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Genetic fingerprinting of date palm (*Pheonix dactylifera* L.) by using ISSR and cpDNA sequences

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Abstract

The date palm (*Phoenix dactylifera* L.) is the most important fruit bearing crop in arid regions of the Middle East and North Africa. The knowledge on genetic structure of date palm is limited and understanding of genetic variability within date palms is important for the efficient utilization of the genetic resources in breeding. Therefore, in the present study, genetic analysis of 14 date palm cultivars collected from 28 localities in Iran was performed to provide prospective of genetic variability and population structure. In addition, publically available sequencing data of chloroplast genome available for 47 cultivars was also explored. The results showed a good level of genetic variability among the cultivars. Grouping of the cultivars was not related to their geographical distribution and no correlation was observed between genetic distance and geographical distance of date palms. Population structure analysis revealed the presence of a low degree of common shared alleles between date palms cultivars that indicate probable local adaptation in structuring the genetic content of date palms.

Key words: *Phoenix dactylifera*, ISSR, cp-DNA, fingerprinting

Introduction

The date palm (*Phoenix dactylifera* L.), a member of Arecaceae family is considered as the most important fruit-bearing crop in arid regions of the Middle East and North Africa. Date palms grow mainly in the desert oases, river valleys and well-irrigated farms. About 3000 date varieties or cultivars are cultivated worldwide. Wide range of diversity existed among the date cultivars for fruit moisture and sugar content, as well as in flowering time. The date palm is one of the earliest cultivated fruit trees pollinated by humans, it is cultivated in south of the Mediterranean Sea, the Middle East, south Asia (Iran) and the Atlantic coast of North Africa in the west; they comprise a date palm oasis (Jaradat 2004). The date palm has also been introduced in the USA (California), Peru, Australia and many other countries (Zehdi-Azouzi et al. 2015).

Date palm is the second most important crop of Iran after pistachio comprising of 400 cultivars. The fruit yield ranges from 100 to 400 kg per tree per annum depending on the cultivation conditions (Jaradat 2004). These cultivars are adapted to local environmental conditions and vary in the quality of fruit. Date palm play an important role in the diet and social life of communities in Middle East (Jaradat 2015).

Limited information is available on genetic variability and population structure of date palm cultivars (Jaradat 2004; Jaradat 2015). For a successful date palm industry, accurate estimates of genetic diversity and its partitioning, especially for fruit quality traits, within and among available gene pools, are important (Jaradat 2015). Publically available date palm genetic resource includes several breeding lines, cultivars, landraces, and wild relatives. Different causes shaped the present day genetic structure and gene pools of date palm; these include human activities, natural selection, clonal propagation, stalk exchanges, and local traits adaptation (Jaradat 2015).

Date palm production has shifted from traditional cultivation in rich and diverse agro systems to intensive monocultures. This development has led to severe genetic erosion, with the loss of cultivars and the overall impoverishment of date palm agro-biodiversity (Jain et al. 2011). Moreover, it is important to find tolerant cultivars for cultivation in arid areas therefore, a global evaluation of the genetic diversity in current date palm accessions is necessary.

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Various molecular markers have been used to study genetic diversity and molecular characterization in date palms. These include simple sequence repeat (SSR) markers (Elmeer et al. 2011; Arabnezhad et al. 2012; Zehdiet al. 2015), Random amplified polymorphic DNA (RAPDs) (Sedra et al. 2008; Bahraminejad and Mohammadi-Nejad 2015), chloroplast DNA sequences (Al-Qurainy et al. 2011), use of circular plasmid like DNAs for molecular characterization (Guettouchi 2017), enzymatic polymorphism (Bennaceur et al. 1991), amplified fragments length polymorphism (AFLP) (Rhouma et al. 2009), inter-simple sequence repeats (ISSR) (Karim et al. 2010; Wafa et al. 2018), and random amplified microsatellite polymorphism (RAMPO) (Rhouma-Chatti et al. 2011). ISSR molecular markers are consistent and cheap (Sheidai et al. 2013), while cpDNA can be used in cultivar differentiation as they include many mutations (Al-Qurainy et al. 2011).

Recently, Hajian and Hamidi-Esfahani (2015) carried out genetic diversity analysis in a few elite Iranian data palms, but detailed information is needed for efficient use for future conservation and breeding program. The present study was, therefore, conducted to analysis the genetic diversity in 14 date diverse palm cultivars using a combination of ISSR and cpDNA (PsbA) molecular markers.

