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REGULAR ARTICLE

Phylogenetic relationships among date palm (*Phoenix dactylifera* L.) cultivars in Syria using RAPD and ISSR markers

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is an important fruit tree in Syria. Determination of genetic relationships among date palm cultivars is of major importance for characterization of date palm germplasm, breeding programs, and conservation purposes. Genetic polymorphism in 23 date palm cultivars in Syria representing 18 female and five male cultivars was assessed using RAPD and ISSR markers. Results revealed that the average polymorphism detected by the RAPD assay (58.5%) was higher than that observed for ISSR (50.6%). Since there was a good degree of fit with a 0.82 r matrix correlation value between RAPD and ISSR data, we only analysed the combined data of both techniques for revealing phylogenetic relationships among cultivars and male trees considered. The percent disagreement values (PDVs) mean average ranged from 0.19 for the two (yellow and red) `Kabkab` cultivars to 0.31 for `Khastawi`. In the dendrogram constructed for cultivars analyzed with the genetic distance based on the combined data of ISSR and RAPD, two main clusters were observed. The first cluster contained three cultivars ('Deglet Noor', 'Maktoom', and 'Khastawi') and one male tree with an average PDV value of 0.30, and the second cluster contained the remaining cultivars and male trees with an average PDV of 0.20. Generally, grouping of cultivars and male trees examined was to a large extent independent of the trees' sex and the cultivar geographical origin.

Keywords: DNA-based markers, genetic characterization, PCR, polymorphisms.

INTRODUCTION

Dates are dioecious perennial monocotyledon fruit trees that belong to the *Arecaceae* family [1]. Date palm (*Phoenix dactylifera* L., 2n=2x=36) is one of the most important domesticated crops in the Near East and North African countries [2]. It is believed to have originated in Mesopotamia [3], and was certainly cultivated as early as 4000 B.C. [4]. Traditionally, date palm has been vegetatively propagated from offshoots produced by elite individual trees [5].

Date palm is cultivated either for fruit production, medical uses or many other purposes (religious, feed, brooms and wood) [6] mainly in North Africa but also in South Asia, USA and Australia. It covers a surface of about 800.000 ha and it is important for the life of about 100 millions of inhabitants [7]. Adawy et al. [8] referred to the great socio-economic importance of date palm as it represents a source of income and nutrition to oases inhabitants, and creates favourable conditions for improving secondary crop culture like barley, alfalfa and cloves as forage.

Date palm is an important tree in Syria, where the cultivation area exceeded 369 ha in 2009 (FAO statistics) and the number of productive trees reached 72,600 trees [9]. It is mainly distributed in the north-eastern regions and some inland areas of Syria. Date palm is grown for propagation purposes in nine centers in the country; of which Palmyra and Al-Bookamal`s are the leading centers. The functions of these centers are to 1) collect native or introduced cultivars and new lines of palm that are adjusted to the environmental conditions in the region to act as mother orchids, and 2) expand the tree cultivation quantitatively and qualitatively in Syria.

Investigation of genetic variation and phylogenetic relationships among date palm cultivars in Syria

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would be useful for their conservation [10] and genetic improvement. Traditionally, morphological characteristics (leaves, spines, and fruit characters) have been used widely for the detection of variation of date palm cultivars (e.g. [11]). A committee of Syrian experts has recently completed the morphological characterisation of date palm cultivars propagated in Syria (in preparation). However, the evaluation of inter-varietal genetic diversity on the basis of morphological markers, mainly those of the fruit, is difficult [12] because a large set of complex phenotypic data [13] is required. Added to that, informative morphological characters can be observed only in mature trees, and they vary due to the environmental effects [5]. In a recent study, Hammadi et al. [14] confirmed that when they observed a high diversity in leaves characteristics among date palm cultivars they analyzed.

The employment of isozyme polymorphisms as descriptive markers for date palm characterization has been illustrated in several studies (e.g. [15]). Biochemical classification was also proved useful by Quafi et al. [16] for the taxonomy and classification of palm cultivars based on analysis of flavonoids from acid hydrolysates. Recently, Munshi and Osman [17] used sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to analyze total proteins of fresh leaves for determination of molecular phylogeny of some date palm cultivars in Saudi Arabia. The authors reported that no major differences in banding patterns of the protein of the studied cultivars were recognized. Although the analysis of biochemical markers is easy to apply, its drawbacks include the organ-specificity of these markers, and the dependence of their expression on environmental conditions, and the often-limited amount of detectable polymorphism [18].

