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GENETIC CHARACTERIZATION OF SOME DATE PALM (*PHOENIX DACTYLIFERA*) MALE TREES USING RAPD AND ISSR MARKERS

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Abstract

The current study aimed to investigate genetic diversity among four selected date palm males (Hayani, Meghal, Fardh and Ghannami). Study of genetic diversity was performed using ISSR and RAPD markers of pollens genome. The total number of amplified ISSR bands ranged from 4 to 13 fragments. The highest number of bands was obtained using Primer (HB-15), while the lowest number was obtained by Primer (844-B). The percent of polymorphism revealed by different ISSR primers ranged from 61.53 to 90 % with an average of 76.97 %. While the total number of amplified RAPD bands ranged from 6 to 12 fragments. OP-A09 primer produced the highest number of fragments (12) and OP-D05 primer generated the lowest number (6). The percentage of polymorphism revealed by different RAPD primers ranged from 36.36 to 91.66 % with an average of 61.15%. Five ISSR and three RAPD primers produced unique bands. The maximum number of unique bands was identified in Ghannami and Hayani (8 bands). The genetic similarity index ranged from 0.026 to 0.622. The highest genetic similarity was between Hayani and Fardh male while the lowest was detected between Hayani and Ghannami males.

Key words: Male palm trees, Genetic diversity, Molecular markers, Fingerprint, RAPD, ISSR.

Introduction

Date palm (*Phoenix dactylifera* L.) has a special significance for its economic, historic and social values in Egypt. It is widely cultivated in arid regions of the Middle East and North Africa (Al-Khayri, 2001). Artificial pollination is necessary for successful date palm fruiting, source of pollens is one of the most important factors affecting production and fruits quality of date palm cultivars. There is a direct effect of pollens type on fruit set, yield, fruit physical and chemical characteristics (Farag *et al*., 2012). Most of the available pollinating date palm males are mainly originated from seed propagation, and varied greatly in blooming date and pollen quality (Maryam *et al*., 2016). Selection of suitable male parent as pollinators is important for improving the quantity and quality of dates (Rizk *et al.,* 2007). Therefore, it is important to select and identify superior male palm tree as a standard pollen source for date palm growers. Development of suitable genetic markers of date palm genotypes would be of a major importance in improvement programs and genetic conservation (Al-Khalifah and Skari, 2003). A variety of morphological and biochemical markers have earlier been employed for identification of date genotypes however, these traits are greatly influenced by environment and the developmental stages of the plant (Gomez-Vidal *et al*., 2008 and Salem *et al.*, 2001). DNA markers represent an efficient tool for estimating the genetic variability and the genetic relationships among closely related genotypes of date palm (Hussein *et al*., 2005). The random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR) and microsatellites have been employed for germplasm characterization of different date palm cultivars (Abdulla and Gamal, 2010, Aladadi *et al*., 2018, González *et al*., 2002 and Mirbahar *et al*., 2014). The RAPD analysis will help to resolve the ambiguity regarding the identity of narrowly-distinguishable cultivars and to assess genetic diversity for the conservation of date palm germplasm in Saudi Arabia (Al-Khalifah *et al*., 2012) and Egypt (Soliman *et al*., 2003) ISSR is believed to be one of the most efficient techniques that reveal high polymorphism and determines genetic diversity in date palm (Zehdi *et al*., 2004, Munshi and Osman, 2010, Hussein *et al.,* 2005, Adawy *et al.,* 2004 and Karim *et al*., 2010 and Elmeer *et al*., 2017).

Table 1: List of ISSR and RAPD primers and sequence used in this study.

Primer Name ISSR	Sequence	Primer Name RAPD	Sequence
844-A	$(CT)_8AC$	OP-A01	CAGGCCCTTC
844-B	$(CT)_8GC$	OP-A09	GGGTAACGCC
17898-A	$(CA)_6AC$	OP-A10	GTGATCGCAG
17898-B	$(CA)_6GT$	OP-A18	AGGTGACCGT
HB-9	$(GT)_6GG$	OP-D05	TGAGCGGACA
HB-15	$(GTG)_3GC$	-	-

Table 2: ISSR fragments for the four palm tree pollens based on the six ISSR primers.

