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METAXENIA : INFLUENCE OF POLLEN ON THE MATERNAL TISSUE OF FRUITS OF TWO CULTIVARS OF DATE PALM (*PHOENIX DACTYLIFERA* L.)

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Abstract

The effect of five pollen samples of date palm (*Phoenix dactylifera* L.) on the maternal tissues of fruits in two cultivars, namely 'Barhy' and 'Nabtet-Saif' were evaluated. Significant variations in percentage of abnormal fruit setting, fruit size, shape, fruit-weight, seed-weight, percentage of pericarp and fruit ripening time were observed. In the female cultivar 'Barhy' the 'Kacst' pollen reduced the formation of abnormal fruits to a significantly lower level while in 'Nabtet-Saif' the 'Fouzan' pollen controlled the abnormal fruit setting to a certain level. With 'Heet' pollen both 'Barhy' and 'Nabtet-Saif produced smaller fruits while 'Fouzan' and 'Muzahmiya' pollen grains resulted in the production of bigger fruits in 'Barhy'. In 'Nabtet-Saif' better results were obtained with 'Dilim' pollen. In 'Barhy' lower seed-weight was produced by 'Dilim' pollen and in 'Nabtet-Saif' by 'Heet' pollen. But in both the cultivars higher seed-weight was produced by 'Fouzan' pollen. All strains of pollens used in this study had greatly influenced the stages of maturity. The 'Heet' pollen promoted early maturity of fruits in both cultivars, but 'Dilim' pollen delayed maturity in 'Barhy' female and 'Muzahmiya' pollen delayed ripening in 'Nabtet-Saif'. The genetic variations in the pollen grains can serve as the basis for selection of males to improve yields, fruit quality and alter fruit ripening time depending on the market demand.

Introduction

Date palm (*Phoenix dactylifera* L.) cultivation is the main source of agriculture economy in many countries of arid regions of West Asia and North Africa. Large distribution of this highly out-breeding, dioecious species brought about nearly 3000 cultivars popular to the date palm growers of the different regions of the world (Zaid and Wet 1999a). Most of these cultivars are known only to some specific localities and their distribution is confined to these areas due to many reasons. Saudi Arabia is one of the leading date producing countries that harbours about 450 cultivars (Bashah 1996). Genetic purity of these cultivars is maintained through off-shoot multiplication. With the advent of tissue culture techniques, massive expansion of elite cultivars of date palms has been made possible; and transportation of the planting materials of these cultivars to distant places became easier. In the last decade, several small and large scale growers have adopted this innovative technique of propagation to expand their farming.

In vitro propagation through tissue culture ensures that all progeny plants remain phenotypically and genotypically similar to their progenitors. However, among TC-derived date palms, several off-types are detected that include variegation, variation in leaf structure and overall plant growth, non flowering, formation of abnormal flowers (Cohen *et al*. 2004), supernumerary carpels (Al-Wasel 2000, Djerbi 2000) and seedless parthenocarpic fruits (McCubbin *et al*. 2000). These somaclonal variations are caused by genetic and or epigenetic alterations generated during the *in vitro* process (Kaeppler *et al*. 2000).

A normal female date flower has three carpels and after pollination only one develops into normal fruit and others degenerate. Failure of effective pollination leads to the formation of triple

parthenocarpic fruits of no economic value (Reuveni 1986, Zaid and Wet 1999b). High degree of occurrence of these multicarpel fruits in TC-derived date palms that can be detected only during fruiting stage *i.e*. after 5-7 years of planting cause severe economic loss to farmers. One of the reasons of this fruiting abnormality may be pollination failure due to interclonal pollen incompatibility. Djerbi (1995) have observed that some date cultivars had better yield when pollinated with selected males rather than with others.

