Effect of salt stress on some defense mechanisms of transgenic and wild potato clones (*Solanum tuberosum L.*) grown *in vitro*

Amal A. Mohamed*[1], Mohamed A. Matter[2] and Mahmoud M. Saker[2]
[1]Plant Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt
[2]Plant Biotechnology Department, National Research Centre, Dokki, Cairo, Egypt
*Corresponding author: amin_amal@yahoo.com

Abstract: Genetically modified plants (GMPs) proved to be a promising solution for many of biotic and abiotic stresses which affect food production. Great attention was given to the evaluation of growth, nutritional quality and safety of GMPs before release and commercialization. In this study, we targeted physiological and biochemical evaluation of transgenic potato plantlets under salt stress conditions. *In vitro* evaluation of salinity effects on transformed and non-transformed clones was investigated with four NaCI (0, 30, 60 and 90 mM). On exposure to NaCl for one month, the shoots height, fresh and dry weight of transformed clone showed significant decrease than those of non-transformed clone. Different biochemical parameters such as proline accumulation, lipid peroxidation, pigments content, ascorbate, and glutathione contents were tested in order to put forward the relative tolerance of both clones to salinity. Constitutive levels of these parameters were almost not similar between both clones. Additionally, electrophoretic analysis of total soluble protein (SDS-PAGE) and some isoenzymes such as acid phosphatase and esterase have revealed that plant subjected to NaCl showed induction in the synthesis of new polypeptides and isoformes. Our finding suggest that, the response of potato plantlets to salt stress may be accomplished by increasing the capacity of antioxidative system and the synthesis of new protein and isoenzymes which could be in turn contribute to select a salt resistant in potato.
*[*Amal A. Mohamed, Mohamed A. Matter and Mahmoud M. Saker. Effect of salt stress on some defense mechanisms of transgenic and wild potato clones *(*Solanum tuberosum L.*) grown* in vitro. Nature and Science 2010; 8(12):181-193]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Ascorbate, glutathione, isoenzymes, proline, NaCl stress, tissue culture, transgenic potato.

Abbreviations: AP: acid phosphatase; DTT: dithiothreitol; EST: esterase; KDa: kilodalton; PAGE: polyacrylamide gel electrophoresis; PMSF: phenylmethanesulphonyl fluoride; ROS: reactive oxygen species; MS media: Murashige and Skoog basal media; SDS: sodium dodecyl sulfate.

Introduction

Plants often face the challenge of several environmental conditions which exerts adverse effects on plant growth and development. One of the major environmental factors limiting the productivity of crops is salt stress which negatively affects the metabolism of plants and causes important modification in different biochemical and molecular processes (Allakhverdiev et al. 2000). Salt stress can trigger various interacting events including the inhibition of photosynthetic enzymes activity, and the decomposition of membrane structures (Meloni et al. 2003). Understanding the biochemical and molecular basis of salt-stress signaling and tolerance mechanisms is essential for breeding and genetic engineering of salt tolerance in crop plants (Zhu 2001). Several studies have shown that salt stress may induce osmotic stress, oxidative stress and protein denaturation in plants, which lead to cellular adaptive responses and accumulation of compatible organic solutes such as soluble carbohydrates, amino acids, proline, betaines, etc., (Hasegawa et al. 2000; Munns 2002). In addition to their role in cell water relations, organic solute accumulation might help towards the removal of free radicals, and stabilization of macromolecules and organelles, such as proteins, protein complexes and membranes (Bohnert and Shen 1999; Bray et al. 2000). Metabolic imbalances caused by ionic toxicity and osmotic stress, under salinity may also lead to oxidative stress and cause accumulation of reactive oxygen species (ROS) and free radicals (Azevedo-Neto et al. 2006; Ashraf 2009). Plants employ antioxidants compounds (e.g., ascorbate, glutathione, α-tocopherol, and carotenoids) and detoxifying enzymes (e.g., superoxide dismutase, catalase, and enzymes of ascorbate-glutathione cycle) to combat oxidative stress (Ashraf et al. 2010). The activity and expression levels of the genes encoding detoxifying enzymes are probably enhanced by ROS under abiotic stresses. Transgenic plants overexpressing ROS scavenging enzymes, such as superoxide dismutase (Alscher et al. 2002), ascorbate peroxidase (Wang et al. 1999), and glutathione S-transferase/glutathione peroxidase (Roxas et al. 2000) showed increased tolerance to osmotic, temperature, and oxidative stresses. Another approach to

understand the molecular basis of salinity tolerance is to identify stress induced changes in the protein expression. Among the studies done in the effect of salt stress on protein synthesis, osmotin a 26 KDa "stress protein" isolated from potato plants adapted to NaCl were quoted (Zhu et al. 1995). The breeding for salt stress tolerance using protein pattern have proved some difficulty because of the large number of stress-associated genes. However, accumulation levels of late-embryogenesis-abundant (LEA) proteins correlate with stress tolerance in various plant species suggesting protective roles under osmotic stress (Vinocur and Altman 2005). Transgenic rice plants engineered to over-express a barley (LEA) gene exhibit better stress tolerance under 200 mM NaCl stress than wild-type plants (Xu et al. 1996). Therefore, the tolerance of plants to salt stress is extremely complex events and various mechanisms appear to be involved. Application of agriculture biotechnology has opened new insights in the development of plants which can tolerate environmental stress and might prove to be a faster track towards improving crop varieties (Ozturk et al. 2002). Potato (*Solanum tuberosum* L.) is one of the most popular and widely consumed vegetables grown worldwide. Potato is considered as moderately salt sensitive compared with other crops (Maas and Hoffman 1977). Although potato was one of the first crops to be transformed by *Agrobacterium tumefaciens* (Sheerman and Bevan 1988) and transgenic plants have been generated in several potato cultivars (Heeres et al. 2002). In Egypt, potato yield is greatly reduced due to infestation with insect pests such as potato tuber moth (PTM), *Phthorimaea operculella* Zeller. In addition, potato plants that harboring different crystal *(cry)* protein genes isolated from many *Bacillus thuringiensis* (Bt) strains have been generated (Davidson et al. 2002; Saker 2003). *In vitro* evaluations of NaC1 effects on potato genotypes were proposed as alternatives to the costly, labor-intensive and sometimes problematic field based traits (Potluri and Devi Prasad 1994). The present study was conducted *in vitro* to evaluate the mechanisms involved in salt stress tolerance of two potato clones (transformed and non-transformed) using different parameters such as; growth, proline accumulation, lipid peroxidation, pigment content, and antioxidant compounds together with the protein and some isoenzymes expression.