DNA-based markers provide useful information on genetic diversity of plant cultivars, as they remain unaffected by environmental factors and the developmental stage of plants. Such markers also provide a nearly unlimited potential to uncover differences at the molecular level [19], and have been revealed as powerful tools [20] to provide information on the relatedness of cultivars that are difficult to distinguish morphologically such as those of date palm. Various molecular markers such as restriction fragment length polymorphisms (RFLPs, [21]), simple sequence repeat (SSR, [22]), the representational difference analysis (RDA) [23], and amplified fragment length polymorphism (AFLP, [24]) have been developed to molecularly characterize date palm cultivars.

RAPD [25] can generate many useful genetic markers for the analysis of genetic diversity and phylogenetic relationships in closely related groups [20]. It is possibly the simplest test technically [17] and the fastest of all recently applied DNA-based markers for the detection of genetic variation within date palm (e.g. [26]). Hence, several studies implied RAPD for the molecular characterization of date palm of Tunisia [20], Morroco [5], Saudi Arabia [12, 27, 17], Egypt [(28, 29, 26] and Algeria [30]. The authors proved the efficacy of RAPD markers for the estimation of phylogenetic relationships among date palm cultivars.

ISSR (‘anchored SSR-PCR’) [31] markers are also believed to be simple, rapid, inexpensive, and highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature [32]. ISSR is believed to be one of the most efficient techniques that can rapidly reveal high polymorphism and determine genetic diversity in date palm. Zehdi et al. [33] and Karim et al. [34] reported the employment of ISSR as informative markers to investigate the phylogenic relationships among a set of Tunisian date palm cultivars. Similarly, ISSR technique was proved efficient for determinating the molecular phylogeny of date palm cultivars from Saudi Arabia [35, 17], and Egypt [8, 26].

Munshi and Osman [17] suggested that utilization of RAPD and ISSR markers could potentially have the high priority for studying genetic diversity and molecular characterization of date palm germplasm. The objective of this study, therefore, was to assess the genetic relationships among five prominent male date palm genotypes and all native and introduced date palm female cultivars clonally propagated in Syria. To our knowledge, the results of the present study have produced the first informative DNA-based markers for the genetic characterization of date palm cultivars analyzed here.

MATERIAL AND METHODS

Plant material and DNA preparation

A set of all commercial palm cultivars (18 female cultivars) domesticated in Syria and five prominent male genotypes, listed in Table 1, has been analyzed. Among these cultivars, only three ('Ashrasi', 'Maktoom', and 'Khastawi') are native Syrian. The remaining cultivars have been recently introduced from Tunisia, Morocco, KSA, UAE, Iran, Egypt, and Iraq. The plant material consisted of young leaves provided by "Al-Boukamal" date palm propagation center for all cultivars and male trees except for the material of `Deglet Noor`, that was obtained from a private orchid, and male 5 which was taken from the date palm propagation center in Palmyra. For each genotype, young leaves were taken from 10 adult trees that were randomly chosen and sampled. Collected leaves were washed three times in sterile distilled water, immersed in liquid nitrogen, and kept at -60 ºC until use.

Total genomic DNA was extracted from the frozen leaves according to the CTAB method of Doyle and Doyle [36]. Recovered DNA pellets were dried under the laminar flow and then resuspended in 100 µL of doubled distilled and sterilized water. The integrity of DNA was evaluated by using agarose electrophoresis, and DNA concentrations were determined using a GeneQuant spectrometer (Amersham Biociences, UK). A working concentration of all DNA samples was set at 10 ng μL^{-1}. A pool of DNAs extracted from samples of each genotype was prepared and used as a template that represents each of these genotypes [8] for later amplifications.

RAPD analysis

RAPD analysis was performed on targeted samples using 35 decamer oligonucleotide primers (Table 2) (Operon Technologies Inc., USA). PCR reactions were carried out in 25 µL volumes containing 7.5 mM Tris-HCl (pH 9 at 25 ºC), 50 mM KCl, 2 mM (NH4)2SO4, 3 mM MgCl2, 0.6 mM of each dNTP (Vivantis, Malaysia), 80 ng primer, 1.5 units of Taq polymerase (Biotools, Spain), and 20 ng of genomic DNA. Amplification was performed using a Genius Hybaid thermal cycler (Techne, UK). Samples were subjected to an initial denaturation at 94 ^{o}C for 1 min, followed by 45 cycles of denaturation at 94 ^{o}C for 10 sec, annealing of primers at 35 ^{o}C for 10 sec, and extension at 72 ^{o}C for 70 sec. A final extension cycle was performed at 72 ^{o}C for 2 min. Generated amplification products were size separated by standard horizontal electrophoresis in 1.2% ethidium bromide (Fluka, Germany)-stained agarose (EuroClone, Italy) gels in 0.5X Tris Borate EDTA (TBE). A 1-Kb DNA ladder (Fermentas, Lithuania) was used to estimate the approximate molecular weight of amplification products.

Table 1. Names of date palm female cultivars and male genotypes used and their origin as provided by the Ministry of Agriculture in Syria.

