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Morphological, Physicochemical, and Molecular Evaluation of Twenty-Three Date Palm Males Growing in Aswan Governorate

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Abstract: An essential element of date palm production is the pollen source. The present study was conducted during two successive seasons of 2020 and 2021 to evaluate some males of Aswan date palm. Herein, morphological, physicochemical, and molecular characteristics of 23 date palm males were evaluated to determine the most superior and promising males for pollinating the female 'Bartamoda' cultivar. It is obvious that 'Male No. 22', recorded almost the highest value for morphological characteristics, pollen viability, crude protein, total amino acids, and mineral content. In contrast, 'Male No. 5', showed the lowest content of crude protein, total amino acids, and mineral content compared to the other males that were grown in the rest of the locations in Aswan government. All ISSR primers were highly polymorphic, with a value of 100%, highlighting the necessity of employing such primers when investigating date palm diversification. Two unique bands were among the 43 total bands that were produced. The genetic coefficients with a mean of 61.5% and the first two primary axes were able to explain roughly 52.30% of the genetic variance across the genotypes of date palms. Those results suggested that all genotypes tested are efficient for the fertilization of 'Bartamoda' inflorescences and can be used as pollinizer in commercial orchards of this cultivar.

Keywords: Bartamoda, Date palm, ISSR, *Phoenix dactylifera* L., Pollen, Viability.

Introduction

Date palm (*Phoenix dactylifera* L.) is the most important fruit in Egypt. It belongs to the family Arecaceae, which has about 200 genera and more than 2,500 species (Johnson, 2011). Date palm is a dioecious, perennial, and monocot plant, it contains 18 chromosomes (2n = 36). Its genome has recently been sequenced, showing a length of 671 Mb and including 41,600 genes (Al-Mssallem *et al*., 2013). Since the date palm is a dioecious plant, it has male and female trees, which are usually cross-mechanically pollinated by humans for commercial production and better fruit quality (Ghnaim & Al Muhtaseb, 2006). In Egypt, the number of fruitful female palms is about 14,379,648 produced 1770603 million tons and planted in approximately 134126 feddan according to (FAO, 2022).

The most important dry date varieties are Bartamoda, Barakawi, Abrimi, Sakouti, Gondaila, Gargoda, and Shamia. These varieties grow well in Aswan and Qena

Governorates where the heat requirements are adequate. The pollen source is a key factor in determining the quality of date palm fruit. New date palm males are needed for domestic and international markets because different sources usually have different pollen viability (Bacha *et al*., 1997**)**. The new genetic resources of date palms are important for crop improvement programs in different climate conditions. The term "viability" refers to the capacity of the pollen to germinate and grow normally (Stanley & Linskens, 1974). High-viable pollen will improve the fruit set and, ultimately, will produce a higher yield. *In vitro* germination is a valuable technique to determine the viability of the pollen grains and to identify the best pollinators for the pollination process (Ibrahim *et al*., 2014). The male palm trees pose a particular problem; they result from seeds and often constitute unique genotypes. The quality of pollen varies from one male palm tree to another. Males are highly variable in their growth, vigor, flowering time, spathe characteristics, and pollen quality Therefore, it is of vital importance to select superior males for crossing and breeding purposes (Moustafa *et al*., 2010).

Molecular markers are commonly used in plant genetic relationships and diversity because they are not affected by the environment (Mirzaei, 2021). The advent of various molecular techniques has led breeders, based on data produced by markers, to estimate genetic diversity among genotypes. Currently, molecular markers have been employed along different loci of any genome that produce a high level of polymorphism, such as random amplification of polymorphic DNA (RAPD) (Mgendi *et al*., 2010; Abubakar *et al*., 2011), inter simple sequence repeat (ISSR) (Crespel *et al*., 2009; Verma & Rana, 2011), simple sequence repeat (SSR) (Zhang *et al*., 2006; Crespel *et al*., 2009; Kumar-Ganesan *et al*., 2014), amplified fragment length polymorphism (AFLP) (Muluvi *et al*., 1999; Yang *et al*., 2013), restriction fragment length polymorphism (RFLP) (Eiadthong *et al*., 2000), sequence-related amplified polymorphism (SRAP) (Li & Quiros, 2001), target region amplification polymorphism (TRAP) (Hu & Vick, 2003), conserved region amplification polymorphism (CoRAP) (Wang *et al*., 2009), and start codon targeted polymorphism (SCoT) (Collard & Mackill, 2009). Several studies have been reported on the application of RAPD or ISSR marker techniques for studying genetic relatedness and diversity analysis with different date palm genotypes in different regions (Haider, 2017; Srivashtav *et al*., 2013; Mustafa *et al*., 2014). The ISSR marker system detects polymorphisms in inter-microsatellite DNA regions without any prior sequence knowledge (Zietkiewicz *et al*., 1994). Primers are based on a repeat sequence and amplify the sequence between two microsatellites, resulting in many amplification products per primer, offering good reproducibility at a low cost. Molecular characterization is an important step in tracking the success of the cultivars studied, which would help to introduce, select, and improve the existing date palm varieties. Moreover, genetic evaluation of date palm cultivars in various regions is crucial for date palm production, mainly due to the great diversity of cultivars and hybrids available for planting.

The goal of this study is twofold: (a) to evaluate the morphological, chemical, and genetic characteristics of 23 date palm males grown in Aswan government; and (b) to let date palm growers know which of them is promised and suited for pollinating date palm cultivars and for breeding purposes as well.

