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Evaluation of Some Date Palm Male Types Using Morphological and Molecular Markers

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FIVE LOCATIONS of mature male palm trees belong to three governorates (Alexandria, Behira and Sohage) were subjected to evaluate some physical characteristics and certain chemical constituents to determine a superior type as promising ones for pollinating the female flowers to incorporate those types in the breeding programs.

It is obvious that locations of El-Behira governorate recorded almost the highest value for morphological characteristics. The same trend was observed for pollen viability and mineral content. The Tahta location which belongs to sohage governorate usually showed a high content of carbohydrates. On the other hand, males of Rasheed (El-Behira governorate) recorded the highest protein percentages. However, there were no significant differences between those males and the other males that grown in the rest of locations. Amino acids content varied from one location to another. Moreover, one primer of RAPD marker (OPC 13) has the potential to identify three genotypes and only four primers have the ability to characterize four genotypes.

Date palm *(Phoenix dactylifera* L.*)* is one of the most important and oldest fruit tree, it has been cultivated by man since antiquities. It is considered to be the oldest tree with a great genetic diversity (Popenoe, 1973, Zohary and Spiegel-Roy, 1975).

In Arab Republic of Egypt, such a crop always ranking superior over many other fruit crops due to the nutritional value of the fruits, which makes it available to the consumers at a relatively low cost. The total area devoted to date palm increased gradually until it reached about 56 thousand feddans occupied by about 6.6 million female trees produce about 14% of the overall fruit production (Ibrahim & Kholif, 2004 and Badawi *et al.*, 1994). The total number of female date palm trees reached about 12039424 trees were cultivated in 86787 feddans produce about 1313696 ton (Agricultural Statistics, 2008). As a matter of fact, dates are dioecious; *i.e.* male flowers are born on a given tree and female flowers born on a separate one, and pollination is usually accomplished by wind, however, under cultivation the female flowers are pollinated by artificial means (hand). However, artificial hand pollination represents a great task for the date growers, and such a practice should be done using the most viable pollens in order to get the highest fruit set and highest gross yield (El-Hammady *et al*., 1977, Khalifa *et al*., 1980, El-Makhtoun, 1981 and Hamdy, 1982).

In date palm breeding programs, cultivars identification still relies on morphological characteristics. However, many cultivars can not be rapidly distinguished by morphological indices, particularly if they are closely related. Furthermore, phenotype identification based on morphological traits is subject to environmental variation (Nielson, 1985). The field of molecular biology has provided tools suitable for rapid and detailed genetic analysis of higher organisms including agriculture species (Williams *et al*., 1990). The most fundamental of those tools are DNA markers. RAPD (Randomly Amplified Polymorphic DNA marker) method, based on amplification of multiple, random segments of the genome using arbitrary primers (Welsh & McClelland, 1990; and Williams *et al*., 1990). The above mentioned technique was utilized to detect phylogenetic relationships between some selected male types using morphological and molecular markers.

The aim of the present study is to evaluate the effect of zone of plantation on some physical characteristics and certain chemical constituents of the spathes, pollens and estimate the genetic relatedness of these genotypes using RAPD markers and subsequently, determine the superior types as promising ones for pollinating the female flowers, to incorporate those types in the breeding programs.

Materials and Methods

The selected types were grown at private orchards located at El-Nahda, Alexandria Governorate, Rasheed and Kafer El-Dawar, Behira Governorate, El-Maragha, Tema and Tahta, Sohage Governorate.

Morphological and chemical studies

Physical characteristics

At the flowering time (middle of March to the end of April), four mature spathes were randomly collected from each male tree type (thirty years old), to measure some morphological characteristics of spathes, inflorescences, strands and pollens in the laboratory of Pomology Department, Faculty of Agriculture, Alexandria University. The following characteristics were measured and recorded:

Spathe

1- The average weight of the spathe (gm) 2–The average width of the spathe (cm)
3–The average length of the spathe(cm) 4–The average weight of the spathe cover (gm)

Inflorescence

The average weight of the inflorescence for each male type (gm), also, the average width and length for the inflorescence of each selected type were measured (cm) and recorded.

Strand

Some strands from each inflorescence that represent each male were separated and subjected to determine

1– Average length (cm) 2– Average number of strands per inflorescence

3– Average number of flowers per strand

Pollen characteristics

1–Average pollen weight per spathe was recorded according to Nasr *et al.* (1986).