Number	Cultivar/male name	Geographical origin (i.e. introduced from)
1	Deglet Noor	Tunisia
2	Medjool or (Mujhoolah)	Morocco
3	Zahidi	Iraq
4	Birbin	Iraq
5	Ashrasi	Native Syrian
6	Maktoom	Native Syrian
7	Khastawi	Native Syrian
8	Barhee	KSA
9	Khalas	KSA
10	Khadrawy	KSA
11	Nabtat-seyf	KSA
12	Lolo	UAE
13	Gish Rabi	UAE
14	Khineze	UAE
15	Zaghloul	Egypt
16	Shahabi	Iran
17	Kabkab (yellow)	Iran
18	Kabkab (red)	Iran
19	Male 1	Syria
20	Male 2	Syria
21	Male 3	Syria
22	Male 4	Syria
23	Male 5	Syria

ISSR analysis

Using 15 selected primers (Table 3), ISSR [37] was carried out on all date palm samples in a 25 µL reaction volume containing 7.5 mM Tris-HCl (pH 9 at 25 ºC), 50 mM KCl2 mM (NH4)2SO4, 3 mM MgCl2, 0.6 mM of each dNTP (Vivantis), 1.5 units of Taq DNA polymerase (Eurobio, France),

Table 3. Names and sequences of primers used for ISSR and the number of polymorphic fragment lines and fragments generated.

	Primer name	Sequence (5`-3`)	No. of fragment lines	No. of polymorphic lines	% Polymorphic lines	No. of fragments amplified	No. of polymorphic fragments	% Polymorphic fragments
	A1	CACACACACACARR	8	7	88	84	61	73
	A7	CACACACACACARM	9	8	89	92	69	75
	A10	CACACACACACARK	9	9	100	117	117	100
	A13	CACACACACACARS	9	8	89	129	106	82
	A16	CACACACACACAR	5	3	60	50	4	8
	A31	AGCAGCAGCAGCR	6	4	67	87	41	47
	A34	AGCAGCAGCAGCY	7	6	86	75	52	69
	A37	AGCAGCAGCAGCM	13	12	92	114	91	80
	A40	AGCAGCAGCAGCK	7	2	29	130	15	12
	A41	AGCAGCAGCAGCK	5	1	20	95	3	3.2
	A44	AGCAGCAGCAGCS	3	0	0	69	0	0
	B13	CAA CAA CAA CAA CAA	8	5	63	97	28	29
	B16	GAC AGA CAG ACA GAC A	3	3	100	41	41	100
	C22	AGAGAGAGAGAGAGAGT	17	16	94	120	97	81
	C29	CAG CAG CAG CAG CAG	3	0	0	69	0	0
Sum			112	84	977	1369	725	759.2
Average			7.5	5.6	65.1	91.3	48.3	50.06

20 ng of genomic DNA, and 200 pmol of each primer (Invitrogen, UK). Amplification reactions were subjected to a cycle of 94 ^{o}C for 5 min, followed by 40 cycles, each of which consisted of 94 ^{o}C for 10 sec, 50-58 ^{o}C (depending on the GC content of the primer, Table 2) for 10 sec, and 72 ^{o}C for 10 sec and a final extension step at 72 ^{o}C for 7 min. The PCR products were separated on 2.5% agarose gels in 0.5 × TBE buffer stained with ethidium bromide. A 100-bp DNA ladder (Biotools) was used to estimate the approximate molecular weight of DNA bands for each PCR product. Electrophoresis was performed at 85 V for 2.30 h. In order to reduce the possibility of cross contamination in RAPD and ISSR amplification reactions, a master reaction mixture was routinely prepared and a negative control was used. This control consists of the reaction mixture excluding any DNA. Further, RAPD and ISSR analyses were repeated twice for all samples, and only clear bands produced in both replicates were scored as mentioned below.

Visualisation of amplification products and data analysis

Amplification profiles generated from RAPD and ISSR were photographed under UV light, screened, and compiled into a binary data matrix. Only distinct, reproducible and well-resolved fragments (bands) were recorded numerically as (1) when present or (0) when absent. Fragments with the same mobility were considered as identical, irrespective of fragment intensity. Based on the collective data generated from RAPD and ISSR markers, the unweighted pair group arithmetic average (UPGMA) method and percent disagreement values (PDV) [38] of the STATISTICA program [39] were used to construct the binary data matrix and the dendrogram. The RAPD and ISSR matrices were subjected to the Mantel Test to verify the level of conformity between the data generated.

