Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars

II- RAPD and ISSR profiling

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ABSTRACT

Two types of molecular markers RAPD and ISSR were employed to assess the genetic polymorphism within and among four Egyptian date palm cultivars (Samany, Hayany, Siwi and Zaghloul). The DNA samples from ten individual trees representing each of the four cultivars were investigated using ten decamer primers. All the tested primers exhibited intravarietal polymorphism as well as intervarietal variation. Moreover, the DNA samples of the ten trees representing each cultivar were bulked to produce a single sample.The bulked samples revealed RAPD profiles comprising all the common bands present in the different profiles exhibited by each cultivar. The ten primers revealed twelve unique polymorphic RAPD markers among the four cultivars. In addition, seven ISSR primers were used with the four bulked samples. Six primers revealed twenty four unique markers characterizing the different cultivars. Moreover, ten seedlings derived from in vitro culture of the cultivar Zaghloul were investigated to evaluate their genetic stability (true to type).

***Key words**: Date palm, RAPD, ISSR, molecular markers.*

INTRODUCTION

The genus Phoenix, which includes the date palm (*Phoenix dactylifera L.*) is the sole member of the tribe Phoeniceae of the monocotyledonous family palmae (Moore, 1963 and Bailey Hortatorium, 1976). Phoenix is widespread in the tropical and subtropical areas of southern Asia and Africa. The date palm is adapted to areas with very hot summers with little rain and low humidity but with abundant underground water. These conditions are found in oases and river valleys in the arid subtropical deserts of the Middle East.

In Egypt, date palm is one of the most important fruits and widely distributed in

different districts. There are 3 main types of dates based on fruit moisture content, i.e., soft, semi - dry and dry cultivars (Moustafa, 1998). The importance of the date palm tree is due to the economical as well as the nutritional value of its fruit. Egypt lies in the fruit largest producer among Arab countries (680.000 tons), FAO (1996).

During the last 20 years, results have shown that date palm can be propagated by tissue culture both by organogenesis (Poulain *et al.*, 1979 and Drira, 1983) or by somatic embryogenesis (Letouze and Daguin, 1989). Currently, the process of somatic embryogenesis seems to be more attractive for an industrial production but the date palms derived from somatic embryos must be true - to- type.

The advent of molecular biology techniques has provided DNA - based markers for detection of genetic polymorphim. RAPDs (Randomly amplified polymorphic DNA) and ISSR (Inter simple sequence repeats) markers are currently widely used for genotype identification to evaluate genetic integrity (Isabel *et al.*, 1993) and analysis of somaclonal variants (Hashmi *et al.*, 1997). In this research we used RAPD and ISSR markers, to assess the polymorphism among the four Egyptian date palm cultivars (Samany, Zaghloul, Siwi and Hayany) and to identify additional unique markers characterizing each cultivar. Moreover, an attempt has been made to evaluate the genetic stability of some Zaghloul tissue culture derived plantlets.

MATERIALS AND METHODS

Plant material

Four Egyptian date palm cultivars (Samany, Siwi, Zaghloul and Hayany) were used in this study.

Isolation of genomic DNA

DNA extraction was performed according to the protocol of Porebski *et al.* (1977). After purification, the DNA was quantified using agarose mini gel electrophoresis as described by Sambrook *et al.* (1989).

RAPD analysis

RAPD analysis was performed as previously described by (Hussein *et al.* 1998 and 2000) with minor modifications. A set of 15 decamer primers were initially employed in RAPD analysis, ten primers produced reproducible and scorable RAPD profiles, the sequence of these ten primers are illustrated in Table (1). Reactions were performed in 50 µl volume. The reaction components were: 1x reaction buffer, 0.2 uM of dNTPs, 0.2 uM primer, 0.5 unit of Taq polymerase, 40 ng of genomic DNA, in sterile distilled water. Amplification of the DNA was performed in a Perkin Elmer thermal cycler 2400 programmed to fulfill 40 cycles The temperature profile in the different cycles was as follows: an initial strand separation cycle at 94°C for 5 min followed by 38 cycles including a denaturation step at 94°C for 1 min,an annealing step at 37°C for 1 min and a polymerization step at 72°C for 2 min. The final cycle was a polymerization cycle at 72°C for 7 min.