Although fertilization and fruit set are the two major results of pollination, there is still another interesting but uncommon effect called metaxenia: the direct influence of pollen on the maternal tissues of the fruit (Janick 1979). In date palms in addition to causing pollination and fruit set, the pollen is not only affecting the size of fruits and seeds but also controlling the time of ripening (Swingle 1928). The direct influence of pollen on the maternal tissues of the fruits were recorded in many cultivars of date palms (Nixon 1927, 1928, Swingle 1928, Whittlesey 1933, Schroeder and Nixon 1958, Pereau-leRoy 1958, Comelly 1960, Ahmad and Ali 1960, Al-Delaimy and Ali 1969, Nixon and Carpenter1978, El-Hammadi *et al.* 1977, El-Sabrout 1979, Higazy *et al*. 1983, El-Ghayati 1983). Most of the male palms available are of seedling origin with great variations in their pollen quality. These pollen grains can bring about a lot of variations in the productivity, size, quality and maturity of date fruits in combination with different female cultivars.

In an attempt to control the high degree of formation of abnormal fruits in tissue culture derived date palms, five different types of pollen grains were tested for pollination in two well-known and economically viable date palm cultivars of Saudi Arabia, namely 'Barhy' and 'Nabtet-Saif'. The objectives were to study the differential behaviour of date fruits in response to five pollen samples, such as, reduction in the percentage of abnormal fruit settings, variations in size, fresh weight, pericarp formation and on the maturity and ripening of fruits.

Materials and Methods

Five elite male date palm trees were selected for pollen collection, from five different farms which are spatially separated by approximately 20 km. These pollen samples are named 'Heet', 'Dilim', 'Fouzan', 'Kacst' and 'Muzahmiya', indicating their place of collection. Mature male inflorescences were cut immediately after the breaking of spathe and kept in paper bags and later transferred to shade and moisture free area for drying. Spathe was removed carefully and bunches were spread over newspaper. Bunches were frequently changed from paper to paper to avoid moisture logging. After one day of drying the strands were separated out from the rachis and again spread over newspaper for further drying. To avoid any possible mixing of pollen each sample was dried separately.

Pollen samples taken from each lot were subjected to morphological studies to find out any possible variations. Pollen grains were stained with 1% safranin mixed with glycerine jelly and studied under microscope. Length and width of pollens were measured using ocular micrometer scale under magnification of 100 ×. Replicates were taken from four fields of each slide. Pollen viability was also examined following the protocol of Moreira and Gurgel (1941). A small amount of pollen was placed on a slide with one - two drops of 1% acetocarmine solution and gently heated for a few seconds. The slides were examined under microscope with 100 × magnification. Pollen stained dark red were considered viable, while the colour-less or light stained ones were regarded as non-viable.

Female trees were selected from two farms near Riyadh, Saudi Arabia. In Farm-A tissue culture derived 'Barhy' and in Farm-B, TC-derived 'Nabtet-Saif' cultivars were grown in large numbers. A survey conducted during the last season indicated that almost all trees of these farms are producing more than 60% of multicarpel fruits (visual analysis; data not included). During March, 2005 when the first flush of female inflorescence came out, five bunches were selected from each tree for the pollination experiment. One bunch on each palm was pollinated by one of the five pollen samples and all the five pollens were used in the same tree. The experiment was replicated in five trees. All selected bunches were of same stage of maturity i.e. tip of the spathe cracked and allowed a few flowers to expose. Each bunch was opened manually and bagged before pollination to avoid contamination. Pollination was done one by one, while keeping the other inflorescences bagged. Pollination was done by inserting 4-6 strands of male spikes in an inverted position inside the female inflorescence and tying the whole bunch using a plastic string. Some pollen grains were also dusted over the bunch and then covered with thin brown paper bag. After pollination the pollinator washed his hand thoroughly to avoid any possible mixing of pollen grains. After three weeks of pollination, bags were removed and bunches were released to allow free growth.

The first data on multicarpel fruit formation was taken eight weeks after pollination. Ten strands were randomly selected from each bunch and the number of normal and abnormal fruit-lets was counted. Percentages of abnormal fruits were calculated out of the total number of fruits produced. Then all the abnormal fruits were removed, allowing more room for the normal fruits to grow.