2 – Viability determination, two general methods assaying pollen viability were used A–Germination test and B– Microscopic examination following staining test. Both of these techniques were achieved according to Ibrahim (1989)

Chemical determination

Pollen grains samples were taken to study some biochemical properties in the Laboratory of Pomology Department and the Biotechnology Laboratory of the Horticulture Research Institute.

Mineral content: For determination nitrogen, phosphorus and potassium, 0.1 gm of dried pollen grains materials were digested by sulphuric acid and hydrogen peroxide according to Evenhuis and DeWaard (1980).

Carbohydrates: Total sugars were determined according to Malik and Singh (1980). Reducing sugars and starch were determined by Dubois *et al*., (1956). Non reducing sugars were calculated by the difference between the total sugars and the reducing sugars. Total carbohydrates were calculated as a summation of the total sugars and other carbohydrate constituents. The results were expressed as gm/100gm or percent on dry weight basis

Proteins: Crude protein (N × 6.25) was determined according to the A.O.A.C method (1981).

Amino acid content: Two steps were achieved to determine the amino acid content

a- Acid hydrolysis (sample preparation)

Seventy-five milligrams of each composite sample of pollen grains were treated with 10 ml of constant-boiling HCL containing mercopto-ethanol (5µl / 10ml acid) in hydrolysis tube which were kept in a vacuum oven at 110 °C for 22 hrs and were cooled to the room temperature. The amino acid content in protein hydrolyzate was detected using Amino Acid Analyzer according to Moore *et al*., (1958). The concentrations of amino acid were calculated as grams per 100 grams of protein or percent on dry weight basis.

b- Separation

Backman Amino Acid Analyzer Model 119CL was used for amino acid determination in pollen grains materials of the tested date palm genotypes.

DNA fingerprint

Total genomic DNA was extracted from young and fresh leaves of date palm tested genotypes, using modified CTAB protocol (Porebski *et al.*,1997). RAPD fragments were amplified according to (Machado *et al.*, 1996). Amplification reaction for RAPD consisted of 1.5mM 10X buffer, 1.5mM $MgCl_2$, 100µM dNTPs (25µM each),0.2 µM primer, 1U Taq polymerase, 50 ng of DNA and sterile water up to 25 µl . RAPD amplification was conducted as follow: an initial step at 94^{o}C for 5 min. 40 cycles for each cycle 1 min. at 94^{o}C, 1 min. at 37^{o}C and 2min. at 72^{o}C and final extension step at 72^{o}C for 5 min.

Statistical analysis

The data obtained throughout this study were statistically analyzed by the analysis of variance as a complete random design as explained by Snedecor and Cochran (1981).

Results and Disscussion

Morphological and Physical Characteristics

Pollen characteristics

The recorded data Table 1 of both seasons clearly showed, males of Kafer El-Dawar recorded the lowest values of pollen weight per spathe in the first and second seasons. Meanwhile, the other males had intermediate average of pollen grains weight with no significant difference between each other in most cases.

TABLE 1. Weight of the pollen grains per spathe and pollen viability tests of different date palm male types during 2009 and 2010 seasons.

Location	2009			2010		
	Pollen weight / Spathe (gm)	Pollen viability		Pollen weight / Spathe (gm)	Pollen viability	
		Stained (%)	Germinated (%)		Stained (%)	Germinated(%)
El Nahda	24.87	94.00	66.18	28.55	93.44	65.49
Rasheed	17.85	94.41	69.12	19.18	94.27	71.72
Kafer EL-Dawar	12.37	89.34	54.47	16.65	88.54	55.66
ELmaragha	17.00	93.00	59.68	18.48	93.89	60.70
Tema	13.00	88.60	62.47	15.37	84.72	63.67
Tahta	20.00	90.49	61.23	22.65	91.26	59.36
LSD (0.05)	4.33	3.75	5.45	5.82	2.39	3.03

For pollen viability, it is obvious that males of Rasheed recorded significantly the higher values in both seasons (69.12 and 71.72 % respectively). In the contrary males of Tema recorded the lowest value in both season (88.60and 84.72% respectively) when direct germination test used (Table 1). However, the viability using the acetocarmine staining test revealed that males grown in Rasheed had significantly the highest viability percentage in both season (94.41 and 94.27 % respectively).

Spathe

Average weight of the spathes of the different male types were highest for those male types selected from Rasheed and lowest for males from Tema region (Table 2) in the second season. Moreover, the data of 2009 and 2010 seasons revealed that the males grown in Rasheed area had significantly higher average spathe width (19.25 and 18.25 cm, respectively) than all the other males grown in the other zones. Meanwhile, Males of Tema gave significantly lowest average width in the both seasons (11.50 and 12.00 cm respectively).

