

MOLECULAR GENETIC DIVERSITY AND EFFICIENT PLANT REGENERATION SYSTEM VIA SOMATIC EMBRYOGENESIS IN SWEET POTATO (*Ipomoea batatas* (L.) Lam.)

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Sweet potato (*Ipomoea batatas* (L.) Lam.) belongs to *Convolvulaceae* family, it is one of the most important food crops and ranks the fourth among the food crops after rice, potato and wheat and the seventh in the world in terms of total production (FAOSTAT, 2008). It contains remarkable vitamin A (Woolfe, 1992) and a staple source of calories. Sweet potato ranks the third among the ten major crops of the world in calorie content (Boukamp, 1985). These aspects are important to agriculturists, ecologists and economists interested in developing sustainable system for production of food in the tropics (Hagenimana *et al.*, 1999). Sweet potato productivity is limited by a number of both biotic and abiotic stress factors (Guo *et al.*, 2006). One of the most important limiting factors for sweet potato production is viral diseases. Up to 20 different types of viruses have been identified to infect sweet potato (Loebenstein *et al.*, 2003). Although, soil salinity is one of the abiotic factors that affect sweet potato production and expansion of cultivation worldwide including Africa where it is a staple food (Dasgupta *et al.*, 2008).

Genetic variation is the source for plant breeding; also biotechnology provides an easy access to innovative genetic variation to support breeding. Tissue culture is the most potent part of biotechnology and is mainly employed in sweet potato germplasm maintenance, besides the production of somaclonal variants and the development of transgenic plants (Nadia *et al.*, 2010). Somaclonal variation can be determined by the analysis of phenotype, chromosome number and structure, proteins, or direct DNA evaluation of plants (Jain, 2001; Smykal *et al.*, 2007).

Studies on the regeneration of sweet potato have predominately focused on the somatic embryogenesis method for plant regeneration (Feng *et al.*, 2011). Production of suitable somatic embryogenic tissues is very commonly cultivar-specific and many sweet potato cultivars were found to be still recalcitrant (González *et al.*, 2008; Feng *et al.*, 2011). Due to these difficulties, few studies have focused on organogenesis in sweet potato regeneration with some success (Morán *et al.*, 1998; Luo *et al.*, 2006; González *et al.*, 2008).

The use of biochemical markers based on total seed and tuberous protein and enzyme proved to be a useful and inexpensive method of developing genetic markers for detection of genetic analysis of several plant species. Isozyme reveal differences among storage proteins or enzymes expressed by different alleles at one or more gene loci (Oppong-Konadu *et al.*, 2005). Total protein profiles and isozymes have been used in genetic diversity studies in sweet potato to identify duplicates in germplasm collections (Saha *et al.*, 2000).

Molecular markers are also tools used for the detection of variability at the DNA level. Among them, RAPD marker (Random Amplified Polymorphic DNA) is the most accessible and has been applied to a wide range of plant crops to assess genetic diversity, fingerprint clone, evaluate genetic relationship between accessions or cultivars and detect accession collection (Williams *et al.*, 1990; Rafalski and Tingey, 1993). Principal Coordinate Analysis (PCoA) was used for estimating the genetic similarity and quantitative variation in crops (Rahim *et al.*, 2008; Sesli and Yeğenoğlu, 2010). This analysis discriminated the genotypes into various groups based on the geographic origin comparatively in a better way than the UPGMA clustering (Selvaraj *et al.*, 2010).

The main objectives of this study were the establishment of an efficient and rapid system for plant regeneration through somatic embryogenesis of an

commercial Egyptian sweet potato cultivar cv. Abees to enhance future breeding programs, and also the assessment of the genetic diversity of the parental and somaclonal variants through biochemical (isozyme) and molecular (RAPD) markers.

MATERIALS AND METHODS

The present study was conducted at the laboratories of the Department of Genetics and the Experimental Farm, Faculty of Agriculture, Kafr Elsheikh University, Egypt.

Plant material and explant preparation

Healthy and virus-free plant material from Abees cultivar was *in vitro* micro-propagated using nodal cutting system. Cultures were routinely subcultured every six weeks on MS medium (Murashige and Skoog, 1962) containing 3% sucrose, 0.2% phytigel and maintained in controlled room culture at temperature of $25\pm 2^{\circ}\text{C}$ for 16 h/8 h light/dark cycle. Stem segments without buds from the *in vitro* manipulated plants were used as explants.

Induction of somatic embryogenesis and plant regeneration system

Callus was initiated from stem segment explant of sweet potato Abees cultivar in three different callus induction media to evaluate their effects on regeneration efficiency. The three tested media containing Murashige and Skoog salts and vitamins supplemented by 3% g sucrose,

0.2% g phytigel as a solidifying agent and pH at 5.8. The first callus induction medium (M1) contained 100 mg/l myo-inositol supplemented with 2 mg/l 2,4-D, the second medium (M2) contained 100 mg/l myo-inositol supplemented with BAP (8 mg/l), on the contrary, the third medium (M3) was supplemented with myo-inositol (100 mg/l), IAA (2 mg/l) and BAP (4 mg/l). Cultures were then incubated in dark in controlled room culture at temperature of $25\pm 2^{\circ}\text{C}$ for 2-3 weeks.

