ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 46(4): Ge. 1-6, (2008).

THE GENETIC VARIAITIONS BETWEEN *SOLENOSTEMA ARGHLE TISSUE CULTURE DERIVED PLANTS*

BY

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ABSTRACT

Random amplified polymorphic DNA (RAPD) markers were used to evaluate the genetic stability of micropropagated plants of *Solenostema arghle*. Eight arbitrary decamers were used to amplify DNA from *in vitro* plant material to assess the genetic fidelity. All RAPD profiles from micropropagated plants exhibited high similarity together. No variation was detected within the micropropagated plants. The utilization of RAPD markers both for the assessment of genetic stability of clonal materials and to certify genetic stability throughout the systems of micropropagation.

Key wards: *Solanostema arghle*,micropropagation,RAPD,

INTRODUCTION

Solanostema arghle (family Asclepiadaceae) is one of the economical and important medecinal plants grown in South Sinai. *Solanostema arghle* grow in a small area in Dahab and naturaly propagated by seeds. But because of the overgarzing of this plant specie it failed to propagate naturally by producing seeds so that it became endanger and it ought to keep this genetic resource. These plants are containing medecinal derivatives.such as argelosides which have been isolated by Perrone *et al.* (2006), flavonoids by Elbatran *et al.* (2005). and glycosides (argelosides) have been isolated by Plaza *et al.* (2005a and b). They also isolated seven new 15-Keto pregnane glycosides (Stemmosides E-K). These plants should be propagate via tissue culture techniques because of the randum collection it became indenger.

In vitro culture techniques provide an alternative means of plant propagation and a tool for crop improvement (Vasil, 1988). Plantlets derived from *in vitro* culture might exhibit somaclonal variation (Larkin and Scowcroft, 1981) which is often heritable (Breiman *et al.*, 1987). Other reports claim that useful morphological, cytological, and molecular variations may be generated *in vitro* (Larkin *et al.*, 1989). Any system which significantly reduces or eliminates tissue culture generated variations can be of much practical utility.

Randomly amplified polymorphic DNA (RAPD) markers were recently shown to be sensitive for detecting variations among individuals between and within species (Carlson *et al.*, 1991, Potter and Jones, 1991, and Roy *et al.*, 1992). Bouman and Kuijpers (1994) found intraclonal RAPD polymorphism amongst micropropagated *Begonia* plants but at a lower frequency than phenotypic variations and without any correlation with the phenoltype. RAPD markers have been used successfully to assess genetic stability among somatic embryos in spruce species (Isabel *et al.*, 1993; 1996) and among micropropagated plants of poplar (Rani *et al.*,1995). Shenoy and Vasil (1992) reported that micropropagation through meristem culture is generally associated with low risk of genetic instability because the organized meristems are generally more resistant to

genetic changes that might occur during cell division or differentiation under *in vitro* conditions. The present study was undertaken to determine the genetic stability of the micropropagated plants of *Solanostema arghle* using Random Amplified Polymorphic DNA (RAPD) markers.

MATERIAL AND METHODS

Solanostema arghle (family Asclepiadaceae) is one of the economical and important medecinal plants grown in South Sinai. *Solanostema arghle* grow in a small area in Dahab and naturaly propagated by seeds. But because of the overgarzing of this plant specie it failed to naturally propagate by producing seeds so that it became endanger and it ought to keep this genetic resource Six plants from *Solenostema arghle* tissue culture derived plants were included in this study. Tissue culture unit started from the year 2003- 2006 to propagate *Solanostema arghle* (family Asclepiadaceae). Total DNA was extracted from these plants. This work was achieved in the laboratory of Tissue Culture Unit, Genetic Recourses Department, Desert Research Center (DRC).

