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GENETIC STRUCTURE AND DIVERSITY OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS IN IRAN REVEALED BY REMAP GENOTYPING

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The date palm (*Phoenix dactylifera* L.) is the most important fruit-bearing crop in arid regions of the Middle East and North Africa. About 3,000 date varieties or cultivars are known worldwide that differ in flowering time, several agronomic traits, and fruit-related traits including moisture and sugar content. *Phoenix dactylifera* is the second most important horticultural crop of Iran that is cultivated mainly in the southern part of the country. It has about 400 known cultivars in Iran and therefore comprises an important part of the whole world date palm genetic resources. We have no detailed information on its population genetic structure. The present study was an attempt to provide the population genetic data on 14 date palm cultivars for the first time. The present study tried to identify genetic diversity of a few cultivars and provide data on their genetic structure with REMAP molecular marker. The results revealed a moderate level of genetic diversity both among and within the studied cultivars. We obtained mean genetic polymorphism of 20.8%.

Key words: *Phoenix dactylifera*, REMAP, genetic diversity

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one the most important fruit-bearing crops in arid regions of the Middle East and North Africa, but it has also been introduced in California, Peru, Australia, and other countries (Zehdi-Azouzi *et al.* 2015). About 3,000 date varieties or cultivars are known worldwide that differ in fruit-related traits. Extensive genetic diversity has been reported in date palm cultivars, but very little is known about the genes controlling important agronomic traits and the population structure of these important crop cultivars throughout the world (Jaradat 2015, Jaradat and Zaid 2004).

Date palm trees are very productive and the fruit yield maybe as high as 100–400 kg per tree per year depending on the date palm cultivars and in each major date-producing country. Cultivars throughout the oases of the Middle East derive their importance from their local adaptation to climatic, edaphic and socio-economic conditions and quality of their fruit. In addition to its local and regional commercial value, the date palm plays an important role

in the diet and social life of communities across the oases of the Middle East (Jaradat 2015).

To have a prosperous date palm industry, it is necessary to determine its genetic diversity and partitioning, especially for fruit quality traits, within and among gene pools in this centre of origin and centre of diversity (Jaradat 2015, Saboori *et al.* 2019, Sharifi *et al.* 2018).

Date palm has several breeding lines, cultivars, land races, and wild relatives, altogether comprising the genetic resources of date palm. Up to now the genetic structure and gene pools of date palm have been formed by human activities, natural selection, clonal propagation, stalk exchanges, and local traits adaptation (Jaradat 2015, Saboori *et al.* 2019, Sharifi *et al.* 2018).

Since the date palm production has shifted from traditional cultivation in rich and diverse agrosystems to intensive monocultures, this has led to severe genetic erosion, with the loss of cultivars and the overall impoverishment of date palm agro-biodiversity (Jain *et al.* 2011). Consequently, evaluation of genetic diversity within date palm cultivars and resources becomes an important step for optimum utilization of genetic variability and date palm conservation (Jaradat 2015, Saboori *et al.* 2019, Sharifi *et al.* 2018). In addition, as the date palm growth undergoes biotic and abiotic stress, identifying the tolerant varieties within date palm germ plasm will be of tremendous importance.

Date palm is the second most important horticultural crop in Iran following pistachio; it is cultivated mainly in the southern part of the country. It has about 400 known cultivars in Iran and therefore comprises an important part of the whole world date palm genetic resources. Recently, molecular investigation of Iranian date palms has been started which is concerned with few elite cultivars (Hajian and Hamidi-Esfahani 2015). Therefore, we need to study the other cultivars to broaden the knowledge of date palm genetic variability within our country. Various molecular markers have been used to study the genetic diversity in date palms in different countries. Both multilocus neutral molecular markers as well as gene sequences have been used (e.g., Al-Qurainy *et al.* 2011, Aladadi *et al.* 2018, Arabnezhad *et al.* 2012, Bahraminejad and Mohammadi-Nejad 2015, Elmeer *et al.* 2011).

