EVALUATION STUDIES ON SEEDLINGS OF SOME DATE PALMS GROWN IN EGYPT 2-EVALUATION AND SELECTION OF BARHI DATE PALM MALE SEEDLINGS UNDER ALEXANDRIA AREA CONDITIONS

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ABSTRACT

Ten different date palm male genotypes were selected for evaluating the recommended pollinator for Barhi date palm cultivar grown in Egypt. This investigation was conducted during 2015 and 2016 seasons, at the Experimental station at El-Sabahiya, Alexandria Governorate Egypt. Spath weight with and without cover, length and width of spath with and without cover, space without strand per spath, number of strands and number of flowers per strand as well as pollen grains germination and viability percentages were determined for each male genotype. Data showed that two male trees (No. 6 and No. 7 genotypes) were superior in their morphological characters compared with the other male palms. On the other hand, there were no major differences in pollen grains viability percentage. The results of the molecular analysis of genomic DNA of the ten male palm seedlings showed that the total number of amplicons amplified by the nine primers was 184 with an average of 20.44 / primer. The polymorphism ranged between 75.0% and 100%, with an average of 92.54% polymorphism. Fifteen of these amplicons were monomorphic and 169 were polymorphic. Genetic similarity value ranged between 8.3% and 11.2%. The highest value (11.2%) was between genotypes No. 5, No. 1 and No. 3. The lowest value was recorded between genotype No. 10 and genotype No. 8. It is obvious that genetic similarity between the tested strains were very low, which may be attributed to their origin as seeds from open pollination.

Key words: Date palm male, evaluation, selection, fingerprint

1.INTRODUCTION

The date palm (Phoenix dactylifera L.) is one of the oldest and most important fruit trees, in Egypt and the worled. It is a monocot that has been widely cultivated for its fruits dioeciously and diploid (2n = 2x = 36) (Marsafari and Mehrabi, 2013). (Hossein et al. 2015). Artificial pollination is necessary for successful fruiting. In some date cultivars, better fruit set results from pollen of certain males than others, due to compatibility between male and females. Many investigators proved that pollen grains from different male date trees did not only influence the size and shape of seed (Xinia), but also has a direct effect on fruit set, yield and fruit physical and chemical characteristics (Metaxinia) (Ream, 1976). El-Hammady et al. (1977) reported that

pollen source was found to affect fruit and seed characteristics and it exhibited metaxinic effect depending on the female genotype used, so the growers now begin to realize the need selection of males for pollination. Al-Hamoudi et al. (2006) evaluated four different date palm male genotypes to use as a pollinator for Barhi date palm cultivar grown in Egypt. Moustafa et al. (2010) studied fifty seedling palm males in order to select the suitable and most promising males to be used in pollinating date palm females. Also, Hafez et al. (2014) selected three different date palm male types namely Abo Rawash, Rashid and El Nubaria, which could be recommended to use as pollinators for Samany date palm cultivar grown at Giza Governorate.

Benamor *et al.* (2014) stated that the percentage of germination for the good quality pollen is higher than 75%, and the viability characteristics vary considerably from one type to another. They also stated that a high heterogeneity between the various genotypes of males contains specific characteristics.

To understand the genetic relationship among and within date palm varieties, RFLP, RAPD, SSR and AFLP markers have been used widely and eficiently to analyze the genetic diversity within and among date palm cultivars in many middle east countries such as Egypt (Soliman et al., 2003; Saker et al., 2006); Oman (Al-Ruqaish et al., 2008); Morocco Baaziz 2000; Sedra et al., 1998); Suadi Arabia (Al-Khalifah and Askari, 2003); Tunisia (Trii et al., 2000; Zehdi et al., 2004a,b); Sudan (Elshibli and Korpelainen, 2007). Some studies observed that the apparent phonotypical differences among some cultivars were not reflected in the polymorphism of the molecular markers. Obviously, many more markers should be isolated from cultivars to enhance breeding and evolutionary studies.

The aim of this study was to select a suitable pollination of Barhi cultivar under the conditions of El-Sabahiya station, Alexandria Governorate, Egypt, which represent a good genetic potential in order to multiply them vegetative and eliminate the inferior male genotypes.