To obtain somaclonal variants, well-proliferated calli were incubated for two weeks in the same callus induction medium. Cultures were transferred to controlled room culture under fluorescent light for 16 h/8 h light/dark cycle for another week. For shoot induction and multiplication, calli from the different callus induction media were transferred to regeneration medium comprising complete MS medium supplemented with 100 mg/l myo-inositol and 6.0 mg/l BAP. The following characteristics were recorded:

- 1- **Callus induction percentage:** (number of explants produced callus/number of initiated explants x 100).
- 2- **Callus type:** it was scored as no callus (0), compact (1), intermediate (2) and friable (3).
- 3- **Percentage of calli with shoots:** (number of shooted calli/number of initiated calli x 100).
- 4- **Number of shoots/callus:** (number of regenerated shoots/number of shooted calli),

- 5- **Root induction percentage:** (number of rooted calli/number initiated calli x 100).

Hardening of in vitro plantlets

Regenerated shoots, 3-5 cm or longer, were excised from the callus and cultured singly in glass jar for more rooting and shoot elongation on hormone free half MS medium supplemented with 15 g/l sucrose and 2 g/l phytigel. Regenerated healthy plantlets, with a well-developed rooting system (~10-15 cm tall), were gently removed and washed carefully to remove phytigel and then transferred to small pots containing 2:1 (v/v) sterile mixture of peatmos:sand. Each pot was enclosed in a polyethylene bag after watering to maintain a high relative humidity, and maintained in controlled room culture under fluorescent light for 16 h/8 h light/dark cycle at $25\pm 2^{\circ}\text{C}$. After seven days, polyethylene bags were pored gradually and subsequently removed for 15 min. every day for the next seven days, then for half an hour for the next five days, then the bags were totally removed and the plants were transferred to bigger clay pots in green house for growing until maturity and flowering. Each somaclone was cutted and cultured in the land for evaluation under hard conditions of drought, salinity, and temperature (cooling and heating).

Data collection and statistical analysis

All data were analyzed statistically as complete randomized design in three replicates (n=5) and the mean values ob-

tained from the treatments were compared using Duncan's multiple range test (Duncan, 1955) at the 5% and 1% significant levels using SXW statistical software.

Assessment of somaclonal variations

Biochemical analysis was carried out to determine somaclonal variations among the regenerated plants which have been developed on different culture media. The 98 regenerated somaclonal variants were maintained *in vitro* for subsequent evaluation of biochemical investigation. They were divided into two groups for isozyme evaluation. The first group contained 70 somaclonal variants, while the second group contained 28 somaclonal variants and they were evaluated for peroxidase (PRX) and esterase (EST) isozymes, respectively. The non-regenerated "Abees" original donor plants were used as control in this study.

Isozyme analysis

For extraction of isozymes, leaf samples were prepared according to Aboulila (2009) with some modifications. Homogenate was prepared from 0.5 g fresh leaves from each sample, crushed in liquid nitrogen in an ice chilled mortar and pestle with 1.0 ml of ice cold extraction buffer [0.2 M Tris-HCL buffer PH 8.5 containing 20% sucrose, 5mM dithiotritol (DTT), 0.03% (v/v) β -mercaptoethanol, and 4% (w/v) polyvinyl-polyprolidone]. The extract was centrifuged twice at 12,500 rpm for 30 min. at 4°C and the supernatant was separated. Polyacrylamide gel electrophoresis

(PAGE) described by Laemmli (1970) was used for qualitative analysis of enzymes. Peroxidase bands were detected as described by Scandalios (1964) in 0.25% benzidine dihydrochloride and 0.30% hydrogen peroxide. While EST isozyme bands were detected on the gel by using α -naphthylacetate as substrate and subsequent color was developed with fast blue RR salts (Scandalios, 1969).

Stained gels were placed in a light box to determine their isozyme banding patterns. The visual bands were recorded and the gels were photographed. The data were expressed as means \pm standard error of mean. The percentage amounts for isoforms of each enzyme were compared using SXW software and the significance was set at $P < 0.05$. The number of bands was recorded and their relative mobilities (Rf) were obtained using Gel Analyzer software (2010a).

Molecular analysis

The 98 obtained regenerated plants which were exposed for isozyme analysis were transferred to another greenhouse for testing their ability for growing under stress conditions of drought, salinity, cooling and heating. Many of these regenerated plants were not able to grow under stress conditions and died. Out of these regenerated plants only 15 plants were very healthy and grew very good under the stress conditions and subcultured in the normal soil under the normal environmental conditions. These 15 somaclonal variants were subcultured once again and also proved to be healthy

and normal plants. Out of these 15 somaclonal variants, only four (S1, S2, S3 and S4) were chosen to be applied for molecular genetic analysis using five RAPD primers.