Among DNA markers, there are ubiquitous retro elements in the plant genome like REMAP (retro transposon-microsatellite amplified polymorphism). The REMAP is produced by amplifying the fragments between a retro transposon insertion site and a microsatellite site and employed in fingerprinting, linkage analysis, mapping, analysis of genome evaluation and genetic diversity. REMAP describes the profile of a population, discriminate between species or genotypes and analyse population diversity (Kumar *et al.* 2010). In the present study we used 9 primers of REMAP molecular markers to investigate the genetic diversity and genetic structure of 14 date palm cultivars. This is the first report on date palm by REMAP molecular marker.

MATERIALS AND METHODS

Plant material – In total 75 plants were studied in 14 cultivars of the most famous cultivars of *Phoenix dactylifera* in Iran (Table 1).

DNA extraction – For molecular studies, the fresh leaves were collected from the plants in the studied area and were dried in silica gel powder. The genomic DNA was extracted using CTAB-activated charcoal protocol (Križman *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.

REMAP assay – In this study, 14 cultivars of *Phoenix dactylifera* were determined using 9 REMAP (primer combinations of ISSR (AGC) 5GA, (GA) 9T, (CA) 7GT) and retro transposon primer (Nikita, 3LTR, 5LTR1 & 2). PCR reactions were performed in a 25-μL volume containing 10 mM Tris-HCl buffer at pH 8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Bioron, Germany), 0.2 μM 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 following program: 5 min for initial denaturation step at 94 °C, 30 s at 94 °C, 1 min at 57 °C, and 1 min at 72 °C. The reaction was

Table 1
Phoenix dactylifera cultivars in ISSR studies

Cultivar's numbers	Cultivar	Province	Locality	Longitude	Latitude	Altitude
1	Mazafati	Kernan	Mahrooyeh	571339	280809	677.4
2	Kalooteh	Kernan	Jiroft	574259	284147	705.3
3	Khale zohrei	Kernan	Roodan	571009	272650	190.6
4	Holeileh	Kernan	Kahnooj	574636	280255	536.1
5	Mordarsang	Kernan	Mahrooyeh	571339	280809	677.4
6	Khazab	Kernan	Minab	570305	271118	31.4
7	Holoo	Kernan	Minab	570305	271118	31.4
8	Khenizi	Kernan	Minab	570305	271118	31.4
9	Negar	Kernan	Mahrooyeh	573913	280809	677.4
10	Shahani	Kernan	Faryab	571332	280518	659.1
11	Male isolate	Kernan	Jiroft	574259	282741	705.3
12	Unknown	Kernan	Faryab	571332	280518	659.1
13	Alimehtari	Kernan	Faryab	571332	280518	659.1
14	Kharook	Kernan	Jiroft	571332	280518	659.1

completed by a final extension step of 7 min at 72 °C. The amplification products were visualised 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). The experiment was replicated 3 times and constant ISSR bands were used for further analyses.

Data analyses – The REMAP bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). The number of private bands versus common bands was determined. Genetic diversity parameters like the percentage of allelic polymorphism, allele diversity (Weising *et al.* 2005), Nei's gene diversity (He), and Shannon information index (I) (Weising *et al.* 2005), were determined. We used GenAlex 6.4 for these analyses (Peakall and Smouse 2006). The Nei genetic distance (Weising *et al.* 2005) was determined among the studied cultivars and was used for the grouping of the genotypes. Genetic differentiation of the studied cultivars was studied by AMOVA with 1000 permutations as performed in GenAlex 6.4 (Peakall and Smouse 2006). The grouping of the species was done by PCoA plot and WARD clustering (Podani 2000). Dentrented correspondence analysis (DCA) of REMAP marker in *Phoenix dactylifera* trees studied was carried out with PAST version 2.17 (Hammer *et al.* 2012). Genetic structure of the cultivars 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. A Markov chain Monte Carlo simulation was run 20 times for each value of K (1–13) after a burn-in period of 105. Data were scored as dominant markers and analysis followed the method suggested by Falush *et al.* (2007). For the optimal value of K in the studied cultivars we used the Structure Harvester website (Earl and von Holdt 2012) to perform the Evanno method (Evanno *et al.* 2005). The choice of the most likely number of clusters (K) was carried out by calculating an ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values, as described by Evanno *et al.* (2005). The Mantel test (Podani 2000) was performed to study the association between genetic distance and geographical distance of the studied cultivars by PAST ver. 3.14 (Hammer *et al.* 2012).

