

SELECTION OF GOOD MALE DATE PALM POLLINATORS FOR IMPROVING BOTH YIELDS AND FRUIT QUALITY, USING MORPHOLOGICAL CHARACTERIZATION AND GENETIC RELATIONSHIPS OF POLLINATORS USING ISSR MARKERS

Zeinab A.M. Abo-Rekab*; T.M. El-Kafrawy** and Ghada A.A.**

* Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

** Central Laboratory of Date Palm Researches and Development, Agricultural Research Center, Giza, Egypt.



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Prof. Dr. M.R.A. Nesiem,
Cairo Univ.

Prof. Dr. Wafaa T. Saeed,
Hort. Res. Inst., ARC.

ABSTRACT: This investigation was carried out during three successive seasons (2011, 2012 and 2013) at Siwa Oasis, Matruh Governorate and Tamiya District, Fayoum Governorate. The investigation included three types of experiments; the first was concerned with the evaluation of twenty chosen seedling palm males in order to select the most promising males. Five male pollinizers were selected according to spathe number, flowers number/spathe, start and duration of spathe burst, pollen grains weight, pollen grains viability (at Siwa Oasis in 2011 and 2012 seasons). The numerical selection of selected male palms showed that pollinizer No. 5 (98.9 units), No. 14 (98.8 units), No. 7 (83.6 units), No. 12 (81.9 units) and No. 13 (81.8 units).

The results indicated that the five selected pollinators increased the yield of both Seewy and Barhee cultivars in both two seasons (2012 and 2013) grown at Tamiya District, Fayoum Governorate. Pollinizer No. 12 induced an early harvesting in both Barhee and Seewy cultivars, while pollinizer No. 7 gave the highest yield in both Seewy and Barhee cultivars as well as moisture percentage. Meanwhile, pollinizer No. 12 gave the highest values of total sugar content and TSS % in Seewy cultivar. The results indicated that pollinizer No. 13 gave the highest dry matter %. Also, the results indicated that pollinizer No. 14 gave the highest fruit dimensions as well as both fruit weight and flesh weight. The pollinizer No. 5 gave the lowest seed weight in both two seasons. The selected five pollinators seedling were genetically discriminated by inter-simple sequence repeats for characterization and construction of genetic linkage maps and the molecular genetic diversity among the five selected pollinators. The results indicated that pollinizers No. 12 and 14 recorded the highest similarity (100%), while the lowest similarity (0.0%) was detected between pollinizer No. 7 and No. 13.

Key words: Selection, male, date palm, pollinators, ISSR analysis.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an important commercial crop of many countries of the world and plays an important role in the economical

development of these countries. The date palm has probably been cultivated in what is known today as Southern Iraq (Mesopotamia) since 3500 B.C. or earlier, and little later in Egypt. In Egypt, date palm has been discovered since prehistoric times

and can be grown under unfavorable environmental conditions in all governorates. There are three main types of dates based on moisture content; soft, semi-dry and dry types.

According to the latest statistical estimates of Ministry of Agriculture (2012), the number of date palm trees in Egypt is about 1253488 produced yearly about 1400072 tons of soft, semi-dry and dry fruits. Fayoum Governorate presents one of the main areas of date production in Egypt, especially semi-dry date cultivars. In Fayoum Governorate, the number of date palm trees is about one million with a yearly production of about 61.560 tons of fruits, there of about 130 thousand seedling male palms. Date flowers tend to set parthenocarpically if they are not pollinated and all three carpels remained and developed to three small fruits with no value. So, pollination is one of the major practices to insure good fruit production. Artificial pollination is considered the major mean for commercial date production (Kotb, 1993). The pollination efficiency is affected by several factors and consequently fruit set is highly dependent on these factors. The pollination time of flowering period of male palm, the type of pollen, pollen viability and amount as well as the female flowers, respectively are considered the main factors involved in this process (El-Salhy *et al.*, 1997). Males are highly variable in their growth, vigor, flowering time, spathe characteristics and pollen quality (Mohamed, 2004). Date palm growers use pollen that is readily available. As a result, yield and fruit quality of palms differ greatly from one to another. The growers are now beginning to realize the need for selection of males (El-Salhy *et al.*, 1997). Recently developed techniques based on polymerase chain reaction (PCR), offer a new tool for genetic analysis and construction of linkage maps. Genetic diversity is the bases for successful crop improvement and can be estimated by different methods which include the use of agronomic characterization and DNA finger printing (Becelaere *et al.*, 2005). One of the

most widely used applications is the ISSR technique which depends on the identification of markers linked to traits of interest without the necessity of mapping the entire genome. In order to overcome some of the production problems of date palm under Fayoum conditions, this investigation is aimed to study the following points:

- 1- Evaluate different male trees and selecting the suitable and highly potent of them to be used in pollinating female palm trees of Seewy and Barhee cultivars, using vegetative fruiting and ISSR as a tool for identification and for determination of genetic diversity among the different seedling males of date palm.
- 2- Study the effect of the selected pollen palm males on fruit-setting, yield, fruit physical and chemical characteristics, as well as fruit maturity and time of Seewy and Barhee date cultivars grown in Fayoum governorate.

MATERIALS AND METHODS

This investigation was conducted in Siwa Oasis, Matruh Governorate in three successive seasons; 2011, 2012 and 2013. The first part of the present investigation included 20 chosen seedling palm males in order to select the most promising males. Evaluation of the selected males based on their vegetative growth and morphological aspects of trees and spathes, as well as pollen grains viability and weight. The second with evaluation using inter-simple sequence repeats (ISSR) to identify the genetic diversity among selected males. Five pollinizers were selected as grown vigorously and marked to complete investigation in two seasons (2012 and 2013). Moreover, the third goal was studying the effect of selected pollen grains on yield and fruit quality of female Seewy and Barhee palm at Tamiya District, Fayoum Governorate. The male spathes were collected once spathe crashing and strands were separated individually and dried at room temperature to avoid high moisture.

