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IMPACT OF SALT STRESS ON CONCENTRATIONS OF Na^+, Cl^- AND ORGANIC SOLUTES CONCENTRATION IN PEA CULTIVARS

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Abstract

To study the salt tolerance potential in pea cultivars (*Pisum sativum* L.), an experiment was conducted with nine local pea cultivars: Samarina Zard (SZ), Olympia (OL), Early Green (EG), Climax (CL), 2001-20 (2001), Meteor (M), Euro (E), 9200-1 (9200) and 9800-5 (9800). The plants were exposed to two NaCl treatments: 0 and 75 mM NaCl. At the end of the experiment, growth parameters, Cl^- and Na^+ in leaves and roots, and proline, quaternarium ammonium compounds, total free amino acids and total soluble sugars in leaves were measured. Saline treatment reduced the total biomass in all the pea cultivars. Thus, salt tolerance, based on growth reduction relative to the control treatment, was similar in all nine pea cultivars. However, regardless of the salt treatments, the cultivars EG, SZ, 9200, 9800 and CL were more vigorous among the nine cultivars. The cultivar 2001 had the highest leaf Na^+ and Cl^- concentrations these were the lowest in 9200. In the nine cultivars studied, an increase was noted in the leaf proline, free amino acids, QAC compounds and total soluble sugars with increase in the root zone salinity. Leaf proline and amino acids concentrations were negatively correlated with the leaf Na^+ concentration suggesting that the synthesis of this organic solute is linked with the osmotic process adjustment rather than Cl^- and Na^+ toxicity.

Introduction

Salinity is one of the serious environmental problems that causes severe reduction in plant growth and crop productivity in irrigated areas of arid and semi-arid regions of the world (Cicerk & Cakirlar, 2002). Pea (*Pisum sativum* L.) has been classified as having medium salt tolerance (Waheed *et al.*, 2007; Noreen *et al.*, 2007), whereas the earlier growth stage is more sensitive to salinity than subsequent stages (Karajol & Naik, 2011). Salt-tolerance in pea plant can also vary among species as Cerda *et al.*, (1982) found useful variation in the salt tolerance of pea cultivars which provide 50% of the maximal yield between 6 and 10 dS m^{-1}. In plants salinity may affect the rate of germination, seedling growth and yield (Bonilla *et al.*, 2004; Okcu *et al.*, 2005; Ashraf *et al.*, 2012), due mainly to the Na^+ ion toxicity causing a range of osmotic, metabolic and physiological problems (Tester & Davenport, 2003;). Moreover, some effects of high salt in pea plants are also caused by the deficiency of other nutrients like B and Ca which occurs due to interference of salt ions with the uptake of these nutrients (Silberbush & Ben-Asher, 2001).

Plants may respond morphologically and physiologically to adopt the saline habitat (Sakamoto & Murata, 2002; Balal *et al.*, 2011). One of these physiological responses consists of increasing the concentration of solutes in the cells, process known as osmotic adjustment, to maintain the cellular turgor which keeps continuation of the water uptake and growth (Kausar *et al.*, 2012). Salinity causes a decrease in osmotic potential of the soil solution and plant's access to soil water becomes limited because of the decrease in total soil water potential (Awan *et al.*, 2012). As the soil dries, the concentration of salts in the soil solution increases, with a concomitant further decrease in osmotic potential. In order to ensure water uptake from a saline soil, plants must osmotically adjust themselves. This can be effected either by taking up significant amounts of salts and distributing them within plant tissue, or synthesizing organic solutes (Ashraf *et al.*, 2012). Halophytes (salt loving plants) generally have a higher ability to store high amounts of salt ions in plant tissues without affecting cell processes (Flowers, 2004) whereas in contrast, glycophytes synthesize organic osmotica, and try to prevent excess salt uptake and accumulation in plant tissues for normal functioning of cell processes. Whether halophytes or glycophytes, most of the plants utilize a combination of these strategies (Soneoka *et al.*, 1999; Ashraf *et al.*, 2006)). Although increased uptake of salt ions may contribute to osmotic adjustment, Na^+ and Cl^- may become toxic in most of the plants (Ashraf *et al.*, 2010). One such mechanism, more important and prevalent in plants, is the accumulation of certain organic metabolites of low molecular weight, collectively known as compatible solutes such as sugars, amino acids, proline, and quaternary ammonium compounds such as glycinebetaine (Athar *et al.*, 2009; Balal *et al.*, 2011). These compatible solutes are uniformly neutral with respect to the perturbation of cellular functions, even when present at high concentrations (Serraj & Sinclair, 2002). Accumulation of these organic solutes helps the plants to retain water within cells and protect cellular compartments from injuries caused by dehydration or thus maintains turgor pressure during water stress (Ashraf *et al.*, 1994; Waraich *et al.*, 2011). Moreover, these compounds stabilize the structure and functions of certain macromolecules (Balal *et al.*, 2011).