Materials and methods

A total 130 plants of *Phoenix dactylifera* were studied in 14 cultivars collected from 28 localities (Table 1) in Iran.

DNA extraction

For molecular studies, the fresh leaves were randomly collected from arbitrarily selected plants in the studied area and were dried in silica gel powder. The genomic DNA was extracted using CTAB-activated charcoal protocol (Kri• man et al. 2006). The extraction procedure was based on activated charcoal and poly vinyl pyrrolidone (PVP) for binding of polyphenolics during extraction and under mild extraction and precipitation conditions. This promoted high-molecular-weight DNA isolation without interfering contaminants. Quality of extracted DNA was examined by running on 0.8% agarose gel.

ISSR genotyping assay

A set of ten ISSR primers, UBC 807, UBC 810, UBC 811, UBC 834, CAG(GA)7, (CA)7AC, (CA)7AT, (CA)7GT (GA)9A, and (GA)9T, commercialized by the University of British Columbia, were used. PCR reactions were performed in a 25 μL volume containing 10 mM Tris-HCl buff er at pH 8, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM of each dNTP (Bioron, Germany), 0.2 ìM of a single primer, 20 ng of genomic DNA, and 3 U of Taq DNA polymerase (Bioron). Amplification reactions were performed in a Techno thermo cycler (Germany) with the conditions: 5 min for initial denaturation step at 94°C, 30 s at 94°C, 1 min at 53.5°C and 1 min at 72°C. The reaction was completed by a final extension step of 7 min at 72°C. The amplification

Table 1. *Phoenix dactylifera* cultivars and localities for ISSR studies

Cultivars numbers	Cultivar	No. of locality	Locality
1	Mazafati	1	Mahrooyeh
		2	Kahnooj
		3	Bam
		4	Jiroft
2	Kalooteh	5	Jiroft
		6	Mahrooyeh
		7	Kahnooj
		8	Roodbar
3	Khale zohrei	9	Roodan
4	Holeileh	10	Kahnooj
		11	Faryab
		12	Minab
5	Mordarsang	13	Mahrooyeh
		14	Kahnooj
		15	Jiroft
		16	Roodan
		17	Roodbar
6	Khazab	18	Minab
7	Holoo	19	Minab
8	Khenizi	20	Minab
		21	Roodan
9	Negar	22	Mahrooyeh
10	Shahani	23	Faryab
11	Male isolate	24	Jiroft
		25	Faryab
12	Unknown	26	Faryab
13	Alimehtari	27	Faryab
14	Kharook	28	Jiroft

products were visualized by running on 2% agarose gel followed by ethidium bromide staining. The fragments size was estimated by using a 100-bp molecular size ladder (Fermentas, Germany).

Chloroplast DNA sequences analyses

Cp-DNA (trnH-psbA sequences) sequences of 47 date palm cultivars were obtained from NCBI (National Center for Bioinformatic Information) and used to differentiate the studied cultivars. The cultivars accession numbers have been provided in Table 2.

Data analyses

The ISSR bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). The number of unique bands versus common bands was determined. Genetic diversity parameters like, the percentage of allelic polymorphism, allele diversity, Nei's gene diversity (He), and Shannon information index (I) (Weising 2005), were determined. The Nei genetic distance (Weising 2005) was determined among the studied populations and was used for the grouping of the genotypes. Genetic differentiation of the studied populations was studied by AMOVA with 1000 permutations as performed in GenAlex 6.4 (Peakall and Smouse 2006). The Mantel test (Podani 2000) was performed to study the association between genetic distance and geographical distance of the studied populations. We also used Mantel test to investigate the agreement of results between ISSR data and geographical locality. For grouping of the plant specimens, the UPGMA (Unweighted paired group using average), Neighbor Joining (NJ) and PCoA (Principal coordinate analysis) were used (Podani 2000). PAST version 2.17 (Hammer et al. 2012) was used for these analyses.