Dusting the female inflorescence by striking 10 male strands with hand on it. Six date palm trees were about 20 years old grown on clay soil, uniform in growth and vigor, subjected to the usual horticultural management. Three spathes on each palm were selected and pollination was made 3 days after spathe cracking. Prior to cracking, female spathes were covered by paper bags within 3 days from cracking. Pollinated female spathes were still covered until fruit set was insured. Experimental treatments were arranged in a randomized complete block design with three replications (one palm tree represented each replicate).

Date male morphological characteristics:

- 1- Palm height and girth: The height and the trunk girth for each male palm used were measured in meters.
- 2- Leaves number: The number of leaves per male palm was counted and recorded.
- 3- Leaf morphology: Morphology of leaves was studied on four full grown leaves per palm (one leaf from each side). The measurements included:
 - a. Leaf length (m) and number of pinnae.
 - b. Average length of pinnae and spine (cm) were determined as follows: 8 leaflets (4 from each side) at either the top, middle and the base of rachis were randomly collected and measured as well as pinnae width was measured.
- 4- Spathe male morphology: Some morphological characteristics of spathes of date palm males used in pollination were recorded at blooming. Five matured spathes were collected from each male palm used in pollination for the following spathe characteristics:
 - a. Spathe number.
 - b. Spathe length (cm).
 - c. Spathe width (cm).
 - d. Spathe weight (kg).

- e. Strand length (cm).
- f. Strand No./spathe.
- g. Flower No./strand.
- h. Weight of pollen grains (g).

- 5- Pollen grains characters: Five mature spathes of every male were collected as soon as they crashed and were dried at room temperature.
 - a. Pollen viability: It was tested with aceto-carmen-staining. One drop of 1% aceto-carmen was placed on a microscope slide, and then a small amount of pollen was dispersed and microscopically were examined. Colorless or unstained pollen grains were considered non-viable. Ten counts at different fields were examined and readings were recorded to determine the percentage of pollen viability according to El-Salhy *et al.* (1997).
 - b. Pollen germination: This test was carried out using a medium containing 7% sucrose + 10 ppm boron + 1% agar in petri dishes. Fresh pollen grains were dusted on the previous medium and petri dishes were placed in an incubator at 27°C for 24 hours. So, pollen grains were microscopically examined for germination from each petri dish (Diab, 2012).
- 6- The number of fruit set per strands was counted using 10 strands per spathe after thirty days from pollination. The percentage of initial fruit set was calculated using the following equation:

$$\text{Initial fruit set (\%)} = \frac{\text{Average No. of set fruit per strand}}{\text{Average No. of set fruit + Average No. of flower scars}}$$

- 7- Evaluation of the tested male seedling date palm was calculated on the basis of 100 units which were divided among the five following characters, i.e. the spathes number, flowers number/palm, pollen grains weight, pollen grains viability and

pollen grains germination (20 units for each). The following equation was used to determine these characters:

$$\text{Characters} = \sum B/A \times 20$$

Where: A= The highest value recorded for studied character among all treatments.

B= Value recorded for the specific characters for considered treatments.

8- Bunches of both Seewy and Barhee date palm cultivars were picked at optimum commercial harvesting time under Fayoum Governorate conditions (i.e. the second week of both August and September).

- The average bunch weight (kg) was determined for each pollinizer as well as the yield of each palm was recorded in terms of weight in kilograms.
- At harvesting date, fifteen fruits from each bunch were picked at ripe stage (Khlal) and divided into three replicates each containing five fruits.
- The obtained sample were studied in the laboratory to determine the fruit physical and chemical characteristics: The fruit flesh and seed weight were obtained in (g) by weighing each replicate (five fruits) and the average was calculated. Also fruit dimensions (axial and equatorial diameter) for the individual fruits were measured by a vernier caliper.
- Concerning moisture and dry matter contents, fruit samples were cleaned and date flesh was cut into pieces and dried at 60-65°C for 48 hours (Dawson and Aten, 1963 and Mawlood, 1980).
- The total soluble solids (TSS) content was determined in fruit juice using a hand refractometer (A.O.A.C., 1995).
- Total and reducing sugars percentages were determined in juice according to

A.O.A.C. (1995). The non-reducing sugars were calculated from the difference between total and reducing sugars. In addition, titratable acidity was determined in well mixed fruit juice (5 ml) by titration against sodium hydroxide (0.1 N) using phenolphthalein as an indicator, as described by A.O.A.C. (1995).

- Acidity percentage was calculated as g citric acid/100g. f.w.
- Also, tannins content was determined by using the Indigo Carmen indicator described by Balbaa (1981).

Inter simple sequence repeat DNA-PCR (ISSR-PCR) procedure:

- 1- ISSR-PCR reactions were conducted using five primers. Amplification was conducted in 25 ml reaction volume containing the following reagents: 2.5 ml of dNTPS (2.5 mmol), 2.5 ml MgCl₂ (2.5 mmol) and 2.5 ml of 10x buffer, 3.0 ml of primer (10 pmol), 3.0 ml of template DNA (25 mg/ml), 1 ml of Taq polymerase (1 µ/ml) and 12.5 ml of sterile H₂O.
- 2- The PCR products were separated on a 105% agarose gel and fragments sizes were estimated with the 100 bp ladder DNA marker (Fermentas) with molecular size of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp.

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94°C for 4 min followed by 45 cycles of 1 min at 94 °C, 1 min at 57°C, and 2 min at 72°C. The reaction was finally stored at 72°C for 10 min.

PCR reactions were conducted using 5 arbitrary 10-mer primers. Their names and sequences are shown in Table (1).

Table 1. List of the primer names and their nucleotide sequences used in the study for ISSR procedure.

No.	Name	Sequence	No.	Name	Sequence
1	HB08	5' GAG AGA GAG AGA GG 3'	4	HB13	5' GAG GAGGAG GC 3'
2	HB09	5' GTG TGT GTG TGT GC 3'	5	HB15	5' GTG GTGGTG GC 3'
3	HB11	5' GTG TGT GTG TGT TGT CC3'			

Statistical analysis:

All obtained data was tabulated and statistically analysed according to Snedecor and Cochran (1980) using the new L.S.D test for distinguishing the significance differences between various treatment means. The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity coefficients (Dice, 1945) and implemented in the computer program SPSS-10.