Sixteen weeks after pollination, 10 strands of fruits were randomly collected from each bunch representing all replicate trees. Fruits detached from the strands of each bunch were sorted-out according to their stages of maturity and their numbers were recorded. From each bunch 30 fruits at 'beser' (greenish-yellow colour of the fruit) stage were selected and their length and diameter were measured using a Vernier Caliper. Fresh-weights of fruits were also recorded and then seeds were removed from each fruit and weighed separately. Percentage of flesh produced per fruit was calculated upon the fresh-weight of fruits. Data were analyzed statistically by analysis of variance and least significant differences (LSD) according to Snedecor and Cochran (1973). Samples collected from each tree for each pollen sample were treated as replicates.

Results and Discussion

Morphological analysis of five pollen samples showed that size-wise they are of three groups (Table 1). 'Fouzan' had significantly larger size followed by 'Dilim' and 'Heet' while 'Kacst' and 'Muzahmiya' showed significantly smaller size. Length-width ratio indicates that 'Kacst' and 'Muzahmiya' are spherical (Fig. 1a) and 'Heet', 'Dilim', 'Fouzan' are elliptical in shape (Fig.1b). Pollen viability test showed that 'Heet' and 'Dilim' have significantly low viability while others have more than 94% viability.

Table 1. Size and viability of the pollen grains.

Parameters	Heet	Dilim	Fouzan	Kacst	Muzahmiya	LSD (p=0.05)
Length (µm)	20.73 b	20.68 b	21.94 c	18.56 a	18.64 a	0.55
Width (µm)	16.32 a	16.43 a	16.96 b	18.51 c	18.55 c	0.42
Length: width	1.27 b	1.25 b	1.29 b	1.00 a	1.01 a	0.04

Viability (%)	83.73 a	84.62 a	94.82 b	95.42 b	96.62 b	1.84

Values followed by the same letter in a horaizontal row are not significant at 5% level.

Interaction between different pollen and 'Barhy' female cultivar had brought about lots of variation in the fruit characteristics (Table 2). Occurrence of abnormal multicarpel fruits was very high in the tissue culture derived palms (Fig. 2a) as in the cases of previous years, when compared to the normal fruits in off-shoot derived plants (Fig. 2b). In the cultivar 'Barhy', 'Kacst' pollen reduced the formation of multicarpel fruits to a significantly lower level (Fig. 2c) while other pollens showed a little variation, which were not significant.

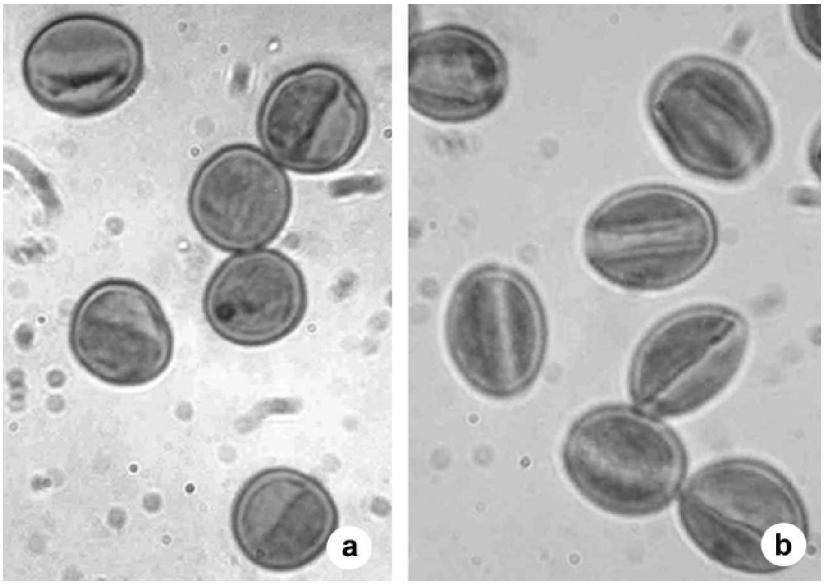


Fig. 1. Two types of pollen grains used for pollination. (a) Spherical as in Kacst and Muzahmiya. (b) Elliptical as in Heet, Dilim and Fouzan.