Inflorescence

The data of Table 2 clearly show that the males of El-Nahda had significantly the highest average weight of the inflorescence in both seasons (1.36 and 1.40 kg respectively) as compared with the other males that grown in the rest of locations. However, the lowest value was recorded by Tema region. On the other hand, the males selected from Rasheed gave the higher values of the average inflorescence width in both 2009 and 2010 seasons (18.73 and 16.25 cm respectively).

It was obvious that males of El-Nahda recorded significantly the highest average of the inflorescence length in both seasons (105.50 and 99.25 cm respectively) in comparison with those of the other males of different locations. On the contrary, males of Rasheed gave the lowest inflorescence length in both seasons (65.25 and 66.75 cm respectively)

Strand

It is clear that the males grown in Kafer El-Dawar produced the highest significantly average strand length in both experimental seasons (23.50 and 24.85 cm respectively) as compared with the obtained values of the other male types. In the meantime males of Tahta had significantly the lowest strand length than those other male types in both seasons (14.30 and 15.15). Moreover, in El-Nahda a significantly highest number of strand per inflorescence (260 and 255 strands respectively) was observed, however, there were no significant differences regarding such a trait between males of El-Nahda and those of Tahta, Kafer El-Dawar, El-Maragha and Rasheed in the first seasons. Almost the same trend was noticed in the second season (table 2). Moreover, the highest number of flower per strand was recorded by the males of El-Nahda (74.66 and 75.01 respectively) while males of Rasheed gave the lowest value in both seasons (49.95 and 52.21. respectively).

The results relating to morphological characteristics are in harmony with those obtained by Ashour *et. al.,* (2004), Ibrahim & Kholif (2004); Alsaikhan (2006) and Rabie (2007). It's clear that the great variation regarding the physical characteristics of studied male types could be due to the differences between the genetic constituents of the different male types.

Chemical studies

Carbohydrates

As shown in Table 3, pollens of the male type of Tahta contained the highest percentage of starch in 2009 and 2010 seasons (11.32 and 11.94 % respectively) and the differences were statistically significant as compared with those of the other male types. Meanwhile, the total sugars percentages in the pollens of the different studied date male showed that a highest total sugar percentages in 2009 for pollens of the males that grown in tahta (6.20%) while, the highest value recorded in 2010 season was found in the pollen of the males that grown in Rasheed (7.85%).

In the second season, pollens of the male type grown in Rasheed contained the highest percentage of reducing sugars (4.24 %), the statistical analysis showed that the differences between such a value and those of the rest of males were highly significant. However, non reducing sugars revealed that pollens of Rasheed male type recorded the greatest content (3.61 %) in the second season, the differences were significant in comparison with those males of Kafer El-Dawar, Tema and Tahta.

Regarding the percentages of total carbohydrates in the pollens of the studied males, it is obvious that pollen of Tahta male type contained the highest value (17.25 and 17.04 %), in 2009 and 2010 seasons respectively. Bacha *et al.* (1997) determined the chemical compositions of pollen grains of 13 date palm male types and found that the starch percentages ranged between 8.10 and 9.24, carbohydrates content ranged from 10.5 to 13.1 %., reducing sugars ranged from 0.053 to 0.177 % and non-reducing sugar in date ranged from 1.18 to 3.89 %. Moreover, Hassan (2011) studied the chemical composition and nutritional value of palm pollen grains; he reported that the value of the carbohydrate content in the pollen was 13.41 %.

Proteins

As shown in Table 3 males of Rasheed recorded the remarkable highest protein percentages in both of the studied seasons (31.73 and 30.69 % respectively). However, there were no significant differences between those males and the other males that grown in the other locations. Human and Nicolson (2006) found that, the value of crude protein content in the date palm pollens ranged between 12 – 16%. Moreover, Campos *et al.* (2008) reported that protein content of pollen grains ranged between 10–40 g/100 g dry weight. In addition, Hassan (2011) stated that the protein content of date palm pollens reached 31.11 g / 100 g dry weight of palm pollen grains.

Mineral content

The obtained data (Table 4) showed that pollens of Rasheed males had significantly highest nitrogen content in both seasons (5.08 and 4.91 % respectively).

TABLE 2. Physical characteristics of the spathes, inflorescence of the different date palm male types during 2009 and 2010 seasons.