RESULTS AND DISCUSSION

1- Vegetative characteristics:

Data in Table (2) described the vegetative characteristics of the twenty investigated male palms. Data cleared that great variability in vegetative traits of various males. Palm heights were differed, the values were ranged from 4.91 to 10.72 m where male No. 12 was the tallest (10.72 m) and the shortest male was No. 20 (4.91 m). Meanwhile the corresponding girth of palm were ranged from (1.46 to 2.16 m) where male No. 12 was the biggest (2.16 m) as compared to the other male palms. Also, data in Table (2) indicated that the annual leaves number per palm was variable, where the annual leaves number was ranged from 13 to 18 leaves/palm. Regarding the leaf characteristics, data in Table (2) showed that the leaf length ranged from 3.66 to 4.11 m in the different male palms. The spines number

per leaf was ranged from 20 to 21 spines/leaf. Also, the pinnae number per leaf were ranged from 193 to 209 pinnae/leaf, while length and width of pinnae were ranged from (42.3 to 52.8 cm) and (2.46 to 3.34 cm), respectively. These findings might be due to increase either the leaf number or leaf area which reflected on a pronounced increase in leaf surface and enhanced the vegetative growth and both viability and germination of pollen grains. These results agreed generally with those found by Diab (2012).

2- Morphological spathe characteristics of different male palms:

Data in Table (3) indicated that great variability in the morphological traits of the spathes of various male palms. The spathe number ranged from 15 to 34 spathes/palm in the different males. The recorded spathe length and width ranged from 79 to 150 and 10 to 19 cm, respectively corresponding to the average of the two studied seasons. The corresponding spathe weight ranged from 1.5 to 3.65 kg whereas for strand length ranged from 195 to 497 cm. Also, strands number per spathe ranged from 72 to 140 strand per spathe. Data in Table (3) indicated that the average number of flowers per strand varied from 50 to 130 flowers/strand. The corresponding weight of pollen grains varied from 280.5 to 1630 g/spathe.

Also, data in Table (3) indicated that beginning of the spathe crashing started from 5th Feb. to 22nd Feb. Data also revealed that end of spathe crashing was from 10th March to 24th March. These results are in agreement with El-Sabrouit (1979); Nasr *et al.* (1986); Marzouk *et al.* (2002) and Diab (2012).

Table 2. Vegetative characteristics of twenty males of date palm used in pollination. Data represented the average of the two studied seasons (2011 and 2012).

Characters Males	Height (m)	Girth (m)	No. of annual leaves/palm	Leaf length (m)	Spines No. /leaf	Pinnae No. /leaf	Pinnae length (cm)	Pinnae width (cm)
Male 1	5.69	1.80	15	3.84	22	197	42.3	2.63
Male 2	6.45	1.78	14	3.74	21	193	44.2	2.69
Male 3	6.56	1.60	13	3.95	22	195	45.6	2.66
Male 4	7.48	1.90	14	3.91	21	199	51.3	2.73
Male 5	10.65	2.00	18	3.82	25	203	50.6	3.30
Male 6	6.93	1.83	16	3.99	20	201	42.1	2.81
Male 7	8.80	1.94	17	4.00	24	205	48.2	3.21
Male 8	6.33	1.52	16	3.73	23	195	43.1	2.84
Male 9	5.74	1.46	15	3.62	22	197	44.2	2.94
Male 10	8.30	1.64	15	3.76	22	191	45.7	2.61
Male 11	7.00	1.61	14	3.69	21	193	46.4	3.10
Male 12	10.72	2.16	18	3.96	24	207	52.6	3.34
Male 13	9.80	1.87	17	3.89	26	209	52.8	3.16
Male 14	9.93	1.92	17	4.11	27	199	51.9	3.28
Male 15	6.20	1.58	16	3.77	24	201	47.5	2.95
Male 16	6.40	1.63	15	3.80	23	193	49.2	2.46
Male 17	7.35	1.75	16	3.83	25	197	45.3	2.56
Male 18	7.15	1.72	14	3.79	22	199	47.8	2.86
Male 19	6.83	1.68	13	3.85	21	201	43.5	2.93
Male 20	4.91	1.59	14	3.66	23	193	42.8	2.58

It was evident from foregoing results that the seedling males differ greatly in the morphological characteristics of the spathes and strands. These results are in agreement with Nasr *et al.* (1986); Marzouk *et al.* (2002) and Diab (2012). They stated that no two seedling palms are similar and of course they had different growth characteristics of spathes in seedling males.

3- Pollen grains characteristics:

The viability percentage of twenty date pollinators are presented in Table (3). It was cleared that viability percentage was averaged from 89.9 to 100%. The corresponding germination percentage of the twenty pollinators were attained from 85 to 100%. It could be concluded that the germination percentage took a similar trend to that of the viability percentage. Generally, it was noticed that there was a positive relationship between pollen viability and

germination percentage. These results are in line with those established by Hamdy (1982), El-Salhy *et al.* (1997) and Diab (2012). They found that the pollen grains of the date palm males showed wide variation in both viability and germination percentage.

4- General Evaluation:

The general evaluation of males date palm seedlings are summarized in Table (4). The male No. 5 and No. 14 rank at the first in the general evaluation as it attained the maximum accumulative units (98.9 and 98.8 units, respectively) as compared to the other males. Male No. 7 gave the second, it attained 83.6 units. The third rank was gained by the males No. 12 and No. 13, as they attained 81.9 and 81.8 units, respectively). In general, there were five males, which were found to be good pollinators (M5, M14, M7, M12 and M13).

Table 3. Morphological spathes and strands characteristics as well as viability and germination percentage of twenty different male palms used in pollination. Data represented the average of the two studied seasons (2011 and 2012).