ISSR analysis

ISSR analysis was carried out in a total reaction volume of 25µl containing 2.5µl 10 x buffer, 5µl of 5x Q solution, 2.5µl (2mM dNTPs), 4µl (10 pmol primer), 0.2µl Hot start Taq polymerase and 4µl DNA (30ng). The PCR temperature profile was composed of an

initial denaturing cycle at 95°C for 15 min followed by 10 touch down cycles (95°C/30 sec, 65-55°C/1 min, 72 °C/90 sec), this was followed by 30 cycles (95 °C/30 sec 55°C/1 min, 72°C/90 sec), and then a final extension cycle at 72°C for 7 min. The sequences of the 7 ISSR primers are presented in Table (1).

Scoring of the data

Scoring of the data for both RAPD and ISSRs was performed from 1% agarose gels, where clear and distinct amplification products were scored as (1) for presence and (0) for absence of bands. The scoring did not consider differences in intensity of the bands among profiles from different samples.

RESULTS AND DISCUSSION

Molecular markers are efficient tools for cultivar identification and estimation of relatedness through DNA fingerprinting. In the present investigation, two types of molecular markers namely, RAPD and ISSR, were employed to assess the genetic polymorphism *within* and *among* four Egyptian date palm cultivars (Samany, Hayany, Siwi and Zaghloul).

Genetic polymorphism as detected by RAPD markers

In a previous work (Hussein *et al.*, 2002 under publication) using 5 decamer primers with the same date palm cultivars, we demonstrated the presence of inter- as well as intravarietal variation. To assess the genetic variability, and to confirm the previous results, the DNA samples from 10 individual trees representing each of the four cultivars (Samany, Hayany, Siwi and Zaghloul) were further investigated using additional primers. Fifteen random decamer primers from Operon B set were initially screened. Each reaction was repeated twice to confirm RAPD banding patterns. Any fragment thought to be artifact, based on the controls or those too difficult to score with certainty were not included in the data set. Out of the 15 tested primers, only ten revealed reproducible and discernible RAPD profiles, therefore, these primers were selected for further investigations. As shown in Fig. (1), all the tested primers exhibited intravarietal polymorphisms as well as intervarietal variations, thus confirming our previous results.

To investigate intervarietal polymorphism among the four date palm cultivars, the different RAPD profiles within each cultivar and in the different cultivars were compared (Fig. 2). RAPD markers that are characteristic for each cultivar were identified as the common markers among the different amplification profiles of the same cultivar. In general, the size of amplified DNA fragments by the ten primers ranged from 2800 to 310 base pairs. The number of bands varied, from 5 to 13 bands depending on the primer and cultivar tested.

The ten primers revealed 12 polymorphic RAPD markers among the four cultivars ranging in size from 2100 bp to 770 bp. Table (2) shows the polymorphic markers across the four date palm cultivars. Moreover, the DNA samples of the ten trees representing each cultivar were bulked to produce a single sample and the four bulked DNA samples representing the four cultivars were reanalyzed using the ten decamer primers. Comparing RAPD profiles of the bulked samples produced by each of the 10 primers with the profiles of the individual samples of each cultivar, it was deduced that the bulked samples revealed all the common bands present in the different profiles exhibited by each cultivar and similar RAPD markers were detected. The cultivar Hayany was character-

ized by the absence of the $OPB01_{2100bp}$ and $OPB13_{1078bp}$ markers which were present in all the other cultivars, while Siwi was characterized by a unique positive marker ($OPB05_{1700bp}$) and a unique negative marker ($OPB10_{1050bp}$). The cultivar Zaghloul was characterized by the absence of $OPB05_{1300bp}$ and $OPB14_{1353bp}$ which were present in the other 3 cultivars. The cultivar Samany was distinguished by the presence of three positive markers ($OPB04_{872bp}$, $OPB06_{2100bp}$ and $OPB09_{2000bp}$) and also by the absence of the markers $OPB09_{1900bp}$, $OPB16_{770bp}$ and $OPB20_{1800bp}$ which were present in the other 3 cultivars. In this context, Isabel *et al.* (1993) and Letouze` *et al*, (1998) reported that RAPD technique can be routinely applied for varietal identification and gives reproducible results. Moreover, Saker and Moursy (1998) used the RAPD technology to evaluate the genetic polymorphism among the same cultivars and found that only six of the ten studied primers revealed polymorphism. While, Letouze *et al.* (1998) differentiated 13 different date palm cultivars using 5 decamer primers which provided 14 polymorphic markers.

