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Identification of Genetic Variation of Male and Female Date Palm (*Phoenix dactylifera* L.) Cultivars Using Morphological and Molecular Markers

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Abstract
Date palm (*Phoenix dactylifera* L.) is a monocot, dioecious and high longevity tropical plant greatly considered due to its high economic importance in Middle East. This investigation was carried out to detect genetic relations among 34 cultivars of Iranian date palm, both males and females, using ISSR markers. Accordingly, total DNA was extracted using CTAB method and was subjected to PCR amplification using 12 ISSR primers. Analysis of ISSR data indicated that PIC value varied from 0.3695 to 0.4998 with average of 0.4497. The highest PIC value belonged to $(CT)_{10}$ G primer (0.4498). Totally, 112 alleles were recognized. According to morphological attributes and ISSR markers, the 34 studied date cultivars divided into eight main groups and different sub-groups, and no significant correlation between grouping made by morphological and molecular markers. Unlike ISSR markers that produced a clear-cut among male and female cultivars, no distinct border was seen among male and female cultivars using morphological attributes. Furthermore, PCA analysis confirmed the grouping made by ISSR markers. In conclusion, ISSR markers have been useful to separate date palm cultivars.

Keywords: DNA, Date palm, ISSR, PIC, Iran.

Introduction

Date palm is one of the holiest and old fruit trees for human (Hashempour, 1999). Due to high resistance to drought and undesired environmental and soil conditions, date palm is one of the few plant species that can be cultivated and developed in warm climates and barren lands. Date palm is in the fifth rank of areas under cultivation and 5.5% of the total horticultural productions in Iran (Anonymous, 2016). Date fruit is one of the main agricultural products and the most important sources of incomes in Iran. Genetically, unknown originality of plant cultivars is one of the gardener's challenges for producing seedlings in nurseries.

Cross-pollination in date palm has result in increased genetic diversity in this species and its potential to tolerate unfavorable conditions during its growth and development. The Middle East and North Africa are the two main date producing regions that have a rich date germplasms as well. Date cultivars are divided into three

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groups based on ripening time, including early-ripening, mid-ripening and late-ripening. Furthermore, there is another classification for date cultivars in which the cultivars are divided into four groups based on fruit texture including soft, semi-soft, semi-dry and dry. Monoculture farming in date palm in some regions has been led to genetic bottleneck of date palm reservoirs (Hamza et al., 2012).

Genetic diversity defined as the total number of genetic characteristics in the genetic makeup of a species, which is essential for population survival and adaptation to changing environments. It also provides fundamental information for plant breeding (Naghavi, 1384). Accordingly, knowledge of germplasms diversity and genetic relationship among date palm cultivars can be an essential issue for date breeding. Regarding to cross-pollination in date palm and influence of pollen properties on date fruit and date stone quality and quantity, determination and use of suitable pollen is a primary step in improving date quality (Khajepour-Tadovani et al., 2016; Rezazadeh et al., 2013). It seems that male and female individuals of the same or close date palm cultivars produce dates with higher quality compared with genetically separated ones. Therefore, study of genetic diversity among male and female individuals of various date palm cultivars in a region and identification of their genetic affinity can be an effective approach to choose the proper pollen for each female cultivar.

There are many morphologically different male and female date palm cultivars in Hormozgan province (Iran) which have not been classified genetically by yet. On the other hand, over the past few years, several high quality male cultivars have been imported to Hormozgan province from other countries (planted in Agricultural Research Station), which needs to be identified in point of affinity with the native cultivars. In previous studies, only morphological attributes of the leaf, fruit, stone and pit of stone were evaluated (Zehdi et al., 2004b), while the related issue needs to be further studied.

Nowadays, determining genetic originality of horticultural crops has been reached up morphological level and it exploits modern biotechnology methods for DNA genotyping (Zehdi et al., 2004b). Although, there are about 5000 date fruit cultivars worldwide (Yusuf et al., 2015), the gardeners prefer to cultivate only high quality date varieties. For example, Hamza et al. (2012) reported that excessive tendency to cultivate DegletNour in Tunisia has reduced the genetic diversity of date germplasms in this country. This approach known as monoculture system is caused resistance decline against biotic and abiotic stresses in date palm.

ISSR markers are simple, rapid, low-cost and repeatable because of its primer length and annealing temperature (Gurcan et al., 2009), in which DNA segments are amplified among two identical microsatellite repeats oriented in opposite directions. The technique employs microsatellites, usually 16-25 bp long, as primers in a single primer PCR reaction, that it have been utilized for various fruit trees such as date palm (Karim et al., 2010; Khanam et al., 2012) and Citrus (Hassanzadeh Khankahdani et al., 2018). Karim et al. (2010) used seven ISSR primers to estimate genetic diversity among 10 accessions of date palm varieties in Tunisia and suggested use of more primers to clear-cut among date palm varieties. By now, several molecular markers including RAPD (Sedra et al., 1998; Al-Khalifa and Askari, 2003), ISSR (Zehdi et al., 2002), RAMPO (Rhouma, 2008), AFLP (Rhouma et al., 2007; Snoussi et al., 2001), RFLPs (Corniquel and Mercier, 1994; Sakka et al., 2003) and SSR (Zehdi et al., 2004a) have been used to study the genetic diversity of date palms.