Character	Spathe number	Spathe length (cm)	Spathe width (cm)	Spathe weight (kg)	Strand length (cm)	Strands No./spathe	Flowers No./strand	Weight of pollen grains (g)/spathe	Viability of pollen grains (%)	Germination of pollen grains (%)	Beginning of spathe crashing	End of spathe crashing	Duration of spathe burst
Males													
Male 1	15	118	13.5	1.85	210	125	65	324.5	90.0	85.0	20/2	18/3	26
Male 2	18	100	10.0	1.50	195	130	80	280.5	91.0	87.0	22/2	18/3	24
Male 3	18	109	16.5	3.00	400	123	65	896.5	96.0	91.5	20/2	17/3	25
Male 4	17	130	18.0	3.00	417	128	62	795.5	95.0	90.5	19/2	18/3	24
Male 5	34	120	18.9	3.65	408	135	119	1630.0	100.0	100.0	5/2	15/3	37
Male 6	23	122	18.5	3.00	410	140	130	980.5	89.9	85.5	15/2	20/3	33
Male 7	30	79	18.5	2.75	274	140	108	1500.5	99.5	99.2	8/2	11/3	31
Male 8	15	90	14.5	1.85	275	125	50	308.0	92.0	88.0	13/2	22/3	37
Male 9	20	100	15.0	2.65	290	120	72	594.5	91.0	86.5	12/2	23/3	39
Male 10	18	116	13.5	2.65	397	87	62	410.5	93.5	90.0	14/2	21/3	35
Male 11	21	150	15.5	2.85	344	115	98	750.0	94.2	90.5	13/2	19/3	34
Male 12	29	122	17.8	3.10	417	120	100	1530.8	100.0	99.7	8/2	10/3	30
Male 13	28	111	18.5	3.50	490	120	126	1550.0	99.0	99.0	10/2	12/3	30
Male 14	33	119	19.0	3.25	497	138	130	1602	98.5	98.0	5/2	10/3	33
Male 15	19	100	15.5	2.25	390	160	125	395.0	95.0	94.5	14/2	17/3	31
Male 16	20	90	17.5	2.25	290	72	90	480.0	94.0	94.0	15/2	23/3	36
Male 17	27	90	16.0	1.85	258	138	72	600.0	95.5	91.5	14/2	18/3	32
Male 18	26	108	17.0	2.20	287	107	80	530.5	92.3	89.5	21/2	24/3	31
Male 19	19	95	17.0	1.95	195	116	71	321.5	93.5	89.5	20/2	21/3	29
Male 20	18	104	16.5	2.10	300	123	65	333.0	94.0	88.0	19/2	24/3	33

Table 4. General evaluation of selected male date palm seedlings.

Character	No. of spathe/palm	No. of flowers/palm	Start of spathe burst	Weight of pollen grains (g)/palm	Viability of pollens grains %	General evaluation of males
Index units specified	20	20	20	20	20	100 units
Male Seedling						
M1	8.8	4.6	5.0	3.9	18.0	40.3
M2	10.6	6.7	4.6	3.4	18.2	43.5
M3	10.6	5.5	5.0	11.0	19.2	51.3
M4	10.0	4.9	5.3	9.8	19.0	49.0
M5	20.0	18.9	20.0	20.0	20.0	98.9
M6	13.5	10.7	6.7	12.0	17.98	60.88
M7	17.7	15.1	12.5	18.4	19.9	83.6
M8	8.8	3.5	7.7	3.8	18.4	42.2
M9	11.8	6.7	8.3	7.3	18.2	52.3
M10	10.6	5.2	7.1	5.0	18.7	46.6
M11	12.4	9.6	7.7	9.2	18.8	57.7
M12	17.1	13.5	12.5	18.8	20.0	81.9
M13	16.5	16.5	10.0	19.0	19.8	81.8
M14	19.4	20.0	20.0	19.7	19.7	98.8
M15	11.2	11.1	7.1	4.9	19.0	53.3
M16	11.8	8.4	6.7	5.9	18.8	51.6
M17	15.9	9.1	7.1	7.4	19.1	58.6
M18	15.3	9.7	4.8	6.5	18.5	54.8
M19	11.2	6.3	5.0	3.9	18.7	45.1
M20	10.6	5.5	5.3	4.1	18.8	44.3

These units are the sum of the units presenting the inclusive evaluation of spathes number/palm, flowers number/palm, start and duration of spathes burst, pollen grains weight/spathe, pollen grains viability. These results are in agreement with those found by Mohamed (2004) and Diab (2012).

5- Fruit set percentage and fruit physical characteristics:

Data presented in Table (5) showed the average fruit set, fruit weight, flesh weight, seed weight, fruit length and fruit diameter of both Seewy and Barhee cultivars.

a. Fruit set (%):

The obtained data indicated that fruit set percentage was significantly affected by different male pollinators for both Seewy and Barhee cultivars in both seasons. Pollinizers No. 5, 12 and 14 gave the highest fruit set percentage as Seewy recorded (100, 100 and 100% for No. 5, 12 and 14,

respectively). Also, Barhee recorded 97.67, 98.17 and 99.17% from pollinizers No. 5, 12 and 14 respectively.

b. Fruit physical characteristics:

▪ **Fruit weight:**

The same trend was noticed in Seewy fruit weight (18.5, 19.17 and 19.54 g for pollinizers No. 5, 12 and 14 respectively). Meanwhile, Barhee gave the highest fruit weight 14.26, 14.05 and 14.74g from pollinizer trees No. 12, 13 and 14 respectively.

▪ **Flesh weight:**

It was noticed from the obtained data that during the two seasons in both Seewy and Barhee cultivars the flesh weight exhibits similar trends for the fruit weight.

▪ **Seed weight:**

The data of seed weight indicated that there were significant differences in both

Table 5. Effect of pollinizer male on fruit set and fruit physical characteristics of both Seewy and Barhee date palm cultivars during 2011 and 2012 seasons.