RESULTS

RAPD analysis

When RAPD was performed on date palm genotypes targeted, all tested primers generated reproducible and easily scorable amplification profiles (e.g. Fig. 1). There were 280 DNA fragment lines, out of which 220 lines were polymorphic (78.6%). For nine primers, all fragment lines were polymorphic. Remaining primers showed polymorphic lines that ranged from 0 (OP-I06) to 92% (Amersham 4). The total average of polymorphic lines was 6.29% per primer. Primers produced multiple band profiles with a number of amplified DNA fragments ranging from 33 (Amersham 3) to 166 (OP-D03). Fingerprinting using RAPD revealed a total number of 3358 unambiguous fragments with an average of 95.9 fragments per primer. In profiles generated, the sizes of the fragments ranged from 300- to 3000-bp. The number of polymorphic fragments ranged from 0 (primer OP-i06) to 138 (primer OP-Z18). The total number of polymorphic fragments produced by all primers was 2001, with an average of 57.2 polymorphic fragments per primer (Table 2).

ISSR analysis

The 15 primers used for ISSR produced reproducible and scorable patterns (e.g. Fig. 2). The amplification profiles were screened for the presence of polymorphisms among the genotypes analysed. A total of 1369 fragments were generated. Primer A40 yielded the highest number of fragments (130 bands), while the lowest number of fragments was observed for primer B16 (41 fragments). Of all fragments amplified, 725 were polymorphic (53%). The number of polymorphic fragments ranged from 0 (only monomorphic fragments, primers A44 and C29) to 177 (primer A10). Primers A10 and B16 yielded 100% polymorphic fragments.

The total number of fragment lines obtained was 112, out of which 84 lines were polymorphic (75%). Of the 15 primers used, two (A10 and B16) had all lines polymorphic (100%). Remaining primers showed polymorphic lines that ranged from 0% (A44 and C29) to 94% (C22) with a total average of 59.8% per primer. In profiles generated for all primers, the sizes of the fragments ranged from 200- to 2000-bp.

The results of the present study revealed that the average polymorphism detected by the RAPD assay (58.5%) was higher than that observed for ISSR markers (50.6%). This was also evidenced through the number of cultivars that could be identified by each technique through unique fragments. RAPD data allowed the discrimination of five cultivars ('Deglet Noor', 'Kabkab' (yellow), 'Medjool', 'Khastawi' and 'Birbin') and

Table 2. Names and sequences of primers used for RAPD and the number of polymorphic fragment lines and fragments generated.

Primer name	Sequence (5`-3`)	No. of fragment lines	No. of polymorphic lines	% Polymorphic lines	No. of fragments amplified	No. of polymorphic fragments	% Polymorphic fragments
Amersham 1	GGTGCGGGAA	6	5	83	87	64	74
Amersham 2	GTTTCGCTCC	4	3	75	45	45	100
Amersham 3	GTAGACCCGT	3	2	67	33	10	30
Amersham 4	AAGAGCCCGT	13	12	92	159	136	86
Amersham 5	AACGCGCAAC	10	8	80	137	91	66
OP-B07	GGTGACGCAG	7	2	29	130	15	12
OP-B09	TGGGGGACTC	16	14	88	126	80	63
OP-C08	TGGACCGGTG	6	5	83	39	16	41
OP-C09	CTCACCGTCC	12	12	100	98	98	100
OP-C10	TGTCTGGGTG	8	4	50	104	12	12
OP-D03	GTCGCCGTCA	9	2	22	166	5	3
OP-D08	GTGTGCCCCA	4	3	75	49	26	53
OP-E02	GGTGCGGGAA	4	1	25	85	16	19
OP-E03	CCAGATGCAC	3	1	33	50	4	8
OP-E07	AGATGCAGCC	7	3	43	115	23	20
OP-F03	CCTGATCACC	4	2	50	61	15	25
OP-F04	GGTGATCAGG	8	7	88	70	47	67
OP-F08	GGGATATCGG	7	2	29	126	11	8.7
OP-i02	GGAGGAGAGG	8	7	88	118	95	81
OP-i103	CAGAAGCCCA	9	6	67	113	44	39
OP-i06	AAGGCGGCAG	3	0	0	69	0	0
OP-i08	TTTGCCCGGT	7	6	86	66	43	65
OP-i20	AAAGTGCGGG	5	3	60	90	44	49
OP-N04	GACCGACCCA	13	13	100	116	116	100
OP-N09	TGCCGGCTTG	13	13	100	103	103	100
OP-O04	AAGTCCGCTC	10	7	70	158	89	56
OP-O08	CCTCCAGTGT	6	6	100	86	86	100
OP-O12	CAGTGCTGTG	10	10	100	68	68	100
OP-R01	TGCGGGTCCT	12	12	100	115	115	100
OP-R09	TGAGCACGAG	7	6	86	56	33	59
OP-R14	CAGGATTCCC	9	9	100	95	95	100
OP-Z 04	AGGCTGTGCT	5	3	60	100	54	54
OP-Z 07	CCAGGAGGAC	5	4	80	53	30	57
OP-Z 11	CTCAGTCGCA	15	15	100	134	134	100
OP-Z 18	AGGGTCTGTG	12	12	100	138	138	100
Sum		280	220	2509	3358	2001	2047
Average		8	6.29	71.7	95.9	57.2	58.5

