Pak. J. Bot., 46(4): 1507-1514, 2014.

PLANT REGENERATION FROM *IN VITRO*-SELECTED SALT TOLERANT CALLUS CULTURES OF *SOLANUM TUBEROSUM* L.

ZAHOOR AHMAD SAJID AND FAHEEM AFTAB*

Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan.
**Corresponding author e-mail: faheem.botany@pu.edu.pk; Tel: +92-42-35857587*

Abstract

This research work reports *In vitro* direct selection of salt-tolerant callus cultures and subsequent plant regeneration in two potato cultivars (Cardinal and Desiree). Results have shown more than 50% reduction in relative fresh callus mass in the two potato cultivars exposed to 120 mM NaCl. Callus morphology correspondingly changed from off-white to blackish-brown at 120 mM to acutely-necrotic at 140 mM NaCl. Regeneration potential of recurrently-selected callus cultures (100 mM NaCl-treated) on salt-free regeneration medium (MS + 2.64 µM NAA and 1.00 µM TDZ) was not much different as compared to the control (non-selected ones). Regenerated plants from salt-tolerant callus cultures of both the cultivars after selection were transferred to soil and organic matter (50:50, *v/v*) for acclimatization in the greenhouse. It was observed that the recurrently-selected plants had higher fresh/dry weight and number of tubers compared with the control ones in both the cultivars. Likewise the protein, peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activities have shown an increasing trend in salt-treated plants of both the cultivars. The results from this study highlighted a strong possibility for the selection of salt-tolerant callus lines of potato followed by an efficient plant regeneration and further acclimatization.

Introduction

Soil salinity is one of the most important abiotic stresses limiting the productivity of agricultural system around the world (Mahajan & Tuteja, 2005). Salinity affects all the major life processes; viz., growth, photosynthesis, protein synthesis, and lipid metabolism. Investigations on tolerance of saline environments frequently point to restricted ion accumulation and synthesis of organic solutes as major adaptations leading to salt-resistance in glycophytes (Ashraf & Foolad, 2007; Kanwal *et al*., 2013: Radica *et al*., 2013). Moreover, salt tolerance is known to be a multigenic trait and after exposure to stress, changes that occur at the cellular level are due to an altered gene expression and thereby resulting in accumulation of proteins involved in stress tolerance (Bohnert & Jensen, 1996; Iba, 2002). In addition to these interrelated and co-existing impacts, salinity also produces an oxidative stress (Panda & Upadhyay, 2004) due to rapid and transient accumulation of reactive oxygen species (ROS) like superoxide and/or hydroxyl radical and singlet oxygen. These ROS cause pigment co-oxidation, lipid peroxidation, membrane destruction, protein denaturation and/or DNA mutation (Mittler, 2002). Plants have to opt for a specific protective mechanism to lessen the harm initiated by these ROS. Antioxidants (superoxide dismutase, catalase and peroxidase, etc) that are produced in response to above-mentioned factors are thus of great significance (Alhdad *et al*., 2013). Superoxide dismutase is a major scavenger of O_2^- and its enzymatic action results in the formation of H_2O_2 and O_2. Peroxidase decomposes H_2O_2 by oxidation of co-substrates, such as phenolic compounds and/or antioxidants, whereas catalase breaks down H_2O_2 into water and molecular oxygen (Mittler, 2002).