Table 2. Fruit characteristics of 'Barhy' in response to five different sources of pollens.

Parameters	Pollen samples					LSD (P = 0.05)
	Heet	Dilim	Fouzan	Kacst	Muzahmiya	
Occurrence of abnormal fruits (%)	51.32 b	45.31b	38.91b	12.39a	36.71 b	17.41
Fruit length (cm)	3.42 a	3.51 ab	3.61 b	3.51 ab	3.61 b	0.14
Fruit diam. (cm)	2.65 a	2.76 a	2.83 b	2.70 a	2.77 b	0.12
Length width of fruits	1.29 b	1.27 a	1.27 a	1.32 c	1.30 b	0.02
Fresh weight(g) of fruits	13.5 a	14.04 ab	14.98 b	14.41ab	14.21 ab	1.11
Seed weight (g)	1.18 b	1.11 a	1.25 c	1.20 bc	1.18 b	0.07
Percentage of pericarp	91.19 a	92.07 b	91.53 ab	91.64 ab	91.62 ab	0.57

Values followed by the same letter in a horaizontal row are not significant at 5% level.

'Heet' produced comparatively smaller size of fruits, while 'Fouzan' and 'Muzahmiya' produced bigger and 'Dilim', 'Kacst' resulted in intermediate forms. In length-width ratio they are of three significant groups; 'Heet' and 'Muzahmiya' shared one group, 'Dilim', 'Fouzan' shared another and 'Kacst' stood apart. In 'beser' stage 'Fouzan' resulted significantly higher fresh-weight and 'Heet' produced fruits with significantly lower fresh weight. Different sources of pollen also resulted significant variations in the mean seed weight. Weight of the seed, produced as a result of combination between 'Barhy' female and 'Dilim' pollen, was significantly low whereas the pollen 'Fouzan' produced higher seed-weight. Mean seed weight produced by the pollen samples 'Heet', 'Kacst' and 'Muzahmiya' were not significant among them. Types of different pollen had also a clear effect on pericarp formation. 'Dilim' pollen produced a significantly higher percentage of pericarp, the edible portion, per fruit while the 'Heet' pollen produced lower percentage of pericarp. Other pollen samples also produced variations but the results were not significant.