Genetic structure of the populations was studied by model-based clustering as performed by STRUCTURE software ver. 2.3 (Pritchard et al. 2000). We used the admixture ancestry model under the correlated allele frequency model. The Markov chain Monte Carlo simulation was run 20 times for each value of K (1-13) for 20 iterations after a burn-in period of 10^5. All other parameters were set at their default values. Data were scored as dominant markers and analysis followed the method suggested by Falush et al. (2007). The STRUCTURE analysis was followed by Evanno method (Evanno et al. 2005), as peformed by STRUCTURE Harvester online tool (Earl and von Holdt 2012). For cp-DNA, the sequences were aligned by MUSCLE program as implemented in MEGA 7. NJ and Maximum Parsimony phylogenetic trees were constructed by MEGA7 software (Tamura et al. 2012). Kimura distance was determined for date palm cultivars based on cp-DNA sequences by Darwin ver.6.

Table 2. The accession numbers of *Phoenix dactylifera* cultivars in cp-DNA studies

No.	Accession number	No.	Accession number	No.	Accession number
1	KY768792- 1	17	KY88136- 1	33	KY768780- 1
2	KY768791- 1	18	KY88137-1	34	KY768777- 1
3	KY768788- 1	19	KY88137-2	35	KY88131- 1
4	KY768786- 1	20	KY88134- 1	36	KY88130- 1
5	KY88132- 1	21	KY88140- 1	37	KY88141- 1
6	KY88147-1	22	KY768785- 1	38	KY88142-1
7	KY88148- 1	23	KY768784- 1	39	KY88145-1
8	KY88144-1	24	KY768783- 1	40	KY88146- 1
9	KY768789-1	25	KY768779- 1	41	KY88149- 1
10	KY768789-2	26	KY88143- 1	42	KY768790-1
11	KY768787- 1	27	KY88135- 1	43	JN854235-1
12	KY768781- 1	28	KY768782- 1	44	JN854230-1
13	KY88139- 1	29	KY768778- 1	45	JN854232-1
14	KY88138- 1	30	KY88133- 1	46	JN854233-1
15	JN854233-2	31	JN854229- 1	47	JN854234-1
16	JN854228- 1	32	JN854231-1		

Results

Genetic diversity by ISSR markers

We obtained 34 ISSR bands (Loci) in total (Table 3). The highest mean number of bands occurred in cultivars Kaloochand Mazafati. The studied cultivars showed the occurrence of both unique as well as common bands.

The highest value for effective no. of alleles as well as genetic diversity due to cultivars (HS) occurred in cultivars, Mazafati and Kalootech. Of the 34 ISSR loci obtained, 25 had significant discrimination power among the studied cultivars ($P < 0.01$). NJ clustering (Fig. 1a) of the studied samples revealed that date palm cultivars are almost distinct in their genetic content as their studied specimens were grouped together in a separate cluster. Very few cases of intermixed plants were observed in the studied cultivars.

STRUCTURE bar plot (Fig. 2) of these cultivars

Table 3. Genetic diversity parameters used to evaluate *Phoenix dactylifera* cultivars (cultivars numbers are according to Table 1)

Cultivars	Num	Eff. Num	Hs
1	1.618	1.349	0.216
2	1.706	1.311	0.211
3	1.059	1.028	0.024
4	1.618	1.288	0.189
5	1.529	1.154	0.100
6	1.059	1.047	0.039
7	1.206	1.165	0.137
8	1.206	1.110	0.082
9	1.029	1.024	0.020
10	1.088	1.071	0.059
11	1.176	1.110	0.078
12	1.029	1.024	0.020
13	1.206	1.165	0.137
14	1.265	1.212	0.176

Num = Mean no. of alleles, Eff. Number = Effective no. of alleles, and Hs = Genetic diversity due to population

revealed more detailed information on genetic structure of the studied date palms. For example, plants of Mazafati cultivar were collected from 4 localities (localities 1-4 in Fig. 2). They differed in their genetic content as revealed by different allele combinations (differently coloured segments). Plants in the localities 2 and 3 are more similar in their genetic structure than plants in localities 1 and 4. The same holds true for cultivar Kaleiteh (numbers 5-8 in Fig. 2). Plants in locality 6 are much different from the other localities. We have some degree of genetic difference among plants in Holeileh cultivar (Numbers 10-12) as well as in Mordarsang cultivar (Numbers 13-17).

PCoA plot obtained after 99 permutations, grouped the studied date palm cultivars in 3 main groups. The cultivars, 1-4 (Mazafati, Kaleiteh, Khale-Zohrehi, and Holeileh, respectively) comprised the first group. They were placed in the upper right corner of the PCoA plot. Cultivars 5-7 (Mordarsang, Khazab, and Holoo, respectively) formed the second group, as they stood in the right lower corner of the PCoA plot. The rest of cultivars were placed in the third group, located in the left side of the PCoA plot. This result is also supported by K-Mean clustering presented bellow.