Materials and methods

Plant materials and salt treatments:

Transformed potato (*Solanum tuberosum L.* cv. Desiree) line harboring the coat protein gene of Potato Virus Y (CP-PVY), were obtained in a previous work done by (Saker 2003) at Plant Molecular Genetics Laboratory; Center of Excellence for Advanced Sciences (CEAS), National Research Centre (NRC), Egypt. Non transformed Desiree control line was also used. Plantlets of both potato clones were grown on MS media (Murashige and Skoog 1962) having 3% sucrose, 0.8% agar and the pH of the medium was adjusted to 5.8 and autoclaved at 121ºC for 20 min. Cultures were maintained and sub-cultured in growth chamber under 16/8 h light/dark photoperiod with 150 µmol m^{-1} s^{-1} illumination at 25 ±1ºC. *In vitro* grown plantlets were propagated by sub-culturing with four weeks interval. Four levels (0.0, 30, 60 and 90 mM) of NaCI were used with five replicates per jars and each jar was inoculated with five bud nodes for 30 days and the experiments were repeated three times. After four weeks, some physiological and biochemical parameters were measured.

Growth parameters:

Ten plantlets from each group were taken at random; the shoot height (cm) and shoot fresh weights were measured. The shoots were then dried in a forced oven at 70 ^{0}C for 72 h, and the dry weights were recorded.

Proline content:

Proline content was estimated following the method of Bates et al. (1973). Leaf samples (0.5 g) were homogenized in 3% w/v sulphosalycylic acid and the homogenate was filtrated. The resulting solution was treated with glacial acetic acid and acid ninhydrin. The mixture was boiled for 60 min in water bath, and then the reaction was stopped by using ice bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and calculated (µg g^{-1} fw).

Lipid peroxidation:

Lipid peroxidation level was measured as the content of malondialdehyde (MDA) according to the methods of Buege and Aust (1978). Content of MDA, which is an end product of lipid peroxidation, was determined using thiobarbituric acid (TBA) reagent. MDA concentration was calculated from the absorbance at 532 nm and measurements were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Chlorophyll a, chlorophyll b and total carotenoids determination:

The photosynthetic pigments (chlorophyll a, b and total carotenoids) were determined according to

the method of Lichtenthaler (1987). Chlorophyll extract was prepared from fresh leaves (0.5 g) by grinding in a cold mortar and pestle together with 10 ml of ice cold 80% acetone. The chlorophyll a and b were measured using UV-Visible Spectrophotometer at 662 nm and 644 nm respectively. As well as, total carotenoids was measured at 470 nm. A solution of 80% acetone was used as a blank. The concentration of chlorophyll a, chlorophyll b and total carotenoids were calculated (µg g^{-1} fw).

Ascorbic acid (AsA) determination:

ASA were extracted in 5% metaphosphoric acid. The homogenate was centrifuged at 10,000 ×g for 15 min at 4ºC. The ascorbate content was measured in the supernatant using 2,4-dinitrophenolindophenol. The absorbance of red color was measured at 520 nm according to Omaye et al. (1979). The concentration of ascorbic acid in the extracts was calculated using ascorbic acid as standard and the results were expressed as µg g^{-1} fw.

Glutathione (GSH) determination:

Glutathione was extracted by grinding 0.5 g leaves tissue in 1 ml ice-cold 5% (w/v) sulphosalicylic acid solution. After centrifugation at 10,000×g at 4°C for 30 min, the supernatants were collected and immediately assayed. Glutathione was measured with Ellman's reagent as described by Tukendorf and Rauser (1990). Briefly, 300 µl of the supernatant was mixed with 1.2 ml of 0.1 M phosphate buffer solution (pH 7.6). After a stable absorbance reading of 412 nm was obtained, 25 µM 5, 5'- dithiobis (2-nitrobenzoic acid) (DTNB) was added, and the increase in absorbance at 412 nm was monitored ($\sum$412 = 13.6 mM^{-1} cm^{-1}).

Protein extraction:

For SDS-PAGE, leaf tissues of each clones were ground to powder under liquid nitrogen and melted in ice-cold extraction buffer (50 mM Tris-HCl, pH 8, 10 mM NaCl, 1% SDS, 5% 2-mercaptoethanol, 0.1 mM PMSF, 0.1 mM DTT), followed by centrifugation at 10,000 ×g at 4 ^{o}C for 15 min. Protein content of the clear supernatants obtained after centrifugation were determined using Bradford method (Bradford 1976) with bovine serum albumin (BSA) as the standard. Extracts were stored at -20 ^{o}C until used.

One-dimensional SDS-PAGE:

Proteins, 30 µg of each sample, were separated by SDS-PAGE according to the method of Laemmli (1970). The separation was performed with a 10% separating gel and a 4% stacking gel using protein vertical electrophoresis unit (Hoefer Scientific Instruments, SE 6152, San Francisco, Calif.). Electrophoresis was started at 10 mA constant current until the tracking dye entered the separating gel and continued at 25 mA until the tracking dye reached the end of the gel. Protein subunit bands were stained with Coomassie blue R- 250 by standard techniques. The protein marker from Sigma was used. The molecular weight of standard protein (in KD) as follows: 200, 150, 100, 85, 70, 60, 50, 40, 30, 25, 20, 15 and 10 KD (13 bands).

Isozymes analysis:

Isozymes were analyzed using the native polyacrylamide gel electrophoresis (Native-PAGE) 10%, according to Pan et al. (1991). The gels were run for 2 h at 10ºC and 30 mA. The following enzymes systems were detected: AP (acid phosphatase), and EST (esterase), then enzymes staining were performed according to the procedures described previously by Posvec and Griga (2000).

Statistical analysis

All data are reported as mean ± standard deviation (S.D.) for the three independent samples (n=3). Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package. The least significant difference (LSD) at $P \leq 0.05$ level was calculated.

Results

Plant growth:

After exposure of plantlets to different concentrations of NaCl (0, 30, 60 and 90 mM) for 30 days, morphological changes were observed and the growth of both potato clones was gradually decreased by increasing NaCl levels. However, plantlets growth was less affected by 30 mM NaC1 containing MS media and generally, it was almost similar to control levels whereas 60 and 90 mM NaCl concentrations, significantly reduced plantlets growth compared with the control (Fig 1). Additionally, NaCl stress treatments gradually decreased the shoot height, shoot fresh weight and shoot dry weight, when compared to those plantlets grown under control condition (Table 1). At 30 mM NaCl, non transformed line yielded higher shoot height (20.4 cm) and shoot fresh weight (60.9 mg) than transformed line, which recorded (12.5 cm) for shoot height and (47.1 mg) for shoot fresh weight respectively. The control was always superior compared to the treatments employed for all the growth traits and clones.

