ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 8 (1): 126-131, 2009
ISSN 1682-296X

Molecular Phylogeny Of Qatari Date Palm Genotypes Using Simple Sequence Repeats Markers

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Abstract: The objectives of the present study is to analyze the genetic diversity among 15 different cultivars of date palm at the experimental farm of Qatar University using Simple Sequence Repeat (SSR) markers and find out the genetic similarity and/or diversity among the well known Qatari date palm cultivars. DNAs were extracted from the young fresh leaves. Among 16 primer pairs tested for their ability to generate expected SSR banding patterns in Qatari date-palm genotypes, 10 primers successfully produced clear single bands in most of the studied genotypes. So, far, six SSR primers did not amplify clear bands in our genetic materials even using different PCR conditions. The amplified SSR band sizes ranged from 100-300 bp. A total of 40 alleles with an average of 4 alleles per locus were scored. Similarity coefficient matrix was computed to cluster the data and to draw precise relationships among the fifteen studied Qatari date palm genotypes.

Key words: Date palm, SSR markers, molecular phylogeny

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) (2n = 2x = 36) is dioecious, perennial, monocotyledon fruit trees that belong to the family of Arecaceae. Date is the major fruit crop of arid climate region in countries of Middle East and North Africa. It is the most important fruit tree 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).

Date palms are generally propagated by separating the offshoots produced by individual trees. This method maintains the genetic integrity of date palm cultivars. Offshoots are produced in limited numbers during a date palm's life span (Zaid and De Wet, 2002). Seeds are breeding material with long backcrossing cycles. The first flowering of the trees takes place at the age of about 5-7 years (Baaziz, 2000; Zaid and De Wet, 2002). Therefore, the biological characteristics of date palm trees render it very difficult to compensate for the rapid decline of trees due to natural disasters. Extensive effort has been made to propagate date palms through tissue culture (Zaid and De Wet, 2002).

The characterization of date palm used to be done based mainly on fruit characteristics (e.g., shape, weight, color, aspect of skin, consistency and texture) and the morphology of leaves, spines. These characters are known to be strongly affected by environmental conditions and have limited discriminatory power. Accordingly, this has led to some cultivars with similar morphological characters being given the same varietal name.

Recently developed techniques, based on DNA markers and Polymerase Chain Reaction (PCR), offer new tools for genetic analysis and the construction of linkage maps. DNA markers are used 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.

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

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(Al-Ruqaish *et al*., 2008), Morocco (Sedra *et al*., 1998), Suadi Arabia (Al-Khalifah and Askari, 2003), Tunisia (Trifi *et al*., 2000; Zehdi *et al*., 2004a, b) and Sudan (Elshibli and Korpelainen, 2007).

Microsatellites, or Simple Sequence Repeats (SSRs) are short stretches of repeated DNA, found in most genomes, which show exceptional variability in most species. This variability has made SSRs the genetic marker of choice due to their abundance, polymorphism and reliability compared to other types of DNA markers for the vast majority of applications, including fingerprinting, analysis of genetic structure and for investigating evolutionary links between species and populations. Microsatellites regions are abundant throughout the eukaryotic genome and highly polymorphic in length and are interspersed. However, it was only with the development of SSR markers that reliable, co-dominant and comparable molecular data on date palm populations could be generated (Billotte *et al*., 2004).

To our knowledge, no detailed research has been conducted to analyze phylogenetic relationships among date palm cultivars native to the state of Qatar using DNA markers. The objective of the present research was to analyze the genetic diversity among 15 different cultivars of date palm grown at the experimental farm of Qatar University using SSR markers. This study will try to answer whether there is a genetic similarity or diversity among the well known Qatari date palm cultivars based on DNA markers.

MATERIALS AND METHODS

Plant material: Fifteen Qatari Date palm cultivars derived from mature *in vitro* culture were used in this study (Table 1). These cultivars are now 10 years old and are grown in Qatar University Experimental Farm at the North part of Doha. They are considered to be the most common Date palm cultivar in Qatar. One tree representing each cultivar was chosen randomly and used for leaf sampling.