Primer	Size of fragments (bP)	Total number of bands	Mono-morphic bands	Poly-morphic bands	Poly-morphism %
44-A	1789 – 300.5	9	1	8	88.88
844-B	605.7 – 326.3	4	1	3	75.00
17898-A	732.8 - 262.9	8	2	6	75.00
17898-B	881.8 – 217.7	7	2	5	71.42
HB-9	1843.3 – 508	10	1	9	90.00
HB-15	2280.2 – 316.8	13	5	8	61.53
Total	-	51	12	39	-
Average	-	8.5	2	6.5	76.97 %

Table 3: ISSR fragments for the four palm tree pollens based on the six ISSR primers.

Primer	Size of fragments (bP)	Total number of bands	Mono-morphic bands	Poly-morphic bands	Poly-morphism %
OP-A01	549.8 – 261.7	11	7	4	36.36
OP-A09	616.6 – 229.3	12	1	11	91.66
OP-A10	596.6 – 221.5	8	4	4	50
OP-A18	641.6 – 322.4	9	2	7	77.77
OP-D05	241.9 – 144.1	6	3	3	50
Total	-	46	17	29	-
Average	-	9.2	3.4	5.8	61.15%

The aim of this study was the use of ISSR and RAPD markers to estimate of genotypic diversity between some male palm trees namely (Hayani, Meghal, Fardh and Ghannami).

Materials and Methods

Plant materials

The current study was carried out during the period from 2016 to 2017 on four male palm trees namely (Hayani, Meghal, Fardh and Ghannami), the studied genotypes were propagated by offshoots. Pollen grain samples were collected from mature trees during April. Samples of Hayani was collected from a private orchard in Bilqas, Dakahlia Governorate while the other genotypes were collected from a private orchard located at (64 Km) of Cairo, Alex desert road. Samples analysis was carried out in the Biotechnology Laboratory of the Horticulture Research Institute, Agriculture Research Center, Egypt.

DNA Extraction

Total genomic DNA was isolated from 200mg fresh pollen grain samples using the DNeasy Plant Mini Kit following the manual instructions (Qiagen© Germany).

ISSR and RAPD Amplification Conditions

PCR reactions were conducted using five RAPD (OP-A01, OP-A09, OP-A10, OP-A18 and OP-D05) and six ISSR (844-A, 844-B, 17898-A, 17898-B, HB-9 and HB-15) primers (Table 1). Amplification was conducted in 25 µl reaction volume containing 2.5 µl of dNTPs (2.5 mM), 2.5 µl $MgCl_2$ (2.5 mM) and 2.5 µl of 10x buffer, 3.0 µl of primer (10 pmol), 3.0 µl of template DNA (25 ng/µl), 1 µl of Taq polymerase (1U/µl) and 12.5 µl of sterile dd H_2O. The reaction was performed on thermo cycler (TC-512© Techne UK) programmed as follows; one cycle initial denaturation step at 94°C, for 4 min. followed by (35 cycles for ISSR and 40 cycles for RAPD) of denaturation step for 1 min. at 94°C, annealing step for 1 min. at 57°C and extension step for 2 min. at 72°C then final extension step for 12 min. at 72°C for one min. The PCR products were separated on 1.5% agarose gels and fragments sizes were estimated with the 50 and 100 bp ladder markers (Thermo Fisher Scienific, USA). Bands were detected using Bio Rad Gel Doc™ XR + imaging system with Image Lab™ (USA).

Data analysis

Bands were precisely measured by Gel documentation System software and scored for each genotype. Data were then computed with the SPSS-16 program to produce a genetic distance matrix which assesses the similarity on the basis of the number of generated bands using Dice similarity coefficient (Sokal and Sneath, 1963).

Results and Discussion

ISSR-PCR Analysis

ISSR analysis of the palm tree pollens genomic DNA is shown in (Fig. 1). The total number of amplified bands among tested primers ranged from 4 to 13 fragments. The highest number of bands was obtained using Primer (HB-15), while the lowest number of bands was obtained by Primer (844-B). The size of the amplification products ranged from 217.7 to 2280.2 bps. All the tested primers

