from 0.007 mg/100g for white nectarines to 10.30 mg/100g in papaya (Ben-Amotz and Sishler, 1998; Gil et al., 2002). Therefore, dates can be considered a moderate source of carotenoids compared to other fruits.

Phenolic Acids

The content of total phenolic acids (free and bound) in date varieties are listed in Table 5. Free and bound phenolic acids were extracted using three different procedures (methanol, alkaline and acid) within the same tube. A total of nine phenolic acids were detected, of which five consisted of hydroxylated derivatives of benzoic acid (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, and syringic acid) and four were cinnamic acid derivatives (caffeic, *p*-coumaric, ferulic, and *o*-coumaric acid). The Khalas variety was richest in total phenolic acids (63.41 mg/100g) followed by Fard (35.42 mg/100g) and Khasab (20.24 mg/100g). Ferulic acid was the predominant phenolic acid in date varieties. Regnault-Roger et al. (1987) studied phenolic acids in dried Tunisian dates and found eight phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid and ferulic acid). Although this study showed a similar phenolic acid profile, the concentrations of phenolics was much higher than those reported earlier. Recently, Mansouri et al. (2005) studied phenolic profiles of seven different varieties of ripe date fruits grown in Algeria and found that all varieties contained *p*-coumaric acid, ferulic acid, and sinapic acid as well as some cinnamic acid derivatives, but these were not quantified. Many factors (such as location, environmental characteristics and fruit maturity) have been reported to influence the content and variability of phenolic compounds within the same fruit type (Goncalves et al., 2004). The contents of phenolic acids in dates examined in this study were comparable with those previously reported for other fruits (Mattila and Kumpulainen, 2002; Alasalvar et al., 2005). It has been reported that caffeic acid, sinapic acid, ferulic acid, and *p*-coumaric acid are more antioxidative than protocatechuic acid, syringic acid and vanillic acid (Cuvelier et al., 1992). Because dates were found to be a good source of

the more active phenolic acids, they may be considered as a good source of natural antioxidants. Besides their naturally occurring antioxidant properties, phenolic acids can also influence product flavor and color.

CONCLUSIONS

The results presented in this work suggest that date varieties serve as a good source of dietary fiber and natural antioxidative compounds that could potentially be used in food and nutraceutical formulations. Although it is difficult to assess, the Khalas variety, which is considered to be of premium quality, had higher antioxidant activity, total carotenoids and phenolic acids than other varieties studied. The observed differences among varieties may relate to the existing differences in their moisture content and/or sun-drying induced changes. Although determination on a dry weight basis may overcome the issue of moisture difference, fresh weight calculation was deemed to be more appropriate from a consumption viewpoint, and this clearly represents the nutritional benefits of these fruits.

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Tables

Table 1. Proximate composition (g/100g) and caloric value of dried dates[a].

Component	Fard	Khasab	Khalas
Moisture	18.5 ± 0.23 [b]	16.5 ± 0.17 [c]	12.6 ± 0.25 [d]
Protein	1.47 ± 0.02 [b]	1.61 ± 0.03 [c]	1.68 ± 0.17 [c]
Fat	1.41 ± 0.18 [b]	0.98 ± 0.16 [c]	0.52 ± 0.15 [d]
Ash	1.49 ± 0.04 [b]	1.59 ± 0.03 [c]	1.79 ± 0.02 [d]
Carbohydrates	77.13 ± 0.47 [b]	79.32 ± 0.39 [b]	83.41 ± 0.59 [b]
Energy	278 [b]	281 [b]	301 [d]

[a]Data are expressed as mean ± SD ($n = 3$) on a fresh weight basis. Means ± SD followed by the same letter, within a row, are not significantly different ($p > 0.05$).

Table 2. Dietary fibre content in dried dates (g/100g)[a].

Dietary fibre	Fard	Khasab	Khalas
Insoluble	6.73 ± 0.14 [b]	7.36 ± 0.23 [c]	5.89 ± 0.07 [d]
Soluble	1.27 ± 0.12 [b]	1.08 ± 0.12 [c]	0.37 ± 0.02 [d]
Total	8.00 ± 0.23 [b]	8.44 ± 0.17 [c]	6.26 ± 0.07 [d]

[a] Data are expressed as mean ± SD ($n = 3$) on a fresh weight basis. Means ± SD followed by the same letter, within a row, are not significantly different ($p > 0.05$).

Table 3. Selenium contents of dried dates (mg/100g)[a].

Varieties	Fard	Khasab	Khalas
Selenium (Se)	0.380 ± 0.011 [b]	0.356 ± 0.020 [c]	0.528 ± 0.038 [d]

[a] Data are expressed as mean ± SD (n = 3) on a fresh weight basis. Means ± SD followed by the same letter, within a row, are not significantly different ($p > 0.05$).

Table 4. Contents of antioxidants, phenolics and carotenoids in dried datesa.

	Fard	Khasab	Khalas
Total antioxidants (µmol of TE/g)	9986 ± 765 [b]	8212 ± 515 [c]	12543 ± 339 [d]
Total phenolics (mg of FAE/100g)	343 ± 7 [b]	217 ± 2 [c]	339 ± 3 [b]
Total carotenoids (mg/100g)	1.19 ± 0.11 [b]	0.92 ± 0.11 [c]	2.91 ± 0.05 [d]

[a] Data are expressed as mean ± SD (n=3) on a fresh weight basis. Means ± SD followed by the same letter, within a row (Fard, Khasab, and Khalas), are not significantly different ($p > 0.05$).

Table 5. Content of total phenolic acids in dried dates (mg/100g) [a].

Phenolic acids	Fard	Khasab	Khalas
Gallic acid	1.60 ± 0.02 [b]	nd	3.09 ±0.16 [c]
Protocattechuic	8.34 ±0.16 [b]	6.48 ±0.32 [c]	nd
p-hydroxybenzoic	nd	nd	nd
Vanillic	3.82 ±0.08 [b]	2.18 ±0.02 [c]	6.4 ±0.06 [d]
Caffeic	nd	nd	7.57 ±0.45
Syringic	9.24 ±0.76 [b]	nd	7.09 ±0.02 [c]
p-coumaric	1.41 ±0.14 [b]	1.71 ±0.16 [c]	14.19 ±0.31 [d]
Ferulic	11.01 ±0.25 [b]	7.95 ±0.15 [c]	18.36 ±0.43 [d]
o-coumaric	nd	1.92 ±0.02 [b]	6.71 ±0.11 [c]
Total	35.42 ±2.25 [b]	20.24 ±0.69 [c]	63.41 ±4.45 [d]

[a] Data are expressed as mean ± SD (n = 3) on a fresh weight basis. Means ± SD followed by the same letter, within a row (Fard, Khasab, and Khalas), are not significantly different ($p > 0.05$). nd, not detected.

Some Physical and Chemical Properties of Three Varieties of Libyan Soft Dates

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Abstract

This work presents the results of a study on some physical and chemical properties of three varieties of Libyan soft dates (called Rutab in Arabic) widely distributed in coastal regions, namely Tabouni, Ammi and Brunsi. The fruits ranged in weight from 11.25 g for Tabouni to 17.69 g for Brunsi, while their lengths ranged between 3.30 cm for Tabouni and 4.32 cm for Brunsi. Fruit diameter ranged from 1.65 cm in Ammi to 2.19 cm in Tabouni. The pH values in these varieties were 5.32, 6.20 and 7.02 for Tabouni, Ammi and Brunsi, respectively, while the acidity levels reached 0.28, 0.21 and 0.03 % as citric acid, respectively. It was found that the differences in moisture content between the three studied varieties of Libyan soft dates were significant ($p < 0.05$). All varieties had distinctly high contents of moisture, but the highest moisture content was recorded in Brunsi (54.12 %), followed by Tabouni (48.04 %) and the lowest was in Ammi (43.29 %). Content of total sugars varied between the varieties. The highest concentration was found in Ammi (49.49 %) followed by Tabouni (38.54 %), while the lowest concentration was recorded in Brunsi (35.87 %). Results indicated that there were significant differences in protein concentration between the three varieties: the highest concentration was found in Tabouni (2.69 %), followed by Ammi (1.62 %) then Brunsi (1.47 %). The lipid contents were markedly low in all varieties and ranged from 0.18 to 0.39 %. The ash content ranged from 1.22 to 1.37 %, while the fiber contents were relatively high and reached 9.70 % in Tabouni. These results confirmed the high nutritional value of these soft date varieties, and their high content of moisture compared to other dates. The high moisture content of soft dates is thought to accelerate their spoilage and deterioration.

INTRODUCTION

Date palm trees are widely distributed along the coastal area of Libya, including the major cities of Sabratha, Al-Zaweah, Tripoli, Tajoraa, Al-Khoms, Zlaiten, Musrata, Tawargaa and Darnah. The number of palm trees in these regions reaches more than 2.5 million, of which more than 40% are productive. Date palm trees are also distributed in the central regions including Al-Wahat (oases) and Al-Joufra. These areas contain about 750 thousand fruiting palm trees including the semi-dry and semi-soft varieties of Dejlat noor, Hamrawee, Khadhray, Saeide, Adhwee, Abble and Bestian. In addition, the southern areas which include large cities like Sabha, Al-Shatt, Mourzog, Wadi Al-Hyatt and Ghatt contain about 2.1 million fruiting palm trees, most of which are dry and semi-dry varieties. The most famous among these varieties are Tasfert, Talees, Adhwee, Aureeg, Taghyatt, Teen and Yeedy (Ahmed, 2000).

Dates are consumed in the coastal areas at the beginning of the production season when they are at the Khalal (Balah) and Rutab stages. The varieties which are consumed during the Rutab stage are Tabouni, Ammi and Brunsi. This is due to their good flavor, the relative decrease in sugar content and increase in moisture content compared to dates at the final stage of ripening. These varieties are also characterized by the lower percentage of tannins which are the compounds thought to be responsible for the astringent taste.

Rutab varieties are considered to have fruit with a short shelf life because they contain an elevated moisture content which make them a suitable medium for the activities of microorganisms. They are also highly vulnerable to insect attacks which

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

accelerate spoilage and decay. These undesirable changes cause substantial economic loss for the producing and exporting countries. In Libya the coastal regions experience elevated relative humidity, which ranges between 60–70 %, and the falling of autumn rains in quantities exceeding 50 millimeters during the production season and these factors exacerbate the problem. Accordingly, the surplus production is dried for pressing and the manufacture of date syrup (Rutab). Because of the scarcity of information associated with Libyan Rutab varieties, this work was conducted to study some of the physical characteristics and the chemical composition of three economically important Libyan Rutab varieties, namely Tabouni, Ammi and Brunsi, which represent the most important and widely consumed of the local date varieties.

MATERIALS AND METHODS

Sampling

Samples were collected randomly from the available Rutab varieties in the coastal region, which were Tabouni and Ammi from Al-Khoms city and Brunsi from Suq-Al-Goumaa region in Tripoli city. The samples were picked directly and packaged aseptically and separately in high density polyethylene containers (Al-Ahmar, 2004). Directly after sampling, containers were transferred to the laboratory, under refrigerated conditions, for physical and chemical analyses.

Determination of the Physical Characteristics of Fruits

Ten fruits of each variety were randomly selected and weighed using a sensitive balance. A caliper was used to measure the length and diameter of fruits, then the mathematical mean and standard deviation were calculated.

Chemical Analyses

For assessment of moisture content, total sugars, sucrose, protein, lipid, ash and crude fibers, the standard approved methods (AOAC, 1995) were used. The pH values were measured using a pH meter, JENWAY 3030.

Acidity was assessed as citric acid by titration with 0.1 N NaOH and calculated according to the formula:

$$\% \text{ Acidity} = \frac{\text{Volume of titrated NaOH} \times 0.007005}{\text{Weight of sample}} \times 100$$

Statistical Analysis

Results obtained were statistically analyzed using the complete randomized design (CRD). Duncan multiple-range test was carried out to differentiate means using SAS system (Statistical Analysis System) from the University of Colorado according to the mathematical model:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + O_{ijk}$$

Where :
μ is the population mean
A_i is the effect of variety
B_j is the effect of storage temperature
$(AB)_{ij}$ is the effect of interaction of factors
and O_{ijk} is the experimental error (Al-Sahooke and Waheb, 1990)

RESULTS AND DISCUSSION

Physical Characteristics

Mean weight of fruits, fruit body and pit, in addition to length and diameter of fruits from Tabouni, Ammi and Brunsi date varieties are presented in Table 1. The weight of fruits ranged from 11.25 g for Ammi fruits to 17.64 g for Brunsi fruits. This increase was reflected in the relative weight of fruit body and pit. The length of fruits ranged from 3.30 cm for Tabouni to 4.32cm for Brunsi variety. Fruit diameters were from 1.65cm in Ammi to 2.19cm in Tabouni fruits. These figures indicated that Brunsi fruits are of an elongated shape and they were the tallest and heaviest in comparison to fruits from the two other varieties, Tabouni and Ammi. By contrast, Tabouni fruits tended to be more rounded in shape as indicated in Figures 1-3.

Chemical Composition

1. Acidity and pH. The results showed that the mean values of relative acidity in Tabouni, Ammi and Brunsi fruits were 0.28, 0.21 and 0.03 % as citric acid, respectively. The pH values were 5.32, 6.20 and 7.02, respectively. Hamad et al. (1986) and Rygg (1948) both reported that pH values of high quality date fruits lay between 6.56 to 6.72. Compared to their results, current results showed that pH values in Tabouni fruits were lower, not exceeding 5.32, and Ammi fruits showed a pH of 6.20. However, the aforementioned studies tested the fruits in the fully ripened stage (Tamr), not in Rutab stage as in the present study, and they did not assess the relative acidity.

2. Moisture Content. The results presented in Table 3 show the means of the major components in Tabouni, Ammi and Brunsi fruits. This table indicates the significant differences (at $P < 0.05$) in moisture content between the three Rutab varieties. All three varieties were characterized by a high moisture content, which was the highest in Brunsi fruits (54.12 %), followed by Tabouni fruits (48.04 %) and finally Ammi fruits which contained 43.29 % moisture.

3. Concentration of Total Sugars and Sucrose. The results presented in Table 3 indicated significant differences (at $P < 0.05$) in the contents of total sugars and sucrose between all three varieties. The maximum concentration of total sugars was recorded in Ammi fruits (49.49 %) and this was reflected clearly in the sweetness of this variety compared to the other two varieties. The second highest content was in Tabouni fruits (38.54%) and the lowest concentration of total sugars was 35.87 % in Brunsi fruits. The variations in sugar content among the date varieties depended mainly on genetic differences. On the other hand, sucrose contents were low in all three varieties and reached 0.88, 0.49 and 0.25 % for Ammi, Brunsi and Tabouni varieties, respectively. This was due primarily to the conversion of most sucrose during the Rutab stage to monosaccharides (glucose and fructose) as a result of the action of the enzyme invertase during the ripening period which is induced by the higher moisture content and warmer temperatures.

4. Protein. The results presented in Table 3 indicated significant differences (at $P < 0.05$) in protein concentrations between the three Rutab varieties, which ranged from 1.47 to 2.69 %. The highest protein content was recorded in Tabouni fruits (2.69 %), while the protein content in Brunsi and Ammi varieties were 1.62 and 1.47 %, respectively.

5. Lipid. Lipid contents in Tabouni, Ammi and Brunsi varieties were markedly low and ranged from 0.18 and 0.39 %. The results presented in Table 3 indicated significant differences (at $P<0.05$) in lipid concentrations, and it was evident that Brunsi fruits had the highest percentage of lipids (0.39%), followed by Ammi (0.21%) and finally Tabouni fruits which contained only 0.18 % lipids.

6. Fibers. Results of the present study indicated an increased fiber content in the three Rutab varieties. Table 3 showed significant differences (at $P<0.05$) in fiber concentrations, and it was clear that Tabouni fruits had the highest content of fibers (9.70%), followed by Brunsi (7.53 %) and finally Ammi fruits which contained 4.64 % fiber .

7. Ash. The ash contents in the three Rutab varieties ranged from 1.22 to 1.37 %. Table 3 indicated significant differences (at $P < 0.05$) in ash concentrations, where Ammi and Brunsi fruits had the highest contents of ash, 1.37 and 1.36 %, respectively, followed finally by Tabouni (1.22 %).

RECOMMENDATIONS

Considering the local dietary patterns, the increased interest by consumers in the dietary importance of dates and the increased consumption of Rutab fruits, the following course of action is recommended:

1. Encourage companies and small production units to improve the packaging and preservation techniques of good date varieties during Rutab stage.
2. Establish an artificial ripening unit for Rutab in one of the coastal regions, where the elevated relative humidity leads to increased spoilage rates during development from Khalal (Balah) stage into Rutab stage.
3. Perform specific chemical and microbiological studies on all Libyan Rutab varieties.
4. Design, perform and support research plans that address the problems in collecting and handling Rutab crops, in addition to determination of the optimum conditions for preservation and storage of important varieties in every production area.
5. Establish standard and reliable specifications for determining the quality of date fruits ready-to-consume during the various ripening stages.

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Tables

Table 1. Some physical characteristics of Tabouni, Ammi and Brunsi fruits at Rutab stage.

Variety	Fruit weight (gm.)	Body weight (gm.)	Pit weight (gm.)	Fruit length (cm.)	Fruit diameter (cm.)
Tabouni	13.8± 0.06	12.32±0.10	1.49±0.02	3.30±0.25	2.19±0.01
Ammi	11.25±0.18	9.66±0.06	1.58±0.09	3.86±0.09	1.65±0.18
Brunsi	17.64±0.12	15.56±0.12	2.08±0.05	4.32±0.06	2.14±0.06

***** The values are means ± standard deviation (SD)

** Means which carrying similar letters in the same column are not different significantly (at $P < 0.05$)

Table 2. Mean values of pH and acidity in Tabouni, Ammi and Brunsi fruit at Rutab stage.

Variety	pH	Acidity (%)
Tabouni	5.32	0.28
Ammi	6.20	0.21
Brunsi	7.02	0.03

Table 3. Mean values of major components in Tabouni, Ammi and Brunsi fruits at Rutab stage.

Component (%)	Variety		
	Tabouni	Ammi	Brunsi
Moisture	48.04[a]	43.29[b]	54.12[c]
Total sugars	38.54[a]	49.49[b]	35.87[c]
Sucrose	0.25[a]	0.88[b]	0.49[c]
Protein	2.69[a]	1.47[b]	1.62
Lipid	0.18[a]	0.21[b]	0.39[c]
Fiber	9.70[a]	4.64[b]	7.53[c]
Ash	1.22[a]	1.37[b]	1.36[b]

* Means which carrying similar letters in the same column are not different significantly (at $P < 0.05$)

Figures

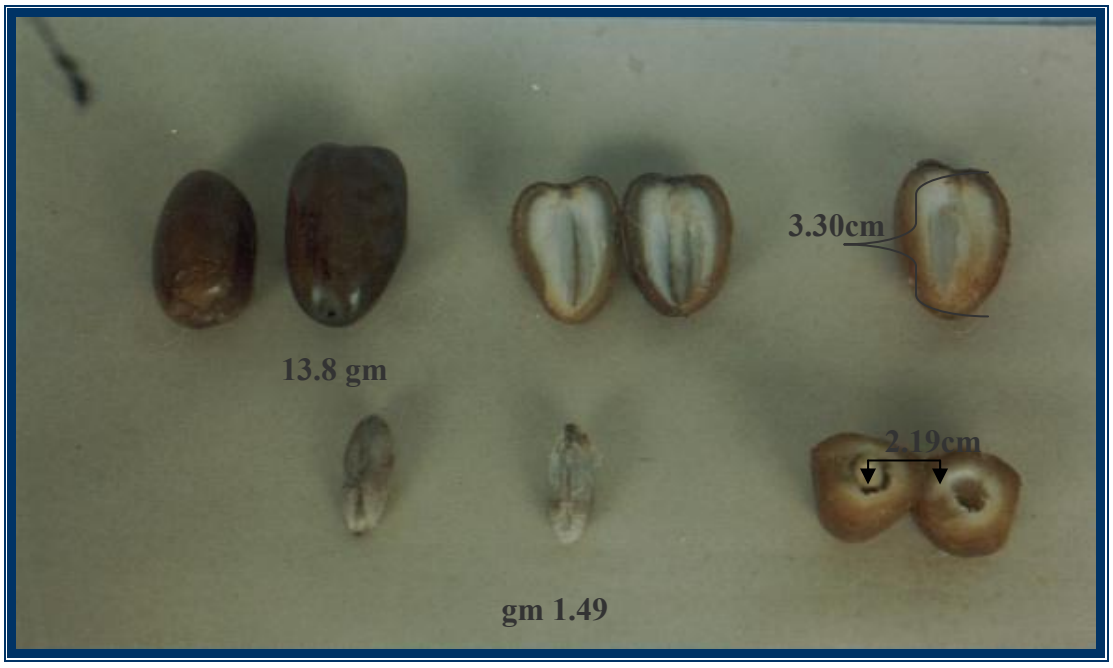

Fig. 1. General fruit morphology and pit shape in Tabouni variety at Rutab stage.

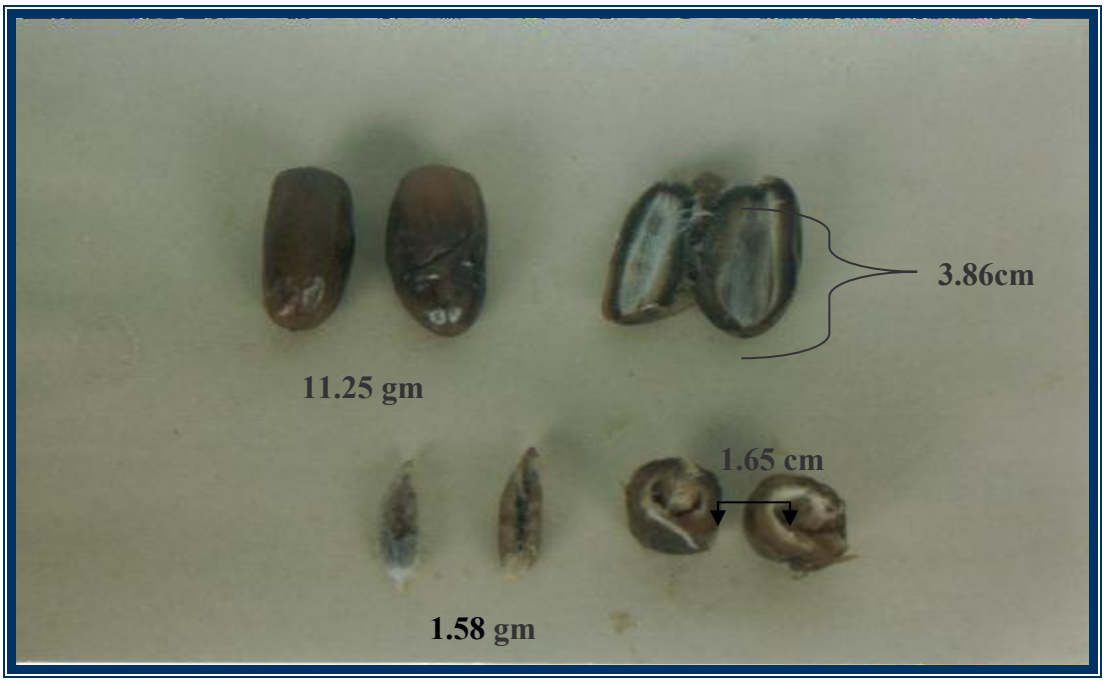

Fig. 2. General fruit morphology and pit shape in Ammi variety at Rutab stage.

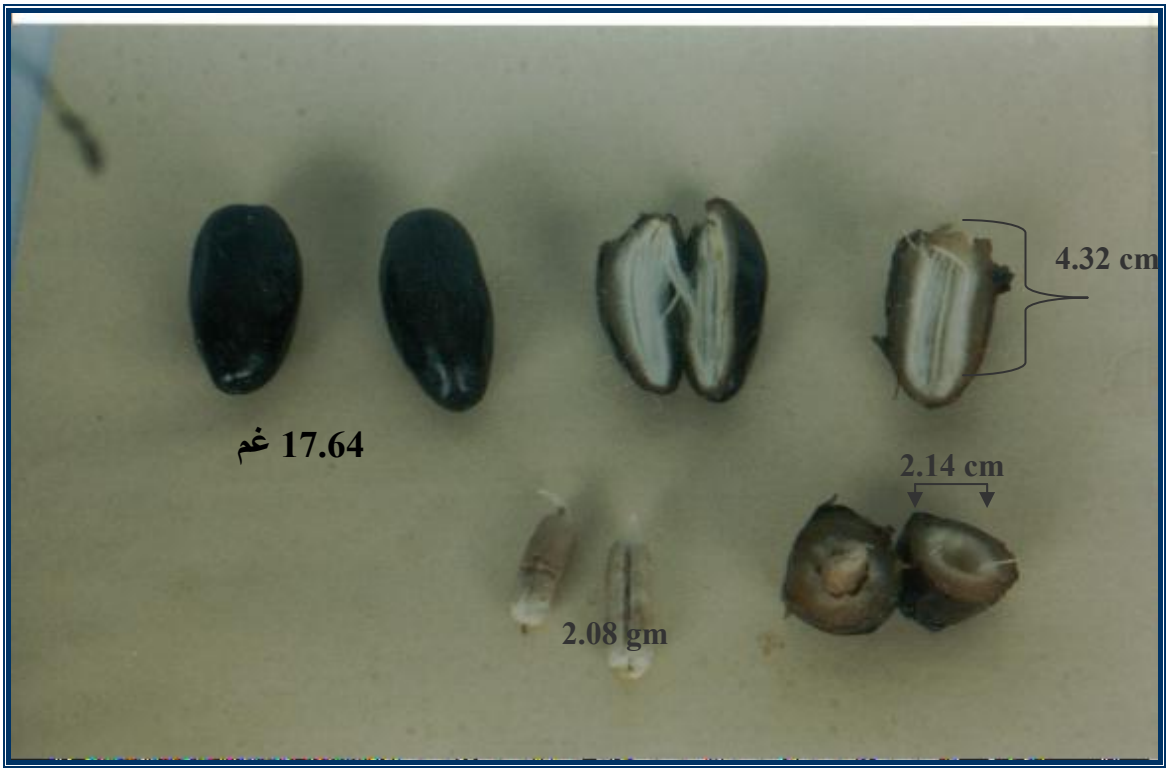

Fig. 3. General fruit morphology and pit shape in Brunsi variety at Rutab stage.

Effect of Mineral Fertilizer and Organic Peat on the Physical Characteristics of Khalas and Khassab Fruits

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Abstract

Thirty date palms of each of Khalas and Khassab cultivars at 6-7 years of age were selected randomly in Al-Fairooz Farm in Khabourah, Batina region. The aim was to improve fruit production and quality by fertilizer application. Experimental treatments were applied in a factorial design and included: organic peat alone with 1000g urea divided into 5 and 4 equal doses mixed with 500g triple superphosphate and 800g K_2SO_4 and with or without micronutrients. The control included no organic or mineral fertilizer. Mineral fertilizer tended to increase Khassab strand weight and decrease fruit number. The highest number and yield was associated with the smallest length and diameter and was produced by NPK + micronutrients and organic peat. In Khalas no significant differences were observed in fruit number, weight and size, but NPK 5 or 4 nitrogen doses with micronutrients and organic peat produced the highest yield. Four nitrogen doses with phosphate and K_2SO_4 increased Khalas yield by 22% over the control and 24.7% over organic peat. The same treatment increased Khassab yield by 77.5% over the control and 130% over organic peat. On the other hand, five nitrogen doses increased the yield of Khalas by 17.8% over the control and 20.4 % over organic peat. On Khassab it increased the yield by 63% over the control and 112.4% over peat. Five nitrogen doses in Khalas produced higher weight of strand, number of fruit per strand, weight and length of fruit and yield than 4 doses. It produced lower strand weight, fruit length and diameter, and higher yield and number of fruit per strand than 4 doses on Khassab. In both cultivars, number and weight of fruit/strand and weight of fruit were lower in Rutab than Bisir stage. However, diameter was reduced and yield was increased in Khassab, whereas in Khalas both fruit length and yield were reduced from Bisir to Rutab.

INTRODUCTION

The date palm *Phoenix dactylifera* is considered the most important crop in the Sultanate of Oman. It is an important element of the national economy, and in our heritage and social life. The consumption of dates by an Omani is 30-60 kg per year (MAF, 2004), indicating its importance in the Omani diet. Date palms occupy 35,227 hectares across the country, which is almost seventy-five percent of the area allocated to tree crops and 50% of the total cropped land of the Sultanate (MAF, 2004). There are about eight million date trees grown, consisting of more than 300 date palm cultivars, only 30 of which produce high quality dates. This is a clear indication of the problem the date industry is facing in the country (MAF, 2004). The most prevalent view in Oman is that the quality standards of dates (fresh and dried) are deficient.

Previous studies by the MAF indicated two reasons for low date quality. Firstly, farmers are not sufficiently aware of quality or the horticultural practices required to make improvement. Secondly, there is a lack of compliance with quality post-harvest and marketing operations (MAF, 2004). Most farmers do not use mineral fertilizers. Results of the 1994 Agricultural Census (MAF, 1994) showed that 74% of date growers in Batina

Proc. III[rd] IC on Date Palm
Eds: A. Zaid et al.
Acta Hort 736, ISHS 2007

region, used only manure as fertilizer, 26% cultivated their orchards and 13.7% used mineral fertilizers. The soil type in Batina is sandy and gravelly, therefore it requires frequent fertilization with organic matter and minerals because of rapid leaching. Date palm fertilization produced variable results according to location, type of fertilizer, cultivar and time of application. Organic manuring can play a valuable role as organic materials supply all plant nutrients, but are often limited. Many researchers reported that organic manure does provide the palm its full requirements to produce a good yield (Bacha and Abo-Hassan, 1983; Al Baker, 1982; Al Juburi et al., 1993; Kassem et al., 1997; Soliman and Osman, 2003; Abdullah et al., 1987; El- Hamaady et al., 1994). Most of the nutrients required for future high-yielding agriculture will have to come from mineral fertilizers, which provide a range of nutrients, not merely one or two (FAO, 1995). Bacha and Abo -Hassan (1983), Al Juburi et al. (1991), Kaseem et al. (1997) and Soliman and Osman (2003) reported an increase in date palm yield with the application of nitrogen (N) at a critical concentration. They reported that higher N than the critical concentration tended to deviate the palm toward vegetative growth. Others reported increased yield by application of potassium (Abdullah et al., 1987). Soliman and Osman (2003) reported an increase in bunch weight and total yield with the application of high N and K, whereas fruit weight, length and diameter were increased by lesser amounts of N with high K.

The main objective of this work was to study the effect of organic and mineral fertilizers on the physical characteristics and yield of Khalas and Khassab date cultivars grown in Al-Khaborah, Batina, Sultanate of Oman. The work was carried out through an integrated plant nutrient system (IPNS) approach (combined organic and inorganic fertilizer).

MATERIALS AND METHODS

Thirty date palms of each of Khalas and Khassab cultivars growing on Al Fairooz farm at Al Khabourah, in Batina area, Sultanate of Oman, were selected in a completely randomized design. They were fertilized with 4 equal doses of 1000g urea (46% N), 800g K_2SO_4 (42.6%K) in March, May, July and October; and 500g triplesuperphosphate (20.24% P) applied as 200g in March and 100g in each of May, July and October. Urea was also applied in 5 equal doses in March, May, August, October and December, with K_2SO_4, 200g each in March, May, July and October; supplemented with 200g in March, and 100g in each of May, August and October. The macronutrients were supplemented by 20g/palm micronutrients (Fetrilon – Combi 2) and/or 80L peat moss. Treatment combinations are shown in Table 1. The experiment was run for 3 years. Each treatment was replicated 3 times. Fruit samples were collected weekly in the Bisir and early Rutab stages (3 strands of each replication). The samples were kept in an ice-filled chest at harvest and sent to the laboratory. The physical characteristics of fruits were immediately determined in the laboratory, including number and weight of fruits/strand using digital balance (Precisa XB 620C), fruit length and diameter were measured by Varnier Caliper and weight. Total yield was calculated by summation of all samples of harvested Rutab and the weight of the final harvest was calculated. SPSS software was used for statistical analysis of the results. Means were separated by Duncan's multiple range test (Montgomery, 2001).