Treatments (A)	Fruit Set (%)		Fruit Weight (g)		Flesh Weight (g)		Seed Weight (g)		Fruit Length (cm)		Fruit Diameter (cm)	
	1 st season	2 nd season										
Control	74.03	74.96	4.05	4.24	4.00	4.18	0.09	0.11	2.73	2.86	1.58	1.62
M5	98.17	98.83	15.81	15.98	14.89	14.99	1.42	1.49	3.66	3.87	2.26	2.35
M7	97.23	98.43	14.92	15.77	14.11	14.86	1.31	1.41	3.76	3.87	2.21	2.31
M12	98.58	99.08	16.07	16.71	15.23	15.78	1.33	1.44	4.08	4.14	2.29	2.46
M13	96.65	97.53	15.25	16.24	14.51	15.32	1.24	1.42	3.94	4.06	2.19	2.31
M14	99.01	99.58	16.78	17.14	15.98	16.08	1.31	1.56	4.20	4.29	2.52	2.63
New L.S.D. (A)	4.71	3.26	5.93	6.77	3.66	5.11	0.28	0.61	0.42	0.43	0.56	0.45
Cultivars (B)	95.46	95.78	15.67	16.55	14.43	15.12	1.23	1.43	3.86	3.97	1.89	2.03
Seewy (B1)	92.43	93.70	11.96	12.14	11.80	11.95	1.00	1.04	3.59	3.72	2.46	2.53
Barhee (B2)	2.72	1.88	3.42	3.91	2.11	2.95	0.16	0.35	0.24	0.25	0.32	0.26
New L.S.D. (B)	74.50	75.83	5.21	5.50	5.10	5.38	0.11	0.12	3.07	3.19	1.64	1.66
Control × B1	73.57	74.09	2.89	2.99	2.89	2.99	0.07	0.09	2.39	2.53	1.52	1.59
Control × B2	100.00	100.00	18.31	18.50	16.63	16.69	1.68	1.81	3.59	3.72	1.88	1.97
M5 × B1	96.33	97.62	13.31	13.15	13.15	13.28	1.15	1.17	3.72	3.96	2.64	2.73
M5 × B2	99.43	99.70	16.76	18.18	15.33	16.59	1.43	1.60	4.00	4.06	1.93	2.07
M7 × B1	95.03	97.17	13.07	13.35	12.88	13.13	1.19	1.22	3.51	3.67	2.48	2.54
M7 × B2	100.00	100.00	18.10	19.17	16.62	17.94	11.47	1.62	4.17	4.23	1.84	2.15
M12 × B1	97.17	98.17	14.03	14.26	13.84	14.01	1.19	1.25	3.98	4.04	2.73	2.77
M12 × B2	98.83	99.13	16.66	18.43	15.36	16.83	1.30	1.60	4.01	4.17	1.84	2.02
M13 × B1	94.47	95.83	13.84	14.05	13.65	13.80	1.19	1.25	3.87	3.94	2.53	2.60
M13 × B2	100.00	100.00	18.95	19.54	17.54	17.70	1.41	1.84	4.34	4.42	2.19	2.32
M14 × B1	98.02	99.17	14.61	14.74	14.41	14.46	1.20	1.28	4.07	4.16	2.86	2.95
M14 × B2	6.66	4.61	8.38	9.57	5.17	7.23	0.40	0.87	0.60	0.61	0.79	0.64
New L.S.D. (A×B)												

seasons. The control in Barhee cultivar gave the lowest seed weight, but Barhee seed weight for pollinizer No. 5 gave the lowest seed weight (1.15 and 1.17 g in the first and second seasons, respectively).

These results agreed generally with those found by Nasr *et al.* (1982); Osman and Soliman (2002) and Diab (2012).

▪ **Fruit length:**

Regarding the effect of different male pollinators on fruit length, data in Table (5) and Fig. (1) indicated that pollinizers No. 7, 12, 13 and 14 gave the highest values of fruit length in both seasons for Seewy cultivar.



Fig. 1. Effect of pollinizers on fruits of both Seewy and Barhee cultivars as well as control (un-pollinated) fruits.

Osman and Soliman (2002) reported that fruit length was affected by type of pollen; these data are in partial agreement with those reported by Soliman (1999) on Samany and Sakkoty cultivars.

▪ **Fruit diameter:**

Also data in Table (5) showed also that significant differences in fruit diameter in both seasons. Pollinizer No. 14 gave the highest fruit diameter in both seasons for both Seewy and Barhee cultivars. These results agree with those found by Soliman (1999), Osman and Soliman (2002) and Diab (2012).

6- Fruit chemical characteristics:

Data presented in Table (6) showed the average moisture percentage, dry matter, total soluble solids (TSS), reducing, non-reducing sugars and total sugars, total acidity and tannins percentage.

a. Moisture (%):

Regarding the moisture percentage, the data obtained indicated that there were significant differences among all treatments for Seewy and Barhee cultivars in both two seasons. Pollinizer No. 7 gave the highest moisture percentage for the Barhee cultivar in both two seasons.

Table 6. Effect of pollinizer male on fruit chemical characteristics of both Seewy and Barhee date palm cultivars during 2011 and 2012 seasons.

