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Molecular identification and genetic diversity of Iranian date palm (*Phoenix dactylifera* L.) cultivars using ISSR and RAPD markers

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Abstract

In the present study genomic profiling of fifteen native cultivars of date palm collected from south and southwest of Iran studied to determine their genetic affinity and allelic diversity by ISSR and RAPD-PCR molecular methods. For each cultivar, genomic DNA profiling of the five genotypes of each cultivars was carried out using 10 RAPD and 14 ISSR primers, exhibiting 132 and 162 amplicons, representing a level of polymorphism of 92.4% and 95.67%, respectively. The genetic relationships among cultivars were estimated in terms of similarity using Dice coefficients. The genetic similarity ranged from 94.5% to 99.3% and from 94.1% to 99.4% for RAPD and ISSR, respectively. Both types of markers showed the highest genetic similarity between Gantar and Deyri cultivars and similarly both type of markers showed highest genetic distance of Gantar and Deyriwith Khazravi and Kabkab cultivars. A dendrogram was constructed using NJ analysis. On the basis of this analysis, the populations were divided into four clusters. Analysis of molecular variance (AMOVA) revealed that 95% (ISSR) and 97% (RAPD) of variability was partitioned among individuals within populations. These results represent the efficiency of ISSR and RAPD markers to evaluate genetic relationships of the date palm cultivars studied here.

Keywords: AMOVA; Gene diversity; genetic similarity; polymorphism; clusters analysis.
Abbreviations: AMOVA- Analysis of Molecular Variance; ISSR- Inter Simple Sequence Repeat Amplification; RAPD- Random Amplified Polymorphic DNA; POL-Polymorphism percent; PIC- Polymorphism information content; MI- Marker index; NJ- Neighbor Joining.

Introduction

Date palm (*Phoenix dactylifera* L.) is a perennial, dioecious, highly heterozygous, monocotyledonous plant, with a very slow growth rate and a late reproductive phase (Adawy et al., 2004). Palm tree is an excellent candidate for cultivation in arid and semi-arid regions of the world due to its high tolerance to environmental stresses (Adawy et al., 2004). It is of great economic importance to oasis agriculture and creates favorable conditions for improving secondary crops (Adawy et al., 2004). In spite of its economic importance, improvement programs in this crop are very limited in Iran. Therefore, the development of accurate fingerprint characterizing the different genotypes of dates would be of great value in improvement of this important crop. Some molecular markers as ISSR (Ahmed, 2010; Adawy et al., 2004; Al- Hadidi, 2010), RAPD (Adawy et al., 2004; Ben Abdallah et al., 2000; El-Rayes., 2009; Trifi et al, 2000), AFLP (Adawy et al., 2004; El-Khisin et al., 2003), and SSR (Al- Hadidi, 2010) were used to describe genotypes of date palm. In Saudi Arabia RAPD fingerprint was used to investigate the genetic diversity of 5 varieties. Of 20 primers, only 12 primers were replicated and 64 bands were produced. The profile was used to distinguish variety (Al-Moshile et al., 2004). Based on two by two comparisons of the products, the genetic similarity was calculated in the region 70 to 85%. Cluster analysis and dendrogram were done by UPGMA and the cultivars were divided into two groups (Al-Moshile et al., 2004). Moghaieb et al. (2010) investigated the genetic diversity and sex determination in 6 genotypes of male and female date palms by RAPD and ISSR markers. Polymorphism amount of the markers was 60.2 and 73% for RAPD and ISSR markers, respectively. The cluster analysis showed that unknown cultivars had close relation with Frehi, Oshkingbil varieties. The results of the study showed that RAPD marker is sex-based marker. It was defined that the markers were suitable in identifying date palm cultivars (Moghaieb et al., 2010). Iran due to having various cultivars of date palm is rich in this respect. Therefore, the objective of the current study was to investigate the genetic diversity of 15 cultivars in Iran and the genetic relationships between these cultivars in order to achieve useful information for germplasm resources.