DNA extraction

Genomic DNA was extracted from fresh leaves of sweet potato plants (control) and four selected somaclonal variants using Cetyl trimethyl ammonium bromide (CTAB)-based procedure (Murray and Thompson, 1980) and the DNA was quantified on 1.5% agarose gel using standard DNA ladder.

RAPD primers and amplification conditions

Five 10-mers arbitrary RAPD primers (OPB-05, OPB-06, OPB-07, OPH-01 and OPH-03) were screened for studying genetic diversity among the parental and selected somaclonal variants. Amplification reactions were performed in a 20 µl volume containing 10 µl of master mix (2x PCR Master mix solution (i-Taq™) iNtRON Biotechnology, Korea), 1 µl of primer 10 pmol, 8 µl of double distilled water and 40 ng of template DNA. The reaction mixtures were over-laid with 20 µl of mineral oil per sample. The PCR amplification was performed on a thermal cycler (Perkin Elmer Cetus) programmed for a first denaturation step of 5 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min annealing temperature at 30°C, 1.50 min extension temperature at 72°C. The final stage was kept at exten-

sion temperature of 72°C for 7 min and then held at 4°C until the tubes were removed. Amplification products were analyzed by electrophoresis and detected on Benchtop UV-transilluminator and photographed using photoDoc-It™ Imaging System. The molecular size of the amplified products was determined against 1 Kb DNA ladder with EtBr stain.

Scoring and analysis of RAPD data

Data matrix was calculated from photographs of gels and analyzed using Gel Analyzer (2010a) program. These data assigning 1 to visible bands and 0 to absent bands for each primer and entered in the form of a binary data matrix. From this matrix, Cluster analysis and Principal Coordinate analysis (PCoA) were performed using Nei & Li coefficient's (Nei and Li, 1979) by computational package MVSP 3.1. The data were processed by cluster analysis using the unweighted pair group average method (UPGMA) and plotted in the form of dendrogram using the same software.

RESULTS AND DISCUSSION

Effect of plant growth regulators on induction of somatic embryogenesis and shoot regeneration

This experiment was planned for induction of high frequency of regenerated plants and somaclonal variations from sweet potato Abees cultivar. The results indicated that the highest percentage of callus induction (100%) was obtained

from M3 medium and it did not differ significantly from M2 which gave high percentage of 98.67 % for the same character. While the lowest percentage of callus induction was obtained from M1 medium (92.13%). On the other hand, BAP concentration of 8.0 mg/l in M2 medium was shown to be the best phytohormone in the callus type, percentage of calli with shoots and number of shoots/ callus, while it gave the highest values of 1.60, 100 and 8.20, respectively and they differed significantly from M1 and M3 media (Table 1). M3 callus induction medium exhibited the highest mean value for root induction percentage but did not differ significantly from the other two media. All the used media led to the production of roots directly from callus in this cultivar "Abees" as shown in Fig. (1).

The present study was initiated with the aim of developing a protocol for rapid induction of embryogenic callus from the Egyptian cultivar Abees. Other reports showed that there is a correlation between the quality and quantity of embryogenic callus and subsequent plant regeneration in sweet potato (Al-Mazrooei *et al.*, 1997). Therefore, it was important to establish rapid system for somatic embryogenesis for the Abees cultivar to facilitate regeneration and to improve traits of interest (Anwar *et al.*, 2010).

In the present study, sweet potato plants were regenerated from cultivar Abees within 3-4 months as shown in Fig. (2), on contrast with the reported regener-

ation system by Kreuze *et al.* (2008) who needed 12 months to regenerate Huachano cultivar. The reduction of time to regenerate plants is of high significance since long time culture of callus-derived plants increases the chance for induction of somaclonal variations. Other researchers have reported rapid regeneration of Jewel through organogenesis (Luo *et al.*, 2006; González *et al.*, 2008). They regenerated plants within ten weeks, and encountered the presence of escapes when polymerase chain reaction (PCR) was used to examine transformed plants regenerated through organogenesis. On the other side, the use of somatic embryogenesis showed 100% transformation frequency as reported by many workers among all regenerated plants although the number of regenerated plants was low (Song *et al.*, 2004).

Although the number of plants produced in the present study was high, the protocol demonstrated reproducibility for the reported cultivars since plants were regenerated using embryogenic calli obtained from three different experiments. A reproducible protocol is more important than regenerating a high number of plants in a single experiment (Yu *et al.*, 2007). Low numbers of regenerated plants have been reported in many previous studies with sweet potato cultivars from different geographical areas (Otani *et al.*, 2003).

Assessment of somaclonal variations as revealed by isozyme analysis

The isozyme patterns of PRX and EST were used to determine the genetic

variability among 98 obtained sweet potato plants regenerated from the three-different media.

