BIOLOGIA PLANTARUM 50 (2): 198-204, 2006

Monitoring of cultivar identity in tissue culture-derived date palms using RAPD and AFLP analysis

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Abstract

In the present study, two polymerase chain reaction (PCR)-based methods namely, randomly amplified polymophic DNA (RAPD) and amplification fragment length polymorphism (AFLP) were employed to assess genetic variations, which may appeared, in tissue culture-derived date palm (*Phoenix dactylifera*) offshoots. Analysis of RAPD banding patterns generated by PCR amplification using 37 random primers gave no evidences for somaclonal variations and the percentage of polymorphic bands in a total of 259 scored bands was zero. Meanwhile, analysis of AFLP banding patterns generated using 13 primer combinations pointed to minor genetic variations in the AFLP banding patterns. The percentage of genetic variations (polymorphism) in tissue culture-derived date palm offshoots belonging to cultivars Sakkoty, Gandila and Bertamoda was 2.6, 0.79 and 1 %, respectively, as revealed by AFLP analysis. The low percentage of genetic variations confirms the genetic stability of tissue culture-derived dry date palm cultivars.

Additional key words: *Phoenix dactylifera*, micropropagation, cultivar identification, PCR.

Introduction

Date palm is one of the most successful fruit crops in tropical and sub-tropical habitats. Extensive breeding programs for the selection of superior clones through the traditional methods is a tedious efforts due to the long life cycle and strongly heterozygous nature of date palm, in addition to insufficient and expensive offshoots (planting materials) required for new cultivation (Moursy and Saker 1998). Contributions of biotechnology researches to improve date palm include, *e.g.*, rapid mass micropropagation (Tisserat 1982, Bhansali *et al.* 1988, Dass *et al.* 1989, Zaid *et al.* 1995, Saker *et al.* 1998, Bekheet *et al.* 2001, 2002), physiological responses of drought-stressed cell cultures (Al-Khayri and Al-Bahrany 2004), molecular characterization and fingerprinting (Saker and Moursy 1999, Sedra *et al.* 1998 and Adawy *et al.* 2002, 2005), molecular characterization of the most notorious pests of date palm (Salama and Saker 2002) and monitoring the genetic stability of tissue culture-derived date palm clones (Salman *et al.* 1988, Saker *et al.* 2000, 2002).

However, a routine and precise method for the analysis of tissue culture-derived plants for somaclonal variations has yet to be established. Limited data are available regarding molecular analysis of tissue culture-derived palm plants. In this context, Salman *et al.* (1988) reported similar isozyme patterns and only chromosome polyploidization in one case of *in vitro* propagated date palms, suggesting high genetic uniformity of tissue culture-derived plants. On the other hand, a molecular marker linked to somaclonal variations in oil palm has been identified by Rival *et al.* (1998). Recently, isozyme analysis and activities of peroxidase, polyphenol oxidase and glutamate oxaloacetate and randomly amplified polymophic DNA (RAPD) fingerprints were used to assess somaclonal variations in tissue culture-derived clones of some Egyptian date palm cultivars. The frequency of somaclonal variations was found to be age dependent and RAPD analysis showed genetic variations in approximately 4 % of the analyzed plants (Saker *et al.* 2000). In conclusion, additional work dealing with the

Received 9 August 2004, *accepted* 17 April 2005.
Abbreviations: AFLP - amplification fragment length polymorphism; 2,4-D - 2,4-dichlorophenoxyacetic acid; IAA - indole-3-acetic acid; 2ip - 2-isopentenyladenine; MS - Murashige and Skoog; NAA - α-naphthaleneacetic acid; PCR - polymerase chain reaction; RAPD - randomly amplified polymophic DNA.
Acknowledgement: This study is a part from a project kindly supported by The Arabic Organization for Education, Culture and Science (ALECSO).
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detection of somaclonal variations among tissue culture derived date palms using molecular markers is needed, especially this approaches proved to be effective in other crops such as *Vigna radiata* (Betal *et al.* 2004), *Heritiera* sp. (Mukherjee *et al.* 2003/4) and tea (Singh *et al.* 2004).