Location	First season (2009)										Second season (2010)									
	Spathes				Inflorescence			Strands			Spathes				Inflorescence			Strands		
	Weight (kg)	Length (cm)	Width (cm)	Cover. Wt.(Kg)	Weight (kg)	Length (cm)	Width (cm)	Length (cm)	No. of strand / Infloresce-nce	No. of flowers / strand	Weight (kg)	Length (cm)	Width (cm)	Cover Wt.(Kg)	Weight (kg)	Length (cm)	Width (cm)	Length (cm)	No. of strand / Inflorescence-	No. of flowers / strand
El Nahda	2.33	112.5	13.25	0.97	1.36	105.50	12.32	20.00	260.00	74.66	2.37	111.00	12.50	0.97	1.40	99.25	11.78	21.00	255.00	75.01
Rasheed	2.00	68.75	19.25	0.80	1.20	65.25	18.73	15.50	231.25	49.95	2.05	70.00	18.25	0.88	1.17	66.75	16.25	18.87	227.50	52.21
Kafer El-awar	1.85	96.25	13.50	0.70	1.15	91.25	16.53	23.50	243.75	54.65	2.00	87.5	14.25	0.87	1.13	84.50	15.74	24.85	235.00	59.36
ELmaragha	1.72	80.75	14.00	0.69	1.04	77.25	13.12	18.00	240.00	65.42	1.80	82.00	13.75	0.80	1.00	78.75	14.23	20.00	219.50	68.48
Tema	1.35	97.75	11.50	0.51	0.84	88.50	11.89	15.20	223.00	55.18	1.50	93.00	12.00	0.61	0.89	85.00	12.72	17.40	231.25	63.41
Tahta	1.55	75.00	14.75	0.62	0.92	72.00	16.76	14.30	245.00	58.18	1.75	73.00	15.50	0.76	0.99	69.25	15.65	15.15	230.00	68.61
LSD (0.05)	0.40	14.52	2.77	0.25	0.25	12.73	2.49	3.17	32.50	14.04	0.56	8.62	2.50	0.13	0.31	10.62	2.63	3.20	31.87	13.30

TABLE 3. Chemical constituents of the pollen grains of the different date palm male types during 2009 and 2010 Seasons.

Location	Season 009						Season 2010					
	Starch (%)	Reducing Sugars (%)	Non - reducing Sugars (%)	Total sugars (%)	Total carbohydrates (%)	Protein (%)	Starch (%)	Reducing Sugars (%)	Non - reducing Sugars (%)	Total sugars (%)	Total carbohydrates (%)	Protein (%)
El Nahda	7.48	3.31	2.86	6.17	13.65	23.68	8.95	2.50	3.15	5.65	14.60	22.98
Rasheed	7.91	2.53	2.32	4.85	12.76	31.73	7.15	4.24	3.61	7.85	15.00	30.69
Kafer El-Dawar	8.67	2.54	2.57	5.11	13.78	19.75	9.83	1.96	2.54	4.50	14.33	18.75
ELmaragha	7.05	2.88	2.17	5.05	12.10	17.37	7.20	2.40	2.85	5.25	12.45	20.29
Tema	8.24	2.35	2.11	4.46	12.70	21.73	9.11	1.90	2.55	4.45	13.56	19.61
Tahta	11.32	3.45	2.75	6.20	17.52	19.86	11.94	2.45	2.65	5.10	17.04	18.33
LSD (0.05)	2.19	0.92	0.91	1.58	2.58	7.53	1.78	1.10	0.79	1.23	1.80	5.74

As for the phosphorus content, the obtained data revealed that pollens of the males of Kafer El-Dawar and Rasheed recorded the highest phosphorus content the differences were statistically significant except with those males of El-Maragha and Tahta in 2009 only. Significant differences were noticed between pollens of the male type that grown in Rasheed and all other male types in the second season. On the other hand, it's clear that pollens of El-Maragha males of recorded a considerable highest value of potassium content in the first season (1.75 %), and the differences were statistically significant in comparison with the values of all other male types. These findings were confirmed by Bacha *et al.*, (1997) studied the mineral composition of the pollen of some date palm male types and found that the percent of nitrogen ranged between 2.53 – 2.88 % on the basis of dry weight, as for phosphorus and potassium content, they found the percent varied between 0.82 – 0.94 % and 0.96 – 1.11 % for the two elements respectively.

TABLE 4. Mineral contents of the pollen grains of the different male types during 2009 and 2010 seasons.