Several strategies have been worked out in the past to improve abiotic or biotic stress-resistance in crops. These involve pre-sowing seed treatments, exogenous application of different compounds, breeding, mutation, pooling physiological traits, interspecific hybridization, the use of marker-aided selection and transformation (Ashraf *et al*., 2008). Besides these approaches, methods relying mainly on *In vitro* selection can also be found in the literature (Ochatt *et al*., 1999; Queiros *et al*., 2007). *In vitro* culture techniques are an excellent tool to study the behavior of undifferentiated cells and the whole plants in ambient stress under control conditions. The exploitation of somaclonal variation is also potentially quite useful for *In vitro* selection of cells and tissues against several stresses (Bajaj, 1987; Tal, 1996). However, the potential benefits may only be tied together if a trait is highly amenable to *In vitro* selection, and is expressed and transmitted to the regenerated plants. Despite such limitations, salt-tolerant cell-lines and plants are reported in a range of species, for instance, tomato (Hassan & Wilkins, 1988) wheat, (Barakat & Abdel-Latif, 1996), rice (Lutts *et al*., 1999), potato (Sabbah & Tal, 1990; Burgutin *et al*., 1996; Ochatt *et al*., 1999; Benavides *et al*., 2000; Queiros *et al*., 2007), sunflower (Alvarez *et al*., 2003), and sugarcane (Gandonou *et al*., 2006). Potato is highly amenable to tissue culture and several attempts have been made to get salt-tolerant cell-lines, for instance, successful regeneration of salt-tolerant plants from stable salt-tolerant cell-lines was reported by Ochatt *et al*., (1999). However, the most prominent of the problems seems to be the reproducibility of protocols thus limiting sustainability of acquisition of salt tolerance in potato. Sustainable salt tolerance in potato has thus seldom been achieved (Sabbah & Tal, 1990). A reproducible as well as sustainable production of salt-tolerant potato plants, in particular through *In vitro* means, for the same reason still remains elusive. Generally, two methods (so called 'direct' and 'stepwise' methods of selection) have been adopted for the selection of salt-tolerant cell lines. Selection of salt-tolerant cell lines by direct selection method is considered superior compared with stepwise method of selection (Mc-Hughen & Swartz, 1984; Sabbah & Tal, 1990; Aghaei *et al*., 2008) as it more closely resembles field conditions. Gradual exposure of plants or tissues is generally considered inefficient since several non-tolerant cells with a labile metabolism have enough time to adapt to the gradual rise of salt (Ochatt *et al*., 1999; Miki *et al*., 2001; Queiros *et al*., 2007). It is accepted that long term

selection of cell-lines is not only responsible for the necrosis of 50-95% cells but also the cause of genetic abnormalities that are usually retained by the cell population (Nabors, 1990). Considering the above-mentioned facts, it becomes quite obvious that though methods for the production of salt-tolerant callus lines in potato have been reported, lack of sustainability and even extension of protocols to most potato cultivars is a major limiting factor in the way of harnessing the ultimate benefits this technology may render towards sustainable potato production. The literature survey also indicates that the information regarding mechanism of salt tolerance in general, and in terms of role of proteins and antioxidant enzymes in the selected cell lines of potato in particular, is quite scanty. Interestingly, it is yet debatable as which method of selection is better since evidence for both direct as well as indirect selection strategies exist in the literature. Thus the present research work was undertaken to establish efficient *In vitro* selection strategy to produce salt-tolerant callus lines and subsequent regeneration protocols in two economically important potato cultivars (Cardinal and Desiree). Emphasis was given to developing further understanding of the mechanism of salinity stress with special reference to total soluble proteins, and enzymatic antioxidants (peroxidase, catalase and superoxide dismutase). Another objective of the present study was to investigate the conditions influencing the establishment of plants in saline soil under glasshouse and/or greenhouse environment.

Material and Methods

Plant material and culture conditions: Internodal explants (ca. 1.0 cm) of 60-day-old *In vitro* plants of two potato (*Solanum tuberosum* L.) cultivars (Cardinal & Desiree) were inoculated on Murashige & Skoog (1962) medium (medium C4) supplemented with 18.09 µM 2, 4-dichlorophenoxyacetic acid (2, 4-D) for callus induction during this study. The medium was solidified with 0.7% agar and pH adjusted to 5.7. The cultures were maintained under complete dark condition at 25 ± 2°C. Calluses were sub-cultured after 15 days interval on fresh medium for further proliferation.

Selection of salt-tolerant callus lines: Pre-weighed main callus cultures (60-day-old) of both the cultivars developed on MS (Murashige & Skoog, 1962) medium supplemented with 18.09 µM 2, 4-D were sub-cultured to the same medium but containing different concentrations of NaCl (0, 20, 40, 60, 80, 100, 120 or 140 mM; eight treatments). Ten culture vessels (150 × 25 mm) were inoculated for each treatment and the experiment was repeated thrice. Callus cultures were maintained under dark conditions at 26 ± 2°C. Data were recorded for percentage relative fresh weight growth (PRFWG) and morphology of callus cultures after 90 days of salt treatment. Prior to recording the data, the calluses were sub-cultured after two weeks to their respective salt concentration. The PRFWG of callus cultures was calculated by using a formula; W_1-W_0/W_0 × 100 (where W_1 was fresh weight after 90 days of salt treatment and W_0 being the weight of callus cultures before salt treatment).

Sub-lethal concentration of salt was selected on the basis of decrease in PRFWG. The concentration of salt that resulted in 50% decrease in PRFWG was selected as sub-lethal one for each cultivar. Furthermore, calluses were sub-cultured and maintained on this concentration of salt for 3 months. Recurrent selection was done by transferring the calluses to NaCl-free basal medium (BM) for two successive subcultures, then returned to their respective MS basal medium plus NaCl. The callus cultures that survived and resumed growth for at least two further subcultures were picked and inoculated on optimized regeneration medium (Ochatt *et al*., 1999).

