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Evaluation of salt stress tolerance on some growth and biochemical attributes in *Suaeda fruticosa* L. (Forssk)

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ABSTRACT

A field-experiment was setup with earthen pots in complete randomized block design having ten replicate plants for each salt (NaCl) treatment. Initially the plants were raised by irrigating with tap water and then after their establishment, with NaCl (50, 100 and 200 mM) containing water. Various growth and biochemical characteristics were found to be adversely affected by saline treatments i.e., root length, shoot length, fresh weight, dry weight, amount of protein and antioxidant enzymes. The higher levels of NaCl (100-200 mM) in this investigation drastically suppressed the growth of plants of *Suaeda fruticosa* L. in the form of stunted growth. The overall increase in antioxidant enzyme activities during this study seems to be their scavenging role by neutralizing the reactive oxygen species produced during salt stress episode. It is therefore, suggested from the results of this research work that biochemical characteristics were related to a transferring of plants from being salt sensitive to relatively more tolerant. Apparently lot of work on this salt bush regarding biochemical and physiological characteristics still remains elusive and needs further experimentation under greenhouse as well as field conditions to draw meaningful conclusions.

Keywords: Antioxidant enzyme activities, Halophytes, NaCl, Protein contents, Stress

INTRODUCTION

The total area of the world which is affected by salinity is 831 million hectares (20% of total irrigated land in the world) which include 399 million hectares of saline and 445 million hectares of sodic soil (Zaman *et al.,* 2009; FAO 2014). Soil salinity, a major environmental constraint badly affects the growth as well as productivity of many cash crops and reduces the overall production in the world. It naturally occurs in areas in which salt is already a part of the soil composition. Secondary source of salinity are irrigation with saline water and poor drainage system (Zhu, 2007). Physical condition of soils in which salt contents are greater will become very poor for germination of seeds. It will be less aerated as well as the ability of soil to hold water is also lost. In other words, the soil becomes less fertile. Additionally, too much sodium and chloride ions are lethal to plants. The cations and anions that are correlated with high salinity are Na^+,Ca^{2+}, Mg^{2+}, Cl^-, SO_4, and HCO_3 (Yadav *et al*., 1996).

Most of the halophytes (550 species) are included in *Chenopodiaceae* (Aronson, 1989). Several other families such as *Poaceae, Fabaceae, Asteraceae* also includes halophytes but they count only less than 5% of the species (Aronson, 1989). Salt tolerant plants are distributed in coastal and inland areas of the world (Ungar, 1991). It is reported that plantings of halophyte could remove about 2,646 Kg ha^{-1} of salt per year in the saline habitats (Chaudhri *et al.*, 1964). For their establishment germination is an important stage in the life cycle of plants and during the germination stage salt tolerance is critical for growth of plants (Khan and Ungar, 1996). Generally, response of plants under salinity stress can be studied at three levels i.e., at cell stage, at tissue level and at whole plant level. To develop complete knowledge of salt tolerance in halophytes, it is the pre-requisite to have thorough understanding the mechanism operating at three above mentioned levels. Less is known about the biochemical/molecular mechanisms involved against the various abiotic stresses such as drought, salt and osmotic stress (Zhu, 2001; Seki *et al.,* 2003).

Suaeda fruticosa L. is a succulent obligate halophyte and it produces numerous seeds in a

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growing season. It is widely distributed in the salt marshes and deserts (Stewart, 1974). It is used as forage for camels especially in desert areas (Towhidi *et al*., 2011) and high quality edible oil is also obtained from seeds of *Suaeda fruticosa* (Weber *et al.*, 2012). In halophytes, the potentially damaging reactive oxygen species (ROS) at the cell levels are kept within a narrow but functionally significant range under optimum growing environment by using enzymatic coordinated system like superoxide dismutases (SOD), ascorbate peroxidase (POD) and catalases (CAT) and by non-enzymatic antioxidants for instance ascorbate (ASA; Ascorbic acid) and glutathione (GSH) (Shabala and Mackay, 2011). At whole plant level, it might be a strong antioxidant defense system other than well-organized regulation of ions likely to be a reason of salt tolerance in plants. Further, production of several compatible solutes and the protection of photosynthetic machinery are attributed to salt alleviation in halophytes (Guan *et al*., 2011; Shabala and Mackay, 2011). However, while plant is under high saline environment these mechanisms would become inefficient/less effective which ultimately leads to growth retardation and/or death (Munns and Tester, 2008). Keeping in view the harmful effects of salt and importance of *Suaeda fruticosa* (halophytes) in understanding the mechanism of salt tolerance, a pot experiment was conducted to determine whether application of salts induce certain morphological and biochemical changes in *Suaeda fruticosa*. Such studies are scanty in available literature and need to be carried out on priority basis. Additionally, this study is also helpful/beneficial to bring under cultivation of saline areas by growing most suitable salt tolerant plant species.