RESULTS AND DISCUSSION

Effects of Fertilizer

1. Khalas. Data in Table 1 show the effect of fertilizer on the physical characteristics of Khalas fruits. There were no significant differences in all of the measured physical characteristics. However, application of NPK plus micronutrients with or without peat, produced higher fruit weight/strand and smaller fruit length and diameter than the organic peat. Also, higher fruit number was produced by 5-N doses, with P and K plus micronutrients, than when organic peat was added with it. This increase in fruit number

was associated with heavier fruit of slightly larger diameter and length/diameter ratio but lower yield. Application of NPK (4 or 5 doses) with micronutrients and organic peat (t10 and t6) produced higher yield than all other treatments (45.15kg/palm, 46.7kg/palm respectively). However, application of urea in 5 doses produced higher average number and weight of fruit/strand, fruit weight length and total yield than 4 doses. Such small increases in physical fruit characteristics using 5 doses compared to 4 doses can be related to less leaching of N and K, with 5 doses making more of them available (Al Juburi, 1995; Albakr, 1982). From these results it can be seen that application of NPK in 4 doses supplemented with micronutrients and organic peat, increased Khalas yield by 22.2% compared to the control and 24.8% compared to treatment with organic peat. Whereas, when applied in 5 doses urea and 4 doses P and K, the yield increases were 17.8% and 20.4%, respectively.

2. Khassab. Data in Table 2 show the effect of fertilizer on the physical characteristics of Khassab fruits. Application of NPK with or without micronutrients and organic peat resulted in significant increase in yield, number of fruits and fruit weight/strand than the organic peat. The results confirmed that date palm requires mineral and organic fertilizers (Bacha and Abo-Hassan, 1983). The highest yields (96 kg/palm and 99 kg/palm) were obtained with applications of 5-N with P, K and 4–N doses with P, K alone and combined with organic matter and micronutrients, respectively. Application of 5-N doses resulted in greater increase than 4-N doses on yield by 14.98% and number of fruits by 12.37% and fruit length over diameter ratio by 1.86%. However, application of 4-N doses resulted in greater increase than 5-N doses on weight of fruits/strand (5.97%), fruit weight (12.37%), length (1.03%) and diameter (3.11%). Considering the results from Khalas and Khassab (Tables 1 and 2) it can be seen that 5-N doses with 4 doses of P and K resulted in slight increases over the 4 NPK doses on Khalas fruit weight and number/strand, fruit weight, length and length/diameter (L/D) and yield, but reduced fruit diameter. In contrast, it caused reductions on Khassab fruit weight/strand, fruit weight and length, but increased yield, length/diameter (L/D) and fruit number and reduced diameter. The observed increases in the physical characteristics are compatible with reports by Hussein et al. (1972) and Soliman and Osman (2003). Moreover, application of NPK in 4 doses supplemented with micronutrients and organic peat resulted in increased yields of Khalas and Khassab by 22.2% and 77.5% over the control and 24.8% and 130% over organic peat, respectively. Whereas application of urea in 5 doses and P, K in 4 doses increased the yield of Khalas and Khassab by 17.8% and 63.1% over the control and 20.4% and 112% over organic peat, respectively. These results are compatible with those of Khudairi cultivar (Bacha and Abo-Hassan, 1983). Moreover, the results indicated that Khalas and Khassab can produce higher yields using 4 doses without causing drastic effect on other fruit physical characteristics. It is clear that application of NPK in 4 doses produced higher yields than 5 doses when compared to the control and organic peat. A possible explanation of the effect of 4-N doses is that the date palms received more N (750g) compared to 5 doses (600g). This resulted in increased vegetative growth (probably chlorophyll) and consequently higher photosynthetic productivity. Likewise, nitrogen enhances cell division and protein synthesis (Hussein et al., 1971; Nixon and Carpenter, 1978). Such results reflect variability in the response of the two cultivars (Khalas and Khassab) to fertilizer treatments. Especially Khassab is known as a late cultivar and the effect of the 4N dose could possibly be insufficient to influence later developmental stages. Whereas, the 5N dose resulted in increased yield and reduced fruit drop. These results made it difficult to recommend one treatment on the basis of fertilizer effect on both cultivars. However, in both cultivars NPK with micronutrients and organic peat produced the best results.

Interaction Effect for Fertilizer and Year-Khalas

1. Yield. There was an interaction effect of fertilizer x year on date palm yield ($P<.001$) (Fig. 1). In 2003, it was consistently lower (20.643kg). The highest yield was obtained using 5-N doses with P, K + micronutrients + organic peat (t6) in 2004. Four-N doses (t7,

t8, t9, t10) produced lower yields than 5-N doses (t6 and t5). The lower yields in 2003 can be attributed to three different reasons, particularly in regard to Khalas cultivar: early fruit drop before full ripening, late pollination in that year, alternate bearing phenomenon, considering year 2003 as an off year for Khalas. The alternate bearing phenomenon in this year was also noted on Khalas and Khassab with pollination experiments (El Mardi, personal communication).

2. Fruit Weight. The interaction effect of fertilizer x year on single fruit weight (P=.053) (Fig. 2) was slightly significant. Except for fertilizer treatment of 5-N doses with P, K with micronutrients (t6), all fertilizer treatments and the control produced heavier fruits in year 2003. Whereas, in 2004, all treatments produced lower fruit weights except 5-N doses with P, K with micronutrients and organic peat and 4-N doses with P, K with micronutrients (t9). Interaction for fertilizer and year effect showed an inverted pattern on yield and single fruit weight. The heavier fruit weight corresponded to lower yield in 2003, the lower fruit weight corresponded to higher yield in 2004.

3. Fruit Length. There was an interaction effect or fertilizer x year on fruit length (P=.043) (Fig. 3). Length of fruits was consistently lower in year 2004, with the exception of the organic peat treatment (t2) and 4NPK doses with organic peat (t8). Fruit lengths in 2004 and 2002 were very close in treatments t2 and t8 (3.767, 3.761cm), respectively. The association of low fruit weight and reduced length with high yield is a common phenomenon in date palm due to competition among the fruits for nutrients and food as observed in several thinning experiments (El Mardi, 2002; Mustafa, 1993; Nixon, 1935).

Interaction Effect for Fertilizer and Year- Khassab

1. Fruit Weight/Strand. The interaction effect of fertilizer x year on fruit weight/strand was significant (P=.001) (Fig. 4). Weight of strand was consistently lower in year 2003. Except for 5–N doses P, K with micronutrients and organic peat (t5), strand weight was consistently higher in 2004 (270.8g) than in 2002 (227.2g). The highest (359.868g) strand weight was produced by application of 4-N doses of P, K treatment (t7). The highest fruit weight was produced by application of 4-N doses P, K in 2004.

2. Number of Fruits/Strand. The interaction effect of fertilizer x year on number of fruits/strand was significant (P=.003) (Fig. 5). Number of fruits/strand was consistently lower in 2003 than for previously reported reasons, except for application of 5-N doses P, K with micronutrients (t5), in which fruit number/strand in 2004 was consistently lower than in 2002.

3. Fruit Weight. The interaction effect of fertilizer x year on fruit weight was significant (P=0.006) (Fig. 6). Fruit weight in 2004 was consistently lower than in 2003. The highest fruit weight was produced in 2003 (t9) and 2002 (t7): 15.06g and 15.08g, respectively. Highest fruit weights in 2003 were a result of reduced competition for the available food because of the smaller numbers of fruit as indicated above.

4. Fruit Diameter. The interaction effect of fertilizer x year on fruit diameter was insignificant (P=.070) (Fig. 7). Fruit diameter was consistently lower in 2004. In 2003 consistently larger fruit diameter was achieved in all treatments, except t7 and the control. In 2002 fruit diameter was lower than in 2003 except in 4-N doses P, K treatment (t7) and the control (2.38 cm). Five doses of urea with (2.28cm) or without (2.27cm) organic peat produced the most comparable diameters, to the shape of Khassab fruit. These interaction effects indicated that high number and weight of fruits/strand caused reduction in fruit weight and diameter. Therefore, high yield in Khassab is more related to number of fruits than to fruit weight. This trend is similar in Khalas fruits, but the reduction was in fruit length and not in diameter for Khalas fruits.

Interaction Effects for Fertilizer and Stage

1. Fruit Weight/Strand. The interaction effect of fertilizer x stage on fruit weight/strand of Khassab was insignificant (P=.065) (Fig. 8). However a trend could be seen. Fruit weight/strand was consistently lower in Rutab stage than in Bisir stage. This is the natural fruit behavior as it moves toward more mature stages. The heaviest fruit/strand (293.46g)

was produced in treatment 3 with NPK (5-N doses) and the lowest weight in the Rutab stage using peat (t2): 145g. Fruit weight/strand in Bisir stage followed the succession (3>6>5>4) (7>10>8>9) and in Rutab stage it was (5>3>4>6) (7>8>9>10).

CONCLUSION

It is clear that date palms in the Sultanate of Oman do not receive good attention in terms of fertilization and this results in a reduction in date palm yield. Application of only organic peat or manure is not sufficient to optimize date palm production of both Khalas and Khassab cultivars in Batina region. However, a combination of both organic and inorganic fertilizers is recommended for higher yields and better physical fruit characteristics. Application of 1 kg of nitrogen, 500g of phosphorous and 800 g of potassium sulfate with trace elements per palm per year in 4 doses is recommended to maintain good fruit growth and development. It is recommended to apply nitrogenous and potash fertilizers in March, May, July and October, and phosphorous in March and double the amounts of each in May, July and October. Long term trials on the use of mineral fertilizers in Batina are required. Several years may be required for the full effect of fertilizer to be reflected in an increased yield because the growth of leaves and production of inflorescences must be increased first. It would be good practice to provide each year increased nitrogen, phosphorous and potassium supply adequate to sustain good yield and quality. Therefore further investigation is required.

ACKNOWLEDGMENT

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Tables

Table 1. Effect of fertilizer treatments on the physical characteristics of Khalas fruits.

No.	Fertilizer	Fruit / Strand (g)	Fruit No.	Fruit Wt. (g)	L (cm)	D (cm)	L/D	Yield (Kg/Palm)
1	Control	103.1 a	7.5 a	13.46 a	3.82 a	2.4 a	1.59 a	38.31 a
2	Organic	84.39 a	6.5 a	14.65 a	3.92 a	2.45 a	1.6 a	37.48 a
3	NPK (5-N)	101.65 a	7.4 a	14.14 a	3.85 a	2.43 a	1.58 a	38.05 a
4	NPK (5-N)+Org	93.63 a	6.8 a	15.18 a	4.00 a	2.48 a	1.6 a	34.57 a
5	NPK (5-N)+Mic	97.06 a	8.7 a	16.44 a	3.89 a	2.43 a	1.63 a	34.42 a
6	NPK (5-N)+Org +Mic	85.07 a	5.91 a	14.38 a	3.86 a	2.37 a	1.54 a	45.16 a
7	NPK (4-N)	92.1 a	6.58 a	15.14 a	3.73 a	2.41 a	1.54 a	31.19 a
8	NPK (4-N)+Org	98.35 a	7.45 a	14.44 a	3.77 a	2.43 a	1.55 a	31.97 a
9	NPK (4-N)+Mic	96.44 a	6.79 a	14.13 a	3.92 a	2.44 a	1.6 a	34.23 a
10	NPK (4-N)+Org +Mic	88.53 a	6.41 a	14.78 a	3.86 a	2.44 a	1.58 a	46.75 a
±5-N dose from 4-N dose		+0.53	+5.88	+2.72	+2.09	-0.10	+1.28	+5.57

- Numbers followed by the same letter in the same column are not significantly different at 0.05 levels.
- NPK= Urea, Superphosphate, Potassium sulphate, (4-N) and (5-N) = 4 and 5 Nitrogen doses, Mic = Micronutrients, Org. = Organic peat, L= Fruit length, D= Fruit diameter, and L/D=Fruit ratio of length over diameter.

Table 2. Effect of fertilizer treatments on the physical characteristics of Khassab fruits.

No.	Fertilizer	Fruit / Strand (g)	Fruit No.	Fruit Wt. (g)	L (cm)	D (cm)	L/D	Yield (Kg/Palm)
1	Control	187.8 d	18.39 e	10.72 cd	3.35 a	2.28 bc	1.47 a	55.86 bc
2	Organic	202.76 cd	19.84 de	11.05 c	3.34 a	2.28 bc	1.46 a	44.29 c
3	NPK (5-N)	244.82 ab	23.81 ab	10.89 c	3.54 a	2.28 bc	1.55 a	96.15 a
4	NPK (5-N)+Org	216.11 bcd	21.13 abcde	10.95 c	3.36 a	2.28 bc	1.48 a	72.41 abc
5	NPK (5-N)+Mic	222.99 abc	22.79 abcd	10.22 cd	3.3 a	2.25 cd	1.48 a	87.84 ab
6	NPK (5-N)+Org +Mic	208.42 cd	23.19 abcd	9.4 d	3.26 a	2.18 d	1.5 a	91.1 ab
7	NPK (4-N)	251.54 a	20.38 cde	13.95 a	3.53 a	2.34 bc	1.51 a	61.58 abc
8	NPK (4-N)+Org	223.27 abc	23.31 abc	10.33 cd	3.36 a	2.27 bc	1.48 a	62.5 abc
9	NPK (4-N)+Mic	228.52 abc	20.77 bcde	12.29 b	3.44 a	2.43 a	1.43 a	79 abc
10	NPK (4-N)+Org +Mic	245.67 ab	24.43 a	10.02 cd	3.27 a	2.23 cd	1.48 a	99.14 a
±5-N dose from 4-N		-5.97	+2.28	-12.37	-1.03	-3.11	+1.86	+14.98

- Numbers followed by the same letter in the same column are not significantly different at 0.05 levels.
- NPK= Urea, Superphosphate, Potassium sulphate, (4-N) and (5-N) = 4 and 5 Nitrogen doses, Mic = Micronutrients, Org. = Organic peat, L= Fruit length, D= Fruit diameter, and L/D=Fruit ratio of length over diameter.

Figures

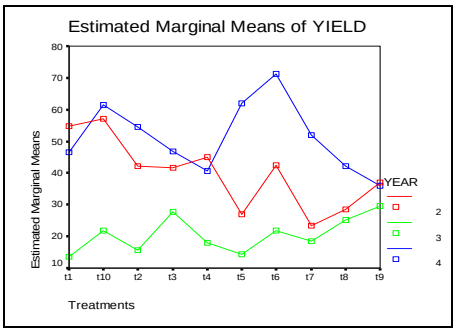

Fig. 1. Interaction effect for fertilizer x year Khalas yield (kg/palm) (P<.001).

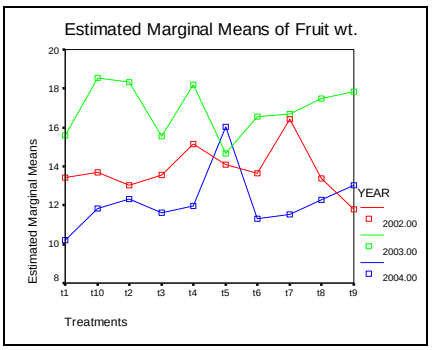

Fig.2. Interaction effect for fertilizer x year on Khalas single fruit weight (g) (P=.053).

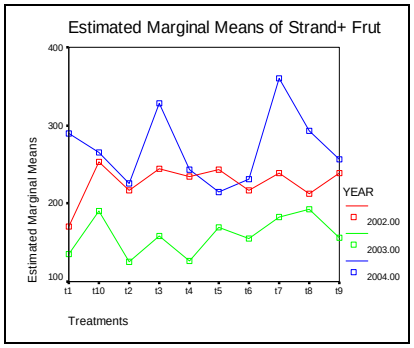

Fig. 3. Interaction effect for fertilizer x year on Khalas fruit length (cm) (P=.043).

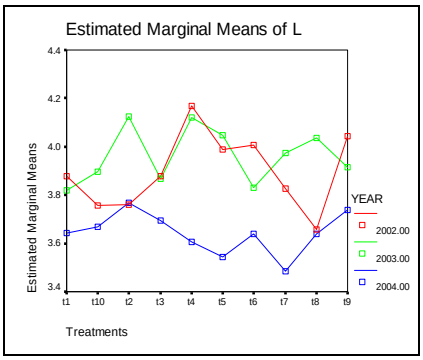

Fig. 4. Interaction effect for fertilizer x year on Khassab fruit weight /strand 9g) (P=.001)

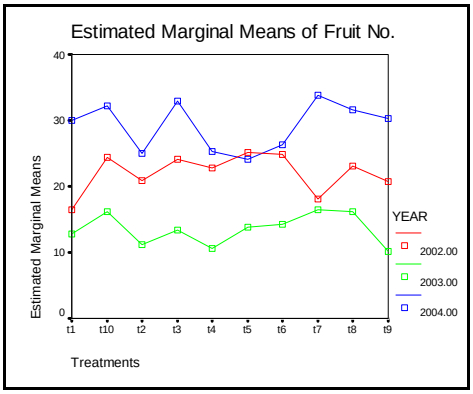

Fig. 5. Interaction effect for fertilizer x year on Khassab no. of fruits (P=.003).

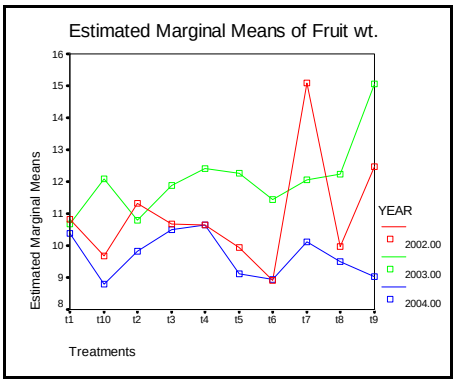

Fig. 6. Interaction effect for fertilizer x year on Khassab single fruit weight (g) (P=.006).

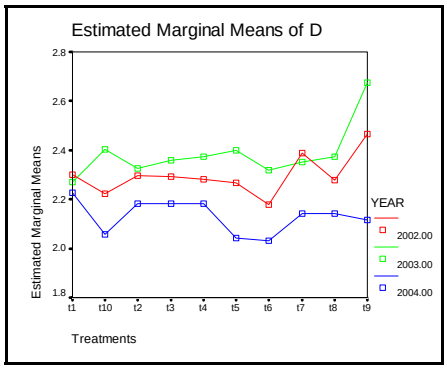

Fig. 7. Interaction effect for fertilizer x year on Khassab fruit diameter (cm) (P<.07).

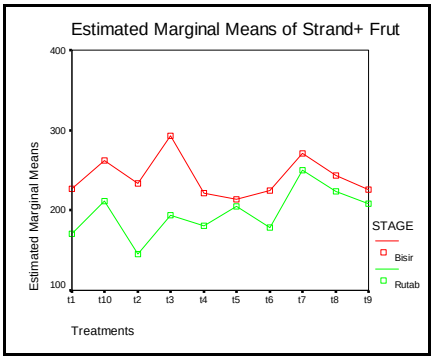

Fig. 8. Interaction effect for fertilizer x year on Khassab fruit weight/strand (g) (P<.065)

Reduced Fat Sesame Paste/ Date Syrup Blend: a Novel Product

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Keywords: fat substitution, rheology, sensory, gums, sesame butter

Abstract

A blend of sesame paste and date syrup is a promising nutritious and healthy product due to the high protein and fiber content of sesame paste and the high mineral and vitamin content of date syrup. This blend is traditionally made in Iran and other Middle East countries. Due to the high fat content of sesame paste, which is of nutritional concern to most consumers, using reduced fat sesame paste in the preparation of this blend will give it a wider consumer appeal. In this study, the rheological and sensory properties of low fat sesame butter (blends of 20% reduced fat sesame paste and date syrup) were investigated. Fat substitutes guar gum, xanthan gum and modified starch were used at three concentrations (0.01%-1.75% depending on the type of hydrocolloid), and at four mixing temperatures (25, 35, 45, 55 C). It was found that low fat sesame butter behaves as a pseudo-plastic foodstuff. The power law model was the best model for describing the flow behavior of all experimental samples. Fat substitution level and temperature in the forward- and backward- measurements influenced the consistency coefficient (k). The consistency coefficient significantly decreased as the mixing temperature increased. The effect of temperature on consistency of low fat sesame butter followed an Arrhenius equation.

INTRODUCTION

Sesame paste is a commodity produced from milled seeds of sesame (*Sesamum indicum* L.), which are dehulled and roasted without adding or removing any of its constituents. This product is popular in Iran and other Middle East and Eastern Asian countries and is known by various names such as Ardeh (in Iran) and Tahineh (in Arabic countries). It is used in preparation of local dishes such as hummus bi tahina, Bakdoonsiyeh and halwa. It has remarkable oil stability and is resistant to oxidative deterioration at ambient temperatures due to the presence of natural antioxidants such as sesaminol, sesamin and tocopherol. Sesame paste has a flavor similar to that of roasted peanuts and contains about 60% oil which is more fluid than peanut butter. It has a high nutritional value which comes from about 25% protein, 8% carbohydrates, 4.5 mg/100g niacin, 1.08 mg/100g thiamin and minerals such as calcium, phosphorous and iron (Sawaya et al., 1985; Abu-Jdayil et al., 2002). On the other hand, date syrup is a product obtained from matured dates (tamr) and is used as such or in the preparation of traditional and industrial foods such as ice cream, confectionery, beverages, alcohol, vinegar etc. (Mohamed and Ahmed, 1981; Lewandowski et al., 1999). Date fruit (*Phoenix dactylifera*) is also a highly nutritious food due to high vitamin and mineral contents. It is rich in calories. It is exceptionally rich in potassium and extremely low in sodium (Al Hooti et al., 2000).

A blend of sesame paste and date syrup is a promising nutritious and healthy product due to the high protein and fiber content of sesame paste and the high mineral and vitamin content of date syrup. Because of the high fat content of sesame paste, which is of nutritional concern to consumers, using reduced fat sesame paste in preparation of this blend will give this popular commodity wider appeal to consumers. Starches and gums are used in low fat foods to replace the oil content. When added to foods, replacements for fat thicken and add bulk by producing a mouth feel similar to that provided by fat. These compounds have also been used for giving additional stabilization to emulsions

(Singh et al., 2000).

In previous studies, the effect of temperature, date syrup ratio and concentration on flow behavior and emulsion stability of non-reduced fat blends were investigated (Habibi Najafi and Alaei).

Testing the emulsion stability and rheological properties of reduced fat blends is considered essential to evaluate the flow behavior and consumer acceptance of the blend.

The objective of this study was to investigate the rheological properties of low fat sesame butter (blends of 20% reduced fat sesame paste and date syrup) using three types of fat substitutes (guar gum, xanthan gum and modified starch) and four mixing temperatures (25, 35, 45, 55 °C).

MATERIALS AND METHODS

Sesame paste was obtained locally (Simorgh Halva Co. Mashhad, Iran). Concentrated date syrup with °Bx 72 was produced in the pilot plant, Department of Food Science and Technology, Ferdowsi University of Mashhad, Iran. Guar and xanthan gum were obtained from Rhodia Co. EU, and modified starch was purchased from Merck Co. Germany. These products were used as fat replacers.

Viscosity Measurement

The viscosity of blends was measured using a Brookfield rotational viscometer, RVDVII model (Brookfield Engineering Laboratories, Stoughton, MA,USA) equipped with the spindle7 at speed of 1-60 rpm. Enough samples in a 600 ml beaker were used to immerse the groove on the spindle with guard leg (Sopade and Filibus, 1995). A thermostatic water bath was used to control the working temperature. The viscosity of blends was measured in the temperature range of 25-55 ºC by increasing (forward measurements) and decreasing (backward measurements) the rpm. The values of the rpm/viscosity were recorded every 0.5 min. The power law model described flow behavior:

$$\tau = k (\gamma)^{n} \quad (1)$$

k is the consistency coefficient (Pa. s^{n}) and n is flow behavior index (dimensionless).

Statistical Analysis

Experimental data were tested by analysis of variance (ANOVA) and means separation was achieved using Duncan's multiple range test at $p<0.05$ level, using SAS institute (1997) software version 6.12.

RESULTS AND DISCUSSION

The relationship between viscosity and rotational speed (rpm) for blends prepared with different levels of guar gum along with the control at ambient temperature is shown in Figure 1. It was evident that an increase in the amount of guar gum resulted in increased viscosity of sesame butter which may have been related to the colloidal nature of the continuous phase as well as the average particle size (Coia and Stauffer, 1987). Fig. 1 also revealed that after a sharp reduction, the viscosity change evened out at high speeds. This can be related to the size of colloidal aggregates as the speed increased (Davis, 1973). The same trend was observed for all other treatments prepared with xanthan and modified starch. The presence of a large number of high molecular weight molecules increased the resistance to flow which, in turn, increased the apparent viscosity of the emulsion system (İbanoğlu, 2002). The flow behavior index (n) and consistency coefficient (k) values were obtained by fitting the rotational speed versus apparent viscosity data to a power law model (Eq. 1), in both forward and backward directions. The above mentioned parameters for all blends at different temperatures are presented in Table 1. The results showed that the shear stress/shear rate relationship is non-linear, indicating that sesame butter blends behave as non-Newtonian fluids. Moreover, the fact that n is less than unity indicated that the experimental blends were a pseudoplastic foodstuff. The relationship between shear stress/shear rate for both forward and backward

directions of blends prepared with different levels of guar gum as shown in Fig. 2 was thixotropic. The same trend was also observed for other fat replacers studied in this research. The difference in consistency coefficient of the experimental blends in forward and backward measurements (Table 1) was an indication of time-dependent behavior of blends. Generally, when a material is sheared at a constant shear rate, the viscosity of a thixotropic material will decrease over a period of time, implying a progressive breakdown of structure. In order to study the thixotropic behavior of blends, constant shear rate (1/s) was applied for 10 min as suggested by other workers (Abu-Jdayil et al 2002). Fig. 3 shows the temperature-dependence of the consistency coefficient, k, both for forward and backward measurements of 0.01% guar gum. Obviously, the consistency coefficient decreased with increasing temperature. The same trend was observed for xanthan gum and modified starch. Fig. 4 shows emulsion stability of sesame butter with addition of guar gum. It was likely that addition of hydrocolloid stabilizers inhibit the coalescence of oil droplets into larger oil droplets which have a tendency to separate from the blends. Results from the analysis of variance indicated that there were significant differences between the control and all experimental samples prepared with different levels of fat substitution. These results demonstrated that addition of polysaccharides (starch and gums) increased the stability of emulsions. Although Sanderson (1981) reported that polysaccharides can be used as a thickener, stabilizer and emulsifier, we used it as a stabilizer. A repulsive protein-polysaccharide interaction may stabilize the emulsion by immobilizing protein-coated droplets in a polysaccharide gel network, or destabilize it by inducing phase separation between the absorbed protein component and the solubilized polysaccharide component. Similar results were obtained for low fat samples prepared using xanthan at 0.02% (Dickinson and Mcclements, 1995).

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Table

Table 1. Rheological parameters of reduced fat sesame butter prepared with different levels of xanthan gum at different temperatures calculated from power law (Eq. 1).

Xanthan		Forward		Backward	
Gum%	T(°C)	k(Pa. s^n)	n	k(Pa. s^n)	n
control	25	108	0.49	95.4	0.57
	35	64.54	0.47	60.3	0.53
	45	49.12	0.44	41.7	0.55
	55	44	0.42	40.6	0.51
0.01 %	25	181.3	0.46	129.5	0.5
	35	126.5	0.44	108.8	0.49
	45	112.3	0.42	90.3	0.49
	55	90.8	0.4	72.3	0.48
0.015 %	25	187.0	0.44	131.2	0.48
	35	149.4	0.41	113.7	0.48
	45	121.9	0.39	96.7	0.47
	55	96.3	0.38	77.4	9.46
0.02 %	25	201.5	0.45	179.8	0.48
	35	168.0	0.41	150.3	0.45
	45	134.0	0.41	119.9	0.45
	55	112.7	0.38	90.7	0.46

Figures

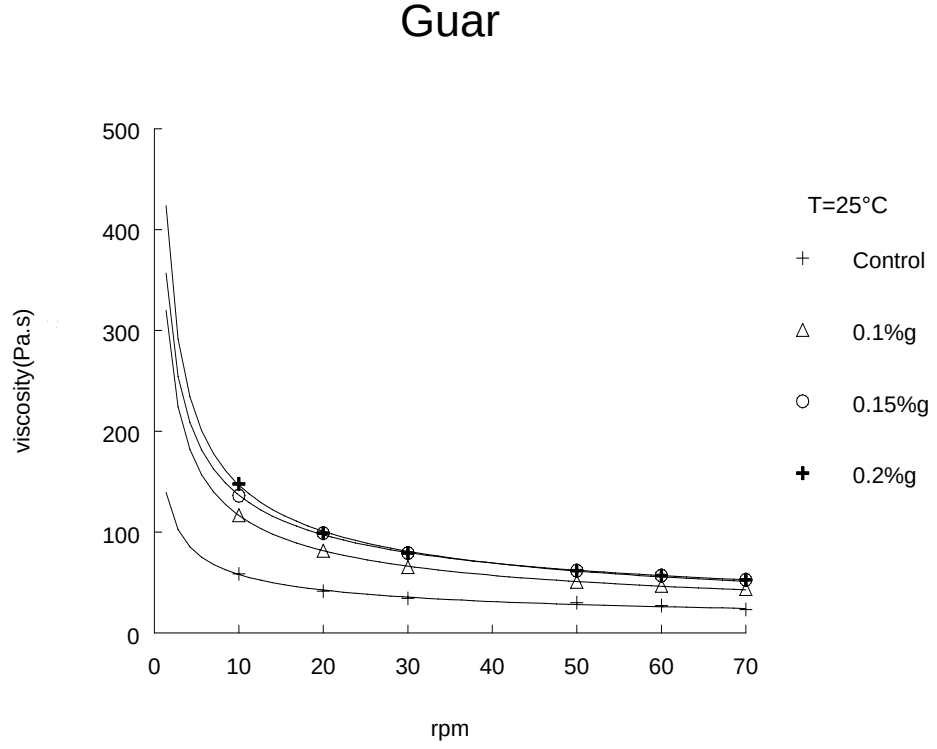

Fig. 1. Flow curves of reduced fat sesame butter prepared with different levels of guar gum at ambient temperature.

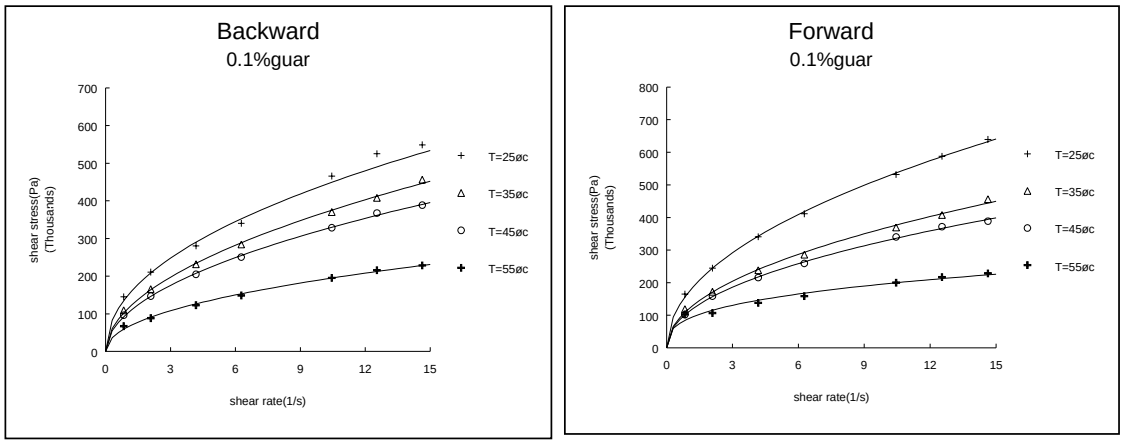

Fig. 2. Relationship between shear stress/shear rate for forward and backward directions for blends prepared with different levels of guar gum.

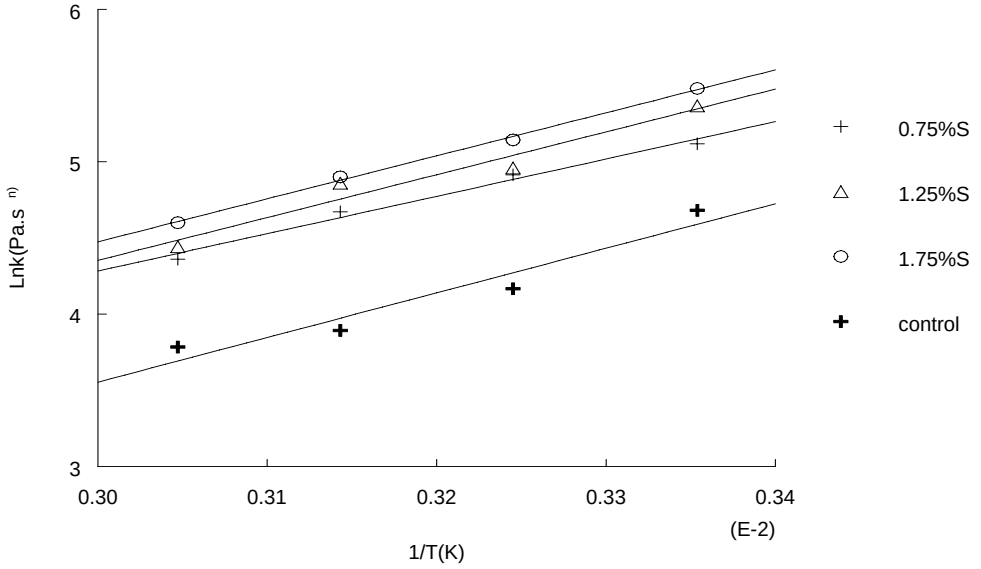

Fig. 3. The temperature-dependence of the consistency coefficient, k, for forward measurement.