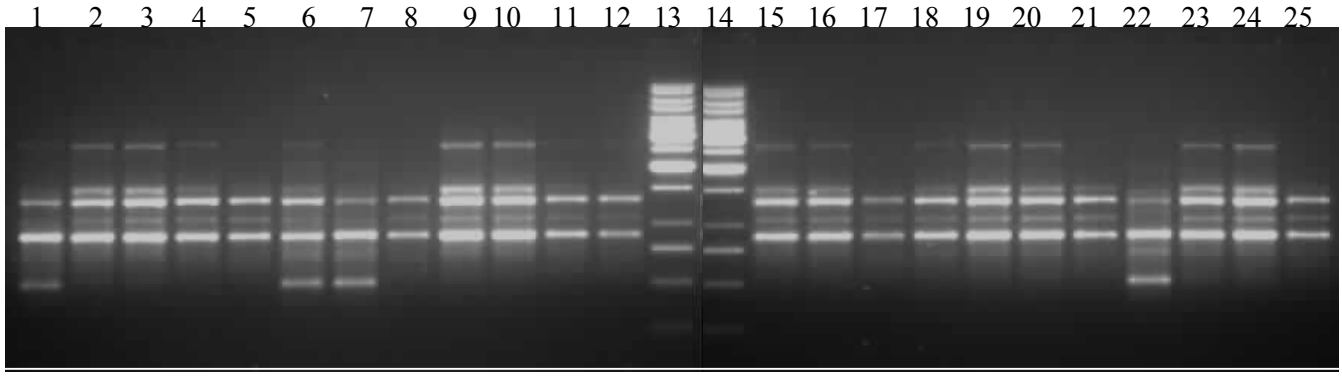

Figure 1. RAPD banding profiles generated using primer OP-F 03 for date palm genotypes (lanes 1-12 & 15-25, respectively) (Table 1). Lanes 13 & 14, standard 1-Kb DNA ladder.

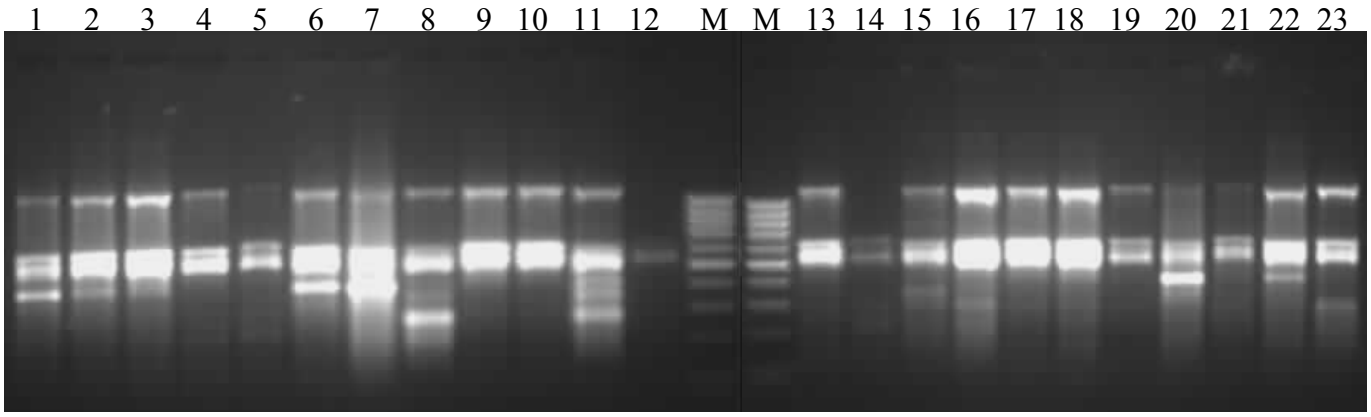

Figure 2. ISSR banding profiles generated using primer B16 for date palm genotypes (lanes 1-12 & 15-25, respectively) (Table 1). Lanes 13 & 14, standard 100-bp DNA ladder.

one male genotype (male 2). The use of ISSR, however, could distinguish only one cultivar ('Khadrawy') and the same male genotype distinguished by RAPD (Table 4).

The Mantel Test that was carried out on RAPD and ISSR matrices to verify the level of conformity between the data generated from both techniques showed that there was a good degree of fit with a 0.82 r matrix correlation value between RAPD and ISSR data which made us combine all data in one matrix (Fig. 4). The PDV mean average for the combined data of all genotypes studied ranged from 0.19 for each of the two 'Kabkab' (yellow and red) cultivars to 0.31 for 'Khastawi'.