Materials & Methods

Plant materials

This study was conducted during two growing seasons of 2020 and 2021 using 23 date palm males grown in different sites in Aswan governorate, Egypt (Table 1). Three healthy and uniformly vigorous male date palms from each site were selected to be used in this study as each male was considered a replicate. The most cultivated date cultivar in Aswan (cv. 'Bartamoda') was used to determine genetic relatedness and genetic similarity with the selected males. All selected date palms were grown in sandy soil, irrigated by the drip irrigation system, and received normal cultural practices. Mature male inflorescences were cut immediately after the breaking of the spathe and kept in paper bags, and later transferred to a shaded and moisture-free area for drying. Spathe was removed carefully, and bunches were spread over a newspaper. Bunches were frequently changed from paper to paper to avoid moisture logging. After one day of drying, the strands were separated out from the rachis and again spread over newspaper for further drying. To avoid any possible mixing of pollen, each sample was dried separately. The pollen grains were separated from the strands using a sieve, and then the pollen grains were transferred to stopper bottles and stored in a dry place at room temperature. Three mature spathes were randomly collected from each male tree at the flowering time (middle of March to the end of April), and brought immediately to the Central Laboratory of Date Palm Research and Development, Agricultural Research Center, Giza, Egypt, where the following characteristics were measured and recorded:

Morphological and physical characteristics

Spathes

The average weight of the spathe (g)

The average length of the spathe (cm)

The average width of the spathe (cm)

Inflorescences

The average length of the inflorescence for each male (cm)

Strands

Ten strands from each inflorescence that represent each male were separated to determine the average length of the strand (cm), the average number of strands per inflorescence, and the average number of flowers per strand

Pollen characteristics

Average pollen weight per spathe was recorded according to Moustafa *et al*. (2010).

Determination of pollen viability by staining and germination tests

Staining test: the dehiscent pollens were lightly tapped on a drop of 1% acetocarmine stain on a slide. Three slides were made for each pollen source. Ten microscopic fields were randomly chosen for each slide, and pollen grains were counted and graded as viable or aborted in two classes. The pollen grains that were darkly stained and round were considered viable, while those that were lightly stained or shrunk were considered aborted. The following formula calculated the pollen viability percentage according to (Iqbal *et al*., 2011)**:**

$$\text{Viability \%} = \frac{\text{Viable pollens}}{\text{Total pollens}} \times 100$$

Pollen germination test: The pollen germination medium used was prepared in distilled water (pH 7.0), 10% sucrose, boric acid 150 ppm, and 2.5 g L^{-1} gelrite in Petri dishes, and Petri dishes were placed in an incubator at 25°C for 24 hours. Pollen germination was examined under a microscope (Nikon ECLIPSE 90i) according to (Moustafa *et al*., 2010). The pollen germination percentage was determined according to the following equation:

$$\text{Germination \%} = \frac{\text{Germinated pollens}}{\text{Total pollens}} \times 100$$

Chemical properties of pollen

Proteins content

Crude protein (N × 6.25) was determined according to the A.O.A.C. (1984).

Amino acids content

The determination of total amino acids (TAA) by ninhydrin reaction was accomplished by 1ml of the sample, which was extracted during the soluble protein estimation, reacting with 1ml of 10% pyridine and 1ml of 2% ninhydrin solution. The optical densities of the colored solutions were then read at 570 nm using a spectrophotometer (Hamilton & Van Slyke, 1943).

Minerals content

Iron (Fe) and zinc (Zn) concentrations were determined by digesting 0.1 g of dried pollen grain materials with sulphuric acid and hydrogen peroxide, as described by Harangozó& Královič, (1996). Calcium (Ca), magnesium (Mg), and potassium (K) concentrations were determined by atomic absorbance spectrophotometry according to the AOAC. (2000).

Molecular analysis using ISSR marker

DNA extraction

Fresh young leaf samples of 23 date palm males along with the female 'Bartamoda' cultivar (Table 1) were collected in April 2021. Collected leaf samples were immediately stored in liquid nitrogen until DNA extraction. DNA of date palm leaves was extracted according to Arif *et al*. (2010) as follows: About 100 mg of a fresh leaf of date palm was put in a sterile mortar with about 50 mg of Sterile sand and 500 µL of lysis buffer (Trizma (1.21 g) + Na_2EDTA (0.4 g) + CTAB (2.0 g) NaCl (8.12 g) PVP (2.0 g) pH8/100 ml). The leaf sample was crushed and allowed to dry at room temperature for about 5 min. The crushed sample was transferred into a 1.5 mL Eppendorf tube and kept in a water bath at 60 °C for 30 min. After that, the tube was centrifuged at 9,500 g for 5 min using a Hettich centrifuge (D-78532 Tuttlingen, Germany). About 200 µL of supernatant was transferred to a new tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The tube was shaken gently from top to bottom for 5 min followed by centrifugation at 9,500 g for 5 min. 200 µL of supernatant was transferred to a new tube and sodium acetate (3.0 M; 20 µL) and 500 µL of cold isopropanol was added. The tube was centrifuged at 11,500 g for 10 min. The supernatant was discarded and 500 µL of 70% cold ethanol and centrifuged at 7,000 g for 5 min. the supernatant was discarded, and the tube contents were air-dried at room temperature. DNA was eluted with 100 µL of TE buffer (1 M Tris base and 0.5M EDTA pH8) and kept at 4 °C for further use. DNA quality was checked by means of absorbance ratios A_{260}/A_{280} through a UV-spectrophotometer where DNA is pure with a ratio A_{260}/A_{280} from 1.8-2.0. Moreover, DNA qualitative check was done using

electrophoresis in 1% agarose gel with ethidium bromide.