Treatments	Moisture (%)		Dry matter (%)		TSS (%)		Total Sugars (%)		Reducing Sugar (%)		Non-Reducing Sugar (%)		Total Acidity (%)		Tanins (%)	
	1 st season	2 nd season														
Control	40.60	39.58	59.49	58.75	24.00	25.52	29.47	30.29	23.96	25.28	5.52	5.19	0.64	0.62	2.61	2.79
M5	39.41	40.72	60.59	59.28	43.79	44.56	75.32	76.41	64.41	65.38	10.91	11.03	0.44	0.42	1.48	1.55
M7	40.93	41.95	59.07	58.05	51.51	43.04	72.87	73.40	61.71	62.36	11.00	11.03	0.47	0.46	1.65	1.70
M12	39.47	40.19	60.53	59.81	48.73	49.55	78.68	79.94	67.62	68.85	11.06	11.09	0.41	0.37	1.44	1.49
M13	38.23	38.76	61.77	61.24	42.42	44.59	73.20	74.54	62.04	63.36	11.16	11.18	0.50	0.49	1.81	1.85
M14	38.44	39.08	61.56	60.92	46.88	47.82	76.85	78.34	65.66	67.07	11.20	11.27	0.41	0.38	1.28	1.30
New L.S.D. (A)	2.30	2.53	1.60	2.11	8.46	9.35	3.77	3.27	16.45	17.56	5.13	4.88	0.22	0.21	0.56	0.58
Cultivars	30.42	31.41	69.61	68.59	44.66	46.15	68.96	70.04	51.85	52.63	17.11	17.47	0.39	0.37	1.46	1.52
(B)	48.61	48.68	51.39	50.76	37.79	38.87	66.50	67.59	63.28	64.80	3.16	2.79	0.57	0.55	1.96	2.05
New L.S.D. (B)	1.33	1.46	0.93	1.22	4.88	5.40	2.18	1.89	9.50	10.14	2.96	2.81	0.13	0.12	0.32	0.34
Control × B1	23.23	23.72	76.93	76.28	25.30	27.63	30.33	31.19	22.37	23.23	7.97	8.33	0.65	0.63	2.79	2.90
Control × B2	57.96	55.45	42.04	41.22	22.70	23.40	28.61	29.38	25.55	27.33	3.06	2.05	0.63	0.60	2.42	2.69
M5 × B1	30.47	32.23	69.53	67.77	48.60	49.37	76.45	77.75	57.93	58.60	18.53	19.15	0.32	0.31	1.10	1.17
M5 × B2	48.35	49.21	51.65	50.79	39.98	39.74	74.19	75.06	70.90	72.16	3.29	2.90	0.55	0.54	1.86	1.92
M7 × B1	31.76	33.17	68.24	66.83	46.70	48.80	75.07	76.13	56.21	56.99	18.86	19.14	0.37	0.35	1.30	1.34
M7 × B2	50.10	50.73	49.90	49.27	36.32	37.28	70.67	70.67	67.20	67.74	3.13	2.92	0.58	0.57	2.00	2.07
M12 × B1	33.70	34.74	66.30	65.26	51.43	51.93	79.70	80.60	60.70	61.36	19.00	19.24	0.29	0.27	1.16	1.21
M12 × B2	45.25	45.64	54.75	54.36	46.03	47.17	77.65	79.27	75.54	76.33	3.11	2.94	0.52	0.48	1.72	1.78
M13 × B1	30.10	30.67	69.90	69.33	46.47	48.43	74.18	75.48	55.04	56.07	19.14	19.41	0.41	0.39	1.46	1.48
M13 × B2	46.37	46.85	53.63	53.15	38.37	40.74	72.22	73.60	69.05	70.66	3.17	2.95	0.59	0.58	2.15	2.22
M14 × B1	33.23	33.96	66.77	65.04	49.43	50.73	78.03	79.11	58.85	59.56	19.18	19.55	0.28	0.25	0.97	1.00
M14 × B2	43.64	44.20	56.36	55.30	44.33	44.90	75.67	77.57	72.46	75.58	3.21	2.98	0.55	0.52	1.58	1.61
New L.S.D. (A×B)	3.26	3.58	2.27	2.38	11.96	13.23	5.33	4.63	23.26	24.84	7.25	6.89	0.31	0.30	0.79	0.82

These data are in partial agreement with those reported by Soliman (1999) and Osman and Soliman (2002).

b. Dry matter (%):

Data showed that significant differences in dry matter content percentage in both seasons. Pollinizer No. 13 gave the highest dry matter percentage (69.9 and 69.3% in both two seasons, respectively) for Seewy cultivar.

c. Total soluble solids (TSS %):

Total soluble solids percentage was significantly increased by different male pollinators in both seasons. Pollinizer No. 12 gave the highest values for Seewy cultivar in both seasons (51.43 and 51.93%, respectively). These results are in agreement with Osman and Soliman (2002) and Diab (2012) who found that total soluble solids percentage significantly increased by different male pollinators.

d. Reducing sugars (%):

The obtained data in Table (6) indicated that the reducing sugars percentage was significantly affected by different male pollinators in both seasons. Pollinizer No. 12 gave the highest values of reducing sugars % in Barhee cultivar (74.54 and 76.33, respectively) in both seasons. These data are in partial agreement with Soliman (1999) on Samany and Sakkoty cultivars and Osman and Soliman (2002).

e. Non-reducing sugars (%):

The non-reducing sugars percentage increased significantly by different male pollinators in the two seasons. Pollinizer No. 14 gave the highest non-reducing sugars (19.18 and 19.55%, for Seewy cultivar). These results are partially in agreement with Osman and Soliman (2002) and Diab (2012).

f. Total sugars (%):

Concerning the effect of different male pollinators on total sugars percentage, data in Table (6) showed a significant increase by different male pollinators. Pollinizer No. 12 gave the highest values of total sugars

percentage in Seewy cultivar of both two seasons (79.70 and 80.60%). These results are partially in agreement with Soliman (1999) and Osman and Soliman (2002).

g. Total acidity (%):

The obtained data indicated that pollinizer No. 13 produced the highest values in both seasons (0.59 and 0.58%) for Barhee cultivar. The present data are in partial agreement with those reported by Osman and Soliman (2002), Diab (2012) and Abo-Rekab *et al.* (2013).

h. Tannins content (%):

Concerning the effect of different male pollinators on tannins %, data indicated that pollinizer No. 13 gave the highest values of tannins percentage in two seasons for Barhee cultivar (2.15 and 2.22%, respectively). These results are in agreement with those reported by Abo-Rekab *et al.* (2013).

Data in Table (7) showed the harvesting date, bunch weight and yield/palm. The obtained data indicated that pollinizer No. 12 induced early harvesting for Barhee cultivar compared with the control and Seewy cultivar. Pollinizer No. 7 gave the highest bunch weight and yield/palm in both two seasons for both two cultivars (Seewy and Barhee). These results are in agreement with those established by Marzouk *et al.* (2002) and Abo-Rekab *et al.* (2013).

7- Identification of ISSR-PCR markers:

ISSR was a class of molecular markers based on inter-tandem repeats of short DNA sequences. These inter repeats were highly polymorphic, even among closely related genotypes due to the lack of functional constraints in these non-functioning regions. Similarly a high level of polymorphism was generated utilizing the two ISSR-PCR primers. A total number of 41 ISSR bands were obtained; of these 41 bands, 23 bands were polymorphic (56.1%) and 18 were monomorphic (43.9%). The highest number of applicants was generated. Polymorphism levels differed from one primer to the other.