Regeneration potential of recurrently-selected callus cultures: After recurrent selection, callus cultures were picked and transferred to an optimized callus regeneration medium (MS + 2.64 µM Naphthaleneacetic acid (NAA) and 1.00 µM N-phenyl-N'- 1, 2, 3-thiadiazol- 5-yl urea (thidiazuron; TDZ). After 60 days of callus inoculation on regeneration medium, different growth parameters of regenerating plantlets (number of days for regeneration, number of shoots per callus culture and number of roots) were recorded.

Assessment of stability of acquired salt tolerance after recurrent selection: Well-acclimatized plants of both the cultivars after recurrent selection were transferred to a mixture of soil and organic matter (50:50, *v/v*) and were irrigated with Hoagland (Hoagland & Arnon, 1950) solution for 30 days. Single plant was placed in each (8 × 8 cm) pot in glasshouse at 25 ± 2°C/16 ± 2°C day/night temperatures in 16 h photoperiod. After 30 days, pots were irrigated with Hoagland solution supplemented with or without 100 mM NaCl to experimental and control ones, respectively, whenever required for 60 days. There were ten replicates for both the control and the experimental plants. Different morphological (number of tubers per plant, fresh and dry weight) and biochemical parameters (protein contents, peroxidase, catalase, and superoxide dismutase activity) were scored at day 60.

Biochemical assay: Plant material (fully expended leaves) was ground in liquid nitrogen into a very fine powder using an ice-chilled pestle and mortar. One-gram ground tissue was homogenized in 2.0 ml of 0.1 M phosphate buffer, pH 7.2 (13.6 g KH_2PO_4 and 17.4 g K_2HPO_4 in 1,000 ml of solution) containing 0.5% (*v/v*) Triton X-100 and 0.1 g of polyvinyl-pyrrolidone (PVP). The homogenate was centrifuged at 14,000 rpm at 4°C for 30 minutes using Sorval RB-5 refrigerated super-speed centrifuge. The supernatant was collected and stored at 0°C for further estimation of protein, peroxidase, catalase and superoxide dismutase activities.

Biuret method of Racusen & Johnstone (1961) was adopted for the estimation of soluble protein contents. For the quantitative estimation of peroxidases (E.C 1.11.1.7) "Guaiacol-H_2O_2" method of Luck (1974) was adopted with certain modifications. Catalase (E.C 1.11.1.6) activity was assayed according to Beers & Sizer (1952) with certain modification. Superoxide dismutase (E.C 1.15.1.1) activity was assayed spectrophotometrically according to Maral *et al*., (1977). Details of methods and modifications were the same as reported earlier (Sajid & Aftab, 2009).

Statistical analysis: The data were subjected to Duncan Multiple Range Test by using SPSS version 20.0.

Results

Selection of salt tolerant callus lines: Table 1 depicts that there was a significant difference with reference to PRFWG and callus morphology between different concentrations of NaCl in callus cultures of both the cultivars. At 0 mM NaCl concentration, callus cultures from both the cultivars were off-white and granular and having efficient proliferation response (Figs. 1 & 2a). Salt-treated callus cultures showed maximum PRFWG (72 and 87%) at 20 mM NaCl in Cardinal and Desiree, respectively. As the concentration of salt was increased in the medium, PRFWG decreased correspondingly and off-white, green callus cultures turned yellow to brown (Figs. 1 & 2 b-e). At 100 mM NaCl, relative fresh weight growth was decreased to 54 and 57% in Cardinal and Desiree, respectively and the morphology of callus cultures changed to greenish-yellow in both the cultivars (Figs. 1 & 2f). Callus cultures were completely necrotic above 100 mM NaCl. It was also observed that callus cultures of Desiree had comparatively better PRFWG as compared to Cardinal at all the tested salt levels. Color of callus cultures in both the cultivars changed to blackish-brown at 120 mM salt level (Figs. 1 & 2g). Thus 100 mM NaCl concentration was identified as sub-lethal because above this salt concentration, calluses turned completely necrotic in both the cultivars. Calluses were sub-cultured and further maintained on this salt concentration for recurrent selection as detailed above.

Table 1. Effect of different NaCl levels (0-140 mM) supplemented to optimized callus proliferation medium on relative fresh weight growth and callus morphology of potato (cvs. Cardinal and Desiree) at day 90.