ISSR primers, polymerase chain reaction (PCR), and gel electrophoresis conditions

A total of eight highly polymorphic ISSR primers were used to perform the date palm molecular analysis as indicated in table (2). The DNA amplifications were performed in a thermocycler (MJ Research, PTC-200). PCR conditions included one cycle of 95 °C for 5 min, followed by 45 cycles of 95 °C for 1 min, 50.5 °C for 45 sec., and 72 °C for 45 sec. Then, 72 °C for 7 min. Finally, 4 °C for overnight. PCR reaction (25 µL) contained 12.5 µL of Master mix (3 mM $MgCl_2$, 30 mM KCl, and 10 mM Tris, (pH 8.3), 2 µL ISSR primer (25 pmol), 2 µL extracted DNA (100-250 ng). The PCR product was electrophoresed in 1.5% agarose gel in 1x TBE buffer at 120 V for 1 hour and stained with ethidium bromide (0.5 $\mu l.ml^{-1}$) (Sambrook *et al.*, 1989). The fragments were photographed using a UV transilluminator (Cleaver Scientific Ltd.). Standard DNA (1 Kbp) was utilized to control fragment sizes and primer runs on two independent gels.

Data analysis

All data were statistically analyzed using analysis of variance (ANOVA) according to Snedecor & Cochran (1989) in a completely randomized design with three replicates per treatment using the statistical package SPSS. Any two means are significantly different if their difference exceeds the LSD value at a 5% significance threshold. Based on the electrophoresis of ISSR results, the amplified bands were recorded for "1" presence or "0" absence. The NTSYS-pc2.02 software (Rohlf, 2004) was used to make the unweighted pair group method of the arithmetic averages (UPGMA) dendrograms, the principal coordinate analysis (PCA), and the similarity matrix, which shows the relationships between genotypes.

Table (1): List of date palm males included in this study and their localities.

Genotype	ID	Site in Aswan	Geographic location
Male 1	M1	El Alfeya	24°02′46″N: 33°04′00″E
Male 2	M2	El Eman	24°05′19″N: 32°53′20″E
Male 3	M3	Antar	24°05′19″N: 32°53′20″E
Male 4	M4	Hager El Arab	24°05′16″N: 32°54′45″E
Male 5	M5	El Aqula	24°05′19″N: 32°53′20″E
Male 6	M6	El Qarah	24°05′18″N: 32°53′20″E
Male 7	M7	Kom Mir	24°12′29″N: 32°38′10″E
Male 8	M8	El Masry	24°05′38″N: 32°54′03″E
Male 9	M9	El Kharaza	24°05′02″N: 32°53′37″E
Male 10	M10	El Awadly	24°05′21″N: 32°53′44″E
Male 11	M11	El Bosaileya	24°04′58″N: 32°54′15″E
Male 12	M12	Kelh El Gabal	24°06′06″N: 32°53′20″E
Male 13	M13	El Ramady	24°06′21″N: 32°53′05″E
Male 14	M14	Abou El Rish	24°06′22″N: 32°53′59″E
Male 15	M15	El Mansoureya	24°05′16″N: 32°53′32″E
Male 16	M16	El Gaafrah	24°05′43″N: 32°52′14″E
Male 17	M17	Baharef	24°05′34″N: 32°54′04″E
Male 18	M18	Daraw	24°24′13″N:32°55′53″E
Male 19	M19	Ballanah	24°21′13″N: 32°56′51″E
Male 20	M20	El Mafalsa	24°04′48″N: 32°53′37″E
Male 21	M21	Hager Edfou	24°58′41″N: 32°52′28″E
Male 22	M22	El Qarah	24°05′18″N: 32°53′20″E
Male 23	M23	El Sheikh Amer	24°05′23″N: 32°52′48″E
Bartamoda*	Bartamoda*	El Alfeya	24°02′46″N: 33°04′00″E

Table (2): List of the ISSR primer names and their nucleotide sequences used in the study.

Primer name	Sequence (from 5` to 3`)	Reference
ISSR-01	5'-$(AG)_8$YC-3'	Ibrahim *et al*. (2020)
ISSR-02	5'-$(AG)_8$YG-3'	
ISSR-03	5'-$(AC)_8$YT-3'	
ISSR-04	5'-$(AC)_8$YG-3'	
ISSR-05	5'-$(GT)_8$YG-3'	
ISSR-06	5'-CGC$(GATA)_4$-3'	
ISSR-07	5'-GAC$(GATA)_4$-3'	Aldhahrani & Althobaiti (2019)
ISSR-08	5'-$(AGAC)_4$GC-3'	

Results & Discussion

Morphological and physical characteristics

Spathes

Data in table (3) showed that all studied spathes were significantly different in their morphological and physical characteristics. The average weight of the spathes of thedifferent male types from (Male No. 6 and Male No. 22) was the highest in both seasons, while the lowest value was for the (Male No. 5) in both seasons. It was clear that (Male No. 22) had the highest average spathe length in both seasons when compared to the other males in Aswan governorate. On the contrary, (Male No. 5) gave the lowest spathe length in both studied seasons. Moreover, the data revealed that the (Male No. 22) had the biggest average spathe width of all the other males grown in the other sites. Meanwhile, (Male No. 5) gave the lowest average width in both seasons.