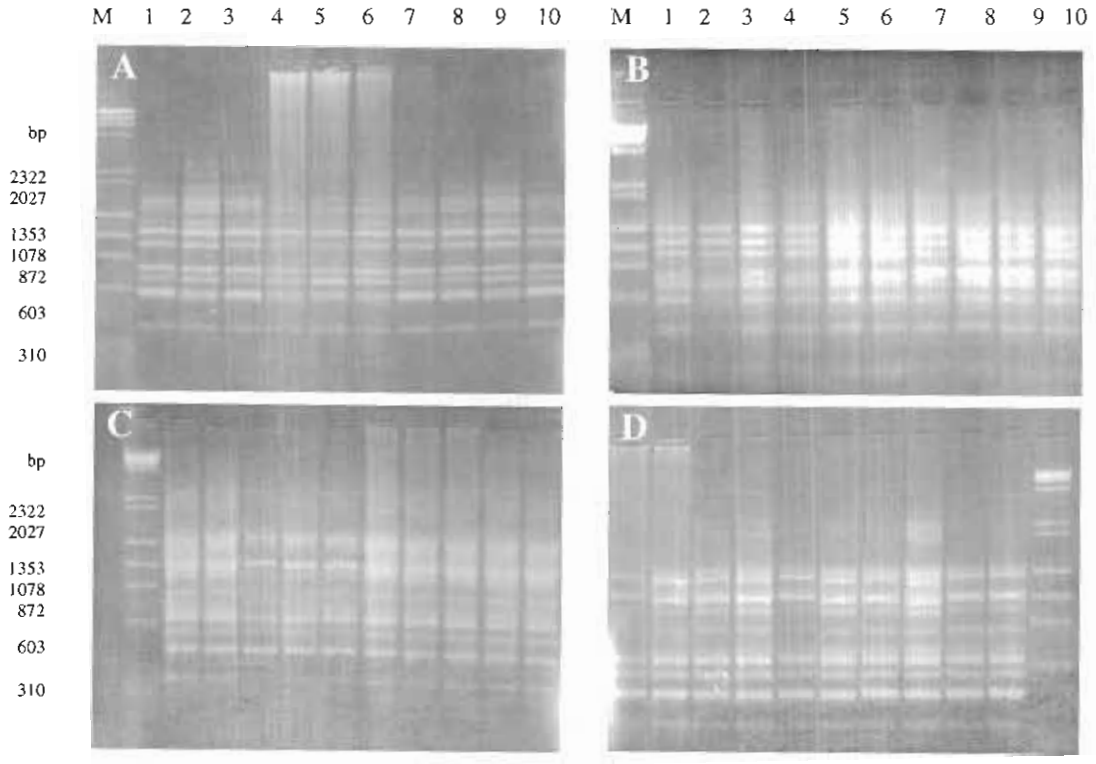

Fig. (1): RAPD profiles for date palm cultivars using different primers OP B20, (A) Hayany, (B) Zaghloul and OP B5 (C) Zaghloul, (D) Hayany, M Lambda Hind III digest, Phi x 174, Hae II digest DNA marker.

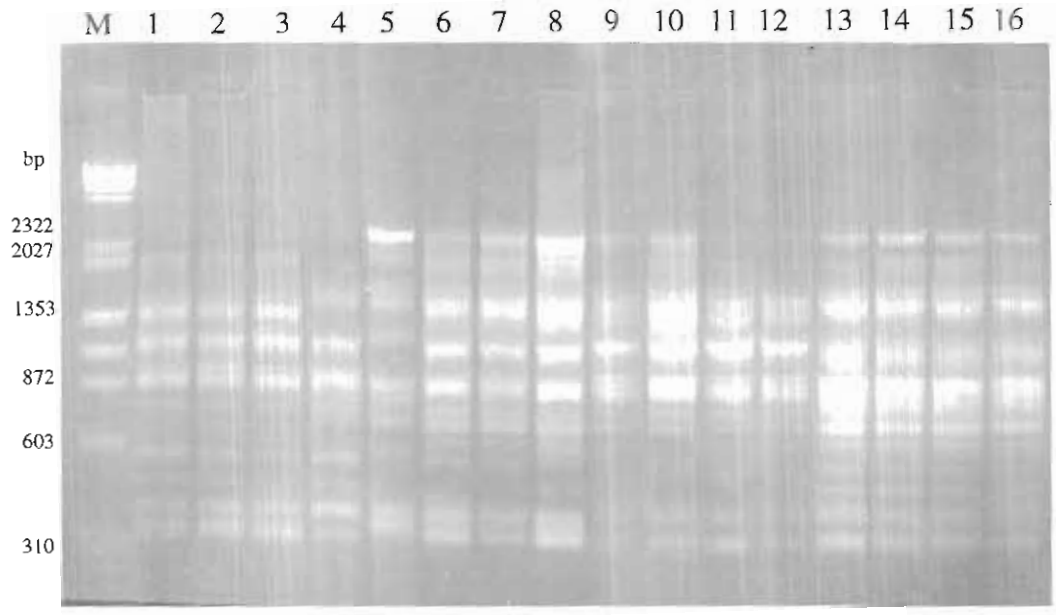

Fig. (2): RAPD profiles of the four date palm cultivars Hayany lanes 1-4, Zaghloul 5 8, Siwi 9 12 and Samany 13 16, amplified by OP B1 primer, M DNA molecular marker, Lambda Hind III digest, Phi x 174, Hae II digest.

