2012 2nd International Conference on Environment and Industrial Innovation
IPCBEE vol.35 (2012) © (2012) IACSIT Press, Singapore

Inter-simple Sequence Repeat (ISSR) Analysis of Somaclonal Variation in Date Palm Plantlets Regenerated from Callus

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Abstract. Date palm (*Phoenix dactylifera*) 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 palms are generally propagated by separating the offshoots produced by individual trees. Somaclonal variation refers to the phenotypic and genotypic variation observed in plants regenerated through cell culture. On the other hand, some somaclonal variations have desirable agronomic and commercial advantages and sometimes lead to the release of new cultivars. Inter-simple sequence repeat (ISSR) was used in this study to detect genetic variation based on DNA markers between the regenerated plantlet and its corresponding callus. Somaclonal variation between callus and more than one regenerated plant was observed in three cultivars: Kubkub, Merziban and Dharlag, respectively.

Keywords: Somaclonal variation, Inter-simple sequence repeat (ISSR), date palm

1. Introduction

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 efforts were made to propagate date palms through tissue culture (Tisserat 1979; Zaid and de Wet 2002).

However, plant tissue culture method leads to somaclonal variation. Typically, somaclonal variation refers to the phenotypic and genotypic variation observed in plants regenerated through cell culture. These variations are heritable, i.e., transmitted through meiosis and are usually irreversible. Generally, somaclonal variation could be a problem and an opportunity as well. In case of micropropagation of valuable elite cultivars, such variation can result in problematic off-types that weaken the commercial value of propagated plants. On the other hand, some somaclonal variations show desirable agronomic and commercial advantages and sometimes lead to release of new cultivars.

Though, whether somaclonal may imply an advantage as a source of variability for new lines, or a disadvantage for the propagation of an elite cultivar, it is important to achieve a rapid and easy method to assess the genetic stability of the propagated plants at the earliest stage of plant growth to save at least 7-10 years and avoid the later economical loss in mass regeneration and propagation.

PCR based markers are widely used to detect genetic variations due to the simplicity of DNA amplification. More recently, the amplification of microsatellites or simple sequence repeats are preferred

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due to the fact that they are co-dominant markers, offering the possibility to display all the alleles at a single locus. Microsatellites are tandem repeats of 1-6 nucleotides and are present in all eukaryotic genomes. The method is very accurate and reproducible, being able to detect any variation within repeats number, determined by addition or deletion of repeat units, or by point mutations.

The purpose of this study was to detect genetic variation based on DNA markers between the regenerated plantlet and its corresponding callus using Inter-simple sequence repeat (ISSR) markers..

2. Materials and Methods

2.1. Plant materials and DNA extraction

Fresh immature callus and regeneration plant derived from five Qatari date palm cultivars (Barhe, Khalas, Kubkub, Merziban, Dharlag) were obtained from the tissue culture laboratory of Biotechnology Center, Ministry of the Environment, Qatar. DNAs was extracted using DNasy extraction kit (Qiagen, Inc., Valencia,CA).

2.2. Inter simple sequence repeat (ISSR) amplification

A total of 16 ISSR markers were used to amplify DNA using genomic DNA of the Date palm samples (Table 1). AmpliTaq Gold PCR Master Mix was used to prepare PCR reactions. Total volume of 20 µl contained approximately 20 -30 ng of DNA as a template (2 µl), 20 ng/ µl (1.0 µl) of primer and 10 µl of AmpliTaq Gold PCR Master Mix including 1 X PCR buffer, 1.5 U Taq DNA polymerase, 0.1 – 1.0 nMdNTP mix, in addition to 7 µl double distilled water to to reach the total volume of 20 µl. PCR amplifications were performed using Hot Start program as the following conditions: A hot start at 95°C for 5 min, followed by 35 cycles of three steps: Denaturation at 94°C for 30 sec, annealing depending on primer for 30 sec, extension at 72°C for 1 min, and a final extension for 7 min at 72°C. The PCR amplified products including all of samples were separated by electrophoresis on 2% agaros gels.