The above mentioned situation encouraged us to develop an efficient, stable and reproducible DNA-based methods for genetic analysis of tissue culture-derived clones of some economically important date palm cultivars grown in south of Egypt and to establish cultivar-specific amplification fragment length polymorphism AFLP fingerprints.

Materials and methods

The fresh leaves of different certified date palm trees of cultivars Sakkoty, Gandila and Bertamoda, *i.e*. the donor mother trees (8 - 10 m), were collected during fruiting season. At the same time, the fresh leaves of tissue culture-derived date palm clones (fourteen offshoots per cultivar) of the above mentioned cultivars were collected.

The tissue culture-derived dry date palm plants were produced by Gadalla (2003) through somatic embryogenesis. The system involves the culturing of shoot tip explants onto Murashige and Skoog (MS) medium supplemented with 10 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D) + 5 mg dm^{-3} α-naphthaleneacetic acid (NAA) + 5 mg dm^{-3} indole-3-acetic acid (IAA) + 3 mg dm^{-3} 2-isopentenyladenine (2ip) + 1 g dm^{-3} charcoal.

Total DNA was extracted from young leaves according to Porebski *et al.* (1997). RAPD was performed as described by Williams *et al*. (1990) with few modifications. PCR reactions were carried out in 0.025 cm^3 volumes containing 25 ng of total genomic DNA, 10 pmol primer, 200 µM dNTP, 2 mM $MgCl_2$, 1× PCR buffer and 2 Units Ampli Taq Polymerase (RTS Taq DNA polymerase). Different decamer oligonucleotide random primers were obtained from *Operon Technologies, Alameda*, USA). Amplification was performed in *Perkin-Elmer 9600* (Foster City, USA) thermal cycler with the following profile: 94 °C for 5 min, 94 °C for 1 min, 36 °C for 1 min, 72 °C for 90 s for 40 cycles with a final extension at 72 °C for 7 min.

AFLP was carried out as described by Vos *et al.* (1995) using the *Gibco BRL AFLP* analysis system, and the AFLP starter primer Kit (Cat No. 10544-013 and 10483-014, respectively). Briefly, genomic DNA (250 ng) was digested with 1 mm^3 of a mixture of EcoRI/MseI (1.25 units mm^{-3}) at 37 °C for 2 h and ligated to EcoRI /MseI adapters with 1.5 mm^3 (1 unit mm^{-3}) of T4 DNA ligase at 20 °C for at least 4 h. After digestion and ligation to adaptors, DNA was amplified in two sequential steps called pre amplification and amplification. For pre amplification reaction, a mixture of 2.5 mm^3 of DNA from the ligation mixture, 20 mm^3 of pre Amp mix II, 2.5 mm^3 of 10× PCR buffer + Mg, and 0.2 mm^3 of Taq DNA polymerase (5 units mm^{-3}) was used. The pre-amplification reactions were performed in *Perkin-Elmer 9600* thermal cycler with a profile as follows: 20 cycles at 94 °C for 30 s, 56 °C for 60 s and a extension cycle at 72 °C for 60 s then soaking at 4 °C. For the selective amplification, preamplified products were employed as templates using two AFLP primers, in a reaction of 20 mm^3 total volume containing 5 mm^3 diluted pre Amp product, 5 mm^3 mixture of EcoRI/MseI primer and 10 mm^3 of Taq DNA polymerase (*RTS Gibco BRL*), PCR buffer plus magnesium and double distilled water. The reaction was carried out using the following cycling parameters: One cycle of 30 s at 94 °C, 30 s at 65 °C, 1 min at 72 °C followed by 12 cycle in which the annealing temperature decreases 0.7 °C per cycle, followed by 23 cycles of 1 min at 94 °C, 30 s at 56 °C and 1 min at 72 °C.

The RAPD-PCR products were resolved on 1.5 % agarose gel in 1× TAE buffer, stained with ethidium bromide and visualized by UV illumination. The AFLP-PCR amplification products were separated in a 6 % denaturing polyacrylamide gel in a *Sequi-Gen Cell* (*BioRad Laboratories*, Hercules, USA). The DNA fragments were visualized by silver staining using the method described by Bassam *et al.* (1991), with minor modifications. PCR amplification products (10 mm^3) was mixed with equal volume of 1× loading buffer (98 % formamide, 10 mM EDTA, 0.025 % bromophenol blue and 0.025 % xylencyanol), denatured for 5 min in boiling water bath and 7 mm^3 was loaded per well. Electrophoresis was performed at 100 W and 50 °C. The developed AFLP plates were scanned either as colour or black white images.