Zehdi et al. (2004b) and Karim et al. (2010) obtained useful information regarding phylogenetic relations among Tunisian date

cultivars using ISSR markers. In addition, Munshi and Osman (2010) showed high efficiency of ISSR markers in determining phylogenetic relations among date cultivars in Saudi Arabia (Munshi and Osman, 2010; Al-Issa et al., 2008) and Egypt (Adawy et al., 2011; Hussein et al., 2005). Cullis (2011) believeed that grouping of different date cultivars using molecular markers is independent of geographical situation and sexuality. Haider et al. (2012) studied phylogenetic relation among different male and female date cultivars using RAPD and ISSR markers and found no sexuality and geographical dependency of the cultivars. In contrast, Mitra et al. (2011) showed sexuality-dependency of the date cultivars using RAPD and ISSR markers. Moghaieb et al. (2010) in an assessment using RAPD and ISSR markers for identification of genetic diversity and sexuality determination of six male and female date genotypes in Qatar found high polymorphism by using ISSR markers (73%) compared with RAPD (60.2%).

Although, no significant correlation was found between molecular and morphological markers (Hamza et al, 2012; Bruschi et al., 2003), in some cases, accompaniment of ISSR and morphological results has been documented. For instance, Hamza et al. (2012) showed that grouping of date cultivars based on the fruit texture, can be successfully done by ISSR markers. Marsafari and Ashraf-Mehrabi (2013) showed potential of ISSR and RAPD markers in diverging 15 date cultivars in south and south-western of Iran. The present investigation aimed to determine genetic relations among commercial male and female date cultivars of Hormozgan province.

Materials and Methods

This study was performed in molecular laboratory of Plant Protection Research department of Agricultural and Natural Resources Research and Education Center of Hormozgan in summer 2016. Plant materials (Table 1) were prepared from date palm collection of Agricultural Research Station of Minab and Hajiabad, Hormozgan, Iran. The samples were prepared from initial and chlorophyll-less leaves.

Morphological attributes

Measurements of all morphological attributes were done on five same age date palms by exploiting a national prescription of distinction, uniformity and stability (DUS) tests for date palms (Maraashi, 2006). Some attributes after measuring were modified based on the date palm age to fade age discrepancy. The evaluated attributes have been summarized in Table 2. According to prescription, there was three and two demonstrations status for male and female spaths, respectively. Spath length was calculated based on flowering time. Inflorescence color had two demonstrations status including bright cream and pink reddish.

DNA extraction

Genomic DNA was isolated using Murray and Thumpson (1980) protocol. Although completely young leaves were sampled for DNA extraction, nevertheless the leaves were so thick that their powdering became impossible even using liquid nitrogen. To overcome the obstacle, the leaf samples following complete washing (because of the presence white powder on the leaves), were disinfected by ethanol (70%) and divided into fine segments using sterile scissors and then powdered using electrical blender. In some references, the leaf samples were powdered using sterile sand or glass powder in presence of liquid nitrogen (Aitchitt et al., 1993). Quantity and quality of the extracted DNA was assayed using NanoDrop spectrophotometer and electrophoresis on 1% agarose gel, respectively.

PCR reaction using ISSR primers

ISSR amplification reactions were prepared to a final volume of 10 µl [25 ng of template DNA, 0.2 mM dNTPs, 0.7 µmol primer 1.0 µl of 10× PCR buffer, 1.5 mM of $MgCl_2$ and one unit of Taq polymerase (Cinnagen, Iran)]. The amplifications were performed on a

PEQStar 96 Universal Gradient 96 wells thermal cycler with reaction conditions programmed as initial pre-denaturation at 94 °C for 4 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing for each primers for 45 s, and extension at 72 °C for 2 min. A final 7 min extension at 72 °C followed the completion of 40 cycles. To evaluate polymorphism of DNA fragments 12 ISSR primers was used. After amplification, the DNA fragments were separated by electrophoresis in 1.5% agarose gel.

Table 1. Name, age and collecting identification of the male and female date cultivars

Cultivar	Sex	Age (year)	Location	Cultivar	Sex	Age (year)	Location
Male1005	Male	28	Minab*	Khenezi	Female	31	Minab
Male1006	Male	28	Minab	Mozafati	Female	28	Minab
Male1007	Male	28	Minab	Barhee	Female	29	Minab
Male1008	Male	28	Minab	Kahrobaei	Female	28	Minab
Male1009	Male	28	Minab	Shahani	Female	31	Minab
Male1010	Male	28	Minab	Almehtari	Female	31	Minab
Male1011	Male	28	Minab	Khasab	Female	28	Minab
Male1012	Male	28	Minab	Mangenas	Female	28	Minab
Male1013	Male	28	Minab	Halili	Female	31	Minab
Male1014	Male	28	Minab	Thoory	Female	29	Minab
MaleFard	Male	29	Minab	Deyri	Female	31	Minab
MaleJervis	Male	29	Minab	Medjool	Female	28	Minab
DegletNour	Female	31	Minab	MaleB1	Male	28	Minab
Hallawi	Female	31	Minab	MaleB2	Male	28	Minab
Mordaseng	Female	31	Minab	Piyarom	Female	28	Hajiabad**
Estaamaran	Female	31	Minab	Zahidi	Female	28	Hajiabad
Kabkab	Female	31	Minab	Khasui	Female	28	Hajiabad

*Agricultural Research Station of Minab placed in 105 km east of Bandar abbas in 27° 6´ N latitude and 57° 5´ E longitude and 27 m altitude above sea level with warm and humid weather in summer and mild and cold in winter.
**Agricultural Research Station of Hajiabad placed in 155 km north of Bandar abbas in 28° 19´ N latitude and 55° 55´ E longitude and 930 m altitude above sea level with warm and dry weather in summer and cold and dry in winter.