Inflorescences

Physical characteristics of date palm male types significantly differed in inflorescence properties as presented in table (4). The average length of inflorescence in different male types was highest for the male selected from El Qarah region (Male No. 22), followed by the male selected from Hager Edfou (Male No. 21) in both seasons. Whereas the lowest value was recorded for the male from El Aqula region (Male No. 5) in both seasons

Strands

Table (4) also cleared that strands characteristics significantly differed among all date palm types. The (Male No. 22) produced the highest average strand length in both experimental seasons, followed by (Male No. 21). In the meantime, (Male No. 5) had the lowest strand lengths than those other male types in both seasons. Also, the higher number of strands per inflorescence and the number of flowers per strands were recorded by the (Male No. 22), and the lowest for a (Male No. 5) in both seasons.

Pollen characteristics

The recorded data in (Table 5) of both seasons clearly showed that the male of El Qarah region (Male No. 22) recorded the highest values of pollen weight per spathe in the first and second seasons. Meanwhile, the other males had an intermediate average pollen grain weight with no significant difference between each other in most cases.

Table (3): Physical characteristics of spathes of date palm males grown in Aswan governorate during 2020 and 2021 seasons

Site in Aswan/Genotype	Spathe weight (g)		Spathe length (cm)		Spathe width (cm)	
	2020	2021	2020	2021	2020	2021
El Alfeya/Male 1	886.00	909.33	41.23	42.71	16.33	17.35
El Eman/Male 2	935.00	996.67	52.37	53.69	16.84	17.84
Antar/Male 3	920.67	977.67	46.34	47.82	16.62	17.48
Hager El Arab/Male 4	752.67	781.33	40.04	40.91	15.23	16.31
El Aqula/Male 5	503.33	561.33	37.41	38.54	11.35	12.37
El Qarah/Male 6	2170.00	2483.33	55.76	56.29	21.25	22.39
Kom Mir/Male 7	1118.33	1949.67	42.68	43.61	17.53	18.56
El Masry/Male 8	1171.98	1216.67	51.26	52.37	17.63	18.68
El Kharaza/Male 9	713.00	745.00	59.33	59.86	15.04	16.10
El Awadly/Male 10	982.00	726.67	56.27	56.97	17.24	18.32
El Bosaileya/Male 11	1334.33	1419.33	51.34	52.65	17.82	18.91
Kelh El Gabal/Male 12	817.67	878.00	40.17	30.96	16.11	17.23
El Ramady/Male 13	1399.33	1706.00	54.92	55.17	19.93	20.95
Abou El Rish/Male 14	829.33	895.00	62.35	63.52	16.21	17.25
El Mansoureya/Male 15	1591.00	1644.33	53.26	54.67	19.63	20.66
El Gaafrah/Male 16	1468.00	1554.33	53.16	54.40	18.14	19.21
Baharef/Male 17	762.67	796.00	53.00	53.60	16.00	17.06
Daraw/Male 18	1425.33	1520.00	52.88	53.93	18.10	19.20
Ballanah/Male 19	403.33	1500.16	52.68	53.46	18.00	19.07
El Mafalsa/Male 20	1788.00	1835.67	63.71	64.43	20.00	21.06
Hager Edfou/Male 21	1866.67	1983.33	63.92	64.85	20.50	21.21
El Qarah/Male 22	2068.33	2225.00	65.02	65.89	25.80	26.53
El Sheikh Amer/Male 23	1982.67	2072.33	61.35	62.89	20.18	21.64
L.S.D	**180.59**	**178.45**	**4.16**	**5.95**	**0.22**	**0.15**

Determination of pollen viability by staining and germination tests

Data in table (5) indicated that the viable pollen percentage of fresh pollen by staining was significantly higher (Male No. 9 and Male No. 22, respectively), giving the maximum percentage in the first season as compared with the other male types. Concerning the second season, (Male No. 22) also recorded 100% of pollen viability by the stained method. Also, the obtained viability percentage for the 23 males by the germination method recorded higher values (Male No. 22) compared to other males in both experimental seasons. These findings are consistent with those of other researchers who suggested that the wide variation in the morphological and physical traits of the investigated male types could be caused by variations in genetic characteristics or by environmental factors (Al-Hamoudi *et al*., 2006; Moustafa *et al*., 2010; Abo-Rekab *et al*., 2014; Aly, 2018).

Table (4): Physical characteristics of inflorescences and strands of date palm males grown in Aswan governorate during 2020 and 2021 seasons