DNA extraction: Young leaf samples were cleaned carefully to remove the waxy layer. Two hundred to three hundred milligrams of leaf sample was cut in small pieces and grinded in liquid nitrogen in a cold Mortar and Pestle (kept at -20°C over night).

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 microliter of DNA from each sample were applied to 0.85% Agarose gel and electrophoreses was done at 100 V for 30 min. The gels were stained in Ethidium bromide and visualized under UV light.

PCR and Microsatellites (SSR) amplification: A set of 16 date-palm specific SSR primer pairs developed by Billote *et al*. (2004) (Table 2) was tested. PCR reactions were performed in a total reaction mixture of 25 μL containing: 20-30 ng of total genomic DNA (1 μL) as template, buffer (GeneAmp, Applied Biosystems), 0.2 mM of dNTP PCR mix (GeneAmp, Applied Biosystems), 0.625 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 sec at 95°C, 90 sec at 52-60°C and 90 sec 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 analysis: Microsatellite 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). The matrix was then computed to draw phylogenetic diagrams.

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 SSR primers used in this study developed for Date palm by Billotte *et al.* (2004)

			Primer sequence (5'-3')	
Primer No.	Primer name	Motif repeat	Forward	Reverse
1	mPdCIR010	(GA)22	ACCCCGGACGTGAGGTG	CGTCGATCTCCTCCTTTGTCTC
2	mPdCIR015	(GA)15	AGCTGGCTCCTCCCTTCTTA	GCTCGGTTGGACTTGTTCT
3	mPdCIR016	(GA)14	AGCGGGAAATGAAAAGGTAT	ATGAAAACGTGCCAAATGTC
4	mPdCIR025	(GA)22	GCACGAGAAGGCTTATAGT	CCCCTCATTAGGATTCTAC
5	mPdCIR032	(GA)19	CAAATCTTTGCCGTGAG	GGTGTGGAGTAATCATGTAGTAG
6	mPdCIR035	(GA)15	ACAAACGGCGATGGGATTAC	CCGCAGCTCACCTCTTCTAT
7	mPdCIR044	(GA)19	ATGCGGACTACACTATTCTAC	GGTGATTGACTTTCTTTGAG
8	mPdCIR048	(GA)32	CGAGACCTACCTTCAACAAA	CCACCAACCAAATCAAACAC
9	mPdCIR050	(GA)21	CTGCCATTTCTTCTGAC	CACCATGCACAAAAATG
10	mPdCIR057	(GA)20	AAGCAGCAGCCCTTCCGTAG	GTTCTCACTCGCCCAAAAATAC
11	mPdCIR063	(GA)17	CTTTTATGTGGTCTGAGAGA	TCTCTGATCTTGGGTTCTGT
12	mPdCIR070	(GA)17	CAAGACCCAAGGCTAAC	GGAGGTGGCTTTGTAGTAT
13	mPdCIR078	(GA)13	TGGATTTCCATTGTGAG	CCCGAAGAGACGCTATT
14	mPdCIR085	(GA)29	GAGAGAGGGTGGTGTTATT	TTCATCCAGAACCACAGTA
15	mPdCIR090	(GA)26	GCAGTCAGTCCCTCATA	TGCTTGTAGCCCTTCAG
16	mPdCIR093	(GA)16	CCATTTATCATTCCCTCTCTTG	CTTGGTAGCTGCGTTTCTTG

RESULTS AND DISCUSSION

Among the 16 primer pairs tested for their ability to generate expected SSR banding patterns in Qatari data-palm genotypes, 10 primers successfully produced clear single bands in most of the studied genotypes. So, far, six SSR primers did not amplify clear bands in our genetic materials even using different PCR conditions.