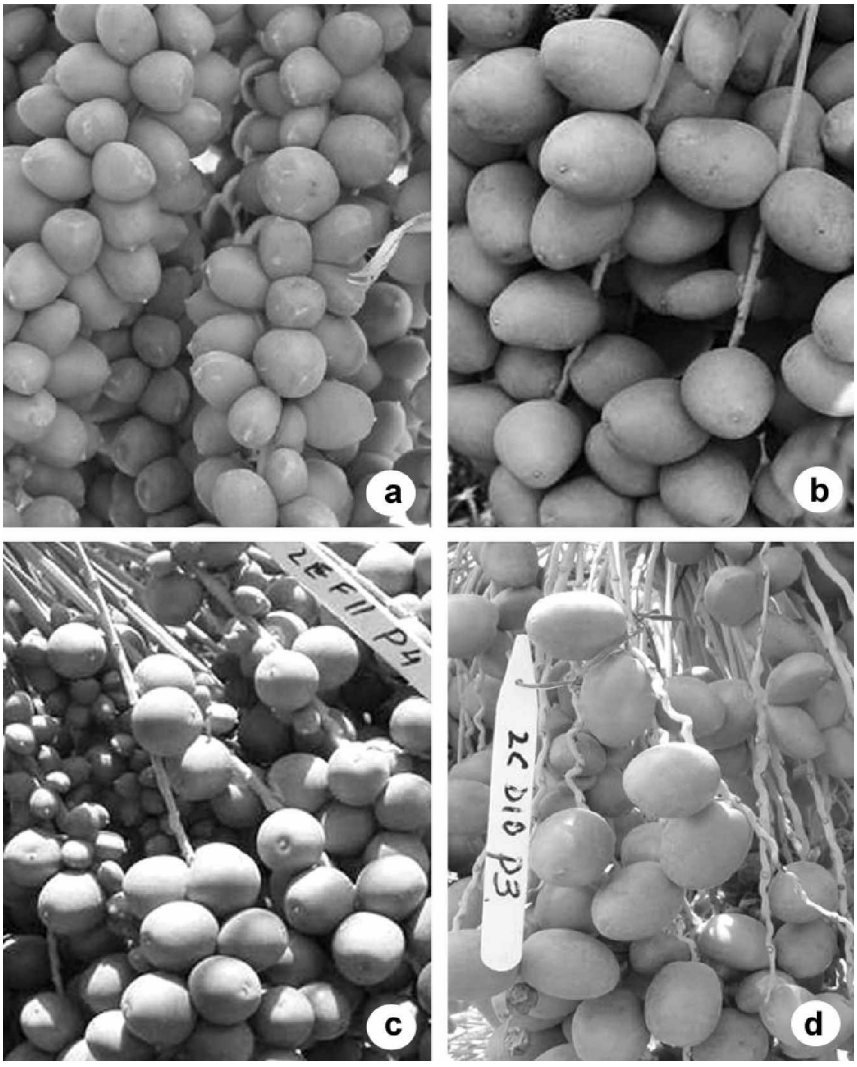


Fig. 2. Abnormal fruit formation and their control by two types of pollen grains. (a) High degree of abnormal fruit formation in TC derived plants. (b) Normal fruits in off-shoot derived plants. (c) Control of abnormal fruit setting in 'Barhy' by 'Kacst' (P4) pollen. (d) Control of abnormal fruit setting in 'Nabtet- Saif' by 'Fouzan' (P3) pollen.

Table 3 indicates that different pollen samples can bring about variations in the maturity period of fruits. Sixteen weeks after pollination the 'Heet' and 'Muzahmiya' pollen samples produced significantly higher percentage of greenish-yellow fruits ('Beser') while 'Dilim' produced higher percentage of green ('Kemri') fruits. 'Fouzan' and 'Kacst' pollen samples also produced higher percentage of 'Beser' fruits than other two stages of fruit maturity, but their differences were not significant. These results showed that the pollen 'Heet' and 'Muzahmiya' caused early maturity and 'Dilim' delayed fruit ripening in 'Barhy' cultivar.

Table 3. Effect of five different sources of pollens on fruit ripening of 'Barhy' date cultivar.

Colour of fruit	Percentage of fruits at different stages of maturity					LSD (P = 0.05)
	Heet	Dilim	Fouzan	Kacst	Muzahmiya	
Green	28.79 aA	51.70 bB	32.97 aA	32.75 aA	26.71 aA	17.12
Greenish- yellow	51.90 abB	28.57 aA	42.33 abA	38.37 abA	56.27 bB	14.39
Yellow	19.28 aA	19.00 aA	24.67 aA	28.82 aA	18.90 aA	14.16
LSD (P = 0.05)	10.38	17.98	19.27	18.29	17.76	

Values followed by the same letter in a horaizontal row are not significant at 5% level.
Values followed by the same capital letter in the same column are not significant at 5% level.

Different strains of pollen also caused several variations in the fruit characteristics of 'Nabtet-Saif' cultivar (Table 4). Among the pollen, only 'Fouzan' showed comparatively lower percentage of abnormal fruits (Fig. 2d), while others showed little variations in the percentage of multicarpel fruits, which were not significant. The fruit-size was significantly reduced when pollinated with 'Heet' pollen and showed better results with 'Dilim' pollen. The size of the fruits produced as a result of pollination with 'Fouzan', 'Kacst' and 'Muzahmiya' pollen was not significantly different. Length-width ratio also showed the same trend of variations as in the case of fruit-size. Regarding the fresh-weight of fruits only the 'Muzahmiya' pollen produced higher values while the other pollen samples produced fruits with no significant differences in their fresh-weight. Lowest mean seed-weight was produced with 'Heet' pollen and highest value was resulted with 'Fouzan' pollen. Other pollen samples did not produce significant differences in the mean seed-weight. Percentage of pericarp production was lowered significantly with 'Fouzan' pollen followed by 'Dilim' pollen, while the other three produced higher percentage of pericarp, but the differences were not significant.

