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Determination of inter- and intra-specific genetic variations among Qatari date palm cultivars using inter simple sequence repeat (ISSR) markers

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The separated offshoots from individual trees are mainly used for date palms propagation, which maintains the genetic integrity of date palm cultivars such as the fruit morphology and quality, however, some variations are observed. The objectives of the current study were to determine the genetic similarity or diversity among and within the well-known Qatari date palm cultivars using different 18 primers of inter simple sequence repeat (ISSR). Five common and the most cultivated date palm cultivars in Qatar were selected including Khalas, Sheshy, Rezezy, Barhee and Khanezy from three different locations (Al-Shamal, Al-Khour and Al-Rayan). All primers had amplified polymorphic bands in the studied cultivars either among the cultivars or within each cultivar in different cultivated areas. These results reveal the existence of genetic variations among the studied cultivars as well as within each cultivar, supporting the observed variation in some morphological and quality characters for different trees that are grown in different environments and derived from the same cultivar. Results reported from this study will help date palm community in Qatar for intera- and inter- fingerprinting different cultivars leading to identification of new lines or cultivars that are of a high quality and thus may be patented as unique Qatari cultivars.

Key words: Date palm, inter simple sequence repeat (ISSR), genetic variations.

INTRODUCTION

Date palms (*Phoenix dactylifera* L.) (2n = 2x = 36) are dioecious, perennial, monocotyledon fruit trees that belong to the family Arecaceae. This tree is considered the most important cultivated fruit trees with 71% from the total planted area of fruit trees in Qatar. The total cultivated area is approximately 1366 ha (containing 335765 trees bearing fruits and 146955 non-productive trees). Most cultivated date palm areas are located in the north and middle area of Qatar State where there are favorable environmental conditions and deep profile soil with low salinity when compared with other parts of the country (Abufatih et al., 1999).

The tremendous advantage of the date palm tree over other trees is attributed to its minimal requirement inputs, its long-term productivity and the multiple production purposes. The separated offshoots from individual trees

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Table 1. List ofQatari date palm cultivars and their collection sites.

Cultivar	Al-Shamal (A)	Al-Khour (B)	Al-Rayan (C)
Khalas	1	1	1
Sheshy	2	2	2
Rezezy	3	3	3
Barhee	4	4	4
Khanezy	5	5	5

are mainly used for date palms propagation, which maintains the genetic integrity of date palm cultivars such as the fruit morphology and quality. Offshoots are produced in limited numbers during a date palm's life span where the first flowering of the trees takes place at the age of about five to seven years. This phenomenon makes the biological characteristics of date palm trees very difficult to compensate for the rapid decline of trees due to natural disasters.

Due to the nature of date palms as dioecious trees intra-varietal variations are expected due to different sources of pollen grains. Numbers of methods are currently available for analysis of either inter- or intra genetic variation in the most cultivated date palm cultivars. These methods have relied mainly on the availability of genetic markers. Genetic markers have the ability to represent variation at a particular site on the genome which is heritable, easy to assay and can be followed over generations. Thus, molecular markers are of great value in genetic research as they can determine the genetic variation among and within different genotypes. Recently, developed techniques, based on DNA markers and polymerase chain reaction (PCR), offer new tools for genetic analysis in different areas including varietal fingerprinting and estimation of the relatedness between different genotypes discernment of evolutionary relationships.

Morphology of leaves, spines and fruit characters are the primary descriptors that were used to differentiate between the cultivated date palm varieties (Hammadi et al., 2009; Sedra et al., 1993, 1998, Ben Salah, 1993; Ben Salah and Hellali, 2004). With the identification of molecular markers and their associated specificity, further assessment of the genetic diversity among the date palm cultivars is warranted in order to accrete and refine the existing morphological classification system. While several molecular assays could be used to assess the genetic diversity, each method differs in its principle, application, the degree of polymorphism detected, cost and time required.

