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METAXENIAL EFFECTS ON MORPHOLOGICAL ATTRIBUTES IN DATE PALM CVS. HILLAWI AND KHADRAWY

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Pollen grains cause the great diversity and variations in size, quality and ripening time in date palm. In this regard nine pollen sources were selected to study the differential behaviour of pollen parents on fruit characteristics of two female cultivars i.e. Hillawi and Khadrawy. Selected male spathes were excised and stored for anther dehiscence, and made controlled pollinations. Physical characters including ripening time, bunch weight, fruit length, fruit width, fruit area, pulp weight, seed weight were highly variables. Among nine pollen sources M8 was superior because of positive association with most of the variables. Fruits developed from M8 pollinations were prominent with high bunch weight, more sweet in taste (high total sugars), good fruit size, and reduced seed weight and ripening time. M7 male retained good fruit length and width, and reduced ripening time and seed weight. Strong positive correlation was found among most of the traits. All the new combinations developed from pollinating the two date palm cultivars with nine pollen sources were categorized by principle component analysis. Our results showed that physical traits were very appropriate for studying the metaxenial effect that can be used for further breeding targets like yield and quality traits.
Keywords: Metaxenia, cultivars, fruit traits, ripening, breeding, PCA, morphology, pollen

INTRODUCTION

Date palm is among the oldest cultivated plants in history of mankind, being used as food for 6000 years (Amer, 1994). Dates due to its significant nutrition, environmental and aesthetic benefits, in addition to health and economic values are being used since generations. Dates are rich source of protein as compared to other fruits such as apples, oranges, bananas and grapes (0.3%, 0.7%, 1.00% and 1.00% proteins, respectively) (Al- Showiman, 1998). Currently, it is cultivated in the Middle East, North Africa, parts of Central and South America, Southern Europe, India and Pakistan (Al-Shahib and Marshall, 2003). Pakistan is the largest producer of dates after Egypt, Saudi Arabia, Iran, Algeria and Iraq, with a production of 600 thousand metric tons (FAO, 2012). Different factors affect the date palm growth (Ata *et al*., 2012). In Pakistan important dates producing provinces are Sindh (Khairpur, Sukkur), Balochistan (Makran, Panjgoor), Khyber Pakhtunkhwa (D.I. Khan) and Punjab (Jhang, Muzafargarh, Bahawalpur, D.G. Khan) (Markhand *et al*., 2010).

Date fruit is a single seeded berry with fleshy mesocarp and fibrous endocarp (Mansour, 2005) and constitute about 85% to 90% of total date fruit weight (Hussein *et al*., 1998). Naturally date palm is wind pollinated but artificial pollination is very critical agricultural practice to ensure the good fruit production and quality (Asif *et al*., 1986). One male tree can pollinate fifty female trees and fruits are produced only by female trees. Usually female date flower contains three carpels and after effective pollination only one develops ordinarily into fruit while other ablates. Triple parthenocarpic fruits of economic values develop due to ineffective pollination (Reuveni, 1986). Palms that have different origin show great variations in pollen quality. Some date palm cultivars had rigorous yield when selected males were used for pollination rather than random (Djerbi, 1995). Fertilization and fruit set are consequences of pollination as there is an interesting but uncommon effect known as metaxenial, the direct influence of pollen on maternal tissues of date palm (Janick, 1979). Pollen not only control the fruit set, size of fruits and seeds but also effect the time of ripening (Swingle, 1928). Pollen grains can cause a great diversity and variation in size, quality, yield and maturity of date fruits. Date palm growers use readily available pollen due to which fruit size, productivity and quality of palms varies from year to year (Osman *et al*., 1974). As the pollen has more precised and definite effect on the parts of seed and fruit lying outside the embryo so it is requisite to select and identify the superior male plants. Metaxenic effect to accelerate the fruit maturity at significant level is established along with high temperature as dominant factor (Farag, 2005). Time of ripening is an important consideration especially in those areas having very short period of warm temperature during date palm growth and development and threatened by rain or frost.