Site in Aswan/Genotype	Inflorescence				Strands			
	Length (cm)		Length (cm)		No. of strands		No. of flowers/	
	2020	2021	2020	2021	2020	2021	2020	2021
El Alfeya/Male 1	20.16	21.33	19.58	20.64	141.33	152.33	52.67	56.33
El Eman/Male 2	26.45	27.52	22.15	23.17	156.33	168.00	55.00	57.33
Antar/Male 3	25.93	26.97	20.10	21.20	150.00	162.00	54.67	56.33
Hager El Arab/Male 4	19.60	20.63	14.34	19.41	115.00	125.67	45.00	46.67
El Aqula/Male 5	18.43	19.25	14.15	15.26	106.67	116.67	40.00	43.33
El Qarah/Male 6	28.63	29.73	25.39	26.52	218.00	234.67	114.00	119.00
Kom Mir/Male 7	25.63	26.73	19.84	20.91	166.00	173.67	60.33	64.67
El Masry/Male 8	26.02	27.31	20.37	21.43	172.79	181.33	61.98	68.00
El Kharaza/Male 9	29.64	30.66	25.68	26.75	112.00	123.33	43.00	45.33
El Awadly/Male 10	29.51	30.59	25.59	26.67	160.33	141.00	58.33	62.33
El Bosaileya/Male 11	26.09	26.22	21.58	22.64	176.33	187.00	66.67	73.00
Kelh El Gabal/Male 12	20.14	21.11	19.11	20.17	128.00	135.33	48.33	50.67
El Ramady/Male 13	28.33	29.31	25.26	26.32	202.67	212.67	85.00	92.00
Abou El Rish/Male 14	30.37	31.78	26.08	27.21	137.33	148.00	50.33	55.33
El Mansoureya/Male 15	28.07	29.22	24.63	25.74	199.00	214.00	79.33	85.33
El Gaafrah/Male 16	27.91	28.84	24.42	25.51	195.33	205.67	72.67	76.33
Baharef/Male 17	27.66	28.72	23.81	24.91	116.33	124.00	46.33	49.00
Daraw/Male 18	27.25	19.76	23.69	24.83	188.00	194.33	47.33	78.67
Ballanah/Male 19	26.67	27.83	23.11	24.33	183.00	195.83	46.67	74.27
El Mafalsa/Male 20	30.72	31.81	26.43	27.64	204.67	212.33	96.00	101.33
Hager Edfou/Male 21	31.53	32.73	27.18	28.27	207.67	221.33	102.33	108.00
El Qarah/Male 22	32.63	33.93	28.15	29.25	239.00	248.00	119.67	127.33
El Sheikh Amer/Male 23	30.53	31.63	25.81	26.93	211.33	211.00	110.33	114.67
L.S.D	4.13	5.15	1.82	0.06	20.85	20.27	20.12	20.82

Chemical properties of pollen

Proteins content

Male No. 22, had the highest protein percentage (35.30 g.100 g^{-1} DW), as shown in table (6). On the other hand, the (Male No. 5) recorded the remarkably lowest protein percentage. However, there were significant differences between those males and the other males that were grown in the other locations. The value of crude protein content in date palm pollen was found in the literature to range between 12 and 16% by Human & Nicolson (2006). Also, according to Campos *et al*. (2008), the protein content of pollen grains varied between 10 and 40g.100 g^{-1} dry weight. Moreover, the protein content of date palm pollen was 31.11g.100g^{-1} of the dry weight of the pollen grains Furthermore, these results are in agreement with previous results reported by Bujang *et al*. (2021).

Table (5): Weight of the pollen grains per spathe and pollen viability of date palm males grown in Aswan governorate during 2020 and 2021 seasons.

Site in Aswan/Genotype	Pollen weight/spathe (g)		Pollen viability			
			Stained (%)		Germinated (%)	
	2020	2021	2020	2021	2020	2021
El Alfeya/Male 1	31.38	33.61	98.67	99.00	93.33	94.67
El Eman/Male 2	31.92	34.57	98.67	99.67	91.33	93.33
Antar/Male 3	31.74	33.94	98.00	99.33	81.67	84.67
Hager El Arab/Male 4	30.26	32.23	99.67	100.00	97.33	97.67
El Aqula/Male 5	28.31	30.72	99.33	99.67	65.67	95.66
El Qarah/Male 6	50.26	52.63	98.67	99.67	96.67	98.00
Kom Mir/Male 7	32.72	35.44	97.33	97.00	91.33	95.33
El Masry/Male 8	33.63	45.74	99.08	99.33	93.20	92.67
El Kharaza/Male 9	29.89	31.84	100.00	99.33	96.00	98.33
El Awadly/Male 10	32.18	35.14	97.00	98.33	95.33	97.33
El Bosaileya/Male 11	33.58	36.93	96.67	98.67	94.33	95.33
Kelh El Gabal/Male 12	30.65	32.93	99.00	100.00	93.00	97.33
El Ramady/Male 13	40.80	42.64	99.67	96.00	85.67	89.33
Abou El Rish/Male 14	30.94	33.26	99.67	100.00	91.00	92.00
El Mansoureya/Male 15	38.14	40.35	97.67	98.33	97.00	97.67
El Gaafrah/Male 16	35.45	38.07	97.33	97.67	94.67	97.33
Baharef/Male 17	30.58	32.83	96.33	97.00	95.67	96.00
Daraw/Male 18	34.72	37.25	99.33	69.33	97.00	97.33
Ballanah/Male 19	34.05	37.47	98.33	99.33	95.00	95.65
El Mafalsa/Male 20	41.55	43.39	99.00	99.00	97.00	97.33
Hager Edfou/Male 21	42.83	46.39	99.67	99.33	96.00	97.67
El Qarah/Male 22	53.96	55.72	100.00	100.00	97.00	98.00
El Sheikh Amer/Male 23	47.76	50.53	98.67	99.33	96.00	97.33
L.S.D	5.02	5.35	NS	N.S	N.S	N.S

Amino acids content

Table (6) also represented the amino acid content of pollen grains of the different date palm types which significantly differed in the 2020 and 2021 seasons. Pollens of (Male No. 22) showed the highest value of total amino acids in both studied seasons. On the other hand, (Male No. 5) recorded the lowest value of total amino acids in both studied seasons. These results are in line with Bacha *et al*. (1997) and Abo-Rekab *et al*. (2014) who indicated that pollen grains' amino acids differed from one cultivar to another.

Minerals content

The obtained data (Table 6) showed that the pollens of (Male No. 22) had significantly higher values of iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), and potassium (K). The pollens of (Male No. 5) recorded considerably lower values of mineral content (Fe, Zn, Ca, Mg, and K) than those of other male types. Several authors, including Stanley (1971), Bacha *et al*. (1997), and Bujang *et al*. (2021), validated the findings on mineral content.