Table 4. Fruit characteristics of 'Nabtet-Saif' cultivar in response to five different sources of pollens.

Parameters	Pollen samples					LSD
	Heet	Dilim	Fouzan	Kacst	Muzahmiya	(P=0.05)
Occurrence of abnormal fruits (%)	79.73 b	78.89 b	66.92 a	81.27 b	80.93 b	11.92
Fruit length (cm)	3.47 a	3.86 c	3.63 b	3.69 b	3.65 b	0.14
Fruit diam. (cm)	2.81 a	2.91 bc	2.85 ab	2.95 c	2.92 c	0.07
Length width of fruits	1.23 a	1.32 c	1.27 b	1.25 ab	1.25 ab	0.03

Fresh weight of fruits (g)	15.86 a	16.67 a	15.84 a	16.83 ab	17.92 b	1.15
Seed weight(g)	0.80 a	0.96b c	0.99 c	0.89 b	0.91 b	0.06
Percentage of pericarp	94.94 c	94.26 b	93.64 a	94.65 bc	94.88 c	0.43

Values followed by the same letter in a horaizontal row are not significant at 5% level.

Selection of different strains of pollen grains used in this study was based on farmers' practical experience. Morphologic studies of pollen grains conducted also supported this selection process by providing variations in the length, width, length-width ratio and percentage of viability. These results were in concurrence with the findings of Al-Tahir and Asif (1983), Shaheen *et al*. (1986) and Tisserat and De Mason (1982).

The effect of pollen in bringing down the percentage of abnormal fruit formation was evident in both the cultivars. In the female cultivar 'Barhy' the 'Kacst' pollen reduced the formation of abnormal fruits to a significantly lower level, while in 'Nabtet-Saif' the 'Fouzan' pollen controlled the abnormal fruit setting to a certain level. The variability in the percentage of abnormal fruit setting in both cultivars with different sources of pollen shows the variation in the degree of inter-clonal incompatibility between the strain of pollen grain and female cultivar. Failure of pollination or fertilization leads to the formation of multi carpel fruits, especially in tissue cultured date palms. Variations in fruit settings, with different pollen sources, reported by earlier workers (Al-Delaimy and Ali 1969, El-Hammadi *et al*. 1977, Nixon and Carpenter 1978) also support these findings. It is known that the incompatibility depend upon a recognition reaction between specific incompatibility proteins carried by the pollen grains and the matching proteins produced in the stigmas or styles (Raven *et al*. 1981). This recognition reaction between the pollen and stigmas resulted in variation of degree of acceptance, which ultimately resulted in the production of varying percentage of abnormal fruits.

Table 5. Effect of five pollens on fruit ripening of 'Nabtet-Saif' date cultivar.

Colour of fruit	Percentage of fruits at different stages of maturity					LSD (P = 0.05)
	Heet	Dilim	Fouzan	Kacst	Muzahmiya	
Green	13.37 aA	33.76 aA	29.01 aA	18.19 aA	64.02 bB	24.71
Greenish-yellow	26.02 abB	30.78 abA	33.41 bA	26.21 bA	14.49 aA	17.89
Yellow	59.73 bC	33.11 aA	37.55 abA	55.70 abB	23.95 aA	22.86
LSD (P = 0.05)	11.10	20.68	18.28	9.63	12.17	

Values followed by small letter in a horaizontal row are not significant at 5% level.
Values followed by the capital letter in a column are not significant.

With 'Heet' pollen both 'Barhy' and 'Nabtet-Saif produced smaller fruits while 'Fouzan' and 'Muzahmiya' pollen grains resulted in the production of bigger fruits in 'Barhy'. In 'Nabtet-Saif' better results were obtained with 'Dilim' pollen. Similar type of variations in the fruit-sizes were recorded by Swingle (1928), Osman *et al*. (1974), El-Sabrout (1979) and El-Ghayati (1983) in date palm cultivars.

