African Journal of Biotechnology Vol. 10(65), pp. 14311-14318, 24 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1713
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic stability among date palm plantlets regenerated from petiole explants

Reda E.A. Moghaieb[1], Abdel-Hadi A. Abdel-Hadi[1]* and Mohamed Reda A. Ahmed[2]

[1]Department of Genetics and Genetic Engineering Research Center (GERC), Faculty of Agriculture, Cairo University, Giza, Egypt.
[2]Plant Tissue Culture Unit, Desert Research Center (DRC), Ministry of Agriculture, Egypt.

Accepted 7 September, 2011

The regeneration capacity between the shoot tip and petiole explants that excised from two date palm (*Phoenix dactylifera* L.) cutivar namely Unknown and Ferhi was compared. It was noted that the shoot tip explants started to swill after several subculture when placed on Murashige and Skoog (MS) medium supplemented with 100 mg l^{-1} 2, 4-D, 3 mg l^{-1}6-Benzyladenine (BA) and 3 g l^{-1} activated charcoal while embryogenic calli was induced by culturing the petiole explants on MS medium supplemented with 1 mgl^{-1} 2, 4-D. The data indicate that callus induction efficiency decreased with increasing 2, 4-D in the culture media. The embryonic calli were transferred into shoot inducing medium containing 1 mgl^{-1} BA and 1 mgl^{-1} naphthaleneacetic acid (NAA). The developed protocol for plant regeneration using petiole explant is repeatable and takes less time (four months) compared with the shoot tips as explants which take much time (3 years). Genetic similarity between the mother plants of the Unknown and Ferhi cultivars and several plants regenerated *in vitro* from both cultivars were examined by random amplification polymorphic DNA (RAPD) analysis using 10 random primers. Each primer generated a unique set of amplification products ranging in size from 200-2600 bp. The data indicate that the regenerated plants from the Unknown and Ferhi cultivars showed 36.2 and 37.8 % polymorphism and sharing 63.8 and 62.2% of similarities, respectively with their mother plants. The level of genetic similarity between regenerated plants and their mother plants as revealed by RAPD analysis indicate that this regeneration protocol is promising and can be used to produce transgenic date palm plants expressing economically important genes.

Key words: Date palm, somatic embryogenesis, genetic stability, RAPD anaysis.

INTRODUCTION

The date palms (*Phoenix dactylifera*, L.), is an Arecaceae family member, and is one of the most economically important woody plants cultivated in Middle East and North Africa (Sghaier-Hammami et al., 2009). It is especially so in the Arab region and the area bordering the Mediterranean Coast. The statistical records of the year 2006 indicate that the cultivated area of date palms was estimated as 1.18 million hectares in the world (FAO, 2006). Annual production of dates was estimated as 6.7 million tons. Plant tissue culture techniques have been employed to clone a wide range of plants andeconomically important palms for example, coconut, oil and date palms (Cheikh et al., 1989). Date palms can be propagated via seeds, vegetatively propagated via offshoots or via mass production in a sterile artificial laboratory environment using *in vitro* techniques. Vegetative propagation via offshoots derived from the axillary buds of the palm tree has been traditionally used as the main process for plant production. Unfortunately, this traditional procedure is limited by both the numbers of offshoots produced from a superior selected plant and the development of useful offshoots from a single plant, which occurs only during the juvenile phase of the palm's life (Kunert et al., 2001). Only about 20 offshoots are produced during the first 10 to 15 years of the tree life (FAO, 2006).

The date palm micropropagation process, like other

*Corresponding author. E-mail:abdelhadi1974@yahoo.com.Tel: 002 010 5337325. Fax: 00202 35717355.

large-scale commercial plant production processes, carries a number of risks. Off-types, that is, non true-to-type and genetically not identical to the mother plant, may be among the resulting plants. They simply can be the result of hardening errors and not originate from a change in the genetic make up of the plant (Kunert et al., 2001). In previous work, we could identify an unknown date palm cultivar grown at Matroh Governorate (Egypt) compared with the other known cultivars grown in the same area based on RAPD and Inter Simple Sequence Repeat (ISSR) analyses, the data indicate that the unknown cultivar was closely related to the cultivar Frehi and Oshkingbil (Moghaieb et al., 2010). For genetic conservation of the unknown genotype and its nearby cultivar Frehi in this study an efficient *in vitro* culture conditions was optimized. The regeneration capacity and the efficient induction of the somatic embryogenic calli of shoot tip and petiole explant was compared. The reported protocol here will be useful for improving the efficiency of transgenic date palm production. Genetic stability in tissue-cultured date palm was examined by RAPD analysis.