Peroxidase isozyme analysis

The electrophoretic banding patterns of PRX isozyme for 70 of the selected 98 (from 1 to 70) sweet potato somaclonal variants and their original Abees cultivar (control) are shown in Fig. (3 A, B, C, D and E). The PRX isozyme pattern contained numerous bands, and its complexity could be related to the fact that sweet potato is allohexaploid. Most of the tested regenerated plants exhibited differences in both number and activity of bands. The detection of PRX patterns in the parent and somaclonal variants was distributed as seven common bands in the parent (lane C) and somaclones (lanes from 1 to 70) as shown in Fig. (3). The mean percentage of appearance of PRX isoforms and relative mobility (RF) were shown in Table (2).

The seven bands were polymorphic with different activities and the mean percentage of appearance of the seven bands ranged from 49.32 to 93.32. In addition, the main band (PRX-1) with (RF= 0.079-0.101) and mean percentage of appearance of 93.32% as shown in Table (2) was found in the original cultivar and all somaclones, except somaclones No. 5, 11, 12, 18 and 20. This band showed, over expression in somaclone No.7 (Fig. 3A). Also, most of the tested regenerated plants exhibited differences in both num-

ber and activity of bands and showed number of bands more than their original cultivar. These alterations in isozyme patterns which were detected in peroxidase zymograms might reflect the changes in genetic materials produced by tissue culture.

Esterase isozyme analysis

Isozyme patterns of EST were determined and analyzed in the parent and 28 (from 71 to 98) selected somaclones regenerated from Abees cultivar as shown in Fig. (4 A and B). In respect of the EST zymograms, a view of sub-patterns was observed among the different tested regenerates. No. monomorphic bands were detected in all of the 28 regenerated plants in addition to the parent. The three exhibited bands were polymorphic, while they were absent from some regenerated plants and their parent and presented in different intensities. One of them (EST-2) was found in the parent and the other two bands (EST-1 and EST-3) were found in some of regenerated plants (somaclones) as new bands.

In this work, an increase in PRX and EST content was observed in all somaclonal variants when compared with control. Similar results were obtained by Lara *et al.* (2003) using PRX as marker for monitoring genetic stability and/or variability, caused by *in vitro* cultures in potato. They also observed a high enzymatic activity in the analyzed material. Iglesias *et al.* (1995) reported that bio-

chemical polymorphism as well as resolution and repeatability in PRX isozyme of leaf tissue was better than that observed in shoot and root samples of potato cultivars.

Peroxidase isozyme has been widely used as genetic marker, since it presents different isoforms in most vegetative tissues (Lara *et al.*, 2003). Peroxidase isozymes also play an important role in the biosynthesis of cell wall components, as well as cellular differentiation (Christensen *et al.*, 1998) and their relationship with resistance to adverse biotic and abiotic factors (Dalisay and Kuc, 1995).

Assessment of genetic diversity using RAPD assay

A total of five RAPD primers were evaluated for their ability to prime PCR amplification of sweet potato genomic DNA of cultivar Abees (parent) with its four somaclonal variants (S1, S2, S3 and S4). A total of 68 RAPD loci were amplified from different plant genotypes. Most of the PCR products were in the molecular size range of 217-3990 bp with an average of 13.6 loci per RAPD primer (Fig. 5). Out of the 68 loci scored, 26 loci (38.24%) were found to be polymorphic and 42 loci (61.76%) were found to be monomorphic in nature with an average of 5.2 polymorphic loci per primer (Table 3). The polymorphism% ranged between 18.18 (for OPB-05) and 75% (for OPB-07). Moreover, all primers produced positive and negative unique DNA bands,

except OPB-05 for negative unique bands and OPB-07 for positive unique bands.

The high level of diversity found in genotypes of sweet potato may be associated with spontaneous mutations, knowing that sweet potato presents a high frequency of somatic mutations and is very common in species selection (He *et al.*, 2006). Many studies have reported that DNA fingerprinting techniques are the best techniques to distinguish related genotypes from each other and for analysis of genetic similarity (Spooner *et al.*, 2005; Solis *et al.*, 2007). In a study on sweet potato using 18 RAPD primers, a total of 150 bands were scored, out of them 145 were polymorphic using 18 RAPD primers (Moulin *et al.*, 2012); and with 15 primers, 86 fragments were produced, which were 100% polymorphic (Zhang *et al.*, 1998). Molecular analysis using RAPD technique is also being used to detect somaclonal variation in various crops. Similar work was reported by Bordallo *et al.* (2004) using RAPD to detect somaclonal variation in five commercial potato cultivars, Achat, Baraka, Baronesa, Bintje and Contenda using twenty arbitrary sequence primers.