Individual PDV values among genotypes ranged from 0.08 for 'Nabtat-seyf' and 'Lolo', which were thus being recorded as the two most closely related cultivars, to 0.37 that was attained by each of the following four pairs: 'Maktoom' and 'Khadrawy', 'Khastawi' and 'Khadrawy', 'Khastawi' and 'Lolo', and 'Khadrawy' and male 2, which were considered as the most genetically distant from each other.

The dendrogram constructed (Fig. 3) for genotypes analyzed was in very good agreement with the PDV matrix. Two main clusters were observed. The first cluster contained three cultivars ('Deglet Noor', 'Maktoom', and 'Khastawi') and male 2 with an average PDV value of 0.30. In this cluster, the two cultivars 'Maktoom' and 'Khastawi' were the closest to each other with a mere 0.13 PDV value. The second cluster included the remaining 15 cultivars and four male trees with an average PDV of 0.20. This cluster was further divided into two sub-clusters; the first sub-cluster contained the three male genotypes 3, 4 and 5, and the second sub-cluster included 15 cultivars and male 1. The latter sub-cluster was further divided, at an average PDV value of 0.19, into two groups; one containing only two cultivars, namely 'Medjool' and 'Zahidi' and the second containing male 1 and the remaining 13 cultivars which included the most closely related cultivars studied. i.e. 'Nabtat-seyf' and 'Lolo' (PDV=0.08), followed by red and yellow 'Kabkab' (PDV=0.10), and 'Birbin' and 'Ashrasi' (PDV=0.11).

In general, grouping of genotypes examined revealed that they were clustered independently of the genotype sex. Thus, although three (3, 4, and 5) out of the five male genotypes (all native Syrian) fell in one sub-cluster, the other two male genotypes (1 and 2) were closely grouped with female cultivars. Although there were few instances were very closely related cultivars originated from the same country [e.g. the two 'Kabkab' cultivars (Iran), and 'Birbin' and 'Ashrasi' (Syria)], for most cultivars however clustering was independent from their geographic origin. For instance, the sister cultivars `Nabtat-seyf` and 'Lolo' have different geographical origins (KSA and UAE, respectively).

DISCUSSION

Despite the outstanding agronomic and socio-economic significance of date palm, attempts to improve knowledge about the biodiversity of date palm in Syria have been limited to the phenotypic description of those cultivars. However, a deeper insight of the genetic diversity of date palm is becoming an urgent priority [5] to guide the use of this diversity in improvement programs of date palm. Results generated by Mitra et al. [40] indicated the effectiveness of RAPD and ISSR marker systems for demonstrating genetic relationships among date palm genotypes. This study portrays the molecular characterization of all date palm female cultivars propagated in Syria using RAPD and ISSR markers, and the investigation of phylogenetic relationships among them and their genetic relatedness to five prominent male date palm genotypes.

Results revealed that the overall polymorphism among date palm genotypes identified by RAPD markers was higher than that observed for the ISSR markers, suggesting that RAPD markers are very effective for assessing the molecular polymorphism of this crop [12, 17]. This may be explained by the fact that RAPD is less specific than ISSR because it uses shorter primers that need lower annealing temperature which makes annealing of primers more random.

Because RAPD and ISSR amplify different parts of the genome, the data generated from both analyses were combined in order to obtain more balanced values for genetic variation among genotypes targeted and an equilibrated dendrogram representation of their relationships. This allowed us to explore the DNA polymorphism in the collection of genotypes analyzed, and generate many polymorphic markers ensuring a good coverage of the genome [5, 20].

Table 4. Unique fragment(s) that identified one cultivar.

Cultivar	Technique/primer	Approximate size of unique bands (bp)
Male 2	RAPD/Amersham4	1500-2000
	RAPD/O12	750
Deglet Noor	RAPD/B9	500-250
Kabkab (yellow)	RAPD/C10	1000-1500
Medjool or (Mujhoolah)	RAPD/E7	500-750
Khastawi	RAPD/i3	750-1000
Birbin	RAPD/R9	500
Male 2	ISSR/A13	1250
Khadrawy	ISSR/B13	300