Proline accumulation:

It was clear from table (2) that, a gradual increase in the accumulation of proline was observed in both clones at all stress regimes. A more pronounced increase was observed in the transformed line compared to non-transformed line. However, at 60 mM NaCl, the level of proline content was significantly increased in both clones and were higher in transformed line (203.1 µg g^{-1}fw) than in non-transformed line (269.4 µg g^{-1}fw) . Also, at 90 mM NaCl, the level of proline was increased by 342.3% and 179.6 % of the control (100%) in transformed and non-transformed line, respectively.

Lipid peroxidation:

Salt stress caused a significant increase in the levels of malondialdehyde (MDA) accumulations, caused by cell membrane lipid peroxidation, in both clones compared to control treatment (Table 2). MDA reached to the highest level at 90 mM NaCl treatment in both clones, but the rate of increment was higher in non-transformed line (1.11µmol MDA g^{-1} fw) than the transformed line (0.72 µmol MDA g^{-1} fw). Also, the increasing level at 30, 60 and 90 mM NaCl salts were 146.9, 225.0 and 346.9 % in non transformed; and 166.7, 242.0 and 342.9 % in transformed line , respectively compare to the controls level (100%).

Chlorophylls content:

Data in table (3) showed that NaCl-stress caused significant reduction ($P<0.05$) in the content of chlorophyll a, chlorophyll b and total carotenoids (Cx+c) however, these reduction was more pronounced in non- transformed line. Generally, it was clear that the values of chlorophyll a, chlorophyll b and total carotenoids for transformed line treated with different concentration of NaCl were higher than the values of non- transformed line at the same levels of NaCl.

Antioxidant compounds (Ascorbate and glutathione):

Ascorbate (AsA) content in leaves of stressed plantlets tended to increase gradually with increasing of salt levels in the growth media (Table 4). It is well known that, ascorbic acids acts as antioxidant in detoxification of ROS. The highest values were recorded in transformed line, while the lowest values were in case of non-transformed one. Similarly, in the same table, the glutathione content was also affected significantly due to salt stress. The glutathione (GSH) content in leaves of non-transformed line increased by 276.3% and 337.7% at 60 and 90 mM NaCl respectively compared with that of untreated control one (100%). However, in leaves of transformed line, the GSH content showed a marked increase under NaCl treatments; the values reached to 263.4 % and 298.3% at 60 and 90 mM NaCl respectively, compared to control one (100%).

Protein profile:

Salt stress caused an induction in the synthesis of some new polypeptides in potato plantlets compared to control one (Fig. 2). Generally, the electrophoretically separated protein in NaCl-treated plantlets as compared with control revealed (i) quantitative decline in certain proteins, (ii) rise in levels of other proteins, (iii) some proteins remained unchanged, and (iv) *de novo* induction of specific proteins. Levels of proteins with molecular weights of 150, 100, 60, 80, 70, and 20 KDa polypeptides in both clones, were common bands under controlled treatments. Application of NaCl in the media at 60 and 90 mM NaCl caused changes in the levels of proteins with molecular weights of 60 and 70 KDa for non-transformed clones (lanes 3 and 4) however, 25 and 30 KDa for transformed line (lanes 7 and 8). These alterations ranged in molecular weight from as low as 20 KDa to as high as 200 KDa. From the general picture of stress proteins emerging from this work, one point is noteworthy, more protein alterations were scored in transformed line than non-transformed for stress tissue, and it is possible that this differential response in both clones reflect their relative sensitivities to stress conditions.

Isozymes activity:

The electrophoretic profiles of AP and EST isozymes showed that isozymes activity was affected by NaCl treatments and a differential AP and EST isozymes profiles between both clones (Fig. 3 and 4) were observed.

Acid phosphatase (AP): At 30 and 60 mM NaCl, when protein extracts were separated by native electrophoresis and monitored for AP activity, some isozymes band were disappeared (lanes 2 and 3) compared to 90 mM NaCl (lane 4) in non-transformed line. However, in transformed line other distinct bands in varying amount were observed at 90 mM NaCl treatment (lane 8) compared to control treatment (lane 5), this variation seems to have correlation with salt stress in the medium (Fig 3).

Esterase (EST): Electrophoretic patterns of esterase isozyme showed differences in density and number of bands among control and treated both clones (Fig. 4). Both two clones generated 5 distinct bands which could be easily resolved into four separate zones of enzyme activity from anodal side to cathodal side of the gel. Therefore, we recommend that these enzymes

could be used for identifying potato clones when grown under salt stress.

Discussion

Plant growth:

Generally, growth results from the interaction of all the metabolic processes within the plant and is expressed as increment in dry mass, volume, length or area of cells. In the present study, salt stress retarded the plantlets growth of potato clones by inhibiting root/shoot length and dry mass production, etc. The shoots of transformed line showed more reduction in fresh weight, and dry weight when compared to non-transformed line with increasing salt stress. Reduced tissue growth in stressful medium is a typical phenomenon that has been interpreted as a change in metabolism initiated to resist stress. In addition to poor extension growth of roots, the shoots become yellow, while under control conditions, no growth depression was observed in both lines. This observation is in conformity with earlier reports of Munns (1993). The author reported that growth parameters were found to be important indicators for screening of salt tolerance. In addition to shoot height, the results revealed that leaf damage was due to the accumulation of excessive toxic ions (Na^+ and K^+) in the cell vacuoles. The accumulation of excessive amounts of toxic ions in leaf cell vacuoles reduces carbon fixation (Barlass and Skeene 1981). Whereas, reduced shoot growth under salinity is associated with reactive oxygen species (Rodriguz et al. 2002).

Proline accumulation and lipid peroxidation:

Proline accumulation is a common metabolic response of higher plants when subjected to salt stress, and has been the subject of numerous works. For example, proline content have been reported to increase under NaCl stress in *Phaseolus aureus* (Misra and Gupta 2005), *Morus Alba* (Ahmad et al. 2007), *Sesamum indicum* (Koca et al. 2007). Proline has several functions during stress: e.g. osmotic adjustment (Voetberg and Sharp 1991), osmoprotection (Kishor et al. 2005), free radical scavenger and antioxidant activity (Sharma and Dietz 2006). A positive correlation between magnitude of free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials between mulberry cultivars (Ramanjulu and Sudhakar, 2000). Salt stress has been widely shown to cause an induction of lipid peroxidation. Determining the MDA content and hence, the extent of membrane lipid peroxidation, has often been used as a tool to assess the degree of plant sensitivity to oxidative damage (Blokhina et al. 2003). Also Koca et al. (2007) showed that lipid peroxidation was higher at 100 mM NaCl in salt sensitive cultivar of *Sesamum indicum* than salt tolerant one. Our data showed that MDA reached to the highest level at 90 mM NaCl treatment in both clones but, the rate of increment was higher in non-transformed line than the transformed one. Lipid peroxidation was shown to remain unchanged in plants tolerant towards salinity or drought (Egert and Tevini 2002). The better protection in transformed line seems to be resulted from the most efficient antioxidative system while significant increase in MDA level in leaves of transformed appeared to be derived from decreased activities of the antioxidative enzymes system.