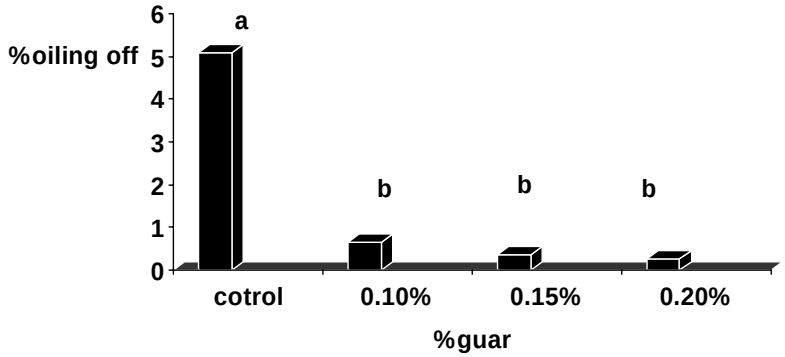

Fig. 4. Emulsion stability of sesame butter prepared with addition of different levels of guar gum.

Chromatographic Separation of Fructose from Date Syrup

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Keywords: Ion chromatography

Abstract

The objective of this study was to develop a process for separating fructose from a mixture of sugars containing essentially fructose and glucose that were obtained from date palm fruits. The extraction procedure of date syrup from fresh dates gave a yield of 86.5% solids after vacuum drying. To separate fructose from an aqueous solution of date syrup, solutions (20, 30 and 40 percent by weight) were introduced into a chromatographic column filled with Dowex polystyrene strong cation exchange gel matrix resin Ca^{2+} and divinylbenzen, functional group, sulfonic acid, particle size 320 micro-meter, with a flow rate of 0.025 and 0.05 BV/min., under 30°C and 70°C column temperature. After the date sugar solution batch, a calculated quantity of water was supplied into the column. Glucose was retained by the resin more weakly than fructose and proceeded faster into the water. Three fractions were collected: a glucose-rich fraction, return fraction, and fructose-rich fraction. The return fraction was based on when the peaks of fructose and glucose were reached, which could be determined by means of an analyzer (polarimeter) which was based on the property of glucose and fructose solutions to turn the polarization level of polarized light. A high yield of fructose was obtained at 70°C column temperature with flow rate of 0.025 BV/min and date syrup solution containing 40% sugar concentration. Because a low recovery was obtained using date syrup solutions having sugar concentrations of 20 and 30%, a 40% concentration was used. However, with the 40% date syrup, the average concentrations of glucose and fructose in the return fractions were increased, and could be used to dilute the thick date syrup solution.

INTRODUCTION

Date is known to be a rich source of the sweeteners, glucose and fructose. This characteristic has prompted the development of extraction procedures of fructose from dates. Mohamed and Ahmed (1983) analyzed date syrups and showed that fructose and glucose were the major sugar components. Al-Eid et al. (1999) found that date syrups contained more fructose than glucose. Fructose could be separated from dates to yield value-added products. Date is an ideal raw material because of its high fructose content, plentiful supply, and year round availability. However, practical methods need to be developed for producing nutritive sweeteners such as fructose from dates.

Traditionally, fructose has been obtained from sucrose syrup produced by the cane and beet industries or from syrup produced by enzymatic depolymerisation and hydration of starch-based carbohydrates such as corn and potatoes. The proportion of fructose in these syrups is then increased to 55-90% w/w by chromatographic separation method (Barker et al., 1984).

Classically, separation of sugars is done with resins in the calcium form, while the separation of sugar/nonsugar is achieved with resins in the potassium form. Resins in the calcium form simultaneously produce two kinds of separation. Firstly, they act as a molecular sieve. Large molecules are not able to enter the resin beads and are more or less excluded. Secondly, separation is related to the stability difference of the sugar-calcium complex: only polyols and certain sugars (fructose, galactose) are capable of forming such a complex. The other components (sucrose, glucose) do not form this complex with

the resin, thus explaining the separation (Paillat et al., 2000). Cation exchangers (Ca^{2+}) with pure water as the eluent are commonly employed (Verhaar and Kuster, 1981). In the "Ca-column" system, the main function of the ion exchangers is to immobilize Ca^{2+}, while separation is the result of the different complexing abilities of the polyols with Ca^{2+}. However, the degree of divinyl benzene cross-linking is also important, polymeric sugars being better separated at low degrees and mono- and dimeric sugars at high degrees of cross linking.

Fructose separation can be performed using an anion exchanger with borate as eluent (Verhaar and Dirkx, 1977), a cation exchanger (Ca^{2+}) with water as eluent (Ladisch and Huebner, 1978) or unmodified silica with acetonitrile containing a trace of water (Olst and Joosten, 1979). In these three systems the analysis time needed is about 30 min and only the first system yields complete separation (Verhaar and Kuster, 1981). Some studies report the use of anion-exchange resins in the bisulphate (HSO_3^+) form to separate fructose-glucose mixtures on analytical scale columns. This form is known to retard glucose while fructose is carried with the mobile phase (Barker et al., 1984). Saska et al., (1992) reported that the separation of fructose on the Dowex Monosphere 99 CA resins at 70^{o}C appeared to be superior to that on Duolite resins at the same temperature. The chromatographic processes for the separation of sugars utilizes beds of cation exchange resign in the calcium (Ca^{2+}) form, which is known to form a weak complex with fructose, and results in preferential retardation of fructose while glucose is carried away with the mobile phase (Boehringer, 1967; Barker et al., 1984). Advantages of these processes are cheap eluent, high column capacity and stability and complete elution of all sugars injected (Angyal et al., 1979).

Glucose equilibrium does not depend on separation temperature; glucose is probably not adsorbed but only penetrates the pores. Fructose partition coefficients are temperature dependent; retention of fructose is lower in the chromatographic column at higher temperature (Viard and Lameloise, 1992). The adsorption of fructose in preference to glucose by ion-exchange materials is due to a greater tendency of fructose to form complexes with the metal counter-ions contained therein. In practical applications, calcium forms of ion-exchange adsorbents are preferred because of their low cost, their non toxic nature, and their strong complexion ability (Lloyd and Nelson, 1984). Adsorption arises from unstable complexes between sugars and Ca^2+ sites; it is a physical adsorption process, and so it is assumed to be sufficiently fast to cause no significant contribution to the overall kinetic resistance (Viard and Lameloise, 1992).

Barker and Joshi (1991) carried out a continuous chromatographic separation of inverted beet molasses resulting in a fructose rich product and a product containing glucose and other non-sugars. They used a semi-continuous countercurrent chromatographic refiner, consisting of ten 10.8 cm diameter by 75 cm long stainless steel columns packed with a calcium charger 8% cross-linked polystyrene resin. They used feed of approximately 20% w/w total solids and separation temperature of 60^{o}C. Their results revealed that cations present in beet molasses displaced calcium ions from the resin resulting in poor separation of the glucose and fructose.

The objective of this study was to provide a process for separating fructose from a mixture of sugars containing essentially fructose and glucose obtained from date palm fruits. The process would be based on the use of calcium form of polystyrene sulfonated cation-exchange resin cross coupled with divinyl benzene as separating media.

Experimental Design

One date variety *(*Khalas) was used in this project. One resin was chosen based on the fact that separation of fructose on the Dowex Monosphere 99 CA resins at 70^{o}C appeared to be superior to that on other resins. Two separation temperatures (30^{o}C and 70^{o}C) were chosen, based on literature and manufacturer recommendations. Three different sugar concentrations of date syrup solutions (20, 30 and 40%) as well as their flow rate (0.05 and 0.01 Bed Volume (BV)/min) were examined. The experimental design was a 3 x 2 x 2 factorial experiment.

MATERIAL AND METHODS

The following parameters were controlled in the chromatographic procedure: sugar concentration of date syrup, separation temperature and flow rate of date syrup. The dependant variables were elution volume (ml) and time (min) to fructose-rich fraction, return fraction, and glucose-rich fraction, as well as glucose and fructose concentrations in each fraction.

Extraction and Color Removal of Date Syrup

Sugars of date were extracted by heating equal amounts of date (Khalas variety) and water at 80^{o}C for 30 min. The mixture was filter pressed to obtain an impurity free sugar solution, and concentrated by vacuum drying at 70°C to 86.5°Brix. This sugar solution usually contained fructose, glucose and small amounts of sucrose.

The color substances of date syrup were removed by mixing the syrup solution with 2% activated charcoal (Fisher Scientific, Fair Lane, NJ, USA), and then centrifuging (10,000 RPM) at 4^{o}C for 15 min. This technique could be easily applied to large-scale processes.

Chemical Analysis of Extracted Date Syrup

The syrup was analyzed for protein (Kjeldhal*, Nx6.25*), and total ash according to A.O.A.C. standard methods of analysis (1992). Moisture content was determined using Abbe Refractometer at a constant temperature (20°C). The reading was converted to moisture content (percent by weight) according to Saudi Arabian Standard organization (1987). Total soluble solids (Brix) were determined by means of Abbe Refractometer. The extracted date syrup sample was analyzed for glucose, fructose, and sucrose using a Varian HPLC equipped with (RID 6A) refractive index detector according to Aleid et al. (1999). The mineral composition was determined using an atomic absorption/flame emission spectro-photometer AA-670 (Shimadzu Corporation, Spectro-photometric Instruments Plant, Analytical Instruments Division, Koyoto, Japan) according to A.O.A.C. standard methods of analysis (1992).

Description and Wetting Procedure of the Resin

Separation of fructose from the obtained sugar solution was carried out according to Sigma Aldrich (1997) and Verhaar and Kuster (1981).The resin type was a Dowex polystyrene strong cation exchange gel matrix resin Ca^{2+} and divinylbenzen, functional group, sulfonic acid. Particle size of 320 micro-meter with total exchange of 1.5 meq/ml and capacity of 4.5 meq/g, pH range of 0-14, and maximum operating temperature of 150ºC were used.

A weighed amount of resin (1g per ml column contents) was placed into the separation chromatographic column (B-685 Medium-Pressure Chromatography Column, Buchi Labottechnik AG, Post Fach, CH-9230 Flawil, Switzerland). The eluent peristaltic pump (Buchi Chromatography B-688 Pump) was connected and deionized water was pumped through the column to wet the resin.

Separation Steps

Date syrup (elute) was diluted in deionized water to 20, 30 and 40% total sugars, and passed through a 2-micron cellulose filter. The volume of the date syrup solution introduced into the column was 5% of the BV. The eluent was deionized water. Eluent was heated to more than the column temperature to expel any dissolved gases that were present. The column used was a Buchi double jacketed chromatographic column with dimensions of 460mm length and 36mm internal diameter. The calculated cross section area was 10.17cm^{3}. The column temperatures were 30^{o}C and 70^{o}C.

The calculated amount of date syrup solution (5% of the column BV) was introduced into the column with a dry material content of 20, 30 and 40 percent by weight and with a flow rate of 0.025 and 0.05 BV/min. After the date sugar solution, a calculated quantity of deionized water (elution) was added to the column with the same flow rate

0.025 or 0.05 BV/min. The elution time was calculated from the time the water pump was switched on. Slower flow rates may be desirable for good resolution. The solution flowing out of the column was directed to a fraction collector (Buchi B-684 Fraction Collector) at the rate of 60sec/test tube. When separation was completed, the column was rinsed with deionized water at a flow rate of 0.025 or 0.05BV/min. Collected column effluents (in 60 second portions) were analyzed for fructose and glucose concentrations using Boehringer Mannheim Enzymatic glucose and fructose kit. Manufacturers of these kits provide detailed instructions on how to carry out the analysis. This method comprised a series of steps to determine the concentration of both D-glucose and D-fructose in food.

RESULTS AND DISCUSSION

Chemical Composition of Date Syrup

Composition and principle characteristics of the extracted syrup are presented in Table 1. The extraction procedure of date syrup gave a yield of 86.5% solids after vacuum drying. Date syrup was extracted by means of pressure collection or heat extraction followed by filter press (Mustafa et al., 1983). The effect of extraction method on the chemical and physical properties of date syrups was studied by El-Shaarawy et al. (1989). They found that autoclaving dates at 15 psi for 10 minutes in 2.5 times their weight of water was the best extraction method. However, a dark color as well as an over-cooked flavor occured.

Date syrup had 81% total sugars (41% fructose, 39% glucose and 1% sucrose). It was clear that the amount of total solids was related mainly to sugar content due to high sugar concentration in the syrup. Glucose and fructose were the only monomers of the reducing sugars. In general, date syrup in this study exhibited more fructose than glucose (Table 1). Mohammed and Ahmad (1983) analyzed date syrups and showed that fructose and glucose are the major sugars. The physical and proximate analysis of date fruits from 25 cultivars have been studied by Sawaya et al. (1983). They found that reducing sugars were the dominant sugars in dates.

The protein content of the syrup was 2.2% which concurs with data from El-Shaarawy et al. (1989) on protein content of syrups from Khalas variety. The nutritional value of some Egyptian soft date cultivars was evaluated by Hussein et al. (1989). They found that the percentage protein content was between 3.21 and 2.93% on a dry weight basis.

The mineral composition of date syrup showed a high amount of K, some Na and low amounts of Ca and Mg, as well as trace amounts of Mn and Fe. Sawaya et al. (1983) reported a high amount of K, low amount of Na, some Ca, P and Mg, a low level of Fe, Cu and Zn, and traces of Mn and F. The extracted syrup showed low Na to K ratio (10.18). Rygg (1977) reported that the low Na to K ratio in date, may be of dietetic importance, especially for those who have a restricted Na intake. Among the micronutrients, Mn was not detected and Fe was present in trace amounts. The mineral profile of date syrup in ppm were 50 for Na, 509 for K, 20 for Ca, 37 for Mg, and 2 for Fe.

Chromatographic Separation of Glucose and Fructose

Figure 1 presents the fractions removed out of the column. The Y-axis represents the dry material content (g / liter) of the different fractions, and the X-axis shows the time in minutes. The glucose-rich fraction I, the return fraction II, and the fructose-rich fraction III, were successively collected from the column. The return fraction is based on when the peaks of fructose and glucose were reached, which could be determined by means of an analyzer (polarimeter). The zero point represented the introduction of the sample into the column.

1. Values of D-glucose / D-fructose. A process for separation of the mono-saccharides fructose and glucose from an aqueous solution of date syrup was carried out by column chromatography. Twelve experimental separation runs were performed. The best result was obtained under conditions corresponding to run number 10, in which 70°C column

temperature and 0.025 BV/min flow rate with date syrup solution containing 40% sugar concentration were used. The purity degree of glucose and fructose, in the fractions taken out of the column from run number 10 were 73% glucose in the glucose-rich fraction and 80% fructose in the fructose-rich fraction, based on dry material content (Table 2). The purity of glucose and fructose solutions is very important in influencing the effectiveness of the process. Barker and Joshi (1991) observed that as the run progressed, the purity of the two products reached a maximum and then decreased rapidly. This was because molasses, the raw material used in their experiment, contains 5.8% potassium and 1.7% sodium expressed as %w/w on dry substance. These ions displaced the calcium from the resin reducing the amount of calcium ions with which the fructose could form a complex. Therefore some of the fructose would have been carried with the glucose in the mobile phase. However, the atomic absorption analysis in our experiment showed that date syrup had high amounts of potassium (509 ppm), which might cause a similar effect on the separation process of fructose from date syrup.

To achieve a higher degree of separation between the glucose and fructose fractions, a return fraction was collected to dilute the date syrup solution that was added to the column. The glucose and fructose content of the return fractions ranged from 41.57 to 60% and 40 to 58.43 respectively (Table 2). Barker and Joshi (1991) and (Barker et al., 1984) described a highly complicated method in which several fractions were recalculated.

2. Effect of Elution Volume. The elution time and volume for the twelve experimental separation runs are presented in Table 3. The data revealed that total elution volume for the three main fractions increased with increasing date syrup concentration. The elution volume of the glucose-rich fraction was less than that of the fructose-rich fraction, while the return fraction scored the lowest. Increasing the flow rate from 0.025 BV/min did not show an increase in recovery of glucose, in the glucose-rich fraction, as well as fructose in the fructose-rich fraction. The separation of glucose and fructose occurred faster, but less sharply, by increasing the flow rate considerably. However, the volume of glucose and fructose-rich fraction solutions affected the evaporation cost.

3. Recovery of Glucose and Fructose. The weight recovery of glucose and fructose in the glucose-rich fraction, return fraction, and fructose-rich fraction is shown in Table 4. The recovery was calculated on the supply of date syrup (dry sugar weight) into the column. The fructose recovery by weight in fructose-rich fraction at 30^{o}C was higher than that at 70^{o}C. However, the opposite was true for the recovery of glucose in glucose-rich fraction. Viard and Lameloise (1992) worked on the modeling of the chromatographic separation of glucose and fructose from invert sugar feedstock by adsorption on calcium gel type C^{+2} ion exchange resins. The temperature study using Duolite resin (C204) showed that the the influence of temperature was less marked for glucose, especially in the case of Doulite resin which showed no difference between diffusivities at 30^{o}C and 60^{o}C. For another resin (Dowex monosphere resin C328), greater diffusivities were obtained for both glucose and fructose at 60^{o}C than at 30^{o}C. The glucose and fructose solutes had intra-particle diffusivities of the same order of magnitude, with glucose diffusing slightly faster than fructose. This behavior was very noticeable at low temperatures. Separation of sugars with this cation exchanger (Ca^{2+}), with water as eluent, is generally performed at elevated temperatures (Ladisch et al., 1978; Scobell et al., 1977). Owing to a higher rate of mass transfer, the peak widths decrease. At lower temperatures, double peaks of the different sugar anomers occur, which fuse at higher temperatures due to the increased mutarotation rate (Angyal et al., 1979). On the other hand, components are more strongly adsorbed (exothermic adsorption) at lower temperatures and, therefore the resolution can be improved if the influence of the lower mass transfer rate is small and the effect of mutarotation is suppressed (Verhaar and Kuster, 1981).

The fructose-rich fraction in run number 10 gave a fructose purity of 80% representing 37.89% of the total sugar supplied into the column compared with that of run 11, which scored 44.2%, of the total sugar supplied into the column with less fructose purity in the fructose-rich fraction (68.76%) (Tables 2 and 5).

The purity and recovery of glucose that was collected in this process, mainly as a by-product, has not been discussed in detail. The focus of this study was the collection of fructose from date syrup, because fructose is considerably more important economically than glucose.

The dry material content of the return fraction should not be higher than 25% by weight, and preferably not higher than 15% by weight when calculated from the date syrup solution supplied. Percentage of the recovery of glucose and fructose in the return fraction with date syrup supply of 40% into the separation column was 6.7 and 21.05% for runs 12 and 10, respectively, while runs 9 and 11 scored a recovery of glucose and fructose of 15 and 7.14%, respectively (Table 5).

4. Effect of Date Syrup Concentration. Increasing the supplied date syrup concentration from 20 to 40% caused the recovery of fructose, in fructose-rich fraction to be increased. The same effect was observed for glucose recovery in glucose-rich fraction. Percentage of the recovery of glucose and fructose from glucose-rich fraction, return fraction, and fructose-rich fraction was shown in Table 5. In respect to eluted glucose and fructose concentrations, there was no fixed relationship between recovery of sugars and their purity.

CONCLUSIONS

In the process of separating fructose from glucose, the uniform supply of the solution onto the resin surface is very important (Melaja, 1972). This problem was encountered at high date syrup concentration (40%) with high flow rate 0.05 BV/min. The temperature in the column was 30^{o}C or 70^{o}C, but lower temperatures could also be used, e.g. room temperature, however the capacity would decrease accordingly. Saska et al., (1992) reported measurement of the adsorptive properties of a Dowex Monosphere 99 CA resin (Ca^{2+} form; Dow chemical, Midland, MI, USA) for glucose and fructose under conditions of high concentrations.

The economic value of fructose is approximately three times that of sucrose. Fructose syrups, manufactured from corn, are rapidly replacing sucrose in the sweetener markets in both Japan and USA ("High Fructose Corn Syrup") (Barker and Joshi, 1991). Fructose is marketed as a solution, in which case the degree of purity should be no less than 80%. Therefore the fructose solution obtained from the column is a finished product conformin to the requirements of commercial quality. The results achieved in these small-scale experiments have shown that separation of fructose and glucose from date syrup could be highly economically.

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Tables

Table 1. Chemical composition of date syrup.

Moisture %	13. 5
Total Solids %	86. 5
Fructose %	41
Glucose %	39
Sucrose %	1
Ash %	1.5
Protein %	2.2
Pectin %	1.8

Table 2. Average percentage concentration of glucose and fructose in glucose-rich fraction, return fraction, and fructose-rich fraction.

Run	Date Syrup Concentration %	Flow Rate ml / min.	Column Temperature C	Average Percentage of Glucose and Fructose					
				Glucose Rich Fraction		Return Fraction		Fructose Rich Fraction	
				Glucose % *Gg*	Fructose % Gf	Glucose % Rg	Fructose % Rf	Glucose % Fg	Fructose % Ff
1	20	5.5	30	79.29	20.71	56.70	43.30	25.93	74.07
2	20	5.5	70	79.06	20.94	60.00	40.00	41.78	58.22
3	20	11	30	73.70	26.30	54.84	45.16	30.38	69.62
4	20	11	70	73.29	26.71	59.65	40.35	39.80	60.20
5	30	5.5	30	74.50	25.50	52.00	48.00	29.88	70.12
6	30	5.5	70	75.28	24.72	46.00	54.00	33.07	66.93
7	30	11	30	68.06	31.94	57.64	42.36	36.75	63.25
8	30	11	70	66.76	33.24	49.22	50.78	28.40	71.60
9	40	5.5	30	72.90	27.10	45.76	54.24	23.00	77.00
10	40	5.5	70	72.90	27.17	52.45	47.55	20.00	80.00
11	40	11	30	68.62	31.38	49.26	50.74	31.24	68.76
12	40	11	70	63.84	36.16	41.57	58.43	25.63	74.37

Gg = Average percentage of glucose in glucose-rich fraction. ***Gf*** = Average percentage of fructose in glucose-rich fraction.
Rg = Average percentage of glucose in return fraction. ***Rf*** = Average percentage of fructose in return fraction.
Fg = Average percentage of glucose in fructose-rich fraction. ***Ff*** = Average percentage of fructose in fructose-rich fraction.

Table 3. Elution time and volume of glucose-rich fraction, return fraction, and fructose-rich fraction.

Run	Date Syrup Concentration %	Flow Rate ml / min.	Column Temperature C	Glucose Rich Fraction		Return Fraction		Fructose Rich Fraction		Total Elution Volume Tev
				Elution Time / min.	Elution Volume / ml *Evg*	Elution Time / min.	Elution Volume / ml ***Evr***	Elution Time / min.	Elution Volume / ml ***Evf***	
1	20	5.5	30	7.5	41.25	6	33	16	88	162.25
2	20	5.5	70	10	55	4	22	10	55	132
3	20	11	30	4	44	2	22	12	132	198
4	20	11	70	4	44	1	11	8	88	143
5	30	5.5	30	10	55	2	11	20	110	176
6	30	5.5	70	12	66	2	11	18	99	176
7	30	11	30	6	66	1	11	16	176	253
8	30	11	70	6	66	1	11	9	99	176
9	40	5.5	30	12	66	6	33	22	121	220
10	40	5.5	70	12	66	8	44	18	99	209
11	40	11	30	8	88	2	22	18	198	308
12	40	11	70	12	132	2	22	16	176	330

Evg = elution volume (ml) for glucose-rich fraction. ***Evr*** = elution volume (ml) for return fraction.
Evg = elution volume (ml) for fructose-rich fraction. ***Tev*** = total elution volume.

Table 4. Weight recovery of glucose and fructose in glucose-rich fraction, return fraction, and fructose-rich fraction.

Run	Date Syrup Con. %	Flow Rate ml / min.	Column Temp. °C	Sugar Supply (g) *Sg*	Weight Recovery (g) of Glucose and Fructose (gram)					
					Glucose Rich Fraction		Return Fraction		Fructose Rich Fraction	
					Glucose (g) (*Gg* x *Evg* x *Sg*) ÷ *Tev*	Fructose (g) (*Gf* x *Evg* x *Sg*) ÷ *Tev*	Glucose (g) (*Rg* x *Evr* x *Sg* ÷ Tev	Fructose (g) (*Rf* x *Evr* x *Sg*) ÷ *Tev*	Glucose (g) (*Fg* x *Evf* x *Sg*) ÷ *Tev*	Fructose (g) (*Ff* x *Evf* x *Sg*) ÷ *Tev*
1	20	5.5	30	2.34	0.47	0.12	0.27	0.21	0.33	0.94
2	20	5.5	70	2.34	0.77	0.20	0.23	0.15	0.42	0.57
3	20	11	30	2.34	0.38	0.14	0.14	0.12	0.47	1.09
4	20	11	70	2.34	0.53	0.19	0.11	0.07	0.57	0.87
5	30	5.5	30	3.51	0.82	0.28	0.11	0.11	0.65	1.54
6	30	5.5	70	3.51	0.99	0.33	0.10	0.12	0.65	1.32
7	30	11	30	3.51	0.62	0.29	0.10	0.06	0.90	1.54
8	30	11	70	3.51	0.88	0.44	0.11	0.11	0.56	1.41
9	40	5.5	30	4.68	1.02	0.38	0.32	0.38	0.59	1.99
10	40	5.5	70	4.68	1.08	0.40	0.52	0.47	0.44	1.77
11	40	11	30	4.68	0.92	0.42	0.16	0.17	0.94	2.07
12	40	11	70	4.68	1.19	0.68	0.13	0.18	0.64	1.86

Sg = Supply of Date Syrup (dry sugar weight g) into the column.

Table 5. Percentage of recovery of glucose and fructose in glucose-rich fraction, return fraction, and fructose-rich fraction .

Run	Date Syrup Con. %	Flow Rate ml / min.	Column Temp. C	Percentage of Average Recovery* Glucose and Fructose					
				Glucose Rich Fraction		Return Fraction		Fructose Rich Fraction	
				Glucose % Recovery (g) ÷ Sg	Fructose % Recovery (g) ÷ Sg	Glucose % Recovery (g) ÷ Sg	Fructose % Recovery (g) ÷ Sg	Glucose % Recovery (g) ÷ Sg	Fructose % Recovery (g) ÷ Sg
1	20	5.5	30	20.16	5.26	11.53	8.81	14.06	40.18
2	20	5.5	70	32.94	8.73	9.99	6.66	17.41	24.26
3	20	11	30	16.38	5.84	9.06	5.02	20.25	46.42
4	20	11	70	22.55	8.22	4.59	3.11	24.49	37.04
5	30	5.5	30	23.28	7.97	3.25	3.00	18.68	43.83
6	30	5.5	70	28.23	9.27	2.88	3.38	18.60	37.65
7	30	11	30	17.76	8.33	2.50	1.84	25.56	43.99
8	30	11	70	25.04	12.47	3.08	3.17	15.98	40.28
9	40	5.5	30	21.87	8.13	6.86	8.14	12.65	42.35
10	40	5.5	70	23.05	8.53	11.04	10.00	9.47	37.89
11	40	11	30	19.60	8.97	3.52	3.62	20.08	44.21
12	40	11	70	25.54	14.46	2.79	3.91	13.66	39.64

* The recovery calculated based on the supply of date syrup (dry sugar weight) into the column.
Sg = Supply of Date Syrup (dry sugar weight g) into the column.

Maintaining the Soft Consistency of Date Paste

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Keywords: Date processing, archeological properties

Abstract

Experiments were carried out to overcome the solidification of date paste manufactured from indigenous date cultivars. A steam treatment was devised. Two Omani date cultivars, namely Bunarinja and Fardh, were exposed to steam for 3 different lengths of time (5, 10, or 15 minutes), wrapped in cellophane sheets and stored at room temperature. Moisture, pH, compression force, colour space, and water activity were monitored for 11 weeks. Results obtained for Bunarinja paste after 10 minutes steaming were: moisture ranged from 17.06-19.8, pH from 5.4-5.66, compression force from 98.67-157.33g/cm^2, water activity (A_w) from 0.503-0.513, and colour lightness (L*) from 24.8-31.7. Fardh paste moisture content ranged from 23.71-18.8, pH from 5.01-4.87, compression force from 61.0-96.6g/cm^2, water activity (A_w) from 0.616-0.589, and colour lightness (L*) from 24.8-31.7. The control results showed a moisture range from 16.39-16.8, pH from 5.66-5.42, compression force from 243.3-1333g/cm^2, water activity (A_w) from 0.466-0.499, and colour lightness (L*) from 24.8-31.7. Exposure to steam for 10 minutes was recommended based on tested properties in addition to sensory evaluation preference. The treatment was up scaled to a pilot experiment using a steam cabinet, which meets the requirements of food processing equipment. As a result, a particular type of steam cabinet for conducting the treatment commercially has been recommended and distributed to date packaging units.

INTRODUCTION

Date paste is one of the most desirable and widely processed local date products in Sultanate of Oman. Small date processing units, which have been subsidized by the Ministry of Agriculture and Fisheries, are now converting their processing plants from manual to mechanical production.

The dryness and hardness of date paste was reported by regional ministry staff to be the main problem facing the producers of the product. However, few of them have attempted to overcome this problem by adding water or oil because this leads to a low quality final product. Previous studies have also reported on the changes in mechanical properties of untreated date paste with the passing of time (Al-Hamdan and Hassan, 2003).

This study was conducted over 11 weeks of two seasons (2001-2002 and 2002-2003). The aim was to develop a simple treatment to sustain the soft texture of date paste when stored for direct consumption or for use in pastry products.

MATERIALS AND METHODS

Treatment of Date Paste with Steam

Date pastes were created from 2 cultivars: Bunarinja and Fardh. Two hundred grams of fresh dates were de-stoned, using a Nazhat destoner. They were spread on a stainless steel sieve, covered and steamed for 5, 10 or 15 min. The steam was generated from a boiling water pan beneath the sieve.

The steamed dates were minced using a Kenwood-mincer, pressed into cubes and packaged in a Junior-minipack-thermal shrinkage machine.

Control treatments consisting of date pastes made from the 2 cultivars without the steam treatment were set up for comparison.

Weekly Measurements

Acidity (pH-values) of the filtrate from 2g samples suspended in 20 ml dist. was calculated using a HACH-portable pH-meter. The average of three readings was considered.

Moisture percentage of 5g of the treated paste was obtained by moisture-balance OHAUS MB2000. The average of three readings was considered.

Compression force of a 500g date paste block was obtained using a Fudoh Rheometer. The average of three readings was considered.

Color space (L*,b*,a*) systematic measurements were obtained by applying NF333-portable spectrophotometer (chromometer) directly to three different points on the paste surface. The average of three reading was considered.

Water activity (A_w) values were obtained using a Novasina water activity measuring instrument. The average of three reading was considered.

RESULTS AND DISCUSSIONS

Moisture Percentage

The moisture percentage of the untreated Bunarinja paste showed a steady trend throughout the experiment, while the treated samples gave fluctuating values with a clear increasing tendency (Fig. 1). The highest moisture percentage of 19.8% occurred in the treatment that had been steamed for 10 minutes, and this is below the maximum acceptable value of 26% (Gulf standard).

The Fardh date pastes showed a more stable moisture percentage throughout the experiment in all treatments and the control (Fig. 2).

pH Measurements

The pH measurements of most treatments remained relatively stable with only slight fluctuations, which was acceptable due to the conditions of measuring. The pH-value is an indication of deterioration in foodstuffs with a high sugar content, due to yeast growth and enzyme activity. The steady pH readings indicated the safety of the steamed paste throughout the eleven weeks. The pH values of Bunarinja paste showed a slight tendency to decrease, but remained within the narrow range of 5.37 and 5.73 (Fig. 3). Fardh paste showed a more stable value in all treatments and remained within the range of 4.87-5.01 (Fig. 4).