RESULTS

REMAP assay: We obtained 42 REMAP bands (Loci) in total (Table 2). The highest mean number of bands occurred in cultivars 1 and 2 (28 and 24 bands, respectively). Some of the cultivars had private bands while a few common bands occurred in the studied cultivars. These are shared alleles among these cultivars. In general, almost similar values of mean effective alleles occurred in the cultivars studied (Table 2), but a higher value of mean gene diversity

Table 2
REMAP bands in date palm cultivars studied

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. of different bands	24	28	12	22	22	16	21	17	14	13	15	12	16	16
No. of different bands with a frequency ≥5%	24	28	12	22	22	16	21	17	14	13	15	12	16	16
No. of bands unique to a single population	4	2	0	2	0	0	0	0	0	0	1	0	1	0
No. of bands found in 25% or fewer populations	3	6	3	3	1	1	1	0	0	0	0	0	1	0
No. of locally common bands found in 50% or fewer populations	7	13	5	8	8	5	6	4	2	2	1	1	3	3

Table 3
Population genetic diversity parameters in date palm cultivars studied based on REMAP markers

Pop	N	Na	Ne	I	He	%P
1	10	1.095	1.266	0.256	0.166	52.38
2	11	1.238	1.237	0.236	0.149	57.14
3	5	0.333	1.011	0.016	0.009	4.76
4	6	0.857	1.225	0.192	0.130	33.33
5	10	0.810	1.150	0.138	0.090	28.57
6	3	0.476	1.091	0.065	0.046	9.52
7	3	0.690	1.119	0.105	0.071	19.05
8	6	0.595	1.102	0.093	0.061	19.05
9	3	0.381	1.033	0.028	0.019	4.76
10	3	0.381	1.031	0.034	0.021	7.14
11	6	0.524	1.098	0.087	0.058	16.67
12	3	0.310	1.023	0.016	0.012	2.38
13	3	0.548	1.109	0.094	0.063	16.67
14	3	0.595	1.129	0.117	0.078	21.43

(He = 1.66) occurred in the cultivars number 1. Populations No. 1 and 2 also showed the highest level of genetic polymorphism (57% and 52%, respectively; Table 3). DCA (Detrended Correspondence Analysis) (Fig. 1) revealed that REMAP molecular markers

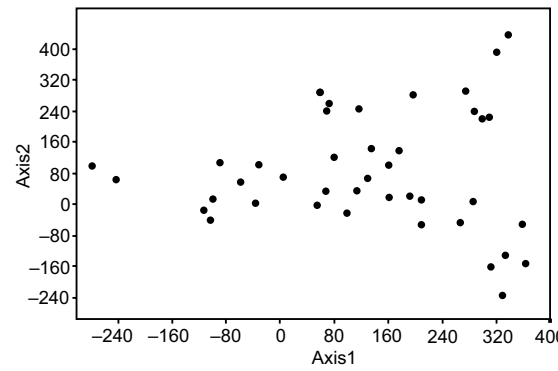

Fig. 1. DCA plot of REMAP molecular markers showing scattered distribution of these loci that indicates they are not closely linked and are independent loci

Table 4
Discriminating power of REMAP loci in date palm cultivars studied. (Sample sizes = 75)