Chlorophylls contents:

Chlorophyll a, chlorophyll b and total carotenoids (Cx+c) contents were decreased under salt stress in non- transformed and transformed clones. Our results were in accordance to Benavides et al. (2000) who showed a reduction of the photosynthetic assimilation associated to a reduction of chlorophyll content and measured a 23% decrease in chlorophyll content in salt-sensitive potato clones compared to tolerant ones. Also, Fedina et al. (2006) reported only a slight reduction of the chlorophyll fluorescence in barley under salt stress conditions. It was found that chlorophyll level of both clones were quite similar at 30 salt application. Turhan and Eris (2004) observed that the variation in chlorophyll amount caused by salt applications in strawberry plant is not important statistically. However, in our study, the variation in chlorophyll amount caused by salt applications both in transformed and non-transformed clones is important statistically.

Antioxidant compounds (ascorbate and glutathione):

Plant antioxidant defense systems and their roles in protecting plants against stresses have attracted considerable interest. The present study showed that AsA content of the potato notably increased upon salinity stress treatments, especially at 90 mM NaCl. The highest values recorded in transformed line, while the lowest values were in case of non-transformed line. Thus, these results seem to support the hypothesis that tolerance to oxidative stress plays an important role in adapting plants to adverse environmental conditions (McKersie et al. 1996; Smirnoff 1998). The same trend also was reported by Yu and Liu (2003) in wild soybean. The increased AsA content is a stress-protecting mechanism of plants under salinity conditions (Shalata et al. 2001). A high level of endogenous AsA is essential for maintaining the non-enzymatic scavenging system that protects plants

from oxidative damage due to salinity stress (Shigeoka et al. 2002). Total glutathione contents in salt-stressed potato clones were increased compared to control. The highest values recorded in transformed line, while the lowest values were in case of non-transformed line. GSH plays an important role in the response of plants to oxidative stress due to the generation of active oxygen species (Huckelhoven and Kogel 2003), and some heavy metals (Grill et al. 1985). A regulated balance between oxygen radical production and destruction required, if metabolic efficiency and function are to be maintained either in normal or stress condition. A constitutive high antioxidant capacity under stress conditions with plant resistance to that particular stress. Consequently, the mechanism that reduces ROS species and increase antioxidant enzyme system in plant are an important role in imparting tolerance in plant under environmental stress (Mittler 2002). We observed that GSH content was enhanced under 90 mM NaCl, and significantly increase GSH content, suggesting that under severe stress conditions, GSH of the potato plantlets plays a protective role in salinity tolerance.

Protein profile:

The protein patterns of two potato clones grown at different concentration of NaCl were analyzed. The separated bands of protein subunits were photographed and presented in Fig. (2). In general, both clones under all treatments contained three major distinct common bands with high molecular weight, 20, 80 and 100 KD. The pattern of high molecular weight band indicated that, there was a particular strong induction of biosynthesis of (100 KD) proteins in both clones under different salt treatments. Also the transformed line exhibited a new faint band with MW ≅30 KD (lane 8) at 90 mM salt stress treatment. Consequently these newly synthesized bands indicated that salt stress induced related gene(s) to produces these salt inducible proteins. One possible explanation for appearance of some proteins under salt stress is that the gene (s) responsible for certain proteins had been completely enhanced as a result of stress. This may be applied to the protein that stained high densely under stress (ELObeidy et al. 2001). Generally the results of SDS-PAGE analysis could reveal two different genetic mechanisms i.e., salt stress resulted in the overexpression of some gene and / or *de novo* induction of gene expression. This is in addition to the occurrence of a new protein band with molecular weight of 26 KDa which was unique to the salt exposed cells. El-Farash et al. (1993) studied the expression of 12 different proteins, which were induced in salt stressed tomato plants. They reported that the expression of these proteins was genetically regulated, depending on the salt concentrations as well as the genetic differences in tomato plant. In general, these patterns may give a remarkable marker to relay the discrimination between treated and untreated clones since one could consider the presence of new bands as an adaptive band for stress treatment.

Isozymes profile:

The utilization of multiple isoforms of enzymes is one of the primary control mechanisms of cellular metabolism in plants. However, little data is available on the regulation of antioxidant enzymes isoforms expression and salt stress tolerance (Gueta-Dahan et al. 1997). It is well known that enzymes that have multiple intracellular distributions are present as different isozyme forms in different cell compartments (Damondara and Venkaia 1984). Therefore, the analysis of the activity of individual acid phosphates (AS) and esterase (EST) isozymes is important, because it can help to understand how each stress affects the different sub-cellular compartments (Scandalios 1993). Generally, the induction of new isoenzymes and the change in the isoenzyme profile is considered to play an important role in the cellular defense against oxidative stress, caused by salt stress. Our focus was to observe the response of hydrolytic isoenzymes to increasing salinity stress, In order to clarify the protective mechanisms of the antioxidant enzymes against salt stress, the changes in the activation and inactivation of isozyme profiles in potato plant subjected to salt stress were detected. The activity and resolution of AP and EST showed strong activity and acceptable resolution. These results indicated that salt stress increased the accumulation of the AP enzyme. In our study, the intensities of AP were enhanced by NaCl; these results are in agreement with the findings of Shih and Kao (1998) who reported that salt stress increased AP activity. Moreover salinity increases EST isozyme, the highest numbers of esterases isoenzymes were detected under the highest NaCl concentration (Hassanein 1999). The evaluation of the protein decay due to salt stress could be a marker of the sensitivity of the concerning cultivars towards NaCl together with the activities of isozymes may be a good indicator for selecting salt tolerance. In conclusion: genetic improvement of salt tolerance has become an urgent need for the future of agriculture in arid and semiarid regions. Transformed potato line harboring the coat protein gene of potato virus y (CP-PVY) can be effective in preventing diseases caused by homologous and closely related viruses. At the same time, the new polypeptide formed in transformed line may play an important role in salt tolerance mechanism. So, many plants response to

various types of stress by changes in the activity of oxidative stress but its enhancement and protein expression vary among plant species and probably could correspond to plant tolerance to different stresses, however many experiments in this field are needed.

Table (1): Shoot height; shoot fresh and dry weights of non-transformed and transformed potato plantlets grown on MS media supplemented with different concentrations of NaCl for 30 days.