Table 7. Effect of pollinizer type on harvesting date, bunch weight kg/palm and yield/palm (kg) of both Seewy and Barhee date palm cultivars during 2012 and 2013 seasons.

Pollinizer	Bunch weight (kg)						Harvesting date						Yield/palm (kg)									
	Seewy		Barhee		Seewy		Barhee		Seewy		Barhee		Seewy		Barhee		Seewy		Barhee			
	1 st season	2 nd season																				
Control	1.13	1.19	2.00	2.20	12 Sept.	13 Sept.	21 Aug.	23 Aug.	21 Aug.	23 Aug.	11.30	11.90	20.00	22.00	11.30	11.90	20.00	22.00	11.30	11.90	20.00	22.00
M5	15.00	15.50	18.80	17.00	24 Aug.	26 Aug.	11 Aug.	10 Aug.	11 Aug.	10 Aug.	150.00	155.00	188.00	190.00	150.00	155.00	188.00	190.00	150.00	155.00	188.00	190.00
M7	16.00	16.40	22.00	23.00	25 Aug.	26 Aug.	10 Aug.	13 Aug.	10 Aug.	13 Aug.	160.00	164.00	220.00	230.00	160.00	164.00	220.00	230.00	160.00	164.00	220.00	230.00
M12	13.80	14.10	21.50	22.00	21 Aug.	23 Aug.	9 Aug.	11 Aug.	9 Aug.	11 Aug.	138.00	141.00	215.00	220.00	138.00	141.00	215.00	220.00	138.00	141.00	215.00	220.00
M13	13.92	14.50	21.80	22.20	27 Aug.	29 Aug.	8 Aug.	10 Aug.	8 Aug.	10 Aug.	139.20	145.00	218.00	222.00	139.20	145.00	218.00	222.00	139.20	145.00	218.00	222.00
M14	13.50	14.63	21.70	22.10	7 Sept.	9 Sept.	13 Aug.	14 Aug.	7 Sept.	9 Sept.	135.00	146.30	217.00	221.00	135.00	146.30	217.00	221.00	135.00	146.30	217.00	221.00

Both primer HB-08 and HB-09 generated 33.33% and HB-11 generated 60.0% which exhibited high polymorphisms. On the other hand, primer HB-15 exhibited low polymorphism (12.5%). The numbers of total amplified fragments, polymorphic, monomorphic and unique bands for each primer for each of five male pollinizers using the five primers are shown in Table (8). There were some specific fragments

discriminated each five male pollinizers from the others as follows: primer HB-08 did not show any specific fragments. Primer HB-09 showed two specific fragments as positive marker. Primer HB-11 showed two specific fragments as one positive marker and the other was negative marker. Primer HB-13 showed one specific fragment as positive marker. Primer HB-15 showed three specific fragments, all of them as positive markers.

Table 8. Total number of bands, polymorphic bands, monomorphic bands and unique band as well as polymorphic % of the 5 selected male palms.

Primer name	Total band	Polymorphic band	Monomorphic band	Unique band	Polymorphic %
HB-08	9	3	6	--	33.33
HB-09	6	2	2	2	33.33
HB-11	10	6	2	2	60.00
HB-13	8	3	4	1	37.50
HB-15	8	1	4	3	12.50
Total	41	15	18	8	

8- Genetic similarity and cluster analysis based on ISSR markers:

The ISSR data were used to estimate the genetic similarity among the five male pollinizers by using UPGMA. Computer analysis are presented in Table (9) and Fig. (2). The highest similarity index (1.0) was recorded between male pollinizer No. 12 and No. 14, while no similarity was detected (0.0) between male pollinizer No. 12 and No. 13. A dendrogram for the genetic relationships among the five male pollinizers was drawn in Fig. (3). The results of ISSR are in harmony with Younis *et al.* (2011) and Abo-Rekab (2013).

Table 9. Genetic similarity matrices among the five male pollinizers accessions as computed according to Dice coefficient from ISSR.

	M5	M7	M12	M13
M7	0.47			
M12	0.96	0.64		
M13	0.80	0.83	0.64	
M14	0.67	0.86	1.0	0.0

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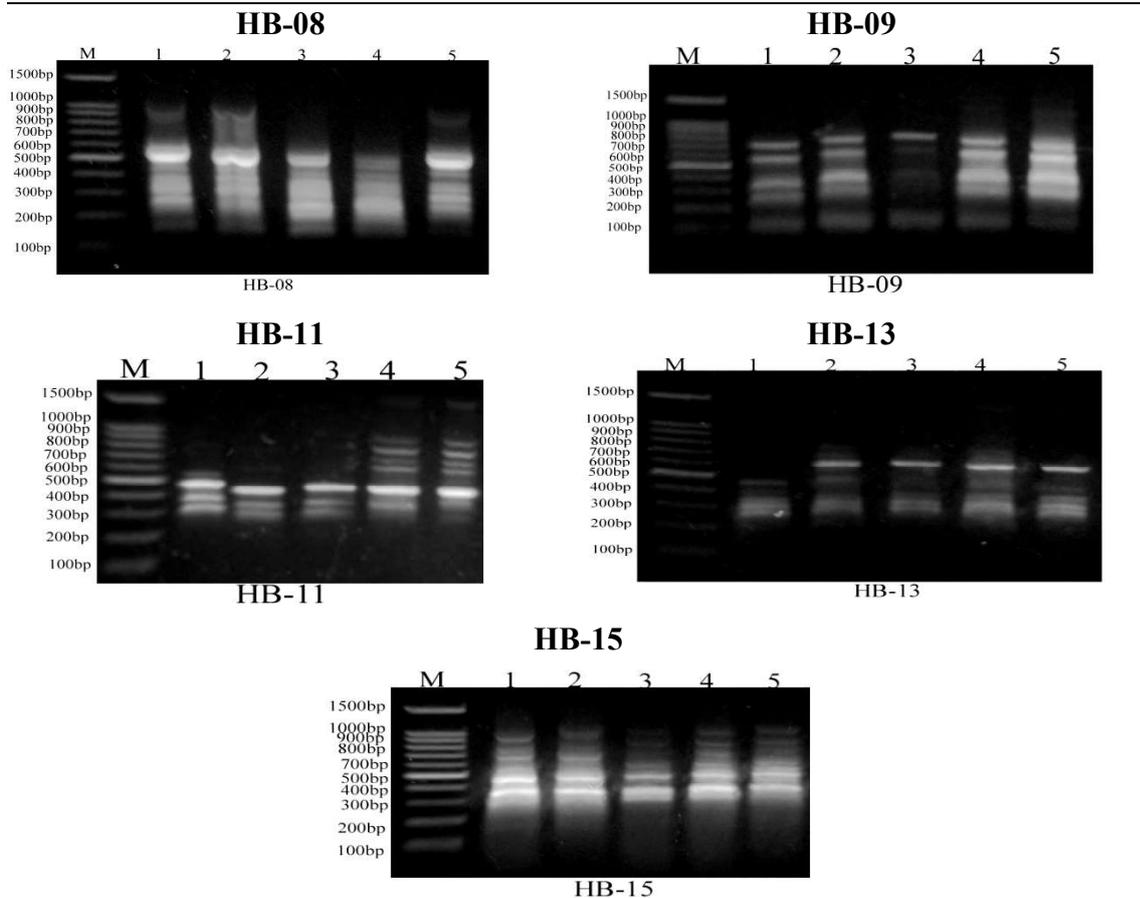


