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GENETIC FIDELITY TESTING IN REGENERATED PLANTLETS OF CRYOPRESERVED AND NON- CRYOPRESERVED CULTIVARS OF *PHOENIX DACTYLIFERA* L.

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Abstract

The genetic fidelity of date palm plantlets (*Phoenix dactylifera* L. 'Sagai and Khalas') derived from somatic embryogenesis was tested before and after cryopreservation with (+LN) and without (-LN) liquid nitrogen. Fifteen randomly selected *In vitro* subcultures were assessed for genetic fidelity using twenty inter simple sequence repeat (ISSR) primers. The maximum similarity recorded between mother plants and plantlets derived from embryogenic calli of date palm cultivar Sagai and Khalas after cryopreservation (+LN) was 100%, whereas the minimum similarity was found to be 97.8 and 97.0%, respectively. The average percent similarity was found to be 98.9 and 98.5 for both cultivars, respectively. The maximum similarity recorded between mother plant and plantlets derived from embryogenic calli of Sagai or Khalas without cryopreservation (-LN) was 100%. However, the minimum similarity was 98.9 and 98.0% with an average of 99.4 and 99% for both cultivars, respectively. Our results indicate that plantlets of these cultivars derived from embryogenic calli after cryopreservation (+LN) and non-cryopreservation showed similarity to their mother plants at the genetic level.

Key words: Genetic similarity, Cultivar, Cryopreservation, Molecular marker.

Introduction

Date palm (*Phoenix dactylifera* L.), family Arecaceae, chromosome number (2n=2X;=36) is a monocotyledonous, perennial and dioecious fruit tree. The tolerance of date palm to environmental stresses made it for ideal for cultivation under severe climatic conditions in semi-arid and arid regions of the world, where no other crops give sufficient economic returns (Kumar *et al*., 2010). Propagation of date palm by seeds produces heterogeneous progeny with poor field performance and lower fruit quality (Naik & Al-Khayri, 2016). Propagation of date palm by offshoots as a safe method to preserve the genetic integrity of the cultivars; however, trees produces limited numbers of offshoots during their life (Taha *et al*., 2003). Therefore, the propagation of date palm by *In vitro* techniques to facilitates large-scale production. This technique has been used by several researchers using various explants sources and different regeneration pathways (El Modafar, 2010; Fki *et al*., 2011; Shareef *et al*., 2016).

Cryopreservation plays an important role in international plant conservation programs and preservation of plant genetic resources in the world (Bajaj, 1995; Benson, 1999). Cryopreservation at (-196ºC) is considered to be the preferred method for conservation of plant genetic resources for the long-term. It also provides the options of backups for long-term preservation of plant species that may be at risk (Kaviani *et al*., 2012). Subculture is not required during the cryopreserved period. Therefore genetic material can remain in storage for an indefinite period (Kaczmarczyk *et al*., 2008). Cryopreservation canbe achieved by using different procedures such as encapsulation-dehydration, pre-culture and dehydration, encapsulation-vitrification, vitrification and droplets (Engelmann, 2004). Conservation of date palm germplasm using traditional methods is difficult because they contain a high amount of pests and pathogens with potential risk for spread. Therefore, cryopreservation is available technique that is most suited for the conservation of date palm germplasm for the long-term (Bekheet, 2011).

Tissue culture and cryopreservation techniques produce and maintain true-to-type plants. Metabolic activities are reduced to zero at temperatures of liquid nitrogen (LN) and after rewarming of cryopreserved germplasm, the plants will be true-to-type (Panis *et al*., 2001). Tissues cryopreserved in LN should remain genetically identical to non-treated tissues can produce normal plants (Dumet *et al*., 2000).

Many researchers did not find any somaclonal variations in regenerated date palms produced by somatic embryogenesis including cultivars; Albrahi (Smith & Aynsley, 1995; Al-Wasel, 1999), Deglet Nour (Othmani*et al*., 2010), Barhee, Khalasah, Zardai, Shishi, Zart, and Muzati (Aslam *et al*., 2015). However, there are a large number of reports showing the absence of any changes in morphology, cytology, biochemistry, or molecular markers in plants stored in LN (Ryynänen &Aronen, 2005; Harding, 2004). The genetic fidelity of regenerated plantlets of four cultivars of date palm including Ajwa, Khodary, Ruthana and Sukary were assessed using the SCoT marker and found minor genetic variations (Al-Qurainy *et al*., 2017). However, plant growth regulators, salts and environmental conditions sometimes produce genetic variations along with somaclonal variations in oat plants and date palm (Skirvin *et al*., 1993; McCoy *et al*., 1982; Moghaieb *et al*., 2011). Cryopreservation sometimes produce genetic variations as observed in various plant species (Ashmore & Engelmann, 1997; Heringer *et al*., 2013; Müller *et al*., 2007).