MATERIALS AND METHODS

Plant materials

In the present study, the efficiency of date palm regeneration from shoot tip and mature zygotic explant was examined. Offshoots about 25 to 30 cm in diameter; 50 to 80 cm in height and 7 to 10 kgs in weight were carefully separated from adult date palm trees (*P.dactylifera* L.). These trees were collected from Matroh Governorate, Egypt.

Shoot tip explant preparation

For obtaining the shoot tips and leaf primordial explants, the leaves and fiber sheath were carefully removed from offshoot acropetally upwards with a hatchet, Hoch sow, serrated knife and sharp knife (always cleaned with antiseptic solution, betadine 10% w/v). The separation of leaves was carried out starting from the base, until the shoot tip material reached 2 to 4 cm in diameter and 6 to 7 cm in length. The shoot tip explants were sprayed in direction the top with ethyl alcohol (70%) then soaked in (1:1000 w/v) benlate for 30 min and then rinsed with sterile distilled water. Then, the explants were soaked in cold sterilized antioxidant solution containing 150 mgl^{-1} ascorbic acid and 200 mgl^{-1} citric acid and kept in a refrigerator for 1 h to avoid culture browning.

Shoot tip culture media

The basic salts and vitamins of Murashige and Skoog (1962) were modified by the addition of NaH_2PO_4 170 mgl^{-1}, KH_2PO_4 170 mgl^{-1}, glutamine 200 mgl^{-1}, adenine sulfate 40 mgl^{-1} and biotin 0.1 mgl^{-1}. Four types of media were used in order to induce callus formation. The media names and composition are presented in (Table 1). The pH of the culture media used in these experiments was adjusted at 5.7 prior to addition of gelrite (2.0gl^{-1}). The explants were cultured on starting medium (M1) for 8 weeks, transfer to (M2) medium for 12 weeks. The explants were transferred to callus inducing medium(M3) 16-20 weeks. The embryonic calli were transferred to shoot proliferation medium (M4) as described in Table (1). All cultures were incubated under controlled condition, where temperature was maintained at 27 ± 2°C. Cultures were incubated under complete dark condition until callus formation occurred.

Date palm regeneration using petioles as explants

Seeds were surface sterilized with 50% sodium hypochlorite (NaOC1) solution (commercial bleach "Clorox" 5.250/0 available chlorine) for 50 min. The seeds were germinated on 0.8% agar medium and were kept at 28°C under complete dark condition for 3 to 4 weeks. Petiole explants were prepared from the germinated seedlings and were used for further regeneration experiment. Four different media composition were used in order to induce callus formation from the petiol explants (CI-1, CI-2, CI-3 and CI-4) as described in (Othmani et al., 2009). The media names and composition are presented in (Table 2). The efficiency of these media for callus induction was compared. The embryonic calli were transferred to shoot inducing medium (SP-1) (Othmani et al., 2009). The culture was kept under 16/8 light/dark condition and the temperature was adjusted to 27°C. The explants were transferred into fresh medium every three weeks. The shoot initiations (2 to 3 in length) were transferred into half strength MS medium for root formation. The plantlets that developed good root system were transferred into plastic pots prior to acclimatization.

Genetic stability among regenerated date palm plantlets in comparison with their mother plant

In order to study the genetic similarities among the regenerated date palm plantlets obtained form the petiole explants excised from both of the Unknown and Frehi cultivars, several regenerated plantlets were analyzed at the molecular levels using RAPD analysis.

RAPD analysis

Total genomic deoxyribonucleic acid (DNA) was isolated from mother plants and also from leaf tissues of several regenerated plants using the CTAB method as described in Rogers and Bendich (1985). Polymerase chain reaction (PCR) reactions were conducted using a set of ten arbitrary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA). These primers and their sequences are presented in Table 3.