Cluster analysis and principal coordinate analysis (PCoA)

Cluster analysis and Principal Coordinate analysis (PCoA) for Abees cultivar and its selected four somaclonal variants of sweet potato were performed based on the relative genetic distances from RAPD data. The PCoA determines

the consistency of the differentiation among the genotypes defined by the cluster analysis (Adhikari *et al.*, 2015). Cluster analysis indicated that the genetic distances between all the sweet potato genotypes were very high; indicating that degree of similarity was low among these genotypes. The UPGMA analysis divided the parent and its somaclonal variants into two separate clusters. The first cluster comprised of S4 only, showing dissimilarity from both the somaclonal variants and the parent. The rest variants and the parent were clustered in the second group (Fig. 6) which contained two sub-clusters, one containing S1 only and the other sub-cluster contained S2, S3 and the parent. S2 showed more genetic similarity to the parent.

Principal coordinate analysis (PCoA) based on RAPD data has managed to divide these genotypes into three groups. The first eigenvector (PCoA axis1) accounted for 59.4 % of the data variation among groups (Fig. 7). The second eigenvector (PCoA axis2) explained 25.7% of the variation. This value indicated that the PCA was succeeded in assessment of genetic diversity and description of heterogeneity within the studied genotypes. These results agree with those reported by (Sonja *et al.*, 2008; Abd El-Aziz and Habiba, 2016).

It was of great interest to mention that S4 somaclonal variant showed best results in all vegetative and yield characters (unpublished data). This somaclonal variant (S4) showed also good re-

sults in the expression of the used isozyme (peroxidase). By molecular studies, S4 was isolated alone in cluster analysis and also specific position in principal coordinate analysis.

In conclusion, the present study had overcome a major setback in regeneration through somatic embryogenesis by successfully reducing the time for regeneration of the popular Egyptian sweet potato cultivar Abees. The culture conditions optimized in this study are currently being used in *Agrobacterium*-mediated genetic transformation of Abees sweet potato with genes that have potential to regenerate transgenic sweet potato resistant to viral diseases. Also, this study concluded that both isozymes (peroxidase and esterase) and DNA fingerprint using RAPD analyses can be used to detect genetic variation caused by somaclonal variation. As well as the analyses based on these techniques such as cluster analysis and principal coordinate analysis are suitable tools for assessing genetic diversity in sweet potato. All of this leads to consider these techniques as important tools for breeding and improvement of sweet potato. It is recommended that somaclonal variant (S4) as source for more genetic studies using DNA sequencing to identify the presence of specific gene(s) in this somaclone.

SUMMARY

Somatic embryogenesis and plant regeneration at high frequency have been restricted to few sweet potato varieties.

For enhancing and accelerating somatic embryogenesis from stem segments of the Egyptian sweet potato cultivar Abees were investigated using three different phytohormones; 2,4-dichlorophenoxyacetic acid (2,4-D), benzyleaminopurine (BAP) and indole acetic acid (IAA). The phytohormone BAP was found to be the best for the induction of embryogenic calli and most studied traits. Data analysis showed a significant variation in three different tissue culture media for all parameters, expect root induction percentage. Two different isozymes; peroxidase (PRX) and α naphthyl acetate esterase (EST) were used and analyzed to determine the genetic variability among the regenerated plants. The two analyzed isozymes successively showed polymorphic variations among the parent and 98 sweet potato plants regenerated from the three different callus induction media. Peroxidase isozyme produced seven polymorphic bands showing genetic variation as compared to the control (Abees cultivar), while esterase isozyme produced only three polymorphic bands. The regenerated plants exhibited somaclonal variations that can be utilized for selection of desired traits in sweet potato. On the other hand, five RAPD primers were used for assessment of genetic diversity in the somaclonal variants compared with control. A total of 68 RAPD loci were amplified with molecular size range of 300–3000 bp with 13.6 loci per each primer. Out of the 68 loci scored, 26 loci (38.24%) were found to be polymorphic and the polymorphism% ranged between

18.18% for (OPB-05) and 75% for (OPB-07). Moreover, all primers produced positive and negative unique DNA bands, except OPB-05 for negative unique bands and OPB-07 for positive unique bands. The same result was confirmed by the cluster and principal coordinate analyses for the positions of somaclonal variant no. 4 which showed high diversity from the parental cultivar.

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Table (1): Effect of different callus induction media on callus induction%, callus type, % of calli with shoots, No. of shoots/callus and root induction% for Abees sweet potato cultivar.

Callus induction media	Callus induction%	Callus type	% of calli with shoots	No. of shoots/callus	Root induction%
M1	92.13±3.08b	1.27±0.12b	71.67± 8.40b	3.27±0.30b	68.33±8.26a
M2	98.67±1.33a	1.60±0.13a	100.00±0.00a	8.20± 0.75a	65.67±9.33a
M3	100.0±0.00a	1.00±0.00b	32.11± 6.07c	2.20± 0.31b	76.33±6.87a
LSD _{0.05}	5.52	0.29	17.09	1.43	23.46
LSD _{0.01}	7.38	0.39	22.84	1.91	31.36

Table (2): Mean ± SE of the percentage amounts and relative mobilities (RF) for the two studied enzymes in regenerated plants (somaclones) of tissue culture-derived sweet potato.