Locus	Ht	Hs	Gst	Nm*
L1	0.4921	0.0694	0.8590	0.0821
L2	0.4990	0.0999	0.7998	0.1252
L3	0.4917	0.0958	0.8052	0.1210
L5	0.4669	0.1300	0.7216	0.1929
L6	0.1787	0.0419	0.7654	0.1533
L7	0.4996	0.1201	0.7595	0.1583
L8	0.1672	0.0349	0.7913	0.1319
L9	0.4729	0.1362	0.7119	0.2023
L13	0.3183	0.0856	0.7312	0.1838
L25	0.2999	0.0551	0.8163	0.1126
L30	0.1327	0.0000	1.0000	0.0000
L35	0.3649	0.0409	0.8881	0.0630
L37	0.3649	0.0409	0.8881	0.0630
L38	0.3514	0.0214	0.9391	0.0324
L39	0.4495	0.0559	0.8756	0.0710
Mean	0.2265	0.0700	0.6911	0.2235
St. dev.	0.0335		0.0030	

Nm* = estimate of gene flow from Gst or Gcs. E.g., Nm* = 0.5(1–Gst)/Gst; (cf. McDermott and McDonald 1993). The number of polymorphic loci is 41. The percentage of polymorphic loci is 97.62.

are not closely linked to each other as these loci are distributed in different positions of DCA plot. This indicates that these markers represent different regions of the genome and are suitable molecular markers for differentiating date palm cultivars.

Discriminating power of the REMAP loci are presented in Table 4. We presented only REMAP loci with at least 0.70 Gst value. The mean Gst value 0.69 for all REMAP loci indicates that these molecular markers have a good discriminating power and can be used in date palm cultivar genetic finger printing.

WARD dendrogram (Fig. 2) of the studied date palms based on REMAP data revealed that date palm cultivars are almost distinct in their genetic content as their studied specimens were grouped together in a separate cluster. However, in WARD dendrogram, in few cases intermixed plants were observed in the studied cultivars. For example, some trees of cultivars 1 and 2 were placed intermixed due to shared common alleles.

PCoA analysis of REMAP data after 1,000 times permutations placed date palm cultivars in 3 major groups (Fig. 3). The cultivars 5–8 comprised the first group, while cultivars 9–14 formed the second group. The cultivars 1–4 were placed close to each other and comprised the third group. Therefore, based on REMAP data, we can differentiate 3 genetic groups within the date palm cultivars studied.

AMOVA based on REMAP data produced significant difference among date palm cultivars studied (PhiPT = 0.56, p = 0.001). AMOVA also revealed that about 44% of total genetic diversity is due to within population genetic

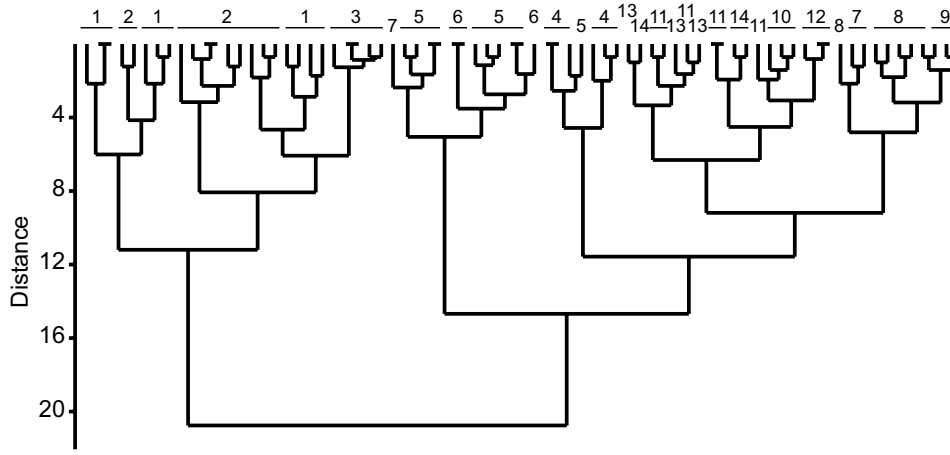

Fig. 2. WARD dendrogram of the studied date palm cultivars based on REMAP molecular markers

variability, while 56% was due to among cultivars genetic difference. This result indicates that not only date palm cultivars differ genetically from each other, but also they have good level of within cultivar genetic variability. Pairwise AMOVA (Table 5) in date palm cultivars produced significant difference mainly between the cultivars 1–6, and also between these cultivars and 7–14. However, it did not produce significant difference between those cultivars which were placed intermixed in WARD dendrogram, for example, between cultivars 10–14.