The PCR mixture

The reaction mixture (20 µl) contained 10 ng DNA, 200 µM deoxynucleotide triphosphates (dNTPs), 1 µM primer, 0.5 units of Red Hot Taq polymerase (AB-gene Housse, UK) and 10-X Taq polymerase buffer (AB-gene Housse, UK). For DNA amplification, a Perkin Elmer thermal cycler (2720) programmed as follow: Denaturing: 95˚C for 5 min 94˚C for 0.45 min. Then annealing (35 cycles) 35˚C for 1 min. Followed by 72˚C for 1 min and 30 s and finally Extension: at 72˚C for 7 min (Adawy et al., 2004). The amplification products were separated in 1% (w/v) agarose gel in 1X Tris/Borate/Ethylenediaminetetraacetic_acid (TBE) buffer and visualized by staining with ethidum bromide. Reproducibility of DNA profiles was determined by replicating all RAPD reactions at least three times using DNA markers. Variations among date palm genotypes across the primers were evaluated from pair wise comparison for the proportion of shared bands amplified (Nei, 1978). The similarity coefficient was calculated by using statistical software package STATISTICA-SPSS (Stat Soft Inc.).

Table 1. Different media composition used for callus and shoot induction from shoot tip explants.

Parameter	Starting medium		Callus inducation medium	Shoot proliferation medium
	M1	M2	M3	M4
MS medium	4.4 gl^{-1}	4.4 gl^{-1}	4.4 gl^{-1}	4.4 gl^{-1}
Sucrose	30 gl^{-1}	30 gl^{-1}	30 gl^{-1}	30 gl^{-1}
Mio inositol	0.1 gl^{-1}	0.1 gl^{-1}	0.1 gl^{-1}	0.1 gl^{-1}
2,4 D	100mgl^{-1}	10mgl^{-1}	50mgl^{-1}	10mgl^{-1}
B.A	3 mgl^{-1}	-	-	3 mgl^{-1}
$Na_2.7H_2po_4$	-	170 mgl^{-1}	-	-
$K_2.7H_2po_4$	-	170 mgl^{-1}	-	-
Thiamin Hcl	-	5mgl^{-1}	-	-
Glutamine	-	200mgl^{-1}	-	-
Glycine	-	2mgl^{-1}	-	-
Adenine sulfate	-	40mgl^{-1}	-	-
IAA	-	1mgl^{-1}	-	-
NOA	-	5mgl^{-1}	-	-
NAA	-	5mgl^{-1}	-	-
Activated charcoal	3gl^{-1}	1gl^{-1}	3gl^{-1}	2gl^{-1}

Table 2. Different media composition used for callus induction and shoot formation from petioles explants

Medium composition (mg l-1)	Callus indication medium				Shoot proliferation
	CI-1	CI-2	CI-3	CI-4	SP-1
MS salts	4,568	4,568	4,568	4,568	4,58
MS vitamins	1	1	1	1	1
Fe –EDTA	65	65	65	65	65
Sucrose	50 000	50 000	50 000	50 000	50000
Myo-inositol	100	100	100	100	100
Glycine	2	2	2	2	2
Glatamine	100	100	100	100	100
KH_2PO_4	120	120	120	120	120
Adenine	30	30	30	30	30
Difco agar	7000	7000	7000	7000	7000
2,4-D	0	1	10	100	0
NAA	0	0	0	0	1
BAP	0	0	0	0	1
Activated charcoal	300	300	300	300	0

RESULTS AND DISCUSSION

Date palm improvement through traditional method is a slow, time and labor consuming process. So the optimization of date palm regeneration system is an important process and is considered as a prerequisite step towards plant transformation. Cell and tissue culture related to variability and selection efficiency are two essential components of the molecular breeding program (Lichtenstein and draper, 1985). The ability to regenerate plants from cultured cells and tissue culture at high frequencies, either through organogenesis or somatic embryogenesis is an important tool to verify the fundamental aspects of plant biology. In the present study with the aim of genetic preservation of the Unknown date palm genotype, plant regeneration capacity between the shoot tip and petiole explants was compared. Shoot tip explants were excised from young date palm offshoot and were introduced in a tissue culture program consisting of starting, callus induction and shoot proliferation steps. It was noted that the shoot tip started to swill after several subculture when placed on MS medium supplemented with 100 mg l^{-1} 2, 4-D, 3 mg l^{-1} BA and 3 g l^{-1} activated charcoal (Figure 1a to f). The regeneration system reported here includes starting stage, callus induction and somatic embryogenesis which

Table 3.Names and sequences of RAPD primers used in genetic stability among regenerated date palm plantlets in comparison with their mother plants.