Molecular analysis using ISSR marker

Twenty-three date palm males were selected as candidate pollinators for the female

Bartamoda cultivar. ISSR-based-PCR approach was devoted to detecting the genetic polymorphisms and genetic distance of 23 date palm males and the female 'Bartamoda' cultivar. The data of the ISSR primers amplified results can be described as a total of 43 bands, with 100% of polymorphic bands, indicating the high ability of those primers to distinguish different date palm genotypes (Table 7). The number of fragments generated per primer varied between 3 and 7, and each of ISSR-01 and ISSR-08 produced one unique band. These markers are useful for genotyping date palms and could be employed in breeding schemes. The percent of polymorphism reported here was similar to that reported earlier using ISSR primers in date palm by Guettouchi *et al*. (2017), but higher than those reported by Srivashtav *et al*. (2013), Abo-Rekab *et al*. (2014), and Haider (2017),. Replication slippage might cause this polymorphism (Powell *et al*., 1996).

Table (6): Protein content, total amino acids (TAA), and minerals content of date palm males pollen grains grown in Aswan governorate

Site in Aswan/Genotype	Protein (g 100 g^{-1} DW)	TAA (g 100 g^{-1} protein)	Minerals content (mg 100 g^{-1} DW)				
			Fe	Zn	Ca	Mg	K
El Alfeya/Male 1	18.60	15.25	239.20	130.04	549.21	310.04	760.20
El Eman/Male 2	19.20	16.33	241.39	132.06	552.66	311.29	765.53
Antar/Male 3	18.78	15.70	240.23	131.12	551.34	310.54	763.21
Hager El Arab/Male 4	17.30	15.12	236.20	125.61	544.53	302.57	751.61
El Aqula/Male 5	13.22	11.17	230.19	120.17	538.71	298.13	745.31
El Qarah/Male 6	32.12	29.43	265.10	138.91	577.63	330.64	783.19
Kom Mir/Male 7	19.68	16.89	234.13	133.51	555.35	317.51	767.25
El Masry/Male 8	20.00	18.17	245.01	134.27	557.23	318.69	769.13
El Kharaza/Male 9	16.50	14.30	235.55	124.35	541.51	301.21	750.34
El Awadly/Male 10	19.45	16.10	242.63	133.02	554.40	315.51	766.40
El Bosaileya/Male 11	20.11	18.35	245.33	134.80	539.61	319.84	770.48
Kelh El Gabal/Male 12	18.09	15.82	238.21	128.71	547.35	306.52	757.18
El Ramady/Male 13	22.93	20.11	251.20	136.77	569.95	327.63	778.61
Abou El Rish/Male 14	18.55	16.20	238.65	129.81	548.62	308.51	659.81
El Mansoureya/Male 15	21.89	19.11	250.63	136.21	567.21	325.83	776.32
El Gaafrah/Male 16	20.70	18.83	250.23	135.46	565.61	324.65	774.30
Baharef/Male 17	17.65	14.93	237.63	126.20	546.60	304.23	754.11
Daraw/Male 18	20.20	18.40	249.36	135.43	563.75	321.28	773.12
Ballanah/Male 19	20.13	18.40	246.34	134.83	561.53	320.37	772.11
El Mafalsa/Male 20	27.23	25.30	252.52	137.02	571.37	328.55	779.35
Hager Edfou/Male 21	29.40	26.18	259.40	137.19	572.24	329.75	780.16
El Qarah/Male 22	35.30	32.11	280.63	139.80	579.81	331.04	785.36
El Sheikh Amer/Male 23	30.99	28.53	260.73	137.52	573.21	330.07	781.24
L.S.D	**1.05**	**1.55**	**0.65**	**0.18**	**0.28**	**0.20**	**0.14**

Table (7): Descriptive data of ISSR primers amplified results

Primer Name	Total scorable band	Polymorphic band	Unique band	Polymorphic %
ISSR-01	7	7	1	100%
ISSR-02	5	5	0	100%
ISSR-03	6	6	0	100%
ISSR-04	5	5	0	100%
ISSR-05	3	3	0	100%
ISSR-06	7	7	0	100%
ISSR-07	6	6	0	100%
ISSR-08	4	4	1	100%
Total	43	43	2	100%

The genetic similarity coefficients ranged from about 32% (between Male 5 from El Aqula and Male 15 from El Mansoureya) to about 91% (between Male 17 from Baharef and Male 18 from Daraw), with a mean equal to 61.5% (Table 8). These results were confirmed by the unweighted pair group method of the arithmetic averages (UPGMA) clusters and the principal coordinate analysis (PCA) analyses (Fig. 1A and B). These analyses illustrated that the 'Bartamoda' cultivar was most closely related to Male 1, which is located at the same location and is the farm's primary pollinator. Interestingly, there was a cluster group of date palm males closely linked to the Bartamoda cultivar and its main pollinator (Male 1). This group included eight males, namely Male 13, Male 15, Male 16, Male 17, Male 18, Male 19, Male 22, and Male 23 (Fig. 1A). It is also worth mentioning that Male 22 revealed the highest values in morphological and physicochemical analyses. The principal coordinate analysis (PCA) plot demonstrated the genetic relationship between the 23 male accessions of date palm and the female 'Bartamoda' cultivar based on their binary genetic distance (Fig. 1B). PCA generally produced similar results to those obtained by UPGMA. Based on the whole set of ISSR marker data, the first two principal axes explained about 52.30% of the total genetic variation among date palm genotypes, of which 20.89% was attributed to the first coordinate and 13.41% to the second coordinate. This study verified that the ISSR marker is a useful tool for date palm varietal identification, allowing all cultivars to be characterized accurately. This knowledge can assist significantly in the selection of optimal pollinizers and help to facilitate further progress in the strategy of date palm breeding.