STRUCTURE plot (Fig. 4) of these cultivars revealed more detailed information on genetic structure of the studied date palms. Date palm trees in the cultivar 1 (Mazafati) showed high degree of within cultivar genetic variability as these trees are colored differently in STRUCTURE plot. The same holds true

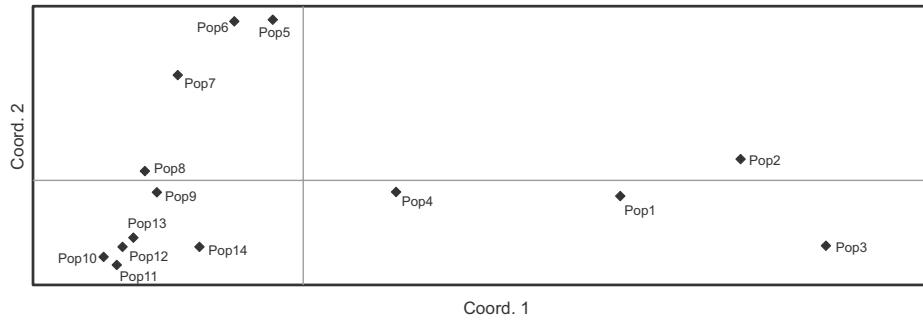

Fig. 3. PCoA plot of date palm cultivars based on REMAP markers

Table 5
Pair-wise AMOVA in date palm cultivars studied (cultivars 1–14 are according to Table 1)

NO	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.000	0.010	0.020	0.010	0.010	0.010	0.010	0.010	0.020	0.010	0.010	0.010	0.010	0.010
2	0.237	0.000	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.010	0.010	0.010	0.020	0.010
3	0.338	0.422	0.000	0.010	0.010	0.040	0.010	0.020	0.040	0.010	0.010	0.010	0.030	0.030
4	0.433	0.516	0.642	0.000	0.010	0.020	0.040	0.020	0.030	0.020	0.010	0.030	0.020	0.030
5	0.512	0.587	0.808	0.582	0.000	0.010	0.020	0.010	0.020	0.010	0.010	0.010	0.010	0.010
6	0.480	0.548	0.909	0.638	0.414	0.000	0.070	0.030	0.120	0.140	0.010	0.110	0.080	0.130
7	0.439	0.514	0.856	0.479	0.380	0.561	0.000	0.020	0.120	0.100	0.010	0.120	0.130	0.080
8	0.465	0.576	0.827	0.540	0.630	0.642	0.509	0.000	0.040	0.020	0.020	0.020	0.030	0.040
9	0.418	0.548	0.919	0.544	0.678	0.802	0.577	0.311	0.000	0.100	0.030	0.130	0.110	0.070
10	0.413	0.562	0.905	0.551	0.694	0.792	0.653	0.491	0.700	0.000	0.030	0.110	0.090	0.130
11	0.446	0.593	0.824	0.570	0.690	0.735	0.607	0.528	0.603	0.250	0.000	0.020	0.020	0.210
12	0.393	0.567	0.937	0.513	0.696	0.839	0.690	0.575	0.786	0.684	0.436	0.000	0.150	0.100
13	0.389	0.532	0.848	0.511	0.631	0.697	0.526	0.572	0.654	0.483	0.233	0.500	0.000	0.370
14	0.357	0.492	0.782	0.395	0.635	0.636	0.474	0.526	0.577	0.333	0.144	0.444	0.111	0.000