Results

RAPD and ISSR amplification

Using 10 RAPD primers, 132 amplicon were amplified; 10 amplicons of RAPD products were monomorphic and rest showed polymorphism among 75 genotypes. The polymorphism of RAPD markers was 92.42% (Table 2.). By 14 ISSR primers, 162 amplicons were produced, 155 bands of which were polymorphism and polymorphism percent (POL) was 95.67% (Table 3.). The size of the RAPD products was ranging from 100 to 2500 base pairs. The number of fragments for each primer was ranging from 6 to 23 bands. The average total number of bands and average polymorphism bands for each primer were 13.2 and 12.2, respectively. Polymorphism information content (PIC) distribution was ranging 0.144 to 0.304 with the average 0.228. The Marker Index (MI) in the present study varied

Table 1. Name, abbreviations and origin of date palm cultivars evaluated.

Number	Name	Collection location	Code	Geographical characteristics of collection location
				Longitude (N), Latitude (E)
1	Shahani	Abadan	A	30°12′ – 48° 24′
2	Majol	Ahvaz	B	31°15′ – 48° 37′
3	Berim	Ahvaz	C	31° 15′ – 48° 37′
4	Estamaran	Ahvaz	D	31° 15′ – 48° 37′
5	Zahedi	Ahvaz	E	31° 15′ – 48° 37′
6	Piyram	Jahrom	F	33′° 53ó - 29˚28
7	Khazravi	Ahvaz	G	31° 15′ – 48° 37′
8	Berhi	Abadan	H	30° 12′ – 48° 24′
9	Deiry	Ahvaz	I	31° 15′ – 48° 37′
10	Mazafati	Bam	J	29° 06ó – 58° 21′
11	Oveydi	Ahvaz	K	31° 15′ – 48° 37′
12	Fersi	Ahvaz	L	31° 15′ – 48° 37′
13	Gantar	Ahvaz	M	31° 15′ – 48° 37′
14	Daglatnoor	Ahvaz	N	31° 15′ – 48° 37′
15	Kabkab	Behbahan	P	30° 35′ – 50° 37′