2. MATERIALS AND METHODS

This investigation was conducted during the two successive seasons 2015 and 2016 on about eight year old trees of Barhi date palm cultivar of, planted at $7x10 \text{ m}^2$ apart in El-Sabahiya Station, Alexandria Governorate, Egypt. Several visits were carried out to select ten uniform, vigorous male date palm trees numbered from one to ten. All palm trees were healthy and subjected to the same cultural practices in both seasons under study.

2.1. Horticultural study

Data collected for the selected males included date of flowering, end of flowering, duration period of flowering (days) and the number of spath per tree. Morphological characters of date palm males were studied including spath weight with and without cover, weight of spath cover, length and width of spath with and without cover and space without strands / spath. The number of strands and the average number of flowers per strand were counted. Strand weight and length with and without flowers were recorded. Pollen viability and pollen germination percentages were recorded for all male palm trees under study.

2.2. DNA Fingerprint

2.2.1. Inter Simple Sequence Repeats (ISSRs)

Total DNA was extracted from young leaves as described by Porebski *et al.* (1997). Nine ISSR primers were used for PCR amplification (Table 1). PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94 °C. Each cycle

Table (1): Sequence of reliable ISSR primers.

Pi m	101.5.
Primer	Sequence
IS1	TAT(CA) ₇ C
IS2	CAC(TCC) ₅
IS3	TTT(TCC) ₅
IS6	(GA) ₈ CG
IS7	ATTA(CA) ₇
IS8	(AG) ₈ CT
IS9	AAC(TG) ₇ T
IS10	(TCC) 5AC
A9	$(AGC)_4AC$

consisted of a denaturation step at 94 °C for 1 min, an annealing step at 36 °C for 1 min, and an elongation step at 72 °C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each, size class was scored as 1 or 0, respectively.

2.2.2. Data analysis of DNA fingerprint

A similarity matrix using the similarity coefficients of Nei and Li (1979), was constructed for ISSR data based on the presence (coded as 1) or absence (coded as 0) of the resulted fragments for each primer. Moreover, the relationships among the different palm males as revealed by dendogram were done using SPSS windows programming (V.10) Schwartz (1978).

2.3. Statistical analysis

The experiment included in this study followed a completely randomized design in factorial experiment. The obtained data was subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1972). Means were differentiated by Duncan's multiple range tests at 5% level (Duncan, 1955).

3. RESULTS AND DISCUSSION 3.1. Horticultural study

Data in Table (2) showed that across 2015 and 2016 the spath weight with cover ranged between 0.77 to 2.32 (kg). Male No.5 had the least weight (0.77 kg), in the first season. In the second season, the fifth male also gave the least value (1.21 kg). Concerning the weight of spath cover, males No.6&7 gave the highest values (870.0 and 957.0 g, respectively in 2015 and 2016). Male No.5 had the least weight (300.0 and 378.0 g) in the two seasons under study. Data also cleared that the male No.6 and male No.7 recorded the highest significant weight of spath without cover, which ranged from1147.0 to 1368.0 g, the least was for male No.5 which had 513.3 g for the first season and 828.3 g for the second season. These findings are in line with those of Nasr et al. (1986) who selected some male date trees based on certain characteristics including weight and size of the spathes.

Table (3) and Fig.(1) showed that the highest value of spath length with cover was for male No.7 which had 91.00 and 98.33 cm for 2015 and 2016 seasons, respectively. The least value was given by male No.8 (52.33 cm), in the first season, and male No.5 (58.67 cm), in the second season. As for the length of spath without cover, results indicated the same trend; the male No.7 was the superior (82.33 and 86.00 cm) for two seasons under study, respectively. In 2015 season, the two males (No. 5 and No. 8) had the least values (47.00 and 44.67 cm, respectively), while in the second season (2016), male (No. 5 and No.8) had the least length (49.67 cm).