Results

Data of RAPD amplification products, generated by PCR amplification using 37 primers, indicated that the total number of amplified bands was 259 and the molecular sizes ranged from 100 to 3000 bp. The average number of amplification products per primer was 8 bands. No distinct polymophic bands were detected and the percentage of polymorphism is zero (Table 1). Based on data extracted from RAPD analysis, it could be concluded that the tissue culture-derived clones contain the same genetic make up of the parent mother trees, *i.e.* true-to type. Although this conclusion was latter on confirmed by AFLP analysis but no one can exclaim that it is

Table 1. Total number of RAPD and AFLP primers, total number of amplification bands (amplicons), number of polymorphic bands and percentage of polymorphism among the three *in vitro* grown date palm cultivars.

	RAPD			AFLP		
	Sakkoty	Gandila	Bertamoda	Sakkoty	Gandila	Bertamoda
Number of primers	37	37	37	13	13	13
Total number of bands	259	259	259	380	380	380
Number of polymorphic bands	0	0	0	10	3	4
Polymorphism [%]	0	0	0	2.6	0.8	1.0

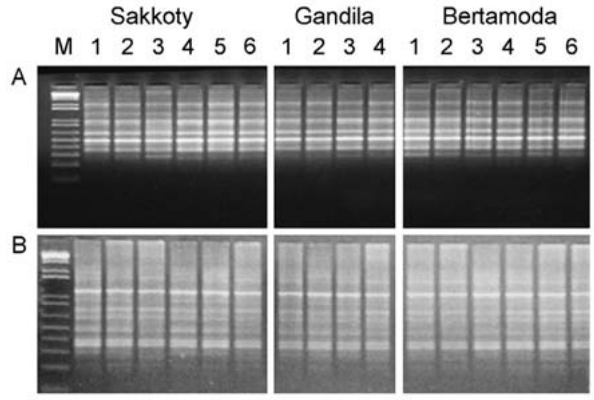

Fig. 1. RAPD banding patterns of donor mother trees (*lane* 1) and different tissue culture-derived date palm clones (*lanes* 2 - 6) belonging to cultivars Sakkoty, Gandila and Bertamoda. The pattern generated by PCR amplification using the random primers OPD8 (A) and OPZ11 (B). The PCR products were separated in TAE buffer using 2 % agarose gel. M - DNA ladder.

accurate because RAPD banding patterns generated by PCR amplifications using 37 primers are failed not only to detect genetic variations among tissue culture-derived clones but also to distinguish among the different three date palm cultivars (Fig. 1).

The thirteen primer combinations used in AFLP analysis (Table 1) in the present study generated 380 polymorphic band, 17 of them were polymorphic (inter cultivar and intra cultivars) and the overall level of polymorphism is 4.47 %. The level of polymorphism in Sakkoty, Gandila and Bertamoda was 2.6, 0.79, and 1.0 %, respectively. From thirteen combinations, five primer combinations, namely Eco ACA/Mse CTG, Eco AGC/Mse CTG, Eco AAC/Mse CTT, Eco ACT/Mse CTC and Eco AGG/Mse CTT, were selected. The selection criteria are the well visible, easy to recognize and score, sufficient and stable polymorphism in AFLP banding patterns. A glance on the developed AFLP banding profiles generated following PCR amplification using these selected primer combinations (Figs. 2, 3) indicates clearly that no one AFLP profile can either identify the three different cultivars or confirm the presence or absence of genetic variations. However, by comparing the overall distribution of polymorphic bands in the five AFLP profiles (Table 2), its possible to distinguish the three different cultivars and to estimate the genetic variations. Data of Table 2 are focused only on 9 informative polymorphic bands, *i.e.* bands which can distinguish among different cultivars as well as detecting genetic variations among different clones.