The DNA stained using fluoroDye. DNA fragments were visualized and documented with the help of Uvitec Geldoc system.

Data analysis

Morphological attributes was analyzed in randomized complete block design with 34 treatments (cultivars) and five replications using SAS 9.1 software and the means were compared using PLSD test ($p<0.05$). For ISSR data, a binary data sheet was prepared by scoring amplified fragments (1 for present and 0 for absent band), A pair-wise similarity matrix was constructed using Jaccard, Dice and simple matching (SM) similarity coefficient. Dendrogram constructed using NTSYS software based on UPGMA algorithm. Polymorphism information content (PIC) was calculated using the formula: $PIC=2f_i(1-f_i)$, where f_i is the frequency of the amplified allele (present band), and $(1-f_i)$ is the frequency of the null allele (absent band) (Roldan-Ruiz et al. 2000). SPSS 16.0 software was used to estimate correlation between morphological and genetic data. Principal coordination analysis (PCA) was performed based on ISSR data using NTSYS software version 2.02.

Results

Morphological analysis

The data regarding morphological attributes of the male and female date cultivars was evaluated using variance analysis and clustering procedures. In all 16 measured morphological attributes, we found significant differences among date cultivars ($p<0.01$). The variation

coefficient (CV) varied from 1.4 in trunk perimeter to 16.4 in leaf's thorny part length/leaf length ratio (data not shown).

Generally, mean comparison of the morphological attributes demonstrated the highest variation in annual longitudinal growth, trunk perimeter, leaf length, average thorn length and leaf end axis perimeter, causing increased number of grouping among date cultivars. The lowest variation was observed in middle leaflet length and leaflet/thorn number ratio (Table 2). In all six parameters about fruit characteristics, the female cultivars showed significant difference ($p<0.01$) and CV varied from 5.3 in fruit length to 11.6 in stone weight (data not shown). Fruit and stone weight showed the highest variation among studied fruit characteristics (Table 2).

Table 2. Grouping of date cultivars derived from mean comparison of morphological attributes

Row	Attribute description	Demonstration status	†Group No.	††Grouping situation
1	Annual longitudinal growth	Measuring	21	a-u
2	Trunk perimeter	Measuring	23	a-w
3	Leaf lamina length	Measuring	19	a-s
4	Leaf lamina width	Measuring	17	a-q
5	Leaf lamina length/width ratio	Compute	15	a-o
6	Leaf's thorny part length	Measuring	16	a-p
7	Leaf length	Measuring	22	a-v
8	leaf's thorny part length/leaf length ratio	Compute	15	a-o
9	Middle leaflet length	Measuring	15	a-o
10	Middle leaflet width	Measuring	11	a-k
11	Middle leaflet length/width ratio	Compute	16	a-p
12	Average thorn length	Measuring	20	a-t
13	Leaflet no.	Counting	16	a-p
14	Thorn no.	Counting	15	a-o
15	Leaflet/thorn no. ratio	Compute	12	a-l
16	Leaf end axis perimeter	Measuring	21	a-u
17	Inflorescence color	Bright cream-Pink reddish	-	-
18	Male spath length	Gladiate- Spindle shaped- Flat spindle	-	-
19	female spath length	Gladiate- Spindle shaped	-	-
	Fruit attributes in female cultivars			
20	Fruit length	Measuring	9	a-i
21	Fruit diameter	Measuring	9	a-i
22	Fruit weight	Measuring	12	a-l
23	Stone length	Measuring	10	a-j
24	Stone diameter	Measuring	9	a-i
25	Stone weight	Measuring	12	a-l

†The number of grouping date palm cultivars derived from PLSD ($p<0.05$); Increasing group number indicate more variation in that parameters among date palm cultivars. ††The range of letters in mean comparison among date palm cultivars using PLSD ($p<0.05$).

Grouping of male and female date cultivars based on morphological attributes

To reach a statistical analysis, after grouping and binary scoring the morphological attributes data, the best correlation coefficient was determined. Accordingly, the highest correlation was observed in Jaccard coefficient (0.647). Therefore, morphological dendrogram was reconstructed using Jaccard coefficient (Fig. 1).

Totally, male and female date cultivars were divided into three main groups including A: consisting DegletNour, Medjool, Male1006; B: consisting MaleB1, Kahrobaei, Mangenas, Deyri and Male1010; C: consisting other male and female

cultivars. There were two subgroups in C group including C1: Piyarom, Male1012, Halili, Almehtari, Estaamaran, Hallawi, Zahidi and Male 1011 and C2: Thoory, Khenezi, Mozafati, MaleJervis, Khasui, Khasab, Barhee, Mordaseng, Male1014, MaleFard, MaleB2, Kabkab, Male1013, Male1009, Male1008, Male1007, Shahani and Male1005. Based on reference line, male and female date cultivars were divided into eight main groups with several subgroups. Results of similarity coefficient among male and female cultivars revealed the greatest similarity between Almehtari and Estaamaran cultivars (0.66) and Khasab and Barhee (0.60) cultivars ranked in the second. Shahani and Male1005; Male1007 and Male1008; Deyri and Male1010 each with the same similarity coefficient (0.50) placed in the third rank. The lowest similarity coefficient was observed between Male1006, DegletNour and Medjool cultivars with other male and female date cultivars (0.16) (Fig. 1).