Fruit-shape of cultivar is a genetically controlled character and normally it does not vary much within the cultivar. Here, in both the cultivars length-diameter ratio showed significant variations as an effect of pollination with different pollen grains. In 'Barhy' female, 'Dilim' and 'Fouzan' pollen produced similar ratio while 'Heet' and 'Muzahmiya' pollen resulted fruits with non significant values of length-diameter ratio. The 'Kacst' pollen with 'Barhy' female produced

significantly higher ratio while in 'Nabtet-Saif' female 'Dilim' showed higher value. El-Wakeel and Ibrahim (1969) and Higazy *et al*. (1983) also noted this kind of variations on the fruit-shape as evidenced by the variations in the length-diameter ratio in their study materials.

Fresh-weight of the fruit also showed considerable variations in both the cultivars as an effect of pollination with different pollen grains. In the cultivar 'Barhy', 'Fouzan' pollen and in 'Nabtet-Saif', 'Muzahmiya' pollen produced fruits with higher fresh-weight. Similar kind of observations were recorded by earlier workers on many date palm cultivars in their respective areas (Nixon 1951, Ahmed *et al*. 1962, Hussein 1970, El-Hamadi *et al*. 1977, Khalifa *et al*. 1979, El-Ghayati 1983 and Higazy *et al*. 1983).

Analysis of the data on fresh-weight, seed-weight and percentage of pericarp of both cultivars showed an interesting feature. Even though the fruits of 'Nabtet-Saif' were larger in size and heavier in fresh-weight, their seed weight was lighter than 'Barhy' and percentage of fleshy pericarp was higher. These parameters were also showed differential responses as the influence of different pollen grains. In 'Barhy' lower seed-weight was produced by 'Dilim' pollen and in 'Nabtet-Saif' by 'Heet' pollen. But in both the cultivars higher seed-weight was produced by 'Fouzan' pollen.

The effect of pollen in controlling the ripening time of date fruits were also clear in both the cultivars. All five types of pollens used in this study greatly influenced the stages of maturity. The four stages of maturity, as reported by Al-Ghamdi (1993) were clear in the fruits of both cultivars (Fig. 3a-h). The 'Heet' pollen promoted early maturity of fruits in both cultivars, but 'Dilim' pollen delayed maturity in 'Barhy' female, and 'Muzahmiya' pollen promoted late maturity in 'Nabtet-Saif' female. In the cultivar 'Barhy' early ripening was induced by 'Kacst' pollen, while in 'Nabtet-Saif' its effect was not significant as in the case of 'Fouzan'-'Barhy' and 'Fouzan'-'Nabtet-Saif' combinations. Al-Delaimy and Ali (1969) found variations in the ripening time of 'Zehdi' date fruit, when 4 different types of pollen grains were used. This study also proved that use of different sources of pollen grains can bring out variations in the ripening time of date fruits.