Enzyme	Isoform (locus)	RF	Mean ± SE
PRX	PRX-1	0.079-0.101	93.32 ± 0.04
	PRX-2	0.155-0.216	77.30 ± 0.09
	PRX-3	0.244-0.349	67.96 ± 0.09
	PRX-4	0.442-0.539	63.96 ± 0.09
	PRX-5	0.560-0.712	70.64 ± 0.10
	PRX-6	0.740-0.928	81.30 ± 0.07
	PRX-7	0.788-0.959	49.32 ± 0.09
EST	EST-1	0.182-0.189	20.00 ± 0.00
	EST-2	0.483-0.482	93.50 ± 0.06
	EST-3	0.710-0.719	40.00 ± 0.00

The significance level was considered when $p < 0.05$.

Table (3): RAPD primer sequences and distribution of amplified fragments in sweet potato Abees cultivar and its selected four somaclonal variants.

Primer name	Primer sequence 5'→ 3'	Molecular size range (bp)	Monomorphic bands	Polymorphic bands				Total number of amplified bands	Percentage of Pol- ymorphic loci
				Polymorphic Without unique	Unique bands		Total		
					Positive	Negative			
OPB-05	TGCGCCCTTC	217-3016	9	-	2	-	2	11	18.18
OPB-06	TGCTCTGCC	285-3489	6	2	3	1	6	12	50.0
OPB-07	GGTGACGCAG	267-2513	2	-	-	6	6	8	75.0
OPH-01	GGTCGGAGAA	316-3990	12	5	2	2	9	21	42.86
OPH-03	AGACGTCCAC	286-3000	13	1	1	1	3	16	18.75
Total			42	8	8	10	26	68	
Mean			8.4	1.6	1.6	2	5.2	13.6	40.96

