Molecular Studies and Soma-Clonal Variations in Some Potato Genotypes

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Abstract

Potato is the fourth most important crop of the world wide annual production approaching 300 million tons. The tuber, the most important part of the plant, is an excellent source of complex carbohydrates, proteins, and vitamins. Salt accumulation is one of the main factors that diminish crop productivity since most of the crops are not halophytic against its toxic effects on plants also lead s to loss chloroplast activity as metabolic changes. The main objective of this study was to examine the powerful tissue culture system with three different potato genotypes. In addition to induce genetic variabilities through soma-clonal variation. Moreover, evaluate saline, and drought tolerance within the obtained clones.Results show that, The Shoot number of the three potato genotypes significantly affected by the two concentrations of sodium chloride (1000 and 2000 ppm).The shoot number of Daimont genotype was highly significant decreased in respons to the salt trearments (3.68 at 1000 ppm and 3.96 at 2000 ppm as compared with the control, 5.91). While shoot length of the three studied potato genotypes was affected by six combinations of plant hormones (auxin cytokinin) especially sponta (3.489). Trehalose,in three concentrations, exhibited an effect on shoot length of the three potato genotypes under study. Results of the molecular genetics study using IssR, showed the presence of polymorphic bands in variants and mutants suggesting that in response to all the studied treatments comparing with control.

Keywords: Potato, Soma-Clonal Variations , NaCl, Trehalose

Introduction

Plants regenerated via tissue culture techniques display genetic variations for different characters which have been named to as somaclonal variations(**Larkin and Scowcroft, 1981),**. it is also called tissue or culture-induced variation (**Kaeppler et al., 2000). Somaclonal variations are generally attributed to pre-**existing genetic variation in somatic cells (**Walbot, 1985**), single gene mutations, aneuploidy, transposable elements, cytogenetic changes and DNA methylation, (**Jain, 2001; Gaj, 2004**). The extent of variation depends on genotype, age of the donor plant, explant type and plant hormones in the culture medium

-(**Peredo et al., 2006**). Genetic variability is essential for breeding and selection of superior genotypes for crop improvement (**Clegg, 1990**). In vegetatively propagated plants, such genetic modifications can be directly incorporated into new varieties (**Jelenic et al., 2001**). In several commercial varieties of potato, tissue culture induced variations were observed in a wide range of characters, such as plant morphology, tuber

-characteristics (**Taylor et al., 1993**), disease resistance (**Matern et al., 1978; Behnke, 1979, and 1980; Cassells et al., 1991; Sebastini et al., 1994**),. isoenzymatic pattern, tuber proteins as well as chromosome number and structure (**Pijnacker and Sree Ramulu, 1990**). The use of In-vitro cultures in biotic and abiotic stress breeding offers several advantages over the In-vivo techniques including, obtaining explants from pre-existing cultures and recovering mutants hence rapidly micro-propagating them under controlled environmental conditions.

Some of the genetic changes are difficult to observe at the morphological or physiological level because of the structural differences in the gene product that may not alter its biological activity sufficiently to produce an altered phenotype. Analyses of secondary metabolites have also been used, but they are limited in their sensitivity (**Morell et al., 1995**). To overcome this problem, DNA markers are conveniently being used to detect tissue culture-induced variations and mutations.

Molecular markers have been used to estimate genetic diversity and examine genetic relationships that exist between cultivars in a range of horticultural crops (**Bradley et al., 1996; Graham et al., 1996; Polzerova, 2001; Yasmin et al., 2006; Ehsanpour et al., 2007; Joshi & Rao, 2009**). ISSR (Inter simple sequence repeats) analysis using in association with primers of arbitrary sequences has been demonstrated to be sensitive in detecting variations among individuals (**Xena de Enrech, 2000**). The advantages of this technique area large number of samples can be quickly and economically analyzed using only micro-quantities of material; b) the DNA amplicons are independent from the ontogenic expression; and c) many genomic regions can be sampled with a potentially unlimited number of markers (**Isabel et al., 1993**).

The present study aimed to produce useful somatic variants both by somaclonal variation and tolerate to salinity,as well as to analyze the genetic variation using ISSR-PCR method.