Many of the molecular markers have been used widely and efficiently to determine the intra- and inter-genetic variations of date palm cultivars. Examples of these molecular markers are isozymes and DNA-based markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) (Torres and Tisserat, 1980; Baaziz and Saaidi, 1988; Al-Jibouri and Adham, 1990; Bennaceur et al., 1991; Corniquel and Mercier, 1994, 1997; Bendiab et al., 1998; Mokhtar et al., 1998; Saker and Moursy, 1998; Sedra et al., 1998; Salem et al., 2001).

SSR and ISSR are used widely as molecular markers. SSR, also known as microsatellites, are tandem repeat motifs composed of one to six nucleotides, which are ubiquitous, abundant and highly polymorphic in all eukaryotic genomes. ISSR is a novel PCR technique that employs repeat-anchored or non-anchored primers to amplify DNA sequences between two inverted SSR. Unlike SSR, ISSR markers do not require a prior knowledge of the SSR targets sequences and highly reproducible due to their primer length, the high stringency achieved by the annealing temperature as well as their abilities to provide highly polymorphic fingerprints (Bornet and Branchard, 2001).

In recent work, SSR markers have been used to assess the molecular characterization and the phylogenic relationships among common Qatari date palm cultivars (Ahmed and Al-Qaradawi, 2009). The results provided evidence of a genetic diversity among the studied cultivars and the ability of SSR markers to assess the genetic diversity in date palm.

Despite the genetic integrity of each well-known date palm cultivar, little differences in fruit morphology and quality have been detected among the individual trees of the same cultivar. Therefore, the objective of the present study was to determine the inter- and intra-specific genetic variation in most common cultivars of date palm that are grown in Qatar. In this study, ISSR molecular markers were used to determine the genetic similarity or diversity within the well-known Qatari date palm cultivars. A further aim of this study was to develop a detailed understanding of the genetic and molecular relationships of Qatari date palm cultivars. Collectively, these data may further lead to identify new lines or cultivars that are of a high quality and thus may be patented as unique Qatari cultivars.

MATERIALS AND METHODS

Plant materials and genomic DNA isolation

Leaf samples of five common date palm cultivars in Qatar (Khalas, Sheshy, Rezezy, Barhee and Khanezy) were collected from three different locations in Qatar: Al-Shamal, Al-Khour and Al-Rayan

Table 2. Fruit morphological characters of date palm cultivars used in this study.

Cultivar	Fruit shape	Fruit dimension length/width (cm)	Fruit color (unripe)	Fruit color (ripe)
Khalas	Rectangular	3.58/2.30	Golden yellow	Hazel color
Sheshy	Oval to ball shape	3.65/2.42	Green brown	Auburn
Rezezy	Conical oval	2.87/2.09	Amber	Red-grayto black
Barhee	Circular	3.17/2.39	Yellow	Bright brown
Khanezy	Oval	3.45/2.22	Red brown	Dark brown

Table 3. List of ISSR primers used in this study.

Primer name	Tm (°C)	Sequence
814	44	(CT)8TG
844A	44	(CT)8AC
17898A	44	(CA)6AC
17898B	44	(CA)6GT
17899A	44	(CA)6AG
17899B	44	(CA)6GG
HB 8	44	(GA)6GG
HB 9	44	(GT)6GG
HB 11	44	(GT)6CC
HB 12	44	(CAC)3GC
844B	44	(CT)8GC
HB 10	44	(GA)6CC
HB 13	44	(GAG)3GC
HB 14	44	(CTC)3GC
HB 15	44	(GTG)3GC
TA-1	60	(AG)10C
TA-2	55	(CT)10G
TA-3	60	(AGG)6

(Table 1). Fruit morphological characters of these cultivars are shown in Table 2. These five cultivars were selected as representatives of genetic diversity of Qatari cultivars when SSR markers were used (Ahmed and Al-Qaradawi, 2009).