In order to standardize the sensory evaluation of the work, measurements of compression, and color space (L*, a*, b*), were carried out. Although, the measured pH–values and moisture percentages indicated the safety of the treated paste, additional measurements of water activity (A_w) were necessary to dissipate any concern about eventual spoilage.

Compression Force

Compression force of Bunarinja paste (control) increased markedly from the first week, i.e. from 243.3g/cm^2 to 843.3g /cm^2. The readings fluctuated within the range of 1150-1350g/ cm^2 until the end of the experiment. Meanwhile, the steam treated pastes generally showed (Fig. 5) a slight increase in the compression force during the eleven weeks. With respect to the other parameters, of color, pH-values and sensory evaluation, the ten minute steam treatment resulted in an acceptable paste.

A similar trend appeared in the changes of the compression force of Fardh paste during the experiment (Fig. 6).

Color Space

The color space (i.e., L*, a*, b*) of steam treated Bunarinja pastes indicated a decrease in lightness value (L*) with the progress of time. On the other hand, no obvious change in a* and b* values was observed. Generally, the total value of the measured color space decreased, which indicated the disappearance of the color hue and an increase in the

darkness for all treatments, including the control (Fig. 7). In Fardh, the L* values (Fig. 8) did not show a clear trend towards darkening, which was probably due to the naturally dark black colour of the Fardh date. Cooling of the paste improved the color by slowing down non-enzymatic browning, but the consistency of the paste became stiffer (Belitz et al., 2004). However, the slight improvement in color for paste made from both cultivars would not justify the cost of refrigeration.

These results led to the conclusion that steam treatment improved the preservation of the paste.

Water Activity (A_w) Measurements

Water activity (A_w) measurements of Bunarinja paste showed a slight increase in the second week, but remained beneath A_w 0.6 (Fig. 9) within the range of 0.502 and 0.526 throughout the experiment. This is within the safe range, which reduces the spoilage potential. However, the control exhibited a steep increase in the second week and subsequently stabilized within the range of 0.497 and 0.499. The obtained values supported measurement that indicates the food spoilage as pH and moisture content.

The trend of A_w values in Fardh paste showed no obvious change (Fig. 10) despite the higher initial value (higher than Bunarinja paste) of 0.6, but showed slight change through the experiment which is little over A_w 0.6, hence within the safe range (Fellows, 1988) for the paste and correlated with the pH and moisture values.

CONCLUSION

The results confirmed the effectiveness of 10 minutes of steaming to both sustain the softness and help preserve the paste. The positive effect is due to thermal sterilizing in addition to enzyme inactivation. The quality of the product is not altered as steam is a natural treatment and no preservatives are added. Steaming for ten minutes is recommended to soften the date paste.

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Gulf standard No. 656/1997 (whole packed dates 'Arabic version').

Figures

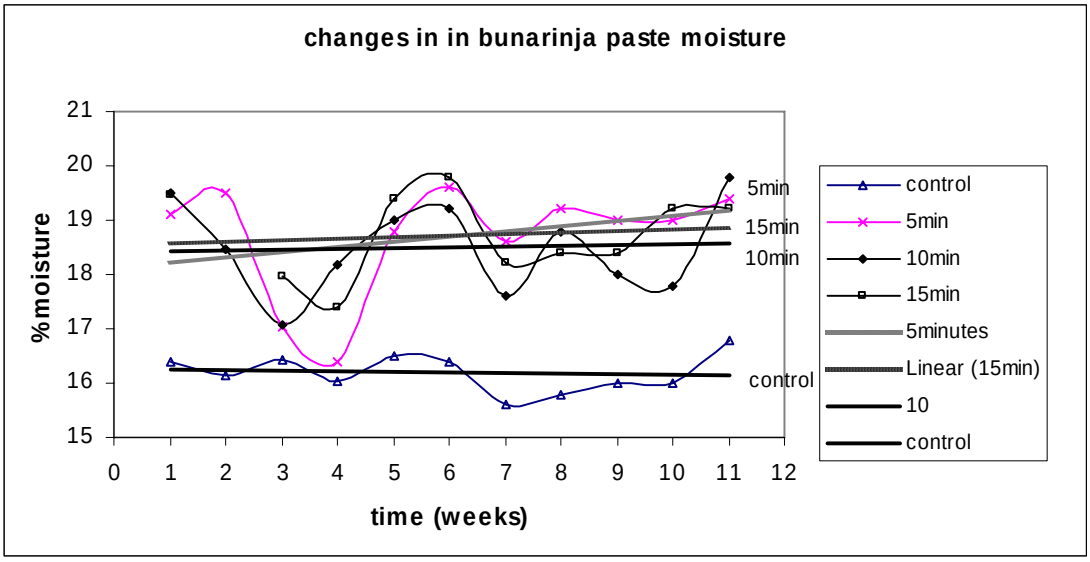

Fig. 1. Changes in the moisture content of Bunarinja date paste.

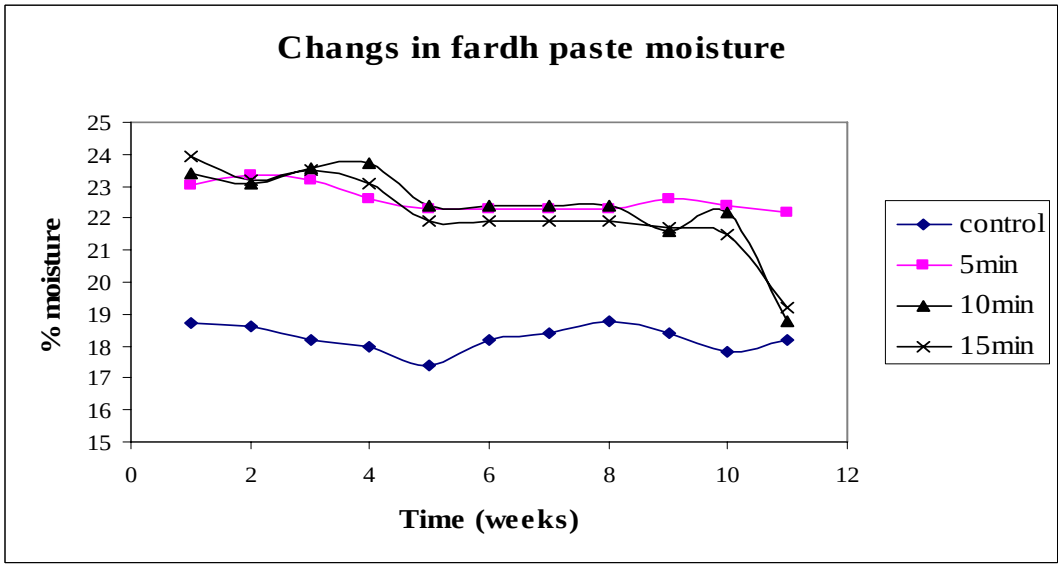

Fig. 2. Changes in the moisture content of Fardh date paste.

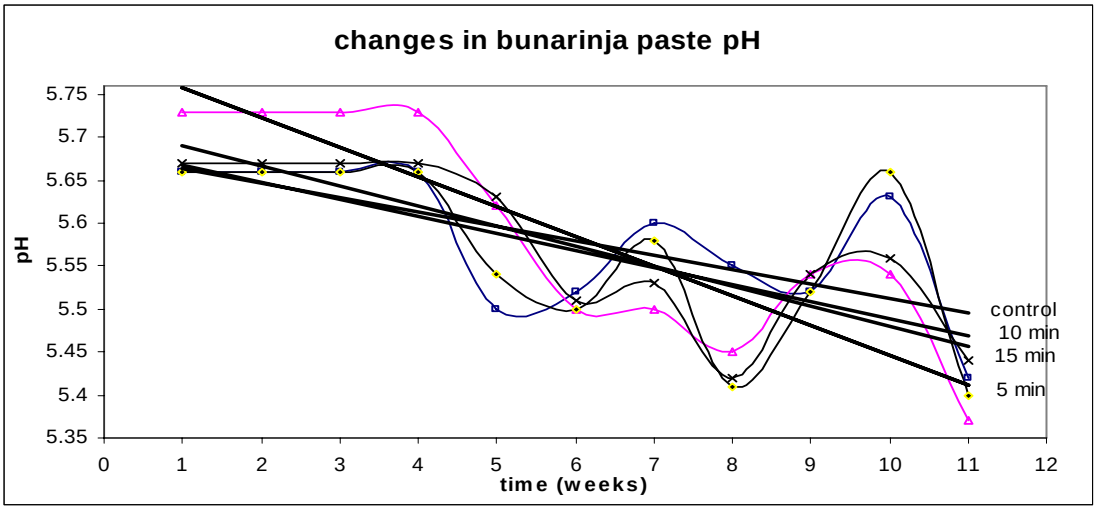

Fig. 3. Changes in pH of Bunarinja paste.

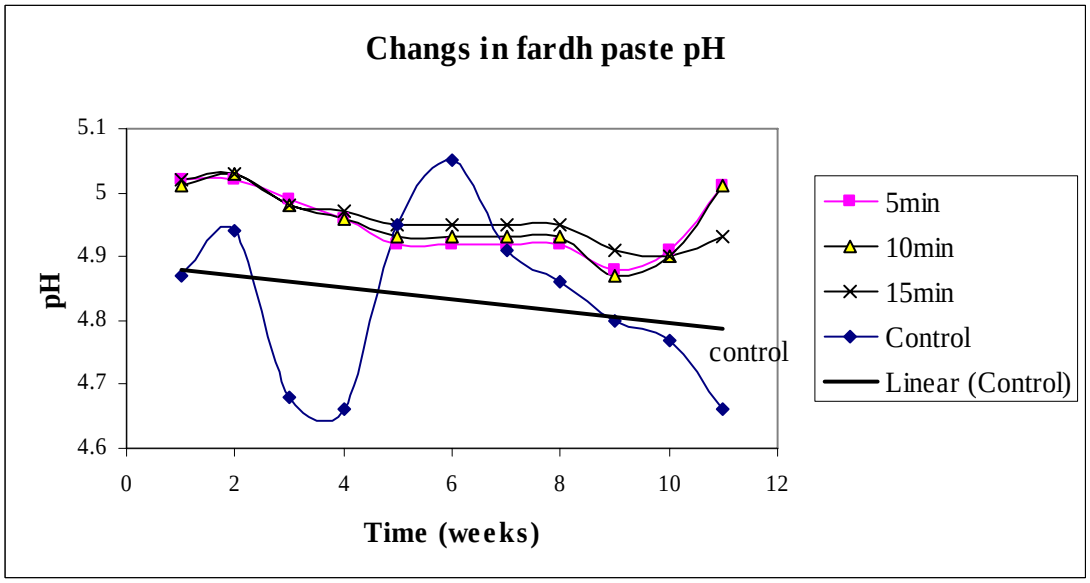

Fig. 4. Changes in pH of Fardh date paste.

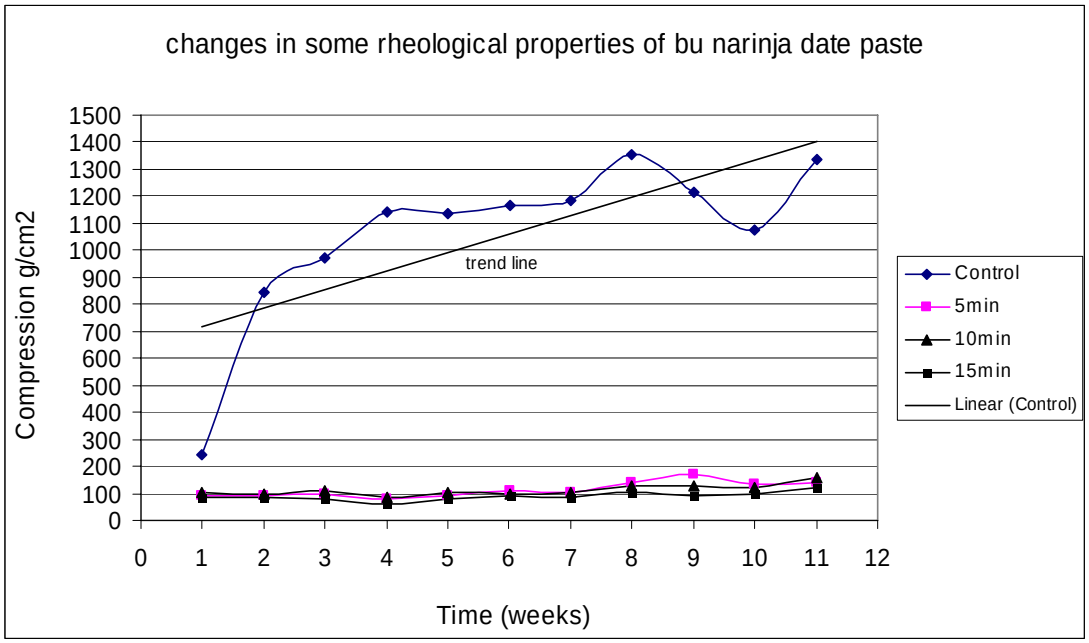

Fig. 5. Changes in some rheological properties of Bunarinja date paste.

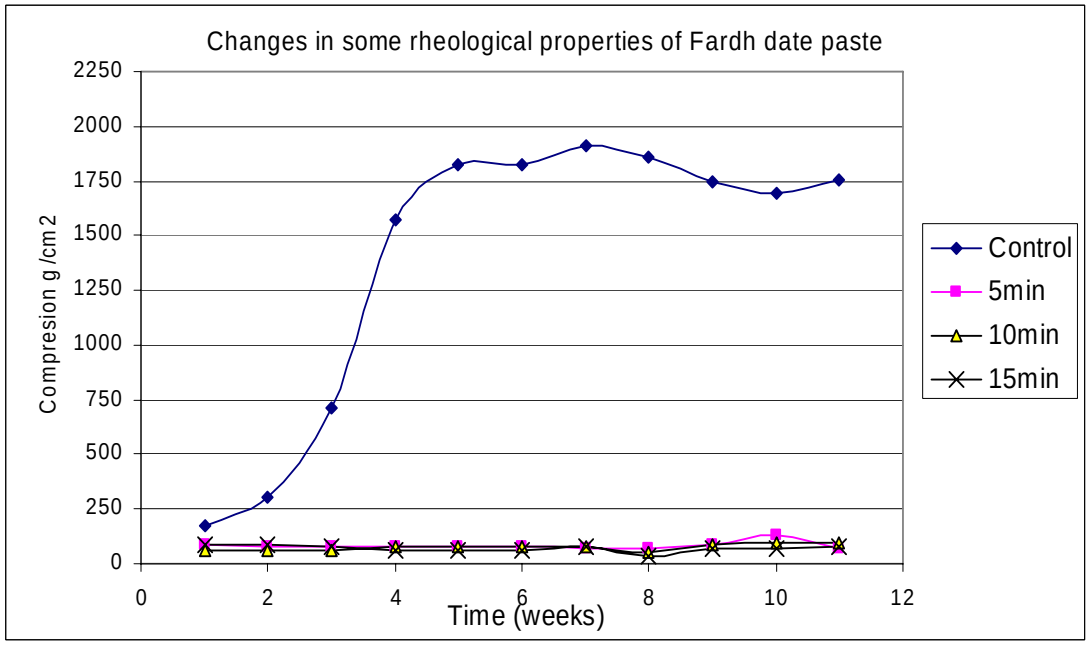

Fig. 6. Changes in some rheological properties of Fardh date paste.

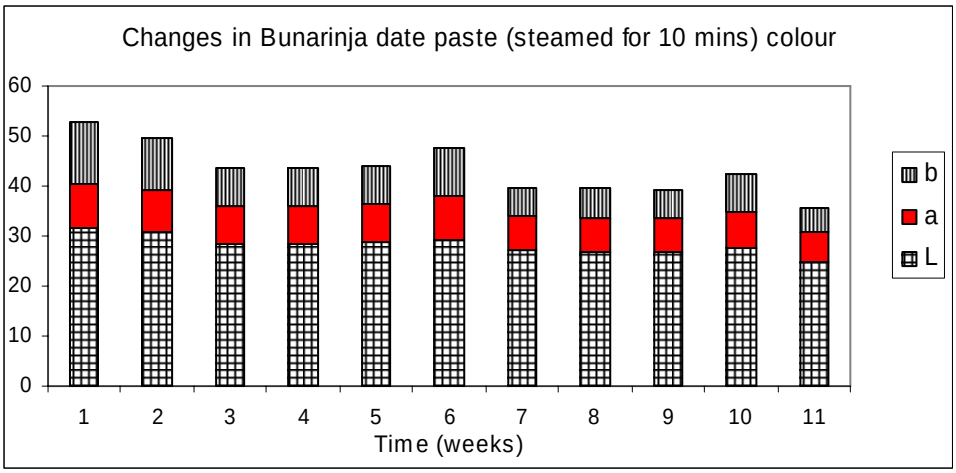

Fig. 7. Changes in the colour of Bunarinka date paste (steamed for 10 min).

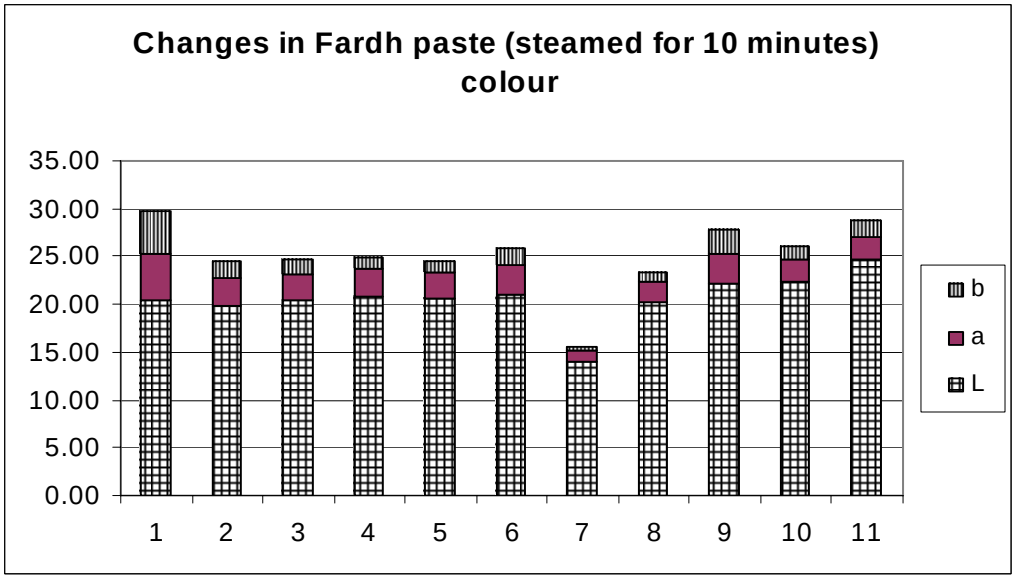

Fig. 8. Changes in the colour of Fardh date paste (steamed for 10 min).

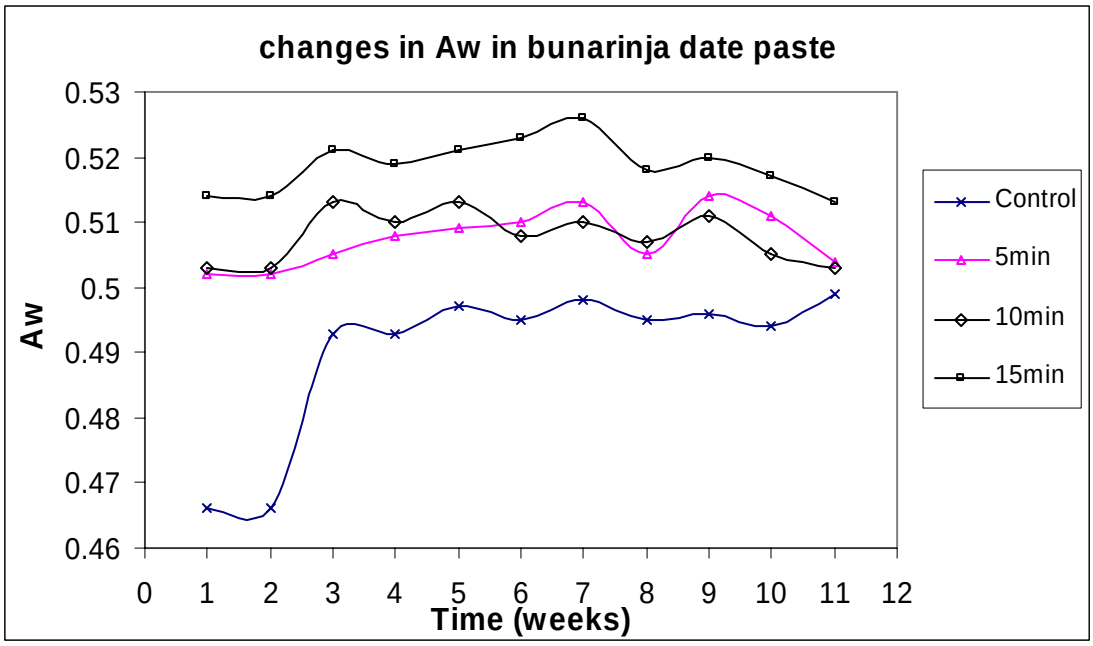

Fig. 9. Changes in the water activity (A_w) in Bunarinja date paste.

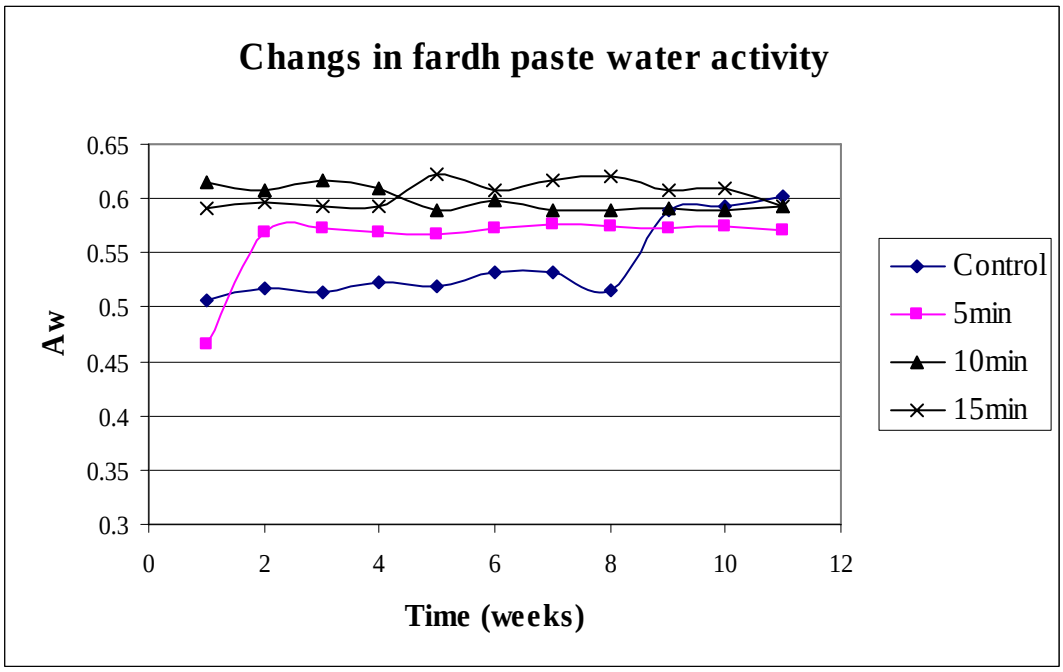

Fig. 10. Changes in water activity (A_w) in Fardh date paste.

Added Value for Date Extracts by Invertase Production

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Keywords: biotechnology, fermentation, ß-fructofurannosidase, *Saccharomyces cerevisiae*

Abstract

Date production occupies an important place in Algerian agriculture. Part of this production remains unsold. In order to productively use this portion a new product was sought that would be easy to market and be of industrial use. We attempted to isolate a yeast (*Saccharomyces cerevisiae*) from the extract of date variety Mech degla that would produce invertase. The results have revealed a good adaptation of the isolated yeast from the beginning of culture, contrary to the commercial yeast which presented low adaptation in the beginning. The growth rate for the enzyme isolated and the commercial enzyme cultivated under the same conditions was estimated at 0.16/hour. The enzymatic activity of invertase produced by *Saccharomyces cerevisiae* cultived on an extract of the date variety Mech degla was better than that produced by *Saccharomyces cerevisiae* cultived on an extract of dates of Ghars. We concluded that the use of this technology could be beneficial to both the yeast (*Saccharomyces cerevisiae)* and date industries.

INTRODUCTION

By-products of the palm date which are rich in fermentable sugars, contain substrates used in the production of numerous substances of high added value. These by-products represent 30 to 50% of the Algerian production.

According to the latest statistics, Algerian production of dates reached 420 000 ton/year of which 116 000 ton/year are considered dates of high added value (FAO, 2003) if they can be recovered and transformed using bioengineering processes.

The use of these dates in the process of fermentation permits the biosynthesis of invertase. This transformation is realized by the common yeast *Saccharomyces cerevisiae* which uses sugar substrates (Haq et al., 2002; Baig et al., 2003).

The sugar content in dates is high and can reach 80%. It contains a mixture of sucrose, fructose and glucose which varies according to the variety of the date (dry or soft) (FAO, 1993; Belguedj, 2002).

Sucrose is a disaccharide composed of a molecule of ß-D-Glucose and of a molecule of ß-D-Fructose bound by a link α-1,4-glycosidique. When this link is broken in a reaction of hydrolysis, an equimolecular mixture of glucose and fructose is produced. This mixture of monosaccharides is called the inverted sugar (Shafiq et al., 2003). Sucrose can be hydrolysed in the presence of invertase to give glucose and the fructose. The name of the invertase is it β-D-fructofurannosidase (EC3.2.1.26).

The invertase produced by the yeast *Saccharomyces cerevisiae*, is mainly used in the agroalimentary industry (candies) where fructose is preferred to sucrose because it is softer and does not crystallize easily. In Algeria, all of this enzyme is imported.

The present work investigated the production of the invertase by *Saccharomyces cerevisiae* from aqueous extracts of dates. The activity of the enzyme and the rate of inversion of sugars were determined for 2 varieties of dates (soft and dry).

MATERIAL AND METHODS

Two varieties of dates (fruit of the palm date, *Phoenix dactylifera*) were used: dry variety 'Mech-degla' and soft variety 'Ghars' (Fig. 1 and 2).

The stump of yeast was isolated from its natural habitat: the date. Isolation was achieved on Sabouraud environment by microscopic identification while determining the shape of the cells and the mode of multiplication. The faculty to the filamentation was realized on PDA environment during one week. The study of the physiological characters was based on the test of sugars, the assimilation of nitrogenous compounds and the reduction of the green bromocrésol on the WLN environment (Larpent et al., 1990; Larpent, 1991; Didier, 1996; Callon, 1997).

The fermentation process was first achieved by a reactivation of the stump in "Carlsberg" environment followed by a prefermentation phase in an adaptation environment rich in nitrogenous compounds (date extract 20%, urea, ammonium dihydrogenophosphate, ammonium sulphate) (Bourgeois and Larpent, 1996).

Biomass production was obtained in an automatic control fermenter type H.W.S MAINZ, according to the method described by Revuzes (1979) and Larpent (1992). The separation of the microbial biomass was obtained by refrigerated centrifugation at 5000 t/mn, using a centrifugator type Sigma 3K20. The residue was dehydrated in an oven of Memmert type.

The study of the enzymatic activity of the *Saccharomyces cerevisiae* that was produced, was conducted in a sucrose solution 65° Brix incubated in a bath at 60°C (Gilbert and Pierre, 1988; Larreta, 1997; Scriban, 1999). Control of the enzymatic reaction was achieved using a polarimeter type Schmidt Haench. The transformation speed of sucrose was determined during the first hour, which permitted the control of sucrose inversion rate during the following 9 hours.

RESULTS AND DISCUSSION

Analysis of the two Algerian date varieties showed that the dry variety was richer in sugars than the soft variety. Higher rates of sucrose were obtained for the variety 'Mech-degla' (Table 1). These results are consistent with those of several authors (Belguedj, 2002; Erskin and al., 2004).

The isolation on the Sabouraud environment showed colonies with the shape and colour of yeast characteristics. Microscopic examination revealed the multiplication mode of yeast cells by budding. The colonies transferred onto the PDA were identified as pseudomyceliums. Incubation during 48 hours at 30°C on the WLN environment showed colonies of green colour, revealing an absence of reduction.

The physiological tests summarized in Table 1 and 2, confirmed that the isolated stump was *Saccharomyces cerevisiae*. Indeed, the test of sugar fermentation showed that the isolated stump from the dates was not able to ferment the lactose and the xylose, which is a characteristic of the *Saccharomyces cerevisiae* species. The inability to use the nitrates and the lactose confirmed that the isolated stump was *Saccharomyces cerevisiae* (Scriban, 1999).

The growth kinetics of the isolated stump showed that it grew better in the environment with 'Mech-degla' extract. This is due to the latency time, which was 4 hours for this variety and 5 hours for 'Ghars' (Fig. 1). 61.0% of sucrose was inverted during one hour (1g sucrose inverted / min.) by the invertase produced in the extract 'Mech-degla', however only 50.3% (0.8g sucrose inverted / min) by the enzyme produced in the extract 'Ghars' (Fig.2).

After one hour, the inversion of sucrose was slow. At the end of 10 hours, 82.3 and 65.2% of inverted sugars were formed, respectively, by *Saccharomyces* in each environment (Fig. 3). Similarly, other studies reporting on the production of the invertase by *Saccharomyces*, showed that higher concentration of inverted sugars in the environment at the beginning of the culture reduced the invertase activity (Egorov et al., 2000; Baig et al., 2003).

CONCLUSION

It was shown that the two varieties of dates are rich in fermentable sugars (sucrose and reducing sugars), however the dry variety contained the higher level of sucrose. These sugars are used by *Saccharomyces cerevisiae* as an organic substrate for invertase production. The results showed that adaptation time of the isolated yeast stump is relatively short and presented good growth requiring only nitrogenous compounds. This yeast growth was better in the environment based on dry date extract 'Mech-degla' and the invertase produced by *Saccharomyces* was more active on this same environment. The rate of bioconversion was equivalent to 80%. It is hoped that this technique can be applied on a commercial scale for the production of invertase, providing greater value for the production of "yeast-dates".

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Tables

Table 1. Sugar content in two varieties of dates * (%).

Composition	Ghars	Mech-degla
Dry matter	80.8	84.9
Total sugars	81.3	79.8
Reducing sugars	71.1	31.8
Sucrose	10.2	47.9

* average values of 5 replicates

Table 2. Test of fermentation of sugars.

Sugars	glucose	sucrose	fructose	ribose	lactose	arabinose	xylose	raffinose
VCO_2	+++	+++	-	++	-	-	-	+
Result	F	F	X	F	0	0	0	F

(F): Fermentation: (0): negative result, (X): Variable or non determined, (+++) : Gas occupies the whole bell, (++): Gas occupies 2/3 of the bell, (+): Gas occupies 1/3 of the bell, (-): Absence of gas.

Table 3. Test of assimilation nitrogenous substrate.

Middles	Results
+ nitrates as unique source of nitrogen	No change of colour
+ urea as unique source of nitrogen	Change in colour of the green to the yellow (+)
+ ammonium as unique source of nitrogen	Change in colour of the green to the yellow (+)

(+) : positive. (-) : negative.

Table 4. Inverted rates of sucrose (%).

Varieties	1 hour	10 hours
Mech-degla	61.0	82.3
Ghars	50.3	65.5

Figures

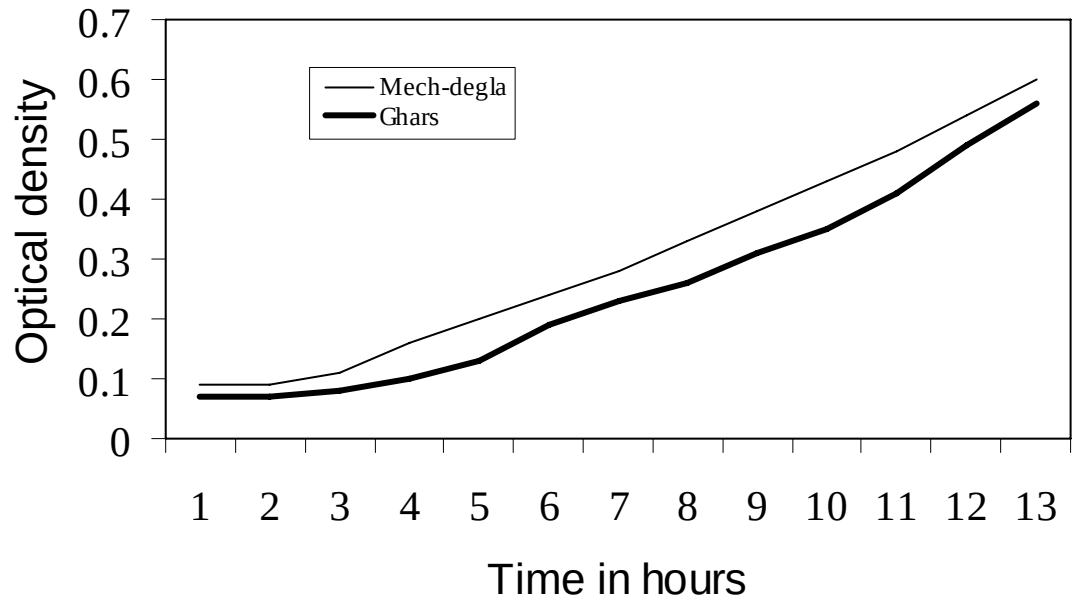

Fig. 1. Evolution of the optical density versus time.