There are great variations within date palm (*Phoenix dactylifera* L.) males in Pakistan but unfortunately it is unexplored due to lack of breeding programs. There is a

need to explore these variations to establish the best pollen parents on the basis of production and nutritional value. Therefore, nine date palm pollen parents were selected to investigate the morphological attributes in two commercial female cultivars with an objective to characterize the superior pollen parent.

MATERIALS AND METHODS

***Pollen collection*:** Nine male date palm trees as pollen parents were selected from three different locations i.e., Horticultural Fruit Garden Square No. 9, Square No 32 and Agriculture Research Institute D.I. Khan (Table 1). Inflorescence from male trees was collected immediately after spathe cracking and placed on paper under shade and moisture free conditions for drying. The papers underneath inflorescence were frequently changed to avoid the moisture. Each sample was handled separately to avoid any possible mixing. After one day flower strands were separated from rachis and spread over paper for further anther dehiscence and finally the pollen powder was stored in paper bags.

***Pollination*:** Three trees of uniform vigor and age of two cultivars, Khadrawy and Hillawi, were treated as female parents at Horticultural Fruit Garden Sq. No. 32. Although these cultivars are genetically different and their fruit is being eaten at different stages but we studied both cultivars simultaneously to compare the effectiveness of pollen parents, as both are major commercial cultivars of Punjab (Pakistan). All the trees were subjected to similar cultural practices in the experimental research area. On each female tree nine spathes were selected for pollination in the month of March. To protect the inflorescence from stray pollen, female spathes were covered with paper bags before opening. In each cultivar, three trees were used as replicates. One spathe on each replicate was pollinated with one male parent. Pollination was performed one by one while keeping the other inflorescence bagged. Pollination was done by inserting 5-6 male strands in inverted position inside the female inflorescence. Some pollen grains were also dusted over the bunch and then covered with paper bag. Pollination was performed manually by climbing over the tree. After four weeks of pollination bags were removed to allow bunches to grow fruit further.

***Fruit analysis*:** Fruits were collected at khalal stage (full size, crunchy) after 4 months of pollination. Ripening time of fruits was examined by observing the total number of days required by the fruit from setting to khalal stage. Ten fruits were selected randomly from each treatment for all physical attributes, weighed on electric balance and mean weight was calculated in grams, and fruit length (mm) and width (mm) was measured with vernier caliper. After pitting, the weight of pulp (g) and seed (g) was recorded.

Data analysis: The experiment was laid out according to two factor factorial in randomized complete block design (RCBD). Multivariance analysis was performed to find principal component value and clustering the accessions on the basis of similarities of various physicochemical characters following XLSTAT program. One way analysis of variance (ANOVA) was also practiced for statistical analysis and significant variations between means were also recorded by Duncan's Multiple Range test at $P < 0.05\%$ on quantitative data.

RESULTS

Duncan's multiple range test was used to discrete the means at 5% confidence interval and their means are presented in Table 2. Different pollen parents caused several variations in fruit characteristics of cultivar Hillawi. Minimum number of days (94 days) was taken by Hillawi when it was pollinated with M8 and maximum (128 days) in open pollinated (M0). Among all the pollen parents M8 (18.83 kg) showed comparatively higher bunch weight followed by M4 (16.67 kg), while others showed less variation. Fruit weight was significantly low in open pollinated (5.38 g) and high with pollen parent M8 (8.43g). Regarding the fruit length, only M8 produced the higher value (38.2 mm) while other pollen parents produced no significant differences in their fruit length. Almost similar trend was found in fruit width and fruit area except open pollinated with low values. Maximum pulp weight was observed in cultivar Hillawi, when pollinated with pollen parent M8 (8.16 g) and minimum with openly pollinated M0 (4.29 g) but other pollen sources showed non-significant differences for pulp weight. The

Table 1. Description of male and female date palm accessions.