K-Means clustering of date palm cultivars, also supported the PCoA groupings and produce k = 3 as the best grouping based on Calinski & Harabasz' pseudo-F (Table 3). This analysis also produced k = 14 based on Bayesian Information Criterion, which indicates that all 14 cultivars differ in genetic content. AMOVA performed among all cultivars produced significant genetic difference among the studied cultivars (PhiPT = 0.56, P = 0.01). It also revealed that 56% of total genetic variability occurred due to among cultivar genetic difference, while 44% was due to within cultivar genetic variability. This is also supported by new genetic differentiation parameters G'st(Nei) = 0.56 (P = 0.001), and G'st(Hed) = 0.62 (P = 0.001). Pair-wise AMOVA almost revealed significant genetic difference among date palm cultivars studied (Table 4).

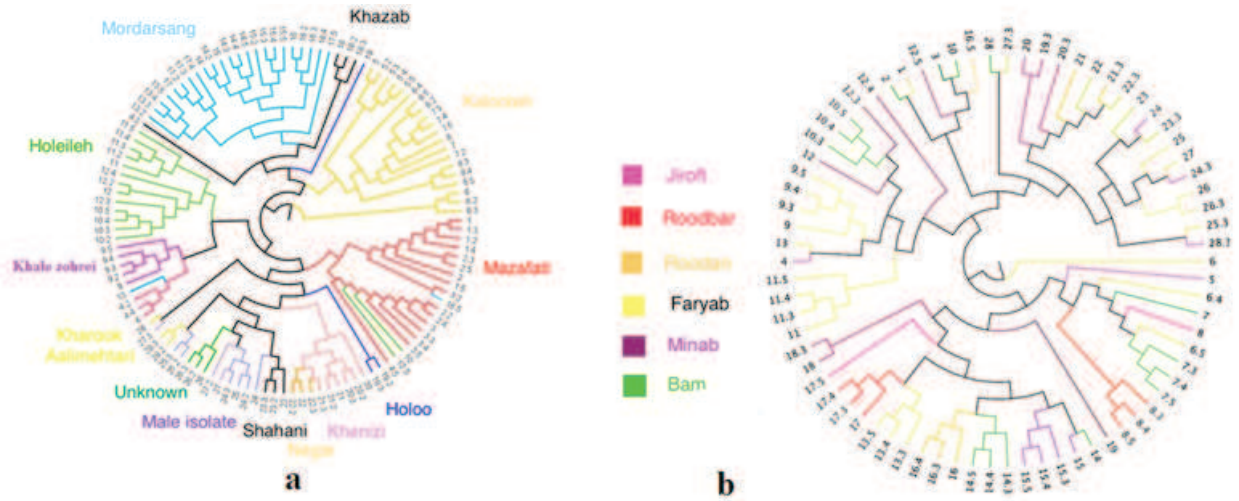

Fig. 1. a = Neighbor-joining tree of 14 *Phoenix dactylifera* cultivars generated using genotypic data developed with ISSR markers, b = UPGMA tree of the studied date palm cultivars based on geographical locality. (Numbers in each locality are different orchards visited)

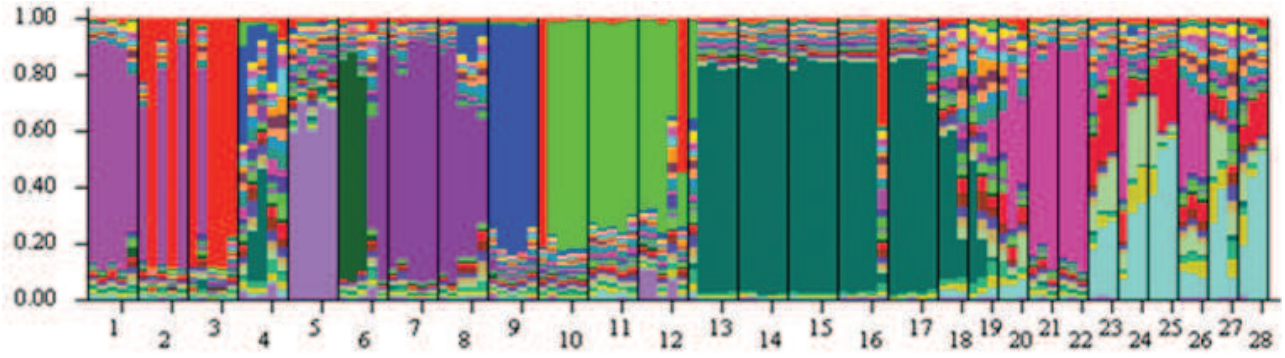

Fig. 2. STRUCTURE plot in 14 cultivars of 28 locality of *Phoenix dactylifera* (cultivars numbers are according to Table 1)