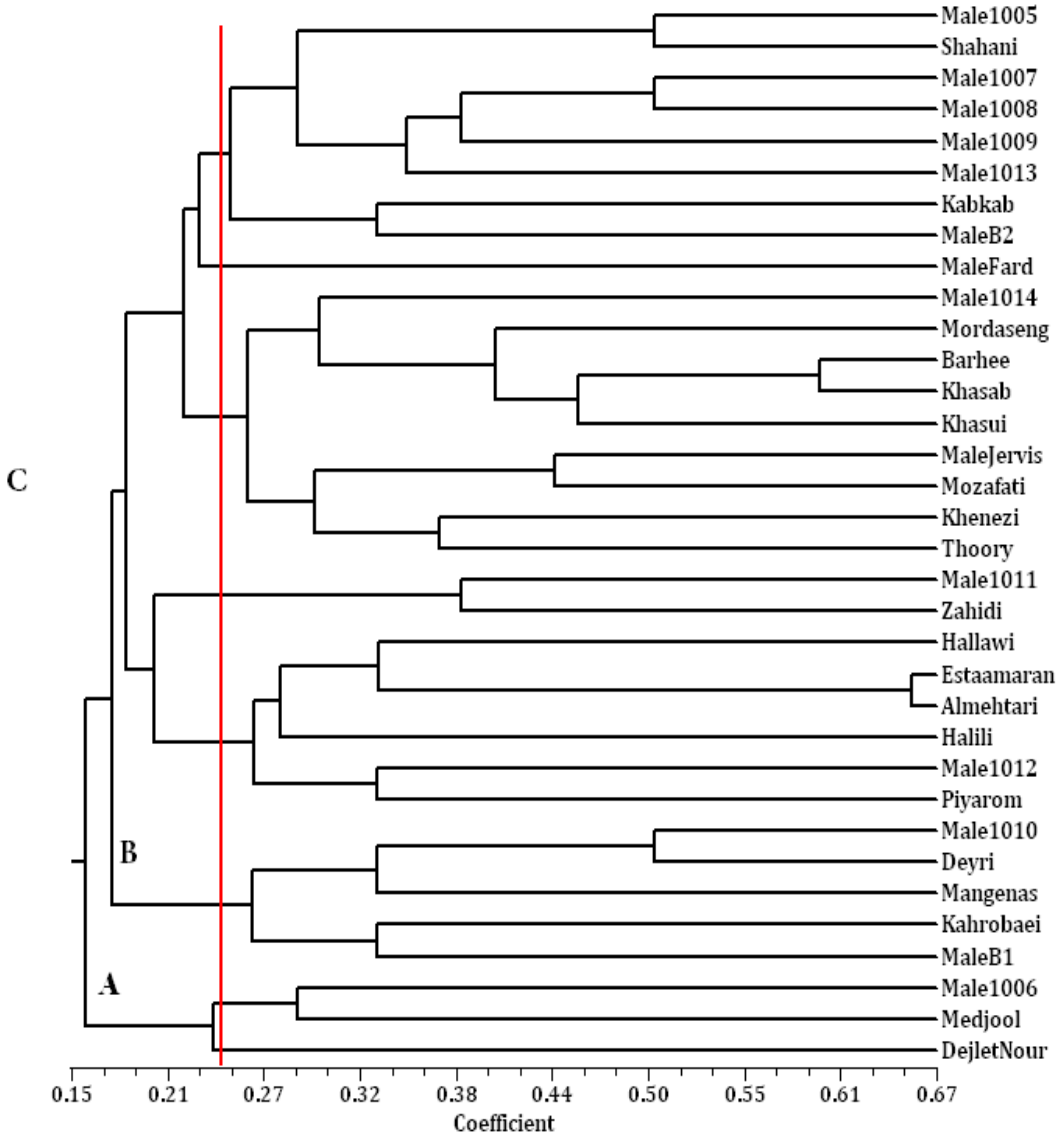

Fig. 1. Morphological similarity among 34 male and female date cultivars. The dendrogram reconstructed using UPGMA clustering method based on the 25 morphological attributes.

ISSR assay

Seven out of 12 primers were polymorphic. In total, 112 bands were produced in which 16 bands were amplified in each primer (Fig. 2). The highest number of amplified bands was observed in DP9 primers (19 bands) and the lowest was in DP4 primer (13 bands). DP3, DP9, DP10 and DP11 showed 100% polymorphism. The lowest P% was observed in DP8 primer (82%). Average of P% in the seven primers was 95.4% (Table 3).

Mean of PIC was 0.4497 for seven primers. The highest PIC was observed in DP6 and DP10 (0.4998 and 0.4997, respectively) and the lowest was in DP9 (0.3695). The maximum and minimum marker index (MI) was observed in DP3 and DP4, respectively (7.603 and 4.722, respectively). Mean of MI was 6.828 (Table 3). The length of amplified bands by seven primers varied from 100 to 3200 bp. According to the correlation coefficients between Cophenetic and similarity coefficients [Dice (0.83), Jaccard (0.84) and SM (0.85)], the highest correlation was obtained by using SM coefficient. But, since the range of similarity coefficient in Jaccard coefficient was wider than the other similarity coefficients and it showed more variation and low difference with SM, hence Jaccard coefficient was used for reconstructing the dendrogram (Figure 3).