S.No.	Male	Collection site	Female	Collection site	Progeny (Hillawi)	Progeny (Khudrawy)
1	M0	Open pollination	H1	Sq.#32 UAF	HM0	KM0
2	M1	Sq.#9 UAF	H2	Sq.#32 UAF	HM1	KM1
3	M2	Sq.#9 UAF	H3	Sq.#32 UAF	HM2	KM2
4	M3	Sq.#9 UAF	K1	Sq.#32 UAF	HM3	KM3
5	M4	Sq.#9 UAF	K2	Sq.#32 UAF	HM4	KM4
6	M5	D.I. Khan	K3	Sq.#32 UAF	HM5	KM5
7	M6	Uni. campus			HM6	KM6
8	M7	Sq.#32 UAF			HM7	KM7
9	M8	Sq.#32 UAF			HM8	KM8

Table 2. Fruit characteristics of cultivar Hillawi in response to nine pollen parents

Parameters	Pollen parents								
	M0	M1	M2	M3	M4	M5	M6	M7	M8
Maturity time (days)	128.6 h	110.6 de	99.6 b	102.3 bc	99.3 b	116.6 f	111.3 de	101.3 b	94.0 a
Bunch weight (kg)	7.9 e	12 d	14.1 cd	14.3 cd	16.6 b	13.6 cd	13.5 c	13.2 cd	18.8 a
Fruit weight (g)	5.3 c	7.4 ab	7.2 b	7.8 ab	7.8 ab	7.1 ab	7.2 ab	7.6 ab	8.4 a
Fruit length (mm)	24.1 c	33.5 b	35.2 b	36.6 ab	36.0 b	33.1 b	34.5 b	37.0 ab	38.2 a
Fruit width (mm)	11.8 d	15.6 c	17.8 c	18.6 ab	17.4 bc	16.9 bc	16.2 cc	18.6 abc	19.3 a
Fruit area mm^2	285.5 d	527.7 c	628.7 c	685.7 ab	628.9 bc	560.5 bc	561.1 c	691.7 abc	737.7 a
Pulp weight (g)	4.2 c	6.2 b	6.1 b	6.6 ab	6.4 b	5.9 b	6.0 b	6.3 b	8.1 a
Seed weight (g)	1.0 b	1.2 a	1.1 a	1.2 a	1.3 a	1.2 a	1.1 a	1.3 a	1.3 a

Means sharing similar letters in the row are statistically non significant (P>0.05)

Table 3. Fruit characteristics of cultivar Khadrawy in response to nine pollen parents

Parameters	**Pollen parents**								
	M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**
Maturity time (days)	139.0 a	118.6 c	126.0 c	121.0 c	112.6 d	129.0 b	128.6 b	113.0 d	107.0 e
Bunch weight (kg)	7.5 e	12.5 d	12.7 cd	13.7 cd	14.9 b	13.1 cd	14.8 c	12.8 cd	16.0 a
Fruit weight (g)	6.2 c	9.5 ab	9.0 b	10.6 ab	9.3 ab	10.0 ab	10.3 ab	10.3 ab	11.2 a
Fruit length (mm)	21.3 d	35.1 ab	33.9 c	35.9 ab	33.8 c	35.2 ab	34.9 b	34.8 b	36.5 a
Fruit width (mm)	16.0 d	24.0ab	21.8 c	24.8 ab	23.1 bc	23.6 bc	23.0 c	23.8 b	25.1 a
Fruit area (mm^2)	340.9 d	850.1 c	744.3 c	892.6 ab	784.0 bc	834.5 bc	807.1 c	830.8 abc	916.9 a
Pulp weight (g)	5.6 d	8.4 bc	7.9 c	9.1 b	8.1 bc	9.0 b	9.1 b	7.8 c	10.0 a
Seed weight (g)	0.6 b	1.0 a	1.0 a	1.5 a	1.1 a	1.0 a	1.1 a	1.3 a	1.1 a

Means sharing similar letters in the row are statistically non significant (P>0.05)

Table 4. Descriptive analysis of physical variables of fruits of date palm cvs. Hillawi and Khadrawy as affected by nine pollen parents

Variable	Minimum	Maximum	Mean	SD	CV (%)
Maturity time (days)	94.0	139.0	116.5	31.8	27.3
Fruit length (mm)	29.2	37.0	33.1	5.5	16.8
Fruit width (mm)	11.8	25.1	18.5	9.4	50.8
Fruit area (mm2)	285.5	917.0	601.2	446.5	74.3
Pulp weight (g)	4.2	10.0	7.1	4.1	57.4
Seed weight (g)	0.6	1.6	1.1	0.7	62.1
Fruit weight (g)	5.4	11.2	8.4	1.7	19.6
Bunch weight (kg)	7.5	18.8	13.4	2.7	19.9

lowest seed weight was recorded in pollen parent M0 (1.08 g) but other pollen parents were at par.