Primer name	Primer sequence
OPA-03	5'-AGTCAGCCAC-3'
OPB-05	5'-TGCGCCCTTC-3'
OPC-11	5`-AAAGCTGCGG-3`
OPE-08	5`-TCACCACGGT-3`
OPG-12	5'-CAGCTCACGA -3'
OPK-02	5'-GTCTCCGCAA -3'
OPM-05	5'-GGGAACGTGT -3'
OPM-10	5'- TCTGGCGCAC-3'
OPM-15	5'-GACCTACCAC -3'
OPN-13	5'- AGCGTCACTC-3'

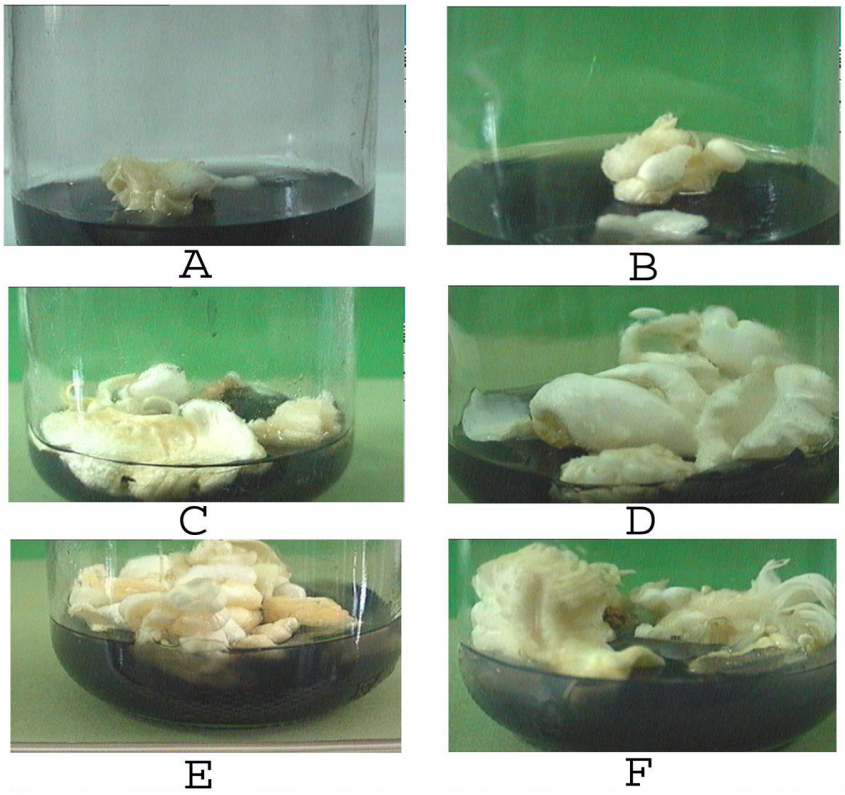

Figure 1. Callus induction on shoot tip as starting explants. A-D show a swilling of explants, while E-F: is an embryogenic callus.

are consistent with Hassan et al. (2008) who reported that a typical somatic embryogenesis protocol for date palm involves a series of consecutive stages beginning with somatic embryo induction, maturation, germination and ending with rooting stage. Aslam and Khan (2009) developed a protocol for an efficient tissue culture cycle (callus induction, callus growth, plant regeneration and root induction) for date palm cultivar 'Khalas' using various plant growth regulators (PGR) in both liquid and solidified MS medium.

The data indicate that somatic embryos formations were achieved by transferring the embryonic calli obtained into shoot proliferation medium (M4). The somatic embryos were directly emerged from the body of callus explants as shown in Figure 2. Costa and Aloufa (2006) studied the effects of 6-benzylaminopurine (BAP) and (IAA) indole acetic acid on the micropropagation of date palm (cv. Khadrawy) by direct organogenesis from

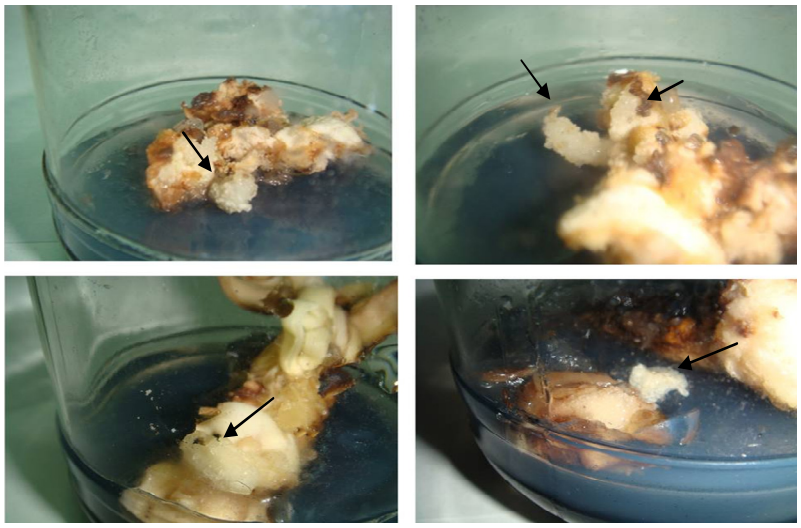

Figure 2. Embryogenesis processes of date palm resulted from transferring the embryogenic calli into shoot inducing medium. The arrows indicate the pro embryo production.