Although salt tolerance of pea plants may depend on the variety but in the scientific literature the information on comparison of different pea cultivars for salt tolerance is scanty. The objective of this study was to determine genotypic differences among the 9 pea cultivars extensively grown in Pakistan, in terms of salt tolerance and to determine if the difference in salt tolerance among pea cultivars is due to the difference in accumulation of organic solutes and Cl^- and Na^+ ions in the leaves.

Materials and Methods

Plant materials: The proposed study was carried out at greenhouse of the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan with 9 pea cultivars [Samarina Zard (SZ), Olympia (OL), Early Green (EG), Climax (CL), 2001-20 (2001), Meteor (M), Euro (E), 9200-1 (9200) and 9800-5 (9800)]. These pea cultivars are typical from various climatic regions of Pakistan. Seeds of all the cultivars were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Physiological and biochemical data were recorded at Plant Stress Physiology Lab of NIAB, Faisalabad, Pakistan. The experiment was laid down in a completely randomized design with three replications. Salinity levels applied were, control (no-salt treatment) and 75 mM NaCl. Seeds were sown in 9 liter, bottom perforated, plastic pots containing sand rinsed with distilled water. After emergence of first true leaf (15 days after germination), the number of plants per pot was adjusted to five and irrigated. Twenty days after sowing, half strength Hoagland nutrient solution [containing 100 g/L KH_2PO_4, 500 g/L KNO_3, 500 g/L Ca $(NO_3)_2.4H_2O$, 200 g/L $MgSO_4.7H_2O$, and micronutrients] was applied to plants as a growth medium. The electrical conductivity of solutions with 0 or 75 mM NaCl treatment was 2 and 9.5 dS/m, respectively. To avoid the osmotic shock, NaCl concentrations were adjusted by gradually increasing 25 mM every two days until desired concentration reached. Each pot (5 plants) was considered as one replicate. Plants were watered daily with sufficient volume to leach from the bottom of all pots. During the experimental period (30d), the diurnal temperature and relative humidity were about 26± 2ºC and 70%, respectively.

Plant growth parameters: Internodal distance for each plant was measured with the help of measuring tape in centimeters (cm). Average of internodal distance was calculated for each treatment. Fresh weight of each plant was recorded with the help of electronic balance. Average fresh weight for each plant and treatment was calculated. Dry weight of whole plant was measured after keeping them in an oven at 72^{o}C for 72 h. At the end of the experimental period, number of leaves and branches per plants were also counted.

Solutes accumulation: After 30 days of imposition of salinity treatment, organic solutes were determined in fresh tissue of leaves. Total free amino acids were extracted with phosphate buffer having pH 7, and quantified according to protocol described by (Hamilton & VanSlyke, 1943). Lucine was used as standard. Quaternary ammonium compounds (QAC) were extracted with 1-2 dichloroethane (cooled at -10^{o}C), quantified according to Grieve & Grattan (1983) and glycine betaine was used as standard. Proline was estimated according to the method of Bates *et al.,* (1973) by extracting with 3% sulfo-salicylic acid. Total soluble sugars were determined according to Riazi *et al.,* (1985).