Early fruit maturity was observed in female cultivar Khadrawy pollinated with pollen parent M8 (107 days) followed by M4 (112.67 days) but M0 (139 days) showed late maturity (Table 3). Bunch weight was most significant in M8 (16.07 kg) but least with M0 (7.5 kg). M0 produced smaller fruits while M8 and M4 produced larger fruits, and intermediate with M5, M and M7. Types of different pollen parents also had a clear effect on pulp weight. M8 (10.04 g) pollen parents produced significantly higher pulp value followed by M6 (9.14 g) but least in M0 (5.63 g). The lowest seed weight was recorded in pollen parent M0 (0.61 g) but other pollen parents did not differ significantly.

Descriptive statistics and correlation analysis for physical variables of fruits of date palm cvs. Hillawi and Khadrawy affected by different pollen parents: Fruit physical variables (maturity time, bunch weight, fruit weight, fruit length, fruit width, fruit area, pulp weight and seed weight) were analyzed to study the comparative effect of different pollen parents in two mother parents of date palm, i.e. Hillawi and Khadrawy. Descriptive statistics indicating the maxima, minima, means, standard deviation and coefficient of variation (CV) is shown in Table 4 which presents high range of physical variations. Some traits presented high CV value like fruit width (50.8%), seed weight (62.1%), fruit area (74.3%), pulp weight (57.4%), maturity time (27.3%) but it was less than 20% for fruit length, fruit weight and bunch weight. Maturity time varied from 94 to 139 days

Table 5. Correlation coefficients among physical variables of fruits of date palm cvs. Hillawi and Khadrawy as affected by nine pollen parents

Variables	MT	FL	FW	FA	PW	SW	FWT	BW
MT	1.000							
FL	0.413	1.000						
FW	-0.886	-0.686	1.000					
FA	0.392	0.696	-0.536	1.000				
PW	0.653	0.774	-0.671	0.692	1.000			
SW	-0.920	-0.621	0.988	-0.562	-0.670	1.000		
FWT	0.128	0.526	-0.093	0.473	0.796	-0.084	1.000	
BW	-0.709	0.179	0.523	0.117	-0.046	0.571	0.400	1.000

Abbreviations: Maturity time (MT), fruit length (FL), fruit width (FW), fruit area (FA), pulp weight (PW), seed weight (SW), fruit weight (FWT), bunch weight (BW)

with the mean value of 116.5 days. All the date palm combinations showed the fruit length variations from 29.3 to 37.0 mm. Fruit width ranged from 11.8 to 25.1 mm with the mean value 18.5 mm. Pulp weight of fruits ranged from 4.2 to 10 g while mean value was 7.1 g. Seed weight ranged 0.6-1.6g. Fruit weight varied from 5.4 to 11.2g. Bunch weight ranged from 7.5 to 18.8 kg with the mean value of 13.4 kg.

Strong positive correlation was resulted among different fruit physical traits (Table 5). The highest positive correlation was found between seed weight and fruit weight (0.988). High correlation was also observed between traits like fruit weight and pulp weight (0.796), pulp weight and fruit area (0.692), maturity time and pulp weight (0.653), fruit weight and fruit length (0.526), bunch weight and fruit width (0.523), fruit weight and fruit area (0.473), fruit length and maturity time (0.413) and bunch weight and fruit weight (0.40).

Contrary to positive correlation occasional negative correlation also existed within these physical traits. The highest negative correlation existed between fruit width and maturity time (-0.886), seed weight and pulp weight (-0.670), fruit width and fruit length (-0.686) and seed weight and fruit area (-0.562).