Materials and Methods

Callus induction: Leaf explants of cv. Cara, Sponta and Diamant were cultured on MS medium(Murashige & skoog) supplemented with phytohormones NAA (1.0 mg/l) and BAP (0.5 mg/l) for the induction of callus. The sub-culturing of main callus was carried out after every 2 weeks using the same medium.

Organogenesis (direct shooting): Leaf explants of cv. Cara, Sponta and Diamant were cultured on MS medium supplemented with different phytohormones, The shoot system of every genotype was treated with different concentration of sodium chloride (1000, 2000, 3000 and 4000 ppm).

Tolerance: The obtained calli were transferred to regeneration medium. The plantlets thus formed were used for further analysis.

DNA isolation: Fresh potato leaves of control plants and selected variants resulted from shoot systems treated with different concentration of sodium chloride (1000, 2000, 3000 and 4000 ppm,) for DNA extraction using the modified CTAB procedure (**Doyle & Doyle, 1990**). Approximately, 100 mg of fresh leaf was ground to a powder in liquid nitrogen, using a mortar and pestle. It was mixed with 2.5 ml CTAB extraction buffer and incubated at 65ºC for 30 minutes. The sample was extracted with 2.5 ml of chloroform / isoamyl alcohol (24:1 v/v) and the aqueous phase was mixed with 2/3 volume of cold iso-propanol. After centrifugation the sample was washed with 0.1 ml cold wash buffer, dried and resuspended in 0.1 ml TE or sterile water. Single stranded RNA was digested with 1.0µg/µl RNase A for 30 minutes at 37ºC and the DNA was quantified by gel electrophoresis.

ISSR analysis: Inter simple sequence repeats profiles were generated by using 7 primers. PCR reactions were performed in total volumes of 25 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 0.01% (w/v) gelatin, 50 µM each of dATP, dTTP, dGTP, dCTP , 0.2 µM primer, 0.5 µg of template DNA and 2.5 units of Taq DNA polymerase (Fermentas Inc. 7520 Connelley Drive, Maryland 21076, USA). Amplification was carried out using GenAmp-2700 thermocyler (Applied Biosystems) programmed as follows: 4 minutes at 92ºC, 1.30 minutes at 72ºC followed by 40 cycles of 45 seconds at 92ºC, 1.30 minutes annealing temperature of 45ºC, and a final extension 5 minutes at 72ºC. Amplicons were analyzed by electrophoresis in 1.2% agarose gel run in 0.5% TBE buffer and detected by staining with ethidium bromide. After electrophoresis, the amplified products were viewed under UV transilluminator and photographed (Dolphin- Doc with ID software, Wealtec Corp. USA).

Scoring and analysis of ISSR data: The ISSR data was analyzed using MSVP version 3.1 software. ISSR profile was made based on number of shared amplification products. Similarities were deduced by the presence (1) or absence (0) of a specific DNA fragment. The data was used to generate Jaccard's (**Jaccard, 1908**) similarity coefficients for ISSR bands and were utilized to generate dendrogram by using un-weighted pair group method of arithmetic means (UPGMA).

Name and Sequence of the primers used in ISSR

primer	sequence
ISSR- 1	5'-AGAGAGAGAGAGAGAGYC-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'
ISSR- 3	5'-ACACACACACACACACYT-3'
ISSR- 4	5'-ACACACACACACACACYG-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR- 6	5'-CGCGATAGATAGATAGATA-3'
ISSR- 7	5'-GACGATAGATAGATAGATA-3'
ISSR- 8	5'-AGACAGACAGACAGACGC-3'
ISSR- 9	5'-GATAGATAGATAGATAGC-3'
ISSR- 10	5'-GACAGACAGACAGACAAT-3'

Results

Potato somaclonal variants of cv. Cara, Sponta and Diamant regenerated from direct shooting pre-subjected sodium chloride treatments with four concentrations (1000, 2000, 3000 and 4000ppm) were selected for further analysis.

Table 1. Shoot length and number of the studied three potato genotypes in response to three selection pressure levels of NaCl (for salinity tolerance).