Table 4. Comparison between the efficiency of four different media on callus induction from petiole explants isolated from young seedlings of the unknown genotype.

Medium	Explants number (shoot segment)	Callus producing explants	Callus induction %
CI-1	27	3	11.1
CI-2	14	4	28.5
CI-3	19	5	26.3
CI-4	20	4	20

petioles explants culture in MS medium supplemented with 4 combinations of IAA and BAP at equal concentrations (0.0, 0.5, 1.0 and 2.0 mg l^{-1}). They found that the growth regulators enhanced aerial part and primary root differentiation. While Ahmed et al. (2009) reported that the clonal plants of date palm (*P.dactylifera* L.) were regenerated from juvenile leaves on regimes consisting of the use of 2, 4-D. In the present study, the petiole explants isolated from young date palm seedling were cultured on four different media to compare their efficiency for callus induction aiming to optimize the best condition for callus induction. The data presented in Table 4 indicated that the CI-2 medium gave the highest callus induction percentage (28.5%) followed by CI-3 (26.3%).

On the other hand CI-4 medium recorded (20%), while CI-1 medium recorded the lowest percentage of callus induction (11.1%). The results indicate that for embryonic callus induction from petiole explants lower concentration of 2, 4-D was better since medium CI-2 contained 1mgl^{-1} 2, 4-D. the callus induction percentage decreased with increasing 2,4-D concentration on the culture media for 1-100 mgl^{-1}(CI-2 and CI-4) as presented in Tables 3 and 4. The embryonic calli explants were transferred into shoot inducing medium containing 1 mgl^{-1} BA and 1 mgl^{-1} NAA. The explants were transferred into the same corresponding media every two weeks for three subculture and then were transferred into MS hormone free medium. The data in Figure 3 indicated the different developmental stages of somatic embryos from the embryogenic calli drived from petiole explants. The germinated plantlets were transferred into half strength MS medium in order to induce root formation. Regenerated plants that developed a good root system were transferred into plastic pots containing soil and then kept in the greenhouse for acclimatization. The developed protocol for plant regeneration using petiole explant is repeatable and takes less time (four months) compared with those system using shoot tips as explants which take much time (3 years).

Genetic stability between mother plant and regenerated date palm plantlets

One of the main goals of the micro propagation technique is to achieve a successful true-to-type propagation of the plant material to be cloned. Clonal fidelity is a major

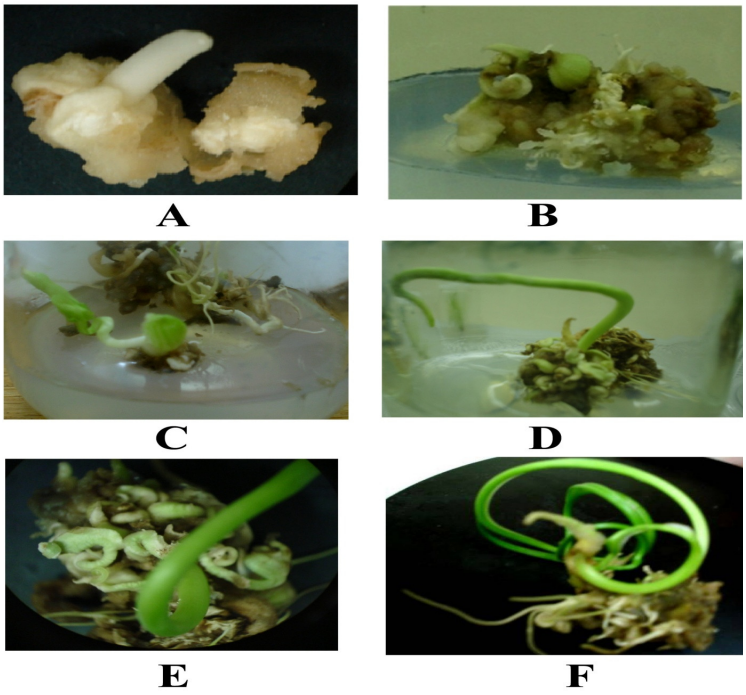

Figure 3. Different developmental stages of date palm somatic embryos; **A** embryonic calli; **b** somatic embryos; **C to E** embryo germination and; **F** whole plant regeneration.