Using isozymes analysis for the detection of genetic changes could be considered a suitable method but it has few applications because the method screens limited regions of DNA (only coding regions) (Kumar *et al*., 2010). Molecular markers (DNA-based) are used for the assessment of genetic fidelity as they are more reliable than other markers. These markers are also useful for the identification and analysis of

different plant species for their phylogenetic relationship. Different molecular markers have been used for testing genetic fidelity including inter simple sequence repeat (ISSR), simple sequence repeat (SSR) (Kumar *et al*., 2010), random amplified polymorphic DNA (RAPD) (Aslam *et al*., 2015) and amplified fragment length polymorphism (AFLP) (Othmani *et al*. (2010). The co-dominant nature of ISSR marker has many advantages as compared to other molecular markers as their analysis requires lower cost, less time and a smaller quantity of DNA (Powel *et al*., 1996). Therefore, the present study was focused to monitor the genetic fidelity of micropropagated and regenerated plants after cryopreservation of date palm using the ISSR markers.

Materials and Methods

The offshoots were collected from Al-Rajhi (Al-Qassim) and the Dirab Agricultural Researchand Experimental Station (KSU, Riyadh) in Saudi Arabia. Young offshoots (2–3 years old) of cultivars Sagai and Khalas (*Phoenix dactylifera* L.) were chosen and separated from healthy mother plant. The tap water was used to wash the shoot tips followed by doubled distilled water. The shoot tips were put immediately immersed into a chilled antioxidant solution (containing 150 mg/L of ascorbic acid and 100 mg/L citric acid) for 24 hours at 4°C in a fridge to reduce browning. Keeping in antioxidant solution, the shoot tips were washed with distilled water three times. Then shoot tips were put in a plastic magenta box containing ethanol (70%) for 1 min. Then shoot tips were surface sterilized by sodium hypochlorite solution at a concentration of 1.6% (30% v/v Clorox, commercial bleach), supplemented with two drops of Tween 20 per 100 mL of sterilization solution for 20 min. Then shoot tips were washed with sterile distilled water three times for 15 min. The explants were dissected and then cultured on modified Murashige and Skoog (MS) media (Murashige and Skoog, 1962) containing (3 mg 2iP/l + 30 mg NAA/l) for cultivars Sagai, and for Khalas (3 mg 2iP/l + 10 mg 2,4-D/l) for proper growth. The MS media was supplemented with sucrose 30 g/l, 120mg/l Myo-inositol, 2 mg/l Glycine, 0.5 mg/l Nicotinic acid, 0.5 mg/l Pyridoxine HCI, 0.1 mg/l Thiamine HCL, sodium dihydrogen phosphate (NaH_2PO_4.H2O) 170 mg/l. Glutamine 200 mg/l, Adenine sulfate 40 mg/l, and plant growth regulators were added according to the propagation stage. The pH was adjusted 5.6 using 0.1 N of NaOH or 0.1N of HCl. Agar was added to MS media at 6-7 g/l for solidification. The modified MS medium was autoclaved for 30 min at 121°C and 1x105 Pa (1.1 kg cm-2) and used at all propagation stages with some modification.

A micropropagation protocol for a large-scale commercial production of cultivar Sagai and Khalas through somatic embryogenesis was employed (unpublished). Calli formed after 1.5-3 months of culture and shifted to a fresh modified MS medium supplemented with 0.1 mg/l 2,4-D for Sagai and 0.1 mg/l NAA for Khalas for embryogenic callus formation (Fig. 1). For somatic embryogenesis, the embryogenic calli were shifted to a fresh modified MS medium free of plant growth regulators supplemented with 30 g/l sucrose for 6 weeks. The somatic embryos were shifted to a fresh modified MS medium supplemented with 0.7 mg IBA/l for germination and plantlets formation. The cultures were incubated at a temperature of 27±2°C, and a photoperiod of 16 h light/ 8h dark under cool-white fluorescent lamps at a photosynthetic photon flux density (PPFD) of 50-60 µmol.m-². s-¹.