MATERIALS AND METHODS

Procurement of the plant material

Suaeda frticosa L. (Forssk) seedlings (ca. 6 cm in height) were procured from the vicinity of Railway track and roadsides of Lahore, Pakistan. A Pot (14″) experiment was conducted at Botanical Garden during the month of August 2019 (average temperature 35 ± 2°C). The seedlings were planted in pots containing 7 kg sand. Initially the plants were raised without any salt treatment for one month (30 days). To check the effect of NaCl on plants, 10 uniform size plants were used for each salt treatment and experiment was carried out thrice. The plants were watered with Hoagland solution (Hoagland and Arnon, 1950) for 30 days and then with distilled water containing various concentrations of NaCl (0, 50, 100, and 200 mM) for next 30 days. Plants were irrigated with respective salt treatment after 7 days interval. After 15 days of experiment, pots were flushed with distilled water to avoid accumulation of salt in the root zone. After 60 days of salt treatment plants were harvested for growth (shoot length/number, root length/number and the average weight of root and shoot), and biochemical (protein contents and antioxidant enzyme activities) parameters.

Growth parameters measurement

The plants were up-rooted after 30-day of NaCl treatment and 60 days of overall plant growth. The length of shoots and number of nodes were taken before harvesting. For biochemical tests leaf samples were also collected. Plants of each pot were harvested and completely washed with running water and dried by using blotting paper. The length (cm) and root of each plant was measured with the help of a measuring tape after 30 days of salt treatment. Then material was dried by using blotting paper and fresh weights of plant shoots and roots were recorded. Plant material was wrapped in aluminum foil and was dried in oven for 5 days at 65°C to get the dry mass.

Biochemical studies

For Biochemical assay, 1 gram of plant material was crushed into powder and then added in 3.0 ml of 0.15 M phosphate buffer (13.6 g KH_2PO_4 and 17.4 g K_2HPO_4 in 1,000 ml of distilled water, pH 7.2) + 0.5% (*v/v*) Triton and 0.15 g of *polyvinyl-pyrrolidone*. This mixture was centrifuged at 15,000 rpm at 4°C for 25 minutes (Sorval RB). The upper portion of (supernatant) this mixture after centrifugation was separated and stored in a refrigerator at 0°C for biochemical assay (Racusen and Johnstone, 1961).

Biuret reagent assay was followed (Racusen and Johnstone, 1961) with minor modifications for the analysis of soluble protein contents. Two test tubes were prepared one consisted of 2.0 ml of Biuret reagent containing 3.8 g $CuSO_4.5H_2O$, 1.0 g KI, 6.7 g Na-EDTA, 200 ml 5N NaOH in 1,000 ml of solution. In this mixture 0.2 ml of supernatant was added. The other consisted of same ingredient with 0.2 ml of de-ionized water rather than supernatant. The optical density was recorded by using *uv*-visible spectrophotometer at 545 nm. The total protein contents were calculated by comparing standard curve of protein, which was prepared by using bovine serum albumin. The equation given below was used for the calculation of protein.

$$\text{Protein contents (mg/g)} = \frac{CV \times TE}{EU \times Wt \times 1000}$$

Estimation of superoxide dismutase, POD and Catalase

Guaiacol-H_2O_2' method was employed with minor modifications for the quantitative estimation of peroxidase activity (Luck, 1974). The assay mixture having 3.0 ml of 0.15 M buffer (Sodium phosphate pH 7.8), 20 mM guaiacol (2-methoxyphenol; 0.05 ml) in solution from, 0.1 ml supernatant and 0.03 ml of 12.5 mM H_2O_2 solution (90% purity). Time required to increase the absorbance (0.1) from (e.g., 0.4 - 0.5) at 240 nm was used to estimate the peroxidases.