The highest significant width of spath with cover (22.00 and 21.73 cm) was recorded by the male No.7 in two seasons. Meanwhile the least values (11.00 and 12.83 cm) were obtained by male No. 5 in the two seasons under study. Concerning the width of spath without cover, the male No. 7 had the highest value (20.43 and 18.57 cm), the male No. 5 had the least values (09.90 and 11.23 cm) in the two seasons, under study, respectively.

On the other hand, the least space without strands per spath was given by the male No. 3 (12.53 cm) in the first season under study while the second male gave the least value (11.83 cm), in the second season.

All these results are in harmony with those found by El-Hammady *et al.* (1977), Al-Hamoudi *et al.* (2006) and Farag *et al.* (2012) who reported that there was a positive correlation between fruit set percentage and

 Table (2): Means of spath weight cover, spath with cover weight and weight of spath without cover for all pollinators in 2015 and 2016 seasons.

Male	Spath weigh (k	t with cover g)	Spath co	over weight (g)	Weight spath of without cover (g)			
number	2015	2016	2015	2016	2015	2016		
1	1.85BC	1.93B	866.7A	886.7AB	983.3BC	1047BCD		
2	1.59CD	1.54D	680.0B	653.3C	913.3BCD	886.7CD		
3	1.68BCD	1.89B	723.3B	833.3AB	956.7BC	1080BC		
4	1.15E	1.62CD	500.0C	666.7C	680.0DE	950CD		
5	0.77F	1.21E	300.0D	378.3D	513.3E	828.3D		
6	2.00AB	2.28A	870.0A	920.0AB	1147AB	1357A		
7	2.19A	2.32A	926.7A	957.0A	1260A	1368A		
8	1.43DE	1.52D	486.7C	586.7C	940.0BCD	933.3CD		
9	1.70BCD	1.98B	726.7B	806.7B	970.0BC	1177AB		
10	1.58CD	1.84BC	720.0B	901.7AB	856.7CD	935CD		

Means in the same column followed by the same letter(s) are not significantly ($p \ge 0.05$) different.

bunch weight obtained at harvest.

Data in Table (4) showed the number of strands per spath of ten male palms under study. In the first season (2015), the highest number was shown by only the male No.7 (190.0), while in the second season (2016), was shown by three males, No. 1, No. 6 and No.7, ranging from 197.0 to 202.7. The male No.5 had the least number of strands per spath (94.3 and 112.7), in the two seasons, respectively. Data indicated that the highest weight of strand was for the male No. 7 (4.77 and 4.53 g), for two seasons under

from 73.13 to 82.47 flowers in the two seasons 2015 and 2016. The least number of flowers per strand was shown by the male No.4 (42.33 and 41.67) in the same two seasons (2015 and 2016).

Regarding the strand length, the obtained results in Table (4) showed that the highest value was for male No.6 (22.67 cm) in the first season. In the second season, there were four males (No. 1, 3, 6 and 7) which had the highest values ranging from 20.83 to 22.83 cm. Data revealed that the least space length without flowers was for the male No.7 (1.70 cm) in season 2015, and

Table (3): Means of length of spath with and without cover, width of spath with and without cover and space without strands/spath for all pollinators in 2015 - 2016.

Mala	Length of	spath with	Length	of spath	Width of	spath with	Width o	of spath	Space	without
numbor	cover (cm)		without cover (cm)		cove	cover (cm)		over (cm)	strands/spath (cm)	
number	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1	81.67AB	86.33B	70.40B	77.67B	15.83CD	16.57DE	15.07C	15.23C	15.30CD	13.67D
2	66.50C	63.00DEF	57.00CD	54.67EF	15.17CD	15.60EF	14.37CD	14.10C	16.17C	11.83E
3	71.33BC	70.33CD	62.67BCD	59.33DE	15.67CD	16.03DE	14.60C	14.90C	12.53F	13.17D
4	74.67BC	73.00C	65.67BC	63.00CD	12.50DE	14.57F	11.40DE	12.77D	19.37B	17.10B
5	53.67DE	58.67F	47.00E	49.67F	11.00E	12.83G	09.90E	11.23E	15.03CD	15.93C
6	62.33CDE	77.00C	55.67D	68.67C	20.00AB	18.77C	18.63AB	17.10B	15.27CD	13.83D
7	91.00A	98.33A	82.33A	86.00A	22.00A	21.73A	20.43A	18.57A	13.23EF	17.07B
8	52.33E	68.00CDE	44.67E	58.67DE	17.33BC	20.40B	15.90BC	18.03AB	16.17C	16.83B
9	65.67CD	76.00C	56.00D	68.00C	16.00CD	16.97D	14.70C	14.83C	14.50DE	15.87C
10	65.67CD	60.67EF	58.67CD	52.67EF	15.23CD	16.37DE	14.27CD	14.10C	20.87A	21.20A