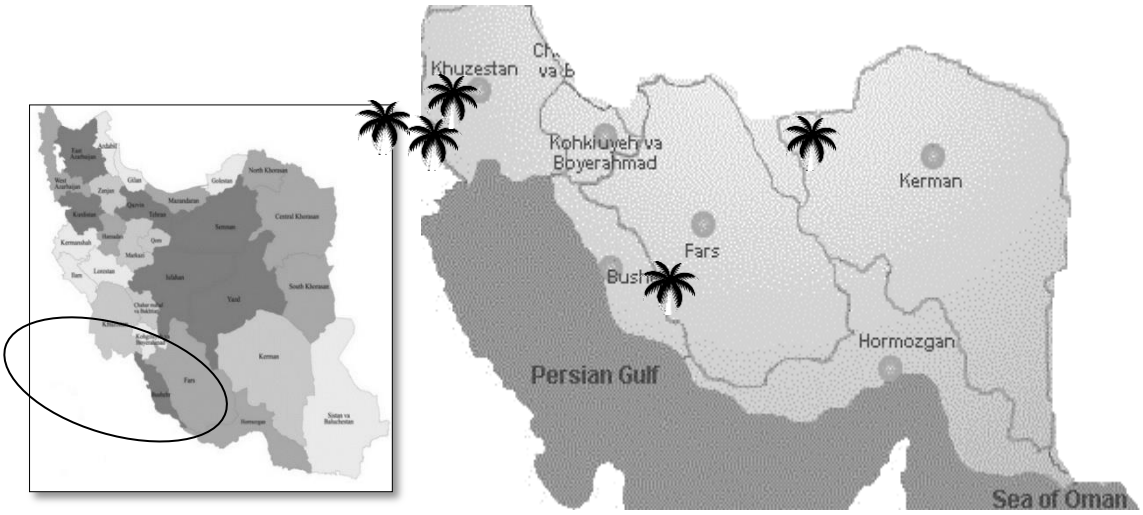

Fig 1. The sites of collected date palm samples in Iran

between 0.864 and 6.992. The maximum marker index was belonging to primer Oligo 211 showing the high separation power of the primer compared to other primers (Table 2.). The size of bands of ISSR primers was 100 to 2250 base pairs. The number of bands for each primer was ranging from 4 to 18 bands. The average total bands and average number of polymorphism bands for each ISSR primer was 11.57 and 11.07. PIC distribution for ISSR primers was 0.222 to 0.309 with average value 0.274. The marker index in ISSR primers was between 0.888 to 4.736, UBC 840 primer by promoting more value of marker index, had highest separation power among the applied primers (Table 3.).

Genetic diversity and heterozygosity index

Genetic diversity indices achieved of RAPD and ISSR primers in various cultivars were shown in Table 4. For RAPD primers gene diversity index was ranging 0.072 to 0.183 with average 0.129 and Shanon index ranging 0.119 to 0.286 with average 0.204. The effective alleles are located in the range of 1.292 to 1.104 with average 1.199. For ISSR primers gene diversity index was ranging 0.059 to 0.188 and Shanon index ranging 0.097 to 0.290. Gene diversity index and Shanon index ranged from 0.188, to 0.097. The effective alleles are located in the range of 1.085 to 1.304. Based on the existing results, the highest and lowest percent of polymorphism gene places, the number of effective alleles, Shanon index and gene diversity index were in Oveydi and Gantar cultivars (Table 4.).

Genetic similarity

The genetic similarity by RAPD marker and Dice similarity coefficient, between the different cultivars ranged 0.945 to 0.993. The highest similarity was noticed between Gantar and Deiry cultivars and the lowest similarity was noticed between Khazravi with each of Gantar and Deiry cultivars (Table 5.). The genetic similarity by ISSR marker and Dice similarity coefficient ranged from 0.941 (Kabkab with each cultivars of Deiry and Gantar) to 0.994 (between Deyri and Gantar) (Tabel 5.).

Cluster analysis

The results of cluster analysis based on NJ method and Dice similarity coefficient for RAPD and ISSR markers were

Table 2. Details of RAPD primers used for PCR amplification of 75 genotypes of date palm.

Prime	Annealing temperature	Primer sequence	Total number of replicated alleles	The number of polymorphism alleles	The size of alleles	POL	PIC	MI
oligo203	34	ʒ-CAC GGC GAG T- ɔ	13	12	150-1500	92.3	0.204	2.448
oligo33	34	ʒ-CCG GCT GGA A- ɔ	15	15	150-1200	100	0.195	2.925
oligo29	34	ʒ-CCG GCC TTA C- ɔ	6	6	300-750	100	0.233	1.398
oligo345	36	ʒ-GCG TGA CCC G-ɔ	13	13	200-1750	100	0.281	3.653
oligo349	36	ʒ-GGA GCC CCC T-ɔ	13	11	200-1000	84.6	0.224	2.464
oligo213	30	ʒ-CAG CGA ACT A- ɔ	11	10	200-1000	90.9	0.209	2.09
oligo214	30	ʒ-CAT GTG CTT G- ɔ	17	13	200-2500	76.4	0.256	3.328
oligo42	32	ʒ-TTA ACC CGG C- ɔ	13	13	300-1750	100	0.230	2.999
oligo342	32	ʒ-GAG ATC CCT C-ɔ	8	6	150-1100	75	0.144	0.864
oligo211	32	ʒ-GAA GCG CGA T- ɔ	23	23	100-2000	100	0.304	6.992

Table 3. Details of ISSR primers used for PCR amplification of 75 genotypes of date palm.