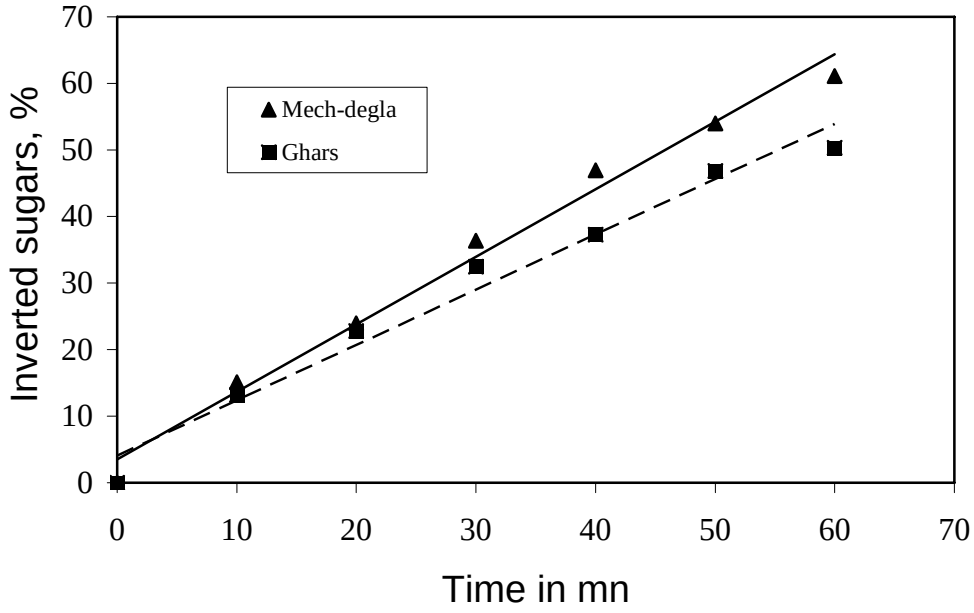

Fig. 2. Invertase activity during one hour.

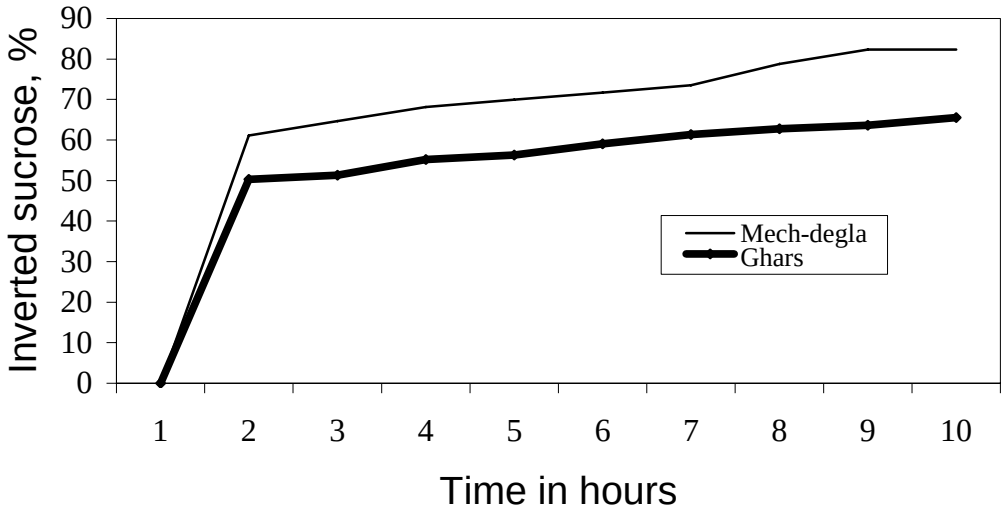

Fig. 3. Inversion sucrose versus time.

Preparation of Caramel Colour from Dates

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Keywords: Dates derived industries, colour measurements

Abstract

Caramel colour was produced by the thermal treatment of date juice (21^o Brix) or dibis (72^o Brix) in the presence of small amounts of ammonia (NH_3) and concentrated sulphuric acid (H_2SO_4). Minute quantities of sodium sulfite (Na_2SO_3) or sodium metabisulfite ($Na_2S_2O_5$) were added to some treatments. Two ml ammonia and 0.1ml conc. sulphuric acid were added to 20g of dibis (72^o Brix). Ethanol was used as an extracting solvent to obtain a clear and applicable caramel colour from the reaction products. The produced colour showed high solubility in water solutions as well as acid stability without the addition of sulfite ion, which has to be monitored. The determined colour hue of the date caramel showed a tendency to the red note. In addition, the measurements of colour space (L*a*b*) confirmed the colour hue. Other specifications of the produced caramel such as pH (4.5-5.5), TSS (76-77), specific gravity (1.39614), viscosity (7770 cps), haze point (2.15 hrs), gel point (2.30 hrs) and resinification (25 hrs) complied with the requirements for diverse food applications. Hence the acid stability test, which had been carried out for three months in acidic solution (pH 2.7) and 10^{o}Brix similar to commercial beverages, showed no precipitation and good colour stability. This finding indicated the kind of the caramel charge, which was most likely negative. The highly viscose hygroscopic caramel colour has been successfully spray dried to powder, using maltodextrin and gum arabic as formulation aids.

INTRODUCTION

In the Sultanate of Oman, the utilization of surplus dates as well as discarded and low-grade fruits is necessary to boost the income from date palm cultivation, and to maintain the role of dates as a vital national resource. The use of such dates in industry to produce products such as caramel colour is promising (Al-Abid, 2005). Caramel is the primary "natural" colourant for foods and beverages, and it forms a significant segment of the overall colour market (around 11%). The market for these colours is thought to be reasonably static, with annual increases of approximately 2-3% (Downham and Collins, 2000). Caramel colour accounts for more than 80% (by weight) of all colourants added to the foods we eat and drink. Annual global consumption exceeds 200,000 t (Kamuf et al., 2003). It is used to add colour from the palest yellow to the deepest brown to beverages, pharmaceuticals, distillery liquids, confectionery and bakery products (Ciolino, 1998).

Caramel colour is produced from the degradation of carbohydrates in acid or alkali in the presence of a catalyst. The manufacture of caramel colour is affected by various conditions such as the carbohydrate material, temperature, pH and the nature of the catalysts. However, many of the production techniques have been registered as patents. Generally, the producing companies keep such information confidential.

This work is an attempt to use dates for the production of caramel colour that can be used in the food and pharmaceutical industries.

MATERIALS AND METHODS

Preparation of Caramel Colour (1)

0.5-3ml ammonia (NH_3) and 0.1ml conc. sulphuric acid (H_2SO_4) were added to 94.4g-100g date juice (21^o Brix) or to 20-75.2g dibis (72^o Brix). 0.025-0.1g sodium sulfite (Na_2SO_3) or 0.025-0.2g sodium metabisulfite ($Na_2S_2O_5$) was also added to some treatments. The mixtures were heated in a water bath at 100^{o}C for 1.5 - 3 hours and stirred occasionally. The product was concentrated under vacuum and dried in a vacuum oven.

Preparation of Caramel Colour (2)

Two ml ammonia and 0.1ml conc. sulphuric acid were added to 20g of dibis (72^o Brix). The mixture was heated while being stirred in a water bath at 100^{o}C for 1.5 hours. One hundred ml ethanol and 50ml water were stirred with the previous reaction product and centrifuged for 10 min. The ethanol was removed under vacuum from the upper phase by rotary evaporator, and the resulting caramel colour was placed in a vacuum oven at 50^{o}C for drying.

Determination of pH

The pH has measured using a glass electrode and digital pH-meter type WTW inolab.

Total Soluble Solids (TSS) and Specific Gravity

Total soluble solids were measured using an Abbé refractometer (Bellingham and Stanley). Specific gravity was measured according to the AOAC method 932.14 (1990).

Determination of Haze and Gel Points

A mixture of 2g caramel and 1g phosphoric acid was heated in a water bath at 100°C. A drop was taken out every five minutes, placed in a tube of distilled water and the solution observed for clarity. When the drop formed a turbid solution, this was recorded as the haze point. The gel point occurred after the haze point, and was the time at which the acid-caramel mixture was no longer fluid.

Resinification

A small amount of caramel colour was sealed in a glass ampule and held at 100° C. The number of hours required for this sample to reach a point where it would no longer flow is the resinification value.

Determination of the Viscosity

Viscosity was determined using a Brookfield DVII+pro viscometer at a temperature of 24.9ºC.

Determination of Colour Hue

The absorbance (abs) changes of 0.15% aq. caramel colour solution (deionized water) was measured at wavelengths of 440nm-620nm using a Jenway 6105 spectrophotometer.

Measurements of Caramel Colour

L^*, b^*, a^* and absorbance for caramel colour solution (0.1%, 10^{o}Brix), which was prepared by method 2, and of commercial beverage (Pepsi) were measured under the same pH conditions using a NF-333 (Nippon Denshoku) and a Jenway 6105 spectrophotometer.

Producing Caramel Colour Powder

The viscose caramel colour was spray dried successfully using maltodextrin and Arabic gum (food grade) as formulation aids. The spray drying was carried out at 130^o C

in dry clean aspiration air using a Büchi mini spray dryer 290b.

RESULTS AND DISCUSSION

Caramel colour prepared by method 1 showed poor water solubility and high precipitation. The attempts to overcome these disadvantages through the addition of sulfite and bisulfate ions were unsuccessful. On the other hand, the colour prepared by method 2 showed high solubility in water and was also stable in an acidic medium. These results encouraged us to proceed with the caramel produced by method 2. In addition, the application of sulfite and bisulfate ions may have created some undesirable residues, which World have had to be monitored and regulated (Ciolino, 1998).

The formation of caramel colour takes place through two major reactions: Maillard and caramelization. In the Maillard reaction, reduced monosaccharides start to react with naturally occurring nitrogen-containing compounds at relatively low temperatures (50^oC), and the rate of reaction is increased by two to three times with each additional 10^oC. This reaction supposedly occurs in dibis manufacturing, particularly through the traditional methods because the previous reactants are available and the other reaction parameters such as temperature, water and suitable pH. Thus, dark colour and an undesirable taste developed due to the formation of melanoids, which are polymers, copolymers and other compounds. In fact, the Maillard reaction has never been thoroughly investigated during the processing of date juice and dibis, which have other reactive constituents such as polyphenols and other phenolic compounds. Hence, one of our fears during the caramel preparation was the domination of the Maillard reaction, which could lead to the formation of a bitter taste and melanoid precipitations. The fact that the dibis contained almost all invert sugar (≈ 50% fructose) reduced the above-mentioned fears because glucose tends to participate in the Maillard reaction more than fructose (Belitz et al., 2004). The conformation equilibrium of fructosa, particularly the relatively unstable part of the β-fructofuranose, facilitates the initial step of of caramelization process through the protonation of the hemi-ketal by the mineral acid, which leads to the formation of aliphatic sugar. *Lobry de Bruyn-van Eckenstein* rearrangement of the formed aliphatic sugar followed by dehydration or β-elimination reaction produces the important intermediate β-dicarbonyl compounds, which undergo several reactions including dicarboxylic cleaving, retro-aldol reaction, aldol condensation and radical reaction to form the colour and flavor of the caramel. Accordingly, the assumed temperature of 150-200°C, which is necessary for caramel colour production from sucrose, was decreased. The reaction of caramelization was directed to produce mainly the caramel colour "sucre couleur", which refers to the complicated composition of polymers, by the addition of acid and ammonia. Since the improved stability and solubility of the caramel colour are necessary for successful application of the product, manufacturers added sulfite ion and modified the processing. According to these additions and modifications the caramel colour was classified as follows: (FNB, 1994; Sethness, 2002; Kamuf et al., 2003)

- Class I is Plain Caramel Colour
- Class II is Caustic Sulfite Process Caramel Colour
- Class III is Ammonia Process Caramel Colour
- Class IV is Sulfite Ammonia Process Caramel Colour.

The caramel colour prepared from dates using method 2, fitted into class III with a wide spectrum of applications (Codex Alimentarius, 2000). The extracted colour "sucre couleur" using ethanol showed high solubility in water solutions without the need to add sulfite ion, which has to be monitored. Method 2 caramel colour had pH values in the range of 4.8, which, together with the high solid contents (TSS: 76-77%, specific gravity: 1.39614) and the sterile production conditions, provided good microbiological stability (Sethness, 2002; Kamuf et al, 2003). In addition, the relatively high specific gravity would probable prevent damage to the colour properties through freezing. Although the prepared colour appeared to be thick, it was pourable with a viscosity of 7770 cps. The caramel colour produced by method 2 met the requirements of soft drink manufacturers

who desire a colour with as low a viscosity as possible while maintaining the desired specific gravity (Sethness, 2002). The resinification test took 25 hrs, which indicated that the the colour would remain free-flowing for more than one year. Concentrated phosphoric acid solution resistance by the prepared caramel colour solution, which is known as the haze point, took 2 hrs and 15 minutes. Extention to the point at which the acid-caramel mixture was no longer fluid, but gel, required 2 hours and 30 minutes (Sethness, 2002).

The acid stability test was carried out for three months, using acidic solution (pH 2.7) and 10^{o}Brix, which is similar to commercial beverages.There was no precipitation and colour was stable. Instability in acid was the main problem in former attempts to prepare dates caramel colour (Barrefeld, 1993). This finding indicated the caramel charge, which was most likely negative. Caramel colour is essentially a sterile product. With a relatively high solids content (76-77%) and low pH, it is usually not subject to microbial attack until diluted.

Colour hue (Fig. 1), characterized by the relationship between the wavelength and absorbance in the visible range, indicated a high absorbance value in the observed colour grading from orange-yellow to red (wavelength; 440-500nm) (Pavia et al., 1979).

The determined colour hue of the date caramel showed a tendency to the red note. The measurements of colour space (Table 1), showed high value for (a*), which confirmed the determined colour hue. Compared to the Pepsi drink, the lightness (L*) value of date caramel colour was found to be much higher, and so was the (b*) value as shown in Table 1.

These differences are due to the colour concentration used and the unknown colour class. Many attempts to convert the caramel produced to powder form failed because of the high viscose hygroscopic nature of the colour. To overcome this obstacle, the colour was formulated with maltodextrin and Arabic gum. The spray dried caramel colour formulation possesses less colour intensity compared to the liquid form.

The results indicate that production of caramel colour from dates is posible and could have a wide range of applications in the food industry.

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Tables

Table 1. Comparison of colour space values of caramel made from dates with Pepsi drink.

Colour space	Pepsi	Caramel Colour
L*	24.10	33.25
a*	22.67	62.67
b*	12.57	22.22

Figures

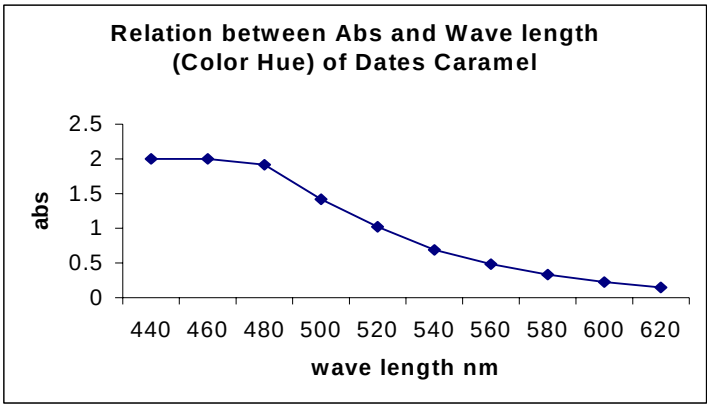

Fig. 1. Relationship between abs and wave length (colour hue) of caramel colour made from dates.

Production and Marketing Problems of Date Palm in the Region of Biskra, Algeria

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Keywords agricultural policy, extension programs

Abstract

This study attempted to identify production and marketing problems facing date producers in the Biskra region of Algeria, with emphasis on the role that could be played by agricultural extension to solve these problems. Particular consideration was given to the differences and interaction between these problems and agricultural policy and their effect on achieving the objectives of the study. A sample of 64 date growers was selected randomly from three major areas in the Biskra region. The research findings are presented and discussed with recommendations on how to improve date production in the region.

INTRODUCTION

The cultivation of palm trees and the production of dates are the most important agricultural activities in the desert of Algeria, and they account for more than half of the arable land. In the past, palm trees have played an essential role in the creation and sustaining of oases with their great ability to adapt to the hard and marginal conditions. The oases became a framework for the creation of living conditions for settlement of the local population, in contrast to the lifestyle of desert residents, who are nomadic. Oases are ecologically, economically and socially important to Algerian Sahara. However, in these areas, illiteracy and poverty are higher, and the standard of living lower, than in the city. Prosperity from the petroleum and gas industries in desert areas has added to the sufferings of this sector, and to the technical problems and diseases of Albewdh. This has led to a mass exodus of the workforce from the palm tree sector, causing a marked deterioration of the palm oases in most regions. Reports reflect strongly the need for agricultural development strategies aimed at the rehabilitation of the palm tree growing areas, with priority being given to financial resources and efforts to address social and economic problems, as well as infrastructure, facilities and institutions.

Research Sample

A random sample of 64 palm farmers was selected, so that net returns per hectare of palm cultivation could be compared. Farmers were selected from the three major areas of agriculture in Biskra: Ain Ben Anoui, Toulga and Adousen. These areas are similar in geography and in the agricultural practices employed.

RESEARCH METHOD

The Geographical Scope of the Search and Its Characteristics

The region of Biskra was selected as a site for this research because it is one of the most important areas of date production in Algeria. The number of palm trees planted in the area is 1,562,424, which occupy 21,262 hectares. The production is estimated at 71,723.2 tones. The area planted with date palms in and around the town of Biskra represents about 25% of the total area of palms in Algeria. Biskra produces 25.2% of the total national production. Table 1 shows that Biskra is ranked second in terms of area cultivated in the south-east states, and ranked second at the national level. The production area ranged between 34.22% and 36.72% of the total area cultivated in the south-east states during 1989 and 2004, respectively. It ranged between 23.7% and 26.39% of the total area cultivated at the national level during 2004 and 1987, respectively.

The general trend of cultivation of palm trees during the period 1985-2004 (Table

1) was an annual increase in production of about 2% of area cultivated in the Biskra region. During the same period the Republic's total production of dates increased by 156,501 tones per annum, an increase of 5.5%. During the period 1985-2004, average production was 71,723.2 tons and ranged between 7.95% 55.78% of the total date production of southern-east towns during 1997 and 2004, respectively. Biskra produced 18.01% and 28.89% of the total national production in 1990 and 2004 respectively. The annual increase in the production of dates was 5,017.7 tons or 7% of the average annual production of dates in the town. The annual increase in productivity of palm trees of 0.80 kg per tree amounted to about 2% of average annual productivity nationally. Average production was 43.10 kilograms per tree during the period 1985-2004 and production ranged between 70.06 and 175.33 during 1990 and 2004, respectively. Table 1 estimates that the annual increase in average production is 2.023 kg, or 47% of the total average of production of the Biskra region.

RESULTS

The Feasibility of Cultivating Palm Trees in the Biskra Region

Feasibility of cultivating palm trees is determined by two key factors. Firstly, palm trees must be able to grow and bear fruit well in the conditions of soil, climate, natural and biological factors. Secondly, date production must be economically viable, and more profitable than growing alternate crops on a farm. This will lead to a rise in the standard of living.

Biskra region was selected for a comparison of different farm systems used in Algeria. It included a sample of 64 farmers who grew palm trees and 64 farmers who had regular plastic houses and grain. Table 2 shows the results of this survey. The net revenue for per hectare production of vegetables in plastic houses was more than the per hectare net revenue of each of the other systems. Second in terms of net revenue was the production system Adagla, followed by mixed production dates, and then grain production. The results in this table suggest that farmers should produce vegetables, and Daglat Nour, because they provide better economic returns. However, comparing returns for palm trees per unit area does not reflect the economic truth because of the vast differences in density of plants in these production systems, which should be taken into account if comparisons between production systems can be real and useful.

Ways to Sell Dates

There are multiple ways of selling dates in the town, and perhaps the most important of these ways are:

1. Sales on Capital Palm Trees. In this case the responsibility of the producer ends at the beginning of the maturity of fruit, and the buyer is responsible for the care of palm trees until maturity. This is done by the owners of large and medium-sized farms and sometimes small ones. This method is preferred as it avoids problems of the guard, preparation, processing costs and risks of the market, that lead to the decline of sale price. The owner of palm trees is unable to show the real value of his produce, and the buyer merchant usually has the advantage. He returns to buy palm trees again, and the price is determined in this way by either auction or bargaining. Major producers use this system. The second system followed by most producers, is based on estimating the quantity of fruit on the trees and the expected price in the next season.

2. Sales Within the Farm. The producer presents the fruit of palm trees for sale when they are fully mature. The buyer bears the cost of collection, sorting, transportation and storage until the dates are sold to consumers.

3. Sales Outside of the Farm. The producers performed all agricultural operations until full maturity. This is in addition to collection, packaging and transport to wholesale markets.

4. Sales Within the Village. This method is followed by small holders. The produce is

marketed within village markets, and sold directly to consumers, local traders or to merchant retailers.

Marketing Channels Used in the Marketing of Date Fruit

The producers in Biskra region sell most of their dates to retailers for the following reasons:

1. Selling to retailers achieved fair to higher prices
2. Retailers are easy to deal with and transportation costs are lower.
3. Retailers buy large quantities.
4. Shortened sales transaction with prompt payment.
5. Avoiding bargaining over the price with consumers.

Sevently-six percent of producers that sold to retailers preferred dealing in cash because:

1. Cash is easy to handle, and its use is clear-cut for both the seller and buyer.
2. The payment of cash ensures there is no disagreement on the price after the completion of the sale. Twelve percent of producers prefer to sell to retailers because of expanding sales and marketing. In addition it develops confidence and cooperation between producers and traders throughout the year. About 15% of the producers who were surveyed, stated that retailers assist in the rapid commercialization of stockroom dates, and in conciliation between the parties collaborating in the market.

Ten percent of the producers who were sampled sell directly to local traders because of the increasing demand by local traders for dates, especially in the harvest season, and, the increasing demand by consumers in the fruit markets.

The community was distributed between the various competing uses. The system for making sale and purchase transactions in a particular place and time must be efficient in meeting supply and demand. It is essential that the parties involved in the marketing process coordinate the targets and decisions for the parties that are involved.

Types of Date Markets

There are multiple types of markets. They vary according to the distance between production areas and consumption areas. The type of intermediaries who handle these areas are different. Usually dates are transferred from the farm to the market of the village, or local market, or the wholesale market, according to the volume of production and the marketing services that are available. The relationship between the size of the domestic demand and domestic production in the region of production matters, but the retail market is closer to consumers. The most important aspects of markets, marketing services and marketing communities in which they operate are investigated.

1. Local Markets. These are one of the most important types of date markets. They are fixed permanently, deal between producer and intermediaries (on behalf of the traders news-stands or wholesalers), or producers and traders directly with discretionary methods. The producer is the weaker partner in terms of his ability to bargain, his access to market data, and his necessity to borrow on his harvest from these communities. The producers or dealers offer relatively modest quantities in these markets.

2. Village Markets. These markets occur regularly in villages, one day a week, supplying the needs of consumers with goods which are purchased from local merchants or producers. Local traders and brokers obtain appropriate quantities of goods from the market and re-sell wholesale to dealers.

3. Wholesale Markets. Wholesale markets of dates and other fruit are found in cities such as Biskra and Graih. These markets have extensive facilities for marketing, but there are some shortcomings which impair their efficiency. The producers or wholesalers supply the produce in wooden or plastic crates, and use trucks to carry loads of different weights. They sell to traders who sell to the various sectors.

4. Retail Markets. These markets are spread across villages and cities, and deal with farmers and traders on the one hand, and with consumers on the other. Dates are sold to consumers in plastic bags or baskets.

The Distribution of Dates Marketed

In the city, consumers obtained dates from several places (Table 4). Of the surveyed sample, 47% of consumers obtained dates from the traditional markets in the cities in which they live, while 22% of consumers obtained dates from special shops that sell dates. About 23% accessed dates directly from the farm, 6% obtained dates from shops, and 2% obtained dates from factories in the city.

Marketing Services

Marketing services and their place of conduct are different for the different types of dates i.e. dry, half dry or wet. The provision of some marketing services by producers can contribute to an increase in their incomes. This brings greater prosperity to them and serves marketing in various ways through picking of fruit, sorting, grading, storage and transport. It may also cover certain types of marketing services such as cooling and freezing of dates in the wet or dry steps of drying. The major marketing services for various types of dates are:

1. Picking Fruit. Fruit vary with the stage at which they are picked. The fruit consumed in the Khalal (Sir) stage represent fruit at the appropriate stage of maturity. Some producers hold fruit on trees according to the availability of workers and their low wages. To separate the mature fruit and keep them in the process of Khalal is Bchammarich. However, this method leads to fruit dropping onto the ground, and if the earth is not covered with thick cloth, the fruit are contaminated by dust and sand, and they cannot be sold to consumers. Fruit from humid regions such as the palm trees of Algars (Hamraia. Aitima) are picked from Alarajin directly. The cultivation of dates that are half dry, like those of Daglat Nour, is increasing in the cities of the south-east. They collect the semi-dry fruit by cutting Alarajin totally or by selecting the desired fruit when the tissues soften.

Certain things are of major concern, notably:

* Harvest stages, separated in steps, in order to take the best harvest, while taking the increased costs into account.
* Failure to leave the dates for a long time on the trees after they dry because of injury to the fruit by insects.
* Fruit that are mature on Alarrajin until the desired stage of consumption will be of better quality than those picked before or after this stage.
* Fruit that are picked before or after the appropriate stage can be treated in ways that improve their quality but they are always of lower quality than those collected at the right stage.

2. Screening and Sorting. This is the next step of the operation after picking, where bad fruit or those which are not good for packaging are discarded. These include fruit which are not mature or are infected by insects or diseases. At this stage fruit are divided into half dry and dry. The screening process also leads to the classification of dates according to their form, size and colour. Screening takes place either manually (by farmers and traders) or through the use of modern equipment and machinery. There is no doubt that the process of sorting and grading is very important in the marketing of the product. The responsibility of the farmer is not confined to the cultivation of the best plants, but is now increased to how he should arrange, classify and sort products to satisfy the needs and wishes of customers. Consumers prefer to purchase items of good quality. The process of sorting and grading according to standards and benchmarks will contribute to the raising of the level of efficiency in marketing.

3. Packing and Packaging. The process of packaging has evolved in recent years and concerns everyone involved in the production, marketing and consumption of dates. It has grown considerably, in relation to the development of industry and the materials used. Generally, there are multiple ways of transporting and packing dates. The traditional way uses packaging that is made from the palm tree leaves and animal leather. The modern ways involve factories and packing in wood, cardboard boxes and Alsilovan. Whether packaging is for the local consumer or for export, one must take into account the

standards and the needs of the consumer. The cost of production must be appropriate and the packaging very clean. There should be consistency between the various components of the overall shape of the packaging, the color graphics and the printing. Other factors that interest the consumers are weight, type, brand, date of production, etc. Good packing allows large quantities to be stored in small spaces for longer times. Storage of dates is important for department stores, as the lack of good condition will lead to waste.. The importance of the topic is growing because one of the stages of maturity of dates requires special storage conditions. Llkhlal storage differs from that of both ripe dates and mature dates. Storage conditions vary according to the quality of dates so what is appropriate for dates tissues does not suit half dry or dry dates. In addition, each class of dates needs appropriate conditions for storage. The process of storage must be appropriate over the whole season. Communities that take the operation of storage into account are rewarded financially as it maintains the supply and market price for producers. So that storage can contribute to increased national income, there are two essential ways of storing dates:

Farm Storage: Dates stored using primitive means will be damaged as a result of exposure of fruit to inappropriate climatic conditions and insect infestation. There are different ways of storing fruit in different production areas. Originally, dates were put in piles after the surface of the soil and gravel had been covered with mats. Then the fruit were covered by mats or plastic for safekeeping from dust, insects and the rain. More recently, methods of storage have evolved and depend on the state of the fruit to be stored. If Alarajin have been picked and contain mostly immature dates, they must be stored until they mature. To achieve the maturity status of full and tender Alarajin, clean stores are required to reduce the incidence of insects.

Modern Methods of Storage: There are several modern techniques for storing dates, including cold storage and freezing, exposure to the sun, vinegar or salt solution.

4. Transport. Transport operations are required to transfer dates to consumers who wish to purchase them in an appropriate manner and at an appropriate time and place. This requires the availability of adequate transport to move the crop to the consumption areas. Transport costs increase the cost of marketing. Many types of transport are used, e.g. animals (Alahmira), cars, trucks, railways and aircraft. In the date season many producers transfer their dates directly to the wholesale markets, because of the increasing demand, and the demand for diverse types of goods. Using the example of Daglat Nour dates, it is difficult to bear the cost of transportation while selling prices are low. The marketing of dates in Algeria remains far from organized. As the harvesting season approaches, wholesalers and middlemen try to monopolize the purchase of the largest quantity of production at the lowest price. Farmers generally lack marketing facilities and modes of transportation, thus traders and brokers easily impose low prices and absorb much of the profits.

The marketing methods (sale on the tree, sale in local markets and selling in the markets of major cities) vary from farmer to farmer. For example, in the areas of Jamaa and Mughir (Ben Zioch Saladin, 1999) 6.51% was sold on the tree, 72.2% were used at home after a screening process, and 8.88% were sold in the local markets. Farmers are prevented from selling their own products in the markets because of the high cost of transport. This situation is good for traders and middlemen, but not for the peasants. It allows traders and brokers to buy the majority of production at the lowest price and sell it at high prices, capitalizing on the efforts of farmers.

Date Sale Prices Applied to Farmers

With the opening of the national department Diwan of dates (O.N.D) in 1997, private monopolies can trade dates and impose prices on farmers. For example, the sale price of Daglat Nour dates in Tougret and Jamaa in 2004, did not exceed 22 DA / kg. The price of Daglat Nour Shamrokh dates was 35 DA / kg, while the price of one kg of Alfraza was 10 DA and 24 DA/kg for Algars. The remaining prices ranged from 5 to 9 DA / kg (5). The sale price of dates was low especially if we consider that the cost of production of one kg of dates is 35.8 DA / kg. This adds to the problems of loss and

stagnation of the commodity and it is sold at the cheapest prices in the form of formulas and speculation that is dominated by monopolies controlled by a few people.

Production and Marketing Problems Faced by Producers of Dates, and their Relative Importance

The survey of problems facing producers in the region of Biskra show that there are multiple and diverse problems varying in nature and levels of importance. Some are on an individual level and others are on the community level. The results of this study show that the problems that are faced by producers of dates in the region are linked to characteristics of individuals and their farms, rather than the characteristics of the local community. The results in Table 5 show that the distribution and productivity problems of date producers are many and diverse. The problems stated by individuals in the research sample vary in nature. Some are economic, others are technological or biological in nature. It became clear from the study that farmers in the region face a number of problems that constitute obstacles to their production. There are 31 problems mentioned by more than 50% of members of the research sample. Because problems are related to one another, solving some may help with others. Table 5 indicates that from five problems which were linked to human resources, three of them were common to 50% of the the sample. Those problems are:

1. Shortage of trained manpower
2. Migration of palm owners
3. Lack of farmers with knowledge and experience.

These problems affect the status of production. There is a lack of trained manpower due to the continued migration from rural to urban areas, where they work for higher wages on projects and in institutions. Consequently, wages rise for the small group that is still available to work on palm trees. This led to an increase in production costs, which resulted in some farmers losing interest in date production, and a deterioration in the industry. When the farmers resorted to untrained labour, lack of experience and necessary knowledge for tasks such as pollination resulted in reduced production. Therefore the development and training of employees in palm tree cultivation and production of dates is very important for the continued production of dates in the region. This underscores the role that could be played by agricultural extension bodies in this area. In addition, there is a need for the provision of machinery and agricultural equipment to alleviate the shortage of labour and high wages. The increased costs in production and lower profits highlight the role that could be played by agricultural officers to help producers understand the best methods of cultivation and production. Considering the substantial drop in the level of knowledge of farmers in the region, a study that followed the production stages of dates was recommended. Four items were reported by more than 50% of the people who were surveyed:

1. The difficulty in obtaining bank loans for agriculture and rural development
2. High interest rates on farm loans
3. Lack of material to expand the cultivation of palm trees
4. Low return on investment.