DNA Extraction and Amplification:

The CTAB (hexadecyltrimethylammonium bromide) method of Dellaporta et al. (1983) was used to extract total DNA . Leaves material of Solanostema arghle were powdered in liquid nitrogen. Amplification of genomic DNA was made on a Perkin Elmer DNA Cycler (BIOMETRA, Germany), using the arbitrary decamers. The eight primers were selected from the primer kit from Operon,their sequences were as follow:

1- OPA15: (5`TTCCGAACCC3`),
2- OP A20: (5`GTTGCGATCC3`)
3- OPB14: (5`TCCGCTCTGG3`),
4- OP CO2: (5`GTGAGGCGTC3`)
5- OPCO4: (5`CCGCATCTAC3`),
6- OP CO7 : (5`GTCCCGACGA3`)
7- OPCO9 : (5`CTCACCGTCC3`),
8- OP C16: (5`CACACTCCAG3`).

A Lampda DNA BstE II (cat. No.AB-0393) digest that contains 14 double stranded fragments of the following sizes (8454, 7242, 6369, 5686, 4822, 4324, 3675, 2323, 1929, 1371, 1264, 702, 224, and 117bp) supplied in loading buffer from AB gene company (www.abgene.com) was used when the PCR products were applied on agarose gel.

Amplifications of genomic DNA of *Solanostema arghle* were performed in 25-µl reaction volumes containing 1.2 units of *Taq* polymerase from Operon Technology, [(10 mM Tris-HCl (pH 9.0), 25 mM KCl, 2 mM $MgCl_2$, 0:2 mM of each dNTP], 24 ng each of random primer and 40 ng of template DNA.The cycle program included an initial 75 sec denaturation at 94°C, followed by 45 cycles of 15 sec at 94°C, 30 sec at 42°C and 75 sec at 72°C, with a final extension at 72°C for 7 min. RAPD fragments were separated electrophoretically on 1.5% agarose gels in 1X TBE buffer, stained with ethidium bromide, and photographed on a UV transilluminator using a digital camera. DNA from each plant was amplified with the same primer more than once, and the banding patterns were compared. Because the RAPD-PCR technology is sensitive to changes in experimental parameters, a total of 8 primers were initially screened against 6 plants selected *in vitro* culture. Fragment sizes were designated as amplified bands, and bands were shared as diallelic characters (present = 1, absent = 0). Those bands amplifying in each instance were scored and included in the analyses.

RESULTS AND DISCUSSIONS

The results were scored as patterns of bands obtained from *Solanostema arghle* micropropagated plants and compared together. Eight different decamers were tested Table (1). Primers produced amplification products that were monomorphic (Figure 1). The size of the monomorphic DNA fragments produced by these primers was ranging from 1874 to 17 bp for OPA15 resulted in 7 bands. The primer OPA20 exhibited 9 bands with size rang from 1620

to15 bp and 1089 to 28bp for OPB14 which resulted in 10 bands .

Primer OPC 02 produced 7 DNA fragments within molecular sizes 712-70bp all samples were highly monomorphic and produced 7 bands, Primer OPC 04 produced 12 DNA fragments and the band size was ranging from 440 bp to 21 bp. But with primer OPC 07 at the size range 809-32bp there were 13 bands, and OPC 09 at the size range 1353 -73 bp there were 13 bands, and finally 10 monomorphic fragments were obtained with primer OPC16 at the band size 640-49bp.

Table (1): Numbers of the monomorphic bands of the PCR products for *Solanostema arghle* tissue culture derived plants.

Primers	R .F.	bands Size range bp	No of bands	Monomorphic bands of samples					
				S 6	S 5	S 4	S 3	S 2	S 1
OPA15	0.33- 0.83	1874-17	7	5	5	5	4	5	4
OPA20	0.37- 0.98	1620-15	9	8	8	6	6	7	5
OPB14	0.42- 0.91	1089-28	10	6	7	9	9	7	7
OPC02	0.42- 0.79	712-70	7	7	7	7	7	7	7
OPC04	0.52 -0.89	440-21	12	7	9	12	12	11	10
OPC07	0.47- 0.91	809-32	13	5	9	12	11	8	6
OPC09	0.44- 0.9	1353-73	13	8	10	10	12	10	9
OPC16	0.58-0.98	640-49	10	3	3	8	9	8	5
Total			81	49	58	69	73	63	53