Prime	Annealing temperature	Primer sequence	Total number of replicated alleles	The number of polymorphism alleles	The size of alleles	POL	PIC	MI
UBC888	52	ɔ- *BDB CAC ACA CAC ACA CA-ʒ	14	13	200-2000	92.8	0.292	3.796
UBC887	51	ɔ- DVD TCT CTC TCT CTC TC-ʒ	12	12	200-1200	100	0.294	3.528
UBC886	52	ɔ-VDV CTC TCT CTC TCT CT- ʒ	18	18	150-2000	100	0.222	3.996
UBC823	52	ɔ- TCT CTC TCT CTC TCT CC-ʒ	7	5	200-1750	71.4	0.307	1.535
UBC826	52	ɔ- ACA CAC ACA CAC ACA CC-ʒ	11	11	250-1350	100	0.246	2.706
ISSR06	52	ɔ-GAG AGA GAG AGA GAG AC-ʒ	15	14	200-1200	93.3	0.261	3.654
UBC841	55	ɔ- GAG AGA GAG AGA GAG AYC-ʒ	13	13	150-2000	100	0.275	3.575
UBC853	53	ɔ- TCT CTC TCT CTC TCT CRT-ʒ	4	4	100-600	100	0.222	0.888
UBC824	52	ɔ-TCT CTC TCT CTC TCT CG-ʒ	5	5	300-1200	100	0.306	1.53
UBC842	55	ɔ- GAG AGA GAG AGA GAG AYG-ʒ	13	11	100-1500	84.6	0.274	3.014
UBC889	51	ɔ- DBD ACA CAC ACA CAC AC-ʒ	12	12	100-1000	100	0.263	3.156
UBC835	55	ɔ- AGA GAG AGA GAG AGA CYA- ʒ	15	14	200-2250	93.3	0.282	3.948
UBC884	51	ɔ-HBH AGA GAG AGA GAG AG-ʒ	7	7	200-750	100	0.309	2.163
UBC840	53	ɔ-GAG AGA GAG AGA GAG AYT-ʒ	16	16	200-2250	100	0.296	4.736

*T, GorC =(B) ،Tor C =(Y) ،G orA =(R)،T,CorA =(H) ،G, C or A =(V) ،T, G orA =(D)

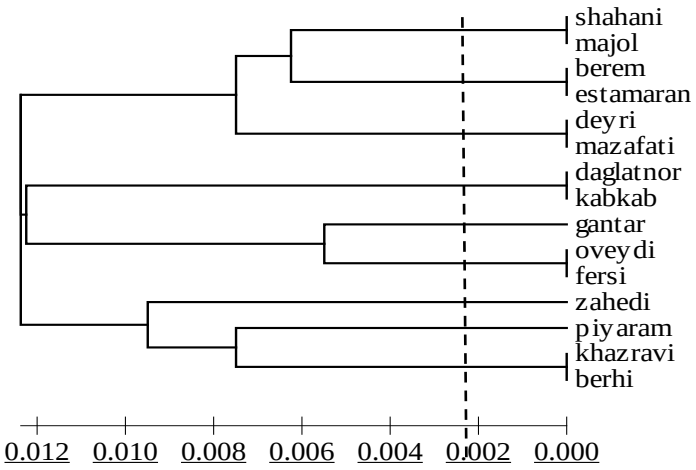

Fig 2. Dendrogram showing grouping of 15 cultivars of date palm based on the genetic distance derived from RAPD markers using NJ analysis.