Genetic polymorphism as detected by ISSRs

The bulked samples of the 4 date palm cultivars were analyzed using 7 Inter simple sequence repeat (ISSR) primers. These included five anchored primers at the 5` end with 3 base pairs and 2 primers composed of non- anchored repetitive sequences as shown in Table (1). The amplification results of the ISSR primers used in this investigation are presented in Table (3). The 7 ISSR studied primers produced good reproducible and scorable patterns and the amplification profiles were screened for the presence of polymorphisms among the 4 date palm cultivars. As shown in Table (3), a total of 53 fragments were generated by the 7 primers, with primer Amic 1 yielding the highest number of products (11 amplicons) and primer A mic7 the least (4 amplicons). The number of polymorphic markers also varied between primers, with primer Mic3 generating only polymorphic bands (100% polymorphism) and primer Amic1 revealing 10 polymorphic bands out of 11 and Amic7 yielding only monomorphic bands. Similarly, Cekic *et al.* (2001) used 10 ISSR primers for screening of polymorphism between two closely related forms of *F. vesca* and stated that the number of polymorphic bands varied between primers. Among the seven studied ISSR primers, six revealed unique markers characterizing each of the four cultivars. The total number of unique ISSR markers was 24 (Table 4). The cultivar Samany was characterized by one positive ($Amic1_{506bp}$) and one negative band ($Amic1_{550bp}$) Hayany was distinguished by 3 positive ($Amic1_{298bp}$, $Amic5_{590bp}$ and $Amic6_{500bp}$)and 6 negative ($Amic1_{280,150bp}$ $Mic3_{396bp}$, $Amic5_{600bp}$, $Amic6_{850bp}$ and $Amic6_{600bp}$) markers. While, Siwi was characterized by 3 positive ($Amic1_{1100bp}$,

Amic6$_{450bp}$ and Amic8$_{350bp}$) and 4 negative (Amic1$_{1200bp}$, Amic6 $_{650bp}$, Amic8$_{800bpand400bp}$) markers. Moreover two positiveAmic8$_{390bp}$ and Mic9$_{396bp}$) and four negative (Amic1$_{344bp}$, $_{120bp}$, Mic3$_{344bp}$ and Mic9$_{298bp}$ unique markers identified the cultivar Zaghloul (Fig. 3). Different authors reported on the usefulness of ISSR for cultivar identification (Kantety *et al.*, 1995,Wolff *et al.*, 1995and Chartess *et al.*, 1996). Kantety *et al.* (1995) added that ISSR is useful in plant breeding programs and identification of polymorphic loci among near isogenic lines. Furthermore, Julie and Pablo (1997) reported that the products generated from 5` anchored primers should exhibit more codominant polymorphism than RAPD.

Table (1): Name and sequences of the primers used in RAPD and ISSR analysis.

Assay type	Primer code	Nucleotide sequences 5` ⟶ 3`
RAPD	OPB01	GTTTCGCTCC
	OPB04	GGACTGGAGT
	OPB05	TGCGCCCTTC
	OPB06	TGCTCTGCCC
	OPB09	TGGGGGACTC
	OPB10	CTGCTGGGAC
	OPB13	TTCCCCCGCT
	OPB14	TCCGCTCTGG
	OPB16	TTTGCCCGGA
	OPB20	GGACCCTTAC
ISSR	A Mic1	CGC(GATA)4
	A Mic5	CGG(CA)7
	A Mic6	GGC(CA)7
	A Mic7	CGA(CAG)5
	A Mic8	GAA(TC)7
	Mic9	(GTG)5
	Mic3	(GACA)4

Table (2): Unique RAPD markers showing polymorphism among the four date palm cultivars, Samany (S), Hayany (H), Siwi (Si) and Zaghloul (Z).