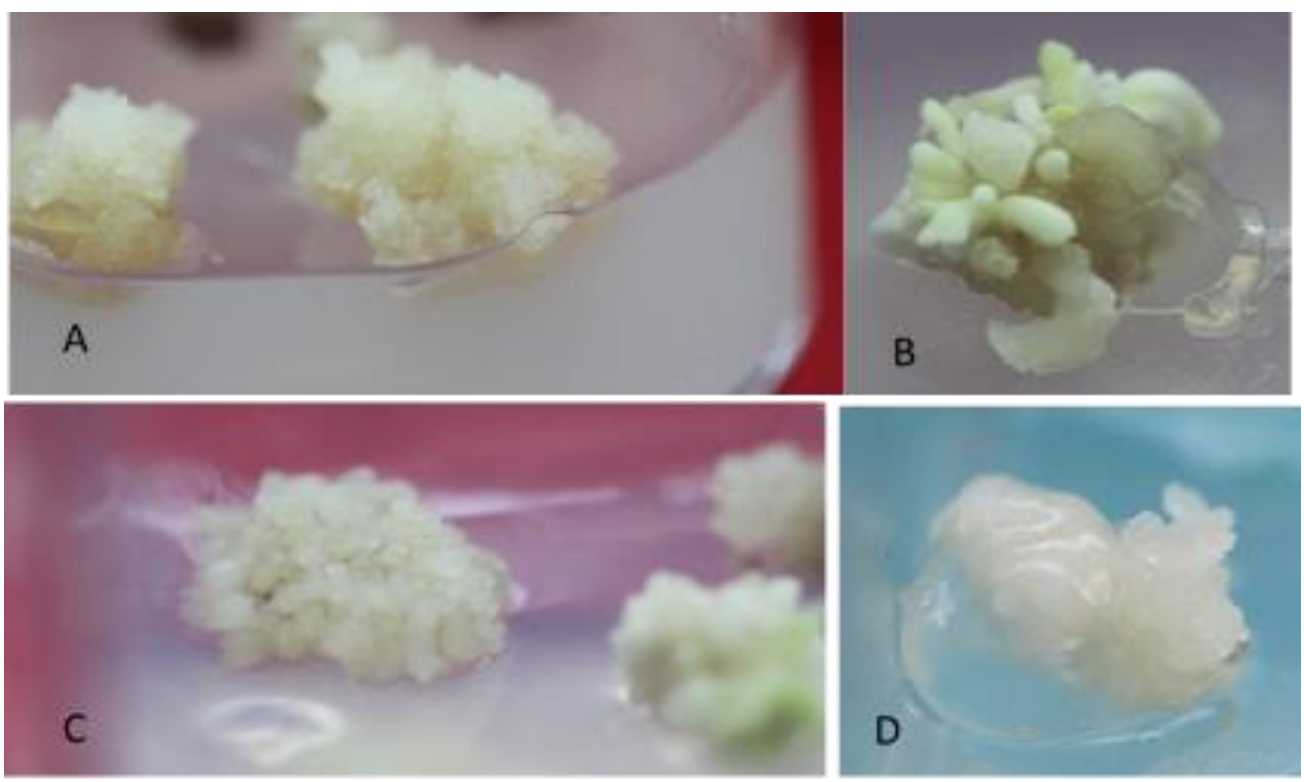

Fig. 1. (A) Embryogenic calli of cultivar Sagai (B)Regeneration of cryopreserved (+LN) embryogenic calli of cultivar Sagai on recovery MS medium using Encapsulation-dehydrationtechnique(C)Embryogenic calli of cultivarKhalas (D)Regeneration of cryopreserved (+LN) embryogenic calli of cultivar Khalas on recovery MS medium using Encapsulation-dehydrationtechnique.