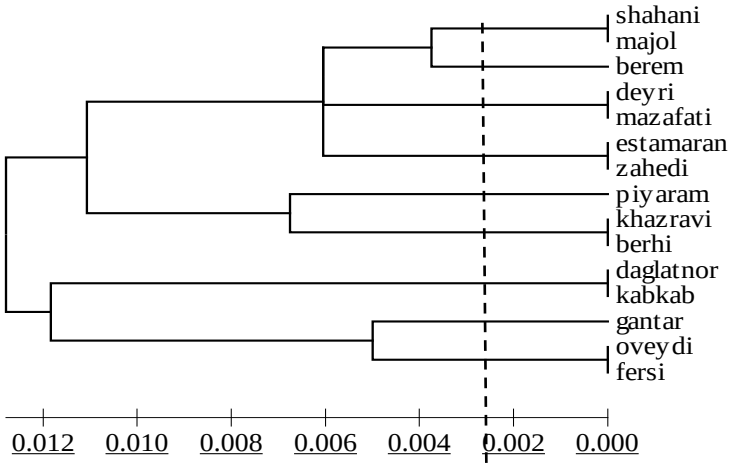

Fig 3. Dendrogram showing grouping of 15 cultivars of date palm based on the genetic distance derived from ISSR markers using NJ analysis.

shown in figures 2 and 3. Dendrogram cut point was related to a point with highest diversity between the cultivars. The obtained dendrogram from RAPD markers was divided into four main clusters; first cluster included cultivars Berhi, Khazravi, Piyaram and zahedi, second cluster included cultivars Fersi, Oveydi and Gantar, third cluster included cultivars Kabkab and Daglatnoor, while the other one included cultivars Mazafati, deyri, Estamaran, Berem, Majol and Shahani (Fig 2.). The obtained dendrogram from ISSR markers was divided into four main clusters; first cluster included cultivars fersi, Oveydi and Gantar, second cluster included cultivars Kabkab and Daglatnoor, third cluster included cultivars Berhi, Khazravi and Piyaram, while the other one included cultivars Zahedi, Estamaran, Mazafati, deyri, Berem, Majol and Shahani (Fig 3.). To investigate the consistency between genetic dissimilarity and dendrogram, the input and output of dendrogram, Cophenetic correlation coefficient was calculated. Based on value 88.4, 87.3%, Cophenetic correlation coefficient (r) in RAPD, ISSR, respectively we can find that there is high correlation between the matrix of dissimilarity and dendrograms by NJ method.

Principal components analysis

All the data obtained using 10 RAPD primers were used in PCA analysis using Dice's coefficients of similarity. Results exhibited that the three first axes (38.15, 17.22 and 15.40, respectively) accounted for 70.77% of the total variability. Fig. 4 illustrates the distribution of the cultivars according to the first two components. Results of ISSR primers exhibited that the three first axes (39.56, 17.89 and 14.80, respectively) accounted for 72.25 of the total variability. Fig. 5 illustrates the distribution of the cultivars according to the first two components (axis1–axis2). In both of type markers, all 15 cultivars formed separate clusters at different space in the two dimensions of the PCA.

Analysis of molecular variance

The AMOVA analysis revealed that the percentages of variances attributable to the differences between and within groups were 3% and 97% for RAPD and 5% and 95% for ISSR, respectively. These results indicated considerable genetic differentiation within the 15 cultivars (in form of population) of studied. The results showed that in other-cultivated species, these results are expected. Because a low level of genes distinguish is seen in the maximum diversity was associated with inside the cultivars (Hamrick, 1999).

Discussion

The results of this study showed clear and high yields of amplified DNA fragments. RAPD and ISSR method appears to be a powerful technique for the analysis of genetic diversity of date palm germplasm. Many oligonucleotide primers are now known for future genetic diversity analysis of date palm and for the identification of available varieties. 10 primers in RAPD and 14 primers in ISSR showed a high number of bands and had been discriminated among the 15 cultivars. RAPD data showed high polymorphism (92.4%) among cultivars. Similarly, Hussein et al. (2004) and Adawy et al. (2004) reported low RAPD polymorphism an Egyptian date palm cultivars (25.2% and 18.9%, respectively). Moghaieb et al. (2010) reported relatively high RAPD polymorphism (60.2 %). The polymorphism revealed of 14 ISSR loci was 95.7 percent. Hussein et al. (2004) and Adawy et al (2004) reported ISSR polymorphism an Egyptian date palm cultivars (28.6% and 64.1%, respectively). Moghaieb et al. (2010) reported high ISSR polymorphism (73 %). RAPD and ISSR markers are dominant molecular markers but ISSR markers showed a higher mean value for the number of effective alleles, Shannon index, Nei's genetic diversity and PIC. This study represents the first attempt to used ISSR markers beside RAPD markers to differentiate Iranian un-worked date palm cultivars. Inter- simple sequence repeats markers have been used in discriminating date palm cultivars and have been introduced as a useful tool in several DNA marker studies (Hossien et al., 2004; Adawy et al., 2004; Moghaieb et al, 2010; Abdulla and Gamal, 2010; Karim et al., 2010; Al-Hadidi, 2010). Both marker types showed the highest genetic similarity between Gantar and Deyri cultivars and RAPD and ISSR markers show highest genetic distance of these cultivars with Khazravi and Kabkab cultivars. Hussein et al. (2004) estimated Dice similarity coefficient between 14 examples of 6 Egypt cultivars by means of RAPD and ISSR markers ranged from 0.995-0.965 to 0.921-