Fig. 2. ISSR products generated with primers HB-08, HB-09, HB-11, HB-13 and HB-15 in five pollinizers.

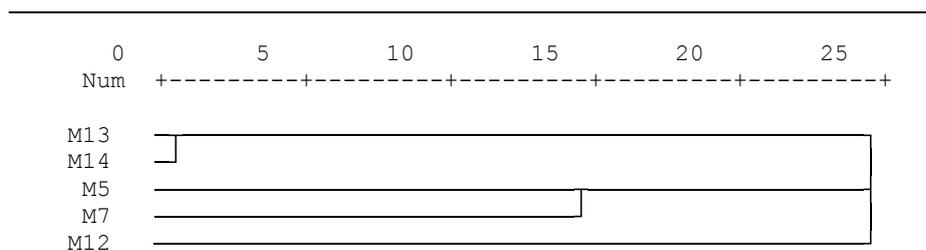


Fig. 3. Dendrogram for the five male pollinizers accessions constructed from the ISSR data using unweightedpair-group arithmetic (UPGMA) and similarity matrices computed according to Dice coefficient.

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إنتخاب أفضل الآباء المذكرة لنخيل البلح لتحسين كلاً من المحصول وبعض صفات الجودة للثمار وتمييز التشابه الوراثي بين سلالات الذكور باستخدام الصفات الطبيعية وتقنية ISSR
زينب أمين محمد أبو ركاب*، طارق محمد حسن الكفراوي** و غادة عادل على**
* معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر.
** المعمل المركزي لأبحاث وتطوير نخيل البلح، مركز البحوث الزراعية، الجيزة، مصر.

أجرى هذا البحث خلال ثلاث سنوات (٢٠١١، ٢٠١٢ و ٢٠١٣) في واحة سيوة بمحافظة مطروح ومركز طامية بمحافظة الفيوم. وقد اشتمل هذا البحث على الآتي: أولاً تقييم ٢٠ نخلة مذكرة تم اختيارها لانتخاب الأفضل منها وقد أوضحت النتائج انتخاب خمسة ذكور والتي تم انتخابها باستخدام التقييم الرقمي والذي اشتمل على عدد الأغريض التي ينتجها الذكر – عدد الأزهار التي يحملها الذكر – موعد خروج أول أغريض والمدة التي يستغرقها الذكر لخروج جميع أغريضه – وزن حبوب اللقاح – والنسبة المئوية لحبوب اللقاح.

وقد تمت هذه التجربة في واحة سيوة وبناءً على هذا التقييم الرقمي أوضحت النتائج أن الملقح رقم (٥)، رقم (١٤) (٩، ٨، ٩٨، ٨ درجة على التوالي) ورقم (٧) (٦، ٨٣ درجة) ورقم (١٢) و (١٣) (٩، ٨١، ٨١، ٨ درجة على التوالي) حيث أنها سجلت أعلى الدرجات حيث تم استخدامها كأباء ملقحة في الجزء الثاني من البحث. أوضحت النتائج أن الخمسة ملقحات المنتخبة زادت كمية المحصول وجودة الثمار في صنف السيوي والبرحي خلال موسمي البحث (٢٠١٢ و ٢٠١٣) باستخدام ٣ مكررات من النخيل المؤنث لكل صنف بمركز طامية محافظة الفيوم وقد أوضحت النتائج إختلاف الذكور في مواعيد النضج حيث أن الملقح رقم (٧) أدى إلى زيادة المحصول وكمية الرطوبة. أما الملقح رقم (١٢) فقد أدى إلى التبريد في النضج في كلا من صنف السيوي و البرحي كذلك أدى لزيادة محتوى الثمار من السكريات الكلية والمواد الصلبة الذائبة في صنف السيوي. أما السلالة المذكرة رقم (١٣) فقد ساعدت على زيادة النسبة المئوية للمادة الجافة. كذلك أوضحت النتائج أن الملقح رقم (١٤) سجل أعلى معدل لأبعاد الثمار وكذلك وزن الثمار واللحم.

أما الملقح رقم (٥) فقد سجل أقل وزن بذرة / ثمرة خلال موسمي الدراسة. وقد استخدمت تقنية الـ ISSR لتمييز التشابه الوراثي بين الخمسة سلالات المذكرة المنتخبة. وقد أوضحت النتائج أن هناك تشابهاً عالياً جداً بين الملقح رقم (١٢) والملقح رقم (١٤) (١٠٠%) بينما لم يكن هناك تشابهاً مطلقاً بين الملقح رقم (٧) والملقح رقم (١٣) حيث أن درجة التشابه بينهما (صفر %).