Leaf and root Cl^- and Na^+ concentration: Sub-samples of leaf and root tissues were extracted with deionized water, and tissue chloride was measured with a Corning 926 chloride meter (Sherwood, UK). Tissue Na^+ concentration was determined by a flame photometer (Jenway PFP-7, UK), after previous digestion in H_2SO_4 and H_2O_2 Methods ( ).

Results

Plant growth parameters: Total plant dry weight data from the control treatment (Table 1) showed that the cultivars more vigorous were EG, SZ, 9200, 9800 and Climax, and the less vigorous was E. Results similar to plant dry weight were observed for number of branches per plant; however, the highest number of leaves per plant was noted in Climax; this parameter being not significant only in the SZ cultivar. Inter-nodal distance also significantly varied with the cultivar in the following order: 9800> 2001> EG> Climax> Olympia> SZ> 9200> M>E.

Table 1. Plant dry weight, number of leaves and branches per plant, and inter nodel distance in different pea cultivars.

Cultivar	Plant dry weight	Leaves plant^{-1}	Branches plant^{-1}	Inter nodel distance (cm)
2001	1.37 c†	70 bcd	8 c	5.27 b
EG	1.91 a	82 b	10 ab	5.17 bc
M	1.41 c	71 cd	9 bc	4.03 g
SZ	1.80 ab	80 abc	10 ab	4.60 e
9800	1.92 a	73 cd	11 a	5.60 a
9200	1.71 ab	70 cd	9 abc	4.27 f
Climax	1.91 a	89 a	10 ab	5.03 cd
Euro	0.75 d	71 bc	6 d	1.77 h
Olympia	1.54 bc	68 d	9 bc	4.87 d
ANOVA	***‡	**	***	***

†Within each column, different letters indicate significant differences at $p<0.05$

‡** and *** indicate significant differences at $p<0.01$ or 0.001, respectively

The salt treatment decreased all the growth parameters measured in this experiment i.e., total plant dry weight, number of branches and leaves per plant, and inter-nodal distance, but significant interaction of cultivar x salt treatment was only noted in the inter-nodal distance and the number of leaves (Fig. 1). In inter-nodal distance, the significant interaction was due to SZ, 6200 and Cl cultivars that had the lowest reduction due to salt treatment, the highest reductions were found in 2001 and E cultivars. As regards the number of leaves per plant, the salt treatment did not reduce this parameter in the EG, 9800 and Cl cultivars but reverse was the case with other cultivars.

Leaf and root Cl^- and Na^+ concentration: There was an increase in the Na^+ and Cl^- concentration in leaves and roots with increasing salinity level in the root medium, but this increase depended on the cultivar as interactions of cultivar x salt treatment were significant (Fig. 2). The highest leaf Cl^- and Na^+ concentration was observed in cultivars 2001 and E, while the lowest was observed in 9200 and Cl, and intermediate values were noted in 9800, EG, M, SZ, and OL. In the root, the highest Cl^- concentration was observed in 2001 and E, the lowest was found in Cl, and the rest of cultivars had intermediate values. Root Na^+ concentration also depended on the cultivar increasing in the following order: 9200 < E < 2001 < M < SZ = OL < 9800 < Cl.

Leaf organic solute concentration: Statistical analysis of the data regarding leaf organic solute concentration showed that the differences between control and salt treatment were highly significant and dependent on cultivar type. Salt treatment increased leaf proline concentration, but this increase was greater in EG, SZ and Cl than in the rest of the cultivars where there was small differences between them (Fig. 3). Similar results were found for free amino acids, with the highest concentrations noted in salinized EG, SZ, 9800 and Cl cultivars. Quaternary ammonium components (QAC) increased with salt treatment in all the cultivars, the highest values found in EG, 9800 Cl and OL cultivars. Leaf total soluble sugar concentration was increased by salt treatment in the following order: M < 2001 = SZ = Eu < 9200 < EG < OL < Cl < 9800.