Shoot number of the three potato genotypes treated with of NaCl (for salinity tolerance)					
NaCL concentrations		Cara	Sponta	Diamont	NaCL Mean
	Control	8.36	7.53	5.91	7.27
	1000 ppm	4.61	4.54	3.68	4.27
	2000 ppm	5.85	5.26	4.52	5.21
	Genotypic mean	5.85	5.26	4.52	
	LSD (0.05)==0.63 Genotype				
	LSD (0.05)=0.37 NaCL				
Shoot length of the three potato genotypes treated with NaCl (for salinity tolerance)					
NaCL concentrations		Cara	Sponta	Diamont	NaCL Mean
	Control	5.79	4.95	4.56	5.10
	1000 ppm	7.03	7.53	4.88	6.48
	2000 ppm	5.93	5.00	5.85	5.59
	Genotypic mean	6.25	5.83	5.10	
	LSD (0.05)=0.63 Genotype				
	LSD (0.05)=0.37 NaCL				
Shoot length of the three potato genotypes response to different concentration of auxin cytokinine (for somaclonal variation)					
Auxin Cytokinine combinations		Cara	Sponta	Diamont	
	1 (…mg/l)	5.83	6.14	4.84	**5.61**
	2 (…mg/l)	6.13	5.88	4.58	**5.53**
	3 (…mg/l)	6.33	4.94	4.37	**5.21**
	4 (…mg/l)	5.78	5.14	4.51	**5.14**
	5 (…mg/l)	6.19	5.99	4.38	**5.52**
	6 (…mg/)	4.81	3.49	4.42	**4.24**
	Genotypic mean				
	LSD (0.05)=0.63 Genotype				
	LSD (0.05)=0.37 NaCL				
Shoot length of the three potato genotypes in response to different concentrations of auxin cytokinine (for somaclonal variation)					
		Cara	Sponta	Diamont	
	1 (…mg/l)	6.77	7.58	5.43	**6.59**
	2 (…mg/l)	6.02	6.23	5.14	**5.80**
	3 (...mg/l)	7.33	5.27	4.17	**5.59**
	4 (…mg/l)	4.978	5.178	5.833	**5.33**
	5 (…mg/l)	6.511	6.522	4.5	**5.84**
	6 (…mg/l)	5.9	4.178	5.511	**5.20**
	Genotypic mean				
	LSD (0.05)=0.63 Genotype				
	LSD (0.05)=0.37 NaCL				

Table 2. Shoot length and number of the three potato genotypes as affected by three selection pressure levels of trehalose (for salinity tolerance)