Table (8): Genetic similarity matrix between 23 male accessions along with the 'Bartamoda' female cultivar based on ISSR markers. (See Table 1 for genotype identification)

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	Bartamoda
M1	1	0.633	0.712	0.56	0.48	0.643	0.528	0.528	0.607	0.549	0.667	0.549	0.698	0.737	0.667	0.655	0.7	0.721	0.7	0.767	0.63	0.702	0.679	0.813
M2		1	0.679	0.591	0.5	0.6	0.681	0.681	0.72	0.622	0.706	0.622	0.807	0.588	0.667	0.692	0.667	0.691	0.704	0.704	0.583	0.628	0.64	0.655
M3			1	0.558	0.698	0.816	0.783	0.739	0.653	0.727	0.8	0.636	0.679	0.64	0.638	0.588	0.679	0.704	0.679	0.566	0.553	0.64	0.653	0.667
M4				1	0.529	0.45	0.487	0.324	0.45	0.571	0.488	0.629	0.511	0.634	0.526	0.476	0.682	0.578	0.591	0.682	0.684	0.537	0.65	0.5
M5					1	0.7	0.595	0.487	0.35	0.457	0.585	0.457	0.426	0.439	0.316	0.381	0.546	0.489	0.5	0.409	0.474	0.439	0.45	0.458
M6						1	0.651	0.558	0.522	0.634	0.638	0.488	0.604	0.553	0.546	0.5	0.56	0.588	0.56	0.56	0.546	0.638	0.565	0.556
M7							1	0.7	0.605	0.632	0.682	0.579	0.64	0.546	0.585	0.622	0.553	0.625	0.596	0.468	0.39	0.591	0.558	0.549
M8								1	0.791	0.526	0.727	0.526	0.64	0.409	0.439	0.578	0.511	0.625	0.553	0.468	0.342	0.5	0.465	0.588
M9									1	0.537	0.681	0.488	0.642	0.426	0.546	0.625	0.56	0.628	0.52	0.56	0.409	0.553	0.478	0.556
M10										1	0.714	0.778	0.708	0.667	0.769	0.651	0.711	0.739	0.711	0.667	0.615	0.714	0.829	0.612
M11											1	0.714	0.704	0.625	0.622	0.612	0.706	0.808	0.745	0.628	0.489	0.583	0.638	0.727
M12												1	0.625	0.619	0.615	0.558	0.667	0.696	0.756	0.622	0.564	0.619	0.781	0.653
M13													1	0.741	0.784	0.836	0.772	0.828	0.807	0.737	0.667	0.778	0.793	0.721
M14														1	0.756	0.694	0.745	0.731	0.745	0.745	0.711	0.708	0.766	0.655
M15															1	0.739	0.708	0.735	0.708	0.667	0.619	0.8	0.818	0.539
M16																1	0.808	0.83	0.769	0.692	0.652	0.816	0.75	0.643
M17																	1	0.909	0.815	0.815	0.833	0.784	0.84	0.69
M18																		1	0.873	0.8	0.735	0.769	0.824	0.746
M19																			1	0.778	0.625	0.706	0.8	0.793
M20																				1	0.792	0.784	0.8	0.759
M21																					1	0.756	0.818	0.539
M22																						1	0.851	0.618
M23																							1	0.667
Bartamoda																								1