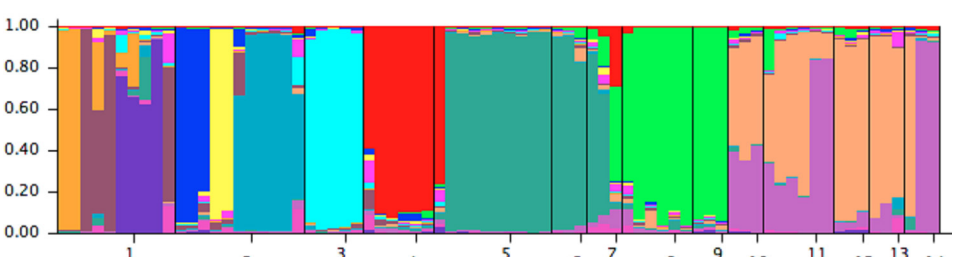

Fig. 4. STRUCTURE plot of date palm trees based on REMAP data (cultivars 1–14 are according to Table 1)

for cultivar 2 (Kalooteh). As stated before in WARD dendrogram, the shared alleles of these two cultivars are pink-coloured segments in some of the trees. The cultivars 3 and 4 (Khale zohrei and Holeileh) showed differently coloured segments and therefore contain different genetic content and allele combination. The cultivars 5–7 share to some degree very similar allele combination. This holds true for the cultivars 7–9 and also for the cultivars 10–14.

Mantel test showed no significant correlation between genetic distance and geographical distance of the date palm cultivars studied ($R2 = 0.008$, $P > 0.05$), Therefore, no isolation by distance (IBD) is present in these cultivars and the genetic difference in date palms is not correlated to geographical distance.

DISCUSSION

It is believed that active conservation and sustainable utilization of existing genetic variability requires a comprehensive evaluation of the genetic variety and population diversity in date palm cultivars (Jaradat 2015, Saboori *et al.* 2019, Sharifi *et al.* 2018).

Date palm is favoured as the main horticultural crop plant in Iran, but no detailed information exists on its population genetic structure. It has been cultivated on a traditional basis in Iran and it is necessary to shift towards modern approaches like biotechnology and molecular breeding. It is also necessary to assess all available cultivars genetically and recognise those with favourable agronomic features. The present study was an attempt to provide population genetic data on 14 date palm cultivars for the first time. The present study tried to identify genetic diversity of a few cultivars and provide data on their genetic structure.

The results here revealed a moderate level of genetic diversity both among and within 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. Marsafari and Mehrabi (2013) used both ISSR and RAPD molecular markers and reported the mean similarity of 94% among date palm cultivars that shows 0.6 mean genetic distance, which is in agreement with our results. The study by Bahraminejad and Mohammadi-Nejad (2015) using RAPD markers on 6 date palm cultivars in Jiroft reported the highest similarity value of 0.58 between the studied cultivars and therefore the genetic distance they report is 0.42 at the highest value.

Aladadi *et al.* (2018) used genomic DNA in zygotic embryo of date palm cultivars and analysed them by both RAPD and ISSR markers. They reported genetic polymorphism percentage ranging between 32.09% and 36.49%, respectively. RAPD markers produced the highest similarity value of 80.0%,

while the lowest value was (59.56%). In the present study, we obtained mean genetic polymorphism of 20.8%. The studied cultivars showed genetic similarity between 0.68 and 0.96, and genetic distance between 0.04 and 0.41. Aladadi *et al.* (2018) conducted an analysis by applying both RAPD and ISSR markers. According to their results, the genetic polymorphism percentage was 32.09% and 36.49%, respectively. RAPD markers had the top similarity value of 80.0%, while the bottom value was 59.56%.

In the present study, we obtained mean genetic polymorphism of 20.8%. The studied cultivars showed genetic similarity between 0.68 and 0.96, and genetic distance between 0.04 and 0.41.

A good level of genetic diversity among the cultivars has been reported by other genetic diversity investigations carried out in other countries. 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 that the mean gene diversity was 0.66. Forty-four percent of the variability is explained at the inter-population level, while 56% of the variability is maintained within individuals. This result is very close to the AMOVA result presented in our REMAP data.