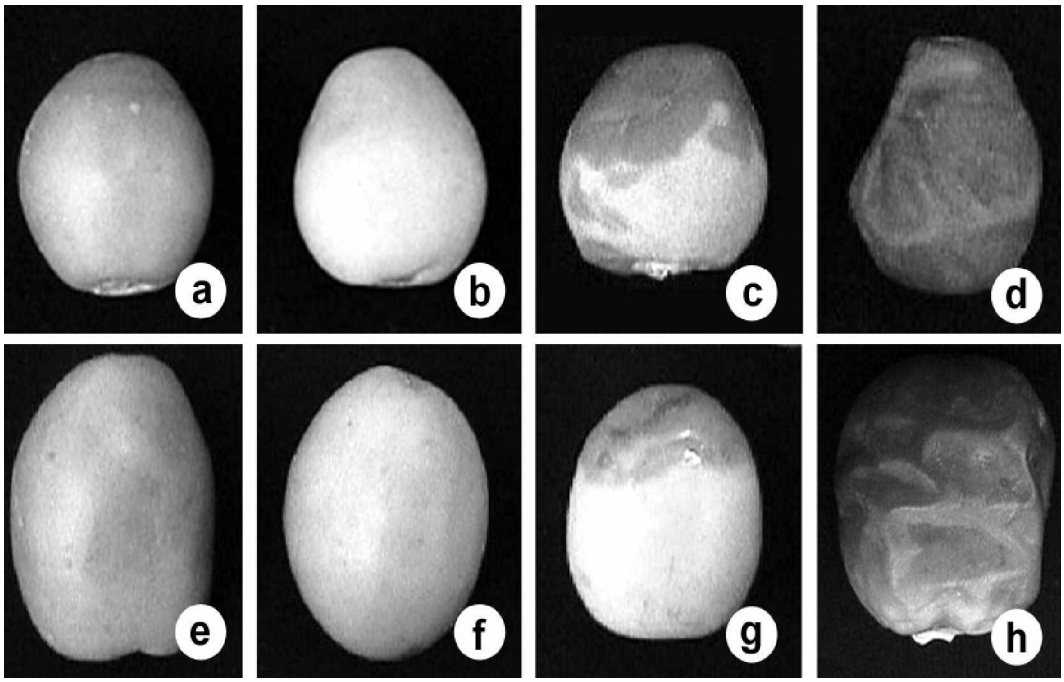
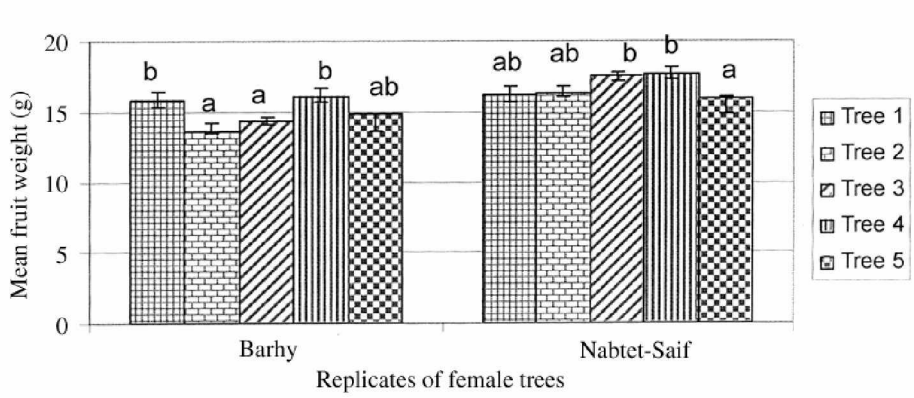


Fig. 3. Four stages of fruit maturity of date fruits during the ripening process. (a - d) Barhy. (e - h) Nabtet-Saif. (a & e) kemri-stage. (b & f) beser-stage. (c & g) rutab-stage and (d & h) tamar-stage.

In angiosperm, concomitantly, with the development of ovule into seed, the ovary or sometimes other parts of flower or inflorescence develop into a fruit. As the ovary develops, its wall, the pericarp, thickens and develops into a fleshy fruit in date palms. The fleshy pericarp of the date fruit being composed of maternal tissues, its characteristic variations in response to different pollen types are of great interest. In almost all parameters studied, along with the influence of pollen grains, the influences of maternal tissues were also significant. This is exemplified in Fig. 4, where the effect of 'Fouzan' pollen on the fresh-weight of fruits of two female cultivars 'Barhy' and 'Nabtet-Saif' is shown as bar diagram. The variations shown by the replicate trees, within the cultivars are of maternal influence or the combined effect of maternal and paternal influences.



Bars having same letter superscript are not significant between the replicate trees.
Mean separation is based on LSD at P = 0.05.

Fig. 4. Efect of 'Fouzan' pollen on the fruit weight of five replicate trees of 'Barhy' and 'Nabtet-Saif'.

Most of the male trees originated by sexual reproduction from seeds harbour high level of genetic variation within populations (Gurevich *et al*. 2005). The large genetic variation represents the expected heterogeneity of male date palms. These variations can serve as the basis for selection of males on the basis of their phenotypic characteristics regarding the potential to improve yields, fruit quality and alter fruit ripening time depending on the market demand.

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