Total genomic DNA was extracted using both commercial DNeasy Plant System (Qiagen, Inc., Valencia, CA) kit and standard CTAB method (Doyle and Doyle, 1987). Further quantification and qualification of isolated DNA was carried out according to Ahmed and Al-Qaradawi (2009).

ISSR amplification

A total of 18 ISSR single primers were designed to amplify ISSR bands using genomic DNA of the date palm as a PCR template (Table 3). PCR reactions were performed in a total reaction mixture of 25 µl containing: 20 to 30 ng of total genomic DNA (1 µl), buffer (GeneAmp, Applied Biosystems), 0.2 mM of dNTP PCR mix (GeneAmp, Applied Biosystems), 0.50 U of Taq DNA polymerase (AmppliTaq, Applied Biosystems) and 0.2 mM of primers. Amplifications were performed in a GeneAmp PCR System 9700 Thermocycler, with the following conditions: a denaturation step of 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 90 s at 44 to 60°C and 90 s at 72°C, and a final extension step at 72°C for 7 min. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed using Gel documentation system.

Data scoring and analysis of ISSR

The only clear and unambiguous bands were considered for the further fingerprinting scoring. Markers were scored for the presence and absence of the corresponding band among different cultivars and within each cultivar grown in different locations. The scores '1' and '0' were given for the presence and absence of bands, respectively. The data obtained by scoring the ISSR profiles of different primers were subjected to cluster analysis. A similarity matrix was constructed using Jaccard's coefficient where the similarity values were used for cluster analysis. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering was done using unweighted pair group method with arithmetic averages (UPGMA) method. Data was analyzed using *Past* software version 1.91 (Hammer et al., 2001) on the basis of Hamming similarity index with 100 bootstrap.

RESULTS

From our initial work (Ahmed and Al-Qaradawi, 2009) that focused on 15 known cultivars in Qatar, we reported a genetic variation among those studied cultivars and further genetic study was warranted to verify if intra-genetic variation exist within the same cultivar grown in different cultivated areas. Here, we employed a new molecular marker tool to detect and identify polymorphisms between these cultivars and within each cultivar grown in different cultivated areas. We selected five cultivars that represent different sub-groups of the most cultivated Qatari cultivars. A total of 1317 bands were generated using 18 ISSR primers for the five studied cultivars in the three locations with average of 73 bands of each primer. The number of amplified bands varied from one cultivar to another and differed from area to area for the same cultivar. The highest number of bands was obtained using Primer 15 (HB 15) for the five cultivars over all locations (104 bands), while the lowest number of bands (29) was scored using Primer 1 (814) (Table 4). It is interesting to note that Khanezy cultivar scored the highest number of amplified bands over all areas and primers (Table 5).

Interestingly, all studied cultivars showed different band numbers in the different cultivated areas as shown in Table 4. Among the five studied cultivars, the polymorphic fragments accounted for 271 in Khalas, 269 in Sheshy, 249 in Rezezy, 239 in Barhee and 289 in Khanezy (Table 5). As expected, genetic variation as a reflection from the number of polymorphic bands within each cultivar

Table 4. Number of amplified bands of 18 ISSR primers from the five studied date palm cultivars grown in different areas.

Primer	Number of amplified bands			Total
	Al-Shamal	Al-Khour	Al-Rayan	
814	6	9	14	29
844A	29	29	34	92
17898A	33	23	34	90
17898B	28	34	32	94
17899A	22	13	28	63
17899B	24	25	26	75
HB 8	25	23	34	82
HB 9	18	20	24	62
HB 11	23	12	11	46
HB 12	36	31	29	96
844B	18	22	26	66
HB 10	4	8	18	30
HB 13	13	13	32	58
HB 14	34	28	35	97
HB 15	38	39	27	104
TA-1	24	23	18	65
TA-2	21	27	28	76
TA-3	21	27	45	93

Table 5. Estimation of different band numbers in the different cultivated areas using genomic DNA of five date palm cultivars.

