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THE GENETIC VARIABILITY OF THE MICROPROPAGATED *SOLENOSTEMA ARGHLE* TISSUE CULTURE DERIVED PLANTS USING RAPD TECHNIQUE

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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 were monomorphic and similar 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 is discussed.

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. 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 propagated 0% 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 morph logical, 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; 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 phenotype. 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.

MATERIALS AND METHODS

Six plants from *Solenostema arghle* tissue culture derived plants were included in this study. 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 (hexadecyltri-methylam-monium bromide) method of Dellaporta *et al.*, (1983) was used to extract total DNA. Leaf material was 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 were performed in 25µl reaction volumes containing 1.2 units of *Taq* polymerase from Operon Technology, [(10 mM Tris-HCl (pH 9.0), 25mM KCl, 2mM $MgCl_2$, 0.2mM of each dNTP], 24ng each of random primer and 40ng of template DNA. The cycle program included an initial 75sec denaturation at 94°C, followed by 45 cycles of 15 sec at 94°C, 30 sec at 42°C and 75sec 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 DISCUSSION

The results were scored as patterns of bands obtained from *in vitro* micropropagated plants and compared together. Eight different decamers were tested Table -1. Primers produced amplification products that were polymorphic (Figure1). The size of the polymorphic DNA fragments produced by these primers was ranging from 1874 to 17 bp for OPA15

resulted in 10 bands with sample 1. The other 5 samples exhibited 9 bands, band size was 1620 to 15 bp for OPA20 which resulted in13 bands, 4 samples produced 12 bands, and 1089 to 28bp for OPB14 which resulted in 11 bands S1, S2 and S3 exhibitd polymorphism with this primer (3 bands).

Primer OPC 02 produced six DNA fragments within molecular sizes 712-70bp all samples produced 5 bands, Primer OPC 04 produced 11 DNA fragments there were polymorphism in 3 bands, but in the case of primer OPC 07 at the size range 809-32bp there were 13 bands, the polymorphism was in 4 bands and OPC 09 at the size range 1353 -73 bp there were 13 bands the polymorphism was in 3 bands and finally there are 10 polymorphic fragments were obtained with primer OPC16 at the band size 640-49bp. Some polymorphisms were observed with amplified DNA fragments profiles Table 1 and Fig -1. In the case of primer OPB14 samples No. 1and 2 showed three polymorphic bands than the other samples. Primer OPC04 showed 3 polymorphic bands with the samples 1 and 2. There were 4 polymorphic bands were observed with each of OPC07 (sample 1) and OPC16 (samples 1, 2 and 6) primers. The sample 1 was the more polymorphic sample in comparison with the other samples as it is obvious in Table 1 and 2.

The genetic similarity matrix (Table 2) shows the relation between DNA amplified samples. There was a high similarity between samples, which was 99%: 85%. These data were agree with Abd Alla (2000; 2007) on date palm and *Balanites aegyptiaca*, he mentioned that there were no genetic instability or changes in the amplified DNA of the micropropagated plant. Also Potter and Jones, (1991) and 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 was similar together. Furthermore, the genome is most probably randomly sampled without the influence of ontogeny. However, only major fragments genetically characterized thro-ugh 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- 1: The band number and percentage of polymorphic bands of the PCR products for the tissue culture derived plants.

primers	bands Size range bp	RF range	Band No	Band no. and percentages of polymorphic band (PPB%)					
				Samples No					
				S1	S2	S3	S4	S5	S6
OPA15	1874-17	0.38-083	10	10	9	9	9	9	9
OPA20	1620-15	037-0.98	13	12	13	12	12	13	12
OPB14	1029-28	0.42-0.91	11	8	9	11	11	10	11
OPC02	712-70	0.42-0.79	6	5	5	5	5	5	5
OPC04	440-21	0.52-0.89	11	8	9	11	10	10	10
OPC07	809-32	0.47-0.91	13	9	11	11	11	10	10
OPC09	1353-73	0.44-.912	13	10	11	12	12	12	11
OPC16	640-49	0.58-0.98	10	6	8	9	9	9	8