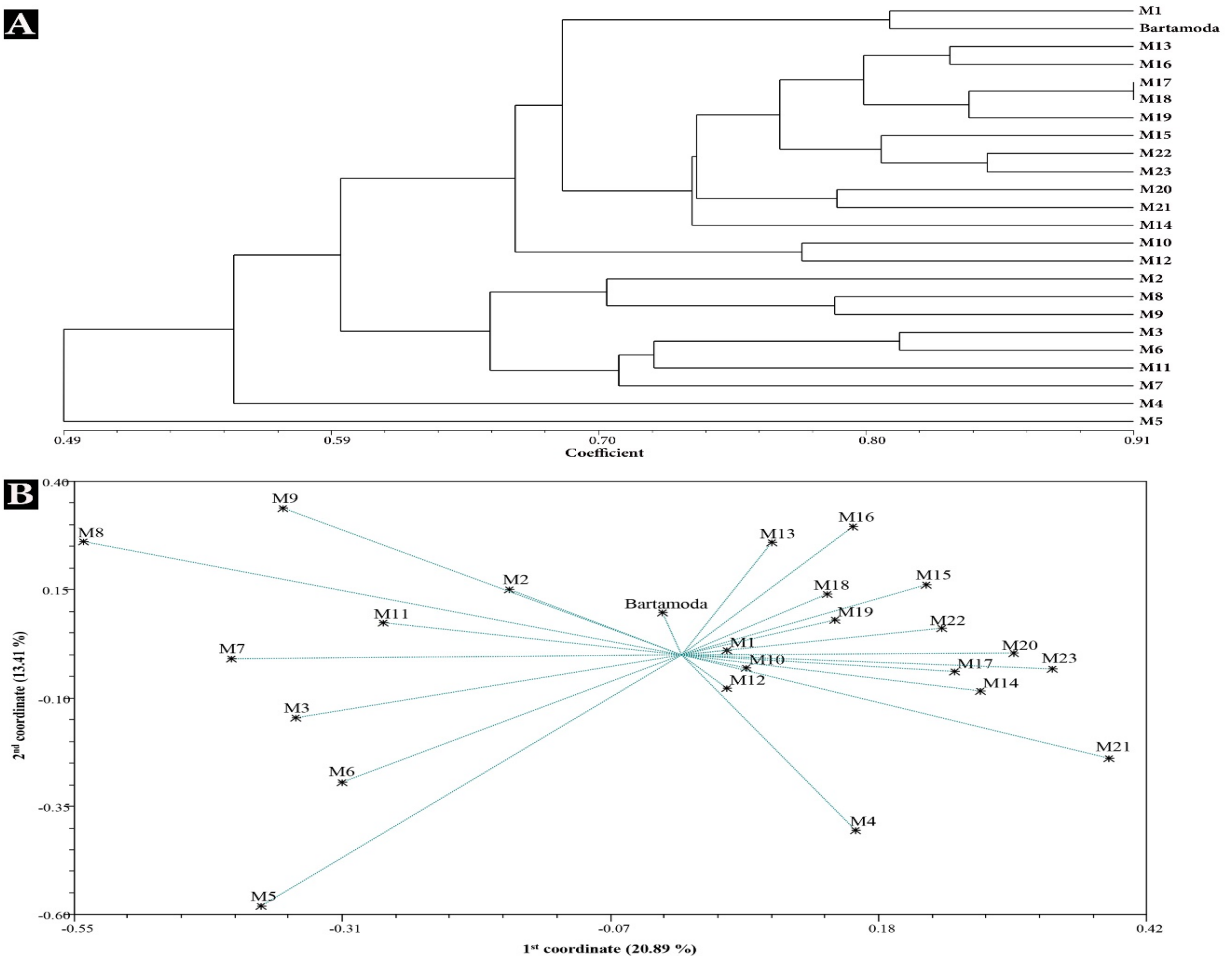

Fig. (1): Molecular evaluation of the studied date palm genotypes. A) UPGMA dendrogram shows the relationships among 23 male accessions and the 'Bartamoda' female cultivar based on the ISSR data. B) Principal coordinate analysis (PCA) plot centroids of the tested date palm males and the female cultivar 'Bartamoda' based on the first two principal coordinates. (See Table 1 for genotype identification).

Conclusions

From the results mentioned above, it is generally noticed that pollen viability and chemical composition differed extremely by the male sources studied in Aswan. The male from El Qarah region (Male No. 22) had the highest values for morphological characteristics, pollen viability, and chemical composition. Also, the ISSR used in this study exhibited a strong ability to distinguish all of the studied date palm genotypes with a high degree of polymorphism. Using eight ISSR primers allowed for the screening of 43 markers, with two specific markers as unique ISSR markers. The increased ability of the ISSR markers chosen for this study will help manage date palm germplasm. More research should be conducted on other cultivars of date palms using the promising pollinators identified in this study.

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Contributions of authors

S.E.: Supervision, conceptualization, methodology, review, and editing.

I.H.: Co-supervision, investigation, visualization, review, and editing.

E.G.G.: Resources, formal analysis, review.

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Conflicts of interest

The authors declare no conflict of interest.

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تقييم الخصائص المظهرية والفيزيائية والكيميائية والجزيئية لثلاثة وعشرون ذكرًا لنخيل التمر النامي بمحافظة أسوان

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المستخلص: مصدر حبوب اللقاح هو عنصر أساسي في إنتاج نخيل التمر. أجريت الدراسة الحالية خلال موسمين متتاليين من 2020 و 2021 لتقييم بعض ذكور نخيل البلح في أسوان. تم تقييم الخصائص المورفولوجية والفيزيائية والكيميائية والجزيئية لـ 23 ذكرًا لنخيل البلح لتحديد الذكور الأكثر تفوقًا والواعدة لتلقيح صنف "برتمودا". من الواضح أن "ذكر رقم 22" النامي بموقع القارة سجل أعلى قيمة للخصائص المورفولوجية وحيوية حبوب اللقاح والبروتين والأحماض الأمينية الكلية والمحتوى المعدني. في المقابل، أظهر "ذكر رقم 5" من موقع العاقولة أقل محتوى من البروتين، والأحماض الأمينية الكلية، والمحتوى المعدني مقارنة بالذكور الأخرى النامية في باقي مواقع محافظة أسوان. كانت جميع البادئات ISSR متعددة الأشكال بدرجة عالية، بقيمة 100٪، مما يبرز ضرورة إستخدام مثل هذه البادئات عند دراسة التنوع البيولوجي في نخيل التمر. كان هناك بندان فريدان من بين 43 باند تم إنتاجها. كان متوسط معامل التشابه الجيني يساوي 61.5٪ ، وأوضح المحورين الرئيسيين الأولين حوالي 52.30٪ من التباين الجيني الكلي بين الطرز الوراثية لنخيل البلح. تشير نتائج هذه الدراسة إلى أن جميع الطرز الوراثية المختبرة فعالة في إخصاب النورات الزهرية لصنف "برتمودا" ويمكن إستخدامها كملقحات في البساتين التجارية لهذا الصنف.

الكلمات المفتاحية: برتمودا، نخيل التمر، *Phoenix dactylifera* L.، ISSR، حبوب اللقاح، الحيوية.