Date palm	Number of amplified band		
	Al-Shamal (A)	Al-Khour (B)	Al-Rayan (C)
Khalas	83	91	97
Sheshy	82	77	110
Rezezy	82	73	94
Barhee	83	78	78
Khanezy	91	85	113

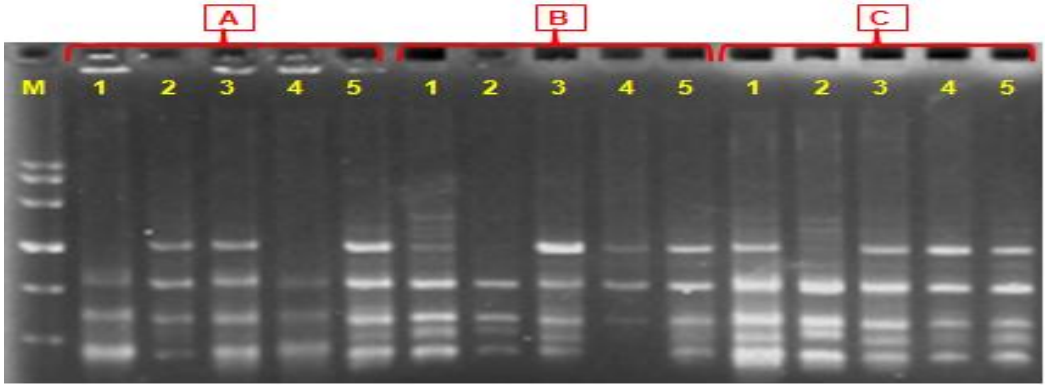

Figure 1. Amplified band-patterns of primer 11 from five studied date palm cultivars in different cultivated areas. Arrows show the polymorphic bands within each cultivar.

was considerably smaller than the inter-specific variation among five cultivars. For example, when Primer 11 (844B) was used, polymorphic band in Khalas appeared in Al-Khour (Area B) and Al-Rayan (Area C) with size of 1209 bp, while it disappeared in Al-Shamal (Area A). One clear band (Figure 1) appeared in area A (Sheshy) and