Means in the same column followed by the same letter(s) are not significantly ($p \ge 0.05$) different.

Table (4): Means number of strands/spath, strand weight, average number of flowers / strand length and space length without flowers for all pollinators in 2015 and 2016seasons.

Male	Male Number of strands/spath		Strand weight (gm)		Average r flowers	number of /strand	Strand len	gth (cm)	Space length without flowers		
number	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	
1	165.3B	199.3A	3.56CD	3.83BC	60.94BC	62.00CD	20.35ABC	21.97A	2.43CDE	2.93BC	
2	111.7CDE	119.3DE	2.70DE	2.27F	64.11AB	67.67BC	18.33BCD	18.37B	3.83B	4.77A	
3	135.3C	159.3BC	2.82DE	2.80EF	58.15BC	60.67D	21.87AB	22.13A	1.90DE	2.77CD	
4	104.3DE	125.7D	4.47AB	4.03AB	42.33D	41.67F	15.43D	16.17C	3.67B	3.43B	
5	94.3E	112.7E	3.61BCD	3.47CD	50.41BCD	52.00E	14.71D	13.50D	2.67C	2.87C	
6	162.3B	197.0A	4.27ABC	4.10AB	77.30A	82.47A	22.67A	22.83A	2.50CD	3.00BC	
7	190.0A	202.7A	4.77A	4.53A	75.58A	73.13B	20.20ABC	20.83A	1.70E	2.30D	
8	127.7CD	151.3C	2.60E	2.80EF	52.21BCD	52.23E	17.73CD	16.33C	4.73A	4.57A	
9	128.0CD	156.0BC	3.10DE	3.23DE	54.60BCD	57.53DE	10.50E	11.77D	1.93CDE	2.97BC	
10	117.7CDE	165.7B	2.83DE	3.07DE	47.06CD	51.93E	17.60CD	17.10BC	3.67B	3.10BC	

Means in the same column followed by the same letter(s) are not significantly ($p \ge 0.05$) different.

study, respectively, the least weight of strand was for the male No. 8 (2.60 g), in 2015 and for male No. 2 (2.27 g).

As for the mean number of flowers per strand, data in Table (4) revealed that the males No,6 and No.7 had the highest number ranging

(2.30cm) in season 2016. These results are in harmony with those reported by Benamor *et al.* (2014) who stated that male palm trees are in a good state for all the descriptive parameters held by farmers (well localized, early and producing a great number of spathes).

Male	Pollen grain strai	ns weight per nd (g)	Pollen vi	ability %	Pollen germination %			
number	2015	2016	2015	2016	2015	2016		
1	0.267AB	0.327B	91.47A	93.03AB	50.67AB	52.50BC		
2	0.160BC	0.200C	89.63A	89.90B	49.94AB	52.40BC		
3	0.303A	0.347B	92.80A	93.03AB	45.15BC	48.07CD		
4	0.167BC	0.197C	91.57A	89.17B	30.07D	42.17E		
5	0.123C	0.153C	91.23A	93.43AB	37.07CD	42.73E		
6	0.343A	0.437A	95.47A	92.97AB	59.65A	60.13A		
7	0.377A	0.363AB	92.67A	95.83A	55.53AB	60.20A		
8	0.127C	0.147C	94.33A	92.10AB	37.43CD	43.83DE		
9	0.103C	0.170C	91.47A	90.60AB	32.68CD	44.20DE		
10	0.143C	0.203C	93.47A	94.37AB	52.83AB	53.97B		

 Table (5): Means of Pollen grains weight per strand, pollen viability and pollen germination percentages for all pollinators in 2015 and 2016 season.