According to ISSR dendrogram, Male1005, Male1006, Male1007, Male1008, Male1009, Male1010, Male1011, Male1012, Male1013, Male1014, MaleFard and MaleJervis were placed in one group (B) and all female cultivars and MaleB1 and MaleB2 were classified in another group (A). Group B consisted of two subgroups b1: MaleFard and MaleJervis and b2: Male1005 to Male1014.

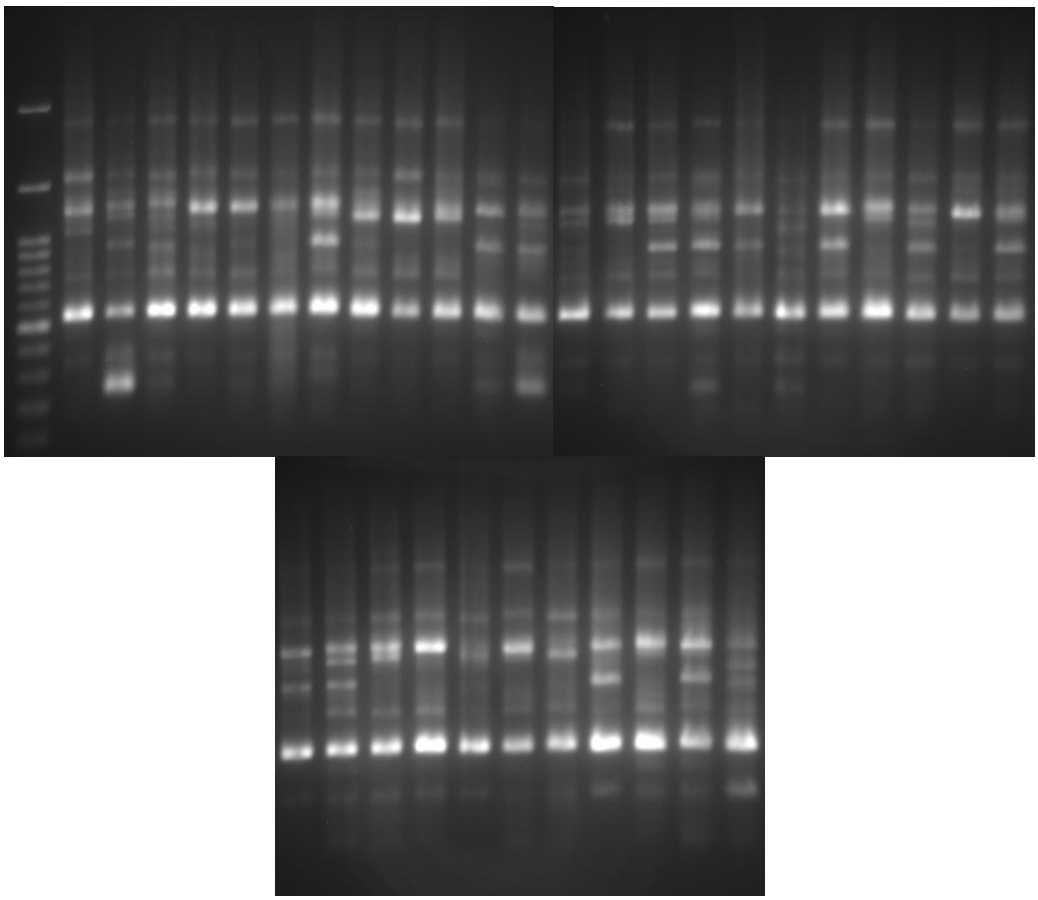

Fig. 2. ISSR profiles amplified from DNA of date palm cultivars using primer DP9.

Table 3. List of the used primers and the complementary information of the ISSR assay

Primer	*Sequence (5′--->3′)	Annealing Temp. (°C)	TBN	NPB	The amplified band length (bp)	P%	PIC	MI
DP1	$(AGG)_6$	55	-	-	-	-	-	-
DP2	$(AG)_{10}G$	60	-	-	-	-	-	-
DP3	$(AG)_{10}C$	60	18	18	450-3200	100	0.4224	7.603
DP4	$(AG)_{10}T$	57	13	12	500-3200	92	0.3935	4.722
DP5	$(CT)_{10}A$	57	-	-	-	-	-	-
DP6	$(CT)_{10}G$	60	16	15	200-3000	94	0.4998	7.497
DP7	$(CT)_{10}T$	57	-	-	-	-	-	-
DP8	$(ACTG)_4$	45	17	14	350-3100	82	0.4879	6.831
DP9	$(GACAC)_4$	55	19	19	100-2600	100	0.3695	7.020
DP10	$(TGGA)_5$	55	14	14	300-2500	100	0.4997	6.996
DP11	$(GACA)_4$	45	15	15	400-2700	100	0.4750	7.125
DP12	$(AG)_{10}$	55	-	-	-	-	-	-
Total		-	112	107	-	-	-	-
Mean		-	16	15.3	-	95.4	0.4497	6.828

TBN: Total band number, NPB: Number of polymorphism bands, P%: Polymorphism percentage, PIC: Polymorphism information content, MI: Marker index. *Primer reference (Karim et al., 2010).