Beers and Sizer (1952) process was employed to measure the catalase activity. The reaction was done by using two types of buffer solutions (A and B). First buffer (A) solution was prepared by adding 50 mM sodium potassium phosphate (pH 7.2), while buffer B was prepared by adding 0.036% H_2O_2 solution in 50 mM potassium phosphate buffer (pH 7.0). Both buffers were mixed for preparing assay mixture having 2.9 ml buffer B and 0.1 ml of supernatant while control sample was prepared by only 3 ml of buffer A. Catalse activity was estimated by determining the time that is required for the absorbance (240 nm) to decline from 0.45 to 0.40 and articulated as U/ml of enzyme. The catalase activity was measured by using formula as below;

$$\text{Catalase activity (units/ml enzyme)} = \frac{3.45 \times df}{Min \times 0.1}$$

Whereas:

df: dilution factor, min: time required for the absorbance (240 nm) to decline from 0.45 to 0.40

For the estimation SOD activity, method of Maral *et al*. (1977) was employed with certain modifications. It was estimated by using spectrophotometer by determining the capability of SOD to inhibit photochemical reduction in nitroblue tetrazolium (NBT). For this two tubes (A & B) were taken, both having 2.0 ml of 1.0 mM sodium cyanide (NaCN), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA and 2.0 µM riboflavin as a substrate. Tube A was used as sample consisting of assay mix + 5.0 µl supernatant. Both test tubes were placed below fluorescent tubes (30-W) for 15 minutes. The absorbance was compared at 560 nm. SOD activity was represented by U/mg of protein.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control sample} - \text{Absorbance of experimental sample}}{\text{Absorbance of experimental sample}} \times 100$$

The enzyme activity was measured as one unit of SOD caused about 50% inhibition.

Statistical analysis

The results were analyzed by ANOVA using the SPSS (version 18.0.0). The mean values were compared by Duncan Multiple Range Test at $p < 0.05$.

RESULTS

Effect of various treatments of salt on growth of *Suaeda fruticosa* L.

A significant reduction in plant growth was observed when *S. fruticosa* healthy plants were provided with higher levels of NaCl (0-200 mM). The data given in Table 1 indicated that after 30 days of treatment, as the NaCl levels increases from 0-200 mM it resulted in a sharp reduction of the studied growth as well as biochemical attributes. As regard to shoot growth of the plants grown on zero concentration of salt (control) after 30 days was 5.90 cm while it was 4.77, 3.74, 2.60 cm at 50, 100, 200 mM NaCl levels, respectively. As the concentration of salt further increased, a sharp decrease in all the studied growth parameters was observed. At 200 mM NaCl shoot growth was reduced and no increase was noticed with reduced root formation.

More or less similar trend in growth was recorded with respect to root length. By increase in the NaCl concentration in the potting mix, there was a gradual decrease both in root growth. Data shown in Table 1 represents that the root length reduced from 4.60 cm (control) to 3.20, 2.60, 0.70 cm at 50, 100, 200 mM salt concentrations. These observations also suggested that perhaps shoot was less affected as compared to root at various concentrations of salt.

In case of number of nodes, a significant difference was observed in all salt treatments after 30 days of NaCl application. The data given in Table 1 indicates that after 30 days of treatment, an increase in the salt (NaCl from 0-200 mM, 4 treatments) correspondingly resulted in a gradual decrease in the number of nodes and internodal distance. The number of nodes was 7.0 in control whereas it was 3.00, 2.40, and 2.00 at 50, 100, 200 mM NaCl level, respectively. By further increasing the concentration of NaCl, a sharp decrease in all the studied growth attributes was observed.

Table 1: Effect on Growth parameters of *Suaeda fruticosa* under NaCl stress

Salt concentration	Length of shoot (cm)	Length of root (cm)	No. of nodes	Fresh weight of shoot	Fresh weight of root	Dry weight of shoot	Dry weight of root
0 (without NaCl)	5.90 ± 0.11[a]	4.60 ± 0.08[a]	7.0 ± 0.03[a]	9.24 ± 0.04 [a]	5.88 ± 0.02[a]	2.52 ± 0.10[a]	1.24 ± 0.13[a]
50 mM NaCl	4.77 ± 0.02[b]	3.20 ± 0.03[b]	3.0 ± 0.02[b]	8.54 ± 0.01[a]	3.56 ± 0.02[b]	1.76 ± 0.12[b]	1.04 ± 0.23[b]
100 mM NaCl	3.74 ± 0.03[c]	2.60 ± 0.04[c]	2.40 ± 0.12[b]	7.02 ± 0.01[b]	3.50 ± 0.03[b]	0.79 ± 0.21[c]	0.22 ± 0.17[c]
200 mM NaCl	2.60 ± 0.041[d]	0.70 ± 0.18[d]	2.0 ± 0.08[c]	5.10 ± 0.03[c]	2.50 ± 0.07[c]	0.82 ± 0.13[c]	0.25 ± 0.18[c]
Significance	*	*	**	*	**	*	*

Values are ± S.E from 10 replicate plants collected after 30 days of salt treatment.