Primers	Approx. length (bp)	S	H	Si	Z
OPB 01	2100	1	0	1	1
OPB 04	872	1	0	0	0
OPB05	1700	0	0	1	0
	1300	1	1	1	0
OPB 06	2100	1	0	0	0
OPB 09	2000	1	0	0	0
	1900	0	1	1	1
OPB 10	1050	1	1	0	1
OPB13	1078	1	0	1	1
OPB 14	1353	1	1	1	0
OPB 16	770	0	1	1	1
OPB 20	1800	0	1	1	1

Table (3): Total number of amplicons and the level of polymorphism among the four date palm cultivars as revealed by ISSRs.

Primers	ID	Total number of amplicons	Polymorphic amplicons	Percentage of polymorphism
Un anchored Primers	Mic 3	6	6	100
	Mic 9	6	2	33
5` Anchored Primers	AMic 1	11	10	91
	AMic 5	10	5	50
	AMic 6	9	5	55
	AMic 7	4	0	0
	AMic 8	7	6	65
Total		53	34	64.1

Table (4): Unique ISSR markers showing polymorphism among the four date palm cultivars, Samany (S), Hayany (H) Siwi (Si) and Zaghloul (Z).

Primers	Approx- band size in bp	Cultivars			
		S	H	Si	Z
AMic 1	1200	1	1	0	1
	1100	0	0	1	0
	550	0	1	1	1
	506	1	0	0	0
	344	1	1	1	0
	298	0	1	0	0
	280	1	0	1	1
	150	1	0	1	1
	120	1	1	1	0
Mic3	396	1	0	1	1
	344	1	1	1	0
AMic 5	600	1	0	1	1
	590	0	1	0	0
AMic 6	850	1	0	1	1
	650	1	1	0	1
	600	1	0	1	1
	500	0	1	0	0
	450	0	0	1	0
AMic 8	800	1	1	0	1
	400	1	1	0	1
	390	0	0	0	1
	350	0	0	1	0
Mic 9	396	0	0	0	1
	298	1	1	1	0