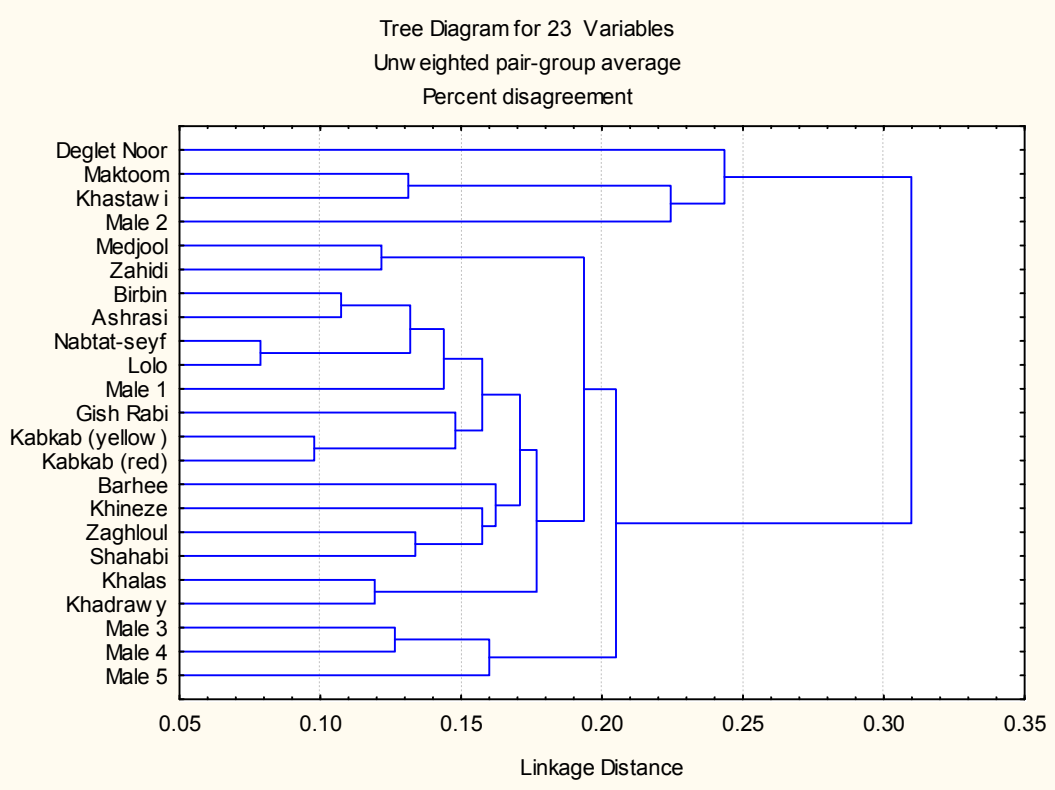

Figure 3. Dendrogram generated for date palm genotypes based on combined data of RAPD and ISSR.

	Deglet Noor	Medjool	Zahidi	Birbin	Ashrasi	Maktoom	Khastawi	Barhee	Khalas	Khadrawy	Nabtat-seyf	Lolo	Gish Rabi	Khineze	Zaghloul	Shahabi	Kabkab (yellow)	Kabkab (red)	Male 1	Male 2	Male 3	Male 4	Male 5
Deglet Noor	0.00																						
Medjool	0.22	0.00																					
Zahidi	0.26	0.12	0.00																				
Birbin	0.32	0.20	0.14	0.00																			
Ashrasi	0.28	0.20	0.15	0.11	0.00																		
Maktoom	0.25	0.25	0.27	0.33	0.30	0.00																	
Khastawi	0.24	0.29	0.29	0.35	0.33	0.13	0.00																
Barhee	0.31	0.23	0.22	0.18	0.18	0.33	0.32	0.00															
Khalas	0.28	0.17	0.16	0.18	0.19	0.29	0.30	0.23	0.00														
Khadrawy	0.32	0.21	0.19	0.18	0.19	0.37	0.37	0.19	0.12	0.00													
Nabtat-seyf	0.31	0.21	0.18	0.13	0.14	0.33	0.36	0.20	0.17	0.14	0.00												
Lolo	0.32	0.23	0.20	0.12	0.13	0.32	0.37	0.20	0.18	0.17	0.08	0.00											
Gish Rabi	0.28	0.20	0.18	0.18	0.17	0.32	0.34	0.22	0.14	0.18	0.16	0.15	0.00										
Khineze	0.28	0.22	0.21	0.17	0.18	0.32	0.33	0.17	0.17	0.16	0.16	0.15	0.17	0.00									
Zaghloul	0.30	0.21	0.20	0.18	0.16	0.34	0.36	0.16	0.20	0.20	0.19	0.20	0.19	0.17	0.00								
Shahabi	0.31	0.22	0.20	0.16	0.15	0.36	0.36	0.16	0.19	0.15	0.14	0.16	0.19	0.15	0.13	0.00							
Kabkab (yellow)	0.30	0.19	0.17	0.16	0.16	0.31	0.34	0.18	0.16	0.17	0.16	0.17	0.15	0.16	0.16	0.13	0.00						
Kabkab (red)	0.28	0.18	0.16	0.13	0.15	0.31	0.34	0.17	0.15	0.17	0.16	0.16	0.15	0.15	0.16	0.15	0.10	0.00					
Male 1	0.28	0.20	0.17	0.14	0.14	0.31	0.35	0.20	0.19	0.19	0.15	0.15	0.17	0.19	0.18	0.15	0.15	0.14	0.00				
Male 2	0.25	0.30	0.30	0.35	0.33	0.23	0.22	0.34	0.31	0.37	0.36	0.36	0.27	0.32	0.34	0.34	0.31	0.31	0.33	0.00			
Male 3	0.26	0.21	0.21	0.22	0.22	0.29	0.29	0.24	0.18	0.23	0.23	0.22	0.16	0.22	0.23	0.23	0.22	0.19	0.21	0.22	0.00		
Male 4	0.22	0.18	0.18	0.20	0.22	0.24	0.27	0.23	0.18	0.21	0.21	0.22	0.19	0.21	0.21	0.20	0.19	0.18	0.19	0.26	0.13	0.00	
Male 5	0.25	0.21	0.20	0.18	0.21	0.32	0.31	0.21	0.22	0.21	0.19	0.20	0.21	0.20	0.18	0.18	0.19	0.19	0.19	0.28	0.18	0.14	0.00
Average	0.28	0.21	0.20	0.20	0.20	0.30	0.31	0.22	0.20	0.21	0.20	0.20	0.20	0.20	0.21	0.20	0.19	0.19	0.20	0.30	0.22	0.20	0.21
	0.28	0.21	0.20	0.19	0.19	0.30	0.32	0.22	0.19	0.21	0.19	0.20	0.19	0.20	0.21	0.20	0.19	0.18	0.19	0.31	0.22	0.20	0.21

