Molecular characterization of date palm (Phoenix Dactylifera L.) using Inter Simple Sequence Repeat (ISSR) markers

Talaat A. Ahmed* and Sara Al-Hadidi,
Department of Biological and Environmental Sciences,
College of Arts and Science, Qatar University, Doha 2713, Qatar
E-mail: t.alfattah@qu.edu.qa

ABSTRACT

To study the genetic diversity among date palm cultivars grown in Qatar, fifteen Date palm samples were collected from Qatar University Experimental Farm. DNAs were extracted from fresh leaves by using commercial DNeasy Plant System Kit (Qiagen, Inc., Valencia, CA). Total of 18 (Inter Simple Sequence Repeat) ISSR single primers were used to amplify DNA fragments using genomic DNA of the 15 samples. First screening was done to test the ability of these primers to amplify clear bands using Date palm genomic DNA. All 18 ISSR primers successfully produced clear bands in the first screening. Then, each primer was used separately to genotype the whole set of 15 Date palm samples. Total of 4794 bands were generated using 18 ISSR primers for the 15 Date palm samples. On average, each primer generated 400 bands. The Number of amplified bands varied from cultivar to cultivar. The highest number of bands was obtained using Primers 2, 5 and 12 for the 15 (470 bands), while the lowest number of bands were obtained by Primers 1, 7 and 8 where they produced only 329 bands. Markers were scored for the presence and absence of the corresponding band among the different cultivars. Data were subjected to cluster analysis. A similarity matrix was constructed and the similarity values were used for cluster analysis.

Key words: Date palm, ISSR (Inter Simple Sequence Repeat), Molecular diversity.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), 2n=36, is a dioecious long-lived monocotyledonous tree, it belongs to the family Arecaceae. Date palm is an excellent crop in arid and semiarid regions of the world with high tolerance to environmental stresses (Adawy *et al.,* 2004). The annual world production of dates has reached 6-8 million mt (metric tons), representing a market exchange value of over 1 billion USD. Date palm is one of the most important horticultural crops in Qatar and is also used as an ornamental or shade plant in parks, gardens and alongside roads. Date Palm plantations represent 71% from the total area planted with fruit trees. Total area cultivated approximately 1366 ha (Containing 335765 trees bearing fruits and 146955 non-productive trees). Most cultivation are in the North and Middle area of the state where environmental conditions are favorable, soil has deep profile with low salinity compared with other parts of the country (Abufatih *et al.,* 1999).

To understand the genetic relationship among and within date palm varieties, RFLP, RAPD, SSR and AFLP markers have been used widely and efficiently to analyze the genetic diversity within and among date palm cultivars in many middle east countries such as Egypt (Soliman *et al.,* 2003; Saker *et al.,* 2006); Oman (Al-Ruqaish *et al.,* 2008); Morocco (Baaziz 2000; Sedra *et al.,* 1998); Suadi Arabia (Al-Khalifah and Askari, 2003); Tunisia (Trifi *et al.,* 2000; Zehdi *et al.,* 2004a,b); Sudan (Elshibli and Korpelainen, 2007).

Development of accurate fingerprint characterizing the common cultivars of date palm would be of great value in improvement of this important crop. Some molecular markers as ISSR (Adawy *et al.,* 2004), RAPD (Adawy *et al.,* 2004; Ben Abdallah *et al.,* 2000; El-Rayes., 2009; Trifi et al, 2000), AFLP (Adawy *et al.,* 2004; El-Khisin *et al.,* 2003), and SSR (Ahmed and Al-Qaradawi, 2009) were used to describe genotypes of date palm. In Saudi Arabia RAPD fingerprint was used to investigate the genetic diversity of 5 varieties. Of 20 primers, only 12 primers were replicated and 64 bands were produced. The profile was used to distinguish variety (Al-Moshile *et al.,* 2004). Based on two by two comparisons of the products, the genetic similarity was calculated in the region 70 to 85%. Cluster analysis and dendrogram were done by UPGMA and the cultivars were divided into two groups (Al-Moshile *et al.,* 2004). Moghaieb et al. (2010) investigated the genetic diversity and sex determination in 6 genotypes of male and female date palms by RAPD and ISSR markers. Polymorphism amount of the markers was 60.2 and 73% for RAPD and ISSR markers, respectively. The cluster analysis showed that unknown cultivars had close relation with Frehi,

The objective of this study is to study the genetic diversity among and within date palm cultivars grown in two different locations in Qatar using 18 (Inter Simple Sequence Repeat) ISSR markers.