As stated before, the STRUCTURE plot revealed the presence of some shared common alleles. To identify the cultivars with shared alleles the population assignment test was performed (Table 5). The high likelihood ratio which is indicative of significant degree of shared common alleles among two populations revealed that gene flow/shared common alleles occur between cultivars 1 and 4, cultivars 4 and 5, 1 and 5, as well as 8 and 11. This is in few individuals in each cultivar. Mantel test showed no significant correlation between genetic distance and geographical distance of the date palm cultivars studied ($R^2 = 0.008$. $P > 0.05$). Therefore, no isolation by distance (IBD) is present in these cultivars and the genetic difference among date palms is not due to geographical distance.

UPGMA clustering (Fig. 1b) Performed among date palms studied based on their localities (six geographical regions) did not group the cultivars based on similar locality. For example, the cultivars collected from Roodbar (localities 8 and 17) were placed in different clusters far from each other. The same holds true for cultivars collected from Minab (Localities 18, 19, and 20), and for ate palms collected from Faryab (Localities 6, 9, 11, 13, and 27). These results indicate that, genetic similarity/or difference of date palm cultivars is not affected by their geographical region. Therefore, we may conclude that the genetic difference of date palms is probably due to local cultivation practice.

Table 3. K-Means clustering of date palm cultivars based on ISSR data (Cultivars numbers are according to Table 1)

k	SSD(T)	SSD(AC)	SSD(WC)	r-squared	pseudo-F	AIC	BIC	Rho
1	563.119	0.000	0.000	0.000	0.000	186.446	752.123	0.000
2	563.119	98.422	464.697	0.175	24.568	165.847	734.225	0.290
3*	563.119	169.346	393.772	0.301	24.729	148.411	719.453	0.381
4	563.119	221.668	341.451	0.394	24.669	133.731	707.401	0.456
5	563.119	259.677	303.442	0.461	24.176	121.987	698.246	0.498
6	563.119	287.463	275.655	0.510	23.360	112.876	691.684	0.539
7	563.119	310.424	252.694	0.551	22.726	104.875	686.192	0.574
8	563.119	330.104	233.015	0.586	22.262	97.611	681.396	0.601
9	563.119	346.219	216.899	0.615	21.749	91.499	677.709	0.620
10	563.119	359.517	203.602	0.638	21.189	86.423	675.015	0.633
11	563.119	375.100	188.019	0.666	21.347	79.462	670.389	0.659
12	563.119	390.105	173.014	0.693	21.728	72.128	665.346	0.682
13	563.119	398.545	164.573	0.708	21.190	68.755	664.215	0.693
14	563.119	407.613	155.506	0.724	20.970	64.645	662.298	0.706

* Best clustering according to Calinski and Harabasz' pseudo-F: k = 3; and Best clustering according to Bayesian Information Criterion: k = 14; SSD (T) = Total sum of squares, SSD (AC) = Among clusters sum of squares, and SSD (WC) = Within clusters sum of squares

Table 4. Pair-wise Fst value and their significant level in date palm cultivars studied (Cultivars numbers are according to Table 1)