Figure 4. Matrix of PDV values generated from combined data of RAPD and ISSR.

The phylogenetic tree constructed here revealed that date palm cultivars examined are not monophelic and provide evidence of divergence among all tested genotypes since they were grouped in clusters. This confirms findings of Sedra et al. [5], Trifi et al. [20], Hamama et al. [41], and Hussein et al. [26]. However, close relationships among cultivars were observed and the genetic polymorphism among them was found to be narrow. Based on RAPD and ISSR, similar scenario was observed for date palm of Saudi Arabia, Morocco, Tunisia, and Iraq [5, 12, 33, 17]. Al-Khalifah and Askari [12] justified that by the exchange of the cultivars between the different plantation areas, clonal propagation of ecotypes, and development of new recombinants by seedling selection and limited sexual reproduction. They also believe that the selection by farmers may represent only a small fraction of date palm germplasm.

Results also showed that the foreign date palm cultivars recently introduced to Syria groves are closely grouped with indigenous ones. This could be explained by the presence of a common genetic origin among the tested cultivars in spite of their great diversity [20]. Al-Khalifah and Askari [12] added that over the years many date palm cultivars have been transplanted to areas other than the area of their origin, and there may have been adapted with different names.

Grouping of genotypes analyzed is not well-defined either according to their sex or geographical origin since the introduced cultivars and the male genotypes did not significantly diverge from the autochthonous female accessions. These results broadly concur with those describing the use of various molecular markers (i.e. AFLP, RAPD, ISSR,

SSR, and PCR-RFLP) for the detection of genetic diversity in date palm originating from Morocco [5], Iraq [10], and Tunisia [33, 42, 22, 43]. This could be due to 1) cultivars interchange between oases and 2) the nature of introduction of the cultivars in the country. Similar scenario was also observed in other species cultivated in oases such as olive [44] and pomegranate trees [14]. Zehdi et al. [33] reported that since all date palm ecotypes are originated by hybridization, it may be assumed that they have a common genetic basis, and cultivars diverged from others by mutational events that arise during selection.

Determination of genetic variability of date palm in Syria can be useful for the selection of possible parents to generate mapping populations as suggested earlier by Munshi and Osman [17]. In a recent study, Haider and Nabulsi [45] characterized same genotypes analyzed in the current study using plasmid-like DNA markers to determine their resistance/susceptibility to the "Bayoud" disease caused by Fusarium oxysporum forma specialis albedinis. The phenotypic description of those genotypes (in preparation), and the molecular characterisation results generated by us and Haider and Nabulsi [45] would be of major importance in date palm improvement programs, germplasm characterization, and conservation to control genetic erosion of this crop. This will also help in the collection and cataloguing of the germplasm in the form of a germplasm bank [8, 46**,** 17].

In conclusion, although the RAPD and ISSR markers generated in this study proved efficient to determine the genetic relationships in date palm grown in Syria, they were not sufficient for their full discrimination. Very recently, Elmeer et al. [47] developed new SSR primers that proved useful for assessment of genetic diversity in date palm. A search is currently in progress in our laboratory to employ those primers for DNA-fingerprinting of cultivars analyzed here.

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