consideration in commercial micro propagation using *in vitro* tissue culture methods (Giri et al., 2004). Somaclonal variation is a spontaneous change that is observed in plants regenerated from *in vitro* culture (Larkin and Scowcroft, 1981). This form of variation has been extensively reported in a variety of plants and provides an additional source of novel variation for, plant breeding and genetics. Tissue culture induces variation in regenerated plants, which is called somaclonal variation (Larkin and Scowcroft, 1981). It can result in a range of genetically stable variation, useful in crop improvement (Skirvin et al., 1993; Jain, 1998), similar to that induced with chemical and physical mutagens. Somaclonal variation is unpredictable in nature and it can be both heritable (genetic) and non-heritable (epigenetic). A better understand for somaclonal variation is necessary to assess the potential usefulness of the tissue culture techniques (Armestrong and Phillips, 1988), and also to develop methods whereby the material can be easily and rapidly screened to reveal any genetic difference from non tissue culture derived controls (Brown et al., 1993; Raimondi et al., 2001).

To detect somaclonal variation in tissue culture derived plants karyological analysis of plant often reveal significant chromosomal changes (Karp and Bright, 1985) but cannot reveal alterations in individual genes. Procedures such as isozyme analysis provide a relatively convenient method for examining biochemical changes although they are usually limited by the number of available markers (Brown et al., 1993). A precise determination of changes in a particular gene sequence resulting from tissue culture can be obtained by restriction fragment length polymorphism (RFLP) analysis. However, this method is time consuming and very expensive, and the results are limited only to gene sequences used as probes (Brown et al., 1993). Molecular markers such as amplified fragment length polymorphisms (AFLP), RAPD and ISSR are believed to be reliable in monitoring variability at the DNA level in plants. RAPD technique was used by several research groups to examine genetic variability and it has been found to be very efficient and reliable (Saker et al., 2000; Sanchez et al., 2003; Othmani et al., 2010). Somaclonal variation was examined among of mother plants, plantlets regenerated from somatic embryo from each of the Unknown and Ferhi cultivars by RAPD using 20 random primers. Among the 20 primers used, 10 have shown polymorphism between accessions. Each primer generated a unique set of amplification products ranging in size from 200 bp to 2600 bp (Figure 4). The data present in Table 5 and Figure 4 indicated that the regenerated plants from the Unknown and Ferhi cultivars showed 36.2 and 37.8% polymorphism and sharing 63.8 and 62.2% of similarities, respectively with their mother plants indicating that the plant regenerated is a result of somatic embryogenesis process.

The similarity percentages between the regenerated plants derived from the Unknown cultivar with their

Table 5. Genetic similarity between regenerated plants from the unknown and frehi cultivars, and their mother plants.

Primer name	The unknown genotype			Frehi		
	Total band number	Number of monomorphic bands	Similarity %	Total band number	Number of monomorphic bands	Similarity %
OPA-03	11	8	72.7	10	6	60.0
OPB-05	8	4	50.0	7	4	57.1
OPC-11	6	3	50.0	7	5	71.4
OPE-08	6	3	50.0	6	5	83.3
OPG-12	5	3	60.0	7	6	85.7
OPK-02	8	5	62.5	8	5	62.5
OPM-05	9	6	66.66	9	6	66.6
OPM-10	6	4	66.7	7	4	57.1
OPM-15	6	5	83.3	5	3	60.0
OPN-13	7	5	71.4	8	5	37.5
Total	72	46	63.8	74	49	62.2