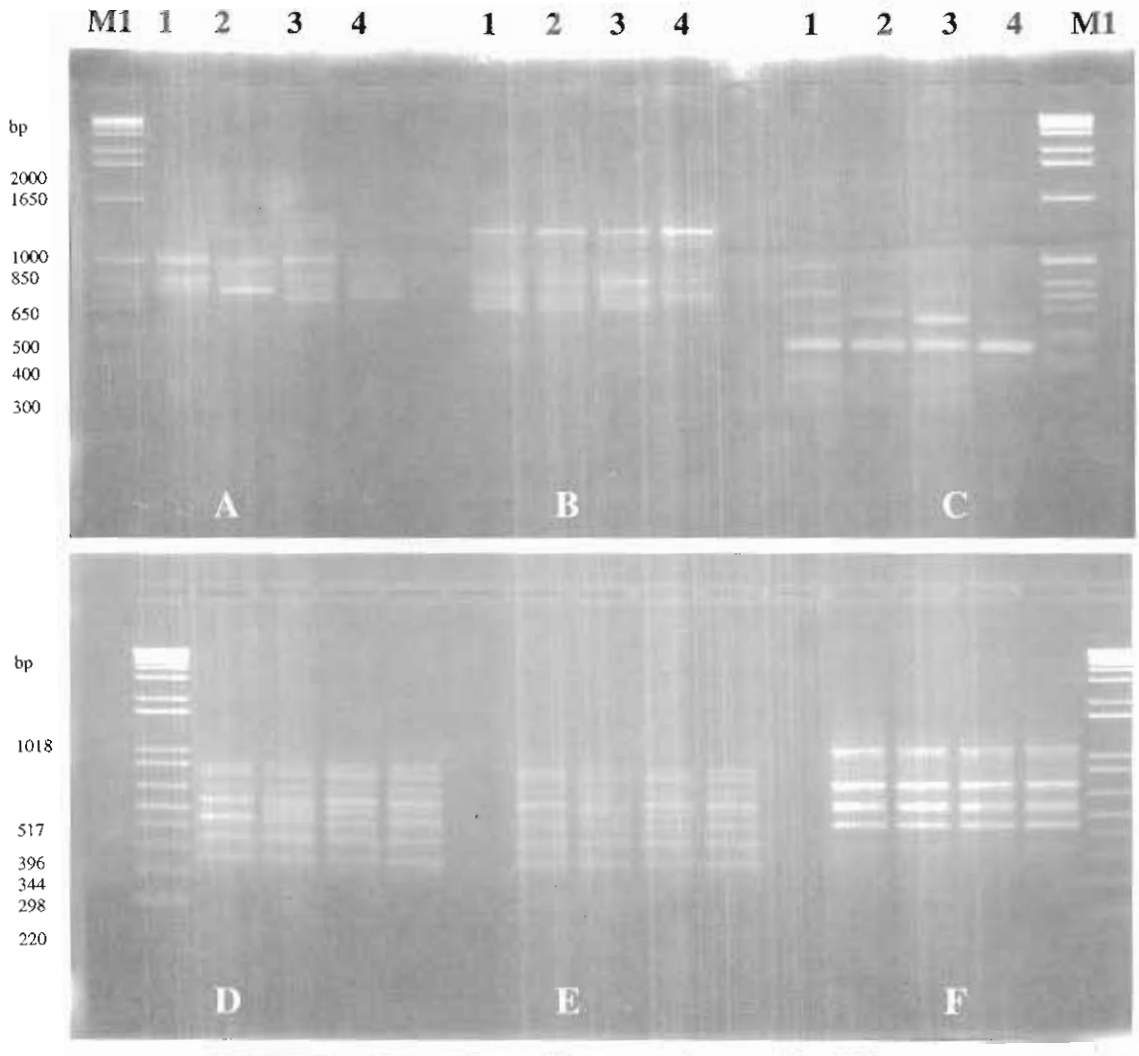

Fig. (3): ISSR profiles of the four date palm cultivars as detected by different ISSR primers (1) Samany, (2) Hayany, (3) Siwi, (4) Zaghloul as detected by (A) Amic5, (B) Amic6, (C) Amic7, (D) Mic3, (E) Mic9, (F) Amic1, M1 and M2, 1 kb plus ladder and 1 kb ladder, respectively.

Genetic stability of somatic embryo-derived plantlets

The tissue culture overcomes the problem of date palm cultivation with traditional methods which prevent rapid crop improvement of the date palm trees. However, the tissue culture protocol should maintain the genotype of the original cultivar, i.e . we must prove that tissue culture - derived plants are true to type. In this respect, Torres and Tisserat (1980) stated that somaclonal variations can not be discovered before the reproduction stage. A callus obtained from clonal tissue is highly heterogeneous (Tisserat and Demason, 1980) and it is highly susceptible to mutation development during somatic embryogenesis. This problem could be avoided if an accurate identification method was utilized at an early stage of propagation. To investigate the genetic stability (true to type) of the clones derived from date palm tissue culture, ten

seedlings derived from *in vitro* culture of the cultivar Zaghloul were subjected to molecular analysis using RAPD markers. RAPD analysis was carried out using 10 random decamer primers from Operon B set, i.e., OP (B01, B05, B07, B09, B11, B12, B13, B14, B15 and B20). These primers were selected since they revealed specific markers characterizing the cultivar Zaghloul (Table 1). The tissue culture RAPD profiles detected by primers OP (B01, B05, B09, B11, B12, B14, B15 and B20) were similar to the RAPD profile of the cultivar Zaghloul. Only two primers showed the absence of some bands in some of the tissue culture- derived plants; for example the absence of band 1100 bp in samples No 2, 4 and 5 in primer OP-B07 and band 1350 bp in some of the samples with primer OP- B13 (Fig. 4). This limited variation could be attributed to the effect of the long period of callus maintenance on the media. Letouze` *et al.* (1998) evaluated somatic embryo- derived plantlets of two cultivars Barhee and Medjool using six RAPD primers for each cultivar at three stages (off shoot, embryo-germinating and acclimatized plantlets). Their results showed that the polymorphism revealed by RAPD markers differed depending on cultivars and primers used, but that for a cultivar and a given primer chosen, no genetic change was detected regardless of the stage.