*Significant and ** non-significant

Table 2: Effect of salt on protein contents and antioxidant enzyme activities of *Suaeda fruticosa*

Treatments	Protein Contents (mg/g tissue)	Peroxidase activity (U/ml of enzyme)	Catalase activity (U/ml of enzyme)	Superoxide dismutase activity (U/mg protein)
Control	2.51 ± 0.006[a]	1.24 ± 0.007[c]	35.43 ± 12.12[c]	16.19 ± 12.12[d]
Salt (50 mM)	0.76 ± 0.014[c]	1.04 ± 0.005[d]	42.36 ± 8.68[b]	30.53 ± 8.68[c]
(100 mM)	0.79 ± 0.009[c]	1.72 ± 0.004[a]	41.30 ± 11.43[b]	47.44 ± 11.43[b]
(200 mM)	0.82 ± 0.004[b]	1.45 ± 0.005[b]	56.50 ± 4.27[a]	58.69 ± 4.27[a]
Significance	*	**	*	*

Values are ± S.E from 10 replicate plants collected after 30 days of salt treatment

*Significant and ** non-significant

Shoot fresh and dry weights

When salt was applied to plants it decreased the fresh biomass of plants as compared to without salt treated ones. Fresh weight of shoot was maximum (9.24 g) of control and that of under 50, 100 and 200 mM salt stress was 8.54 g, 7 and 5 g, respectively. Same pattern was observed in case of shoot dry weight. Shoot dry weight was maximum at 0 mM of salt which was 5.88 g. Shoot dry weight of plants at 50 mM was 2.56 g and by further increasing salt concentration, there was gradual decrease in shoot dry weight.

Root fresh and dry weights

There was little gain in root fresh and dry weights with increasing concentrations of salt stress in *Suaeda fruticosa* plants. Root fresh weight was 2.52 g of control plants and that of under 50 mM salt stress was 1.76 g. There was more decrease in fresh weight with increasing concentrations of salt stress. Fresh weights were 0.79, and 0.82 g at concentrations 100, 200 mM of salt. In the same pattern, there was decrease in root dry weight with increasing levels of NaCl. Dry weight of root of control plants was 1.24 g and that of salt stressed plants was 0.25 g.

Protein content and antioxidant enzyme activities in NaCl Stressed Plants

It was observed that by increasing salt levels in potting mix a gradual decline in protein contents was recorded as shown in Table 2. It is well evident from the table that at 50 mM NaCl, protein contents shown a sharp decrease from 2.51 (control) to 0.76 mg/g. Similarly, protein contents were 0.79., 0.82, mg/g at 100 and 200 mM NaCl, respectively.

Peroxidase (POD) Activity under NaCl Stress

Tables 2 indicated that antioxidant enzyme activities like POD in plants of *Suaeda fruticosa* after 30-days of salt treatment increased as compared to control plants. Changes in POD activity was recorded by increasing salt levels in the potting mix. It is well evident by data presented in Table 2 that peroxidase activity in control (without salt treatment) plants was 1.24 units/ml while in salt-stressed plants it was 1.04, 1.72, 1.45, units/ml of enzyme at 50, 100, and 200 mM NaCl concentrations, respectively.

Effect of salt on catalase activity

Data shows that catalase activity of plants increased significantly under salt stress. In control plants it was 35.43 U/mg of protein but there was a significant increase in catalase activity with increasing salt level in (200 mM) salt stressed plants. Catalase activity was 42.36, 41.30 and 56.5 U/mg of protein noticed at 50, 100 and 200 mM concentrations of salt, respectively.

Effect of salt on superoxide dismutase activity

There was significant effect of salt stress on SOD activity of plants. In control plants its value was 16.19 U/mg of protein but under salt stress its value increased significantly up to 38.26 U/mg of protein. These contents were decreased at 50 mM concentration of salt but at higher concentrations of salt SOD activity were again increased. SOD activity at 100, and 200 mM concentrations of salt were 30.53, 47.44 and 58.69 U/mg of protein, respectively.