Optimized callus proliferation medium (C4) * + NaCl (mM)	Relative fresh weight growth (%)		Callus Morphology***	
	Car**	Ds**	Car	Ds
C4 + 0	100	100	Off-white with yellowish portions, granular	Off-white with green portions, friable
C4 + 20	72	87	Greenish- yellow, friable	Off-white with yellow portions, friable
C4 + 40	70	89	Greenish with brown patches, granular	Off-white yellow, granular
C4 + 60	66	73	Greenish with brown patches, friable	Off-white with brown portions, translucent
C4 + 80	62	63	Off-white with brown portions, granular	Off-white with brown portions, granular
C4 + 100	54	57	Greenish with brown patches, granular	Greenish- yellow, friable, granular
C4 + 120	43	46	Blackish brown, necrotic	Blackish, necrotic
C4 + 140	30	37	Necrotic	Necrotic

*C4: Optimized callus proliferation medium (MS + 18.09 µM 2, 4-D)
** Car: Cardinal, Ds: Desiree
***Callus morphology is based on 30 culture vessels per NaCl treatment at day 90 of initial culturing

Table 2. Growth and biochemical analysis of control and salt-treated plants of potato cvs. Cardinal and Desiree after recurrent selection

Medium (Potting mix*+ NaCl)	Parameter	Cultivar**	
		Car	DS
Control (Potting mix+ 0 mM NaCl)	Number of tubers	9.00 ± 1.550	11.00 ± 0.250
	Fresh weight of tuber (g)	20.00 ± 0.751	21.00 ± 1.250
	Dry weight of tuber (g)	4.68 ± 0.225	4.73 ± 0.225
	Protein (mg/g)	3.35 ± 0.750	3.14 ± 0.157
	POD activity (units/ml enzyme)	2.09 ± 0.553	1.51 ± 0.250
	CAT activity (units/ml enzyme)	5.02 ± 0.625	4.29 ± 0.357
	SOD activity (units/mg protein)	9.24 ± 0.205	9.20 ± 0.265
Saline (Potting mix +100 mM NaCl)	Number of tubers	10.00 ± 0.252	12.00 ± 1.250
	Fresh weight of tuber (g)	24.00 ± 0.115	25.00 ± 0.951
	Dry weight of tuber (g)	5.22 ± 0.345	5.50 ± 0.635
	Protein (mg/g)	3.98 ± 0.259	3.46 ± 0.346
	POD activity (units/ml enzyme)	2.39 ± 0.273	1.93 ± 0.256
	CAT activity (units/ml enzyme)	5.80 ± 0.829	5.51 ± 0.252
	SOD activity (units/mg protein)	9.82 ± 0.215	9.44 ± 0.285

*Potting mix: Soil and organic matter (50:50 *v/v*)
**Cultivar: Car: cv. Cardinal, DS: cv. Desiree
Data are means ± S.E. from 30 replicate cultures at day 30 of salt treatment