It could be observed that the marker Eco ACA/Mse CTG-325 is unique positive marker for the cultivar Gandila and using this marker its possible to distinguish Gandila from the other two cultivars. The marker Eco AGC/Mse CTG-150 is unique positive marker for Bertamoda. Meanwhile, the marker Eco AGG/Mse CTT-230 is a negative marker for Sakkoty. Although, the rest of markers presented in this table are not unique for specific cultivar, they are specific positive markers for two cultivars only and accordingly they are negative markers for the third one (Table 2). For instance the marker Eco ACA/Mse CTG-195 and Eco AGG/Mse CTT-170 are positive markers for Sakkoty and Bertamoda and negative markers for Gandila, similarly Eco AGG/ Mse CTT-230 is a positive marker for Gandila and Bertamoda and a negative marker for Sakkoty. The marker is considered cultivar specific if detected only in the certified mother tree of the cultivars and in most of tissue culture-derived clones of the same cultivar, *i.e.* common across the individuals of the same cultivars. It could be concluded that it is possible to distinguish among the three different date palm cultivars at the DNA level using AFLP banding patterns generated by PCR amplification using the primer combinations presented in Table 2. Figs. 2 and 3 show the AFLP fingerprints of some tissue culture-derived date palm offshoots (offshoots which showed genetic variations, even in one band, from the overall bands generated using the thirteen primer combinations) of Sakkoty, Gandila and Bertamoda and their donor mother trees (control).

The tissue culture-derived clones of cultivars Gandila and Bertamoda are true-to-type to type, meanwhile genetic variations among tissue culture-derived clones of Sakkoty are detected (Table 2). The percentage of genetic variations (polymorphism) detected in tissue culture-derived clones of Sakkoty is 2.6 %, this mean that 97.4 % of the total score-able bands in tissue culture-derived clones of Sakkoty are typical to the donor mother tree. On the other hand, 99.2 % and 99 % of total score-able bands in tissue culture-derived clones of Gandila and Bertamoda, respectively, are typical to donor mother trees

and the percentage of polymorphism is 0.79 % and 1 %. It could be concluded that tissue culture-derived date palm trees grown in Egypt are true-to-type and AFLP analysis can be used to precisely detection of somaclonal variations in tissue culture-derived date palms at early stages of development.