DISCUSSION

It is well evident from literature that salt stress cause severe growth inhibition and at much higher levels it results in loss of crop productivity due to ion imbalance and osmotic stress (Maggio et *al*., 2001). These impacts, on plants cause various types of stresses in plants i.e., oxidative damage, water stress and may end up in decreased growth of plants (Zhu, 2001). The results of the present investigation highlighted the severe reduction in all the studied growth and biochemical attributes of plant under salt stress. The growth parameters for instance length of shoot and root length, node and shoot/root fresh dry weights decreased significantly. The number of shoots increased (stunted growth behavior) at higher levels of NaCl (200 mM) concentration. Multiple shoot formation though was not a good sign as it might indicate that cells are not dividing normally and ultimately form bunch. These results are in line with several workers those have investigated such phenomenon in various plants species. For example Potluri and Devi-Prasad (1993) studied the potato growth at higher level of salt and observed abrupt growth reduction. Another study carried out by Martinez *et al*. (1996) also highlighted same growth reduction of potato plant at higher NaCl levels (100 to 200 mM). This severe decline in growth at higher salt levels was also recorded by Farhatullah *et al*. (2002). Shaterian *et al*. (2005) in a study on potato recorded that growth of plants gradually decreased with an increase of salt levels. The formation of multiple shoots might have been due to drastic effect of stress environment on cell division and elongation (Wang and Nil, 2000).

It was observed during this investigation that 30-days stress in the form of various salt treatments effected shoot/root fresh/dry weight. Our results have indicated that a gradual decrease in fresh/dry weight of shoot when NaCl concentration increased in the potting mix. Our results are very similar to Ochatt *et al.* (1999). They suggested that reduction in shoot fresh/dry weight at higher NaCl might be due to salt toxicity. Liu and Staden (1999) also observed the similar drastic effect on fresh weight of shoot within 28 days of plants. Farhatullah *et al*. (2002) also reported that NaCl injured cells and tissue and limit the growth activities at higher salt concentrations in the medium. They suggested that this reduction of potato plant growth at high salinity level might be due to less uptake of water, ion imbalance and production of oxidative stress (Errabii *et al*., 2007). This can be accomplished with up-regulation of several mechanisms i.e., balance of K^+, Na^+ and Cl^- uptake through the plasma membrane and/or compartmentalization of Na^+ and Cl^- in the cell vacuole (Parida and Das, 2005). It indicated that during stress episode or at higher salt levels, plants generally have adopted to hold up their growth/developmental and metabolism activities (Zhu, 2001). Another possible mechanism might be better management in utilization of resources under nutritional disparity, osmotic and metabolic disorder. Such mechanism adoption in growth and metabolism is not only helpful to conserve energy for their defense mechanism but also inhabit the possible risk of heritable changes (May *et al*., 1998). It has been recorded earlier by many researchers that the occurrences of salt in the potting medium generally retard or even completely restrict the plant growth and development (Lutts *et al.*, 1999).

This study also highlights changes in protein contents and antioxidant enzymes activities under various treatments of NaCl in pot grown *S. fruticosa* plants. Biochemical techniques to manage with stress in plants generally include adjustment of osmotic stress by increasing in several compatible inorganic and organic osmolite and enzymatic as well as non-enzymatic antioxidants (Sairam and Tyagi, 2004). In this study, protein contents of plants increase significantly by increasing concentrations of salts in potting mix. Similar results were also reported in several earlier studies where increases in protein contents were reported because of activation of several proteins that were earlier dormant (Agastian *et al*., 2000). In line to these results increase in protein was recorded in many salt tolerant cultivars i.e., barley, sunflower and rice (Ashraf and Harris, 2004). However, in contrast to this studies are available in literature those indicates that protein contents decrease by increasing concentrations of salts in potting mix. Fidalgo *et al.* (2004) observed a decreasing trend in protein contents by increasing salt treatments. This decline in protein contents was linked to the toxic effects of salt to the plants.