Elshibli and Korpelainen (2008) used microsatellite markers to investigate the genetic diversity in date palms of Sudan. They obtained a high level of genetic polymorphism with an average of 21.4 alleles per locus and expected heterozygosity of 0.841. The results show that the genetic groups of the Sudan cultivars do not fit into a discernible geographic pattern. This is consistent with our Mantel test result. The study by Jaradat (2015) shows that most of the genetic variation was identified among date palm populations; nevertheless, considerable dissimilarities in genetic diversity components were discovered among and within populations. This is consistent with the present study as it verifies the significant genetic discrepancy between the target date palm cultivars. He (Jaradat 2015) also elaborated on the overall partitioning of genetic diversity, according to the phenotypic, biochemical, molecular and fruit quality traits and recommended that date palm cultivars signify a complex gene pool in which historical movement of germplasm, recent introductions and human selection form their genetic structure.

Zehdi-Azouzi *et al.* (2015) show that, the date palm genotypes can be structured into two different gene pools: the first, termed the Eastern pool, consists of accessions from Asia and Djibouti, whilst the second, termed the Western pool, consists of accessions from Africa. These results confirm the existence of two ancient gene pools that have contributed to the current date palm diversity. The presence of admixed genotypes is also noted, which points at gene flows between eastern and western origins, mostly from east to west, following a human-mediated diffusion of the species. In the other study,

the model-based Bayesian method indicated that date palm accessions could be broadly divided into two structure groups, one group with predominantly African accessions and another predominantly Asian. Some germplasm, especially from Tunisia and Iraq, deviated from this generalisation (Hammadi *et al.* 2009). Many accessions in the STRUCTURE-derived groups were found to be genetic admixtures, with gene flow between Asian and African groups. Indian and Pakistani date palms were found to be most closely related to North African germplasm (Chaluvadi *et al.* 2019).

Moreover, the genetic diversity does not have random or uniform distribution in terms of space or time. Genetic diversity is different among oases and populations, or among regions and localities, and several key historical, geographical, ecological, and anthropogenic factors regulate its magnitude and distribution. Date palm cultivars are usually detected according to the plant and fruit morphology, that are not often reliable since these traits are under the influence of environmental conditions or they change along with the developmental stage of the date palm (Jaradat 2015, Jaradat and Zaid 2004). Nevertheless, Jaradat (2015), Jaradat and Zaid (2004), and Hamza *et al.* (2009) show that some of the leaf, leaflet morphological qualitative and quantitative traits of a selected number of cultivars as well as percentage of spinned midrib part, apical divergence angle, maximal pinnae width at the top leaf, percentage of solitary spine, spine length at the middle and maximal spine angle are stable and did not change in reaction to environmental factors or to the tolerance towards stress. These morphological traits can be applied as stable descriptors of date palm.

In conclusion, our work indicates that REMAP molecular markers are efficient for date palm genetic finger printing and can be used to illustrate the cultivars' relationship. We reported moderate genetic variability both between and within date palm cultivars that can be used in future breeding and conservation programs.

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-trnH. – *Int. J. Mol. Sci.* **12**: 6871–6880. https://doi.org/10.3390/ijms12106871

Aladadi, W. M., Moustafa, M. F. and Alruman, S. A. (2018): Genetic variability among seven cultivars of date palm (Phoenix dactylifera L.) based on embryonic DNA of old fruit. – *Kuwait J. Sci.* **45**(1): 108–114.

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.). – *Scientia Hort.* **134**: 150–156. https://doi.org/10.1016/j.scienta.2011.11.032

Bahraminejad, A. and Mohammadi-Nejad, G. H. (2015): Genetic diversity analysis of date palm (Phoenix dactylifera L.) genotypes using RAPD markers. – *Annu Res. Rev. Biol.* **5**(1): 41–47. https://doi.org/10.9734/arrb/2015/11683

Chaluvadi, S. R., Young, P., Thompson, K., Bahri, B. A., Gajera, B., Narayanan, S. and Bennetzen, J. L. (2019): Phoenix phylogeny and analysis of genetic variation in a diverse collection of date palm (Phoenix dactylifera) and related species. – *Plant Diversity* **41**(5): 330–339. https://doi.org/10.1016/j.pld.2018.11.005

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. https://doi.org/10.1111/j.1471-8286.2007.01758.x

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. Gen. Resour.* **4**: 359–361. https://doi.org/10.1007/s12686-011-9548-7