The amplified SSR band sizes ranged from 100-300 bp. A total of 40 alleles with a mean of 4 alleles per locus were scored. The number of alleles per locus varied from 3 (primer 3) to 6 (primer 5). All the 16 SSR primers were checked for amplification using four Date palm DNA genotypes as a first screening. Figure 1 shows an example for first screening using primers 9 and 10 and four different Qatari date palm genotypes. Subsequently, primers that showed clear bands, used to fingerprint the all 15 Qatari date palm genotypes as shown in Fig. 2 for primer 5 as an example.

Among the sixteen SSR tested primers, only ten were used to assess genetic relationships in the tested accessions. These primers which have revealed polymorphic products that were consistently and unambiguously scorable were identified as: mPdCIR016, mPdCIR025, mPdCIR032, mPdCIR035, mPdCIR050, mPdCIR057, mPdCIR078, mPdCIR085, mPdCIR090 and mPdCIR093.

Interestingly, twenty 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 represent 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

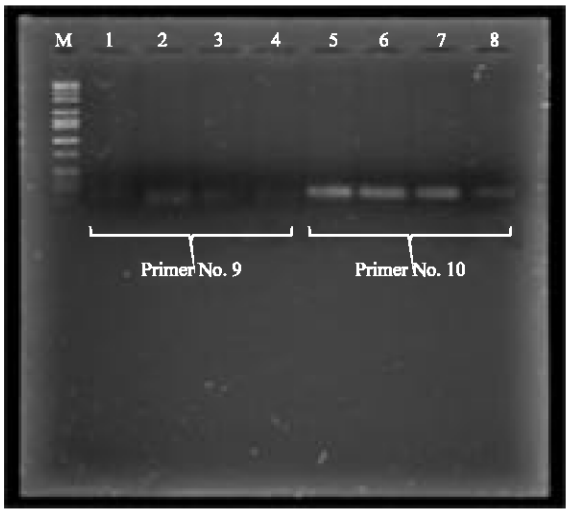

Fig. 1: First check for SSR primers No. 9 and No. 10 using four date palm DNA samples of Zahidi, Hatamy, Helaly and Khalas. M: 100 base Ladder marker

(Hatamy, Helaly, Sheshy, Khadrawy and Thuri) had two unique single bands from two different primers. However, one unique single bands were shown in Succary, Abu Main, Barhee, Naboot Saif and Khanezy.

Band pattern data was converted into a binary data in excel work sheet and was analyzed using SPSS program to calculate similarity coefficient values according to Jaccard (1908). A similarity matrix between Qatari date palm cultivars (Table 3) showed an average similarity coefficient range from 0.000-0.750. The cultivars studied here were highly divergent at the DNA level. The highest similarity coefficient value was observed between Barhee and Sultana cultivars (0.750) which seem to be the nearest two varieties and can be closely regrouped. The similarity coefficient value of 0.000 was obtained between Abu

Table 3: Similarity values matrix among the 15 Qatari date palm cultivars, based on SSR data calculated using Jaccard' s similarity coefficient

Qatari data palm cultivars	Zahidi	Hatamy	Helaly	Khalas	Succary	Anbara	Abu main	Sheshy	Barhee	Sultana	Naboot saif	Khadrawy	Khush zabad	Khanezy	Thuri
Zahidi	0.000														
Hatamy	0.308	0.000													
Helaly	0.273	0.250	0.000												
Khalas	0.143	0.417	0.167	0.000											
Succary	0.273	0.154	0.200	0.400	0.000										
Anbara	0.182	0.273	0.375	0.300	0.222	0.000									
Abu Main	0.077	0.000	0.091	0.077	0.000	0.100	0.000								
Sheshy	0.000	0.143	0.000	0.250	0.083	0.091	0.083	0.000							
Barhee	0.071	0.231	0.182	0.154	0.083	0.333	0.000	0.077	0.000						
Sultana	0.071	0.143	0.182	0.250	0.182	0.333	0.000	0.077	0.750	0.000					
Naboot Saif	0.231	0.214	0.167	0.231	0.167	0.300	0.077	0.154	0.364	0.364	0.000				
Khadrawy	0.077	0.250	0.200	0.273	0.091	0.571	0.091	0.182	0.444	0.444	0.273	0.000			
Khush Zabad	0.143	0.214	0.273	0.231	0.167	0.444	0.000	0.071	0.500	0.500	0.333	0.400	0.000		
Khanezy	0.067	0.133	0.077	0.143	0.077	0.182	0.000	0.154	0.364	0.364	0.333	0.273	0.333	0.000	
Thuri	0.154	0.231	0.182	0.250	0.182	0.333	0.000	0.077	0.273	0.273	0.250	0.182	0.364	0.500	0.000