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	___	0.35	0.42	0.41	0.47	0.35	0.38	0.29	0.36	0.34	0.31	0.32	0.30	0.26
2	0.01	___	0.42	0.46	0.52	0.41	0.42	0.48	0.49	0.53	0.54	0.53	0.50	0.44
3	0.01	0.01	___	0.44	0.75	0.90	0.82	0.77	0.91	0.87	0.79	0.91	0.80	0.70
4	0.01	0.01	0.01	___	./60	0.58	0.47	0.47	0.47	0.47	0.49	0.43	0.46	./35
5	0.01	0.01	0.01	0.001	___	0/42	0.47	0.67	0.69	0.70	0.69	0.69	0.64	0.66
6	0.01	0.01	0.02	0.006	0.01	___	0.62	0.69	0.87	0.81	0.74	0.87	0.69	0.61
7	0.01	0.01	0.01	0.001	0.01	0.10	___	0.55	0.61	0.63	0.59	0.68	0.49	0.43
8	0.01	0.01	0.01	0.001	0.01	0.01	0.01	___	0.25	0.40	0.45	0.52	0.53	0.48
9	0.01	0.01	0.01	0.004	0.01	0.10	0.08	0.06	___	0.74	0.65	0.84	0.68	0.60
10	0.01	0.01	0.01	0.002	0.01	0.09	0.11	0.01	0.09	___	0.25	0.68	0.48	0.33
11	0.01	0.01	0.01	0.001	0.01	0.01	0.01	0.02	0.01	0.034	___	0.46	0.23	0.13
12	0.01	0.01	0.01	0.002	0.01	0.09	0.11	0.02	0.09	0.093	0.01	___	0.50	0.44
13	0.01	0.01	0.02	0.003	0.01	0.10	0.11	0.01	0.10	0.100	0.02	0.11	___	./11
14	0.01	0.01	0.02	0.002	0.01	0.11	0.09	0.01	0.11	0.104	0.16	0.10	0.38	___

Above diagonal = Fst, bellow diagonal; = P value

Table 5. The population assignment test in few individuals in date palm cultivars studied

Individual	Current	Inferred	max_ ikL	home_ Lik	ratio_ Lik
16	Pop1	Pop4	–15.223	–21.961	13.475
18	Pop1	Pop5	–13.386	–19.25	11.313
46	Pop4	Pop1	–9.322	–33.409	48.154
61	Pop5	Pop4	–11.316	–36.171	49.722
80	Pop5	Pop1	–9.078	–30.694	43.232
104	Pop11	Pop8	–9.741	–13.977	8.510

cpDNA assay

CP-DNA sequences of the date palm cultivars studied revealed nucleotide diversity: pi = 133.493, with 269 as the number of segregating sites, 237 numbers of parsimony-informative sites. We obtained Tajima's D statistic: D = 3614.11, p (D > 3614.11) = 0. This indicates that DNA sequences studied evolves. The genetic distance based on cp-DNA obtained varied from 0.00 to 0.70 among the studied date palms. Therefore, cp-DNA is an efficient molecular marker to be used in date palm genetic fingerprinting. Maximum parsimony phylogenetic tree constructed based on cp-DNA (trnH-psbA) sequences, grouped the cultivars almost in 6 major clades with 100% bootstrap value. Therefore, the studied sequence data can be used in date palm cultivars differentiation.

Discussion

Date palm is very important horticultural crop plant of Iran cultivating about 400 cultivars. Most of date palm cultivation is traditional and less effort have been made toward the genetic improvement in Iran. To improve the productivity and to obtain more secure date production, modern approaches like biotechnology and molecular breeding need to be explored. Genetic evaluation of germplasm will be beneficial for effective utilization of the resources. According to Jaradat (2015), a thorough assessment of genetic diversity and population differentiation of *P. dactylifera* are critical for its dynamic conservation and sustainable utilization of the genetic diversity. The present study was an attempt to understand population structure and clonal variation in respective of geographical distribution of 14 date palm cultivars. The results presented here revealed a significant genetic diversity among the studied cultivars. This is in agreement with previous report on the other date palm cultivars of the country by Marsafari and Mehrabi (2013), which used RAPD and ISSR molecular markers and reported 92.4% and 95.67% polymorphism, respectively.

The mean genetic distance obtained among the studied date palm cultivars based on ISSR markers

was 0.35 (Nei genetic distance), while, 0.2 (Kimura's 2-parameter) obtained by cpDNA sequences. However, Marsafari and Mehrabi (2013) reported 0.6 mean genetic distance in date palm cultivars by using ISSR and RAPD molecular markers. This may be due to the different cultivars they studied. Bahraminejad and Mohammadi-Nejad (2015) used RAPD markers in 6 date palm cultivars and reported mean genetic distance value of 0.42. Wafa et al. (2018) used genomic DNA in zygotic embryo of date palm cultivars and analysed by both RAPD and ISSR markers. They reported genetic polymorphism percentage ranging between 32.09% and 36.49%, respectively.