Cryopreservation protocol for date palm: The encapsulation-dehydration cryopreservation protocol was used for embryogenic calli of two cultivars cultured on MS medium. Embryonic calli was grown (two months-old culture), without growth regulators in media containing 0.5 M sucrose (sucrose pre-culture) for 2 days. Further, embryogenic calli were cut into small pieces and suspended in liquid MS medium free of plant growth regulators and calcium chloride. This liquid MS medium was supplied with 0.1 M sucrose and 3% (w/v) sodium alginate. The embryogenic callus clumps were picked separately with some alginate solution using 10 ml sterile pipette and then gently dropped into a liquid MS medium supplemented with 100 mM calcium chloride and 0.1 M sucrose for 15 min for the synthesis of beads. The synthesized beads were transferred to liquid MS media free of growth regulators and supplemented with various concentrations of sucrose (0.1, 0.3, 0.5, 0.75 or 1.0 M), then incubated in rotary shaker providing gentle shaking (100 rpm/min) for 2 days.Thereafter, the beads (synthetic seeds) were dehydrated with air current in a laminar airflow hood for 0, 2, 4, 6 or 8 h on sterilized filter paper at room temperature. The beads were placed in 2 ml sterile and labeled cryogenic vials (each containing fivebeads) and were directly immersed into liquid nitrogen (LN). Then, beads were stored in LN for 48 h. The thawed beads were inoculated onto a solid MS recovery medium containing 0.1 M sucrose and then kept in normal growth conditions. After six weeks, the viability of the calli was evaluated. The regrowth of embryogenic calli in both Sagai and Khalas were assessed until plantlets were obtained.

Genomic DNA extraction and genetic fidelity testing using ISSR marker: The modified hexadecyl trimethyl ammonium bromide (CTAB) method (Khan *et al*., 2007) was employed to get high quality and quantity genomic DNA. Small pieces of date palm leaf tissues (200 mg) were groundin liquid nitrogen with a pestle and mortar. The fine powder was transferred into a 2 ml microtube (Eppendorf), 800 µl of extraction buffer and 10 µl of RNase (10 mg/ml) were added per tube and the tubes were mixed for 10 minutes. The polyvinylpyrrolidone (3 %) and β-mercaptoethanol (20µl) were added to the reaction mixture and mixed by inversion. The mixture was incubated in water bath at 65ºC for 20 min, with inversion every 5 min. After cooling of mixture at room temperature, the equal volume of chloroform and isoamyl alcohol 24:1 was added and mixed for 20 min. The tube was centrifuged for 10 min at 10,000 rpm at room temperature. An equal volume of ice cooled isopropanol was added in the supernatant from the above step. The mixture was kept for 30 min at -20 ºC. Further, DNA pellet was obtained at 4ºC after centrifugation at 10,000 rpm for 10 min. The pellet was washed with cold 70% alcohol and dried at room temperature. Finally, the pellet was dissolved in 200µl TE buffer (Qiagen TE buffer). DNA concentration and purity were determined by Nanodrop spectrophotometer (Nanodrop 8000, Thermoscientific, USA). The purity and quality of the DNA were also checked using 1% agarose gel (1X TBE buffer) stained with ethidium bromide using gel electrophoresis.

The chopped shoot tips were used in tissue culture to produce embryogenic cultures and regenerate plantlets. Leaves samples were kept at -80ºC until use for the genetic fidelity tests with the mother plant. Regenerated plantlets after cryopreservation (using the encapsulation-dehydration method) and regenerated plantlets derived from somatic embryos were taken randomly for the study of genetic fidelity using the ISSR markers.

Forty ISSR primers (Macrogen) were employed for genetic fidelity assessment. Out of 40 primers, 14 primers gave reproducible amplification and were used for further study of genetic fidelity (Table 1). The reaction was performed in 20 µl volume using a PCR master mixture(Solis Biodyne Company- Estonia). 2 µl of template DNA (25 ng/µl), 2 µl of ISSR primer (10 ng/µl), 12 µl of deionized water were added to the above tube and briefly spun for 10 s.

The PCR program was set on a Veriti 96-well Thermal Cycler (Applied Biosystem, Singapore). Initial denaturation performed at 94°C for 5 min, followed by 40 cycles at 94°C for 1 min (denaturation), annealing at 42°C for 1 min, extention at 72°C for 1 min and final extension for 5 min at 72°C. After electrophoresis, the gel picture was taken using Ingenius Syngene Bio-imaging gel documentation system (SynGene).