The samples 1 and 6 were the more polymorphic samples in comparison with the other samples as it is obvious in Table 1 and Table 2.The genetic similarity matrix (Table 2) shows the relation between DNA amplified samples. There was a high similarity between samples which was 93.5% : 72.3%. These data were agree with Abd Alla, 2000) on date palm and *Balanites aegyptiaca*, he mentioned that there were no genetic instability or changes in the amplified DNA of the micropropagated plant. But some variations in the profiles and the similarity matrix may be due to the somaclonal variation that some times occurred in tissue culture process. Also Potter and Jones, 1991; Rani *et al.*; 1995; reported that no polymorphisms or changes in the amplified DNAs were detected after amplification by PCR within micropropagated plants. Similarly, Shenoy and Vasil (1992) reported that micropropagation through meristem culture is generally associated with low risk of genetic instability because the organized meristems are generally more resistant to genetic changes The genetic stability of *Solanostema arghle* shown in this study was in agreement with that obtained by Angel *et al.*(1996) who found that no polymorphism had been shown in cassava (*Manihot esculenta*) plants derived from *in vitro*. In this study, the DNA amplified products exhibited high similarity among all the *in vitro* plants and were similar together. Furthermore, the genome is most probably randomly sampled without the influence of ontogeny. However, only major fragments genetically characterized through segregation analysis should be used as markers. There was disagreement with Rani *et al.* (1995) who found RAPD variations among 23 micropropagated *Populus deltoides* plants originating from the same clone and morphologically similar. Bouman and Kuijpers (1994) also found intraclonal RAPD polymorphism amongst micropropagated *Begonia* plants but at a lower frequency than phenotypic variations and without any correlation with the phenotype.

In conclusion, these results demonstrate that RAPD analysis can be applied to assess the genetic stability between plants derived *in vitro* on an industrial scale as part of crop improvement programs. Also this method might be useful for monitoring the stability of *in vitro* germplasm collections and cryopreserved material.

Table (2): Genetic similarity matrix among the 6 samples of *Solanostema arghle* micropropagated plants as computed according to Dice coefficient from RAPD.

Proximity Matrix						
	Dice (Czekanowski or Sorenson) Measure					
	VAR00001	VAR00002	VAR00003	VAR00004	VAR00005	VAR00006
VAR00001						
VAR00002	0.841					
VAR00003	0.746	0,85				
VAR00004	0.723	0.797	0,935			
VAR00005	0.768	0.86	0,879	0.917		
VAR00006	0.784	0.883	0,869	0.862	0. 914	
This is a similarity matrix						