Potato lines	Proline content (µg g^{-1} fw)			Lipid peroxidation (µmol MDA g^{-1} fw)		
NaCl (mM)	Non-transformed	Transformed	Mean	Non-transformed	Transformed	Mean
0.0	120.5 ± 0.12	79.2 ± 0.06	99.85	0.32 ± 0.02	0.21 ± 0.14	0.27
30.0	152.8 ± 0.22	244.8 ± 0.14	88.80	0.47± 0.014	0.35 ± 0.12	0.41
60.0	203.1 ± 0.05	269.4 ± 0.13	236.25	0.72 ± 0.03	0.51 ± 0.06	0.62
90.0	216.5 ± 0.01	271.1 ± 0.05	243.80	1.11 ± 0.11	0.72 ± 0.04	0.92
Mean	173.23	216.13	194.68	0.66	0.45	0.55
LSD 0.05						
Lines	0.16			0.01		
NaCl	0.23			0.02		
Lines × NaCl	0.32			0.02		

Significant difference at $p \leq 0.05$. The values are mean ± S.D.

Table (2): Proline content and lipid peroxidation in plantlets of non-transformed and transformed lines grown on MS media supplemented with different concentrations of NaCl for 30 days.

Potato lines	Shoot height (cm)			Shoot fresh weight (mg)			Shoot dry weight (mg)		
NaCl (mM)	Non-transformed	Transformed	Mean	Non-transformed	Transformed	Mean	Non-transformed	Transformed	Mean
0.0	21.2 ± 0.20	18. 5± 0.05	19.85	77.5 ± 0.11	58.8 ± 0.15	68.15	9.5 ± 0.17	9.0 ± 0.13	9.25
30.0	20.4 ± 0.31	12.5 ± 0.22	16.45	60.9 ± 0.08	47.1 ± 0.06	54.00	8.8 ± 0.14	8.7 ± 0.05	8.75
60.0	9.5 ± 0.12	6.6 ± 0.05	8.05	57.8 ± 0.02	39.8 ± 0.08	48.80	8.4 ± 0.05	6.9 ± 0.11	7.65
90.0	7.5 ± 0.04	5.8 ± 0.16	6.65	52.6 ± 0.14	37.2 ± 0.06	44.90	7.5 ± 0.07	5.8 ± 0.14	6.65
Mean	14.65	10.85	12.75	62.20	45.730	53.96	8.550	7.60	8.08
LSD 0.05	0.11			0.180			0.23		
Lines	0.15			0.250			0.33		
NaCl	0.21			0.350			0.47		
Lines × NaCl									

Significant difference at $p \leq 0.05$. The values are mean ± S.D.

Table (3): Chlorophyll a (Chla), chlorophyll b (Chlb), and total carotenoids (Cx+c) content in non-transformed and transformed plantlets lines grown on MS media supplemented with different concentration of NaCl for 30 days.

Potato lines	Chla ($\mu g g^{-1}$ fw)			Chlb ($\mu g g^{-1}$ fw)			Cx+c ($\mu g g^{-1}$ fw)		
NaCl (mM)	Non-transformed	Transformed	Mean	Non-transformed	Transformed	Mean	Non-transformed	Transformed	Mean
0.0	314.1 ± 0.01	519.5 ± 0.02	416.80	148.2 ± 0.08	336.9 ± 0.06	242.550	100.6 ± 0.12	180.7 ± 0.21	140.65
30.0	278.2 ± 0.11	440.9 ± 0.12	359.55	114.4 ± 0.13	205.8 ± 0.08	160.100	96.7 ± 0.05	202.9 ± 0.29	149.8
60.0	232.7 ± 0.06	361.6 ± 0.02	297.15	104.9 ± 0.17	195.9 ± 0.11	150.400	87.1 ± 0.09	174.5 ± 0.15	130.8
90.0	224.5 ± 0.04	215.2 ± 0.08	219.85	100.9 ± 0.05	139.5 ± 0.13	120.200	69.5 ± 0.12	162.3 ± 0.17	115.9
Mean	262.38	384.30	323.34	117.10	219.53	168.313	88.48	180.10	134.29
LSD 0.05		0.21			0.15			0.27	
Lines		0.30			0.21			0.38	
NaCl		0.42			0.30			0.54	
Lines × NaCl									

Significant difference at $p \leq 0.05$. The values are mean ± S.D.

Table (4): Ascorbic (AsA) and glutathione (GSH) contents in plantlets of non-transformed and transformed lines grown on MS media supplemented with different concentration of NaCl for 30 days.

Potato lines	Ascorbic content ($\mu g\ g^{-1}$ fw)			Glutathione content (mmol g^{-1} fw)		
NaCl (mM)	Non-transformed	Transformed	Mean	Non-transformed	Transformed	Mean
0.0	1.13 ± 0.08	1.46 ± 0.09	1.30	1.14 ± 0.11	1.75 ± 0.02	1.45
30.0	1.71 ± 0.06	2.7 ± 0.14	2.21	1.65 ± 0.15	3.65 ± 0.07	2.65
60.0	2.09 ± 0.11	3.75 ± 0.04	2.92	3.15 ± 0.17	4.61 ± 0.17	3.88
90.0	3.01 ± 0.13	4.75 ± 0.06	3.88	3.85 ± 0.09	5.22 ± 0.13	4.54
Mean	1.99	3.17	2.58	2.49	3.81	3.13
LSD 0.05						
Lines		0.03			0.32	
NaCl		0.05			0.45	
Lines × NaCl		0.06			0.63	

Significant difference at $p \leq 0.05$. The values are mean ± S.D.