As a result of the changing pattern of life in Algeria in recent times, especially with the rise in oil prices, there is reluctance by many farmers to invest money in date production. While high returns are available on other investments, there are limited financial resources for farmers in the region. It is difficult to obtain loans from the Bank of Agriculture and Rural Development and then the farmer must wait up to seven years in order to receive farm return on capital invested in the production of palm trees. There are negative repercussions on the future of date production in the region. Other facilities are needed to improve and increase production on farms. They should be encouraged to take care of their farms and expand the cultivation of palms and thereby increase date production in the region over the long term. Research findings also show that many problems associated with marketing dates were stated by over 50% of individuals sampled. Those problems included:

1. Low demand for date fruit and inadequate packaging for harvesting
2. Non-availability of packages for collection of crop and the low efficiency of marketing
3. Low prices at the farm level compared to consumer prices.
4. The presence of problems during the sale in terms of quality and value of crop.
5. Low demand for palm remnants and lack of resources and appropriate means of storage.
6. Multi-traders who purchase dates and sell them far from the place of production.

These problems affect other relationships and situations in the region. The lack of storage facilities leads to exposure and perishing of dates and thus reduction of return. There were insufficient markets for selling and lack of traders, thus necessitating the need for a government body or farmer's cooperative to ensure that they have access to remunerative prices, adequate markets in the region that understand production demand, whether locally or externally, and reduced costs of production. The research findings explain that from the three problems associated with the cost of crop production, two of them have been stated by 50% of the people surveyed, i.e. high wages and cost of fertilizer. The problem of rising labour costs is associated with the lack of manpower and limited use of agricultural machinery. Not enough fertilizer is available for expansion and replacement of old trees, and the cost of fertilizer is too high. In spite of the agricultural policy pursued by the Ministry of Agriculture to provide fertilizer through the establishment of nurseries in each of the major regions of the Kingdom, Biskra region is still facing high production costs, which prevent farmers from expanding and replacing old trees. From the viewpoint of farmers establishment of nurseries in the region would help to resolve the problem of fertilizer shortage and high cost. There is no doubt that tackling the problems of employment would also contribute to the solution of the problem Agricultural policies which encourage farmers to produce fertilizer and free distribution to farmers to encourage them to replace old trees and to expand production would contribute to the improvement of the situation in the region.

This research indicates that of the three problems associated with the physical resources in the region, two were stated by over 50% of the people that were surveyed: high salinity and lack of water. The high salinity and shortage of water has had an adverse impact on the production of dates. Calls have been made for research efforts to reduce salinity of the soil and control irrigation and water management, as they are basic factors for production.

Furthermore, the research findings show a number of technological problems associated with biological production of dates in the region, and all were stated by 50% of the research sample:

1. Palm exposure to infection from diseases and pests.
2. Buildings encroaching on plantations, and growing numbers of old plantations.

High Grass and the Lack of Organic Fertilizer

There is insufficient research on palm trees by the scientific and research institutions located across the nation, as well as extension services. It is important that findings and recommendations of researchers are transferred to the farmers if they are to play a key role in addressing the problems. They can be overcome if the farmers are provided with solutions which are commensurate with these problems through education and guidance, appropriate agricultural techniques, and better methods of agriculture.

Research findings indicated the time at which individual problems occur. Of the 4 problems at the community level, three were reported by more than 50% of those surveyed:

1. Lack of local factories for dates and lack of machinery to facilitate operations
2. Inadequate services of the Ministry of Agriculture in the area of prevention.

Due to the absence of local manufacture that moves dates and preserves their quality, while remaining attractive to consumers, purchasing this equipment may lead to increased demand locally and externally. In light of the lack of local demand for dates,the necessity of transfer to non-local factories, and the increased costs of transfer, farmer's

profits are reduced. Therefore the availability of local factories for dates in the region will have a profound impact on the improvement of production and demand, whether locally or externally.

CONCLUSION

The results of this study indicate that the producers of dates in the Biskra region face multiple and diverse problems that have an impact on production in the region. In order to improve the production of dates in the region, those responsible for planning and execution of agricultural and date production, should develop plans that take into account the many associated problems. Agricultural guidance in the region is important, together with education for farmers with emphasis on maintaining the national wealth of palm trees and to guide them in the best methods to raise quality and increase farm profit, i.e. service and harvesting; packing and conservation; protection from damage during storage; and for removal of earth and sand. Interaction between the organisations of agricultural research and local realities is required, in order to appropriately direct the research agendas of those bodies. It would be useful to establish training centers for the work of service to palm trees.

Proposed Economic Recommendations for the Advancement of Date Production in the Biskra Region

For the development of palm trees in the study area, we propose the following recommendations:

1. Attention to irrigation and fertilization to address the decrease in the productivity of the palm trees because of nutrient deficiencies in sandy soils.
2. Development of recommendations for fertilizer type, rates, timing and method of application.
3. Encourage a more active role by State Departments of Agriculture, in terms of both technical advice, supervision, and follow-up funding of palm plantations.
4. Development and modernization of processing of dates, and development of local and foreign marketing and advertising programs to educate consumers concerning the nutritional value of dates.
5. Focus on cultural practices that would increase the total production of dates and to develop products from dry dates.
6. Attention to control of pests and diseases affecting date palms.
7. Identify the technical requirements for effective storage and education of those involved in storage and processing of dates.
8. Staging of exhibitions and special markets in different regions of the Republic to promote dates and the products manufactured from them to both local and international clients.

The implementation of the above recommendations would contribute to the economic development of the date industry. This would have four advantages: the application of scientific methods to improve cultivation; the social benefit of increasing the income of thousands of families who depend on date production; increased employment; and increasing the value of Algeria's currency.

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Tables

Table 1. Estimates of the general trend of cultivated area by palms and production and productivity during the period 1985 / 2004.

Statement	Estimates a	Estimates b	T	Average
Total cultivated area by palm trees (ha)	*66477*	*2200*	*577*	*86284*
Cultivated area by palm trees at the eastern and southern states (ha)	*50375*	*1066.2*	*593.56*	*59971*
Cultivated area by palm trees in Biskra (ha)	*17437*	*425.07*	*52*	*21262*
Total production of dates (t)	*144200*	*15601*	*97*	*284658*
Total production of dates in the South-East (t)	*124200*	*9698*	*24*	*211510*
Total production of dates in Biskra (t)	*26564*	*5017.7*	*78.56*	*71723*
Average production of palm (kg)	*31*	*0.8*	*20.69*	*39*
Average production of palm in the South East (kg)	*30.6*	*2.47*	*2*	*52.85*
Average production of palm in Biskra (kg)	*24.88*	*2.023*	*4.17*	*43*

Source : Data collected and calculated from the data of the Ministry of Agriculture for the period 1985-2004
It was also calculated the general trend in linear form $y = a + b x$
T : The value of T calculated for estimating

Table 2. Comparison of net revenue from the area unit systems in Algeria 2004

The farm system	Net revenue per hectare (DA)
Production system of Degla	484,215.2
The mixed production dates	80,227.8
Vegetable production system inside plastic houses	264,2907.7
The cereal production system	25,402.65

Source : prepared by the researcher, based on a field survey forms were collected from selected areas of the city in 2001

Table 3. The distribution of producers by the research sample in the state of Biskra according to their marketing channels in the marketing of date fruit.

Areas	Retailers				Wholesalers				Local traders	%
	Cash	%	Deadline	%	Yes	%	Not	%		
Among mandate	49	76	8	12	10	15	-	-	6	10

Source: prepared by the researcher
Schedule (No. 3) : The distribution of a particular research producers in the state Biskra according to their marketing channels in the marketing of fruit dates

Table 4. The distribution of dates to consumers in order of importance.

Region / Place	Ain Ben Anoui	Toulga	Total	%
Traditional markets	18	12	30	47
Special Shops selling dates	12	2	14	22
Directly from the farm	13	5	18	23
Shops	2	2	4	6
Factory	1	0	1	02
Total			67	100

Source : Field Study

Table 5. The distribution of the sample research individuals .depending on the nature and type of problems they face in cultivating palm trees

	Quality problems in according its nature and levels	The entire volume sample = 64	
		No.	Ratio
First : problems at the individual level			
	A - Problem related to human resources		
1	Lack of trained manpower	62	97
2	Migration of palm owners	50	78
3	Lack of government supervision indicative at palm trees.	48	75
4	Lack of the necessary expertise to perform the work of vaccination.	44	69
5	Deficiencies in the knowledge and experience of farmers in modern agricultural methods	63	98
	B - Problems related to funding and investment		
6	Difficulty in obtaining loans from the Bank of Agriculture and Rural Development.	44	69
7	High interest rates on farm loans.	42	66
8	Lack of material means to expansion in the cultivation of palm tree	48	75
9	Increase of investment return	40	62.5

C- Problems concerns marketing			
10	Reduced demand for the fruit of dates	50	78
11	Inadequate packaging used in the mobilization of fruit dates	45	70
12	Non-availability of the necessary packages to collect the harvest	48	75
13	Decrease of marketing places capability.	49	77
14	Decrease of selling prices at the farm level compared to consumer prices.	49	77
15	Problems during the sale in terms of quality and value crop.	47	73
16	A decrease in demand for palm residues.	46	72
17	Lack of materials and means of storage	40	63
18	Multiple traders to buy crop	50	78
19	Place of marketing far than the place of production	60	94
D- Problems related of high production costs			
20	High salinity	54	13
21	High fertilizer prices	52	81
22	High prices of fertilizer and equipment	5	8
E-Technological and biological problems			
23	High salinity	40	63
24	Decrease of Water	35	55
25	High water to the surface	5	8
	Technological and biological problems		
26	Palm-exposure to infection diseases	50	78
27	Propagation of bad categuories	60	94
28	Buildings encroachment and installations at the expense of palm trees	52	81
29	Increased numbers and the old palm farms	48	75
30	Large weed	42	66
31	Non-availability of organic fertilizers	26	41
Second : Problems et the regional level			
32	Lack of local factories dates	56	88
33	Lack of machines that facilitate the operations of the service	48	75
34	Inadequate of the services of the Ministry of Agriculture in the area of protection	39	61
35	Lack of roads to the palm farms	2	3

Source : Field Study

Figures

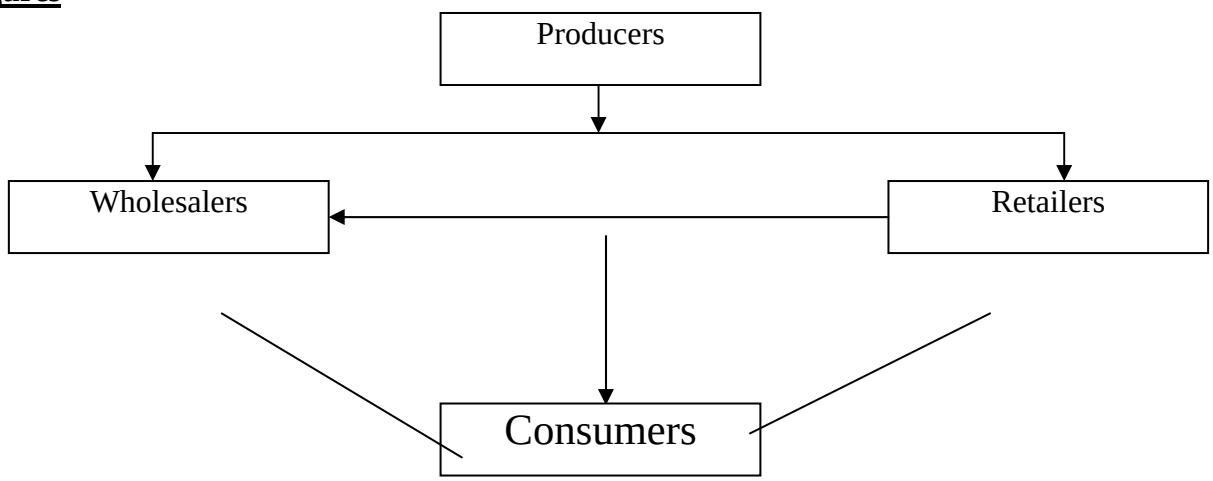

Fig. 1. The first track marketing.

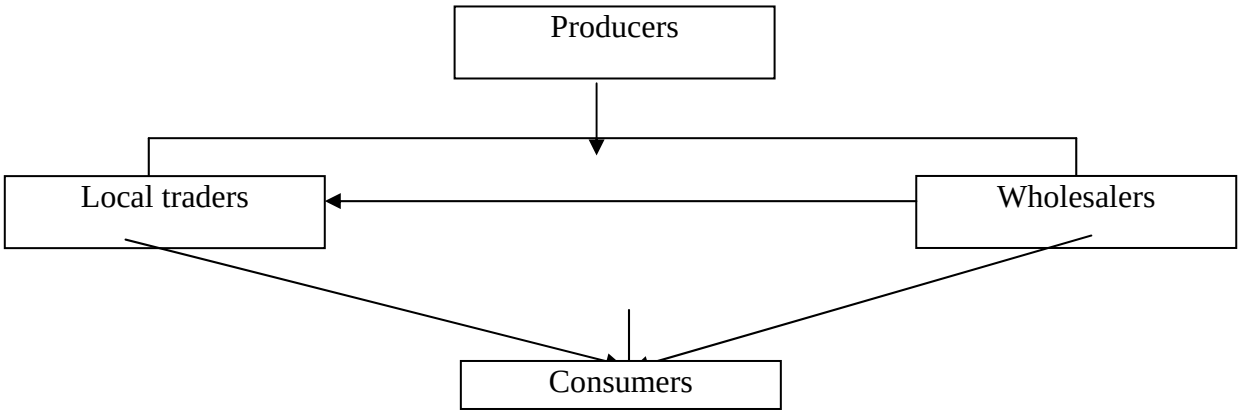

Fig. 2. The second track marketing.

Source : Ibid.

Marketing Problems and Export of Date Trading Varieties in the I.R. of Iran

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Keywords: Sayer, Shahani, Peyaram, Kabkab, marketing margin

Abstract

In this study marketing of date trading varieties was investigated in Khuzestan, Fars, Hormozgan and Bushehr provinces as they are the main areas of date production in Iran. Subjects concerning the marketing of dates and common marketing services were investigated. Marketing margin function was estimated using Mark-Up and Relative Margin models. The effects of socio–economic factors on revenue price of producers was evaluated. The export situation and problems of exporters were investigated. Required data were gathered in two forms: documental in the form of official statistics and a survey through stratified random sampling method during the year 1999-2000. Date varieties were Sayer (Khuzestan), Shahani (Fars), Peyaram (Hormozgan) and Kabkab (Bushehr). Results showed that for each kg of internal consumption of Sayer, Shahani, Peyaram and Kabkab, 300, 570, 4000 and 450 Rials were spent on marketing costs and marketing agent's profit, respectively. For each kg of export Sayer date, 1876.7 Rials were spent on marketing costs and marketing agent's profit. Results also showed that for internal consumption of Sayer, Shahani, Peyaram and Kabkab date varieties the producer's share of the retail price were 85, 65.46, 55.5, and 38.23 percent, respectively. The marketing efficiency results showed that price and technical inefficiencies in marketing routes of trading date varieties were high. The results of estimated marketing cost function based on Mark-Up model in Khuzestan and Fars provinces showed that the marketing margin had a direct relationship with retail and export prices, and an inverse relationship with marketing costs. Also, estimated marketing margin function based on relative margin model in Khuzestan province showed that the marketing margin had a direct relationship with retail and export prices, and an inverse relationship with marketing costs and the value of supplied products. The result of investigations into the effect of socioeconomic factors on revenue prices of producers in Khuzestan showed that factors such as age, level of education, level of production and type of buyer had positive effects on revenue price. Experience as a gardener had no significant effect on revenue price of producers. In Hormozgan, factors such as the possibility of warehousing, packing and sorting, originality of garden income and level of production had positive effects on revenue price of the producers. The export difficulties of date were caused mainly by a weakness in technology in the areas of sorting, packing and standardizing, and by high packing costs, lack of suitable transportation, fluctuations in exchange rate, lack of harmony among exporters and destructive competition, lack of information about global markets and not benefiting from opportunities in these markets, the low knowledge level of exporters and little awareness of them with regard to the scientific principles of international marketing and lack of exporters cash.

INTRODUCTION

One of the most important and essential actions for developing the agricultural sector is paying attention to marketing of farm produce. Unfortunately, in Iran most of the institutes and organizations related to the agricultural sector do not pay much attention to marketing. Their main focus is to expand the hectarage and the yield per hectare. However, increasing production without considering the marketing system has caused decreasing prices for the producer and increasing costs for the consumer. When farmers

should be reaping the result of their efforts, they are facing the situation where the income they earn is less than production costs. The farmer's share of the consumer price is small, consumers voice dissatisfaction at the high prices and middle men and other agents take too much of the profits.

Date is one of the most common horticultural products in Iran. Large expanses of land in some provinces are allocated to its production. Date is one of the exporting commodities of the agricultural sector. The average exporting price worldwide has decreased in recent years, and the average exporting price of Iranian dates has decreased at a higher rate (FAO, 1994-2001). Reduction in the price of dates is a problem for the producing countries like Iran. Sixty percent of Iran's date plantings are located in Khuzestan, Fars, Hormozgan and Busheher. In 2001 they produced 63.1 % of Iran's date production. Because they are the most important provinces for producing commercial date varieties in Iran (Elhampour, 2003), this study was conducted in this area to investigate the marketing problems and establish an understanding of existing conditions.

THEORY AND METHOD OF RESEARCH

Definition of Marketing

Based on Shefered's definition, 'market' is not a special place, but is a group of buyers and sellers with special transaction facilities with each others (Rahmani, 2003). Based on what is inferred by this definition, the crucial point is the quality of relationships that exists among agents. In economic literature, the word 'marketing' has been defined in different ways. Most of them have mooted marketing as all of the selling processes that could sell the commodities sooner and faster. Management of marketing includes analysis, organizing, planning and controlling consumer demands. It accesses the commodities for consumers with the help of services and policies. Marketing is a human activity to satisfy needs and wishes through a transaction process (Crawford, 2004).

Marketing Margin and Marketing Cost

Demand on the farm for agricultural products is a derived demand of the retail market. Product price on the farm is related to its price in the retail market. To suggest the on farm price, buyers usually add the marketing costs, to ensure the final cost would be acceptable to retail consumption. In a competitive market, marketing margin is defined as the difference between consumer and producer prices. It has also been defined as the differences among chains of the marketing system. Based on these definitions 3 kinds of margins, wholesale margin, retail margin and total margin, can be discerned (Crawford, 2004). The relationships between these margins are as follows:

$$MM = RP - PP \quad (1)$$

$$WM = WP - PP \quad (2)$$

$$RM = RP - WP \quad (3)$$

MM, WM, and RM are total marketing margin, wholesale margin and retail margin and PP, WP, and RP are producer price, wholesale price and retail price, respectively.

Marketing Efficiency

Increasing efficiency is a matter of concern for farmers, processing agents, wholesalers, retailers and consumers. Efficiency is vitally important in analyzing marketing system and marketing profit. Inefficient marketing systems cause high costs, large losses, wastage of products and unreasonable prices. High efficiency leads to an increase in income for producers by selling at higher prices or satisfaction of consumers by decreasing buying prices. In other words high efficiency leads to satisfaction of producers and consumers by reducing the difference between selling and buying prices. Some economists believe that marketing efficiency is related to the nature of competition in the market. In other words, a marketing system is efficient when the counted difference of prices is the least and this is true when the market is complete competition. Based on the mentioned definition, Sherivastava and Ranadhir (1995) submitted a method to

determine efficiency. Based on their idea total marketing margin or gross margin has two parts; marketing costs and net margins or profit of marketing agents (Shrivastava and Ranadhir, 1995). According to their definition, they introduced the following relations:

$$GM = P_A - P_B \quad (4)$$

$$MC = CL + CI + CT \quad (5)$$

$$NM = GM - MC \quad (6)$$

GM is gross margin, MC is marketing cost, P_A is the price paid by agent A, P_B is the selling price of previous marketing agent, NM is net margin, CL is labor cost, CI is warehouse cost and CT is transportation cost.

These researchers then divided total inefficiency into two parts: price inefficiency and technical inefficiency, and defined each of them as follows:

$$P_I = (MC/GM) \longrightarrow P_e = 1 - P_I \quad (7)$$

$$T_I = (WC/GM) \longrightarrow T_e = 1 - T_I \quad (8)$$

$$O_I = [(MC+WC)/GM] \longrightarrow O_e = 1 - O_I \quad (9)$$

Where O_I is the total inefficiency of the marketing system and WC is the wastage cost. If marketing cost plus wastage cost equals zero, total inefficiency will be zero and efficiency of the marketing system will be complete, where this indicates a competitive market. If marketing cost plus wastage cost equals GM, inefficiency of the marketing system will be 1 and the marketing system is inefficient.

Determining Factors of Market Margin

In order to determine the market margin, the following models were used; Mark-UP model and Relative Margin model. In the Mark-Up model, the market margin (MM) is considered as a function of retail price (RP) and marketing costs (MC) as follows:

$$MM = F(RP, MC) \quad (10)$$

$$MM = a_0 + \alpha_1 RP + \alpha_2 MC + \varepsilon_i \quad (11)$$

α_i are parameters that should be estimated and εi are error sentences. In the relative margin, market margin is considered as a function of RP, MC and value of products (TR), as follows:

$$MM = F[RP, TR (RP.Q), MC] \quad (12)$$

$$MM = \beta_0 + \beta_1 RP + \beta_2 TR + \beta_3 MC + \varepsilon_i \quad (13)$$

Q is the amount of production and TR is the value of supplied products and β_i are parameters that should be estimated.

Effective Factors on Received Prices of Producers

Analysis of variance was used to determine the effective factors on received prices by producers. In this method every socio-economic variable was classified into two or more levels. Then frequency distribution of prices for these levels was determined. Finally, the average of received prices for each group was calculated and compared together. Duncan's test was used to compare averages of different groups.

Place and Time of Study

This study was performed in Khuzestan, Fars, Hormozgan, and Bushehr provinces of the I.R. of Iran. Necessary data were collected in two forms: official documents on statistical resources, and a survey through stratified random sampling method during the year 1999-2000. Information was collected from agricultural organizations, customs, and management and the Plan Organization of the mentioned provinces. Selection of stratum was based on the number of fruiting palms. One hundred and ninety-five growers and 141 marketing agents (wholesalers, retailers and exporters) were interviewed and filled out special questionnaires. The main commercial date varieties were Sayer (Khuzestan), Shahani (Fars), Peyaram (Hormozgan) and Kabkab (Bushehr).

RESULTS AND DISCUSSION

The results showed that the most important marketing services of date marketing are harvesting, transportation, packing, warehousing, sorting and standardization. The

harvesting method, including picking and collecting of dates, is traditional and the results showed that some dates were wasted in this step. The transportation of dates is done by tractor, truck and van. Collection of Sayer dates is done in wooden and plastic boxes which are transported to packing and exporting firms and retail warehouses. Dates for export were packed in 10, 22 and 30 kg cardboard boxes. For internal consumption packing is done in cardboard and in plastic barrels. The packing of Shahani for internal consumption was done in the unsuitable forms of 3 kg cardboard boxes or 17 kg tins. Pressing dates into this type of packaging results in the accumulation of syrup and loss of quality, severely reducing the marketability. Packing of pitted dates enriched with crushed pistachio or walnuts for export is done in 1 kg, 600, 450 and 100 gram parcels under the names of Danechin, Yaekbarmasraf and Legareo-Talaee. Packing of fresh dates for export is done in 100 gram parcels.

Packing of Peyaram dates varies depending on the type of date. First class dates are packed in 0.330, 0.500, 0.700 and 2 kg parcels. Second class dates are packed in 10 and 12 kg cardboard boxes. Third class dates are packed in gunny sacks or 7 to 30 kg cardboard boxes.

Packing of Kabkab dates is done in 0.8, 3 kg or 17 kg tins.

The absence of storage facilities, such as warehouses and freezers, improper packing and undesirable conditions in existing warehouses (unsuitable ventilation and temperature, activity of insects and microorganisms) play a considerable role in deterioration, and therefore reducing the quality of dates. Also, the shortage of food processors and the high cost of production and harvesting of dates have been difficulties related to production and export of date trading varieties.

The results showed that for each kg of internal consumption of the date varieties Sayer, Shahani, Peyaram and Kabkab, 300, 570, 4000 and 450 Rials were spent on marketing costs and marketing margin profits, respectively. On the other hand, the distance between the internal consumer payment and producer revenue for each kg of dates of Sayer, Shahani, Peyaram and Kabkab were 300, 570, 4000 and 450 Rials, respectively. Of these figures for each kg 0, 270, 1500 and 250 Rials were wholesale margins and 300, 300, 2500 and 150 Rials were retail margins. For each kg of Sayer export date 1876.7 Rials were spent on marketing costs and marketing margin profits. On the other hand the distance between exporter revenue and producer revenue was 1876.7 Rials (Table 1).

Results also showed that for internally consumed date varieties Sayer, Shahani, Peyaram and Kabkab, the producer's share of the retail price was 85, 65.46, 55.5 and 38.23 percent and the retailers share were 15, 18.18, 27.8 and 11.77 percent respectively. For the export of Sayer dates the share of producers and packers and exporting firms was 46.2 and 53.8 percent, respectively (Table 2).

Assessment of the marketing system indicated that price inefficiency in marketing routes was high. The average, maximum and minimum price inefficiency were 0.63, 1 (for the route consumers, wholesalers and producers of Shahani date) and 0.27 (for marketing route consumers and producers of Shahani date), respectively (Table 3). If plans and policies were implemented so that all marketing services are done by producers (through the formation of marketing cooperatives for producers), marketing costs would be reduced and efficiency increased. However, the knowledge of farmers and even administrative and managing staff at agricultural institutes concerning cooperatives and their role is limited. Thus paying attention to education and extension of this subject would be useful. Also, average, maximum and minimum technical inefficiency of the marketing system were, 0.33, 0.66 and 0, respectively (Table 3). It is therefore possible to increase efficiency by reducing wastage costs.

The results of estimated marketing cost function based on the Mark-Up model in Khuzestan and Fars provinces showed that the marketing margin had a direct relationship with retail and export prices, and an inverse relationship with marketing costs. Based on the Relative Margin model, the marketing margin had a direct relationship with retail and export prices, and an inverse relationship with marketing costs and value of supplied

products.

The effect of socio-economic factors on revenue prices of producers in Khuzestan showed that factors such as age, level of education, amount of production, and type of buyer had a positive effect on revenue prices of producers. In Hormozgan, factors such as the possibility of warehousing, packing and sorting, originality of grove income and amount of production had positive effects on revenue price, and factors such as age and education level had no significant effect on revenue price of producer.

The difficulties encountered in exporting dates were largely a weakness of technology in the areas of warehousing, sorting, packing and standardizing, high packing costs and lack of suitable transportation involving refrigeration and freezers. Other problems included fluctuating exchange rates, lack of harmony among exporters, destructive competition, lack of information about global markets and failure to exploit opportunities in these markets, little awareness by exporters regarding the scientific principles of international marketing and lack of exporter's cash. Disharmony and destructive competition among exporters, and the exporting of low quality and low priced dates by some exporters, had an effect on the price of dates in destination countries. Supervision by the Office of Standards is required. With respect to undesirable economic conditions in Iran, and the weak bargaining power of exporters, it seems that harmony and positive relations may prepare the way for getting higher prices and foreign exchange income. An exporters union could play an important role in designing scientific and practical plans. It would also seem possibile to increase the efficiency of the marketing system by improving methods of packing. Also, investment in food processing industries that produce lateral products (syrup, liquid sugar, juice, alcohol, vinegar, etc…) would reduce wastage. Decreasing marketing costs and running advertising programs in global markets, would also assist the Iranian date industry to successfully compete with other date suppliers.

In order to increase date exports and export prices, strict attention must be given to the technology of services after production, like transportation, warehousing, sorting, standardizing and packing. Also, exporters need to be educated to better understand global markets and the method of presence and competition in these markets, reasons for price fluctuations and tastes of international customers.

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Tables

Table 1. The amounts of average margins.

Date varieties	Wholesale Margin	Retail Margin	Packing and exporting Margin	Total Margin
Sayer for internal consumption	-	300	-	300
Sayer for export	-	-	1876.7	1876.7
Shahani	270	300	-	570
Peyaram	1500	2500	-	4000
Kabkab	250	200	-	450

Resource: calculations of research

Table 2. The average share of marketing agents from retail or export price (percent).

Date varieties	Producers	Wholesalers	Retailers	Packing and exporting firms	Total
Sayer for internal consumption	85	-	15	-	100
Sayer for export	53.8	-	-	46.2	100
Shahani	65.45	16.36	18.18	-	100
Peyaram	55.5	16.7	27.8	-	100
Kabkab	38.23	50	11.77	-	100

Resource: calculations of research

Table 3. Various inefficiencies of date varieties.

Date varieties	Marketing routs	Technical inefficiency	Price inefficiency	Total inefficiency
Sayer	Packing and exporting firms, producers	0.66	0.6	0.95
Sayer	Internal consumers, producers	0.53	0.37	0.9
Shahani	Consumers, retailers, wholesalers, producers	0.37	0.84	1
Shahani	Consumers, wholesalers, producers	0.22	1	1
Shahani	Consumers, retailers, producers	0.24	0.73	1
Shahani	Consumers, producers	0	0.27	0.27
Peyaram	Consumers, retailers, wholesalers, producers	0.2	0.78	0.98
Kabkab	Consumers, retailers, wholesalers, producers	0.47	0.45	0.92
Average		0.33	0.63	0.87

Resource: calculations of research.

Financial Stability and Marketing for Date Palm Production in Yemen

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Keywords: production cost, marketing efficiency, long-term investment, net income

Abstract

Long-term investment in date palm is a challenge. Date palm trees have always been important in Yemen because they provide a rich, cheap and locally produced food. Dates have proved to be the best resource to ensure food security during food shortages and crises. The demand for palm tree dates is increasing both locally and abroad because it is a healthy and pure food.

Based on the feasibility study of different palm tree farmers in Wadi Hajer, Hadramout and Wadi Surdud, Governorate of Hodeidah, the following factors were calculated: Benefit-Cost Ratio (BCR), Net Income and Internal Rate of Return (IRR). Market marginal, Efficiency Predicted Marketing (EPM) were calculated. Also the study briefly describes the nature and scope of financial and economic analysis.

In Wadi Hajer, Hadramout the BCR was 1.67, Net Income, 101,238 thousand YR and IRR, 18%. While in Wadi Surdud, Governorate of Hodeidah the BCR was 2.63, Net Income, 335,850 thousand YR and IRR, 20.7%. Marketing analysis indicated that the EPM was 56.31 in Hodeidah Market and 15.79 in Mukalla Market, which means improving market efficiencies will sustain the date palm for long-term investment. Yemen has an economic advantage in marketing its dates, because they are cheaper than the other countries at only about 0.25 US$/kg, while UAE is 3 US$/kg.

INTRODUCTION

In Yemen, cultivation of date palm trees has spread to cover many areas, such as Wadi Hadramout, Wadi Hajer, Tehama, Wadi Surdud, Governorate of Hodeidah, Socotra Island and some other coastal and interior wadis throughout the country. Although Yemen is well-known for its abundance of date palm trees and proven date production of a wide range of cultivars, traditional agricultural practices still prevail. Therefore, there is a real need to revise and modernize the prevailing agricultural system, especially in light of sustaining local food systems, agricultural biodiversity and local livelihoods. Marketing is at the peak of the collective activities practiced by mankind. In the modern era, marketing has became one of the dynamic components of modern civilizations, which may be developed and changed to conform to the needs and prevailing conditions. Accordingly, there is a correlation between marketing and prevailing norms and traditions in communities, and therefore, strong interaction exists. However, some communities are not aware of the close relation between marketing and facilitating ways and means to increase income of the population to break the poverty cycle.