MATERIALS AND METHODS

Plant materials

Forty seven date palm samples representing 15 cultivars from two gene bank collections (Rodat Alfaras Germplasm field and Germplasm field of Qatar University Experimental farm) were collected. 29 samples representing 11 varieties were collected from Rodat Alfaras Farm. Eighteen samples including six varieties were collected from Qatar University Experimental Farm (Table 1).

DNA extraction

DNeasy Plant Mini kit (Qiagen) was used to extract DNA from the Qatari Date palm leaf samples according to the manual instructions of the kit (DNeasy Plant Handbook). Obtained DNAs were quantified and qualified by using agarose gel electrophoresis. Two µl of DNA from each sample were applied to 0.85 % Agarose gel and electrophoreses was done at 100V for 30 min. The gels were stained in Ethidium bromide and visualized under UV light.

PCR amplification and ISSR assay

A total of 18 primers were tested to amplify the isolated DNA. These primers listed in Table 2, and their composition has been arbitrarily established. For PCR amplifications, a 25 µl reaction mixture was used and it contained between 20 and 30 ng of total cellular DNA (1 µl), 60 pg of primer (1 µl), 2.5 µl of 10X Taq DNA polymerase reaction buffer, 1.5 unit of Taq DNA polymerase (Quantum-Appligène, France) and 200 mM of each dNTP. . 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 52 - 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 1.5 % agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed using Gel documentation system.

Data analysis

Bands were precisely measured by Gel documentation System software and scored for each genotype. Each reproducible polymorphic DNA band at particular position on the gel was treated as a separate character and scored as present (1) or absent (0) to generate a binary data matrix.

Data were then computed with the SPSS program to produce a genetic distance matrix which assesses the similarity between any two populations on the basis of the number of generated bands using Jaccard' s similarity coefficient (Jaccard 1908).

RESULTS AND DISCUSSION

Total of 18 (Inter Simple Sequence Repeat) ISSR single primers were used to amplify DNA fragments using genomic DNA of the 15 samples. First screening was done to test the ability of these primers to amplify clear bands using Date palm genomic DNA. All the18 ISSR primers successfully produced clear bands in the first screening. Then, each primer was used separately to genotype the whole set of 15 Date palm samples (An example is shown in Figure 1). Total of 4794 bands were generated using the 18 ISSR primers for the 15 Date palm date palm cultivars. On average, each primer generated 400 bands. The number of amplified bands varied from cultivar to cultivar. The highest number of bands was obtained using Primers 2, 5 and 12 for the 15 cultivars, while the lowest number of bands was obtained by Primers 1, 7 and 8 where they produced only 329 bands. However, variation within each individual cultivar as number of polymorphic fragments was considerably smaller than the inter-specific variation among the studied cultivars.

Interestingly, thirty distinct unique bands were obtained to represent nine date palm cultivars. Four out of seven different sizes bands obtained from primer 3 were appeared in Hatamy, Barhee, Khadraway, and Thuri. Each band represents one cultivar. In the other hand, some cultivars could be represented with single bands amplified with different primers. For example, Zahidi

was represented with single bands obtained from Primer 4, 5 and 15 in 185, 302 and 155 bp, respectively. Six cultivars (Hatamy, Helaly, Sheshy, Khadrawy and Thuri) had two unique single bands from two different primers. However, one unique single band was shown in Succary, Abu Main, Barhee, Naboot Saif and Khanezy.

Band pattern data was converted into a binary data and was analyzed using SPSS program to calculate similarity coefficient values according to Jaccard (1908). A similarity matrix between Qatari date palm cultivars showed an average similarity coefficient range from 0.000 to 0.750. The cultivars studied here were highly divergent at the DNA level. The highest similarity coefficient value was observed between KHUSH ZABAD and KHANEZY cultivars (0.750) which seem to be the nearest two varieties and can be closely regrouped. The following nearest two cultivars are found between ABU-MAIN and SHESHY. All the other cultivars displayed low levels of similarity but still were grouped with each other.