Genetic diversity studies performed on date palm cultivars in the other countries have also revealed a good level of genetic diversity among the cultivars. For example, Elmeer and Mattat (2015) used SSR molecular markers to investigate the genetic diversity in 59 female accessions representing 12 cultivars from different locations in Qatar. They reported the mean gene diversity of 0.66. Forty-four percent of the variability was explained at the inter-population level, while 56% of the variability was due to within individuals. This result is very close to the AMOVA result presented in our ISSR data.

Elshibi and Korpelainen (2008) used microsatellite markers to investigate genetic diversity in date palms of Sudan. They studied 37 female and 23 male accessions and obtained a high level of genetic polymorphism with an average of 21.4 alleles per locus and expected heterozygosity of 0.841. The results indicated that the genetic groups of the Sudan cultivars and/or males do not follow a clear geographic pattern. This is in agreement with the present study which revealed that date palms in different orchards within a single geographical region did not group together and were placed in different clusters.

Investigation performed by Jaradat (2015), revealed that most of the variation estimated for fruit quality traits was found among populations; however, substantial differences in genetic diversity components were found among and within populations. Jaradat (2015) also states that date palm cultivars represent a complex gene pool within which historical movement of germplasm, recent introductions and human selection are shaping their genetic structure. Moreover, the amount of genetic diversity differs between oases and populations, or between regions and localities, and several key historical, geographical, ecological and anthropogenic factors determine its magnitude and distribution.

In conclusion, both ISSR and cpDNA markers revealed genetic variability within 14 date palms of Iran. These findings can be used in future breeding program of date palm in the country.

Authors' contribution

Conceptualization of research (MaS, FK); Designing of the experiments (MoS, MaS, FK); Contribution of experimental materials (MoS, MaS); Execution of field/ lab experiments and data collection (MoS, MaS); Analysis of data and interpretation (MoS, MaS, FK); Preparation of manuscript (MoS, MaS, FK).

Declaration

The authors declare no conflict of interest.

References

Al-Qurainy F., Khan S., Al-Hemaid F. M., Ali M. A., Tarroum M. and Ashraf M. 2011. Assessing Molecular Signature for Some Potential Date (*Phoenix dactylifera* L.) Cultivars from Saudi Arabia, Based on Chloroplast DNA Sequences rpoB and psbA-trnHInt. Int. J. Mol. Sci., **12**: 68-71.

Balasta M. L. F., Perez C. M., Juliano B. O., VIllareal C. P., Lott J. N. and Roxas D. B. 1989. Effects of silica level on some properties of *Oryza sativa* straw and hull. Can. J. Bot., **67**: 2356-2363.

Arabnezhad H., Bahar M., Mohammadi H. and Latifian M. 2012. Development, characterization and use of microsatellite markers for germplasm analysis in date palm (*Phoenix dactylifera* L.). Sci Hort., **134**: 150-156.

Bahraminejad A. and Mohammadi-Nejad Gh. 2015. Genetic Diversity Analysis of Date Palm (*Phoenix dactylifera* L.) Genotypes using RAPD Markers. Annu. Res. Rev. Biol., **5:** 41-47.

Bennaceur M., Lanaud C., Chevallier M. H. and Bounaga N. 1991. Genetic Diversity of the Date Palm (*Phoenix dactylifera* L.) from Algeria Revealed by Enzyme Markers. Plant Breed., **107**: 56-69.

Falush D., Stephens M. and Pritchard J. K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes, **7**: 574-578.

Earl D. A. and Von Holdt B. M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour., **4**: 359-361.

Elmeer K. and Mattat I. 2015. Genetic diversity of Qatari date palm using SSR markers. Genet. Mol. Res., **14**: 1624-1635.

Elmeer K., Sarwath H., Malek J., Baum M. and Hamwieh A. 2011. New microsatellite markers for assessment of genetic diversity in date palm (*Phoenix dactylifera* L.). Biotech., **1**: 91-97.

Elshibli S. and Korpelainen H. 2008. Microsatellite markers reveal high genetic diversity in date palm (*Phoenix dactylifera* L.) germplasm from Sudan. Genetical, **34**: 251-60.

Evanno G., Regnaut S. and Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol., **14**: 2611-2620.