Principle component analysis for physical variables of fruits of date palm cvs. Hillawi and Khadrawy: Factor analysis was based on 8 physical traits between the parents and their progeny. Eigenvalue and cumulative variation are shown as scree plot (Fig. 1). Eigenvalue of first three components dropped sharply but it remained stagnant for other components. Similarly cumulative variations increased sharply for first four components and 98% variations were covered by four factors, so it was adequate to study just first 4 components for 8 physical traits. Principle component analysis showed that that all the traits were summarized I n first four components with 98.64% of total variations (Table 6). Variability of 58.68% was explained with first component which accounted for the fruit weight, seed weight and bunch weight. The second group was featured with 27.80% variability and maximum contribution was from bunch weight followed by fruit weight, fruit length fruit area, and pulp weight. The third component explained the 7.88% variations with heavy loadings of fruit weight, pulp weight, maturity time and fruit width. Fourth component retained 4.28% variability with major contribution from fruit area and fruit weight.

Table 6. Eigen values and cumulative variance of the first 4 components for physical variables of fruit of date palm cvs. Hillawi and Kudrawy as affected by nine pollen parents

Traits	F1	F2	F3	F4
MT	-0.395	-0.296	0.263	0.047
FL	-0.365	0.282	-0.368	-0.515
FW	0.427	0.203	0.200	0.238
FA	-0.335	0.264	-0.395	0.798
PW	-0.409	0.244	0.351	-0.027
SW	0.428	0.228	0.149	0.044
FWT	-0.200	0.513	0.599	0.017
BW	0.158	0.589	-0.308	-0.190
Variability (%)	58.683	27.807	7.876	4.279

Abbreviation: Maturity time (MT), fruit length (FL), fruit width (FW), fruit area (FA), pulp weight (PW), seed weight (SW), fruit weight (FWT), bunch weight (BW)

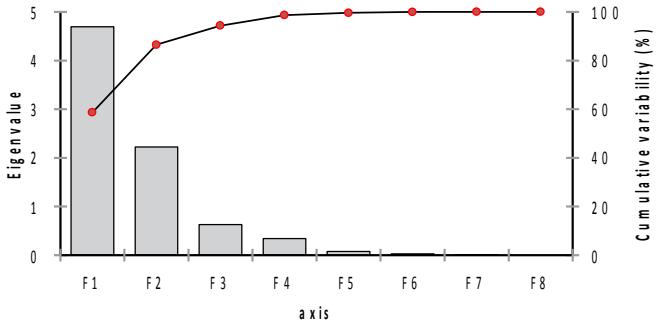
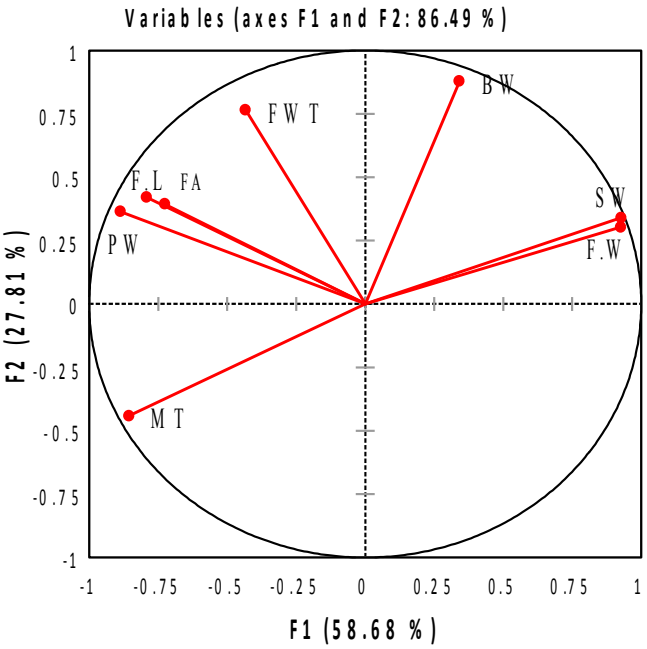


Figure 1. The scree plot for physical variables of fruit of date palm cvs. Hillawi and Kudrawy as affected by nine pollen parents

A linkage map was developed based on variables of first two factors which accounted 86.49% total variations (Fig. 2). This 2D plot explained that fruit physical traits such as fruit width and seed weight, pulp weight and maturity time correlated strongly as compared to other traits.