The Jaccard similarity coefficient matrix was computed to cluster the data and to draw the precise relationships among the fifteen studied Qatari date palm genotypes. The Dendrogram shown in Figure 2, illustrates the divergence between the studied Qatari date palm cultivars and suggests their tree branching.

Hatamy cultivar was in a separate far group compared to the rest of cultivars. ABU-MAIN and SHESHY; HELALY and KHALAS may constitute paired clusters (Fig. 2).

DNA markers are powerful tool to provide information on the relatedness of various clones or varieties that are difficult to distinguish morphologically, thus helping in the management of plant accessions and in breeding programs. Simple Sequence Repeat DNA markers (SSR, or microsatellite markers) is considered the method of choice due to their abundance, polymorphism and reliability compared to other types of DNA markers. However, it was only with the development of SSR markers for date palm (Billotte *et al.,* 2004) that reliable, co-dominant and comparable molecular data on date palm populations could be generated.

The highest levels of polymorphism for ISSRs system compare to other systems also reported in previous studies (Belaj *et al.,* 2003; Russel *et al.,* 1997; Gomes *et al.,* 1998; Maguire *et al.,* 2002; Palombi and Damiano, 2002; Rajora and Rahman, 2003; Ferreria, et al. 2004). This high level of polymorphism, associated with SSR markers, is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage. Replication slippage is thought to occur more frequently than single nucleotide mutations and insertion\deletion events, which generated the polymorphisms detected by RAPD analysis (Powell *et al.,* 1996). The co-dominant nature of SSR markers also permits the detection of a high number of alleles per locus and contributes to higher levels of expected heterozygosity being reached than would be possible with RAPD markers.

CONCLUSION

In this study, ISSR markers have been used to assess the molecular characterization and the phylogenic relationships of Qatari date palm cultivars. Our results provide evidence of a genetic diversity among the studied Qatari date cultivars and the ability of SSR markers to detect the genetic diversity in date palm. We may conclude that all date-palm ecotypes are interrelated in spite of their agronomic divergence. Genetic similarities and Dendrogram could re-group the Qatari date palm cultivars in a way that one cultivar (Abu Main) was excluded from the group due to its dissimilarity with the other cultivars. Two cultivars (Barhee and Sultana) were much closed and could be considered as they came from one origin. Some cultivars were grouped in different similar pairs.

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Figures:

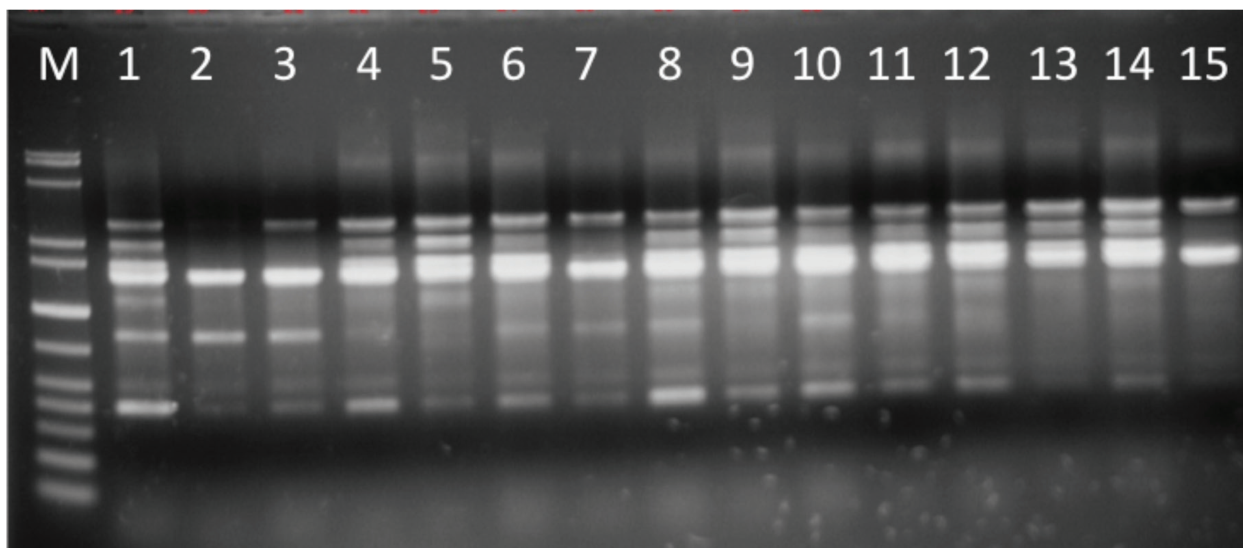

Figure 1. Example of ISSR polymorphism banding patterns in a subset of 15 Qatari date palm genotypes using primers # 10. M: 50 bp. Standard ladder marker; Lanes (1-15): Qatari Date palm genotypes described in Table 1.

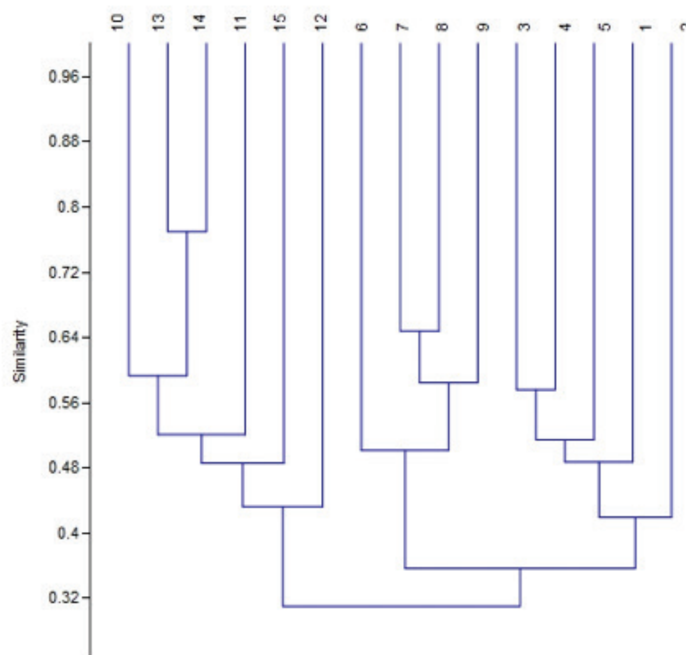

Figure 2. Dendrogram of 15 Qatari date-palm cultivars based on Jaccard genetic similarity coefficient using ISSR data. 1. ZAHIDI, 2. HATAMY, 3. HELALY, 4. KHALAS, 5. SUCCARY, 6. ANBARA, 7. ABU-MAIN, 8. SHESHY, 9. BARHEE, 10. SULTANA, 11. NABOOT-SAIF, 12. KHADRAWY, 13. KHUSH ZABAD, 14. KHANEZY and 15. THURI.

Tables

Table 1: Names of the studied fifteen Qatari Date Palm genotypes

No.	Name
1	ZAHIDI
2	HATAMY
3	HELALY
4	KHALAS
5	SUCCARY
6	ANBARA
7	ABU MAIN
8	SHESHY
9	BARHEE
10	SULTANA
11	NABOOT SAIF
12	KHADRAWY
13	KHUSH ZABAD
14	KHANEZY
15	THURI

Table 2: List of ISSR primers used in this study.

No.	Name	Sequence	Ann. Temp
1	814	(CT) 8TG	55^0 C
2	844A	(CT) 8AC	54^0 C
3	17898A	(CA)6AC	42^0 C
4	17898B	(CA)6GT	42^0 C
5	17899A	(CA)6AG	42^0 C
6	17899B	(CA)6GG	44^0 C
7	HB8	(GA)6GG	44^0 C
8	HB9	(GT)6GG	44^0 C
9	HB11	(GT)6CC	44^0 C
10	HB12	(CAC)3GC	42^0 C
11	844B	(CT)8GC	54^0 C
12	HB10	(GA)6CC	44^0 C
13	HB13	(GAG)3GC	38^0 C
14	HB14	(CTC)3GC	38^0 C
15	HB15	(GTG)3GC	38^0 C
16	TA-1	(AG)10C	62^0 C
17	TA-2	(CT)10G	62^0 C
18	TA-3	(AGG)6	62^0 C