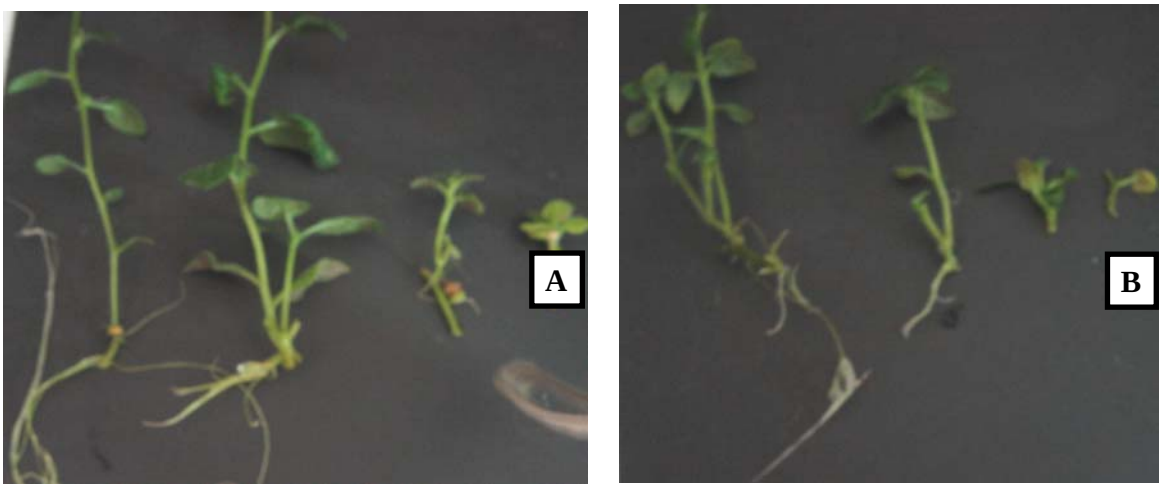

Fig (1): Salinity stress gradually affects the plantlets growth of non-transformed (A) and transformed (B) potato lines grown under different concentrations of NaCI (0, 30, 60 and 90 mM) for 30 days

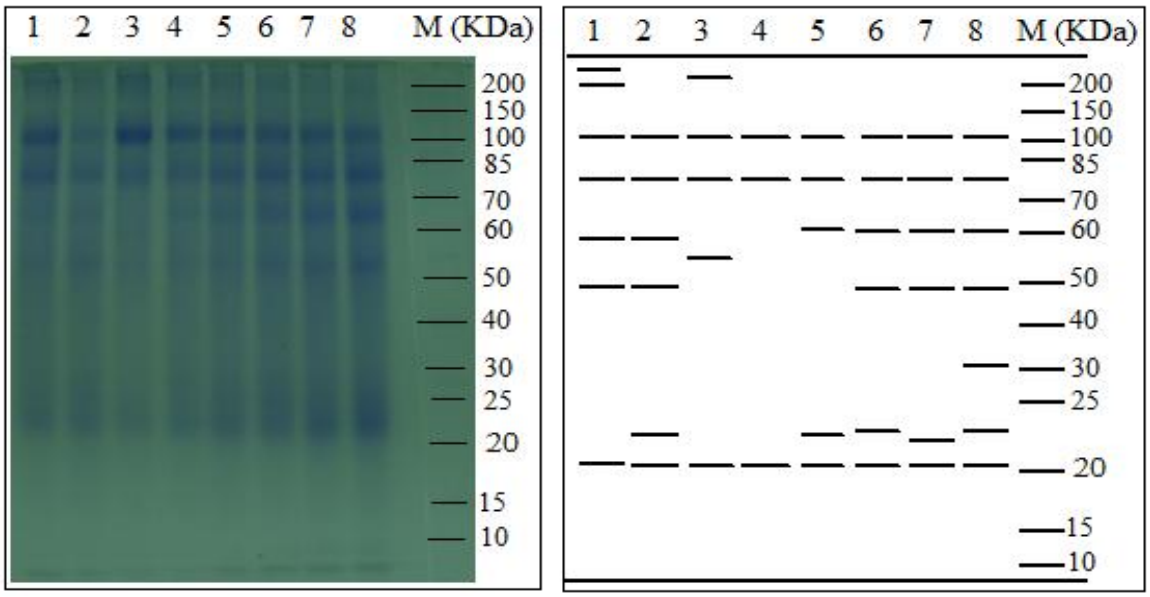

Fig (2): Protein pattern of non- transformed and transformed potato extract. Lanes 1, 2, 3, 4, 5, 6, 7 and 8 from left to right represent proteins extracted from control, 30, 60 and 90 mM NaCl treated non-transformed and transformed potato lines after 30 days of treatment, respectively. Lane M represents the molecular weight marker.

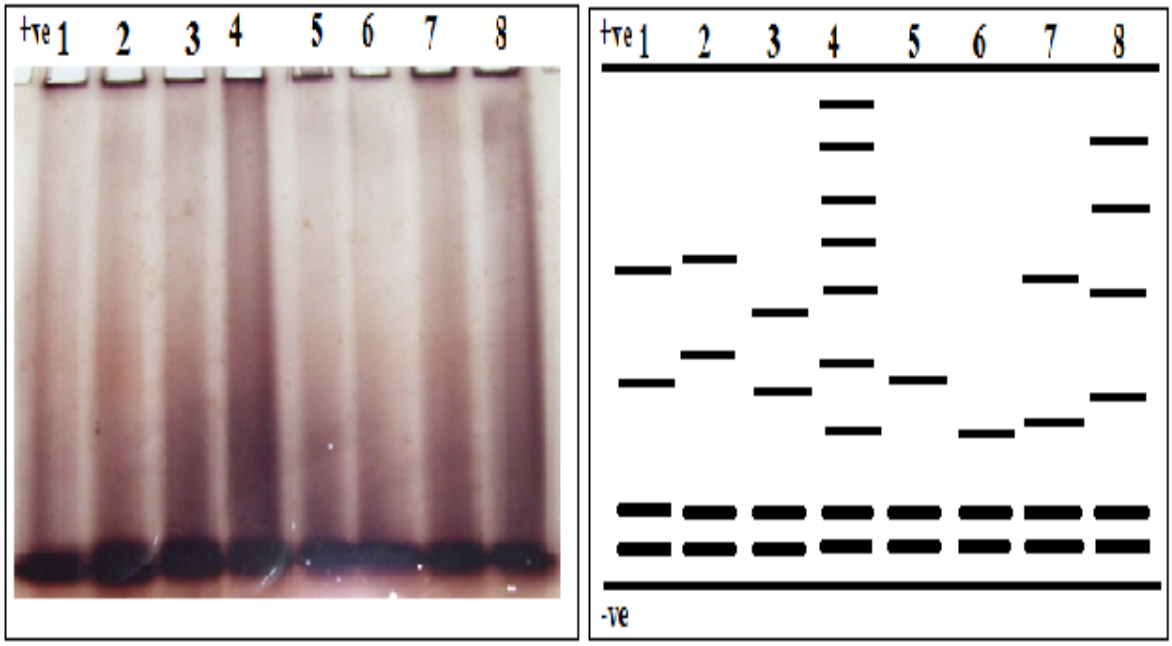

Fig (3): Zymogram of acid phosphatase activity of non-transformed and transformed potato lines whereas:- 1= non-transformed + 0.0 mM NaCl, 2= non- transformed +30 mM NaCl, 3= non- transformed +60 mM NaCl, 4= non- transformed +90 mM NaCl, 5= transformed +0.0 mM NaCl, 6= transformed + 30 mM NaCl, 7= transformed + 60 mM NaCl and 8= transformed + 90 mM NaCl.