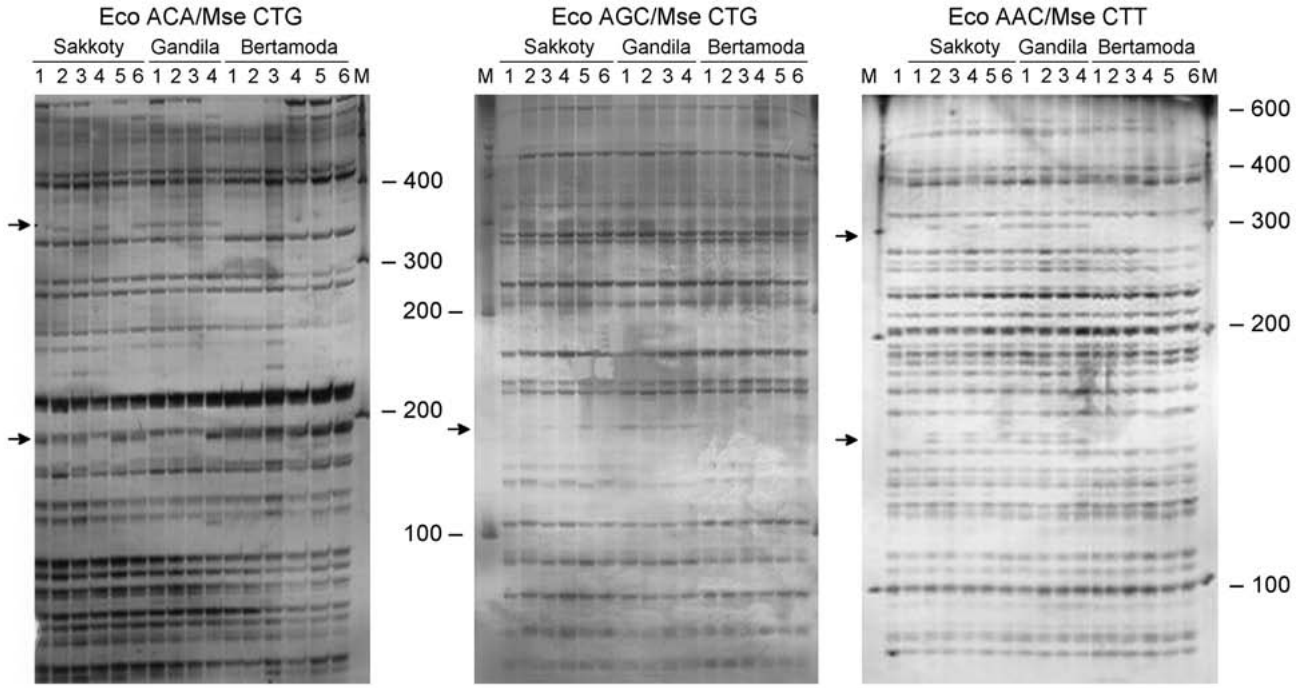

Fig. 2. AFLP banding patterns of donor mother trees (*lane* 1) and different tissue culture-derived date palm clones (*lanes* 2 - 6) belonging to cultivars Sakkoty, Gandila and Bertamoda. The pattern generated by PCR amplification using three different primer combinations. The PCR products were separated using 6 % polyacryamide gel, silver stained and scanned as colour images. M - DNA ladder.

Table 2. Distribution of informative AFLP polymorphic bands (markers + or -) among donor mother trees (C) of cultivars Sakkoty, Gandila and Bertamoda (inter cultivars polymorphism) and among tissue culture-derived clones (TC) of the three cultivars (intra cultivar polymorphism).

Markers	Sakkoty						Gandila				Bertamoda					
	C	TC					C	TC			C	TC				
	1	2	3	4	5	6	1	2	3	4	1	2	3	4	5	6
Eco ACA/Mse CTG-325	-	+	-	+	-	+	+	+	+	+	-	-	-	-	-	-
Eco ACA/Mse CTG-195	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+
Eco AGC/Mse CTG-150	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+
Eco AAC/Mse CTT-300	-	+	-	+	-	+	+	+	+	+	-	-	-	-	-	-
Eco AAC/Mse CTT-80	-	+	-	+	-	+	+	+	+	+	-	-	-	-	-	-
Eco ACT/Mse CTC-290	+	+	-	+	-	+	-	-	-	-	+	+	+	+	+	+
Eco ACT/Mse CTC-120	-	-	+	-	+	-	+	+	+	+	-	-	-	-	-	-
Eco AGG/Mse CTT-230	-	-	-	-	-	+	+	+	+	+	+	+	-	+	-	-
Eco AGG/Mse CTT-170	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+

Discussion

Analysis of the scored phenotypic characters of tissue culture-derived date palm clones, at early stages of development, in the light of the same criteria for the donor parent trees does not gave any evidence for genetic variations at the phenotypic level. Regarding the effectiveness of the two types of molecular markers employed herein to monitor genetic variations in tissue culture-derived date palm clones as well as identifying the cultivars, it was found that AFLP analysis is more effective and can be applied to differentiate closely related cultivars, meanwhile RAPD is less discriminative than AFLP. This conclusion is not in agreement with