Table 4. Gene diversity in the 15 populations of date palm.

Population	Sample size	The percent of gene locations of polymorphism		The number of effective alleles		Shanon index		Gene diversity index	
		ISSR	RAPD	ISSR	RAPD	ISSR	RAPD	ISSR	RAPD
Shahani	5	45.06	43.94	1.194	1.200	0.200	0.201	0.126	0.128
Majol	5	46.30	43.94	1.211	1.210	0.217	0.207	0.139	0.133
Berim	5	32.10	34.85	1.112	1.124	0.130	0.143	0.079	0.087
Estamaran	5	35.80	39.39	1.133	1.153	0.150	0.168	0.092	0.104
Zahedi	5	31.48	34.85	1.121	1.135	0.135	0.181	0.083	0.093
Piyram	5	33.95	37.88	1.161	1.186	0.159	0.181	0.102	0.117
Khazravi	5	54.32	53.79	1.270	1.267	0.255	0.258	0.163	0.167
Berhi	5	45.06	44.70	1.180	1.187	0.194	0.196	0.121	0.123
Deiry	5	30.25	33.33	1.092	1.105	0.114	0.127	0.067	0.075
Mazafati	5	53.09	56.06	1.251	1.255	0.248	0.257	0.158	0.163
Oveydi	5	62.35	61.36	1.304	1.292	0.290	0.286	0.188	0.184
Fersi	5	54.94	53.79	1.257	1.264	0.265	0.258	0.171	0.166
Gantar	5	23.46	28.79	1.085	1.104	0.097	0.119	0.059	0.072
Daglatnoor	5	50.62	52.27	1.218	1.226	0.282	0.233	0.140	0.148
Kabkab	5	59.26	59.09	1.273	1.286	0.282	0.284	0.178	0.183

Table 5. The genetic similarities of various cultivars of Iran local date palm by 132 alleles of 10 RAPD primers (bottom the table) and 162 alleles of 14 ISSR primers (above diameter).