Location	Mineral contents					
	2009			2010		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
El Nahda	3.79	0.44	1.15	3.68	0.40	1.17
Rasheed	5.08	0.38	1.36	4.91	0.50	1.09
Kafer El-Dawar	3.15	0.50	0.88	3.00	0.42	0.70
El-Maragha	2.78	0.47	1.75	3.25	0.38	1.14
Tema	3.48	0.33	0.91	3.14	0.31	0.72
Tahta	3.18	0.42	1.24	2.93	0.37	1.10
LSD (0.05)	1.19	0.09	0.33	0.94	0.08	0.20

Amino acids

Tables 5 & 6 represent the amino acid content of pollen grains of the different date palm types in 2009 and 2010 seasons.

As for the Aspartic acid content in the pollens of the different male types, the data concerning this point in 2009 season were tabulated in a descending order (g/100g protein) for the males of El-Maragha, El-Nahda, Kafer El-Dawar, Rasheed, Tahta and Tema. In 2010 season, it was for Kafer El-Dawar, Rasheed, El-Nahda, Tahta, Tema and El-Maragha respectively. However, threonine content, the obtained results showed that, males of El-Maragha recorded the highest values in both season (4.50 and 4.20 g / 100g protein). On the contrary, males of Tema recorded the lowest values of protein content in both seasons.

For the serine content, it was clear that males of Kafer El-Dawar gave significantly the greatest value in 2009 season (4.90 g / 100g protein). The differences between this male and the males of the other locations, with the

exception of the males of Rasheed and El-Nahda, were significant. Moreover, glutamic acid content in the pollens of the studied males as arranged in a descending order in 2009 season were: 12.76, 11.58, 11.50,

11.41, 10.78, 10.58, 10.28, 9.62, 9.11 and 8.12 (g / 100 g protein) for Rasheed, El-Nahda, Kafer El-Dawar, Tema, El-Maragha and Tahta, respectively. However, in 2010 was for males of Kafer El-Dawar, El-Nahda, , El-Maragha, Tema, Rasheed, and Tahta respectively.

Kafer El-Dawar palm males recoded the highest value of proline content, however, the lowest value was observed by El-Maragha location. Concerning the glycine content, Tahta location revealed the highest value in both seasons, however, the opposite was true for El-Maragha location. A remarkable highest alanine contents was noticed for Rasheed males, while, the lowest contents were for Tahta males. On the other hand the cystine content showed that pollens of El-Nahda male had the highest values of cystine content in both seasons (0.43 and 0.46 g / 100 g protein) and the differences were statistically significant when compared with the values of all other male types.

It is obvious that pollens of El-Maragha male type contained the greatest values of the valine and the methionine in both seasons. For valine, the data of 2009 showed that there were no significant differences between the value recorded for El-Maragha male and the values recorded for the male types of El-Nahda, Rasheed and Kafer El-Dawar. In 2010, the differences were not significant between the value recorded for El-Maragha male and the value recorded for the male types of El-Nahda only. On the contrary, the lowest values found in the pollens of Tahta male in both seasons (3.26 and 3.46 g / 100 g protein).

Pollens of Rasheed male showed the heights value of Isolucine in 2010 (6.30 g / 100 g protein), in the meantime, no significant differences were observed when compared with the recorded values in the pollens of El-Maragha and El-Nahda males. Meanwhile, the leucine content in the pollens of the different male types, the data concerning this point in 2009 season were tabulated in a descending order for Tahta, Rasheed, Kafer El-Dawar, Tema, El-Nahda and El-Maragha males, respectively. However, in 2010, it was for Rasheed, Tahta, Kafer El-Dawar, Tema, El-Nahda and El-Maragha males, respectively.

Regarding the tyrosine content, the obtained results of both seasons indicated that, pollens of El-Maragha male had the highest tyrosine content (16.96 and 15.96 g / 100 g protein), but there were no significantly differences when compared with the recorded values of Rasheed and Kafer El-Dawar pollens in 2009 only. On the other hand, the lowest value of phenylalanine was obtained by

pollens of El-Nahda male (3.09 g / 100 g protein) in 2009, but pollens of Tema male had the lowest phenylalanine content (3.10 g / 100 g protein) in 2010. Moreover, the obtained data revealed that, pollens of Tema male type had the highest histidine content in both seasons (17.24 and 16.53 g / 100 g protein), and significant differences were observed in comparison with that of the rest of the male types.