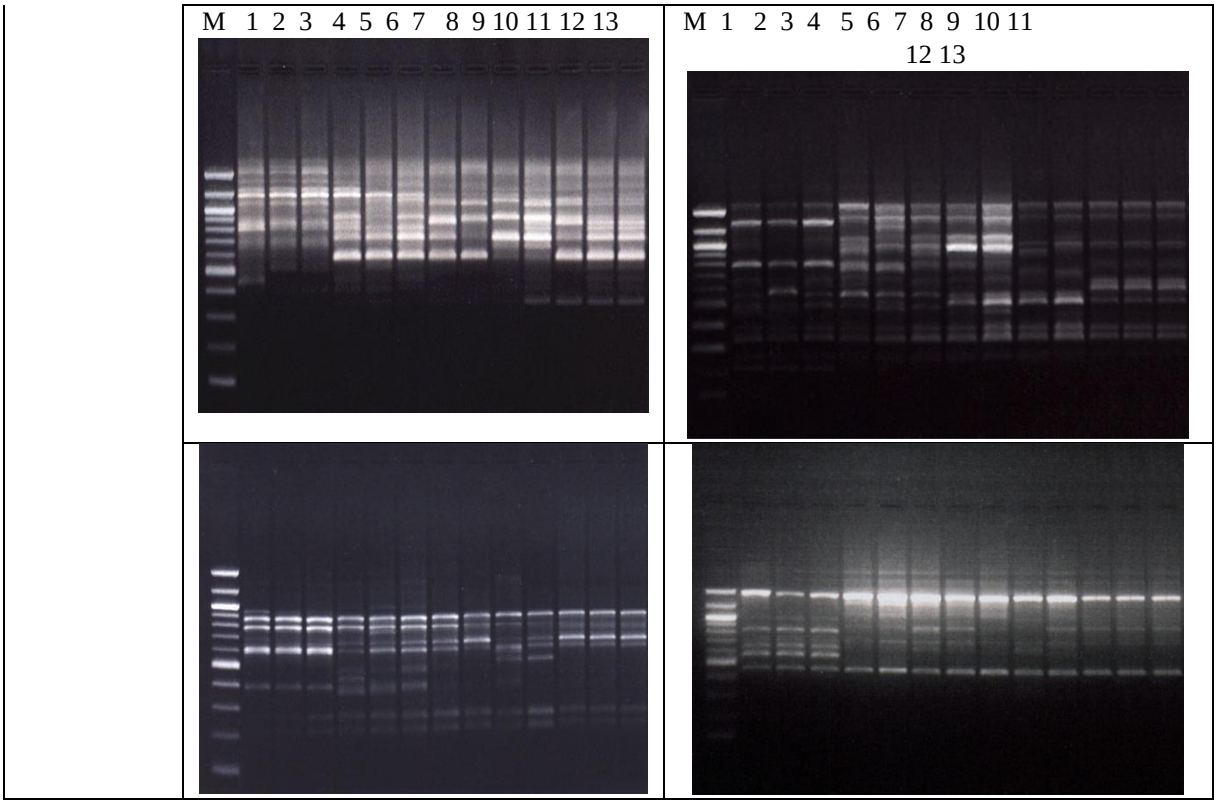
Shoot number of the three potato genotypes as affected by of trehalose					
Trehalose concentrations		Cara	Sponta	Diamont	trehalose Mean
	Control	5.77	6.73	6.57	6.36
	3000 ppm	2.20	4.36	4.60	3.72
	4000 ppm	3.17	6.33	4.00	4.50
	Genotypic mean	3.71	5.81	5.06	
	LSD (0.05)= 1.044 Genotype				
	LSD (0.05)=0.73 Trehalose				
Shoot length of the three potato genotypes as affected by of trehalose					
Trehalose concentrations		Cara	Sponta	Diamont	NaCL Mean
	Control	8.90	9.56	5.30	7.92
	3000 ppm	5.73	8.43	3.60	5.92
	4000 ppm	7.90	3.63	8.40	6.64
	Genotypic mean	7.51	7.21	5.77	
	LSD (0.05)=1.17 Genotype				
	LSD (0.05)=0.52 Trehalose				