Population	Shahani	Majol	Berim	Estameran	Zahedi	Piarem	Khazravi	Berhi	Deyri	Mazafati	Oveydi	Farsi	Gantar	Daglatnoor	Kabkab
Shahani	1	0.985	0.986	0.986	0.986	0.979	0.969	0.985	0.984	0.974	0.978	0.979	0.982	0.986	0.948
Majol	0.985	1	0.978	0.982	0.981	0.976	0.963	0.981	0.981	0.969	0.976	0.978	0.975	0.966	0.948
Berim	0.986	0.979	1	0.990	0.990	0.983	0.954	0.986	0.993	0.967	0.977	0.975	0.993	0.976	0.946
Estamaran	0.985	0.983	0.989	1	0.993	0.985	0.964	0.985	0.992	0.970	0.979	0.975	0.991	0.975	0.946
Zahedi	0.985	0.983	0.990	0.992	1	0.986	0.964	0.988	0.993	0.971	0.976	0.976	0.992	0.976	0.948
Piyram	0.978	0.975	0.981	0.982	0.984	1	0.973	0.982	0.979	0.967	0.973	0.972	0.982	0.979	0.943
Khazravi	0.965	0.961	0.947	0.959	0.959	0.970	1	0.973	0.952	0.973	0.962	0.967	0.952	0.962	0.958
Berhi	0.985	0.981	0.985	0.984	0.988	0.980	0.970	1	0.984	0.976	0.976	0.980	0.985	0.975	0.953
Deiry	0.983	0.980	0.993	0.990	0.992	0.975	0.945	0.983	1	0.966	0.973	0.970	0.994	0.972	0.941
Mazafati	0.974	0.970	0.968	0.969	0.971	0.966	0.970	0.976	0.965	1	0.975	0.972	0.965	0.962	0.964
Oveydi	0.974	0.974	0.974	0.977	0.973	0.970	0.965	0.973	0.969	0.975	1	0.981	0.973	0.972	0.952
Fersi	0.978	0.978	0.975	0.976	0.978	0.974	0.965	0.980	0.970	0.974	0.979	1	0.969	0.971	0.954
Gantar	0.981	0.976	0.992	0.989	0.991	0.979	0.945	0.984	0.993	0.965	0.969	0.970	1	0.976	0.941
Daglatnoor	0.967	0.966	0.973	0.973	0.975	0.977	0.957	0.973	0.969	0.961	0.969	0.972	0.974	1	0.960
Kabkab	0.958	0.965	0.953	0.955	0.956	0.950	0.960	0.961	0.948	0.969	0.956	0.961	0.949	0.963	1

Table 6. Hierarchical AMOVA based on ISSR and RAPD markers.

Change source	Degree of freedom	Sum of square		Mean of square		Predicted variance		Total	
		ISSR	RAPD	ISSR	RAPD	ISSR	RAPD	ISSR	RAPD
Between cultivars	14	298.2123	237.680	21.301	16.976	0.839	0.513	5%	3%
Within cultivars	60	1026.400	864.800	17.107	14.413	17.107	14.413	95%	97%
Total	74	1324.613	1102.480						

100. In another study, the genetic distance between 4 cultivars in Saudi Arabia was 0.66 to 0.85 (Abdulla and Gamal, 2010). NJ trees of the combined data separated the cultivars in four clusters. The 15 cultivars studied were genetically different using morphological characteristics of the fruits (color and form of the fruit). In fact, most of the date palm cultivars grown in Iran can be distinguished at the production stage using morphologic and taste characteristics. The RAPD and ISSR techniques are useful to differentiate the cultivars that cannot be discriminated by the morphology, but most importantly to study the genetic diversity of unnamed genotypes. El-Rayes (2009) was studied characterization of three date palm cultivars based on RAPD fingerprints and fruit chemical composition. In this study a dandrogram was constructed using UPGMA analysis. On the basis of this analysis, the populations were clustered into two clusters: cluster I contained Red Sukkary and Yellow Sukkary cultivars, and cluster II contained Naptet Sukkary cultivar. Askari et al. (2003) in Saudi Arabia classified 7 date palm cultivars into two clusters. Cluster "A" consisted of 3 cultivars viz., Khalas, Barhy and Deglat Noor. Cluster "B" consisted of 3 cultivars viz., Omal, Khashab, Hilwa and Shagra. The cultivar Hilali does not belong to any of the cluster group. Zahedi et al. (2004) investigated 12 ecotype of date by ISSR marker. It was defined that the lowest distance between Zahedi and Gharsmetting was existing. Karim et al. (2010) to investigate the genetic diversity of 10 date palm cultivars via ISSR marker, used dice similarity matrix and UPGMA method to plot dendrogram. In this investigation, Zahedi and Daglatnoor were in one group and Berhi in a different group of other varieties. The results obtained from AMOVA analysis performed between 15 cultivars (populations) showed significant difference within the cultivars studied. The result of AMOVA also supported genetic relationship within cultivars by cluster analysis.
In conclusion, this paper has provided an efficient procedure that can be used routinely to identify date palm cultivars, and can be used to study the genetic diversity of ornamental date palms that are difficult to classify according to their morphological traits and geographical origin.