Means in the same column followed by the same letter(s) are not significantly ($p \ge 0.05$) different.

Data in Table (5) presented the mean of pollen grains weight per strand, pollen viability and pollen germination. Regarding the weight of pollen grains, the three males trees (No.3, 6, and 7) had the highest values ranging from 0.303 to 0.377 g, in the first season. In the second season one male only (No.6) had the highest weight 0.437 g. The males (No.5,8,9 and 10) had the least values of pollen grains weight ranging from 0.103 to 0.203 g in the two seasons 2015 and 2016, respectively.

In the case of pollen viability, data in Table (5) showed no significant differences among ten male trees in the first season (2015), while there were slight differences in the second season. In the same Table, it was clear that the sixth and seventh male trees had the highest values of pollen germination ranging between 55.53 and 60.20%, in two seasons 2015 and 2016. The lowest value of pollen germination for male No. 4 (30.07%) in the first season and by the two males (No. 4 and No. 5), which gave 42.17 and 42.73%, respectively in the second season. Zeinab et al. (2014) selected five male pollenizers according to pollen grains weight and their viability in Siwa Oasis. Benamor et al. (2014) reported that the percentage of germination for good quality pollen is higher than 75% and the viability characteristics vary considerably from one genotype to another. Al-Hammoudi et al. (2006) stated that there were great differences in pollen grains germination percentage in four male trees.

Concerning the period of flowering, Table (6) showed that it was shorter in the first season (2015) than in the second season (2016) for all males palm trees under study. We noticed that

the sixth and seventh male had the longest period of flowering ranging between 69 to 92 days in the two seasons. It was evident that the male palm trees (sixth, seventh and tenth) had the highest number of spathes ranging between 22 and 24 spath per male in two seasons under study. These results are in agreement with those reported by Benamor *et al.* (2014), who considered that the "Male" flowering period is seldom extended until the month of May.

3.2.1. Polymorphism and genetic similarity estimated by ISSR markers

Table (7) summarizes the results obtained from using nine primers of ISSR markers. All of the nine tested primers were reproducible and scorable Fig.(2) using (Ladder molecular weight marker (with a range 100-3000 bp). Four primers produced 100% polymorphic amplicons (1,2, 3 and 5). On the other hand, primers No.8 and 4 recorded the highest percentage of polymorphism (92.9 and 91.3, respectively). The average of percentages was 92.54%. Primer No.6 produced the highest number of amplicons (29), four of these amplicons were monomorphic, while, twentyfive were polymorphic. The total number produced by the nine primers was 184 amplicons, fifteen of which were monomorphic and 169 were polymorphic. In this regard, Ameer et al. (2016) stated that, using of seven ISSR primers for twenty five date palm cultivars produced total scorable bands of 622 with an average of 38.8 bands per primer. Meanwhile, Abd-Alla (2010) mentioned that DNA bands generated with the ISSR primer (HB-15) ranged from 7 to 9. Moreover, primer (HB-10) produced



Fig. (1): Spath of ten seedlings of date palm males under study.

	M	1	2	3	4	5	6	7	8	9	10		M	1	2	3	4	5	6	7	8	9	10
1000 bp 600 bp 500 bp 400 bp 300 bp 200 bp												1000 bp 700 bp 600 bp 500 bp 400 bp 300 bp 200 bp 100 bp											

Fig.(2):Polymorphism detected by ISSR (IS1 and IS3) marker for ten selected date palm males. M: Ladder molecular weight marker.