Guettouchi A., Haider N., Nabulsi I. and Ykhlf. 2017. Molecular characterization of Algerian date palm cultivars using circular plasmid-like DNAs. Indian J. Genet., **77**(1). 170-172. doi: 10.5958/0975-6906.2017.00024.4.

Hammer Ø., Harper D. A. T. and Ryan P. D. 2012. PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica., **4**: 9.

Jain S. M., Al-Khayri J. M. and Johnson D. V. 2011. Date Palm Biotechnology. Berlin, Springer.

Jaradat A. A. 2015. In: Date Palm Genetic Resources and Utilization. (Eds. Al-Khayri J. M., Jain S. M. and Johnson D. V.). Vol 1: Africa and the Americas, PP19-71.

Jaradat A. A.and Zaid A. 2004. Quality traits of date palm fruits in a center of origin and center of diversity. J.F.A.E., **2**: 208-217.

Karim K., Chokri B., Amel S., Wafa H., Richid H. and Nouredine D. 2010. Genetic diversity of Tunisian date palm germplasm using ISSR markers. Inter. J. Bot., **6**: 182-186.

Kri•man M., Jakše J., Barièeviè D., Javornik B. and Prošek M. 2006. Robust CTAB-activated charcoal protocol for plant DNA extraction. ACTA. AGRIC. SLOV., **87**: 427-433.

Marsafari M. and Mehrabi A. A. 2013. Molecular identification and genetic diversity of Iranian date palm (*Phoenix dactylifera* L.) cultivars using ISSR and RAPD markers. A.J.C.S., **7**: 1160-1166.

Peakall R. and Smouse P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes., **6**: 288-295.

Podani J .2000. Introduction to the Exploration of Multivariate Data. Backhuyes: Leiden; p. 407.

Rhouma S., Mohamed Salem A. O., Zehdi-Azouzi S., Chatti K., Rhouma A., Marrakchi M. and Trifi M. 2009. Comparative analysis of genetic diversity in Tunisian date-palm (*Phoenix dactylifera* L.) as revealed by RAPDs and AFLPs. In: ISHS Acta Horticulturae 7rd Symposium on Fruit Breeding and Genetics. Eds. R. Socias i Company, M.T. Espiau, J.M. Alonso.

Rhouma-Chatti S., Baraket Gh., Dakhlaoui-Dkhil S., Zehdi-Azouzi S. and Trifi M. 2011. Molecular research on the genetic diversity of Tunisian date palm (*Phoenix dactylifera* L.) using the random amplified microsatellite polymorphism (RAMPO) and amplified fragment length polymorphism (AFLP) methods. African J. Biotech., **10**: 10352-10365.

Sedra H., Lashermes Ph., Trouslot P., Combes MCh. and Hamon S. 1998. Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. Euphytica, **103**: 75-82.

Sheidai M., Zanganeh S., Haji-Ramezanali R., Nouroozi M., Noormohammadi Z. and Ghsemzadeh-Baraki S. 2013. Genetic diversity and population structure in four Cirsium (Asteraceae) species. Biologia, **68**: 384-397.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S. 2012. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol., **28**: 2731e2739.

Wafa M., Aladadi1 M. F., Sulaiman M. and Alruman A. 2018. Genetic variability among seven cultivars of date palm (*Phoenix dactylifera* L.) based on embryonic DNA of old fruit Kuwait. J. Sci., **45**: 108-114.

Pritchard J. K., Stephens M. and Donnelly P. 2000. Inference of population structure using multilocus genotype Data. Genetics, **155**: 945-959.

Weising K., Nybom H., Wolff K and Kahl G. 2005. DNA Fingerprinting in Plants. in: Principles, Methods, and Applications. (2nd ed.), Boca Rayton, Fl., USA: CRC Press, p. 472.

Zehdi-Azouzi S., Emira Cherif E., Moussouni S., Gros-Balthazard M., Abbas Naqvi S., Luden˜a B., Castillo K., Chabrillange N., Bouguedoura N., Bennaceur M., Si-Dehbi F., Abdoulkader S., Daher A., Terral J F., Santoni S., Ballardini M., Mercuri A., Ben Salah M., Kadri K., Othmani A., Littardi C/, Salhi-Hannachi A/, Pintaud JCh. and Aberlenc-Bertossi F. 2015. Genetic structure of the date palm (*Phoenix dactylifera* L.) in the Old World reveals a strong differentiation between eastern and western populations. Ann. Bot., **116**: 101-112.