PURPOSE

Yemen is classified as a low-income, food deficient country (LIFDC) and imports over 75% of its main staple wheat. Approximately 2.7 million people live below the food poverty line, consuming less than 2,200 calories per capita per day, while 35% of the population (nearly 5 million people) live below the poverty line[1]. The food security status of households is also threatened by degradation of natural resources. Traditional

[1] The World Bank, "Republic of Yemen Comprehensive Development Review for Financial Sector Building Block" Sana'a 2002 (www.worldbank.org/yemen)

agricultural practices are still used and urgently need to be modernized, especially in regard to the cultivation of date palm trees. Dates are a nutritionally rich, cheap and locally produced food. They have proved to be the best source of food security during food shortages and crises. Improved marketing efficiency will help sustain the local food system (Fig. 6).

STUDY METHODOLOGY

The Agro-Economic Survey provides reliable statistics on agricultural production and utilization and is vitally important for formulation and evaluation of development plans. The necessary data concerning the cost of production for several crops including date palm, were collected by a team of 9 staff from Wadi Hajer, Ministry of Agriculture and Irrigation, Mukalla, Hadhramout, who were supervised by the consultancy team (Al-Hebshi and Fawzi Ababneh). Also details concerning crop production in the Surdud Districts Governorate of Odeidah Yemen[2] , for the year 2003 were taken from the survey collected by a team from three local institutions, Bajil extension workers, Al-Kaden Research Station and the Tihama Development Authority (TDA). A survey of 45 farmers was conducted from 9th to 15th August 2003. Farmers were from three different areas (AQM Al Kadan, Al-Nasery, Al-Sareh, Al-Sayyed, Al-Qabaiyah), medal (AQM Al-Saydah, Al Masalah, Al Qabaiyah, Al–Hakamiyeh) and the lower slope of the Wadi (Der Al-Salaam, Al-Khadariyah, Der Shuwel). Farmers were selected from different sized farms. The teams collecting the data faced many difficulties including rain, floods, transportation, and long distances but important data were collected for the analysis.

ANALYSIS AND RESULTS

Costs and Returns

The first step in the analysis was to identify relevant costs and benefits that subsequently could be used to develop cash flow budgets for the project. This study then applied quantitative analysis to compare the production function of palm dates grown on traditional farms and on investment farms in two different Wadis. The first one is in Wadi Hajer Hadramout Governorate and the second in Wadi Surdud Governorate of Hodeidah, Yemen. These costs and returns are reported in Tables 1 and 4. All components are reported on a per-hectare basis.

Internal Rate of Return (IRR)

Although financial management theorists argue that it has shortcomings, the most popular economic criteria for choosing among investment projects is the internal rate of return (IRR). It is widely used by the World Bank and other international financing institutions in their economic and financial analyses (Brigham and Gapenski, 1997).

The internal rate of return is the discount rate that results in a zero net present value for the project. In other words, IRR is the rate that equalizes the net present value of the cost and benefit streams of the project. It is the maximum interest rate that a project could pay for the resources used if the project is to recover its investment and operating costs and still break even. Although there are theoretical difficulties with the IRR, a major advantage of IRR analysis is that estimation of an interest rate to use in discounting costs and benefits to present value is avoided. This measure gives a ranking usually not greatly different from the benefit-cost ratio (Total income/ Total cost) or a ranking of net present values. Ranking projects according to this criteria will indicate in a very general way that one project is better than another, in the sense that it contributes more to the national income as compared to the resources used. If the choice has to be made from a range of alternative acceptable projects under the limited budget constraint, raise the discount rate IRR greater than the cut-off rate can be implemented. Tables 1 and 4 calculated the total

[2] Ghayth Aquatech, Sana'a ROY, updating the feasibilty study and detailed design for sorudud dam project, volume- 1-2-3, Sana'a, January 2004

costs and incomes for dates and bananas which gives the basic information for the cash flow needed to carry out the economic analysis and from Tables 4 and 5 we calculated the IRR in Wadi Hajer Hadramout and Wadi Surdud. The result was satisfactory i.e IRR at 18% and 20%.

Efficiency Predicted Marketing (EPM)[3]

In Mukalla Market EPM for dates and other crops from Wadi Hajer were calculated. In Table 3 it is indicated that the EPM was 15.79% which was considered low.

EPM=[1-(Margin Of Market)/(Margin Of Market + Cost of Market)]*100.

Innovative Farms

Table 4 indicates that the total production cost per hectare of date palm amounted to 205,467 Yemeni Rails. Also, it reveals that the total income was 541,317 YR and net income was 335,849 YR.

Marketing Margin in Surdud

The marketing margins were calculated at the local Surdud Market for the studied crops (Table 6). The marketing margin between farm gate price, price of wholesaler and price of retailer (YR/Kg) are presented.

The EPM was 37% for dates in the local market in Surdud 2003. This marketing margin is reasonable for the stockholders. However, Table 7 indicated that the EPM was 56% in the Hodaidah Markets which shows considerably more efficiency than the local Market in Surdud and the Mukalla Market.

CONCLUSIONS

Table 8 reflects the relative importance of farm surplus in the Wadi Surdud, Hodeidah. Data indicated that BCR, net income per ha (YR, IRR and EPM) are better than the situation in Wadi Hajer, Hadramout, because they have better management and they have a closer relation between marketing, facilitating and production.

Economics Incentives for Marketing Abroad

According to Table 9 Yemen has an economic advantage in marketing its dates, because they are cheaper than those from other countries. The price of Yemen dates is about 0.25 US$/kg, while for UAE the price is 3 US$/kg.

Economics Can Help Inform and Implement Better Utilization and Policies

Farmers in these areas are living under the poverty line[4] and there is no policy at micro or macroeconomic levels to resolve their market problems. It is important that professionals understand the socio-economic (not just financial) implications of their policy decisions in the market.

Week management, corruption and absence of sector police are the main factors causing misallocation of natural resources in Yemen[5]. Appropriately designed and efficiently delivered financial services can play a central role in improving the lives of millions of poor consumers, as well as farmers and other small business communities.

Recommendations include:

(i) Raising awareness across the Arabian countries.
(ii) Bolstering investment in food industries which depend on agricultural products.
(iii) Develop modern preservation technology.

[3] Clark, F. E, Principle of Marketing , New York, the Macmillan company, 1973, p: 56

[4] Al-Hebshi Mohamed & others " CREDIT AND MICROFINANCE SCHEMES", Jurnal Ekonomi Dan Bisin, volum2, Nomor 1 April 2003.

[5] Al-Hebshi Mohamed & others " Estimated Of O&M Expenditures for Spate Irrigation System.", Case Studies from Wadis Zabid, Rima, Abyan, and Tuban- Republic of Yemen, Sana'a 1998

(iii) Setting up a program to regulate the functioning of agricultural subcommittees and reinforce the institutional aspect of quality control and agricultural exports regulation, as well as facilitate the marketing of production locally.

Interest in and support for the agricultural sector will inevitably help strengthen stability of the workforce in agricultural areas and alleviate unemployment among able-bodied people.

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Tables

Traditional Farms

Table 1. Input-output data, gross margin and profit per hectare Hajer Hadramout.

	Unit	الوحدة	Quantity	Price YRL	Value YRL	
Crop produce 1	kg	كجم	5,604.72	45.00	252,212	المنتج الرئيسى
Crop produce 2	kg	كجم	0.00		0	المنتج الثانوى
TOTAL GROSS OUTPUT					**252,212**	**العائد الكلى**
Seed/Seedling	kg/No.	كجم/عدد			0	البذور/الأشتال
Manure	kg	كجم	4,213.02	2.00	8,426	السماد البلدى
Nitrogen	kg	كجم	133.69	50.00	6,685	نيتروجين
Phosphate	kg	كجم			0	فوسفات
Potash	kg	كجم			0	بوتاس
Other (liquid)	kg	كجم			0	أسمدة أخرى
Chemicals:					6,005	المبيدات:
Pesticides	kg	كجم	3.00	2,000.00	6,005	حشرية
Herbicides	kg	كجم			0	عشبية
Fungicides	kg	كجم			0	فطرية
Machinery:					6,178	الآلات:
Land preparation	hours	ساعة	7.72	800.00	6,178	تحضير الأرض
Hired labor:			10.30	1,000.00	17,880	العمالة المؤجرة:
Land preparation	man/day	يوم/عمل	2.38	700.00	1,667	تحضير الأرض
Sowing & planting	man/day	يوم/عمل			0	البذار والزراعة
Fertilization	man/day	يوم/عمل	2.38	700.00	1,667	التسميد
Chemicals	man/day	يوم/عمل	2.38	800.00	1,905	المكافحة
Irrigation	man/day	يوم/عمل	3.16	800.00	2,528	الرى
Harvesting	man/day	يوم/عمل	6.32	1,600.00	10,114	الحصاد
Water Requirements	cu.m.	م 3	5,004.21	10.00	50,042	الإحتياجات المائية
TOTAL VARIABLE COST	**YRL**	**ريال يمنى**			**95,216**	**إجمالى التكاليف المتغيرة**
GROSS MARGIN	**YRL**	**ريال يمنى**			**156,996**	**هامش الربح الإجمالى**
Family labor:					5,316	العمالة العائلية:
Fertilization	man/day	يوم/عمل	4.00	300.00	1,201	التسميد
Chemicals	man/day	يوم/عمل	4.71	300.00	1,413	المكافحة
Irrigation	man/day	يوم/عمل	3.00	300.00	901	الرى
Cultivating	man/day	يوم/عمل	4.00	300.00	1,201	العناية
Harvesting	man/day	يوم/عمل	2.00	300.00	601	الحصاد
Zakat	YRL	ريال يمنى	5%	252,212	12,611	الزكاة
Land rent	YRL	ريال يمنى	15%	252,212	37,832	إيجار الأرض
TOTAL FIXED COST	**YRL**	**ريال يمنى**			**55,759**	**إجمالى التكاليف التابتة**
PROFIT	**YRL**	**ريال يمنى**			**101,238**	**الربح المزرعى**

Sources: Wadi Hajer Survey, Ministry of Agriculture and Irrigation (MA&I) office Mukala, Hadhramout, Yemen, 15 -22/8/2004

Table 2. Internal Rate of Return (IRR) at Wadi Hajer Hadramout.
Analysis of date palm crop for 10 ha in Wadi Hajer (using internal economic rate of return).

Year	Capital Cost (1000YR)	Operation Cost (1000YR)	Rent of land (1000YR)	Outflow or Gross Cost (1000YR)	Output or Goss benfits (1000YR)	Cash Flow (1000YR)	Discount factor (18%)	Present worth (18%)	Discount factor (23%)	Present worth (23%)
1	55,759	95,216	573.920	151548.920	301.5	-151247.420	0.8474576	-128175.78	0.8130081	-122965.38
2		95,216	573.920	95789.920	301.5	-95488.420	0.7181844	-68578.296	0.6609822	-63116.148
3		95,216	573.920	95789.920	301.5	-95488.420	0.6086309	-58117.2	0.5373839	-51313.941
4		95,216	573.920	95789.920	301.5	-95488.420	0.5157889	-49251.865	0.4368975	-41718.651
5		95,216	573.920	95789.920	252,212	156422.080	0.4371092	-40972.725	0.3552012	-33295.024
6		95,216	573.920	95789.920	252,212	156422.080	0.3704315	57943.672	0.2887815	45171.799
7		95,216	573.920	95789.920	252,212	156422.080	0.313925	49104.807	0.2347817	36725.04
8		95,216	573.920	95789.920	252,212	156422.080	0.2660382	41614.243	0.1908794	29857.756
9		95,216	573.920	95789.920	252,212	156422.080	0.2254561	35266.308	0.1551865	24274.598
10		95,216	573.920	95789.920	252,212	156422.080	0.1910645	29886.701	0.1261679	19735.446
11		95,216	573.920	95789.920	252,212	156422.080	0.161919	25327.713	0.1025755	16045.078
12		95,216	573.920	95789.920	252,212	156422.080	0.1372195	21464.164	0.0833947	13044.779
13		95,216	573.920	95789.920	252,212	156422.080	0.1162877	18189.969	0.0678006	10605.511
14		95,216	573.920	95789.920	252,212	156422.080	0.0985489	15415.228	0.0551224	8622.3668
15		95,216	573.920	95789.920	252,212	156422.080	0.083516	13063.753	0.044815	7010.0543
16		95,216	573.920	95789.920	252,212	156422.080	0.0707763	11070.977	0.036435	5699.2312
17		95,216	573.920	95789.920	252,212	156422.080	0.0599799	9382.1837	0.0296219	4633.5213
18		95,216	573.920	95789.920	252,212	156422.080	0.0508304	7951.0031	0.0240829	3767.0905
19		95,216	573.920	95789.920	252,212	156422.080	0.0430766	6738.1383	0.0195796	3062.6752
20		95,216	573.920	95789.920	252,212	156422.080	0.0365056	5710.2867	0.0159183	2489.9798
						18%		3033.2787		-81664.22

Sources: Wadi Hajer Survey, Ministry of Agriculture and Irrigation (MA&I) office Mukalla, Hadhramout, Yemen, 15 -22/8/2004
Sorghum is intercropped in the first four years.

Table 3. Average of crop prices in Mukalla Market.

Crops	Price of Farm (YR/Kg)	Price of wholesaler (YR/Kg)	Price of Retailer (YR/Kg)	Cost/kg	%for Farmer	%for Medd	Marg.of Mark	EPM
Dates	36	50	100	12.00	36.00	64.00	64.00	15.79
Sorghum	166	200	230	21.00	72.17	27.83	64.00	24.71
Millet	166	200	230	35.00	72.17	27.83	64.00	35.35
Watermelon	15	22	39	7.00	38.46	61.54	24.00	22.58
Tomatoes	43		46	10.00	93.48	6.52	3.00	76.92
Mango	60	124	158	10.00	37.97	62.03	98.00	9.26
Banana	25	62	77	10.00	32.47	67.53	52.00	16.13

Sources: Wadi Hajer Survey, Ministry of Agriculture and Irrigation (MA&I) office Mukalla, Hadhramout, Yemen, 15 -22/8/2004
EPM= Efficiency Predicted Marketing. EPM=[1-(Margin Of Market)/(Margin Of Market + Cost of Market)]*100.

Table 4. Date palm margin and miscellaneous budget.

Date Palm	Unit	Quantity	Price YRL	Value YRL
Crop produce 1	Kg	11858	45.65	541,317.70
Crop produce 2				
TOTAL GROSS OUTPUT				541317.7
Seeds	Kg			
Fertilizer Urea	Kg	75	44	3300
Others Fertilizer	Kg	75	90	6750
Manure	Kg	5000	2	10000
Chemicals & Pesticides	Kg	1	2500	2500
Others	Letr	1	200	200
Irrigation	hours	272	100	27200
Land preparation	hours			
Sowing & planting	hours			
Land preparation of animals	ani/day			
Land preparation Labor	man/day			
Sowing & planting Labor	man/day			
Fertilization	man/day	13	350	4550
Chemicals	man/day	7	350	2450
Irrigation	man/day	17.14	350	5999
Weeding	man/day	12	350	4200
lightening / patching	man/day	20	400	8000
Harvesting	man/day	100.3	400	40120
Post-harvesting	man/day	18	400	7200
Transportation				30758
Others				25175
Zakat		5%		27065.885
Cost YR/Ha				205467.885
Net Income	YRL			335849.815

Source: Ghayth Aquatech, Sana'a ROY, Updating the feasibilty study and detailed design for Sorudud Dam Project, Volume- 1-2-3, Sana'a, January 2004

Sorghum is intercropped in the first four years.

Table 5. Internal Rate of Return (IRR).
Analysis of dates palm crop for 10 ha in Wadi Surdud (using internal economic rate of return).

Year	Capital Cost (1000YR)	Operation Cost (1000YR)	Rent of land (1000YR)	Outflow or Gross Cost (1000YR)	Output or Goss benfits (1000YR)	Cash Flow (1000YR)	Discount factor (18%)	Present worth (18%)	Discount factor (23%)	Present worth (23%)
1	265.2	1574.265	573.920	2413.385	301.5	-2111.885	0.8474576	-1789.7331	0.8130081	-1716.9797
2		1267.765	573.920	1841.685	301.5	-1540.185	0.7181844	-1106.1369	0.6609822	-1018.0349
3		1267.765	573.920	1841.685	301.5	-1540.185	0.6086309	-937.40414	0.5373839	-827.67065
4		1267.765	573.920	1841.685	301.5	-1540.185	0.5157889	-794.41029	0.4368975	-672.90297
5		1501.553	573.920	2075.473	2054.25	-21.222	0.4371092	-9.2765503	0.3552012	-7.5382578
6		1634.493	573.920	2208.413	3109.2215	900.809	0.3704315	333.68796	0.2887815	260.13687
7		1736.030	573.920	2309.950	4057.8285	1747.879	0.313925	548.7029	0.2347817	410.36993
8		1906.821	573.920	2480.741	5413.177	2932.436	0.2660382	780.13977	0.1908794	559.7416
9		1906.821	573.920	2480.741	5413.177	2932.436	0.2254561	661.1354	0.1551865	455.07447
10		1802.929	573.920	2376.849	5413.177	3036.328	0.1910645	580.13442	0.1261679	383.08716
11		1802.929	573.920	2376.849	5413.177	3036.328	0.161919	491.63934	0.1025755	311.45297
12		1802.929	573.920	2376.849	5413.177	3036.328	0.1372195	416.64351	0.0833947	253.2138
13		1802.929	573.920	2376.849	5413.177	3036.328	0.1162877	353.08772	0.0678006	205.86488
14		1802.929	573.920	2376.849	5413.177	3036.328	0.0985489	299.22688	0.0551224	167.36982
15		1802.929	573.920	2376.849	5413.177	3036.328	0.083516	253.5821	0.044815	136.07302
16		1802.929	573.920	2376.849	5413.177	3036.328	0.0707763	214.90009	0.036435	110.62847
17		1802.929	573.920	2376.849	5413.177	3036.328	0.0599799	182.11872	0.0296219	89.941849
18		1802.929	573.920	2376.849	5413.177	3036.328	0.0508304	154.3379	0.0240829	73.123454
19		1802.929	573.920	2376.849	5413.177	3036.328	0.0430766	130.79483	0.0195796	59.449963
20		1802.929	573.920	2376.849	5413.177	3036.328	0.0365056	110.84307	0.0159183	48.333303
						20%		874.01368		-719.26489

Source: Ghayth Aquatech, Sana'a ROY, Updating the feasibilty study and detailed design for Sorudud Dam Project, Volume- 1-2-3, Sana'a, January 2004
Sorghum is intercropped in the first four years

Table 6. Marketing prices in the local market in Surdud 2003.

Crops	Price of Farm gate (YR/Kg)	Price of wholesaler (YR/Kg)	Price of Retailer (YR/Kg)	Cost/kg	% for Farmer	% for wholesaler	% of Retailer	EPM
Dates	45.65	62.00	77.00	14.73	59.29	40.71	31.35	36.99
Millet	46.75	50.39	57.90	35.63	80.74	19.26	11.15	76.17
Cawbea	90.42	92.67	103.48	58.69	87.38	12.62	13.06	81.79
Maize	57.75	65.75	75.38	17.93	76.62	23.38	17.63	50.43
Cotton	75.54	78.87	88.20	40.97	85.65	14.35	12.66	76.39
Tobacco	78.17	86.13	109.64	31.60	71.29	28.71	31.47	50.10
Sesame	94.08	111.00	127.60	56.84	73.73	26.27	33.52	62.90
Tomatoes	36.83	45.00	56.47	9.15	65.23	34.77	19.63	25.28
Okra	46.25	60.73	71.41	18.78	64.77	35.23	25.16	26.67
Mango	126.25	174.93	202.70	10.35	62.28	37.72	76.45	19.72
Banana	35.87	50.14	64.71	9.67	55.43	44.57	28.84	26.41
Gwafh	80	108.60	123.97	18.41	64.53	35.47	43.97	18.03
Fodder	6.92	17.30	19.75	4.54	35.02	64.98	12.83	53.45

Source**:** Compiled and Computed from AdDahi, Al Mighlaf and Zaydiyah for the last three months collected by TDA extensions in Bajel.

Table 7. Average of crops prices in Hodaidah Markets and farm gate price in Surdud.

Crops	Price of Farm gate (YR/Kg)	Hodeidah	% for Farmer	% for wholesaler	% of retailer	EPM
Sorghum	39.96	77.5	51.56	48.44	37.54	36.64
Millet	46.75	60	77.92	22.08	13.25	72.89
Datets	45.65	57.1	79.97	20.03	11.43	56.31
Maiz	57.75	70	82.50	17.50	12.25	59.41
Tobacco	78.17	300	26.06	73.94	221.83	12.47
Seasem	94.08	188	50.04	49.96	93.92	37.70
Watermelon	28.08	50	56.17	43.83	21.92	23.26
Tomatoes	36.83	45	81.85	18.15	8.17	52.84
Okra	46.25	110	42.05	57.95	63.75	22.76
Mango	126.25	150	84.17	15.83	23.75	30.35
Banana	35.87	75	47.83	52.17	39.13	19.82
Gwafh	80	110	72.73	27.27	30.00	38.02

Source: Surdud Districts survey, August,2003

Table 8. Financial, economic and market analysis.

	Wadi Hajer, Hadramout	Wadi Surdud, Hodeidah
B/C	1.67	2.63
Net Income per ha (YR)	101238	335850
IRR	18%	20.7%.
EPM	15.79	56.31

Table 9. Marketing prices of dates in Yemen and abroad.

Country	Price of Farm gate (YR/Kg)	Price of Retailer (YR/Kg)
Yemen	45.65 US$/kg	100
UAE®	0.23 3	0.51
Israel	3.5	

Sources: ® Abdelouahhab Zaid, Chief Technical Adviser /Director UNOPS- Date Palm Research & Development Programme, United Arab Emirates, FAO, Rome, 2002
Exchange rate 1 US$ = 195 YR in Sana'a local market 20/12/2005

Figures

Figs. 1, 2, 3 and 4 explain the outcome of the quantitative analysis.

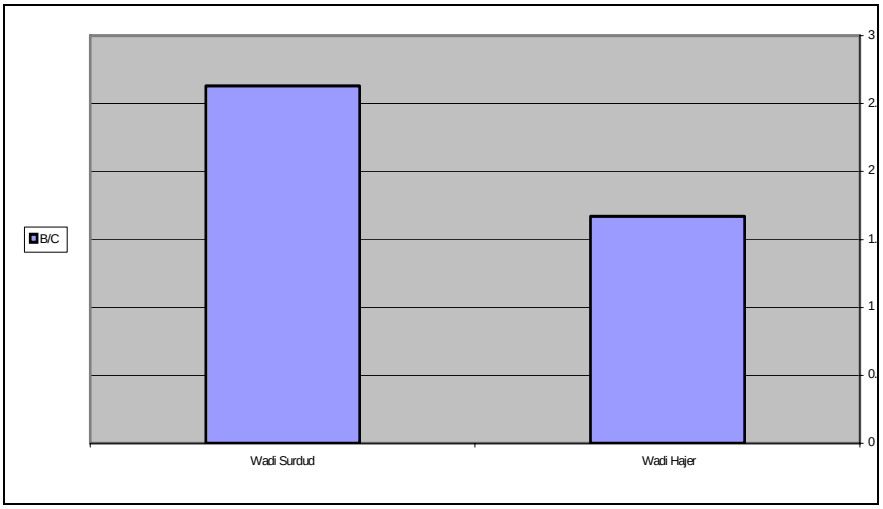

Fig. 1. Benefit Cost Ratio (BCR).

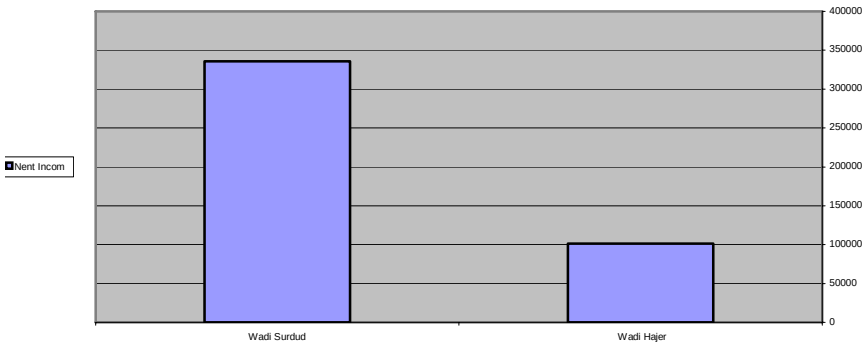

Fig. 2. Net Income.

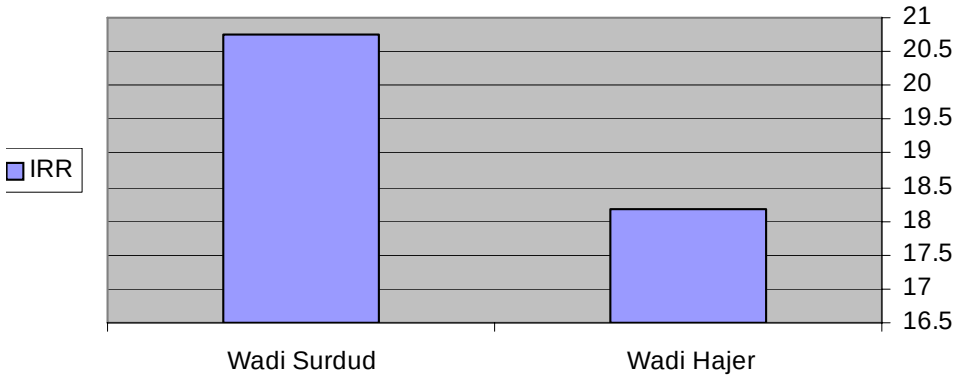

Fig. 3. IRR.

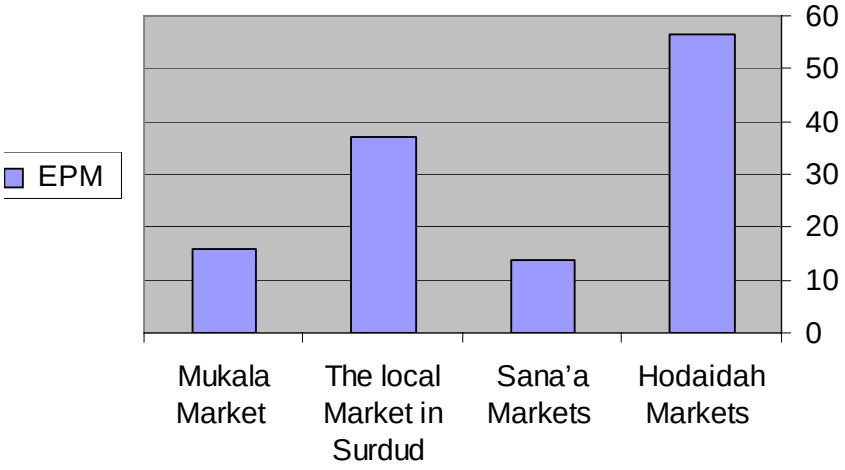

Fig. 4. EPM.

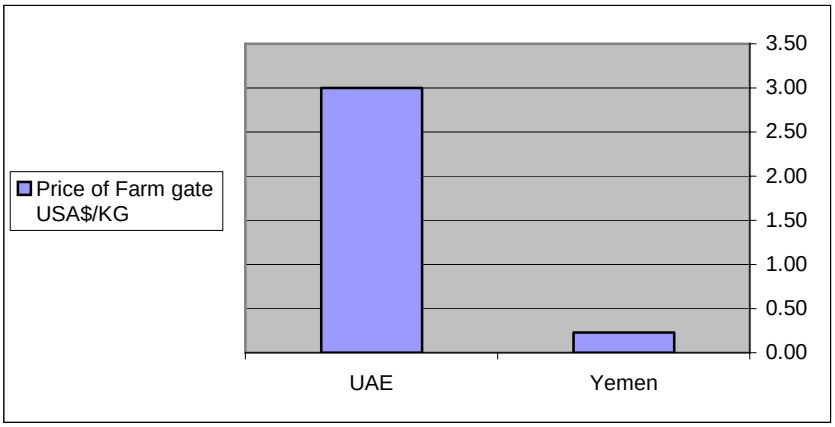

Fig. 5. Price of farm gate USA$/KG.

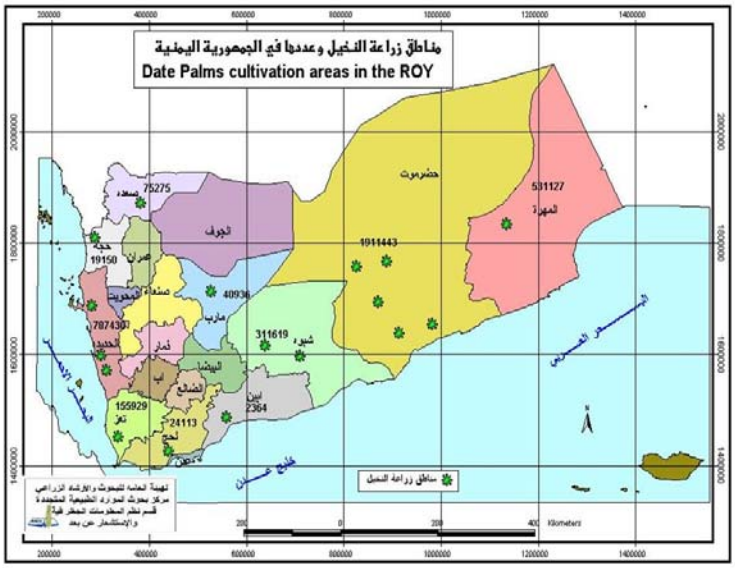

Fig. 6. Local food system.

Shelf Stability of Dhakki Dates as Influenced by Water Activity and Headspace Atmosphere

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Keywords: *Phoenix dactylifera* L., inert atmosphere, quality changes, Pakistan

Abstract

Date palm (*Phoenix dactylifera* L.) has played a vital role in the history of mankind by providing food and shelter to millions of people. Pakistan is the 4th largest date producing country, contributing 11% to world production. A prominent local cultivar 'Dhakki' of Dera Ismail Khan is economically very important as it has large fruit (5cm long, 3 cm thick and 20 g/fruit) with a small pit, fine texture and delicious taste. However, being a late maturing variety, it is prone to environmental stresses as the stormy monsoon season coincidences with the period of fruit ripening. Uneven production and a lack of post-harvest technology are two factors that cause quality deterioration and excessive wastage. In this study, a sorption isotherm was constructed in the range of 0.12 to 0.97 a_w, and water activity determined for Dhakki dates. The stability of Dhakki dates was examined at 0.52 - 0.75 a_w under oxygen, air or nitrogen during storage for 4 months inside tinplate cans at an elevated temperature of 40°C. The quality was evaluated monthly in terms of darkening, pH, and titratable acidity, whereas slime appearance was observed twice daily. The sorption isotherm is sigmoid in shape, and water activity of 0.25 to 0.62 a_w represented the monolayer moisture coverage, whereas 0.61±0.01 a_w was recorded for the freshly ripened Dhakki dates. The deterioration of quality was affected by both water activity and headspace atmosphere. Samples stored with water activities higher than 0.75 a_w deteriorated rapidly with slime formation, whereas those with lower levels displayed proportionately greater stability and at 0.52 a_w, maintained characteristic color and flavor with a semi-dried look. The samples stored under nitrogen afforded greatest stability. The rate of darkening, pH and titratable acidity were about 2.2, 2.8 and 2.7 times higher, respectively, under oxygen than under the nitrogen. The impact of water activity and headspace atmosphere on quality parameters was statistically significant. In order to maintain freshness of the product with extended shelf life the Dhakki dates should be stored under inert atmosphere with a water activity close to 0.61±0.01 a_w.

INTRODUCTION

Few plant species have developed into an agricultural crop so important to human life, as has the date palm (*Phoenix dactylifera* L.). Had the date palm not existed, the expansion of the human race into the hot and barren parts of the ancient world would have been much more restricted. Certainly the date palm imparts close and everlasting association with mankind, and is legendary in the Arabic world. In the Holy Qur'an, it is referred to 20 times. Prophet Muhammad enjoyed his followers to honor it as "a blessed tree", and since then the date palm has become an integral part of Muslim culture. The Arabs say that there are as many uses for dates as the days in a year. Practically all parts of the date palm have a use. Date palm nourishes millions all over the world and contributes significantly towards their development and prosperity, particularly those living in the Arabian deserts. Dates are nutritious being high in carbohydrates, fiber, potassium, and certain vitamins and minerals, but low in fat, and virtually free from cholesterol and sodium. Since dates are an excellent source of energy and stay fresh for several weeks if properly stored, they are used as a staple

diet by some people. During 'Ramadan', which is the annual fasting month for Muslims, the daily fast is broken after sunset with a few dates before taking sips of water.