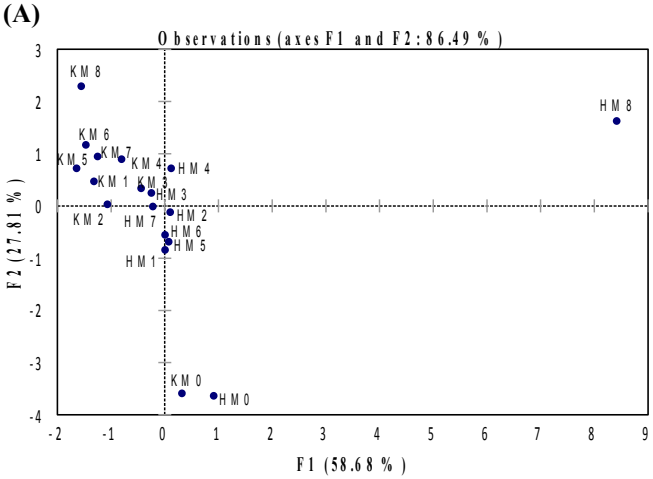
Abbreviations: Maturity time (MT), fruit length (FL), fruit width (FW), fruit area (FA), pulp weight (PW), seed weight (SW), fruit weight (FWT), bunch weight (BW)

Figure 2. PCA analysis based on first two components for physical variables of date palm cvs. Hillawi and Kudrawy as affected by nine pollen parents

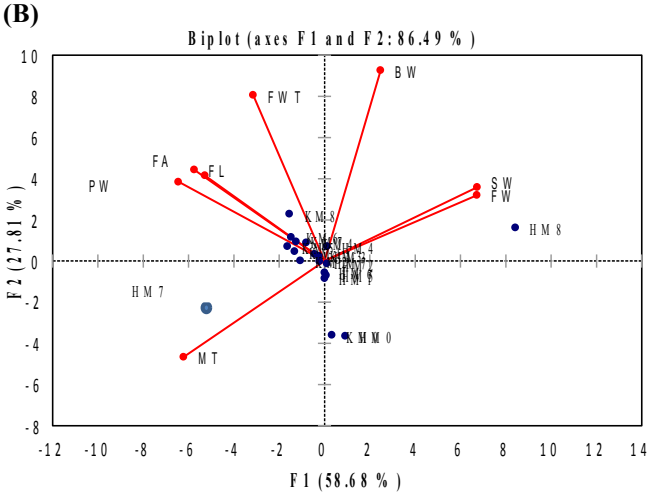
PCA plot (Fig. 3A) divided all the combinations into two groups based on morphological studies. One group included HM1, HM5 and HM6 but rest of the combinations were confined to a single group and showed close resemblance with each other; however, HM8 and KM8 was highly divergent. Similarly HM0 and KM0 were different from other combinations but both have close association for each other.

PCA biplot (Fig. 3B) for all the combinations developed from nine pollen parents and two mother parents showed that HM8 developed from cultivar Hillawi and pollen parent M8 had close association for bunch weight, fruit weight and seed weight. KM0 and HM0 were not positively correlated with any fruit physical trait. HM8 was associated with more fruit width and also some associations for seed weight but KM8 were positively correlated for fruit weight. HM7 related with early maturity but all other combinations piled near the centre of axis and were difficult to differentiate.

(A)



(B)



Abbreviations: Maturity time (MT), fruit length (FL), fruit width (FW), fruit area (FA), pulp weight (PW), seed weight (SW), fruit weight (FWT), bunch weight (BW)

Figure 3. A, B PCA plot (A) and biplot (B) based on first 2 components for physical variables of fruits of date palm cvs. Hillawi and Khadrawy as affected by nine pollen parents

DISCUSSION

Two genetically different female cultivars Hillawi and Khadrawy were selected as female breeding parent. Hillawi is used most preferably at khalal stage while Khadrawy at rutab or tamar stage because it had high phenolic and antioxidant contents at this stage but we studied both cultivars at the same time to compare the effectiveness of pollen parents as both are major commercial cultivars of Punjab (Pakistan).