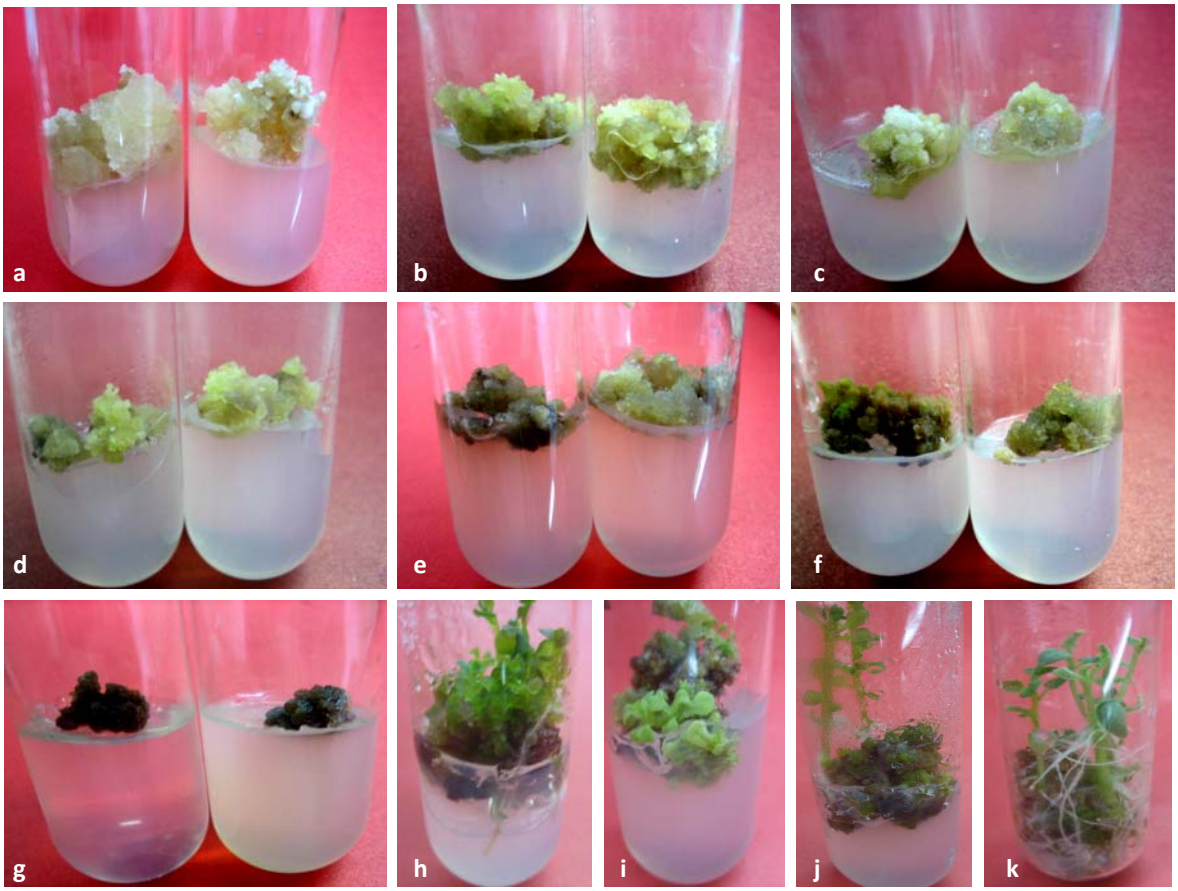

Fig. 1. Callus morphology of potato cv. Cardinal at different concentrations of NaCl at day 90. **(a)** Off-white callus cultures at 0 mM NaCl (1.2x). **(b)** Greenish-yellow callus cultures at 20 mM NaCl (1.2x). **(c)** Green callus cultures with brown patches at 40 mM NaCl (1.2x). **(d)** Greenish, friable callus cultures at 60 mM NaCl (1.4x). **(e)** Callus cultures at 80 mM NaCl (1.3x). **(f)** Green, granular calluses with brown patches at 100 mM NaCl (1.3x). **(g)** Necrotic, blackish-brown callus cultures at 120 mM NaCl (1.4x). **(h)** Regeneration potential of callus cultures at day 60 after transfer to optimized regeneration medium containing 0 mM NaCl after recurrent selection (1.4x). **(i)** Regeneration of callus cultures after 100 mM NaCl treatment at day 60 of transfer to optimized regeneration medium (1.4x). **(j)** Regeneration potential at day 120 of transfer of calluses to optimized regeneration medium containing 0 mM NaCl after recurrent selection (1.4x). **(k)** Regeneration of Cardinal callus cultures after 100 mM NaCl treatment at day 120 (1.5x).

Plant regeneration after recurrent selection: After recurrent selection, it was observed that regeneration potential of callus cultures in medium without salt was better as compared to NaCl-treated (100 mM) ones. Shoot formation via organogenesis was observed in both the cultivars. Shoot initiation was noticed one day earlier in non-treated callus cultures as compared to 100 mM salt-treated callus cultures in both the cultivars. The difference in number of shoots and nodes between treated and non-treated calluses was less sharp in both the potato cultivars. The number of shoots in Cardinal was 9 and 10 and in Desiree 10 and 12 in treated and non-treated callus cultures respectively (Figs. 3 & 4). The number of nodes varied from 16 and 17 in Cardinal and 13 and 14 in Desiree in treated and non-treated callus cultures. The number of shoots per culture vessel was slightly higher in Desiree as compared to Cardinal. The overall vigor of regenerated plants from salt-treated callus cultures in both the cultivars was considerably lower in comparison with the control (Fig. 1 & 2 h-k).

Assessment of stability of acquired salt tolerance in greenhouse: To check the stability of acquired salt tolerance of recurrently-selected plants of both the cultivars, acclimatization was first carried-out in greenhouse and then subjected to salinity stress. A comparison of growth and biochemical features of control and treated plants is given in Table 2. It was observed that number of tubers, fresh and dry weights were not much different in salt-treated plants as compared to plants without any salt treatment (control). The tuber numbers as well as fresh/dry weights of salt-treated Desiree plants were better as compared to Cardinal. Similarly, protein, POD, CAT and SOD activity also showed an increasing trend in salt-treated plants from both the cultivars.