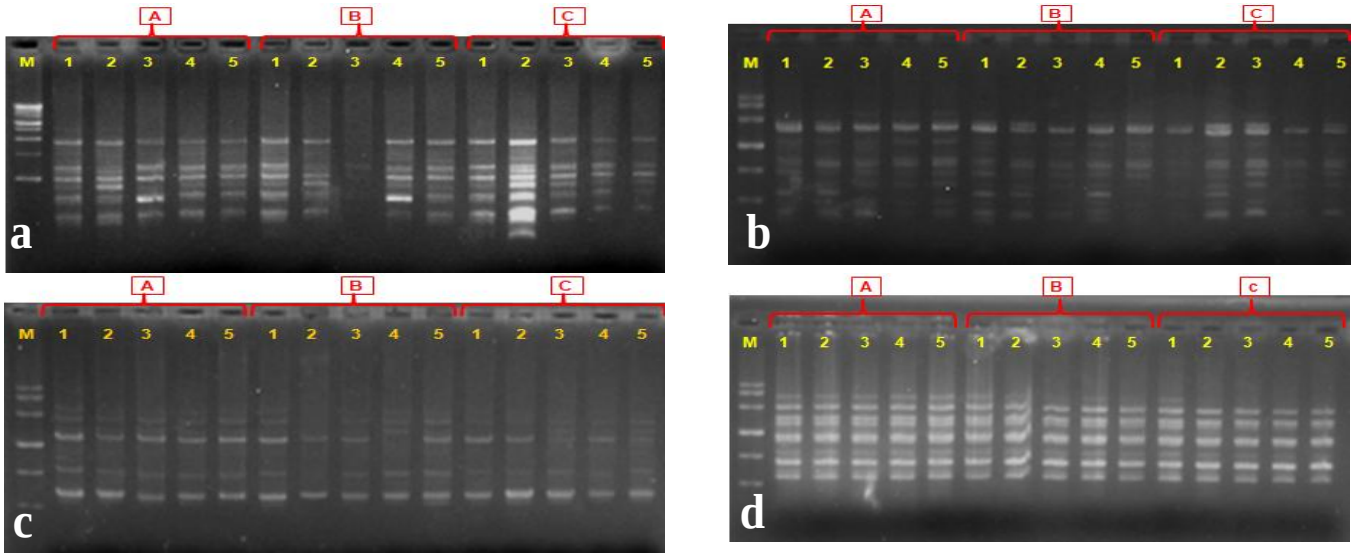

Figure 2. Examples of polymorphic bands that explain the genetic variations among the five cultivars and within each cultivar grown in different locations. Primers 2, 4, 6 and 10 are indicated in bold small letters as a, b, c and d.

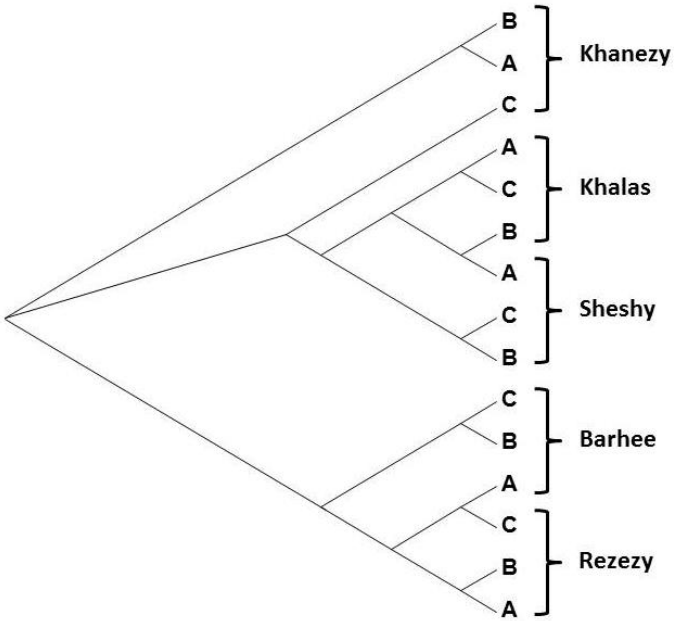

Figure 3. A UPGMA dendrogram based on ISSR data obtained from the studied date palm cultivars grown in the three locations.

disappeared in both Areas B (Al-Khour) and C (Al-Rayan). In contrast, Khanezy did not show polymorphic bands in the three locations.

Additionally, different examples (Figure 2) explain the genetic variations among the five cultivars and within each cultivar grown in different cultivated locations. Primers 2, 4, 6 and 10 are presented in a, b, c and d. The large number of polymorphic fragments at the inter- and intra-specific level demonstrated that there is a high level of genetic variation not only across the five date palm cultivars, but also within each cultivar. This variation is sufficient to permit the assessment of inter- and intra- specific diversity in date palm using DNA marker analysis.

The phylogenetic diagram (phylogram) illustrates the divergence between the studied Qatari date palm cultivars (Figure 3) where it distributed them to three major branches. Mainly, the phylogenetic tree showed three major clusters, the first included two cultivars (Rezeze and Barhee) and the second cluster contained two cultivars (Sheshy and Khalas) while Kanezy was grouped in a separate cluster (Figure 3). A consensus tree derived for five cultivars in three locations, representing 15 Qatari date palm cultivars (Figure 3), exhibited weak clustering relationships with bootstrapping values.

Five sub-grouped were formed clustering the samples that are collected from different areas in Qatar from the same cultivar. These sub-groups were as the following: two for both Rezeze and Khanezy samples from Alshamal and Alkour, two for both Barhee and Sheshy samples from Al-Khour and Al-Rayan and one for Khals samples from Alshamal and Al-Rayan locations. Additionally, two sub-groups were formed from different cultivars collected from different areas as the following: Rezeze sample from Al-Rayan and Barhee sample from Al-Shamal, and Khalas sample from Al-Khour and Sheshy sample from Al-Shamal. Khanezy from Al-Rayan was clustered separately from the rest of the other sub-groups.