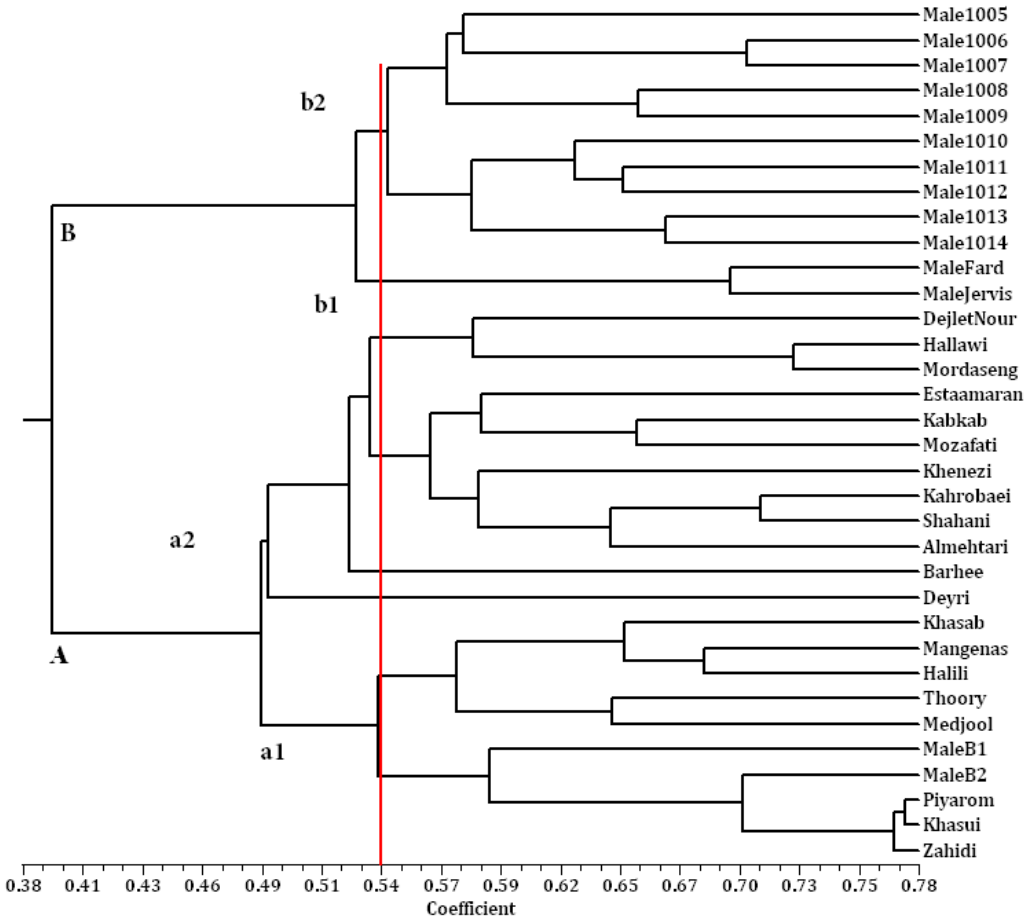

Fig. 3. Reconstructed dendrogram for 34 male and female date cultivars using ISSR markers based on UPGMA algorithm and Jaccard similarity coefficient.

Group A also consisted of two subgroups including a1: Zahidi, Khasui, Piyarom, MaleB2, MaleB1, Medjool, Thoory, Halili, Mangenas and Khasab and a2: Deyri, Barhee, Almehtari, Shahani, Kahrobaei, Khenezi, Mozafati, Kabkab, Estaamaran, Mordaseng, Hallawi and DegletNour. Based on reference line, male and female date cultivars were divided into eight main groups that each consisted of different subgroups (Figure 3).

The greatest similarity coefficient was observed among Piyarom, Khasui and Zahidi (0.77). Mordaseng and Hallawi (0.72) cultivars were placed in the next and Shahani and Kahrobaei (0.0.71) were in the third place. Male1006 and Male1007; MaleJervis and MaleFard; and MaleB2 with three cultivars including Piyarom, Khasui and Zahidi with two-by-two similarity coefficient of 0.70 were in the next place. The lowest similarity coefficient was observed between Male1005, Male1006, Male1007, Male1008, Male1009, Male1010, Male1011, Male1012, Male1013, Male1014 with all female cultivars as well as MaleB1 and MaleB2 (0.39) (Fig. 3).

Results of principle components analysis (PCA)

According to the obtained dimensional equations from PCA, two out of six dimensional equations were more conformity with ISSR results, which is shown in Figure 4. In the both dimensional equations, the male and female date cultivars were divided into two main groups including male cultivars (except MaleB1 and MaleB2) and female cultivars with two male cultivars of MaleB1 and MaleB2.