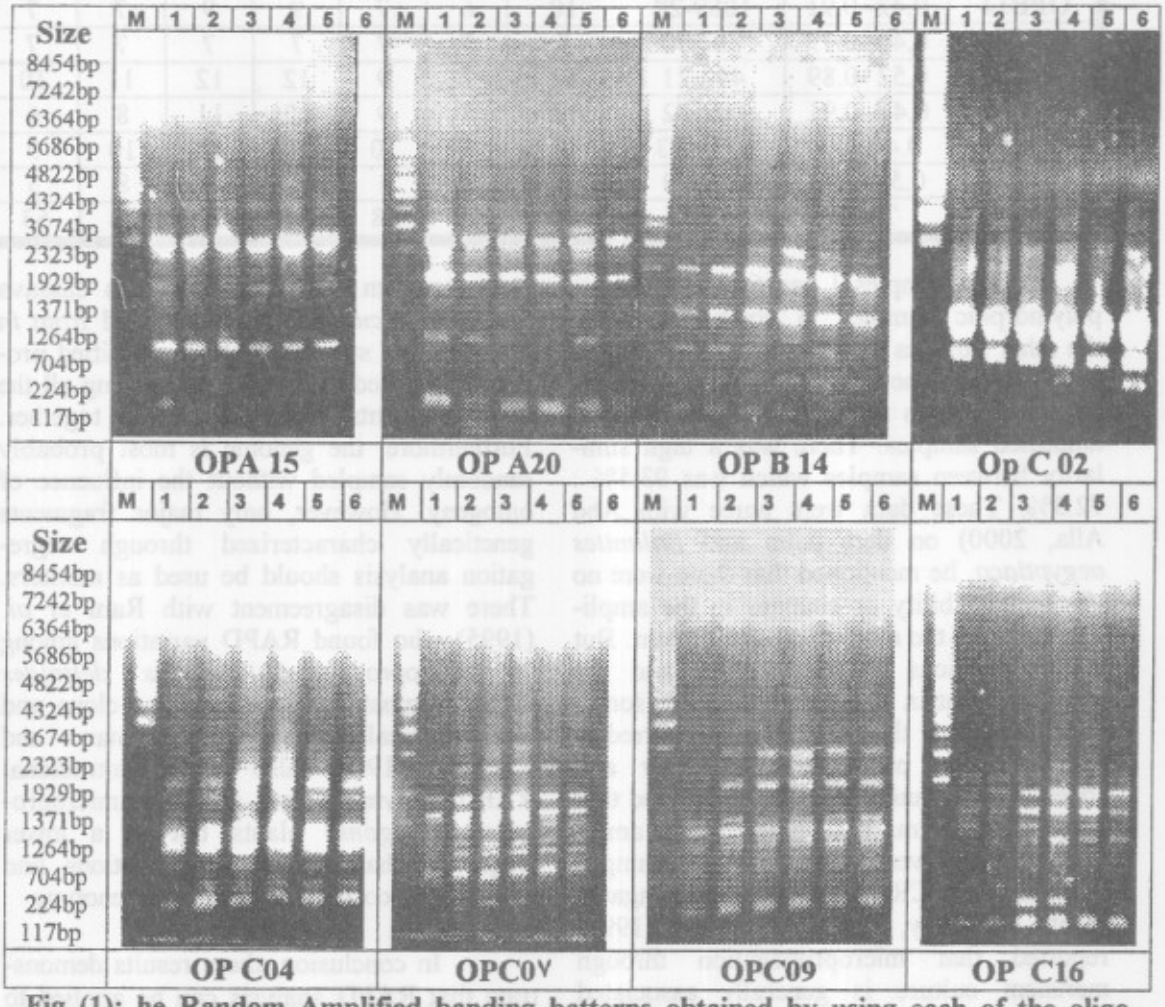

Fig (1): he Random Amplified banding batterns obtained by using each of the oligo primers of Operon for the *Solanostema arghle* tissue culture derived plants as detected.

REFERENCES

Abd-Alla, M.M (2000): Biotechnological studies on date palm tissue cultured plants. PhD thesis Faculty of agriculture Ain Shams Uni.

Angel, F.; Barney, V.E.; Tohme, J. and Roca, W.M. (1996):Stability of cassava plants at the DNA level after retrieval from 10 years of *in vitro* storage. Euphytica 90: 307_313.

Breiman, A.; Felsenburg, T.and Galun, E. (1989): Is *Nor* region variability in wheat invariably caused by tissue culture? Theor. Appl. Genet. 77: 809_814.

Bouman, H. and Kuijpers, A.M. (1994): RAPD-patterns and somaclonal variation in *Begonia*. *In* Abst. 4th Int. Congv. Plant Mol. Biol., Amsterdam, Poster 37.

Carlson, J.E.; Tulsieram, L.K.; Glaubitz, J.C.; Luk, V.W.K.; Kauffeldt C., and Rutledge. R. (1991): Segregation of random amplified DNA markers in F1 progeny of conifers. Theor. Appl. Genet. 83: 194-200.