Materials and methods

Plant Materials

fifteen Iranian date palm cultivars (shahani, Majol, Berim, Zahedi, Piyaram, Kazravi, Berhi, Deyri, Mazafati, Oveydi, Fersi, Gantar, Daglatnoor, Kabkab) of the Abadan, Ahvaz, Behbahan, Jahrom and Bam germplasm collected for molecular studies (Table. 1).

DNA extraction

Best leaves were collected from each plant and five plants per cultivar were subjected to molecular analysis (numbers 1 to 5) and then DNA was extracted by Saghai- Maroof (1984) with a little change. DNA samples of each cultivar were

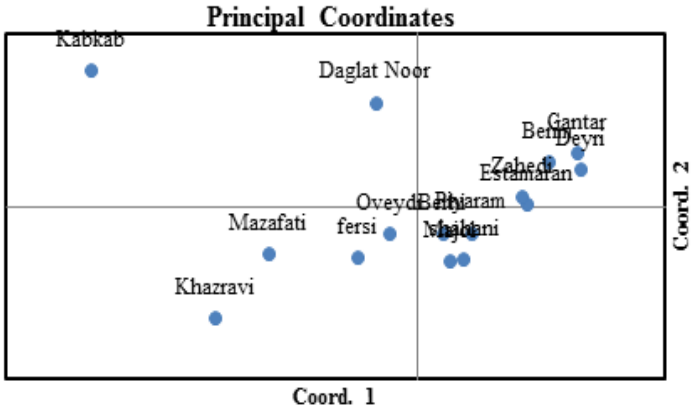

Fig 5. Principle Component Analysis (PCA) ordination based on ISSR data

analysed individually. DNA quantity was evaluated by spectrophotometer and its quality was analyzed by agarose gel 0.8 electrophoresis.

PCR Conditions and Electrophoresis

A set of 10 RAPD primers and 14 ISSR primers were used (Table 2 and 3). Polymerase chain reaction for both RAPD and ISSR markers were performed by thermal cycler Bio-Rad in a 25 µL volume containing: 1.5 µL genome DNA (40-50 ngr DNA), 2 µL buffer 10X PCR, 0.4 µL dNTPs 1mM, 1.2 µL primer, 1.5 (0.3 µL) DNA Taq polymerase and 1.5 µL chloride magnesium 20mM, finally by 12.1 µL distilled water twice sterile, the reaction volume was reached to 20 µL.
The profile used consisted of an initial denaturation for 4 min at 94° C, followed by 35 cycles was as Touchdown. Amplification products were visualized by running on 1.5% agarose gel in 1 X TBE buffer system, followed by ethidium bromide (0.5 µg mL^{-1}) staining. Fragment size was estimated by using a 100 base pairs (bp) molecular size ladder (Fermentas, Germany).

Data analysis

Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands in Excel software. PIC was calculated for each primer by formula

$$PIC = \sum(1 - P_i^2) / n$$

p_i was frequency of ith allele, n the total number of genotypes (Wier, 1990). The marker index was calculated of the product of the number of multiple bands in multiple content indexes (Kumar et al. 2009). To calculate the matrix of genetic distance and analysis to main components of PCA, DARwin 5.0.146 (http://darwin.cirad.fr/darwin) software was used. The cluster analysis was done to plot dendrogram of genotypes and molecular variance analysis to calculate genetic diversity inside and between the populations,

respectively by software MEGA 3.1 (Tamura et al., 2007), GenAlEx 6.41 (Peakall and Smouse, 2006). Principal coordinated analysis (PCA) was also carried out to show multiple dimension of the distribution of the cultivars in a scatter-pot by GenAlEx 6.41 software (Peakall and Smouse, 2006).

Acknowledgements

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