Table 1. Name and sequences of the primers used in *ISSR* analysis.

No.	Primer code	Tm	Nucleotide sequences (5' – 3')
1	ISSBT 2	60.0 ^{0}C	(ACC)6
2	ISSBT 5	52.8 ^{0}C	(AGA GAG)3 AGT
3	ISSBT 6	47.0 ^{0}C	(AGA GAG)2GT
4	ISSBT 7	57.5 ^{0}C	(AGG)6
5	ISSBT 9	43.6 ^{0}C	(CAC ACA)2 AC
6	ISSBT 10	46.2 ^{0}C	(CAC ACA)2 GG
7	ISSBT 12	50.3 ^{0}C	(CAC ACA)2 CAC AA
8	ISSBT 14	50.9 ^{0}C	(CTC TCT)3 CTA
9	ISSBT 15	52.4 ^{0}C	(CTC TCT)3 CTG
10	ISSBT 18	50.5 ^{0}C	(CTC TCT)2 CTC TGC
11	ISSBT 20	41.8 ^{0}C	(CTC)3 GC
12	ISSBT 21	42.3 ^{0}C	(GAG AGA)2 CC
13	ISSBT 22	41.9 ^{0}C	(GAG AGA)2 GG
14	ISSBT 23	46.8 ^{0}C	(GAG AGA)2 GAG AC
15	ISSBT 25	41.0 ^{0}C	(GAG)3 GC
16	ISSBT 30	53.6 ^{0}C	(TCT CTC)3 TCC

3. Results

Calli of five common date palm cultivars and eight regenerated plants were used to track the somoclonal variations during micropropagation process. A total number of about 780 clear DNA bands were obtained through the ISSR analysis using 16 single primers with an average of 48 bands per primer (Fig. 1). Twelve primers could amplify polymorphic bands between callai and their respected regenerated plants in one or more cultivars. On the other hand, four (ISSRBT 7, 18, 21 and 25) ISSR primers did not show any

polymorphic bands at all. Polymorphic bands were expressed in three ways; first by a single band that was found in regenerated plant but disappeared in callus and second a single band that was found in callus but disappeared in regenerated plant (Table 2). As an example for the first case, Khanezy cultivar showed polymorphic bands using three primers ISSRBT 9, 20 and 30 where a single band was found in regenerated plants but disappeared in callus. On the other hand, among five primers showed polymorphic bands in Khalas cultivar, one of them only showed a single band that was found in callus but disappeared in regenerated plant (Table 2). Somaclonal ovations were detected in regenerated plants in both Merziban and Dharlag cultivars using ISSRBT2, ISSRBT5, ISSRBT12, ISSRBT23, ISSRBT6, ISSRBT9, ISSRBT10, ISSRBT15 and ISSRBT22 (Table 2).

Table 2. Screening of polymorphic bands detected by ISSR markers

ISSR Primer	Khanezy		Khalas		Kubkub		Merziban			Dharlag			
	C	R	C	R	C	R	C	R	R	C	R	R	R
ISSRBT2	+	+	+	+	+	+	+	+	-	+	+	+	+
	+	+	+	+	+	+	+	+	-	+	+	+	+
ISSRBT5	+	+	-	+	-	+	+	+	+	+	+	+	+
ISSRBT6	+	+	+	+	+	+	+	+	+	-	+	+	+
ISSRBT9	-	+	+	+	+	+	+	+	+	-	+	+	+
ISSRBT10	+	+	+	+	+	-	+	+	+	+	-	-	-
ISSRBT12	+	+	+	+	+	+	+	-	+	+	+	+	+
ISSRBT14	+	+	-	+	+	-	+	+	+	+	+	+	+
ISSRBT15	+	+	+	+	+	-	+	+	+	-	+	+	+
	-	-	-	-	-	-	-	-	-	-	+	+	+
ISSRBT20	-	+	-	+	+	+	-	+	+	+	-	-	-
ISSRBT22	-	-	+	+	-	-	-	+	+	-	+	+	+
ISSRBT23	-	-	-	+	+	+	+	-	+	+	+	+	+
	-	-	+	-	-	-	-	-	-	-	-	-	-
ISSRBT30	-	+	-	+	-	+	+	+	+	+	+	+	+

C, R; callus and regenerated plants. +, -; present and absent bands respectively.