Pakistan is considered the 4th largest date producing country in the world. Dates are an important cash crop and a good source of foreign exchange earnings. The total cultivated area of all types of dates in Pakistan exceeds 78.1 thousand hectares with an estimated annual production of over 630 thousand tonnes. This constitutes about 11 % of total world production (Anon, 2002). Pakistan exports mostly dried dates worth Rs1.468 billion annually (Anon, 2003).

Cultivation of date palm in the North West Frontier province exceeds 1000 hectares with a production of 6700 tonnes, of which more than 50 % is furnished from the Dera Ismail Khan region. Most of the plantations in Dera Ismail Khan are concentrated in Panyala, Paharpur, Chowdhwan and Dhakki, where summer is hot, causing early ripening of the date fruits. The temperature during June - August normally ranges from 38 to 48°C, rising sometimes above 50°C with about 30-cm annual rainfall. Among the local varieties 'Dhakki' is the most promising cultivar with commercial importance. The date is quite popular for its extra large size (4-5 cm long and 2-3 cm thick), small stone and heavy weight (16-20 g/ fruit). It has fine texture, good flavour (Baloch, 1999) and fetches a high price in the market. However, concurrence of the monsoon season with the period of date ripening causes the the crop to receive heavy damage from rain and insect bites. The losses are even greater in case of Dhakki date, which is a late maturing variety and very susceptible at the mature/ripened stage to the hot humid climate. Moreover, during the peak production period, a large quantity of the fresh fruit is left over, and gluts the local market. Due to lack of appropriate processing and storage facilities the surplus produce is wasted.

A rapid darkening in colour is a common phenomenon when dates are stored in high summer temperatures and humidity, and causes problems for the date industry which must be addressed. Oxidation of phenolic compounds and presence of sugar are the main factors that cause darkening at elevated temperatures (Vandercook et al., 1979). The mechanism of browning in model systems (Hodge, 1953), and in fruits and vegetables (McWeeny et al., 1974; Wedzicha, 1987) has been reviewed thoroughly. Packing under vacuum or inert gases (Rygg, 1977; Mohsen et al., 2003) or applying sulphite treatment (McWeeny et al., 1974) have been suggested to prolong shelf life of high moisture dates and other moist foods. Previously we have reported that Dhakki dates have a water content of about 0.62 (Saleem et al., 1997). Information regarding the effect of storage atmosphere at elevated temperatures as well as water activity on stability of dates in general, and Dhakki dates in particular, is lacking. The objective of the present investigation was to explore the potential of inert atmosphere and optimized water activity of freshly cured Dhakki dates to enhance storage stability at the elevated temperature of 40°C.

MATERIAL AND METHODS

Sample Preparation

Dhakki dates at Khalaal stage with a hardness index of 200–250 mmHg.cm^{-2} (Baloch et al., 2003) were procured from the local market. Well-developed fruits with a good appearance were used while others were discarded. To retain normal color and flavor of the dates during curing/drying the fruits were placed in a wire-mesh basket and dipped (1kg/L) for one minute in potassium metabisulfite (0.5 g/100 ml) solution at 70°C. The treated samples were allowed to drain, placed on stainless steel trays in a single layer loading of 6 kg/m^2, kept in a Pak-made thermostatically controlled dehydrator equipped with a hot air overflow system and then cured and dried at 40°C for 10 h to about 24 % moisture content. The dates were thoroughly mixed to ensure sample uniformity. Samples required for sorption studies were made into pulp after seed removal, whereas whole cured fruits were used for further storage studies.

Sorption Isotherm and Water Activity Evaluation

Pulp was macerated to obtain uniform mash, which was subjected to moisture

contents in the range of 0.12 to 0.97 a_w at 40°C (Table 1). Twenty grams of macerated date was kept inside desiccators each containing saturated salt solutions to maintain required water activity. The sample was weighed twice daily until a constant weight was attained. During equilibration over a 5 day period, the solutions were maintained at saturation by adding respective dry salt or distilled water as required. Equilibrium moisture content (EMC) of samples at each water activity was then determined using A.O.A.C. (1984). A sorption isotherm was constructed by plotting EMC against water activity using MSTATC package. Water activity of the date samples was then determined from the point of intersection with no change in weight of the sample and the sorption isotherm (Spiess and Wolf, 1987).

Storage Studies

1. Evaluation for Slime Appearance. Cured date samples (100g) were maintained at water activities varying from 0.75-0.97 a_w for slime appearance during storage at 40°C in glass desiccators, each having a saturated solution of different water activity. Slime formation was noted twice daily without opening the containers.

Biochemical Studies

The samples were divided into three sets and water activity adjusted to 0.52, 0.58 or 0.75 a_w. Each set was subdivided into three lots for storage under controlled atmosphere of oxygen, air and nitrogen. About 200 g of equilibrated samples were sealed hermetically inside A1 (315 ml) size tin-plated cans fitted with two nozzles (valve-built-in) on cross sides for gas flushing. The cans were evacuated (125mm Hg) for one minute and the required gas was in filled. To retain the required water activity the flushing gas was passed through the desiccators that had a saturated solution to maintain the required water activity.

The process was repeated 4 times before soldering the nozzle outlets. The sealed samples were then incubated for 4 months in an oven at 40°C.

Samples were taken out of the oven every month and analyzed for darkening, pH and titratable acidity. After removing the pits, the dates were cut into small pieces, and ground into a uniform mash. The mash was extracted with distilled water or dilute acetic acid (2 g/100 ml) to measure pH and titratable acidity, and darkening was evaluated. The pH was measured potentiometrically using a digital pH-meter (Model 3010, Jenway England) equipped with a temperature control probe. The titratable acidity (expressed as citric acid, mg/g) was assessed after titrating sample extracts against known (4.0g/L) concentrations of sodium hydroxide using the pH-meter. Darkening was determined on the clarified extract by measuring absorbance at wavelength of 420 nm (Baloch et al., 1973) using spectronic-20 spectrophotometer (Busch and Lamb, USA). The data was analyzed statistically by means of MSTAT-C version 2-10 software package using a completely randomized design (MSTAT-C, 1987). The means were separated by a LSD test using the same package. Linear regressions between measured parameters and time were used to assess the rate of deterioration of quality, and the effectiveness of treatments.

RESULTS AND DISCUSSION

Sorption Isotherm

Date samples stored at water activity of 0.58 a_w or below started losing weight, whereas samples kept under water activity of 0.75 a_w and above gained weight. The loss or gain in weight was rapid during initial equilibration periods, then leveled off after 5 days of equilibration. The equilibrium moisture content (EMC) increased from 10.6% to 95.4% with respective increases in water activity level from 0.12 to 0.97 a_w. Moisture sorption isotherms represent a plot between EMC (%) and corresponding water activity values (Fig. 1). This figure depicts a typical sorption isotherm not segmented distinctly, as frequently reported in theoretical representations. A number of similar isotherms for fruits were reported by Heiss

(1968). The shape of the current isotherm indicated an overlap of moisture layers from one sorption region to the other. The first segment of the isotherm extended to 0.25 a_w with a relatively high rate of water uptake per unit change in water activity. The 2nd portion was larger in size approaching up to 0.6 a_w and appeared to be almost flat in shape. This portion most probably carries a moisture level for monolayer coverage. The last portion was enlarged with the highest slope for water uptake in the region of vapor and capillary water. The isotherm demonstrated how water activity interacts with date moisture levels, and thus helps in predicting the stability of dates during storage at various water activity levels.

Water activity of the sample at zero weight change, calculated from the point of intersection from the plot between water activity and loss or gain in weight (%), was found to occur at about 0.61-0.62 a_w, and appeared to be the water activity of the Dhakki dates. This water activity is within the reported range for dehydrated semi moist fruits (Davies et al., 1976). The water activity of 0.61-0.62 a_w corresponded to 24-25% equilibrium moisture content.

Studies on Slime Appearance

The appearance of slime was observed during storage of samples maintained at water activity levels beyond 0.75 a_w. The period of slime formation decreased rapidly as the level of water activity increased. In samples at water activity close to 1.0 a_w mould growth became visible even prior to storage. A plot of days prior to slime formation against water activity represents the mould-free storage life of dates (Fig. 2). This plot predicts mould-free shelf-life for date fruits at various water activity levels during storage at the elevated temperature of 40°C. Safe shelf life of the fruit rapidly increased with the decrease in water activity. The results indicated that Dhakki dates can be stored well beyond 50 days without showing signs of slime formation, provided they are stored at a water activity below 0.75 a_w.

Biochemical Studies

Taking preliminary studies as well as the sorption isotherm of the date into consideration, three water activity levels of 0.52, 0.58 and 0.75 a_w were selected to further examine the effect of storage on darkening, pH change and titratable acidity of the dates. The chosen limit of water activity covers the water activity of the dates (≈0.62 a_w), extended to the range of semi-dried and moist foods and to the sorption segment intended for storage of freshly cured dates. Further, a temperature of 40°C was selected for storage studies to collect information at an elevated temperature. It is pertinent to note that the selected temperature lies within the range of prevailing summer temperatures during the high production season as well as the rapid deterioration period of freshly ripened dates.

Darkening

Irrespective of environmental factors, darkening continued to increase over time in storage at 40°C (Fig. 3). A maximum amount of darkening (0.089) was displayed by samples under oxygen at 0.75 a_w whereas the minimum (0.059) occurred under nitrogen at 0.52 a_w. Mean values (Table 2) for samples as regard to storage atmosphere are statistically significant ($P < 0.05$). The rate of darkening (12.1×10^{-3} /month) of samples stored with 0.75 a_w under the oxygen was twice that of nitrogen and 1.4 times that observed under air (Fig. 4). The results showed that oxygen accelerated while the nitrogen retarded darkening, compared to air. The samples under nitrogen resisted deterioration and looked normal in color and flavor at the end of the storage period. Those under oxygen appeared dark brown with a smell of burnt sugar, in addition to giving absorbance values close to 0.1 units, an indication that they were at the limit of their shelf life (Baloch et al., 1997; Baloch et al., 2000). Cured dates at tamar stage possessing sugar carbohydrates, amino acids and tannin polyphenolic compounds (Sawaya et al., 1982), showed a continuous rise in browning under any storage conditions, which testifies to the involvement of both oxidative and non-oxidative darkening at 40°C. Similar findings have been reported in the literature (Maier and Metzler, 1965; Maier and Schiller 1960; 1961 a, b). Darkening was reduced by more than 30 % when Dhakki dates were stored

under inert atmosphere at 40°C, whereas a reduction of 20 % was reported for Deglet Noor at 38°C (Maier and Schiller, 1961 a, b).

The darkening was influenced by water activity of the samples. The mean values for water activity (0.52, 0.58 and 0.75) are statistically significant ($P < 0.05$), whereas no significant effects occurred between water activity values of 0.52 and 0.58 under any atmosphere (Table 2). At low water activities stability increased under any atmosphere (Figs. 3, 4). The darkening rate was reduced by a factor of 1.21 - 1.30 when samples were stored at 0.52 instead of 0.75 a_w. The sample stored under nitrogen with the lowest water activity (0.52) was found most stable, and this is consistent with reported observations (Mutlak and Mann, 1984; Saleem et al., 1997).

pH

A gradual decline in pH from 6.3 to 3.58 occurred during storage, with a rapid decline when the samples were stored under oxygen at higher water activities (Fig. 5). A drop in the pH of 2.72, 1.6 and 0.92 units was found for samples under oxygen, air and nitrogen at 0.75 a_w, respectively. A rate of fall (6.75×10^{-1} ΔpH/month, Δ stands for change) in pH corresponding to samples with 0.75 a_w under oxygen was reduced by 1.74 and 3.11 times as a result of change in the atmosphere to air and nitrogen, respectively (Fig. 6). Similar observations were reported for Deglet Noor variety (Maier and Schiller, 1961a). Mean pH values as affected by controlled atmosphere were statistically significant at $P < 0.05$ (Table 2). It was found that storage under inert atmosphere was the most effective technique for controlling deterioration. A continuous fall in pH during storage under atmospheres of oxygen, air or nitrogen demonstrated that both oxidative and non-oxidative mechanisms are responsible for pH changes, and was found for darkening reactions.

Water activity of the samples also played a vital role in governing pH changes, as samples with reduced water activity displayed greater resistance to deterioration. The mean pH values with respect to water activity were statistically significant ($P < 0.05$, Table 2). About 29 - 38 % reduction in the rate of change of pH occurred when water activity was reduced from 0.75 to 0.52 a_w. The same process of quality deterioration were responsible for increased rate of darkening and rate of pH drop as reported previously (Baloch et al., 1977; Rygg, 1977).

Titratable Acidity

A consistent rise in titratable acidity was observed for all samples during storage (Fig. 7). However, the rates were greatly influenced by storage atmosphere and water activity. Mean values of the determinant of both factors were statistically significant ($P < 0.05$, Table 2). Acidity of 22.15×10^{-2} - 22.37×10^{-2} mg/g prior to storage increased to 43.57×10^{-2} - 100.95×10^{-2} mg/g when the equilibrated samples were kept at 0.52 - 0.75 a_w for 4 months at 40°C (Fig 8). The rate of acid formation (19.14 mg/g month) for samples equilibrated with 0.75 a_w and stored under oxygen was about 1.76 and 2.86 times greater than for those under air or nitrogen, respectively, and displayed highly significance differences ($P < 0.05$, Table 2). A minimum of 5.43 (mg/g month) was found with 0.52 a_w under nitrogen (Fig. 8). It is further noted that the samples displaying more darkening produced a greater amount of acidity and pH drop. It is therefore suggested that all such reactions have a common reactive pool from which the determinants are emerging. Present findings are in line with those reported earlier (Saleem et al., 1997; Saddozai et al., 1998).

Since the level of water activity of 0.62 a_w included the segment of sorption isotherm containing capillary moisture range (Fig. 1), it cannot be guaranteed that fruit will remain unspoiled and safe over prolonged storage at 40°C. Deterioration of dates may occur by osmiophilic yeast and zerophillic mould at a water activity as low as 0.62 a_w, and may even be caused by chemical degradation (Brockmann, 1973).

CONCLUSIONS

Sorption isotherm of Dhakki dates was sigmoid in shape, similar to those of foods with high sugar contents. Water activity of Dhakki dates of 0.62 a_w occurred on a section of the isotherm of intermediate moisture, and similar to that of semi-moist foods which experience deteriorative changes of a chemical nature. The darkening and other associated changes responsible for quality degradation of Dhakki dates are a function of storage atmosphere and water activity. The investigated parameters were reduced considerably when the dates were stored under nitrogen and at 0.52 a_w. Since the samples were stored at an elevated temperature of 40°C and at water activity within the range of intermediate moisture limits, the degradative reactions occurred at a rapid rate, and possibly interacted with each other to alter the sequence of the deteriorating process. Higher deterioration rates at higher water activity levels (0.75 a_w) are attributed to the increased mobility of the constituents involved in the deteriorative process, which were further promoted by the oxygen. The study suggests that Dhakki dates should be stored under an atmosphere free from oxygen and at water activity within 0.60-0.61 a_w. Such conditions will provide sufficient stability and ensure for adequate shelf life of Dhakki dates.

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Tables

Table 1. Saturated salt solutions used to provide required water activities at 40°C.

Salts	Formula	A_w
Lithium chloride	($LiCl.H_2O$)	0.12
Potassium acetate	($KC_2H_3O_2$)	0.23
Magnesium chloride	($MgCl_2.6H_2O$)	0.33
Potassium carbonate	(K_2CO_3)	0.44
Magnecium nitrate	[$Mg(NO_3)_2.6H_2O$]	0.52
Sodium bromide	(NaBr)	0.58
Sodium chloride	(NaCl)	0.75
Potassium chloride	(KCl)	0.85
Potassium chromate	(K_2CrO_4)	0.88
Potassium nitrate	(KNO_3)	0.94
Potassium sulphate	(K_2SO_4)	0.97

(Troller and Christian, 1978)

Table 2. Mean values for darkening, pH and titratable acidity of Dhakki dates stored at 40°C for 4-months, as affected by water activity and headspace atmosphere.

Factors ↓ Parameters →		Darkening	pH	Titratable acidity
Water activity (a_w)	0.52	0.053 B	5.58 A	38.28 C
	0.58	0.054 B	5.52 B	40.36 B
	0.75	0.065 A	5.34 C	45.85 A
Headspace atmosphere	Oxygen	0.060 X	5.12 Z	52.67 X
	Nitrogen	0.049 Z	5.79 X	32.02 Z
	Air	0.055 Y	5.53 Y	39.80 Y

Mean values bearing different letters (A – C), (X – Z) in each column for every factor differ significantly (LSD, $P \leq 0.05$).

Figures

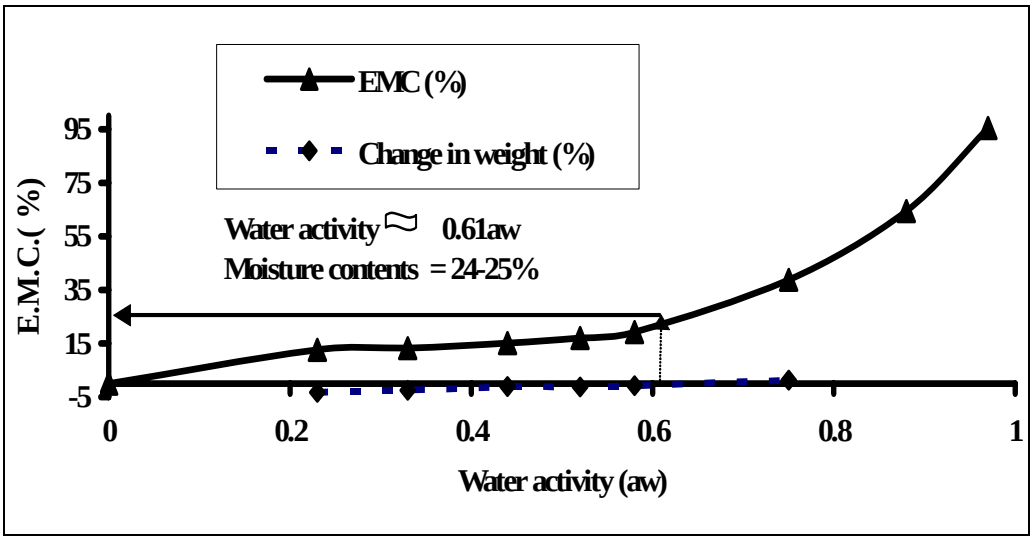

Fig. 1. Sorption isotherm of Dhakki dates at 40 °C.

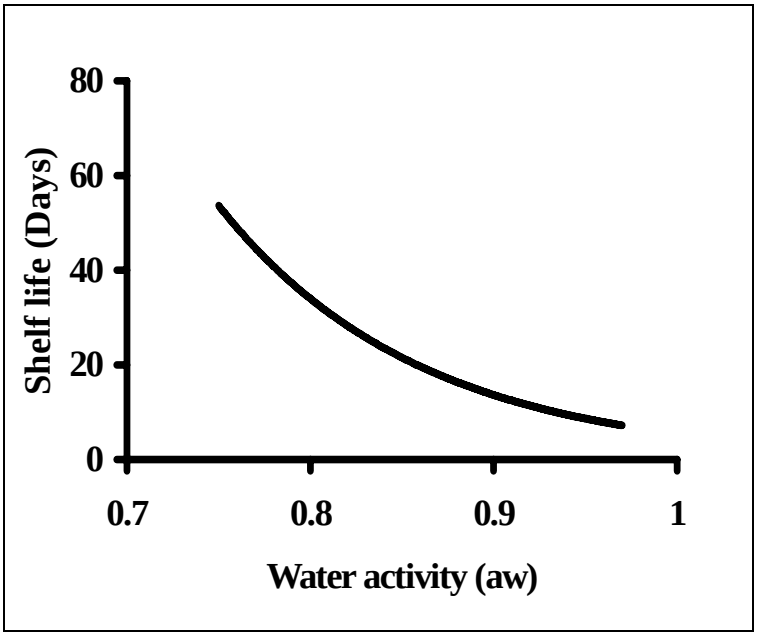

Fig. 2. Shelf life limited by slime appearance during storage at 40 °C with high levels of water activity.

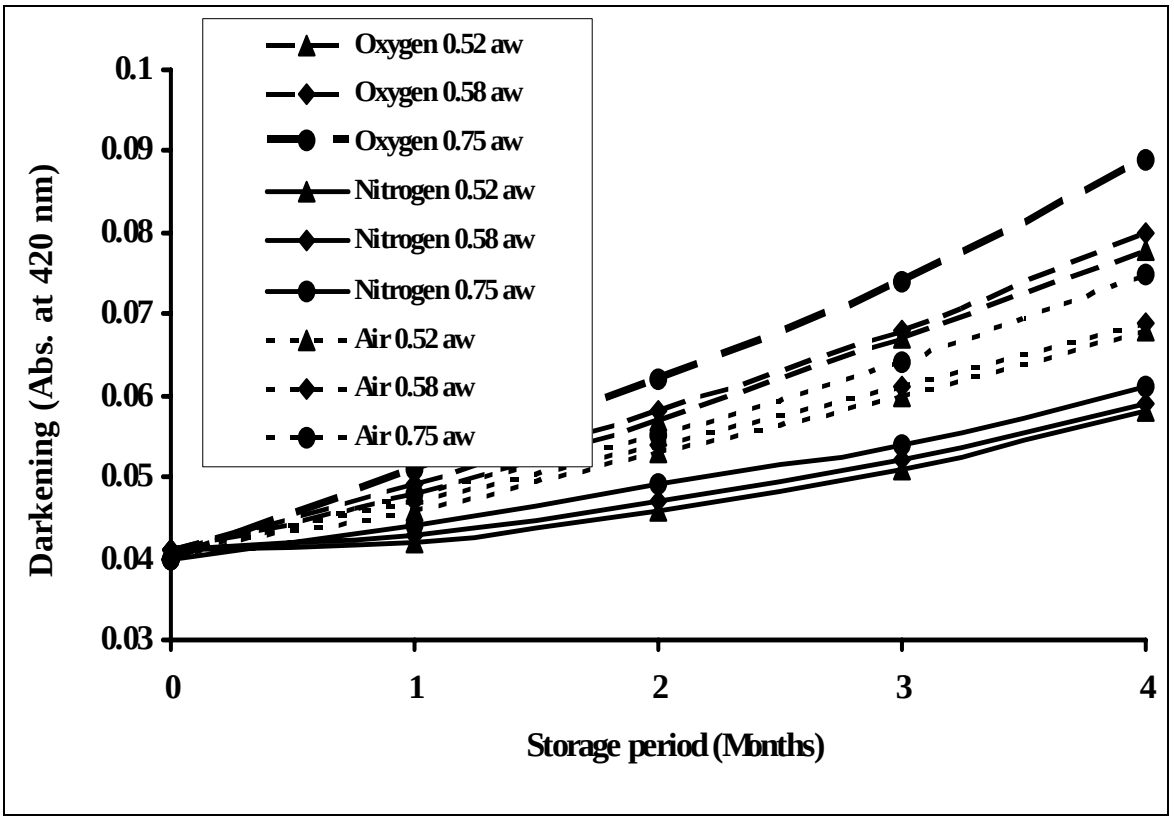

Fig 3. Influence of water activity and headspace atmosphere on darkening of Dhakki dates during storage at 40°C.

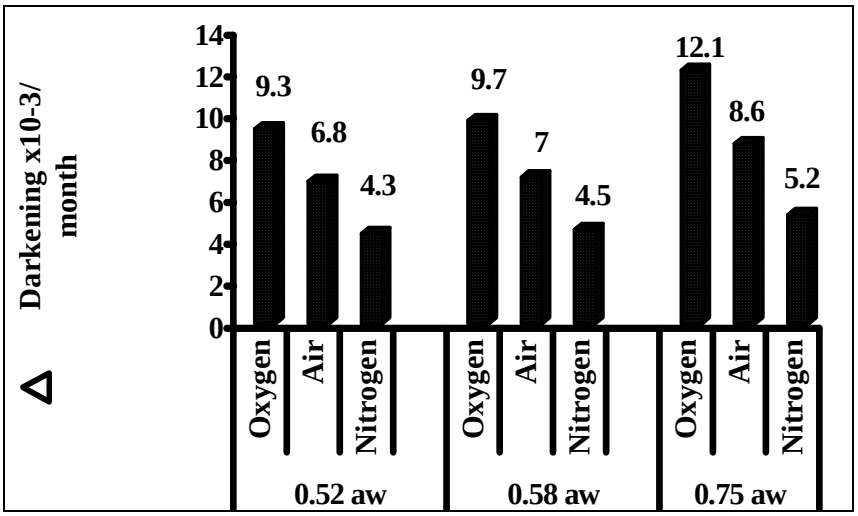

Fig 4. Influence of water activity and headspace atmosphere on rate of change in darkening of Dhakki dates during storage at 40°C.

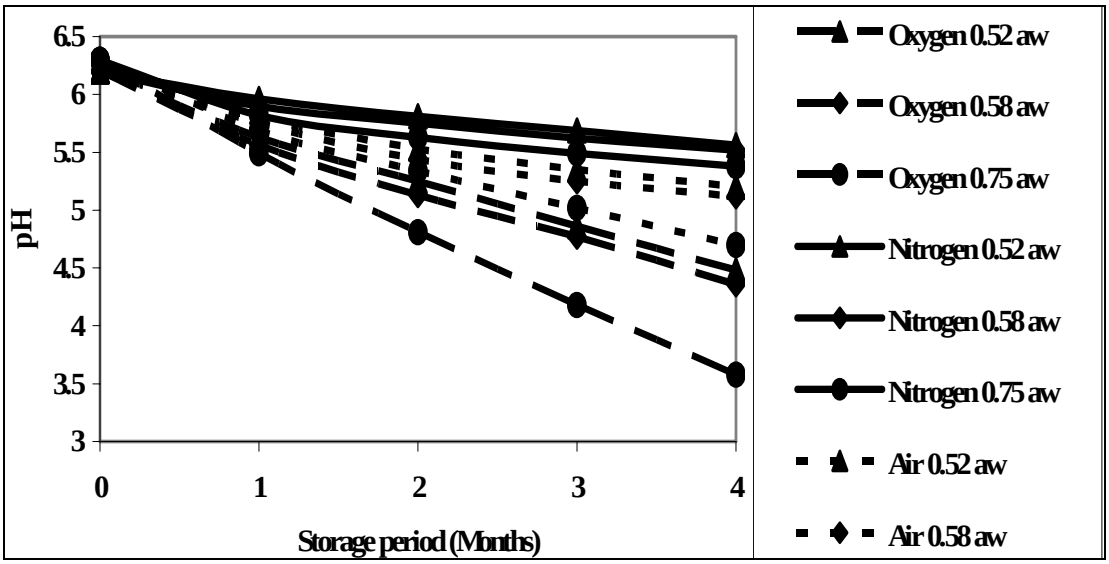

Fig. 5. Influence of water activity and headspace atmosphere on pH of Dhakki dates during storage at 40°C.

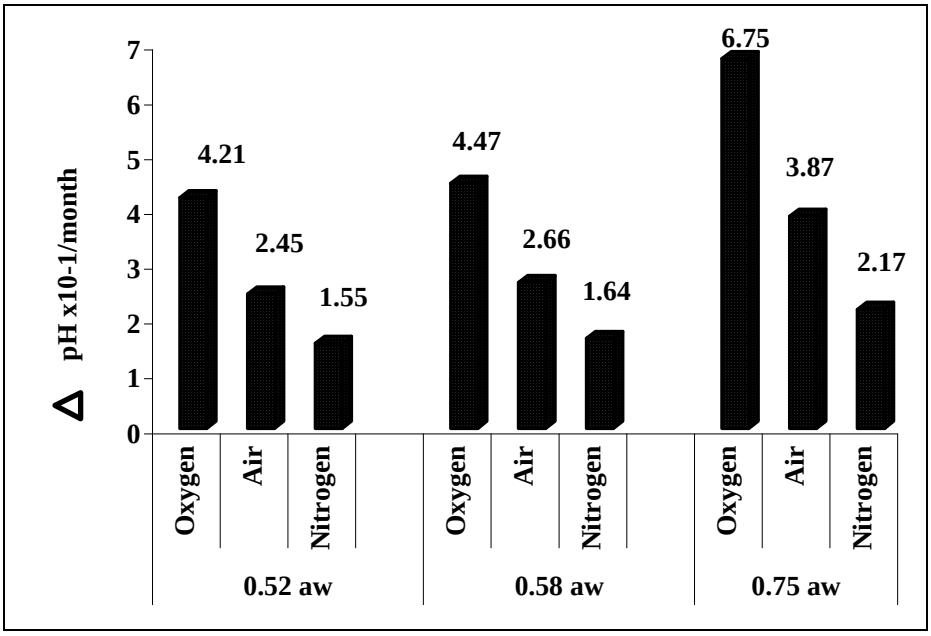

Fig. 6. Influence of water activity and headspace atmosphere on rate of change in pH of Dhakki dates during storage at 40°C.

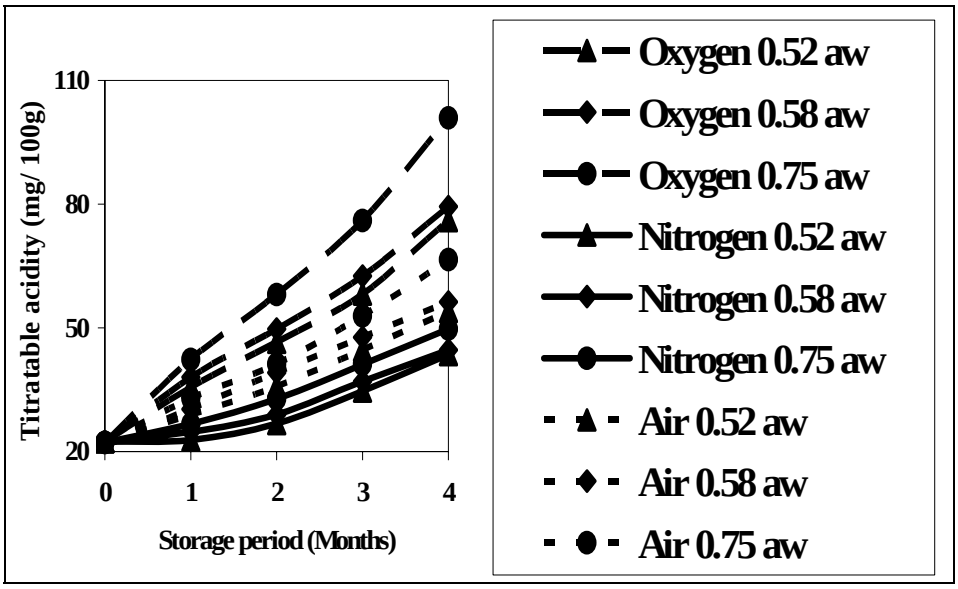

Fig. 7. Influence of water activity and headspace atmosphere on titratable acidity of Dhakki dates during storage at 40°C.

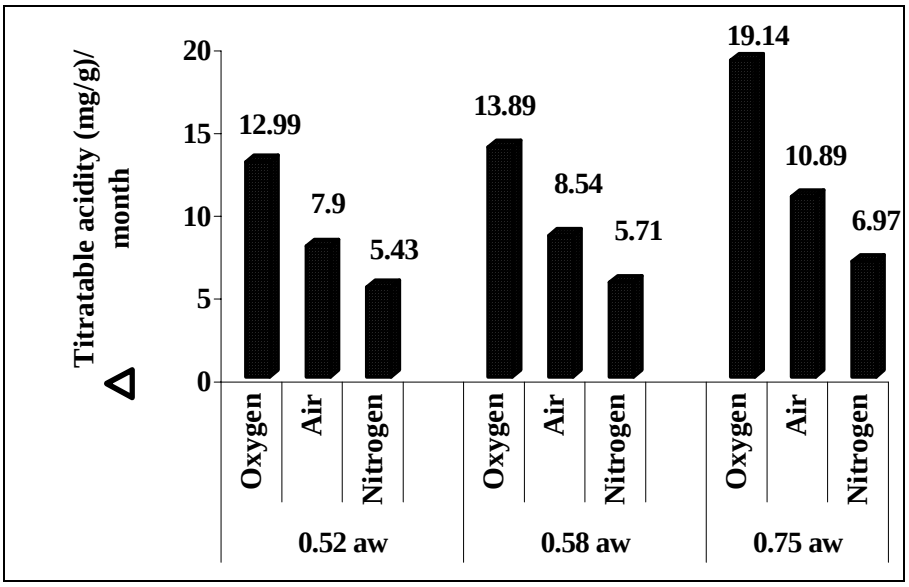

Fig. 8. Influence of water activity and headspace atmosphere on rate of change in titratable acidity of Dhakki dates during storage at 40°C.