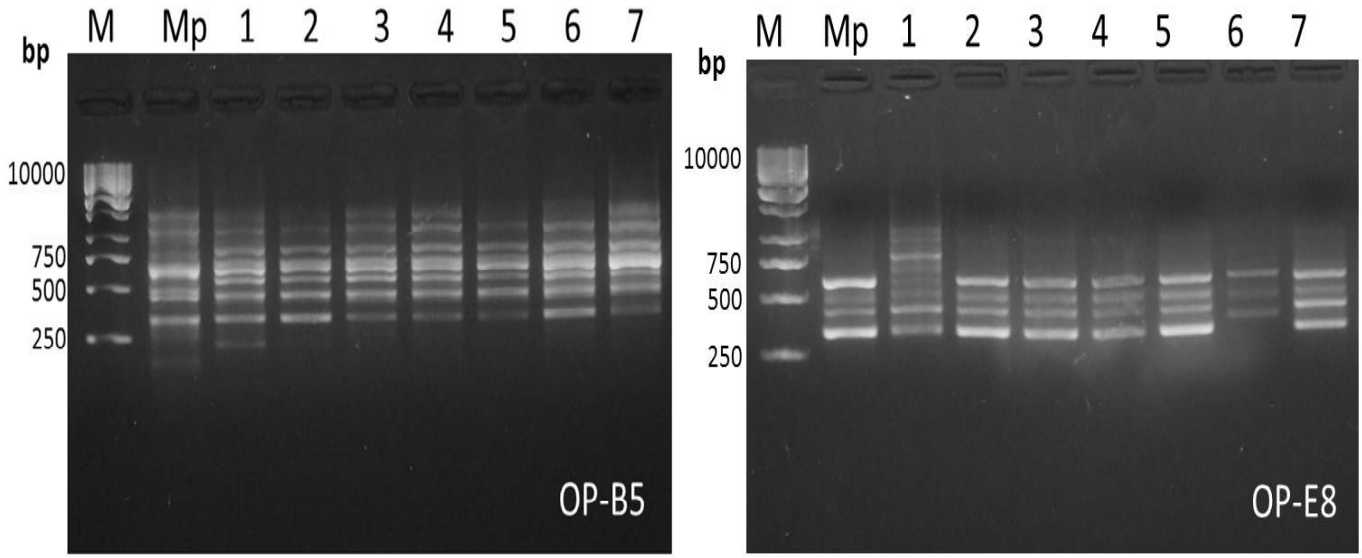

Figure 4. RAPD profile of regenerated date palm plants from the Unknown genotype in comparison to their mother plant. M: 1kp DNA marker, MP: mother plant, Lanes 1 to 7: regenerated date palm plantlets.

mother plants ranged from 50 and 72.7% for the primers OPB-05, OPC-11, OPE.08 and OPA-03, respectively (Table 5). The similarity percentages between the regenerated plants derived from Ferhi cultivar with their mother plants ranged from 37.5 and 85.7% for the primers OPN-13 and OPG-12, respectively (Table 5). The variation observed in the RAPD pattern may be due to different causes including loss/ gain of a primer annealing, due to point mutations or by the insertion or deletion of sequence or transposable elements. Since, even single base change at the primer annealing site is manifested as appearance or disappearance of RAPD bands, it could be suggested that tissue culture conditions can induced varied amount of genetic changes in different regenerated plants (Othmani et al., 2010).

Taken together, it could be concluded that an efficient and reproducible regeneration system of date palm from petiole explants was established. The reported protocol takes less time to obtain regenerated plants compared with the long period needed when apical meristem is used as explants. The level of genetic similarity between regenerants and their mother plants as revealed by RAPD analysis indicate that this regeneration protocol is promising and can be used to produce transgenic date palm plants expressing economically important genes.

Abbreviations

RAPD, Random amplification polymorphic DNA; **ISSR,** inter simple sequence repeat; **PCR,** polymerase chain reaction;

dNTPs, deoxynucleotide triphosphates; **BAP,** 6-benzylaminopurine; **MS,** murashige and skoog medium; **TBE,** tris/borate/ethylenediaminetetraacetic_acid; **PGR,** plant growth regulators; **RFLP,** restriction fragment length polymorphism; **IAA,** indole acetic acid; **AFLPs,** amplified fragment length polymorphisms; **NAA,** naphthaleneacetic acid; **BA,** 6-Benzyladenine.

REFERENCES

Ahmed O, Chokri B, Noureddine D, Mohamed M, Mokhtar T (2009). Regeneration and molecular analysis of date palm (*Phoenix dactylifera* L.) plantlets using RAPD markers. Afr. J. Biotechnol. 8(5): 813-820.

Adawy SS, Hussein Ebtissam HA, Saker MM, El-Itriby Hanaiya A (2004). Intra- and Inter-varietal variation of Upper Egypt date palm cultivars (*Phoenix dactylifera* L.): I. As revealed by RAPD and Inter Simple Sequence Repeat markers. Proceed. Int. Conf. Genet. Eng. Appl. Sharm El-Sheikh, South Sinai, Egypt (April, 8-11, 2004): 165-179.