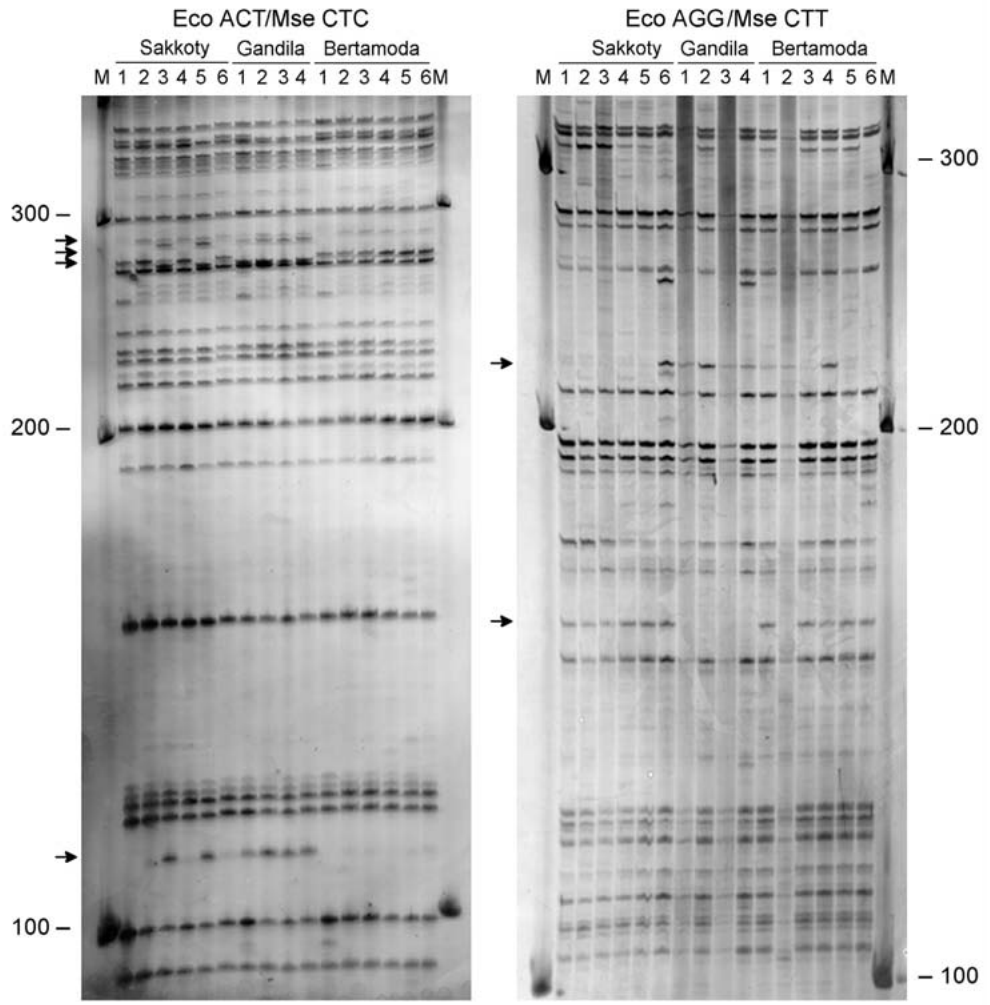

Fig. 3. AFLP banding patterns of donor mother trees (*lane* 1) and different tissue culture-derived date palm clones (*lanes* 2 - 6) belonging to cultivars Sakkoty, Gandila and Bertamoda. The pattern generated by PCR amplification using primer combinations Eco ACT/Mse CTC and Eco AGG/Mse CTT. The PCR products were separated using 6 % polyacryamide gel, silver stained and scanned as black and white images. M - DNA ladder.

some previously published data which pointing to the efficacy of RAPD technique to differentiate not only wild genotypes but also closely related cultivated genotypes (Sharma *et al*. 1995) and to detect somaclonal variations in tissue culture-derived plants (Chowdhury and Vasil 1993). In this context, we also succeeded to detect genetic variations in some fresh date palm plantlets using RAPD but the detected genetic variations were also well-visible, seen and easy to recognize based on morphological criteria (Saker *et al.* 2000). The highly discriminative value and effectiveness of AFLP, compared with other PCR-based markers was previously reported in other crops including rice (Virk *et al.* 2000), barley (Russell *et al.* 1997**)** and potato (McGreger *et al.* 2000, Milbourne *et al.* 1997). Financial considerations play a major role in the choice of marker system for any given application. In this case, it is also accept as true that RAPD is less expensive than AFLP, on the other hand, the apparent sensitivity of RAPD to experimental conditions limits its adoption.