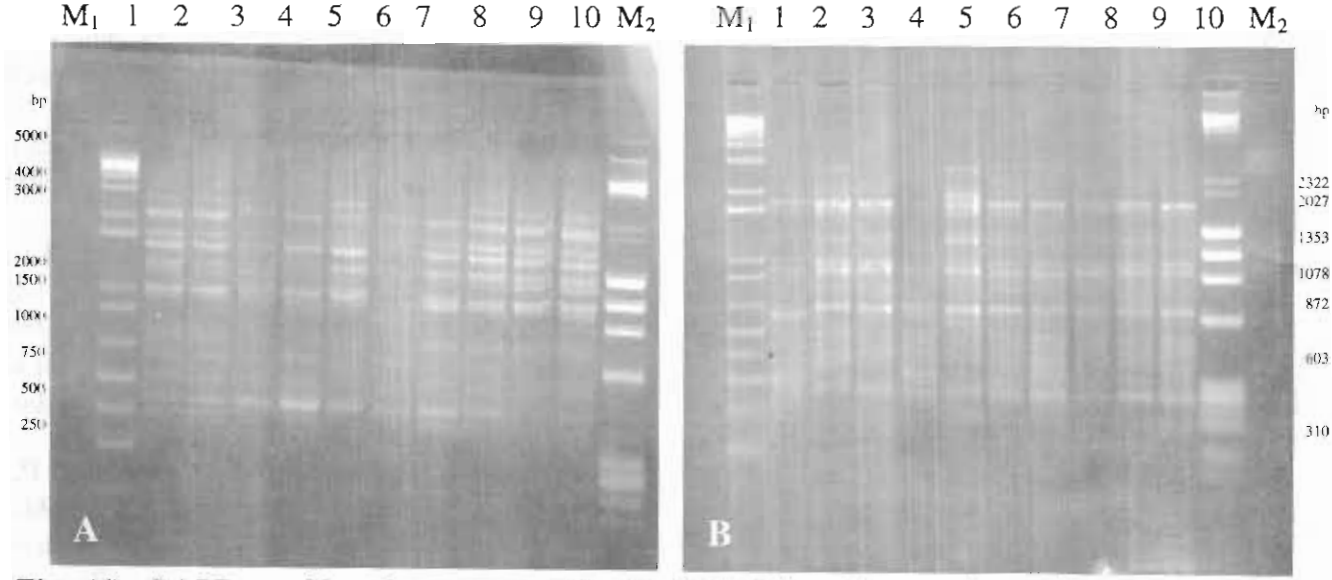

Fig. (4): RAPD profiles of ten plantlets (1 10) derived from tissue culture of cultivar Zaghloul, amplified using primers (A) OP B9 and (B) OP B13, M1 and M2, 1 kb ladder and Lambda Hind III digest, Phi x 174, Hae II digest, respectively.

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REFERENCES

Bailey Hortatorium, L.H. (1976). Hortus Third. Macmillan, New York. (C. F. Krueger R. R. 1998). Proceedings the First International Conference on Date Palms. Al-Ain, United Arab Emirates, March 8-10.

Cekic, C., Battey, N.H. and Wilkinson, M.J. (2001). The potential of ISSR-PCR primer-pair combinations for genetic linkage analysis using the seasonal flowering locus in fragaria as a model. Theor. Appl. Genet., 103:540-546.

Charters, Y.M., Robertson, A., Wilkinson, M.J. and Ramsay, G. (1996). PCR analysis of oilseed rape cultivars (*Brassica napus L.ssp. oleifera*) using 5`-anchored simple sequence repeat (SSR) primers. Theor. Appl. Genet., 92: 442-447.

Drira, N. (1983). Multiplication vegetative du Palmier Dattier (*Phoenix dactylifera* L.) par la culture *in vitro* de bougeons axillaires et de feuilles qui in derivent C. R. Acad Sci.Paris.seri. 111: 1077-1082.

FAO (1996). Food and Agriculture Organization of the United Nations, Production Year Book 50, Table, 66: 152 pp.

Hashmi, G., Huehl, R., Meyer, R. and Krusberg, L. (1997). Plant Cell Reports 16: 624-627 (C.F. Letouze *et al.*, 1998).

Hussein, Ebtissam H.A., El-Itriby, H.A., El-Karim, G.H.A., Adawy, S.S. and El-Assal, S.S. (1998). Detection of genetic variability among *Brassica napus* L. accessions using RFLP and RAPD markers. Egypt. J. Genet. Cytol. 27: 11-24.

Hussein, Ebtissam, H.A., El-Itriby, H.A., Adawy, S.S., El-Karim, G.H.A. and Madkour, M.A. (2000). Identification of molecular markers and genome mapping in *Brassica napus* L. Arab J. Biotech. 3 (2): 165-180.

Hussein, Ebtissam, H.A., Adawy, S.S., El Khishin, D., Moharam, H. and El-Itriby Hanaiya, A. (2002). Genetic variability studies and molecular fingerprinting of some Egyptian Date palm (*Phoenix dactylifera* L.) cultivars. 1-A preliminary study using RAPD markers. Arab J. Biotech. (in press).

Isabel, N., Tremblay, L., Mich, M., Tremblay, F.M. and Bousquet (1993). RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis derived populations of *Picea mariana* (Mill). B.S.P. Theor. Appl. Genet., 86: 81-87 (C.F. Letouze *et al.*, 1998).