Armestrong CL, Phillips RL (1988). Genetic and cytogenetic variation in plants regenerated from organogenic and friable embryogenesis tissue cultures of maize. Crop Sci. 28: 363-369

Aslam J, Khan SA (2009). *In vitro* micropropagation of 'Khalas' date palm (*Phoenix dactylifera* L.), an important fruit plant. J. Fruit Ornam. Plant Res. 17(1): 15-27.

Brown PTH, Lang FD, Kranz E, Lorz H (1993). Analysis of single protoplasts and regenerated plants by PCR and RAPD technology. Mol. Genet. Genomics, 237: 311-317.

Cheikh R, Zaid A, Ait Chitt M (1989). Travaux de recherche conduits sur l'embryogenése somatique chez le palmier dattier. Compte Rendu du Deuxieme Seminaire Maghrebin sur la multiplication du palmier par les techniques *in vitro*. PNUD/INRA, Marrakech 9-12 Oct. Projet FAD/RAB/88/024. pp. 59-69.

Costa NMS, Aloufa MAI (2006). Direct organogenesis in *Phoenix dactylifera* L. through cotyledonar petiole. Pesquisa Agropecuaria Tropical, 36(3): 195-198.

FAO (2006). UN food and agriculture organization (FAO)[1]. http://en.wikipedia.org/wiki/UN_Food_Agriculture_Organisation.

Giri CC, Shyamkumar B, Anjaneyulu C (2004). Progress in tissue culture, genetic transformation and application of biotechnology to trees: an overview. Trees, 18: 115-135

Hassan MM, Gadalla EG, Abd-El-Kareim AH (2008). Effect of sucrose and abscisic acid on *in vitro* growth and development of date palm during rooting stage. Arab J. Biotechnol. 11(2): 281-292.

Jain SM (1998). Plant biotechnology and mutagenesis for sustainable crop improvement. Crop Improvement for Stress Tolerance (Eds. Behl *et al.*). CCSHAU, New Delhi, India, pp. 218-232.

Kunert KJ, Baaziz M, Cullis CA (2001). Techniques for determination of true-to-type date palm (*Phoenix dactylifera* L.) plants. Emirates J. Agric. Sci. 15: 1-16.

Larkin PJ, Scowcroft WR (1981). Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., 60: 197-214.

Lichtenstein C, Draper J (1985). Genetic engineering of plants, In: Glover D.M (ed.), DNA cloning. IRL Press, Oxford, pp. 67-l 19.

Moghaieb REA, Abdel-Hadi AA, Ahmed MRA, Hassan AGM (2010) Genetic diversity and sex determination in date palms (*Phoenix dactylifera* L.) based on DNA markers. Arab J. Biotechnol. 13(2): 143-156

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetic, 89: 583-590.

Othmani A, Bayoudh C, Drira N, Marrakchi M, Trifi M (2009). Somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* L., cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. Plant Cell, Tissue Organ Cult. 97: 1, 71-79.

Othmani A, Rhouma S, Bayoudh C, Mzid R, Drira N, Trifi M (2010) Regeneration and analysis of genetic stability of plantlets as revealed by RAPD and AFLP markers in date palm (*Phoenix dactylifera* L.) cv. Deglet Nour. International Research J. Plant Sci. 1(3): 48-55.

Raimondi JP, Masuelli RW, Camadro EL (2001). Assessment of somaclonal variation in asparagus by RAPD fingerprinting and cytogenetic analyses. Scientia Hortic. 90(1): 19-29

Rogers SO, Bendich AJ (1985). Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol., 5: 69-76.

Saker M, Bekheet S, Taha HS, Fahmy AS, Moursy HA (2000). Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. Biologia Plantarum, 43: 347-51.

Sánchez MC, Martínez MT, Valladares S, Ferro E, Viéitez AM (2003). Maturation and germination of oak somatic embryos originated from leaf and stem explants: RAPD markers for genetic analysis of regenerants. J. Plant Physiol. 160: 699-707.

Sghaier-Hammami B, Valledor L, Drira N, Jorrin-Novo JV (2009). Proteomic analysis of the development and germination of date palm (*Phoenix dactylifera* L.) zygotic embryos. Proteomics. 9: 2543–2554.

Skirvin RM, Norton M, McPheeters KD (1993). Somaclonal variation: has it proved useful for plant improvement. Acta Hort., 336: 333-340.