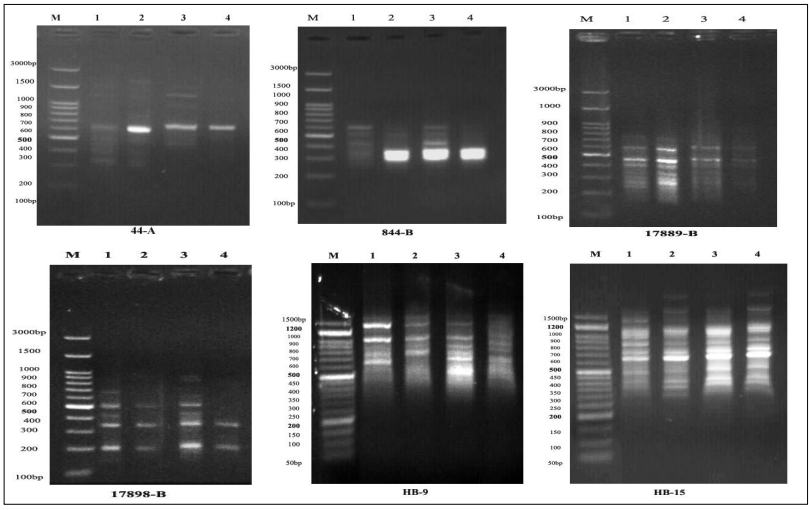

Fig. 1: DNA polymorphism of the four palm tree pollens amplified with six primers using ISSR-PCR (M) DNA ladder marker (bP), 1. Hayani, 2. Meghal, 3. Fardh and 4. Ghannami.

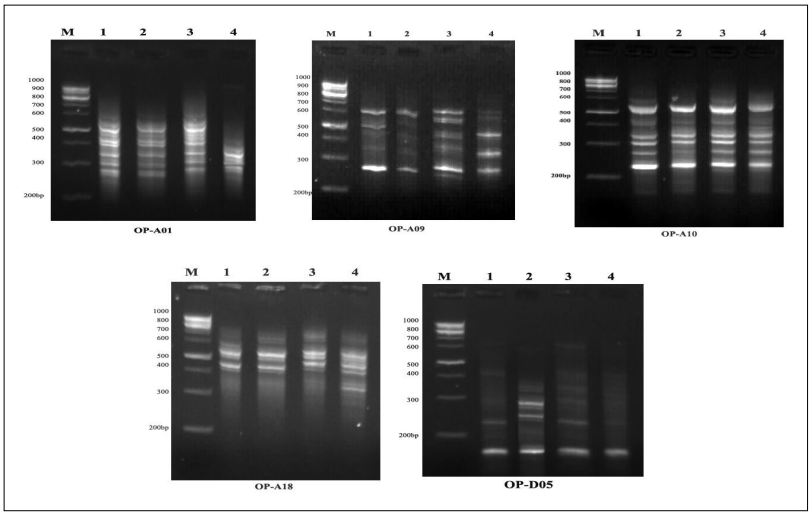

Fig. 2: DNA polymorphism of the four palm tree pollens amplified with six primers using RAPD-PCR (M) DNA ladder marker (bP), 1. Hayani, 2. Meghal, 3. Fardh and 4. Ghannami.

produced polymorphic bands (Table 5) of the total 51 scorable fragments, 39 were polymorphic among the genotypes, while the total number of monomorphic bands was 12. The number of polymorphic bands ranged from 3 to 9 resulting in an average of polymorphism prer primer of (6.5). Primers HB-9 revealed the highest number of polymorphic bands (9) conversely, the lowest number of polymorphic bands (3) was generated by 488-B. The percent of polymorphism revealed by different primers ranged from 61.53 to 90% with an average of 76.97%.

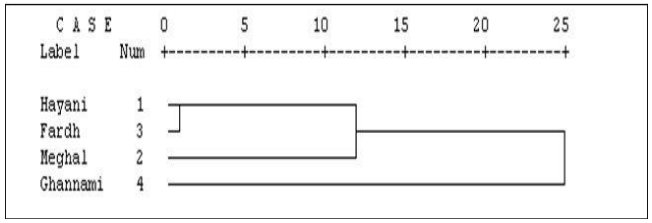

Fig.3: Dendrogram of correlation similarity using average linkage (Within Groups) among the different four male date palm cultivars based on molecular markers results.