It is well evident that under stress plants responses to high salinity by increasing the antioxidant enzymes those neutralize the reactive oxygen species. These enzymes are produced under normal environment but under stress their concentrations increase significantly (Batkova *et al*., 2008). The peroxidase, catalase and superoxide dismutase activities exhibited an increasing tendency by increasing salt in potting mix as compared to plants grown under non-saline environment. These antioxidant enzymes having their essential role in sequestration of reactive oxygen species under stress environment (Rahnama *et al*., 2003). Kumar *et al*. (2008) also recorded similar results in case of SOD, catalse and peroxidase activities in *Jatropha curcas* as compared to non-saline control plants. SOD specifically changes sever toxicity of $O_2^{\bullet-}$ radicals to less toxic H_2O_2 (Scandalios, 1993) and to reduce the effects of H_2O_2 by other enzymes like catalse and peroxidase (Dionisiosese and Tobita, 1998).

It is concluded from this investigation that high salt concentrations (100-200 mM) to pot-grown plants of *Suaeda fruticosa* drastically decreased the growth and development. The results obtained from this investigation in the light of previous literature hints at possible genetic modification at both cellular and whole plant level, those results in increased biochemical (protein and antioxidant enzymes) activities.

REFERENCES

Agastian, P., Kingsley, S.J., Vivekanandan, M. 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica 38, 287–290.

Aronson, J. A. 1985. Economic halophytes: A global review. In *Plants for arid lands*, ed. G. E. Wickens, J. R. Goodin, and D. V. Field, 177–88. London.

Ashraf, M. and Harris, P.J.C. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166, 3-16.

Batkova, P., Paspisilova, J. and Synkova, H. 2008. Production of reactive oxygen species and development of antioxidative system during *in vitro* growth and *ex vitro* transfer. Biol. Plant. 52, 413-422.

Beers, R.F and Sizer, I.W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chemist. 195, 133-140.

Chaudhri, I. I., Chaudri, I. I., Shah, B. H. Naqvi, N., Naqvi, H. and Mallick. I. A. 1964 Investigations on the role of *Suaeda fruticosa* forsk in the reclamation of saline and alkaline soils in west Pakistan plains. *plant and soil* 21, 1 1-7.

Dionisiosese, M. and Tobita, S. 1998. Antioxidant response of rice seedlings to salinity stress. Plant Sci. 135, 1-9.

Errabii, T., Bernard, C., Gandonou, C.B., Essalmani, H., Abrini, J., Idamar, M. and Senhaji, N.S. 2007. Effect of NaCl and manitol induced stress on sugarcane (*Sacchrum* sp.) callus cultures. Acta Physiol. Plant. 29, 95-102.

FAO (2014) www.faostat.fao.org

Farhatullah., Rashid, M. and Raziuddin. 2002. *In vitro* effect of salt on the vigor of potato (*Solanum tuberosum* L) plantlets. Biotechnology. 1, 73-77.

Fidalgo, F., Santos, A., Santos, I., Salema, R. 2004. Effects of long term salt stress on antioxidant defence systems, leaf water relations and chloroplast ultrastructure of potato plants. Ann Appl Biol. 145, 185-192.

Guan, Y., Yao, V., Tsui, K. Gebbia, M., Dunham, M.J., Nislow, C., Troyankaya, O.G. 2011. Nucleosome-coupled expression differences in closely-related species. BMC Genomics 12, 466.

Hoagland, D.R. and Arnon, D.I. 1950. The water culture method for growing plants without soil. California Agricultural and Experimental Statistics Circulation number 347.

Khan, M.A., Ungar, I.A. 1996. Comparative study of chloride, calcium, magnesium, potassium and sodium content of seed in temperate and tropical halophytes. J. Plant Nutr. 19, 517–525.

Kumar, N., Pamidimarri S, D.V.N., Kaur, M., Boricha, G. Reddy, M.P. 2008. Effect of NaCl on growth, ion accumulation, protein, proline contents and antioxidant enzymes activity in callus cultures of *Jatropha curcas*. Biologia 63, 378-382.

Liu, T. and Van-Staden, J. 1999. Selection and characterization of sodium chloride-tolerant callus of *Glycine max* (L.) Merrcb. Acme. Plant Growth Regul. 31, 195-207.

Luck, H. 1974. Methods in enzymatic analysis 2nd edition. Bergmeyer Academic New York, pp 885.

Lutts, S., Kinet, J.M. and Bouharmont, J. 1999. Improvement of rice *callus* regeneration in the presence of NaCl. Plant Cell, Tissue Org Cult. 57, 3-11.