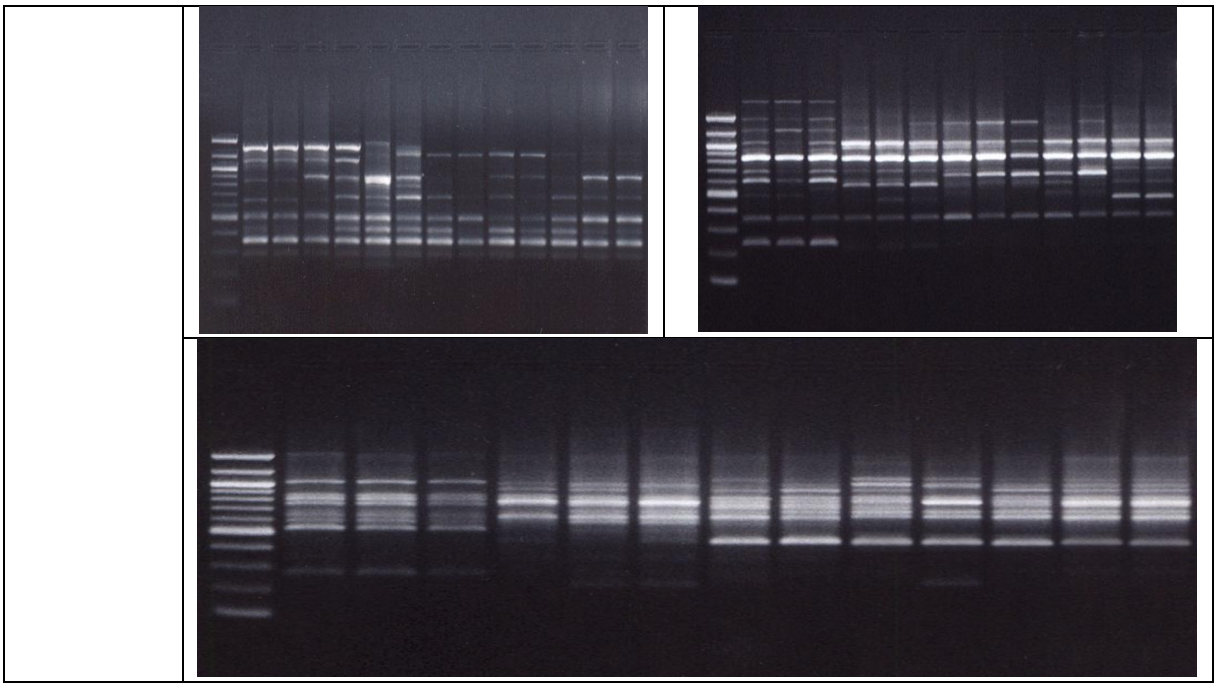
For ISSR analysis, a total of seven primers were evaluated for their ability to prime PCR amplification of potato genomic DNA. The primers revealed 187 clear and easily scorable bands, out of which 140 were polymorphic. The size of the bands that were produced in the PCR reactions ranged from 200-3000 bp, but most of the bands were between 300 and 2000 bp (Fig. 1). The Somatic 5 amplified the maximum number of DNA fragments (129) followed by Somatic Variant 7 (118), Somatic Variant 4 (116), Somatic Variant 7 (115), Somatic Variant 8 and Somatic Variant 9 (108), while Somatic 6 amplified minimum number of bands (95). According to Primer-wise detail of DNA polymorphism detected is given in Table 1, Indicated that quality and quantity of amplification products were sufficient for detection of genetic distance among the potato variants and mutants.

Dendrogram constructed by un-weighted pair group method (UPGMA) with arithmetic averages clustering algorithm from the pair-wise matrix of genetic similarity among somaclonal variants is given in Fig. 2. Different clusters of the dendrogram showed a clear pattern of division among the variants. Two main groups of clusters were identified. In the first cluster, Somatic Variant 6 and Diamont-control are present in one sub-cluster showing genetic similarity while Somatic Variant 10 is present in the second sub-cluster showing genetic distance from them. Second major group consisted of all the rest of the variants and mutants. In this cluster, two sub-clusters comprising Somatic Variant 4, Somatic Variant 5, Somatic Variant 9, Somatic Variant 7 exhibited maximum genetic homogeneity among themselves and clear genetic distances from other mutants, Somatic Variant 7, Somatic Variant 11 and **Somatic Variant 8.**