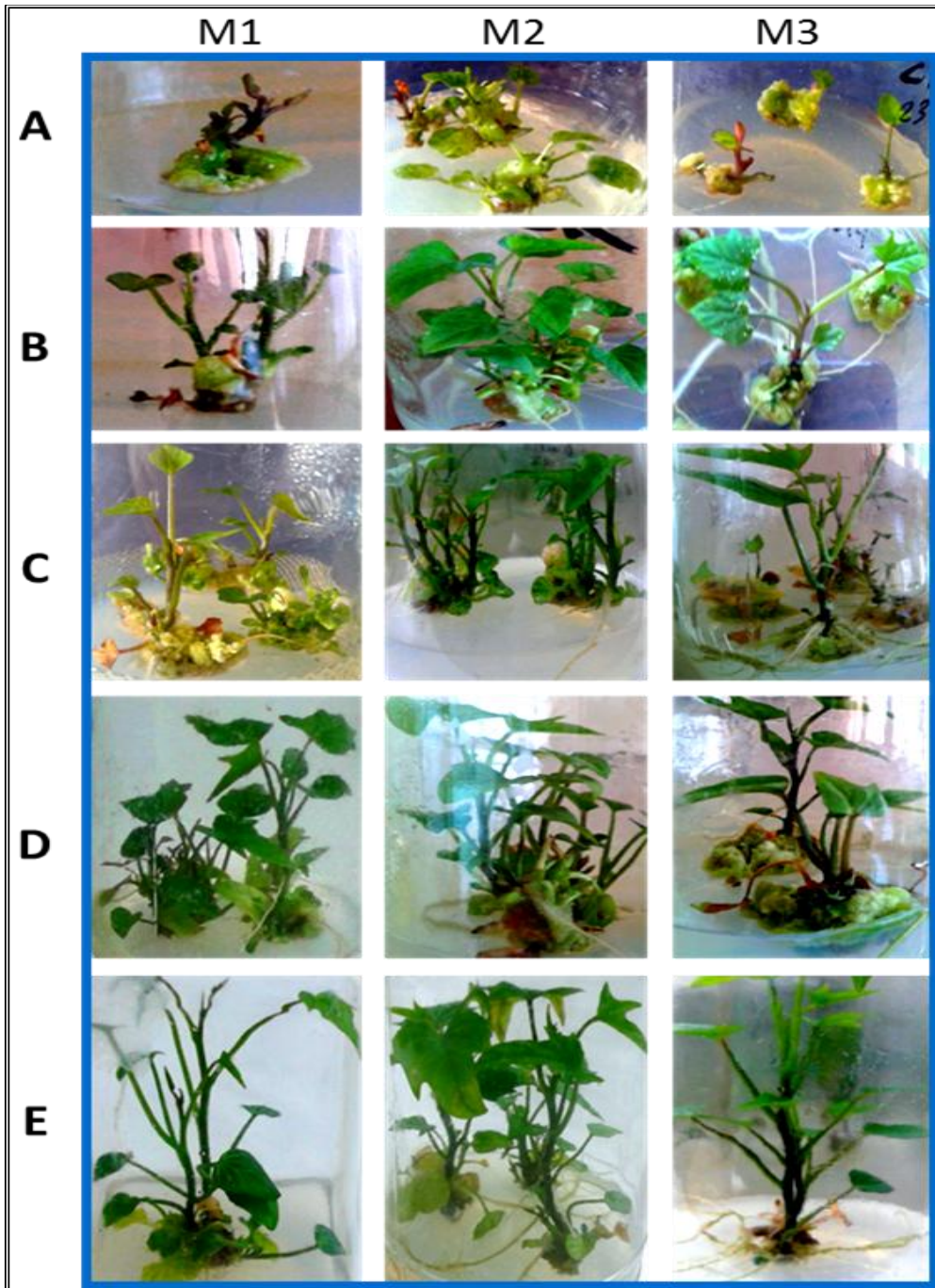


Fig. (1): Schematic illustration of plant regeneration system for sweet potato (*Ipomoea batatas* (L.) Lam.) cv. Abees on the three different callus induction media (M1, M2 and M3). (A) Callus induction, (B) Callus subculture and formation of roots directly from callus, (C) Shoot induction and multiplication on regeneration medium, (D) Shoot elongation and (E) Emergence of amorphous shoots from embryogenic tissue removed from callus and placed directly on hormone free MS medium and plantlets were fully regenerated.



Fig. (2): (A) Growth of regenerated sweet potato plants after transfer from media to small pots containing sterilized soil, (B) Regenerated plants after polyethylene pages half removed, (C) Somaclones in big pots, (D) Big pots after five weeks, (E) Somaclones in green house after two months, (F) Somaclones in green house at maturity after three months and (G) S4, one of the somaclonal variants after sub-culture in land.

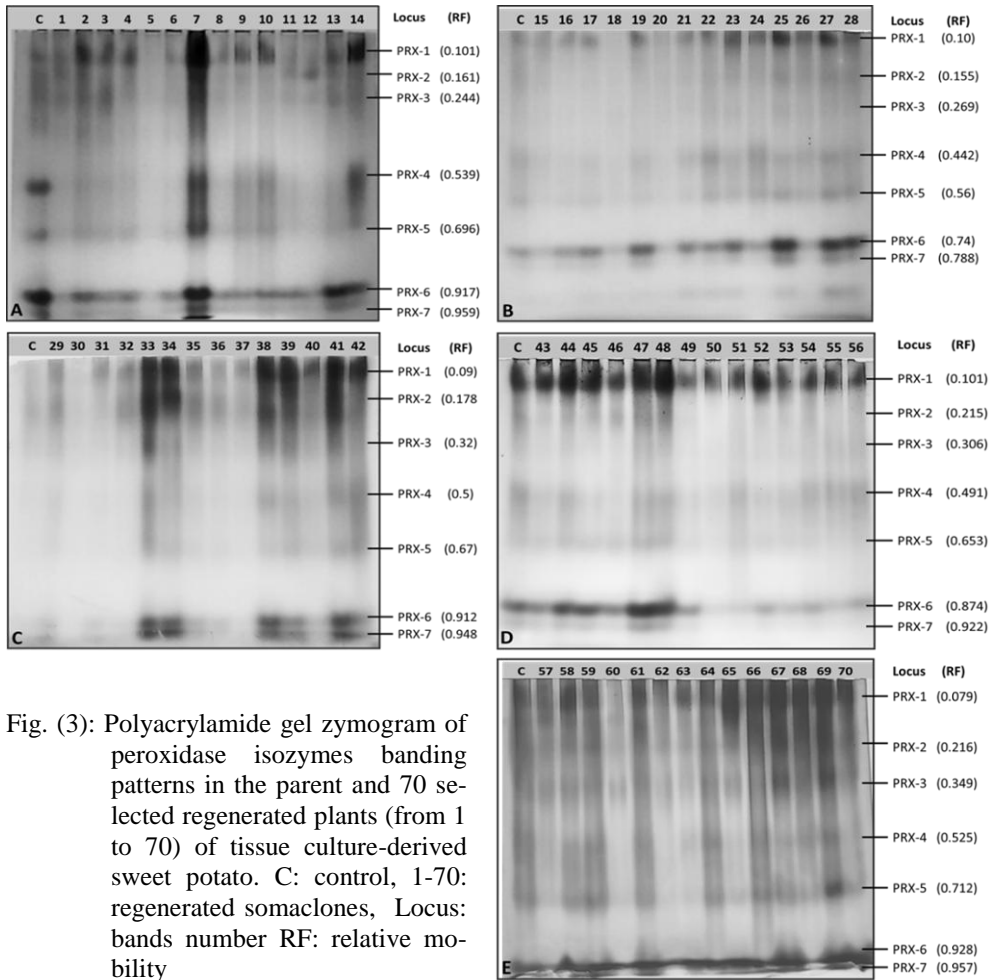


Fig. (3): Polyacrylamide gel zymogram of peroxidase isozymes banding patterns in the parent and 70 selected regenerated plants (from 1 to 70) of tissue culture-derived sweet potato. C: control, 1-70: regenerated somaclones, Locus: bands number RF: relative mobility