Discussion

The concept of *In vitro* selection is to exploit the genetic variation, known to occur in plants by screening cell cultures for resistance to disease, insects, herbicide or any abiotic stress. The procedure of *In vitro* selection typically involves subjecting cells in cultures to a suitable

selection pressure and recovering any variant cell line/s that is/are resistant to that particular stress. These variant lines are then used to regenerate whole plants. During this investigation, a direct recurrent selection procedure was employed to select salt-tolerant callus lines in two potato cultivars (Cardinal and Desiree). Results have shown more than 50% reduction in relative fresh weight in both the cultivars above 100 mM NaCl. Callus morphology correspondingly changed from off-white to blackish-brown above 100 mM to acutely-necrotic at 140 mM NaCl. This decrease in growth of callus cultures at higher salt concentration in potato is considered as a common phenomenon (Benavides *et al*., 2000; Queiros *et al*., 2007). It is therefore perhaps not surprising that such type of growth reduction has also been observed in other plant species, e.g., *Cicer arietinum* (Pandey & Ganapathy, 1984), sugarcane (Gandonou *et al*., 2005), *Chrysanthemum morifolium* (Hossain *et al*., 2007) and *Jatropha curcas* (Kumar *et al*., 2008). Thus under stress conditions, one of the strategies that higher plants in general have probably adopted is to slow down their growth and metabolism (Zhu, 2001, Shahid *et al*., 2013). The other possibility is to better utilize and manage the available resources under nutritional imbalance, osmotic and metabolic disturbances. This reduction in growth not only helps the plants to save energy for defense purposes but also limits the risk of heritable damage (May *et al*., 1998). Change in callus morphology (brownish to black) at higher salt concentrations may directly be linked to cell death at higher salt concentrations.

In the present study, it has been observed that the regeneration potential of recurrently-selected callus cultures (100 mM NaCl-treated) on salt-free medium was not much different as compared to the control ones. Regeneration of selected salt-tolerant callus cultures on salt-free regeneration medium is well documented in the literature (Ben-Hayyim & Goffer, 1989; Jaiswal & Singh, 2001). In contrast to these results, several workers have also obtained regeneration of selected salt-tolerant calluses on salt-containing media (Reddy & Vaidyanath, 1986; Beloualy & Bouharmont, 1992; Ochatt *et al*., 1999). In terms of cultivars, the regeneration response in our studies was more or less similar but with a slight tendency towards better growth parameters in Desiree as compared to Cardinal. The overall vigor (number of shoot and nodes) of the regenerated plants from salt-treated callus cultures was relatively less in comparison with the control.