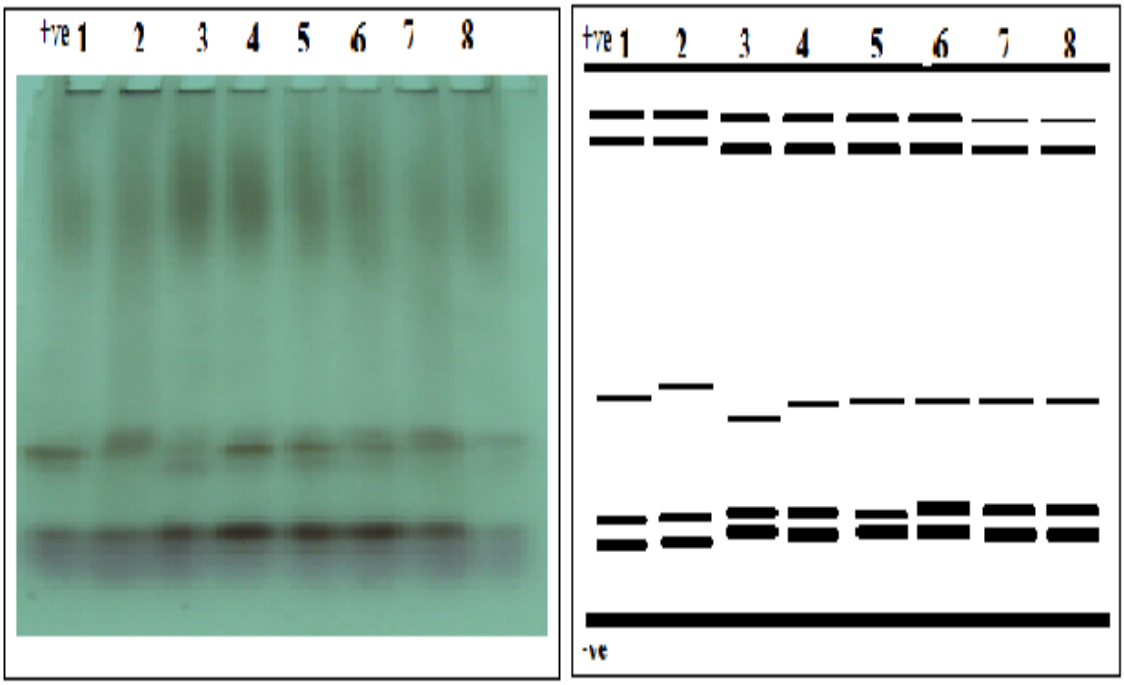

Fig (4): Zymogram of esterase activity of non-transformed and transformed potato lines whereas:- 1= non-transformed + 0.0 mM NaCl, 2= non- transformed +30 mM NaCl, 3= non- transformed +60 mM NaCl, 4= non- transformed +90 mM NaCl, 5= transformed +0.0 mM NaCl, 6= transformed + 30 mM NaCl, 7= transformed + 60 mM NaCl and 8= transformed + 90 mM NaCl.

References

Ahmad P, Sharma S, Srivastava PS. *In vitro* selection of $NaHCO_3$ tolerant cultivars of *Morus alba* (Local and Sujanpuri) in response to morphological and biochemical parameters. *Hort. Sci.* 2007; 34, 115-123.

Allakhverdiev I, Sakamoto A, Nishiyama Y, Inaba M, Murata N. Ionic and osmotic effects of NaCl- induced inactivation of photosystems I and II in Synechococcus sp, *Plant Physiol.* 2000; 123, 1047–1056.

Alscher RG, Erturk N, Lenwood HS. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* 2002, 53, 1331-1341.

Ashraf M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech. Advan.* 2009; 27, 84-93.

Ashraf MA, Ashraf M, Ali Q. Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. *Pak.J.Bot.* 2010; 42, 559-565.

Azevedo - Neto AD, Prisco JT, Enéas-Filho J, Abreu CEB, Gomes-Filho E. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt tolerant and salt-sensitive maize genotypes. *Environ. Exp. Bot.* 2006; *56*, 87-94.

Barlass M, Skeene KGM. Relative NaCl tolerance of grape vine cultivars and hybrids in vitro. *Plant Physiol.* 1981; 102, 147-156.

Bates L S, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. *Plant and Soil.* 1973; 39, 205 – 207.

Benavides MP, Marconi PL, Gullego SM, Comba ME, Tomaro ML. Relationship between antioxidant defense system and salt tolerance in *Solanum tuberosum*. *Aust. J. Plant Physiol.* 2000; 27, 273-278.

Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress; a review. *Annals Bot.* 2003; 91, 179–194.

Bohnert HJ, Shen B. Transformation and compatible solutes. *Sci. Hortic.* 1999; 78,237- 260.

Bradford MM. A rapid and sensitive methods for the quantification of microgarm quintets of protein utilizing the principle of protein–day binding. *Anal. Biochem.* 1976; 72,248-254.

Bray EA, Bailley-Serres J, Weretilnyk E. Responses to abiotic stresses. In: Biochemistry and Molecular Biology of Plants (Gruissem, W. et al., eds), 2000; pp.1158–1203, American Society of Plant Physiologists.

Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods in Enzymolog.* 1978; 52, 302 – 312.

Damondara C, Venkaia B. Studies on isoenzymes of superoxide dismutase from mung bean (*Vigna radiate*) seedlings. *J. Plant Physiol.* 1984; 116, 279-284.

Davidson MM, Jacobs J M, Reader J K, Butler RC, Christina M, Markwich NP, Wratten SD, Conner AJ. Development and evaluation of potatoes transgenic for a cry1Ac9 gene conferring resistance to potato tuber moth. *J.Amer. Soc. Hort. Sci.*, 2002; 127, 590-596.

Egert M, Tevini M. Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (*Allium schoenoprasum*). *Environ. Exp. Bot.* 2002; 48, 43-49.

El-Farash EM, El-Enamy AE, Mazen A. Influence of genotype and NaCl on the levels of growth, proteins, proline, free amino acids, viability and protein regulation in tomato callus cultures. *Physiol. Plant.* 1993; 4, 345-352.

ELObeidy AA, Fayek MA, Mohmoud RA. Quantifying salt tolerance responses of some banana genotypes *in vitro*.1st international conference on: "Biotechnology Applications for the Arid Regions", Eds. Shayji, Y.A.; Siolhu, J.S.; Saleem, M. and Guerinik, K. State of Kuwait – Kuwait. 2001; 135 – 165.