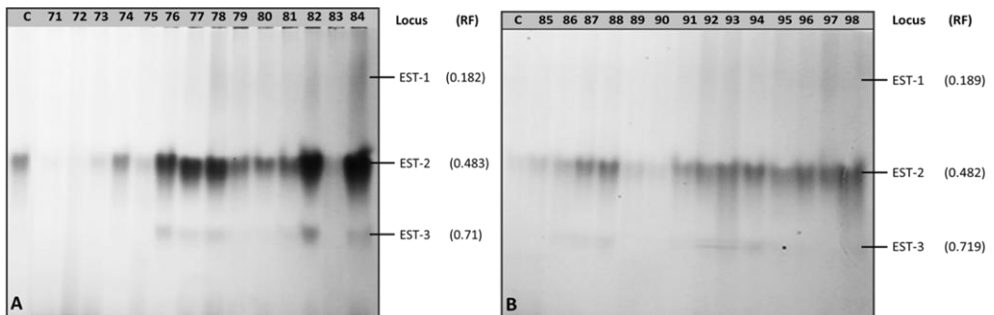


Fig. (4): Polyacrylamide gel zymogram of esterase isozyme banding patterns in the parent and 28 selected regenerated plants (from 71 to 98) in sweet potato Abees cultivar detected with α naphthyl acetate substrates. C: control, 71-98: regenerated somaclones, Locus: bands number, RF: relative mobility.

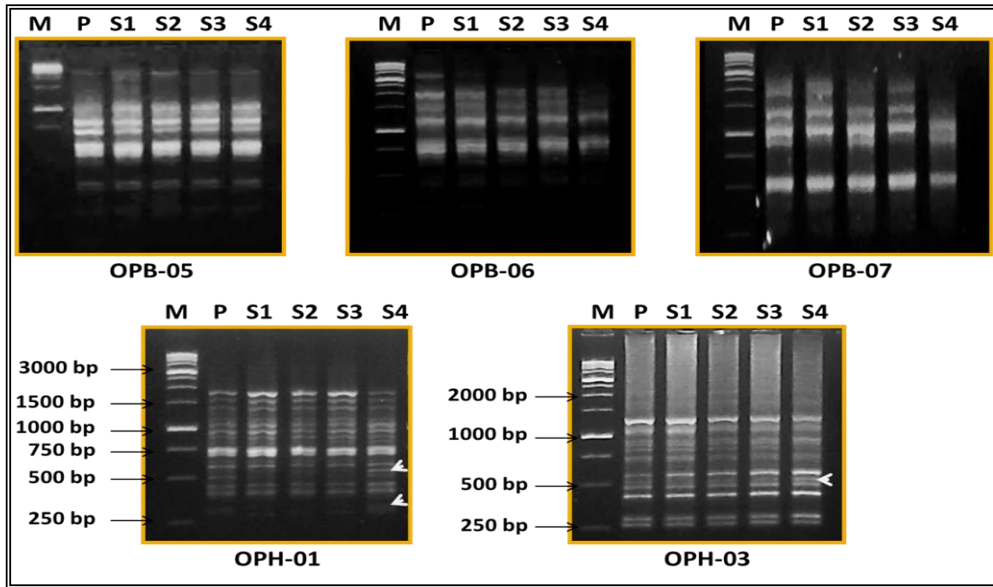


Fig. (5): RAPD fingerprints of sweet potato Abees cultivar (P) and its selected four somaclonal variants (S1, S2, S3 and S4) with five RAPD primers. M is the molecular size marker 1 Kb DNA ladder (250-10000 bp).

Fig. (6): Hierarchical cluster analysis of sweet potato Abees cultivar and the selected four somaclonal variants (S1, S2, S3 and S4) based on variation of RAPD patterns.

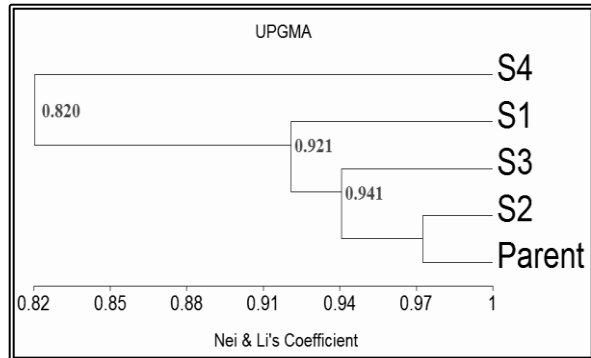


Fig. (7): RAPD marker-based principal coordinate analysis (PCoA) of sweet potato Abees cultivar and the selected four somaclonal variants (S1, S2, S3 and S4).