The similarities in RAPD and AFLP banding patterns in different plants derived from tissue culture may suggest genetic resemblance and it therefore possible to postulate that tissue culture-derived date palm plants are true-to-type and contains similar genetic make up of the parents since they revealed the same banding patterns. The low percentage of genetic variations (poly-morphisms) detected herein and which ranged from 0.79 to 2.6 % in tissue culture-derived date palm plants, as revealed by AFLP analysis, clearly confirms that the tissue culture protocol, which was followed to produce date palm offshoots is suitable for commercial production of true-to-type date palm offshoots. This very low percentage of genetic variations may be due to the fact that embryogenic cells have minimal genetic changes. This agrees with reports of Taylor *et al.* (1995) and

Chowdhury and Vasil (1993). Similar percentage of polymorphism can be detected in traditionally propagated date palm trees of the same cultivar. On the other hand, Adway *et al.* (2002) recorded intercultivar and intracultivar polymorphisms in date palm using RAPD and ISSR. The detected polymorphism may have resulted from concentration of 2,4-D imposed on callus tissue and extended *in vitro* culture (Murashige and Nakano 1966, Karp and Bright 1985). In this context, previously published data (Saker *et al.* 2000, 2002) on the detection of genetic variations among *in vitro* propagated date palm plantlets using biochemical and molecular markers may be also due to the tissue culture system and regime. Moreover, most of the previously mentioned studies on field evaluation of tissue culture-derived date palms rely on some visible morphological criteria and fruit quality, which are a subject for variations, as a result of environmental factors and other agricultural treatments.

References

Adawy, S., Hussein, E., El-Khishin, D., Saker, M., El-Itriby, H.: Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars. II - RAPD and ISSR profiling. - Arab J. Biotechnol. **5**: 225-236, 2002.

Adawy, S.S., Hussein, E., Ebtissam. H.A., Ismail, S.E., El-Itriby, H.A.: Genomic diversity in date palm (*Phoenix dactylifera* L.) as revealed by AFLP in comparison to RAPDs and ISSRs - Arab J. Biotechnol. **8**: 99-114, 2005.

Al-Khayri, J., Al-Bahrany, A.: Growth, water content and proline accumulation in drought-stressed callus of date palm. - Biol. Plant. **48**: 105-108, 2004.

Bassam, J.B., Caetano-Anolles, G., Gresshoff, P.M.: Fast and sensitive silver staining of DNA in polyacrylamide gels. - Anal. Biochem. **196**: 80-83, 1991.

Bekheet, S.A., Taha, H.S., Saker, M.M.: Factors affecting *in vitro* multiplication of date palm. - Biol. Plant. **44**: 431-433, 2001.

Bekheet, S.A., Taha, H.S., Saker, M.M., Moursy, H.A.: A synthetic seed system of date palm through somatic embryogenesis encapsulation. - Ann. agr. Sci. Ain Shams Univ. (Cairo) **47**: 325-337, 2002.

Betal, S., Roy Chowdhury, P., Kundu, S., Sen Raychaudhuri, S.: Estimation of genetic variability of *Vigna radiata* cultivars by RAPD analysis. - Biol. Plant. **48**: 205-209, 2004.

Bhansali, R., Kaul, R., Dass, H.: Mass cloning of date palm plantlets through repetitive somatic embryogenesis. - J. Plant. Anat. Morphol. **5**: 73-79, 1988.

Chowdhury, U., Vasil, I.: Molecular analysis of plants regenerated from embryogenic cultures of hybrid sugar cane cultivars (*Saccharum* spp.). - Theor. appl. Genet. **86**: 181-188, 1993.

Dass, K., Kaul, R., Joshi, S., Bhansali, R.: *In Vitro* regeneration of date palm plantlets. - Curr. Sci. **58**: 22-24, 1989.

Gadalla, E.: Propagation of dry varieties of date palm. - Ph.D. Thesis. Faculty of Agriculture, Cairo University, Cairo 2003.

Karp, A., Bright, J.: On the causes and origins of somaclonal variation. - Oxford Surv. Plant mol. cell Biol. **2**: 199-234, 1985.

McGreger, C., Lambert, C., Geryling, M., Louw, J., Warnich, L.: A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum*) germplasm. - Euphytica **13**: 135-144, 2000.

Milbourne, D., Meyer, R.C., Bradshaw, J.E., Baird, E., Bonar, N., Provan, J., Powell, W., Waugh, R.: Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. - Mol. Breed. **3**: 127-136, 1997.

Moursy, H.A., Saker, M.M.: Date palm problems and the need for biotechnology. - Bull. Fac. Agr. Univ. Cairo **49**: 315-330, 1998.

Mukherjee, A., Acharya, L., Mattagajasingh, I., Panda, P.: Molecular characterization of three *Heritiera* species using AFLP markers. - Biol. Plant. **47**: 445-448, 2003/4.