Table (6): Me	eans of first	spath date,	last spath	date, peri	od day an	d the numbe	er of spathes/	male for a	ıll
р	ollinators in	2015 and 20	16 seasons	s.					
							1		

Male number	First spa	ath date	Last spa	ath date	Period	l (days)	Number of spathes / male		
	2015	2016	2015	2016	2015	2016	2015	2016	
1	25 Jan.	16 Jan.	16 Apr.	15 Apr.	81	90	16	18	
2	21 Nar.	2 Mar.	11 Apr.	9 Apr.	41	38	14	12	
3	2 Feb.	2 Feb.	21 Apr.	15 Apr.	78	73	16	14	
4	1 Mar.	1 Mar.	16 Apr.	1 Mar.	46	61	18	18	
5	1 Apr.	3 Mar.	2 May.	26 Apr.	31	54	14	20	
6	11 Feb.	2 Feb.	22 Apr.	2 May.	70	90	22	23	
7	30 Jan.	25 Jan.	9 Apr.	26 Apr.	69	92	23	22	
8	22 Feb.	2 Feb.	21 Apr.	26 Apr.	58	84	12	10	
9	1 Mar.	2 Feb.	11 Apr.	15 Apr.	41	73	15	18	
10	8 Feb.	12 Feb.	1 Mav.	1 Mav.	82	79	24	20	

Means in the same column followed by the same letter(s) are not significantly ($p \ge 0.05$) different.

Primer No.	Primer name	Monomorphic bands	number of polymorphic bands	Total number of bands	percentage of polymorphic bands
1	IS1	0	25	25	100
2	IS2	0	20	20	100
3	IS3	0	18	18	100
4	IS6	2	21	23	91.3
5	IS7	0	15	15	100
6	IS8	4	25	29	86.2
7	IS9	2	14	16	87.5
8	IS10	1	13	14	92.9
9	A9	6	18	24	75.0
Total		15	169	184	
Mean		1.66	18.77	20.44	92.54%

Table (7): Number of monomorphic	bands, number of	polymorphic band,	total number of	of bands
and nercentage nolymo	rnhism of ten da	te nalm males.		

Table (8): Genetic similarity matrixes computed according to Dice Coefficient from ISSR marker.

D2 10.3	
D3 11.0 10.7	
D4 9.90 9.40 9.90	
D5 11.2 11.1 11.2 10.7	
D6 10.5 10.0 10.5 9.40 10.7	
D7 10.1 9.40 10.5 9.20 10.7 9.40	
D8 9.40 9.10 10.0 8.90 10.2 9.10 8.90	
D9 10.2 9.90 10.6 9.50 10.6 10.1 9.70 9.00	
D10 9.50 9.40 9.90 8.80 9.90 9.00 9.00 8.30 9.10	

(D) Represented male genotypes.

Dendrogram using Average Linkage (Between Groups)



Fig.(3): Dendrogram using average linkage(between groups)

DNA bands with molecular weight of 400-900 bp.

Genetic similarity was estimated according to Dice coefficient (Sneath and Sokal 1973). The genetic similarity ranged from 8.3% to 11.2% (Table 8). The highest genetic similarity 11.2%) was between male No.5 and males No.1 and 3. However, the lowest genetic similarity was recorded between male No.10 and male No.8. It is obvious that genetic similarity between the tested males was very low; this may be attributed to its origin as seeds. In this respect, Hamza *et al.* (2012), observed the highest (48%) and the lowest (5%) genetic distance among date palm cultivars based on ISSR data, interestingly the highest and lowest genetic similarities have divided the date palm cultivars geographically.

3.2.2. Cluster analysis

Dendrogram obtained from UPGMA cluster analysis of genetic distances (Fig. 3) revealed that, all of the tested genotypes were separated into nine clusters. Each cluster includes one male only. However, one cluster grouped males No.8 and10. ISSR has proved successful for assessing genetic diversity within various plant groups for gene mapping and for germplasm identification (Santos *et al.* 2011).

Conclusion

The results clearly indicated that males No.6 & 7 genotype were superior in their morphological characters, in Alexandria area. parameters.

Moreover, fair quality males of the studied palm trees may give a good performance, in other climatic conditions.

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دراسات والتقييم لسلالات بعض النخيل البذرية المنزرع في مصر 2- تقييم وانتخاب ذكور نخيل بلح البرحي تحت ظروف منطقة الاسكندرية بمصر

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ملخص

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