Table 1. List of ISSR primers used in the study.

Primers	Primer sequence (5'-3')	Annealing temperature	Primers	Primer sequence (5'-3')	Annealing temperature
IS_A10	BDB TCC TCC TCC TCC TCC TCC	64	IS_A62	AGA GAG AGA GAG AGA GCT C	57.3
IS_A23	GTG TGT GTG TGT GTG TYC	53.9	IS_A63	AGA GAG AGA GAG AGA GCT A	55.2
IS_A34	AGA AGA AGA AGA AGA AGA AGA	53.5	IS_A64	GAG AGA GAG AGA GAG ACTT	55.2
IS_A53	TGT TGT TGT TGT TGT TGC	53.9	IS_A65	ACA CAC ACA CAC ACA CCTT	55.2
IS_A58	AGT CAG TCA GTC AGT C	48.2	IS_A69	CTC TCT CTC TCT CTC TRG	53.9
IS_A59	ACT CAC TCA CTC ACT C	48.2	IS_A75	CAC ACA CAC ACA CAC ART	51.6
IS_A60	GGG TGG GGT GTG	57.1	IS_A87	CAG CAC ACA CAC ACA CAC	56.1

Table 2. ISSR primers amplified in regenerated and cryopreserved plantlets of the date palm cultivar Sagai.

Primers	Cultivar Sagai						
	Without cryopreservation			After cryopreservation			
	Total no. of scored bands	Polymorphic bands	% Polymorphic bands	Total no. of bands scored	Polymorphic bands	% Polymorphic bands	Size range
IS_A10	4	0	0	4	1	25	200-600
IS_A23	8	0	0	8	0	0	250-1000
IS_A34	11	0	0	11	0	0	150-1000
IS_A53	2	0	0	2	0	0	450-600
IS_A58	8	0	0	8	1	12.5	250-1200
IS_A59	7	0	0	7	0	0	300-900
IS_A60	9	0	0	9	0	0	200-800
IS_A62	7	0	0	8	1	12.5	200-1100
IS_A63	6	0	0	6	0	0	250-600
IS_A64	5	1	20	5	1	20	300-800
IS_A69	8	0	0	8	0	0	200-800
IS_A75	7	0	0	7	0	0	200-1500
IS_A87	10	0	0	10	0	0	250-800
Total	92	1	1.08	93	4	4.30	150-1500

Statistical analysis: Only clear, unambiguous and reproducible bands were scored on the gels as present (1) or absent (0) for each sample. Data were alalysed using NTSys PC version 2.02. Similarity matrix values were calculated (Nei & Li, 1979), and cluster analysis was performed using an unweighted pair group method with an arithmetic mean (UPGMA).

Results

Genetic fidelity of cryopreserved and regenerated plantlets derived from embryogenic calli of date palm cultivar Sagai: Forty primers were tested for evaluation of the genetic fidelity of Sagai plantlets derived from embryonic calli and cryopreserved with LN. Only 13 primes produced scorable and clear amplified products in PCR amplification (Table 2; Fig. 2). Out of the 13 primers, 9 primers gave monomorphic bands in regenerated plantlets from cryopreserved calli, while the remaining primers generated polymorphic bands. The number of bands for each primer varied from 2 (IS_A53) to 11 (IS_A34) and ranged in size from 150 bp to 1500 bp (Fig. 2). All amplified primers produced 93 bands with an average of 7.15 bands per primer (Table 2). Out of the 93 scorable bands, 89 bands were monomorphic (95.70%). However, only four bands were found to be polymorphic.

Out of the 13 primers, 11 primers gave monomorphic bands in plantlets regenerated from calli (without cryopreservation), while the rest generated polymorphic bands (Table 2). The number of bands for each primer varied from 2 (IS_A53) to 11 (IS_A34) and ranged from 150 bp to 1500 bp (Fig. 2). All primers amplified a total of 92 bands with an average of 7.07 bands per primer (Table 2). Out of the 92 scorable bands, 91 were monomorphic (98.92%), and only one primer was polymorphic. The maximum similarity coefficient recorded between plantlets derived from embryogenic calli of Sagai after cryopreservation (+LN) was 1 while, the minimum similarity coefficient was 0.978. Average value of similarity coefficient was 0.989. However, maximum similarity coefficient recorded between plantlets derived from embryogenic calli Sagai without cryopreservation (-LN) was 1 while, the minimum similarity coefficient was 0.989. The average similarity coefficient was 0.994.