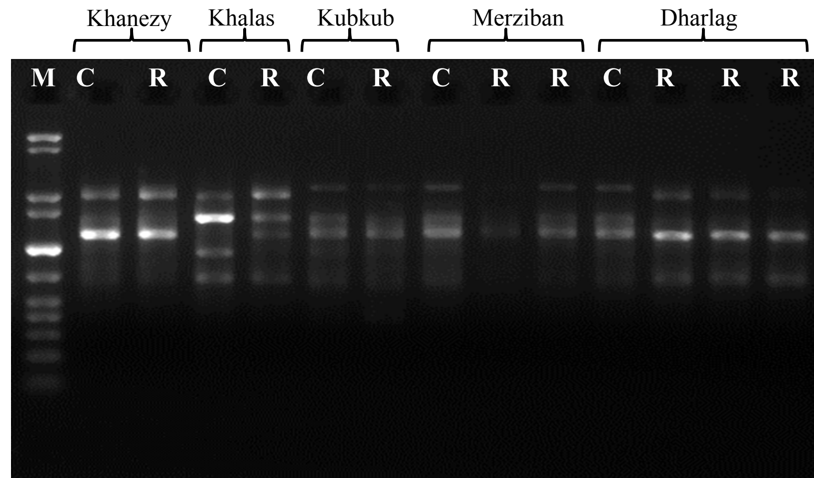

Fig. 1. ISSR pattern obtained with the primer ISSRBT23 in five different date palm cultivars during invitro culture, M, C and R denote for Ladder marker, callus and regenerated plantlet, respectively. The arrow shows the polymorphic bands.

4. Discussion

Over the last years, the employment of molecular markers (e.g. ISSR) contributed to assess the genetic stability of many plant species regenerated from tissue culture process. The frequency of changes detected varies between different species and culture conditions, but it is accepted that somaclonal variation is a common phenomena associated with tissue culture. The selection of explant, age of the culture, genotype, culture conditions and method of plant regeneration are very important aspects for genetic stability of the regenerated plants (Rout et al., 2006).

In this study, genetic variation based on DNA markers between the regenerated plantlet and its corresponding callus was detected in all studied Date palm cultivars. The screening of genetic stability among callus and more than one regenerated plant was possible in two cultivars Merziban and Dharlag.

In a similar previous work on date palm somaclonal variation, Eshraghi et.al. (2005) used RAPD markers to analyze genetic stability of the somatic embryogenesis - derived regenerates (R 1-6) and mother plants in Iranian date palm. The results showed the genetic similarity between mother plant and the callus – derived plantlets was ranged between 94 % (for R1, R2) and 83 %(for R5). Moreover, Cohen et al. (2010) used amplified fragment length polymorphism (AFLP) to characterize the three common date palm off-type phenotypes, where in the variegated trees multiple mutations seem to occur. They found only relatively few mutations were detected among the “low level fruit setting” and dwarf trees. No single specific mutation was found to be associated with these phenotypes. Differences in DNA-methylation patterns were found among the off-type trees. Reduction in the overall DNA methylation level seems to be associated with the “low fruit setting” phenotype.

5. Conclusion

ISSR marker provide a useful technique in the assessment of genetic stability in micro-propagated date palm as well as in their genetic characterization, allowing the differentiation of cultivars with a relatively low number of primers, even those corresponding to a same family.

6. Acknowledgements

The authors appreciate the great help of Ms Amena, Ms Eman, Ms May and Ms Hayat at Biotechnology Center, Ministry of Environment, Qatar. *“This publication was made possible by a grant from the Qatar National Research Fund under its Undergraduate Research Experience Program award number UREP08-048 - 4 - 001. 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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