Some physical and biochemical variables were selected to study the direct influence and efficacy of different pollen parents on two date palm cultivars, i.e. Hillawi and Khadrawy. These variables are important tools to

characterize the best genotypes as pollen parents (Roddriguez *et al.,* 2008). The well defined traits related to fruit quality and fruit yield are economically very important and targeted traits for plant breeders. The findings of this study revealed that morphological traits are reliable for discriminating and estimating the qualitative variables in breeding parents.

Among eight physical traits studied in this experiment, ripening time was one of the most important components. Earlier ripening of date palm is the required factor of Pakistani farmers in order to save the crop from monsoon rains which causes huge economic losses. Maturity time of Hillawi and Khadrawy was influenced significantly by nine pollen parents; however, M8 was most prominent regarding early maturity in both cultivars where as M2 reduced the maturity time in Hillawi and M7 in Khadrawy. Pereau-leRoy (1958) studied the effect of 24 different pollen parents and reported similar results. The results of this study are also in line with Iqbal *et al*. (2012) who declared the M5 pollen the best to bring down the early ripening in date palm cultivars. Similarly the results are favored by Al-Delaimy and Ali (1969) and Nasser and Al-Khalifah (2006) who studied the effect of date palm pollen on maternal tissues.

Bunch weight ranged from 7.90 to 18.83 kg in two varieties in response to different pollen sources. Similar range in bunch weight was determined in Zaghloul cultivar using different pollen sources (Soliman, 2006). Rehaman *et al*. (2005) recorded the maximum bunch weight when pollinated the Saki female parent with Serey pollen. El-Kosary (2009) pollinated the Barhee cultivar with pollen from three different areas and recorded the bunch weight that ranged from 9.7 kg to 11.02 kg. Iqbal *et al*. (2012) contradicted these findings because they found no significant effect of pollen sources on bunch weight of cultivar Dhakki. These findings are also favored by El-Kassas and Mohamed (1984), Hussain *et al.* (2001), Al-Wasfy (2005) and Abdel-Galil *et al*. (2007) who observed increase in bunch weight in various date palm cultivars by using different pollen sources.

Fruit length and fruit weight ranged from 21.3-37.0 mm, 5.4-11.2 mm, respectively, and depicted high variability in fruit area. Shafique *et al*. (2011) reported the variations in fruit length of date palm cultivar Dhakki under the influence of three different pollen parents. Similar positive correlation between pollen parents, fruit length and fruit width was reported by Moustafa (2001), Hani *et al*. (2006) and El-Kosary (2009). Similarly fruit, seed and pulp weight was affected by of different male parents and the physical parameters showed corresponding variations with their respective pollen source. Our data is in agreement with Swingle (1928), Al-Wasfy (2005), Al-Hamoudi *et al.* (2006), Abdel-Galil *et al*. (2007), and Al- Qureshi (2010) for fruit length and width.

Interesting relationships were explained through observed correlations. It was observed that fruit weight was directly proportional to seed and pulp weight, and early maturity. For instance, physical variables showed more negative correlations. On the basis of strong correlations between pulp and fruit weight (0.796), fruit area and pulp weight (0.692), pulp weight and maturity time (0.653), fruit length and fruit weight (0.526), fruit width and bunch weight (0.523) it could be concluded that these factors are equally important and could be used successfully for selection of superior male plants.

The results of PCA showed that most of the variables made their way in first two components including fruit weight, seed weight, bunch weight, fruit length, fruit width, fruit area and pulp weight. Among nine pollen sources M8 (Sq. No.32) was superior because it positively associated with most of the variables. Fruits developed from M8 pollination were prominent with high bunch weight, good fruit size, low seed weight, and reduced ripening time. M7 (Uni. campus) retained the highest fruit length and width, early ripening and low seed weight. This signifies the importance of studied traits not only for the assessment and characterization of superior male parents but are equally important for diversity estimation. Encouraging progress has been made for selection of best male parents regarding their metaxenic effects to boost up the commercial production of dates.

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