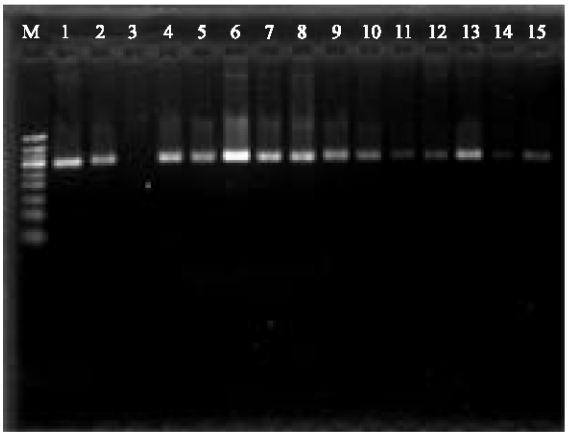

Fig. 2: Examples of SSR polymorphism banding patterns in a subset of 15 Qatari date palm genotypes using primers No. 5. M: 50 bp Standard ladder marker; Lanes (1-15): Qatari date palm genotypes described in Table 1

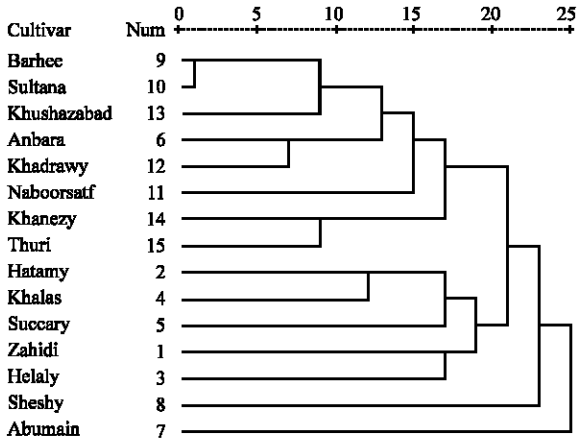

Fig. 3: Dendrogram of 15 Qatari date-palm cultivars based on Jaccard genetic similarity coefficient using SSR data

Main and each of Barhee, Sultana, Khush Zabad, Khanezy and Thuri cultivars, indicating that how far is the relationship between Abu Main cultivar and those cultivars. All the other cultivars displayed low levels of similarity but still were grouped with each others.

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 Fig. 3, illustrates the divergence between the studied Qatari date palm cultivars and suggests their tree branching.

Abu Main cultivar was in a separate far group compared to the rest of cultivars. Barhee and Sultana cultivars were very closed to each other and could be considered one cultivar with different name. The following cultivars: Anbara- khadrawy, Khanezy-Thuri, Hatamy-Khalas, Zahidi-Helaly may constitute paired clusters (Fig. 3).

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 SSRs system compare to other systems also reported in earlier 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; Ferreira *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 codominant 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, SSR markers have been used to asses the molecular characterization and the phylogenic relationships of Qatari date palm cultivars. Present results provide evidence of a genetic diversity among the studied Qatari date Palm 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.

ACKNOWLEDGMENTS

This research project was made possible by a grant from the Qatar National Research Fund and Qatar University Research Fund No. CAS07031. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Qatar National Research Fund.

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