Thus, the obtained results indicate that derived plantlets from embryogenic calli before cryopreservation and after cryopreservation (+LN) of Sagai showed similarity to the mother plants at the genetic level.

Genetic fidelity of cryopreserved and regenerated plantlets derived from embryogenic calli of date palm cultivar Khalas: Genetic fidelity of plantlets derived from embryogenic calli of date palm cultivar Khalas after cryopreservation (+LN) and without cryopreservation was assessed using ISSR markers. Out of 40 primers, 13 primers amplified scorable and clear bands (Table 3). Out of 13 primers, 9 primers amplified monomorphic bands, while the remaining primers generated polymorphic bands from plantlets derived from embryogenic calli Khalas after cryopreservation (+LN). The number of bands for each primer varied from 3 (IS_A53) to 12 (IS_A65) and ranged in size from 150 bp to 1800 bp (Fig. 3). All amplified primers produced a total 105 bands with an average of 8.07 bands per primer (Table 3). Out of the 105 scorable bands, 100 bands were monomorphic (95.24%), and only 5 bands were polymorphic.

Out of the 13 amplified primers, 12 primers produced monomorphic patterns, while only 1 primer amplified polymorphic bands from the plantlets derived from embryogenic calli Khalas without cryopreservation. The number of bands for each primer varied from 3 (IS_A53) to 12 (IS_A65) and ranged in size from 150 bp to 1800 bp (Fig. 3). All amplified primers produced a total of 103 bands with an average of 7.92 bands per primer (Table 3; Fig. 3). Out of the 103 scorable bands, 102 bands were monomorphic (99.03%), while only 1 band was polymorphic.

Table 3. ISSR marker profile in regenerated plantlets of cryopreserved and non-cryopreserved embryogenic calli of the date palm cultivar Khalas.

Primers	Without cryopreservation			After cryopreservation			
	Total no. of scored bands	Polymorphic bands	% Polymorphic bands	Total no. of scored bands	Polymorphic bands	% Polymorphic bands	Size range
IS_A23	8	0	0	8	0	0	150-1000
IS_A34	11	0	0	11	0	0	150-1000
IS_A53	3	0	0	3	0	0	450-650
IS_A58	8	0	0	8	0	0	250-1200
IS_A59	7	0	0	7	0	0	300-900
IS_A60	9	0	0	9	0	0	150-600
IS_A62	8	1	12.5	8	1	12.5	15-1100
IS_A63	8	0	0	8	0	0	150-600
IS_A64	5	0	0	5	0	0	300-800
IS_A65	12	0	0	12	1	8.33	350-1800
IS_A69	5	0	0	7	2	28.57	300-800
IS_A75	8	0	0	9	1	11.11	200-1000
IS_A87	10	0	0	10	0	0	250-800
Total	103	1	0.97	105	5	4.76	150-1800