Pollens of Rasheed had the highest lysine content in 2009 (6.48 g / 100 g protein), however, there were no significant differences when compared with those of El-Maragha, Kafer El-Dawar and El-Nahda males. In 2010 pollens of Kafer E-Dawar male recorded had the highest lysine content (6.50 g / 100 g protein), also there were no significant differences when compared with those El-Nahda, Rasheed and El-Maragha male types. Pollens of El-Maragha male type gave considerable the greatest arginine content in both seasons (5.84 and 5.70 g / 100 g protein),

It seemed that the amino acid contents in the pollens of the studied male types varied from one male to another, such variations could be attributed to the differences in the genetic makeup of the different male types used in the present study and, in some extent, to the diversities in the environmental conditions – especially the nutritional conditions – between the locations where the studied males were grown. In this regard, Campos *et. al.,* (2008) stated that seventeen different amino acids may present in pollen loads proline, glutamic and aspartic acids, lysine and leucine are the predominant amino acids, constituting approximately 55 % of the total amino acids. Moreover, Hassan (2008 & 2011) concluded that, generally, pollen contains all the essential amino acids but the amount may vary from one type to another and the essential amino acids of palm pollen grains were leucine and lysine. Moderate amount of essential amino acids were observed in palm pollen grains, these amino acids were valine, threonine, phenylalanine, histidine and isolucine.

DNA Fingerprint Using RAPD Markers

Polymorphism as detected by RAPD markers

The polymorphism among 12 male date palm genotypes (from 1 to 4 belongs to El-Nahda, from 5 to 8 belongs to Rasheed and from 9 to12 belongs to Tema) was investigated using six RAPD primers. The total number of amplified bands by these six primers was 50 fragments, and the number of amplified DNA fragment by each primer ranged from 7 to 10 fragments. OPL12 amplified the highest number of fragments (10 bands) while, OPD01 and OPL20 produced the lowest number of bands (7). The average number of fragments / primer was 8.3 and the size of these fragments ranged from 250 – 1500 bp.

All the used primers produced polymorphic bands (Table 7). The number of polymorphic was 46 resulting in an average polymorphism / primer of 7.7. Primer OPC13 and OPL12 revealed the highest number of polymorphic bands (9) while, the lowest number polymorphic band (6) was detected by OPM01.

TABLE 5. Amino acids contents of pollens of the different date palm male types (g / 100 g protein) during 2009.

Location	Amino acids*															
	As	Thr	Ser	Glu	Pro	Gly	Ala	Cy	Val	Met	Is	L	T	Phe	His	Ly	Arg
El- Nahda	13.00	3.99	4.44	11.50	3.12	3.90	5.57	0.43	5.00	1.00	5.70	6.00	12.40	3.09	10.24	6.08	4.85
Rasheed	11.94	4.26	4.80	11.58	3.84	4.35	5.85	0.32	4.97	1.38	6.18	6.70	15.08	4.48	12.00	6.48	5.22
Kafer El-Dawar	12.60	4.32	4.90	11.41	3.90	4.34	5.69	0.31	4.90	1.25	5.49	6.50	14.76	4.09	10.64	6.12	4.62
El-Maragha	14.03	4.50	4.21	9.11	1.41	3.17	5.59	0.30	5.49	1.55	6.23	4.70	16.96	4.72	13.88	6.35	5.84
Tema	9.55	2.82	3.60	9.62	1.74	4.06	4.80	0.31	3.80	1.45	4.12	6.28	13.00	3.41	17.24	5.60	5.14
Tahta	9.87	3.34	3.79	8.12	1.71	4.64	4.39	0.39	3.26	1.49	4.89	7.69	13.96	3.77	9.59	5.16	4.84
L.S.D.0.05	2.92	0.87	0.50	0.95	0.50	0.48	0.62	0.04	0.66	0.27	0.40	1.27	0.56	0.62	0.52	0.43	0.39

TABLE 6. Amino acids contents of pollens of the different date palm male types (g / 100 g protein) during 2010.