Fedina I, Georgieva K, Velitchkova M, Grigorova I. Effect of pre-treatment of barley seedlings with different salts on the level of UV-B induced and UV-B absorbing compounds. *Environ. Exp. Bot.*, 2006; 56, 225-230.

Grill E, Winnacker EL, Zenk MH. Phytochelatins: the principal heavy metal complexing peptides of higher plants. *Science.* 1985; 230, 674-676.

Gueta-Dahan Y, Yaniv Z, Zilinskas A B, Ben-Hayyim G. Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in Citrus. *Planta.* 1997; 203,460-469.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Physiol. Plant Mol. Biol*. 2000; 51, 463–499.

Hassanein AM. Alteration in protein and esterase peanut in response to salinity stress. *Biol. Plant.* 1999; 42,241-248.

Heeres P, Schippers-Rozenboom M, Jacobsen E, Visser RGF. Transformation of a large

number of potato varieties: genotype dependent variation in efficiency and somaclonal variability. *Euphytica.* 2002; 124, 13–22.

Huckelhoven R, Kogel KH. Reactive oxygen intermediates in plant-microbe interactions: Who is who in powdery mildew resistance? *Planta.* 2003; 216, 891– 902.

Kishor PBK, Sangam S, Amrutha R, Laxmi PS, Naidu KR, Rao KS. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Curr. Sci.* 2005; 88, 424-438.

Koca M, Bor M, Ozdemir F, Turkan I. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot.* 2007; 60, 344–351.

Laemmli UK. Cleavage of structural proteins during the assembly of the head bacterio-phage T4. *Nature.* 1970; 227, 680-685.

Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymolog*. 1987; 148,350 – 382.

Maas EV, Hoffman GJ. Crop Salt Tolerance – Current Assessment. *J. Irrig. and Drain. Divi. Proc. Am. Soc. Civil Engr*. 1977; 103, 115-134.

McKersie BD, Bowley SR, Harjanto E, Leprince O. Water-deficit tolerance and field performance of transgenic alfalfa over expressing superoxide dismutase. *Plant Physiol.* 1996; 111, 1177-1181.

Meloni DA, Oliva MA, Martinez CA, Cambraia J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. .Exp. Bot.* 2003; 49, 69-76.

Misra N, Gupta AK. Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. *Plant Sci*. 2005; 169, 331-339.

Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 2002; 7, 405-410.

Munns R. Physiological process limiting plant growth in saline soil: some dogmas and hypotheses. *Plant Cell Environ.* 1993; 16, 15-24.

Munns R. Comparative physiology of salt and water stress. *Plant Cell Environ*.2002; 28,239-250.

Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* 1962; 15, 473 – 497.

Omaye ST, Turbull TP, Sauberchich HC. Selected methods for determination of ascorbic acid in cells, tissues and fluids. *Methods Enzymol*. 1979; 6, 3-11

Ozturk ZN, Talamé V, Deyholos M, Michalowski CB, Galbraith DW, Gozukirmizi N, Tuberosa R, Bohnert HJ. Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. *Plant Mol. Biol.* 2002; 48, 551–573.

Pan S, Ye X, Kuc J. A technique for detection of chitinases, B-1, 3-glucanases and protein pattern, after single separation using PAGE or isoelectric focusing. *Phytopathol.* 1991; 81, 970–974.

Posvec Z, Griga M. Utilization of isozyme polymorphism for cultivar identification of 45 commercial peas (*Pisum sativum L.*). *Euphytica.* 2000; 113, 251– 258.

Potluri SDP, Devi Prasad PV. Salinity effects on *in vitro* performance of some cultivars of potato. *R. Bras. Fisiol. Veg.*1994; 6, 1-6.

Ramanjulu S, Sudhakar C. Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. *J Plant Physiol.* 2000; 157, 81–85.

Rodriguz AA, Grunber KA, Taleisinik EL. Reactive oxygen species in the elongation zone of maize leaves is necessary for leaf extension. *Plant Physiol.* 2002; 129, 1627-1632.

Roxas VP, Lodhi SA, Garrett DK, Mahan JR, Allen RD. Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. *Plant Cell Physiol.* 2000; 41, 1229-1234.

Saker MM. Production of biosafe transgenic potato plants with coat protein gene for potato virus Y. *Arab J. Biotech.* 2003; 6, 125-138.

Scandalios JG. Oxygen stress and superoxide dismutases. *Plant Physiol.* 1993; 101, 1-7.

Shalata A, Mittova V, Guy M, Tal M. Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicum pennellii* to salt-dependent oxidative tress: the root antioxidant system. *Physiol. Plant.* 2001; 112, 487-494.

Sharma SS, Dietz KJ. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot.* 2006; 57, 711-726.

Sheerman S, Bevan MW. A rapid transformation method for *Solanum tuberosum* using binary Agrobacterium tumefaciens vectors. *Plant Cell Reports.* 1988; 7, 13-16.

Shih CY, Kao CH. Induction of acid phosphatase in detached rice leaves under stress conditions. *Bot. Bull. Acad. Sin*. 1998; 39, 29-32.

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K. Regulation and function of ascorbate peroxidase isoenzymes. *J. Exp. Bot.* 2002; 53, 1305-1319.

Smirnoff N. Plant resistance to environmental stress. *Curr. Opin. Biotechnol*. 1998; 9, 214-219.

Tukendorf A, Rauser WE. Changes in glutathione and phytochelations in roots of maize seedling exposed to cadmium. *Plant Sci.* 1990; 70, 155 – 166.

Turhan E, Eris A. Effect of sodium cloride applications and different growth media on ionic composition in strawberry Plant. *J. Plant Nutr*. 2004; 27, 1653- 1665.

Zhu B, Chen THH, Li H. Activation of two osmotin-like protein genes by a biotic stimuli and fungal pathogen in transgenic potato plant. *Plant Physiol*. 1995; 108, 929 – 937.

Zhu JK. Plant salt tolerance. *Trends in Plant Science.* 2001; 6, 66–71.

Wang J, Zhang H, Allen RD. Overexpression of an Arabidopsis peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. *Plant Cell Physiol.* 1999; 40, 725-732.

Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotech.* 2005; 16, 123–132.

Voetberg GS, Sharp RE. Growth of the maize primary root in low water potentials. III. Roles of increased proline depositions in osmotic adjustment. *Plant Physiol.* 1991; 96, 1125-1130.

Xu D, Duan X, Wang B, Hong D, Ho THD, Wu R. Expression of a late embryogenesis abundant protein gene, HVA, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol.* 1996; 110, 249–257.

Yu BJ, Liu YL. Effects of salt stress on the metabolism of active oxygen in seedlings of annual halophyte Glycine soja. *Acta Botanica Boreali-Occidentalia Sinica.* 2003; 23, 18-22.

11/12/2010