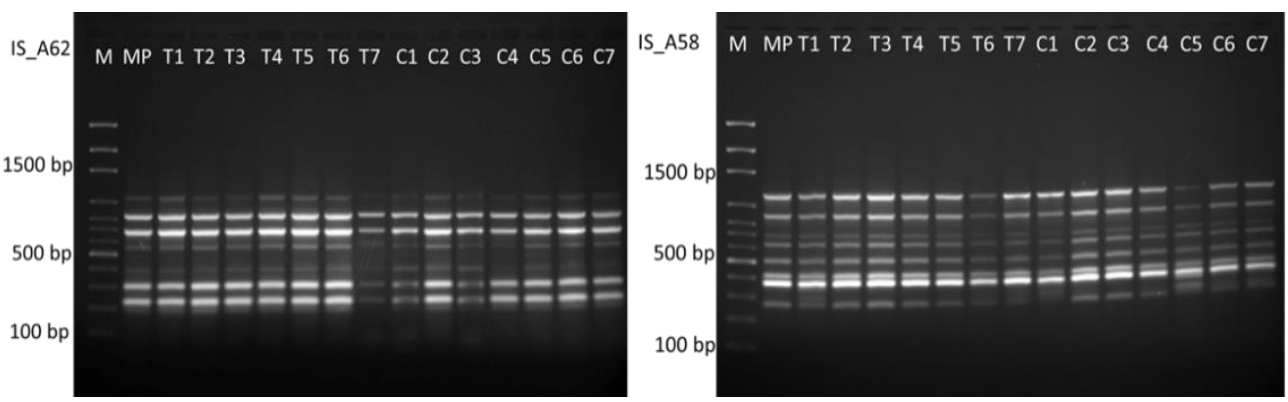

Fig. 2. ISSR marker profile with primers (_A59 and IS_A62) for *Phoenix dactylifera*(Sagai); Lane M-100 bp DNA ladder, Lane MP: Mother plant, Lane T1-T7: Without cryopreservation and lane C1-C7: After cryopreservation.

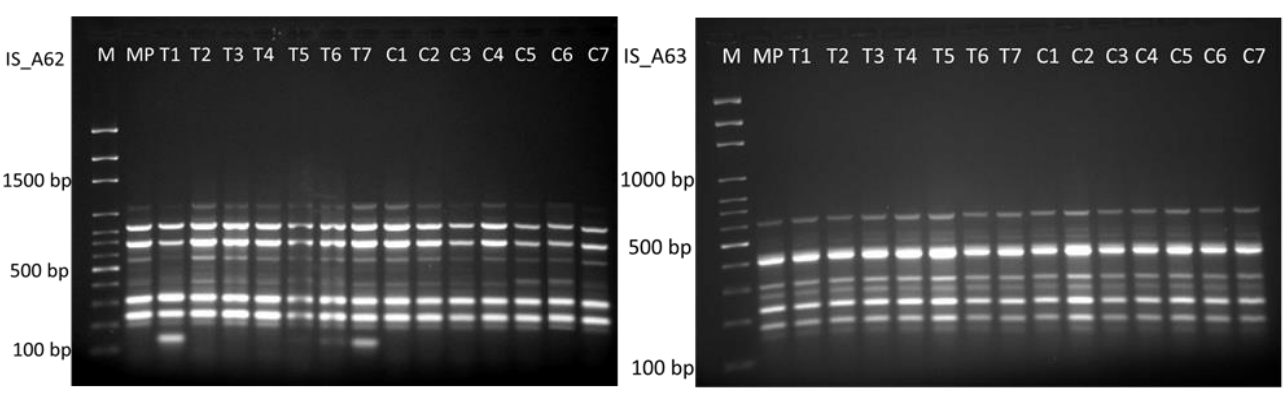

Fig. 3. ISSR marker profiles with primers (A62 and IS_A63) for *Phoenix dactylifera* (Khalas); Lane M-100 bp DNA ladder, Lane MP: Mother plant, Lane T1-T7: Without cryopreservation and lane C1-C7: After cryopreservation.

A similarity coefficient was calculated from the ISSR marker data analysis. Cluster analysis was performed using the UPGMA clustering method. The maximum similarity coefficient recorded between plantlets derived from embryogenic calli Khalas after cryopreservation (+LN) was 1, whereas the minimum similarity coefficient value was 0.970. The Average similarity coefficient was 0.985.However, maximum similarity coefficient recorded between plantlets derived from embryogenic calli Khalas without cryopreservation was 1, while the minimum similarity coefficient was 0.980. The average similarity matrix coefficient was found to be 0.99. Results indicate that plants derived from non-cryopreserved embryogenic calli and after cryopreservation (+LN) of date palm cultivar Khalas showed similarity to the mother plants at the genetic level.

Discussion

Genetic fidelity of plantlets derived from cryopreserved embryogenic calli of date palm cultivars Sagai and Khalas: Analysis of the ISSR banding patterns indicated the low genetic variation in the plantlets derived from embryogenic calli of Sagai and Khalas after cryopreservation (+LN) using the encapsulation-dehydration technique compared to the mother plants. Out of 13 primers, 4 primers gave polymorphic bands with an average of 1 polymorphic band (Tables 2 and 3). The average similarity coefficient recorded between plantlets derived from embryogenic calli of Sagai and Khalas after cryopreservation (+LN) were 0.989 and 0.985, respectively. Thus, the plantlets from both cultivars were similar to the mother plants at the genetic level. However, few variationswere found among them that could be attributed to proliferation *In vitro* or the regeneration process, instead of cryopreservation itself (Harding, 1997). The procedure of cryopreservation may cause stress or damage to plant tissues in a different way. For example,stresses associated with cryopreservation may lead to cryo-selection and enhancement of cold hardiness. Although, the effects of those events on the genome are often unknown, the variations may not be due to cryopreservation per se, but due to the whole process including culture-cryoprotection-regeneration (Harding, 2004). In this study, all samples further rooted normally, which suggests that the cryopreserved embryogenic calli maintained their developmental capability.