Elmeer, K. and Mattat, I. (2015): Genetic diversity of Qatari date palm using SSR markers. – *Gen. Mol. Res.* **14**(1): 1624–1635. https://doi.org/10.4238/2015.march.6.9

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.). – *3 Biotech.* **1**: 91–97. https://doi.org/10.1007/s13205-011-0010-z

Elshibli, S. and Korpelainen, H. (2008): Microsatellite markers reveal high genetic diversity in date palm (Phoenix dactylifera L.) germplasm from Sudan. – *Genetica* **34**(2): 251–260. https://doi.org/10.1007/s10709-007-9232-8

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. https://doi.org/10.1111/j.1365-294x.2005.02553.x

Hajian, S. and Hamidi-Esfahani, Z. (2015): Date palm status and perspective in Iran. – *Date Palm Gen. Res. Util.* **2**: 19–47. https://doi.org/10.1007/978-94-017-9707-8_2

Hammadi, H., Mokhtar, R., Mokhtar, E. and Ali, F. (2009): New approach for the morphological identification of date palm (Phoenix dactylifera L.) cultivars from Tunisia. – *Pak. J. Bot.* **41**: 2671–2681.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D. (2012): PAST: Paleontological statistics software package for education and data analysis. – *Palaeontol. Electron.* **4**: 9.

Jain, S. M., Al-Khayri, J. M. and Johnson, D. V. (2011): *Date palm biotechnology.* – Springer, Berlin.

Jaradat, A. A. (2015): *Biodiversity, genetic diversity, and genetic resources of date palm*. – In: Al-Khayri, J., M., Jain, S. M. and Johnson, D. V. (eds): Date palm genetic resources and utilization. Vol. 1. Africa and the Americas. Springer, Dordrecht, Heidelberg, New York, London, pp. 19–71.

Jaradat, A. A. and Zaid, A. (2004): Quality traits of date palm fruits in a center of origin and center of diversity. – *J. Food Agric. Environ.* **2**(1): 208–217.

Križman, M., Jakše, J., Baričevič, D., Javornik, B. and Prošek, M. (2006): Robust CTAB-activated charcoal protocol for plant DNA extraction. – *Acta Agric. Slov*. **87**: 427–433.

Kumar Biswas, M., Xu, Q. and Deng, X-X. (2010): Utility of RAPD, ISSR, IRAP and REMAP markers for the genetic analysis of Citrus spp. – *Sci. Hortic.* **124**(2): 254–261. https://doi.org/10.1016/j.scienta.2009.12.013

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. – *Austral. J. Crop Sci.* **7**(8): 1160–1166.

McDermott, J. M. and McDonald, B. A. (1993): Gene flow in plant pathosystems. – *Annu. Rev. Phytopathol.* **31**: 353–373. https://doi.org/10.1146/annurev.py.31.090193.002033

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*. – Backhuys Publishers, Leiden, 407 pp.

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

Saboori, S., Noormohammadi, Z., Sheidai, M. and Marashi S-S. (2019): SCoT molecular markers and genetic fingerprinting of date palm (Phoenix dactylifera L.) cultivars. – *Genet. Resour*. **67**: 73–82. https://doi.org/10.1007/s10722-019-00854-x

Sharifi, M., Sheidai, M. and Koohdar, F. (2018): Genetic fingerprinting of date palm (Phoenix dactylifera L.) by using ISSR and cpDNA sequences. – *Indian J. Genet.* **78**(4): 507–514.

Weising, K., Nybom, H., Wolff, K. and Kahl, G. (2005): DNA fingerprinting in plants. – In: *Principles, methods, and applications*. 2nd ed. CRC Press, Boca Raton, 472 pp.

Zehdi-Azouzi, S., Emira Cherif, E., Moussouni, S., Gros-Balthazard, M., Abbas Naqvi, S., Ludeña, 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, J. Ch. and Aberlenc-Bertossi, F. (2015): Genetic structure of the date palm (Phoenix dactylifera) in the Old World reveals a strong differentiation between eastern and western populations. – *Ann. Bot*. **116**: 101–112. https://doi.org/10.1093/aob/mcv068