Murashige, T., Nakano T.: Tissue culture as a potential tool in obtaining polyploidy plants. - J. Heredity **57**: 115-118, 1966.

Porebski, S., Baley, L.G., Baum, B.R.: Modification of a CTAB DNA extraction protocol for plants containing high polysaccharides and polyphenol components. - Plant mol. Biol. Rep. **15**: 8-15, 1997.

Rival, A., Tregear, J., Verdeil, J.L., Richaud, F., Beule, T., Duval, Y., Hartmann, C.: Molecular search for mRNA and genomic markers of the oil palm “mantled” somaclonal variations. - Acta Hort. **461**: 165-171, 1998.

Russell, J.R. Fuller, J.D., Macaulay, M., Hatz, B.G., Jahoor, A., Powell, W., Waugh, R.: Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs, and RAPDs. - Theor appl. Genet. **86**: 975-984, 1997.

Saker, M., Bekheet, S., Taha, H., Fahmy, A., Moursy, H.: Detection of somaclonal variations in tissue culture-derived date palm plants using isozyme analysis and RAPD fingerprints. - Biol. Plant. **43**: 347-351, 2000.

Saker, M., Moursy, H., Bekheet, S.: *In vitro* propagation of Egyptian date palm: I - Morphogenic responses of immature embryos. - Bull. Fac. Agr. Univ. Cairo **49**: 203-214, 1998.

Saker, M.M., Kawshity, S., El-Bahr, M.K., Fahmy, A.: Flavonoid content and isozyme analysis of tissue culture-derived date palm clones. - Arab J. Biotechnol. **5**: 207-216, 2002.

Saker, M.M., Moursy, H.A.: Molecular characterization of Egyptian date palm: II - RAPD Fingerprints.- Arab J. Biotechnol. **2**: 71-78, 1999.

Salma, H., Saker, M.M.: DNA fingerprints of three different red palm weevil forms collected from infested Egyptian date palm orchards. - Arch. Phytopathol. Pflanz. **35**: 1-8, 2002.

Salman, M., Al-Jibouri, M., Al-Quadhy, K., Omar, S.: Isozyme and chromosomal analysis of tissue culture derived date palms. - Date Palm J. **6**: 401-411, 1988.

Sedra, M., Lashermes, P., Trouslot,,P., Comes, M.C., Haman, S.: Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. - Euphytica **103**: 75-82, 1998.

Sharma, S.K., Knox, M.R., Ellis, T.N.: AFLP analysis of the diversity and phylogeny of lens and its comparison with RAPD analysis. - Theor. appl. Genet. **93**: 751-758, 1995.

Singh, M., Saroop, J., Dhiman, B.: Detection of intra-clonal genetic variability in vegetatively propagated tea using RAPD markers. - Biol. Plant. **48**: 113-115, 2004.

Taylor, J., Fraser, A., Ko, L, Henry, J.: RAPD analysis of sugarcane during tissue culture. - In: Terzi, M., Cella, R., Falavigna, A. (ed.): Current Issues in Plant Molecular and Cellular Biology. Pp. 241-246. Kluwer Academic Publishers, Dordrecht 1995.

Tisserat, B.: Factors involved in the production of plantlets from date palm callus cultures. - Euphytica **31**: 201-214, 1982.

Virk, P.S., Zhu, J., Newbury, H., Bryan, G., Jackson, M., Ford-Lloyd, B.: Effectiveness of different classes of molecular markers for classifying and revealing variations in rice (*Oryza sativa*) germplasm. - Euphytica **112**: 275-284, 2000.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M.: AFLP: a new technique for DNA fingerprinting. - Nucleic Acids Res. **23**: 4407-4414, 1995.

Williams, K., Kubelik, A., Livak, K., Rafalski, J., Tingey, V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. - Nucl. Acid Res **18**: 6531-6535, 1990.

Zaid, A., Hughes, H.: Water loss and polyethylene glycol-mediated acclimatization of *in vitro* grown seedlings of 5 cultivars of date palm (*Phoenix dactylifera L.*) plantlets. - Plant Cell Rep. **14**: 385-388, 1995.