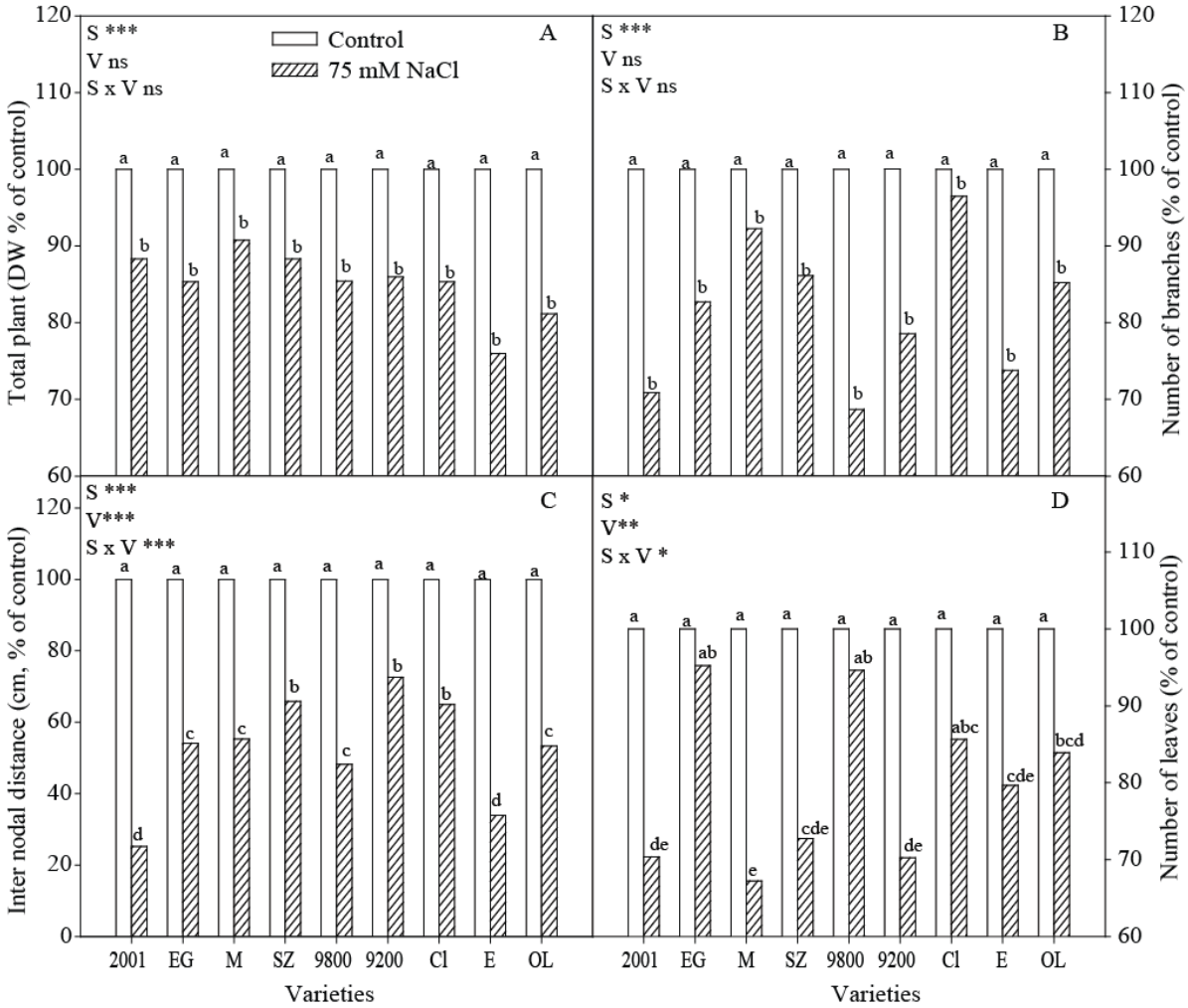

Fig. 1. Effects of salt stress on total plant weight (A), number of brunches (B), inter nodal distance (C) and number of leaves (B) in different pea