RAPD-PCR Analysis

The preliminary screening of the five RAPD primers (OP-A01, OP-A09, OP-A10, OP-A18 and OP-D05) produced polymorphic amplification pattern (Fig. 2). The total number of amplified amplicons among tested primers ranged from 6 to 12 fragments. OP-A09 primer produced the highest number of fragments (12 bands). However, OP-D05 primer generated the lowest number (6 bands). The average number of fragments per primer was (9.2) and the approximate size of these fragments ranged from 144.1 to 641.6 bps. All the tested primers produced polymorphic bands of the total of 46 scorable fragments, 29 of the accessions were polymorphic (Table 3). The number of polymorphic bands varied between 3 and 11 producing an average of (5.8) of polymorphism/primer. The percentage of polymorphism revealed by different primers ranged from 36.36 to 91.66% with an average of 61.15%.

Genotype identification by unique ISSR and RAPD markers

The genotype-specific unique bands of both ISSR and RAPD markers with approximate size are shown in (Table 4) out of the tested primers, 5 ISSR primers and 3 RAPD primers were able to generate unique bands that could differentiate the studied date palm genotypes. However, 844-B, OP-A01 and OP-D05 primer failed to produce any unique marker. The total number of generated unique bands ranged from 2 to 5 bands in ISSR and 2 to 6 RAPD. The maximum number of unique markers was identified in Ghannami and Hayani with 8 bands. However, Meghal were characterized by 6 unique bands.

Dendrogram for the genetic relationship

The genetic similarity ranged from 0.026 to 0.622 (Table 5). The highest genetic similarity revealed by the ISSR and RAPD was between Hayani and Fardh male (0.622) this was followed by Fardh and Meghal palm tree males (0.528), while the lowest similarity index was detected (0.026) between Hayani and Ghannami males.

Table 4: Genotype identification of palm tree pollens by unique positive ISSR and RAPD markers.

	Unique positive ISSR			Unique positive RAPD		
Palmx Males	**Primer**	**Size in bp**	**Total**	**Primer**	**Size in bp**	**Total**
Hayani	44-A	377.2	5			3
	17898-A	732.8		OP-A09	526.8	
	17898-B	657.7			492.5	
	HB-9	927.8		OP-A18	586.4	
	HB-15	1365.7				
Meghal	44-A	1789.0	4			2
	17898-A	677.7		OP-A09	427.6	
	HB-9	1843.3		OP-A18	636.5	
	HB-15	316.8				
Fardh	44-A	1046.7	4		537.9	3
	17898-B	881.8		OP-A09	229.3	
		611.9		OP-A18	462.1	
	HB-9	564.4				
Ghannami	HB-9	508.0	2		602.5	6
				OP-A09	555.6	
				OP-A10	319.4	
	HB-15	549.7		OP-A18	395.3	
					403.7	
					322.4	

A dendrogram for the genetic relationship among the four palm tree pollens was carried out as illustrated in (Fig. 3) which separated cultivars into two groups. The first group included Ghannami male only, while the second group was divided into sub clusters, the first group included Meghal and the second sub cluster included Hayani and Fardh. It is clear that the Hayani and Fardh are closer related than the Meghal this was followed Ghannami palm tree male. As previously reported morphological descriptors have traditionally been used to characterize and distinguish the different accessions (Andrés- Agustin *et al*., 2006 and Pérez de oteyza *et al.,* 1999). However, cultivar identification based on phenotypic traits is labor intensive and can be inaccurate due to the influence of the environment (Zhang *et al.,* 2012). Therefore, molecular markers are being increasingly used to optimize plant genetic resource management, molecular techniques based on DNA markers have proven much more reliable for genetic characterization (Mahar *et al*., 2011). Both RAPD and ISSR markers were efficient in detecting genetic relationship among date palm cultivars. The variation detected among the closely related genotypes indicates the efficiency of DNA markers over the morphological and isozyme markers for the identification and construction of genetic linkage maps (Al-Khalifah *et al*., 2003, Soliman *et al*., 2003, Younis *et al*., 2008).

Table 5: Genetic similarity matrix detected between four palm tree pollens with molecular markers based on spss analysis.

Genotype	Hayani	Meghal	Fardh	Ghannami
Hayani	1.000			
Meghal	0.622	1.000		
Fardh	1.000	0.528	1.000	
Ghannami	0.026	0.034	0.126	1.000

Conclusion

We can come to a conclusion that, molecular markers can be used to differentiate between date palm genotypes. It was clear that molecular studies help us to an early identification of date palm males. Knowledge of the degree of genetic relationship between these cultivars will be important for the germplasm collection, in situ conservation and palm tree breeding programs.

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