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

	VAR0001	VAR0002	VAR0003	VAR0004	VAR0005	VAR0006
VAR0001	-					
VAR0002	0,966	-				
VAR0003	0,96	0,99	-			
VAR0004	0,95	0,98	0,99	-		
VAR0005	0,99	0,96	0,95	0,94	-	
VAR0006	0,9	0,87	0,86	0,85	0,91	-

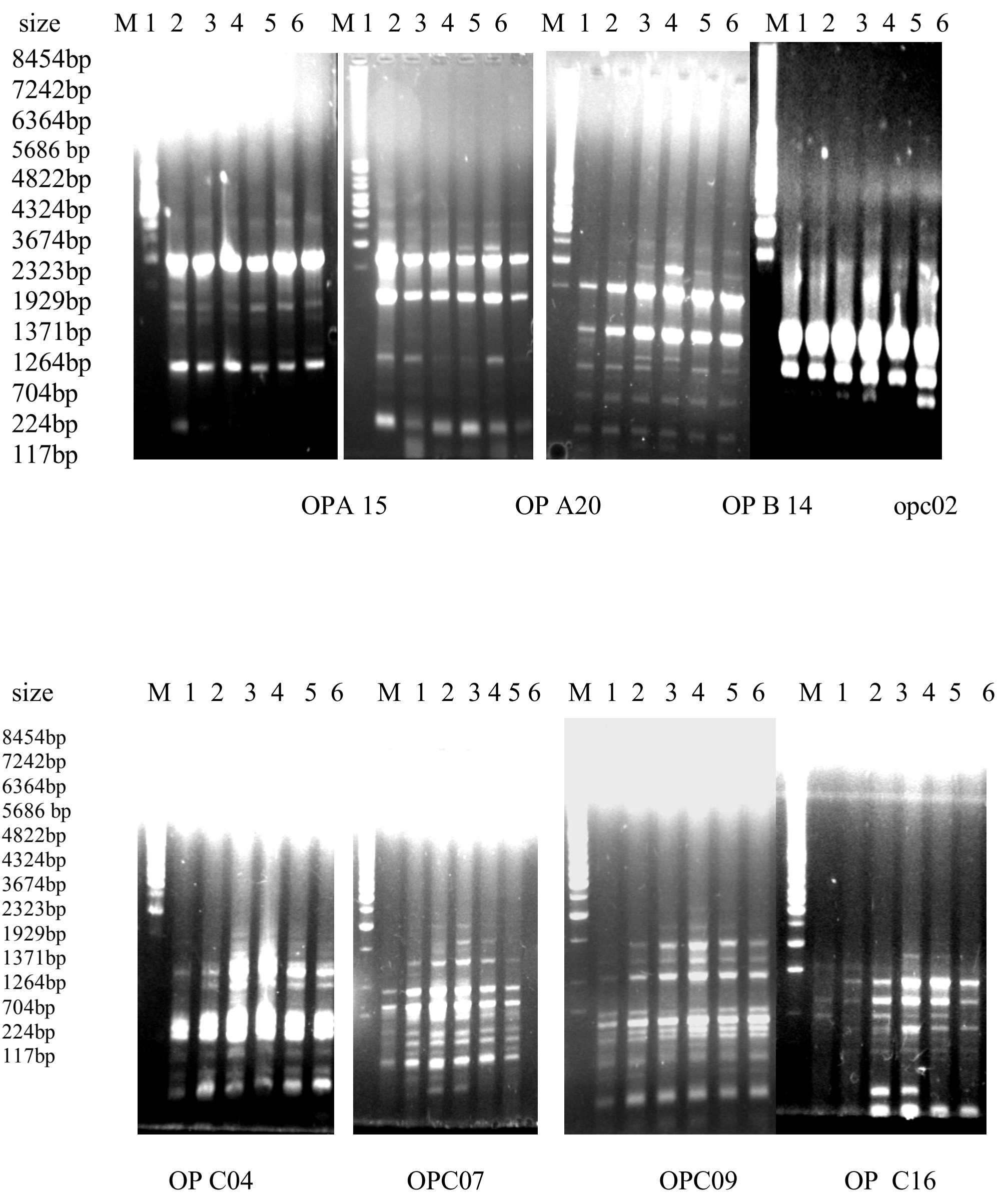

Fig.-1: The Random Amplified banding batterns obtained by using each of the oligo primers of Operon for the ***Solanostema arghle*** tissue culture derived plants as detected.

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