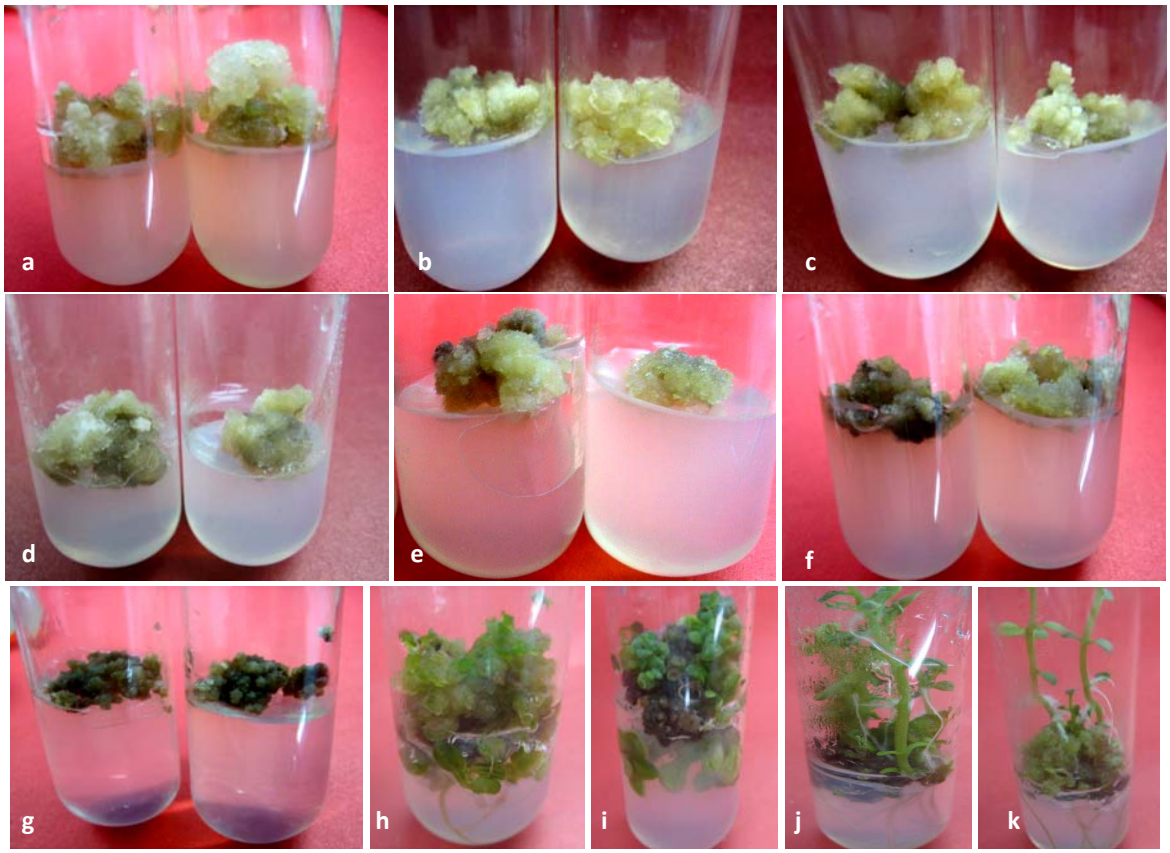

Fig. 2: Callus morphology of potato cv. Desiree at different concentrations of NaCl at day 90. **(a)** Off-white with green portions at 0 mM NaCl (1.2x). **(b)** Off-white yellow ones at 20 mM NaCl (1.4x). **(c)** Off-white, yellow calluses at 40 mM NaCl (1.4x). **(d)** Off-white brown, translucent calluses at 60 mM NaCl (1.2x). (e) Callus cultures at 80 mM NaCl (1.5x). **(f)** Green, granular calluses with brown patches at 100 mM NaCl (1.2x). **(g)** Necrotic, blackish-brown callus cultures at 120 mM NaCl (1.2x). **(h)** Regeneration potential at day 60 of transfer of callus cultures to optimized regeneration medium containing 0 mM NaCl after recurrent selection (1.4x). **(i)** Regeneration of callus cultures after 100 mM NaCl treatment at day 60 of transfer to optimized regeneration medium (1.2x). **(j)** Regeneration potential at day 120 of transfer of calluses to optimized regeneration medium containing 0 mM NaCl after recurrent selection (1.4x). **(k)** Regeneration of Cardinal callus cultures after 100 mM NaCl treatment at day 120 (1.2x).

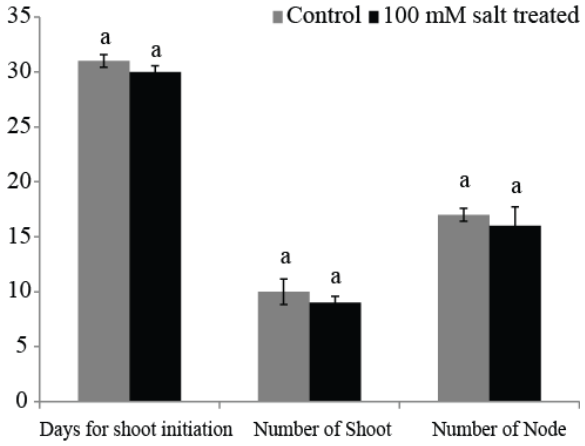

Fig. 3. Regeneration response of salt-tolerant callus cultures of potato (cv. Cardinal) on optimized regeneration medium at day 60 Values represent the means ± S.E. from 30 replicate cultures. Means followed by the same letter(s) are not significantly different at $p \leq 0.05$

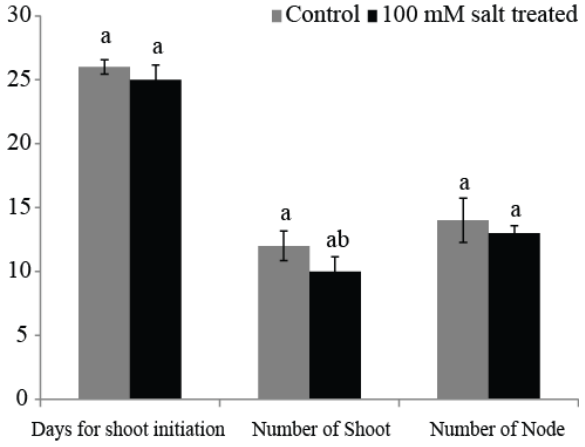

Fig. 4. Regeneration response of salt-tolerant callus cultures of potato (cv. Desiree) on optimized regeneration medium at day 60. Values represent the means ± S.E. from 30 replicate cultures. Means followed by the same letter(s) are not significantly different at $p \leq 0.05$