Maggio, A., Hasegawa, P.M., Bressan, R. A., Consiglio, M. F., and Joly, R. J. 2001. Unraveling the functional relationship between root anatomy and stress tolerance. Funct Plant Biol, 28, 999-1004.

Maral, J., Puget K. and Michelson. A. M. 1977. Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochem. biophys. Res. Commun. 77, 1525-1535.

Martinez, C.A., Maestri, M. and Lani, E.G. 1996. *In vitro* salt tolerance and proline accumulation in Andean Potato (*Solanum* spp.) differing in frost resistance. Plant Sci. 116, 177-184.

May, M.J., Vernoux, T. Leaver, C. Montagu, M.V. and Inze, D. 1998. Glutathione homeostasis in plants: implications for environmental sensing and plant development. J. Experi. Bot. 49, 649-667.

Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651–668

Ochatt, S. J., Marconi, P.L., Radice, S., Arnozis, P.A. and Caso, O.H. 1999. *In vitro* recurrent selection of potato: production and characterization of salt-tolerant cell lines and plants. Plant Cell, Tiss Org Cult. 55, 1-8.

Parida, K.A. and Das, A.B. 2005. Salt tolerance and salinity effects on plants. Ecotox. Environ. Safety 60, 324-349.

Potluri, S. D. P., Devi Prasad, P. V. 1993. Influence of salinity on axillary bud cultures of six lowland tropical varieties

of potato (*Solanum tuberosum*). Plant Cell Tiss. Org Cult. 32, 185-191.

Racusen, D. and Johnstone, D.B. 1961. Estimation of protein in cellular material. Nature 191: 292-493.

Rahnama, H., Ebrahimzadeh, E. and Ghareyazie, B. 2003. Antioxidant enzymes responses to NaCl stress in calli of four potato cultivars. Pak. J. Bot. 35, 579-586.

Sairam RK, Tyagi A 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci. 86, 407–421.

Scandalios, J.G. 1993. Oxygen stresses and superoxide dismutases. Plant Physiol. 101, 7-12.

Seki, M., Kamei, A., Yamaguchi-Shinozaki, K., Shinozaki, K. 2003. Molecular responses to drought, salinity and frost: common and different paths for plant protection. Curr. Opin. Biotechnol. 14, 194–199.

Shabala, A. and Mackay, M. 2011. Ion transport in halophytes. Advan in Botanic. Res. 57, 151–99.

Shabala, S. and Mackay, A. 2011. Ion transport in halophytes. In: Kader JC, Delseny M (eds) Advances in botanical research, vol 57. Elsevier, Amsterdam, pp 151–199

Shabala, S. and Mackay, A. 2011. Ion transport in halophytes. Adv. Bot. Res. 57, 151-199.

Shaterian, J., Waterer, D., De-Jong, H. and Tanino, K.K. 2005. Differential stress response to NaCl salt application in early and late maturing diploid potato (*Solanum* sp.) clones. Environ. Experi. Bot. 54, 202-212.

Stewart, G.R. and Lee, J.A. 1974. The role of proline accumulation in halophytes. Planta 120, 279-289

Towhidi, A, Saberifar, T., Dirandeh, E. 2011. Nutritive value of some herbage for dromedary camels in the central arid zone of Iran. Trop Anim Health Pro. 43, 617–622.

Ungar, I.A. 1991. Ecophysiology of Vascular Halophytes. Boca Raton: CRC Press. 209 pp

Wang, Y. and Nil, N. 2000. Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. J. Hort. Sci. Biotechnol.75, 623-627.

Weber, V.S., Araus J.L., Cairns, J.E., Sanchez, C., Melchinger, A. E.E., Orsani A. 2012. Prediction of grain yield using reflectance spectra of canopy and leaves in maize plants grown under different water regimes. Field Crops Res. 128, 82–90.

Yadav R., Flowers, T.J. and Yeo A.R. 1996. The involvement of the transpirational bypass flow in sodium uptake by high- and low sodium-transporting lines of rice developed through intra-varietal selection. Plant Cell Environ. 19, 329–336.

Zaman, S., Padmesh, S., Bhat, N.R., Tawfiq, H., 2009. Germination characteristics and storage behavior of *Tamarix aucheriana* (Decne.) seeds. Eur. J. Sci. Res. 26,532–538.

Zhu, J.K. 2001. Plant salt tolerance. Trends in Plant Science 6: 66-71.

Zhu, J.-K. 2007. *Plant Salt Stress*: John Wiley & Sons, Ltd.