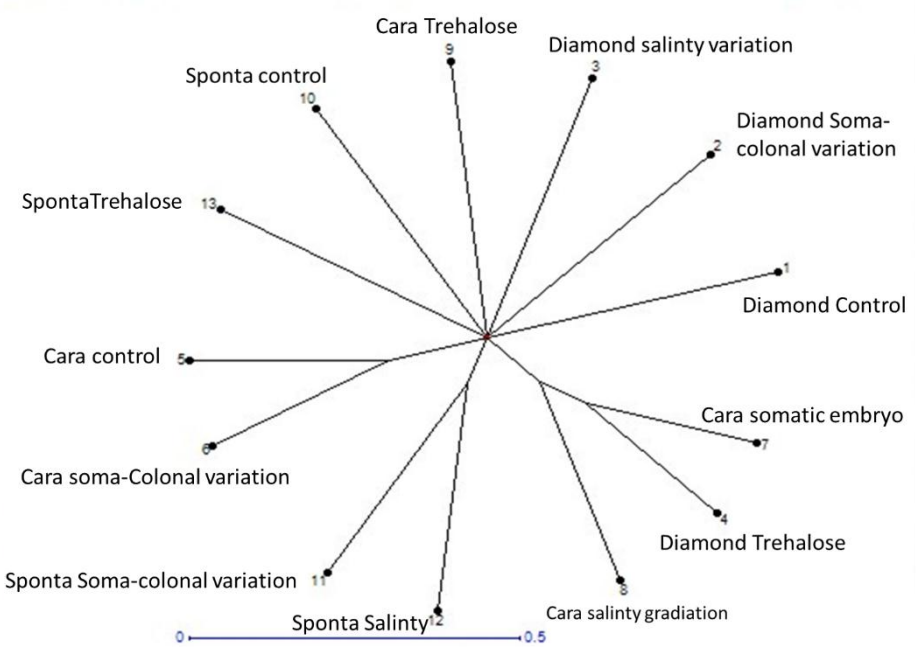
Discussion

Tissue culture-induced changes, including morphological, cytological, biochemical and genetic/epigenetic alterations, have been frequently reported in many plants. However, the mechanism underlying this has called somaclonal variation remains largely unclear (**Peredo et al., 2006; Kaeppler et al., 2000**). The most common factors affecting somaclonal variation are genotype, explant source, in vitro period and culture conditions (**Bordallo et al., 2004**). Unlike epigenetic changes, somaclonal variation which results from altered gene expression is usually irreversible (**Karp 1991; 1995**). The segregation pattern of mutations in the progeny is mostly Mendelian (**Larkin et al., 1984**).





1. **Control of Diamont 2. Somaclone s for Diamont 3. salinty graduation 4. Trehalose for diamont. 5. Control of cara 6. Somaclones for Cara 7. Somatic embryos of Cara 8. salinty graduation 9. salinty graduation 10. Control of Sponta 11. Somaclones for Sponta 12. Salinity graduation 13. Trehalose for sponta**



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Somaclonal variation and induced mutations results in the production of new genotypes with a limited change in the original genome. Thus the combination of *in vitro* culture techniques provides a simple, fast and highly efficient method to improve horticultural crops.

In the present study somaclonal variants were selected on the basis of Salt tolerance. The importance of somaclonal variation in potato is emphasized by several studies describing the improvements in agronomic characters such as disease resistance, changed tuber shape, skin color, shallow eye, tuber size and yield (**Ahloowalia, 1990; Jelenic *et al.*, 2001**).

Among the molecular markers, ISSR-PCR is being used successfully to identify, characterize and estimate genetic divergence of potato cultivars (**Moisan-Thiery *et al.*, 2001; Rocha *et al.*, 2002**). In

this study ISST-PCR method was used to detect genetic variability among the somaclonal variants and induced mutants of potato as compared to control. Some somaclonal variants of potato cv. Diamont regenerated from internode- were genetically analyzed. **Forapani *et al.*, (1999)** and **Moisan-Thiery *et al.*, (2001)** identified respectively, 37 and 57 potato genotypes using only three primers. **Demeke *et al.*, (1993)** identified 36 commercial potato cultivars using RAPD technique with only two primers while **Ghislain *et al.*, (1999) reported that by** using 12 primers 102 polymorphic markers were obtained, in the discrimination of 128 Accesses of andigena potato. Similarly **Collares *et al.*, (2004)** efficiently characterized 27 potato genotypes on the basis of RAPD markers. In our study, 7 arbitrary primers were used producing 187 bands, out of which 140 were polymorphic, and these primers produced more fragments and more amplified polymorphism as compared to these authors.

For detecting genetic variation among the somaclonal variants among three genotypes of potato and their somaclonal variants, **Bordallo *et al.*, (2004)** used ISSR-PCR technique. Likewise **Wendt *et al.*, (2001)** used 10 ISSR primers for the molecular analysis of gamma irradiated regenerated plants of potato cv. Macaca. The primers produced 70 fragments being 24% polymorphic.

Conclusion

The present study clearly showed that genetic variations are present in the plants regenerated via somaclonal variation and also suggested that the ISSR methodology is efficient in detecting somaclonal variation in potato.

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