When well-acclimatized recurrently-selected plants were treated with 100 mM NaCl and compared with control plants to check their acquired salinity tolerance, it was observed that the recurrently-selected plants had higher fresh/dry weight and number of tubers as compared to the control ones in both the cultivars. Similar growth behavior in selected salt-tolerant lines of potato was observed by Ochatt *et al*., (1999) and Queiros *et al*., (2007).

Proteins have been suggested as an important molecular marker for the improvement of salt tolerance using genetic engineering techniques (Pareek *et al*., 1997). In the present study, recurrently-selected plants had higher protein contents as compared to the control (non-selected ones) in both the cultivars. These results were in agreement with the reports of other workers. Cano *et al*., (1998) studied the growth and physiological responses to salinity of two inter-specific hybrids between the cultivated tomato (*Lycopersicon esculentum* Mill.) and its wild salt-tolerant species (*Lycopersicon pennellii*) and compared to those of their parents. They concluded that protein contents increased with salinity in all the genotypes. These salt-induced proteins were also reported in potato plants by Rahnama & Ebrahimzadeh (2004). Recently, Queiros *et al*., (2007) also observed this increasing trend of soluble and insoluble proteins in potato cultures during the selection of salt-tolerant cell lines. These higher protein contents might be attributed to synthesis of stress-induced proteins (Sajid & Aftab, 2012) that may be helpful for maintaining the osmotic imbalance. Salt-responsive proteins were also suggested to be quite valuable for further analysis of general cellular adaptive mechanism to abiotic stress. Salt has two antagonistic effects on protein; firstly they tend to break electrostatic bonds and secondly increase hydrophobic interactions (Melander & Horvath, 1977; Ashraf & Harris, 2004).

It is quite evident from the literature that higher plants generally up-regulate several antioxidant enzymes (POD, CAT, SOD, etc) to scavenge reactive oxygen species (ROS) produced in response to salt stress (Mittova *et al*., 2003; Rahnama *et al*., 2003; Ashraf & Harris 2004; Batkova *et al*., 2008). In the present investigation, recurrently-selected plants had higher POD, CAT and SOD activities as compared to the control ones in both the cultivars. The above-mentioned antioxidant enzymes play a necessary role in detoxification of ROS produced under stressful conditions (Rahnama *et al*., 2003; Hu *et al*., 2012). Quite recently, Kumar *et al*., (2008) reported that SOD activity increased in salt-treated callus cultures of *Jatropha curcas* as compared to non-treated controls. Similarly, an increase in SOD activity was also reported by Sreenivasulu *et al*., (2000) and Cherian & Reddy (2003). SOD normally converts more toxic $O_2^{\bullet-}$ radicals to less toxic H_2O_2 (Scandalios, 1993) and to neutralize H_2O_2 other enzymes such as peroxidase and catalase are produced (Dionisiosese & Tobita, 1998). So the increase in peroxidase and catalase activity in this study seems to be in agreement with such previous reports on these enzyme behaviors. It is, therefore perhaps safer to infer from the results of this investigation that the changes in growth and biochemical parameters were rather comparable to a shifting behavior of plants from being sensitive-to-relatively-more-tolerant-ones.

Conclusion

In conclusion, higher levels of NaCl in this investigation severely suppressed the growth of callus cultures of the two tested potato cultivars. The results from this study also highlight a strong possibility for the selection of salt-tolerant callus lines of potato followed by efficient plant regeneration. The results from this work in the light of contemporary literature indicate a probable genetic modification at the cellular level resulting in an acquisition of salt tolerance that was partly evident in enhanced activity of proteins and antioxidant enzymes. Although a potential NaCl-tolerant callus line was selected and further maintained in this study and the fact that an associated useful biochemical information was gathered, apparently lot of work regarding biochemical and

physiological aspects of salinity tolerance still remains elusive in potato and deserves further experimentation not only under *In vitro* but also in greenhouse and/or field conditions to draw meaningful conclusions.

Acknowledgements

We are thankful to Higher Education Commission of Pakistan for financial support (HEC project 20-143) to FA. The work described here is part of PhD thesis of ZAS.

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(Received for publication 20 January 2013)