Dellaporta, S.L.; Wood, J.and Hicks, J.B. (1983): A plant DNA mini preparation. Version III. Plant Mol. Biol., Rep. 1: 19-21.

El-Batran, S.A.; Abdel-Azim, N.S.; Abdel Shafeek, K,K.A.; Shahat, A.E.A. and Elissery, M.M. (2005): Flavonoidesof Gomphocarpus sinaicus and evaluation of some pharmacological activites. Natural product sciences. 2005; 11(4):233- 239.

Isabel, N.; Tremblay, L.; Michaud, M.; Tremblay, F.M. and Bousquet J.. (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.

Isabel, N.; Tremblay, L.; Michaud, M.; Tremblay, F.M. and Bousquet, J. (1996): Occurrence of somaclonal variation among somatic embryoderived white spruces (*Picea glauca*, Pinaceae). Amer. J. Bot. 83: 1121_1130.

Larkin, P.J. and Scowcroft, W.R. (1981): Somaclonal variation- a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197_214.

Larkin, P.J.; Banks, P.M.; Bhati, R.; Bretell, R.S.; Davies, P.A.; Rayan, S.A.; Scowcroft, W.R.; Spindler, L.H. and Tanner, G.H. (1989): From somatic variation to variant plants: mechanisms and applications. Genome 31: 705_711.

Perrone, A; Plaza, A; Ercolino, S.F; Hamed, A.L; Pizza, C. and Piacento, S. (2006): 14,15-Secopregnane derivatives from the leaves of *Solanostema arghle*. Journal of natural products, Salerno, Italy. 2006; 69(1): 50-54.

Plaza, A.; Perrone,A.; Balestrieri, C.; Balestrieri, M.L.; Bifulco, G.; Carbone,V.; Hamed, A.; Pizza, C.; Piacente, S. (2005 a): New antiproliferative 14, 15 seco-pregnane glycoside from *Solanostema arghle*.Tetraherdon, 2005; 61 (31):7470-7480

Plaza, A.; Perrone, A.; Balestrieri, M.L.; Felice, F.; Balestrieri, C.; Hamed, A.I.; Pizza, C.; Piacente, S. (2005 b): New unusual pregnane glycosides with anti-proliferative activity from *Solanostema arghle*. Steroides,2005;70 (9):594-603.

Potter, R. and Jones, M.G.K. (1991): An assessment of genetic stability of potato *in vitro* by molecular phenotypic analysis and. Plant Sci. 76: 239_248 .

Rani, V.; Parida, A.and Sraina, N. (1995): Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoides* Marsh. Plant Cell Rep. 14: 459-462.

Roy, A.; Frascaria, N.; MacKay, J. and Bousquet, J. (1992): Segregating random amplified polymorphic DNAs (RAPD) in *Betula alleghaniensis*. Theor. Appl. Genet. 85:173-180 .

Shenoy, V.B. and Vasil, I.K. (1992): Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napiergrass (*Pannisetum purpureum* K. Schum). Theor. Appl. Genet. 83: 947_955.

Vasil, I.K. (1988): Progress in the regeneration and genetic manipulation of cereal crops. BioTechnology 6: 397_402.

Welsh, J. and McClelland, M. (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213_7218.

الاختلافات الوراثية لنباتات الحرجل الناتجة من زراعة الانسجة باستخدام التضاعف العشوائى المتسلسل

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أجريت هذه التجارب علي نباتات الحرجل الناتجة من الإكثـار المعملـي لهـذا النبـات المهـدد بالانقراض في الفترة من ٢٠٠٣الى ٢٠٠٦بمعمل زراعة الأنسجة بمركز بحوث الصحراء لدراسة الثبـات الوراثي لها. أثبتت الدراسة على المادة الوراثية المستخلصة من النباتات في الأشـكال المظهريـة للتفاعـل العشوائي المتسلسل باستخدام ثمانية بادئات ذات عشرة قواعد ان هناك درجة عالية من الثبات الوراثي بـين هذه النباتات وبعضها.