DISCUSSION

Several types of molecular markers such as AFLP, RAPD, SSR and ISSR have been used to assess the genetic diversity and the phylogeny among date palm genotypes

(Devanand and Chao, 2003; Torres and Tisserat, 1980; Baaziz and Saaidi, 1988; Al-Jibouri and Adham, 1990; Bennaceur et al., 1991; Corniquel and Mercier, 1994, 1997; Bendiab et al., 1998; Mokhtar et al., 1998; Saker and Moursy, 1998; Sedra et al., 1998; Salem et al., 2001; Zehdi et al., 2004). In this study, we used the ISSR as high polymorphic markers to estimate the intra- and inter-specific genetic variations of five date palm genotypes grown in Qatar. Due to the genetic diversity among the studied cultivars, high inter-specific variations were found, while little intra-specific variations within the same cultivar were detected. Similar results were reported for date palm using isoenzymes where scored percentage of resolution was higher than that observed (Booij et al., 1995; Ould et al., 2001) for plastid DNA haplotypes (Sakka et al., 2003). ISSR markers revealed narrow genetic diversity when they were applied on four important cultivars in Saudi Arabia (Munshi and Osman, 2010). Consequently, our results provided powerful molecular markers that are suitable in cultivar identification. Their transfer to other laboratories over the world would be of great interest to label at a large-scale offshoots, any other plant material at early stage and *in vitro* plantlets. All the primers used in our study amplified large numbers of loci, varying from 6 to 38 per ISSR primer depending on the cultivar and its growing location.

In our ISSR-based study, scored polymorphism was higher than recorded in previous studies using other marker systems. Because the current ISSRs detected high polymorphisms in date palm, we anticipate that the results of ISSR-based markers will be used as useful tools in the management, conservation and improvement of this important tree. Additionally, this ISSR-based study has enhanced our understanding of the genetic status of five date palm genotypes. Based on this information, it will be useful to plan distribution strategies for the studied date palm cultivars that efficiently capture genetic diversity for selection trials and subsequent distribution of clonal planting stock. It is known that high genetic variation is a safeguard against co-evolving biotic factors such as pests and diseases. Hence, ISSR-based assessments will be helpful both in deciding how and where to conserve germplasm and in planning crosses in breeding programs. Moreover, the assessment of genetic variation within cultivars of date palm will assist in predicting achievable genetic gain in breeding programs and it may reflect the genetics stability of progenies obtained from interspecific crosses.

Genetic diversity is necessary especially for long-term crop improvement and enhancement of the plant resistance to important biotic and abiotic stresses. Information from genetic diversity can be used in breeding programs by crossing cultivars with a high level of genetic distance as well as for the introgression of exotic germplasm. Estimating genetic diversity using DNA based-molecular marker techniques are extremely useful for intellectual property protection, particularly in the determination of essential derivation. The genetic diversity estimates based on molecular marker data may be compared with a minimum genetic distance, which indicates that two cultivars are not essentially derived (Lefebvre et al., 2001).

Conclusions

The ISSR marker analysis outlined in this study is easy and readable and adopted as a tool to study the inter- and intra-specific genetic variations in date palm. In this current study, we used 18 ISSR primers and the genomic DNA of five date palm cultivars grown in three different locations. All primers amplified polymorphic bands in the studied cultivars either among the cultivars or within each cultivar grown in different cultivated areas. The results indicate the existing genetic variations not only among well-known cultivars, but also within each cultivar, explaining the variation in some morphological and quality characters from different trees within the same cultivar. In addition, data provide evidence of the possibility of using these powerful markers as descriptors in the certification and the control of origin labels of date-palm material.

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