Location	Amino acids*																
	As	**Thr**	**Ser**	**Glu**	**Pro**	**Gly**	**Ala**	**Cy**	**Val**	**Met**	**Is**	**L**	**T**	**Phe**	**His**	**Ly**	**Arg**
El- Nahda	11.00	4.10	4.31	10.40	3.40	3.80	5.20	0.46	5.51	1.10	5.90	5.60	13.00	3.21	9.23	6.12	4.50
Rasheed	12.00	4.15	4.95	9.58	3.45	4.45	5.65	0.35	4.99	1.35	6.30	6.50	15.09	4.30	12.60	6.41	5.30
Kafer El-Dawar	13.15	4.11	4.30	10.41	3.70	4.15	4.98	0.32	4.87	1.20	5.59	5.97	13.76	4.12	10.17	6.50	4.60
El-Maragha	8.90	4.20	4.14	10.11	1.50	3.60	4.95	0.28	6.00	1.50	5.99	4.75	15.96	4.80	14.17	6.17	5.70
Tema	9.50	2.91	3.15	9.60	1.60	4.30	4.47	0.29	3.70	1.43	4.17	5.70	13.12	3.10	16.53	5.11	5.11
Tahta	10.47	3.66	3.27	7.45	1.70	4.50	4.29	0.37	3.46	1.47	4.70	6.50	12.97	3.79	10.12	5.12	4.60
L.S.D.0.05	2.21	0.76	0.48	1.10	0.60	0.45	0.77	0.05	0.65	0.29	0.46	1.50	0.65	0.67	0.55	0.40	0.41

*Abbreviations : Aspartic acid (As), Threonine (Thr), Serine (Ser), Glutamic (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Cystine (Cy), Valine (Val), Methionine (Met), Isolucine (Is), Leucine (L), Tyrosine (T), Phenylalanine (Phe), Histidine (His), Lysine (Ly), Arginine (Arg):

Therefore, the percent of polymorphism revealed by the different primers ranged from 75% - 100%.

In this respect, Abdalla and Gamal (2010) investigated some date palms using RAPD technique, the obtained fragments ranged from 300 – 3000 bp, and the average number of amplified bands was five and all of the tested genotypes revealed a unique profile.

TABLE 7. The total number of bands; monomorphic bands, polymorphic bands and percentage of polymorphism as revalues by RAPD markers among 12 date male genotypes.

Primers	Total number of bands	Monomorphic bands	Polymorphic bands	percentage of polymorphism
OPC13	9	_	9	100
OPD01	7	_	7	100
OPL12	10	1	9	90
OPL20	7	_	7	100
OPM01	8	2	6	75
OPZ01	9	1	8	88.8
Total	50	4	46	553.8
Mean	8.3	0.66	7.7	92.3

Cluster analysis as revealed by RAPD marker

The Dice RAPD-based coefficient of genetic similarity among the 12 genotypes of male date palm resulted in a dendrogram (Fig.1), which separated genotype 1 from all the other male date palm genotypes, thus demonstrating the distinctiveness of the genetic background of genotype 1 from all the other genotypes. The other male date palm cluster comprised two subclusters. One subcluster was divided into two groups the first included genotype 9. While, the other group was divided into three subgroups. Both of genotype 8 and 7 was grouped together, reflecting common genetic background, meanwhile genotypes 10, 11 and 12 were clustered together in one subgroup. On the other hand, the second subcluster was divided into three groups. Genotypes 1 and 6 were grouped separately from the other genotypes in the second subcluster. However, the third group was divided into two subgroups, one of these subgroups included genotype 4, while the other one included genotypes 3 and 5. Motawei *et al*. (2003), constructed a dandrogram using UPGMA analysis from PAPD marker with five date palm cultivars (Barhi, Nabet Ali, Rothanah, Ajwa and Sokkari), and on the basis of analysis, the populations were clustered into two clusters: cluster I contained Bachi and Ajwa cultivars, and cluster Π contained Nabet Ali, Rothanah and Sokkari cultivars. Whereas, the RAPD based dendrograms clustered the accessions belonging to each of the 3 cultivars Fraihy, Siwi and Gandila in separate groups. However, the reshuffling in the position of the

accessions belonging to the other cultivars in the different dendrograms revealed that they share common genetic background (Hussein *et al*., 2004).

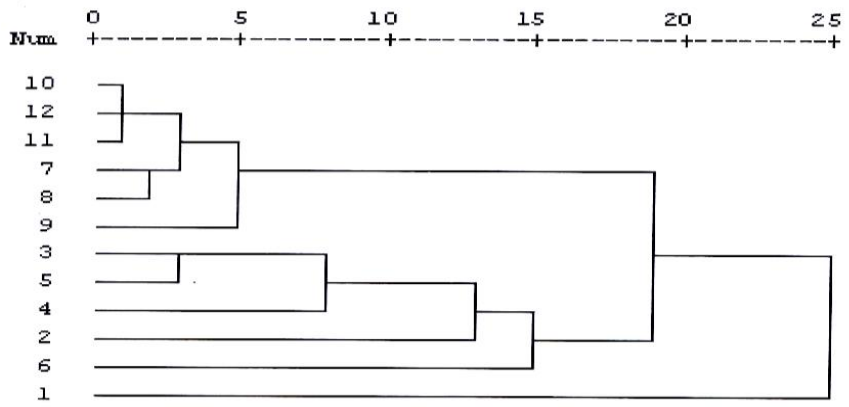

Fig. 1. Dendrogram of correlation similarity using average linkage (Between Groups) among the different male types based on RAPD results.