Julie, M.V. and Pablo, A.S. (1997). Direct amplification from microsatellites, detection of simple sequence repeat-based polymorphisms without cloning. In, G. Caetano-Anolles and P. M. Gresshof (eds). DNA Markers, Protocols, Applications and Overviews.Wiely-Liss, Inc. P 137-150.

Kantety, R.V., Zeng, X., Bennetzen, J.L. and Zehr, B.E. (1995). Assignment of genetic diversity in dent and popcorn (*Zea mays*.L) inbred lines using inter-simple sequence repeat (ISSR) amplification. Molecular Breeding 1: 365-373.

Letouze`, R., Daguin, F., Satour, P., Hamama, L. and Marionnet, F. (1998). Somatic embryogenesis and mass micropropagation of date palm (*Phoenix dactylifera* L.). Characterization and genetic stability of regenerated plantlets by RAPD markers. Proceedings the First International Conference on Date Palms. Al-Ain, United Arab Emirates, March 8-10, pp158-167.

**Letouze`, R. and Daguin, F. (1989).** L`embryogenese somatique:des possibilites nouvelles pour lmicroprodu dattier (*Phoenix dactylifera* L). Compte-rendu du deuxieme seminaire maghrebin sur la multiplication rapide du palmier dattier par les techniques

de culture *in vitro*. *Marrakech* FAO/PNUD/RAB/88/024., pp.144.

Moore, H.E. (1963). An annotated checklist of cultivated palms. Principes 7: 119-189.

Moustafa, A.A. (1998). Studies on pollination of date palms. Proceedings the First International Conference on Date Palms. Al-Ain, United Arab Emirates, March 8-10, pp 39-48.

Porebski, S., Bailey, L.G. and Baum, R. (1977). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and phenol components. Plant Mol.Biol.Reporter, 15(1): 8-15.

Poulain, C., Rhiss, A. and Beauches ne, G. (1979). Multiplication vegetative, culture *in vitro* du palmier dattier (*Phoenix dactylifera* L) C. R. Acad. Agri. Paris, 1131-1157.

Saker, M.M. and Moursy, H.A. (1998). Molecular characterization of Egyptian date palm. II RAPD fingerprinting. Proceedings the First International Conference on Date Palms. Al-Ain, United Arab Emirates, March 8-10, pp 173-182.

Sambrook, J., Fritsch, E.R. and Maniatis, T. (1989). Molecular Cloning :A Laboratory Manual.2nd edition.Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Tisserat, B. and Demason, D. (1980). A histological study of the development of adventitive embryos in organ culture of (*Phoenix dactylifera* L.).Annals of Botany. 46: 465-472.

Torres, A.M. and Tisserat, B. (1980). Leaf isozymes as genetic markers in date palms. Am.J.Bot. 67: 162-167.

Wolff, K., Zietkiewicz, E. and Hofstra, H. (1995). Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. Theor. Appl. Genet. 91: 439-447.

الملخص العربي

تقييم التباين الوراثي وعمل البصمة الوراثية الجزيئية لبعض أصناف نخيل البلح المصري II- باستخدام تكنيك ISSR, RAPD

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تم استخدام نوعين من الواسمات الجزيئية RAPD و ISSR على أربعة أصناف من نخيل البلح (سماني حياني سيوي زغلول) لدراسة التباينات الوراثية بين الأصناف وداخل الصنف الواحد . تم عزل الدنا DNA من عشرة أشجار من كل صنف ، وباستخدام بادئات RAPD أظهرت عشرة بادئات تباينات وراثية بين الأصناف المدروسة علاوة على ظهور بعض التباينات داخل الصنف الواحد . كما تم خلط الدنا DNA المستخلص من العشرة أشجار الممثلة لكل صنف لتكوين عينة مجمعة واحدة واعادة دراسة RAPD على الأربعة عينات المجمعة للأصناف الأربعة ، حيث أظهرت الدراسة تماثلاً في نمط التفريد الكهربائي للـ RAPD بين العينات المجمعة لكل صنف والأنماط المختلفة في العينات الفردية لهذا الصنف . وقد أمكن تحديد ١٢ واسم فريد من نوع RAPD مميز لأصناف نخيل البلح الأربعة بالإضافة إلى ذلك تم دراسة عينات الدنا DNA المجمعة الأربعة باستخدام سبعة بادئات من نوع ISSR حيث أظهرت ستة بادئات وجود ٢٤ واسم منفرد للتمييز بين أصناف نخيل البلح الأربعة ، أيضاً شملت الدراسة تقييم الثبات الوراثي لعشرة بادرات ناتجة من بين زراعة الأنسجة لصنف زغلول باستخدام تكنيك الـ RAPD.