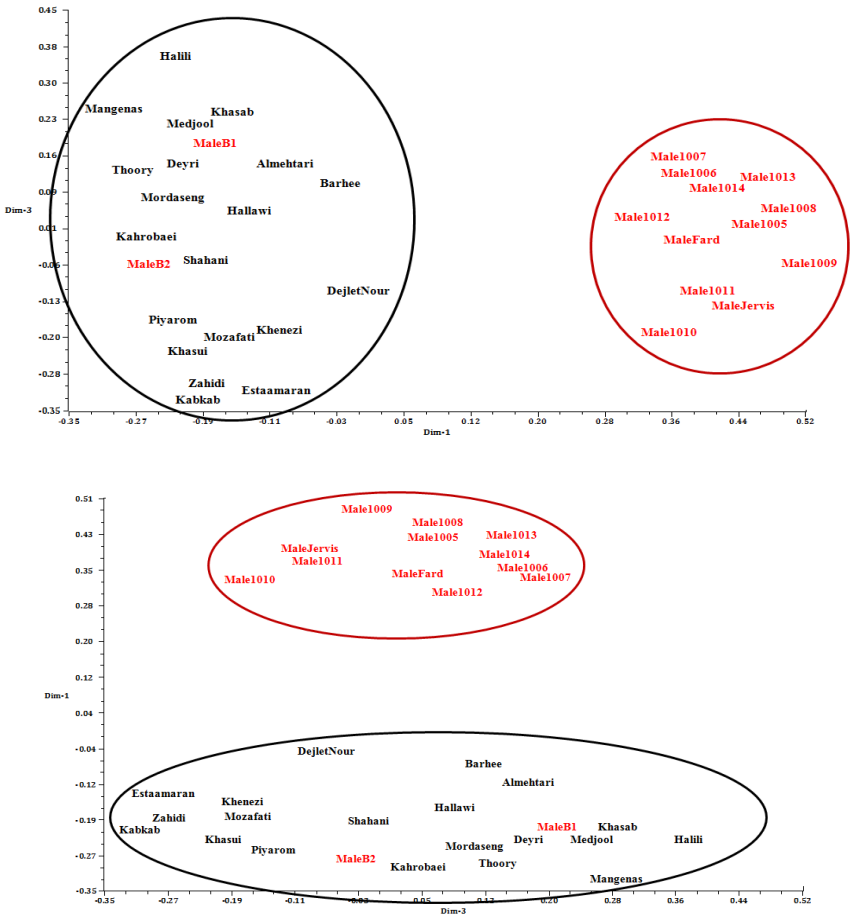

Fig. 4. Dimensional equations Dim-1/Dim-3 and Dim-3/Dim-1 derived from PCA. Female and male cultivars are shown with black and red colors, respectively.

Discussion

ISSR method is one of the effective techniques to clarify date palm genetic diversity (Haider et al., 2012). There are numerous studies about utilization of ISSR markers to assess genetic diversity in date palm (Adawy et al., 2002; Zehdi et al., 2004b; Zehdi-Aziuzi et al., 2009). The ability of ISSR to identify genetic diversity in date palm and its potential to amplify polymorphism fragments have been shown by different researchers which is in line with our results. For instance, Zehdi et al. (2004b) employed seven ISSR primers to appraise genetic diversity of 12 Tunisian date cultivars and they could obtain high polymorphism rate. In their study the number of polymorphic bands was sufficient to separate all cultivars. In another study, Cullis (2011) by using RAPD, AFLP and ISSR markers was able to separate well date palm cultivars. Each of the mentioned markers could be used successfully alone or in combination with each other.

Haider et al. (2012), by reconstructing phylogenic relations using RAPD and ISSR markers among male and female date cultivars in Syria, found that polymorphism rate produced by RAPD (58.5%) was more than ISSR (50.6%) markers. They observed 75% of the amplified bands in ISSR primers were polymorphic and DP11 primer ($GACA_4$) showed 100% polymorphism, which was similar to the results of the present study. The amplicon size varied from 200 to 2000 bp. They found the highest genetic similarity between Medjool and Zahidi cultivars with 0.88 similarity coefficient. In our study, similarity coefficient between Medjool and Zahidi was 0.54. Similarity coefficient among yellow Kabkab, Barhee, Khenezi, Medjool, Zahidi and DegletNour cultivars has been reported between 0.69 to 0.88 (Haider et al., 2012), while similarity coefficient among above cultivars varied from 0.49 to 0.56 in our study. The difference may be because of combination of ISSR and RAPD markers by Haider et al. (2012). However, here we just used ISSR markers.

Moghaieb et al. (2010) studied genetic diversity of date palm using ISSRs. DP3 and DP6 were amongst ISSR primers used by them and in the current study as well. They reported the highest similarity between Khenezi and Thoory cultivars (0.48), which are in line with the results of current study (0.49). Furthermore, they found that range of similarity coefficient among Zahidi, Barhee, Khenezi and Thoory cultivars varied from 0.32 to 0.48. This parameter in the present study varied from 0.49 to 0.54.

Munshi and Osman (2010) suggested RAPD and ISSR markers to evaluate genetic diversity and molecular characteristics of date palm germplasms. Hamza et al. (2012) employed seven ISSR primers to assess genetic variation among Tunisian date cultivars and indicated that the length of the amplicons from 200 to 2500 bp. PO6, PO7 and D9 are some primers used by Hamza et al. (2012) here named as DP3, DP4 and DP6, respectively. They found 60.0, 66.6 and 62.5% polymorphism, respectively, for PO6, PO7 and D9. In our study polymorphism rate for the above mentioned primers was 100, 92 and 94%, respectively. Hamza et al. (2012) reported 73.5% polymorphism in DegletNour cultivar, which in our study was 89.4%.

So far, many ISSR primers have been tested for date palm cultivars (Zehdi et al., 2002; Mitra et al., 2011) and other monocot species such as Bluegrass (Arslan and Tamkoc, 2011) and Durum wheat (Pasqualone et al., 2000). Hamza et al. (2012) used seven ISSR primers to evaluate genetic diversity of date cultivars and obtained totally 43 amplified bands (6.14 bands/primer), which was very low in comparison with other findings (Zehdi et al., 2002; Mitra et al., 2011) as well as with the results of present study. We produced totally 112 amplified bands using seven primers (16 bands/primer). This discrepancy in results can be stemming

from differentiation in the number of genotypes used for study or low genetic affinity of the genotypes studied by Hamza et al. (2012).