Genotype Identification by unique RAPD markers

In the present study, genotype-specific RAPD unique markers could distinguish 4 out of the 12 date palm genotypes. The RAPD primers generating the markers and the markers approximate size are shown in Table (8). Among these four genotypes, genotype 1 was characterized by both positive and negative unique RAPD markers. However, the other three genotypes were characterized by only positive or negative unique markers. Genotype 4 and genotype 6 showed only one unique negative marker at 875 bp, 537 bp with OPC13 and OPL12 primers, respectively. On the other hand, genotype 9 was characterized by two unique positive markers at 540 bp, 355 bp with OPC13. Genotype 1 was characterized by one unique negative marker at 1335 bp with OPZ01, while it characterized by four positive unique markers at 950 bp (OPL20), 1240 bp (OPZ01) and 1450 and 450 bp (OPC13). The primer (OPC13) has the potential to identify three out of the four characterized genotypes. Thus, only four primers characterized four genotypes out of the twelve tasted date palm male, however, if more primers were examined, more markers would be expected.

In this respect, Motawei *et al.* (2003) demonstrated that RAPD polymorphism and reproducibility within five date palm cultivars (Barhi, Nabtel Ali, Rathanat, Ajwa and Sokkri) suggest that RAPD markers can be used successfully for varietals. Identification and for studying the genetic diversity of cultivars. Moreover, Hussein *et al*. (2004) characterized 4 cultivars out of six date palm cultivars (Sakkoty, Bertmoda, Malkaby, Gandila, Fraihy and Siwi) when used 27 RAPD primers.

TABLE 8. Unique positive and negative markers that characterized the studied date palm male genotypes.

Genotype number	Unique positive markers			Unique negative markers		
	Size of the marker band (bp)	primer	Total number of Markers / genotype	Size of the marker band (bp)	primer	Total number of Markers / genotype
1	950 1240 1405 -450	OPL20 OPZ01 OPC13	4	1335	OPZ01	1
4	-	-	-	875	OPC13	1
6	-	-	-	537	OPL12	1
9	540 - 355	OPC13	2	-	-	--

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تقيم بعض الطرز المذكرة لنخيل البلح باستخدام الواسيمات المور فولوجية و الجزيئية

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خمس مواقع لاشجار ذكور النخيل البالغة من ثلاث محافظات (الاسكندرية، البحيرة وسوهاج) تم تقييم بعض صفاتها الطبيعية و الكيميائية لتحديد أفضل الطرز المتميزة لتلقيح أزهار الاناث لمساهمة هذة الطرز فى برامج التربية. من الواضح أن مواقع محافظة البحيرة سجلت ذكورها أعلى قيم للصفات المورفولوجية. كما لوحظ نفس الاتجاه لكل من حيوية حبوب اللقاح والمحتوى المعدنى لطرز الذكور المدروسة. منطقة طهطا والتى تقع بمحافظة سوهاج سجلت ذكورها محتوى عالى من الكربوهيدرات.

من ناحية أخرى، كانت أعلى نسبة بروتينات مسجلة لذكور منطقة رشيد، فى حين لم يكن هناك فروق معنوية لهذة الصفة على مستوى باقى المواقع المدروسة. كما لوحظ اختلاف محتوى الذكور المدروسة فى الاحماض الامينية تبعاً للمنطقة ولنوع الحمض الامينى. بالاضافة لما سبق ، استطاع بادئ واحد أن يميز ثلاث طرز من الذكور المدروسة، كما تمكن 4 بوادىء من تميز 4 طرز من ذكور النخيل المدروسة.

من هذه الدراسة يتضح ان افضل الذكور المدروسة تلك النامية بمنطقة رشيد بمحافظة البحيرة ومنطقة النهضة بمحافظة الاسكندرية وبالتالى يمكن التوصية باكثارها للمحافظة عليها وكذلك باستخدامها فى برامج التربية لتحسين صفات ثمار نخيل البلح كماً ونوعا.