The results of the present study are in line with Helliot *et al*. (2002) who found that 0.3% of AFLP fragments were variable between non-cryopreserved and cryopreserved plantlets. Moreover, they observed that genetic change was not detected using RAPD and AFLP markers among the plants of *Prunusferlenain* regenerated after cryopreservation.Mishra *et al*. (2011) reported that the genetic fidelity of *Picrorhiza kurrooa* grown from encapsulated beads analyzed using RAPD assays, revealed an average similarity coefficient of 0.966 confirming the genetic stability of plants derived from encapsulated micro-shoots after storage for 3 months.Srivastava *et al*. (2009) reported that the similarity coefficients was observed 0.932–0.955 with RAPD marker in *Cineraria maritima* plants grown after storage of encapsulated microshoots. Furthermore, they mentioned that plants derived from encapsulated microshoots showed similarty to the mother plants (control) at genetic level.However, minor genetic variation was found with RAPD primers to examine the genetic fidelity of plantlets derived from cryopreserved date palm (*Phoenix dactylifera* L. 'Zaghlool') (Bekheet *et al*., 2007). No significant genetic variation was found in tissue cultures raised plantlets of date palm, whereas RAPD marker showed genetic variation in only 4% of the analyzed plants (out of 70 regenerated), which were preserved at 25°C for 6-12 months (Saker *et al.,* 2000).

On the other hand, the results of this study are similar to those of Jokipii *et al*. (2004) who analyzed *Populus tremula* L. × *Populus tremuloides* Michx. Genetic similarity was maintained using slow cooling and a vitrification process. Ryynänen and Aronen, (2005) mentioned genetic or phenotypic changes of regenerated silver birch (*Betula pendula* Roth) plants after cryopreservation using RAPD assays. No polymorphism was observed with ISSR or RAPD marker among fresh polyembryoids and polyembryoids survived after post-freezing in *Elaeis guineensis* Jacq. Gantait *et al*. (2015). Moreover, Agarwal *et al*. (2015) reported that the amplification bands were monomorphic across all the micropropagated plants with RAPD, ISSR and SCoT primers in *Alhagi maurorum*, as well as parent plants. Contrary to the findings of this study, genetic variations have been observed in micro-algal stains and strawberry after cryopreservation studied using the AFLP marker (Müller *et al*., 2007; Hao *et al*., 2002).

Genetic fidelity ofnon-cryopreserved plantlets derived from embryogenic calli of date palm cultivars Sagai and Khalas: The plantlets derived from non-cryopreserved embryogenic calli of cultivarswere more stables in regards to genetic fidelity than plantlets of the same cultivar after cryopreservation. Out of 13 primers, two primers (IS_A62and IS_A64) gave polymorphic bands compared to their mother plants (Tables 2 and 3). Also the similarity coefficients (0.994 and 0.99) were similar to the parental plants of cultivar Sagai and Khalas, respectively.

These resultsindicate that true-to-type plants of date palm can be produced by somatic embryogenesis which is the safe mode. However, the results of this study are in close agreement with Kumar, (2010), who mentioned that SSR analysis showed no genetic variation among micropropagated plants of date palm produced through somatic embryogenesis analyzing up to 42 *In vitro* subcultures. These findings are in line with Othmani *et al*. (2010) who reported thatno genetic variation have been found between regenerated plantlets of date palm cultivar, Deglet Nour through somatic embryogenesis, using RAPD and AFLP analysis.

Conclusion

The plantlets regenerated from cryopreserved and non-cryopreserved embryogenic calli were very similar to the parent plants at the genetic level. Thus, the standardized protocol for these date palm cultivars may be used for the cryopreservation of other local cultivars available in the Kingdom of Saudi Arabia.

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