In our study, correlation between morphological and ISSR markers was insignificant ($r=0.025^{ns}$). Although, molecular markers are able to uncover genetic diversity among date palm genotypes, only few investigations have shown correlation between molecular markers and phenotypic parameters (Hamza et al., 2012). For instance, Rhouma-Chatti et al. (2011) did not observed significant differentiation between results of AFLP and RAMPO markers on male date cultivars. Moreover, Hamza et al. (2012) observed significant correlation between genetic outcomes and fruit texture firmness. In contrast, they did not observe significant correlation between molecular analysis and fruit ripening time. In the present investigation, the highest morphological similarity was observed between Almehtari and Estaamaran cultivars (0.66). Based on scientific evidences, these two cultivars have no high similarity with each other. It seems that the planted cultivar as Estaamaran in the collection of date cultivars in Minab Agricultural Research Station, which has been transferred from Date and Tropical Fruits Institute, is not Estaamaran and it has highly close similarity to Almehtari cultivar. The similarity was also confirmed by trunk diameter, height, early-ripening and fruit size.

Eissa et al. (2009) discovered that RAPD and ISSR markers were able to recognize and separate nine date cultivars in Egypt. Mirbahar et al. (2014) observed similarity coefficient equal to 0.95 between Hallawi and Mozafati cultivars using RAPD markers in Pakistan. They reported 79.4% variation among 25 date cultivars. In our study, similarity coefficient between Hallawi and Mozafati cultivars was 0.54 and variation rate among 34 date studied cultivars was 37%. Ahmad and Al-Qaradawi (2010) in evaluation of genetic diversity by using 18 ISSR primers on date cultivars in Qatar found that only three primers amplified clear and polymorphic bands. Apart from climate differences, the assessed cultivars had high affinity together. Moreover, they found that Barhee and Soltana cultivars have same origin. Ahmad and Al-Qaradawi (2010) used two primers of TA-1 and TA-2 that both primers had same sequence pattern with DP3 and DP6 primers, respectively, in the present study. They observed close relationship between Khenezi and Thoory cultivars, while in our study, similarity coefficient between these two cultivars was 0.48.

Arabnezhad et al. (2012) evaluated male and female date cultivars collected from Iran, Iraq and Africa using SSR markers and showed cultivars grouping based on geographical region. They observed high similarity among Medjool, DegletNour and Thoory cultivars from Africa, which was in parallel with our results. Here, we showed close relationship between Medjool and Thoory cultivars.

Recognizing male genotypes in seedling stage is one of the most important obstacles for date palm seedling producers. By dissolving the mentioned challenge we can keep genetically close males and females in seedling stage, otherwise the males with no close relations can be replaced by other genetically close ones. Dhawan et al. (2013) designed a sequence-characterized amplified region (SCAR) primer pair to determine sexuality of date palms. By using this primer and through amplifying a unique fragment of 354 bp just exists in male genotypes, sexuality of date palms in the early stages of growth can be understood. Al-Mahmoud et al. (2012) were able to determine sexuality of date palms in early stages using RFLP technique and restriction enzymes *Bal*I and *Hpa*II with accuracy of 90%.

In the present study, length of the amplified fragments varied from 100 to 3200 bp. Marsafari and Ashraf-Mehrabi (2013) reported amplicons with 100-2250 bp using ISSR markers. Average of the amplified bands per each primer varied

from 4 to 18 whereas in our study, the average of the amplified bands per each primer varied from 13 to 18 bands. Average of total band number and polymorphic band number documented by Marsafari and Ashraf-Mehrabi (2013) were 11.6 and 11.1, respectively, while in our investigation these values were 16.0 and 15.3, respectively. In addition, PIC and MI in Marsafari and Ashraf-Mehrabi (2013) investigation were 0.222-0.309 and 0.888-4.736, respectively, which in our study were 0.3695-0.4998 and 4.722-7.603, respectively. They reported that Dice similarity coefficient between Kabkab and Deyri cultivars was 0.941, while in the present study Jaccard similarity coefficient between both cultivars was 0.49. This indicates that type of similarity coefficient can impact on similarity of the cultivars. In our study, more variation was cleared among cultivars by using Jaccard coefficient and it was from 0.37-0.77. In our study, Cophenetic coefficient between dendrogram and Jaccard similarity matrix was 83%, whereas Cophenetic coefficient between dendrogram and Dice similarity matrix in Marsafari and Ashraf-Mehrabi (2013) was 87.3%. In Marsafari and Ashraf-Mehrabi (2013) investigation, DegletNour and Kabkab cultivars were in a same cluster, while in our study both with similarity coefficient of 0.54 were in a main cluster but in two separated sub-clusters (fifth and sixth). Marsafari and Ashraf-Mehrabi (2013) placed Barhee and Piyarom cultivars in one group, while in the present study both these cultivars with similarity coefficient of 0.49 were placed in different main groups.

Conclusion

Based on analysis of ISSR markers and morphological attributes, the studied date cultivars were divided into eight main clusters and various sub-groups, but no significant correlation was observed between grouping made by molecular and morphological markers. Unlike ISSR markers that produced a clear-cut among male and female cultivars, no distinct border was seen among male and female cultivars using morphological attributes. PCA analysis confirmed the grouping made by ISSR markers.

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