cultivars. Ns, *, **, **** indicate non-significant or significant differences at p<0.05, 0.01 and 0.001, respectively.

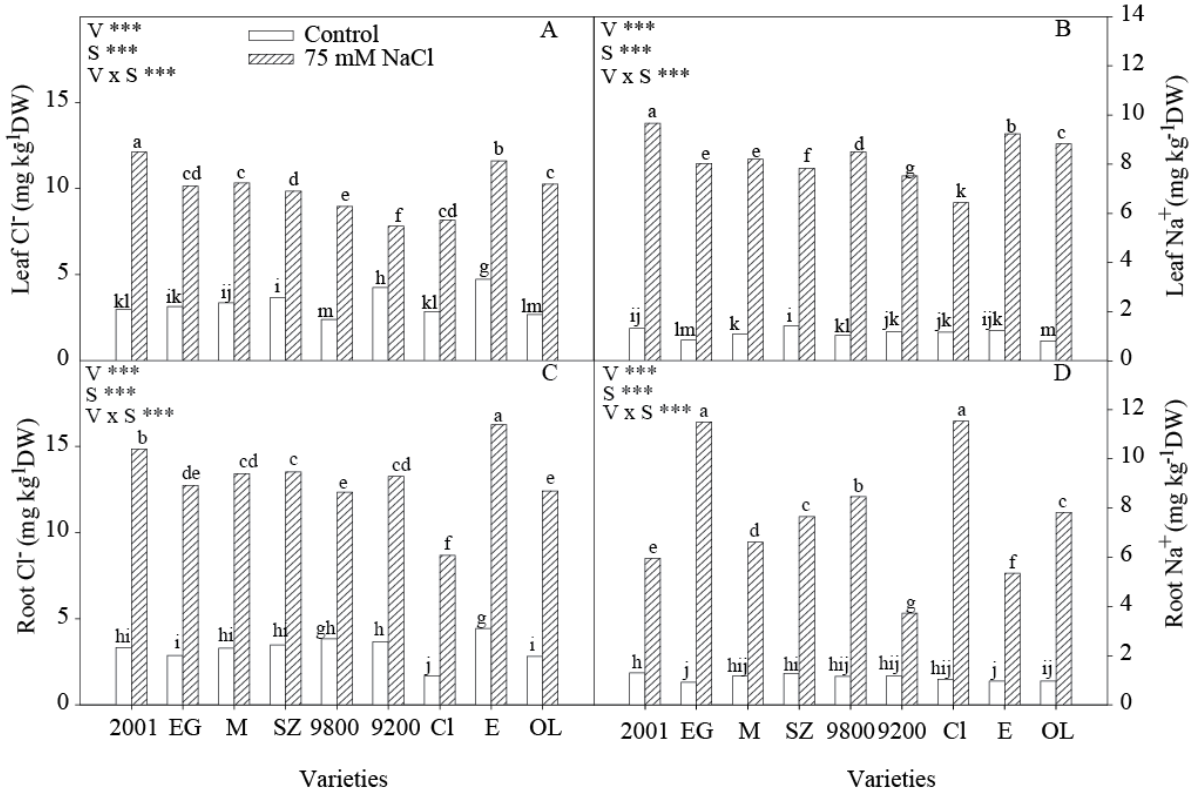

Fig. 2.Effects of salt stress on leaf Cl^- concentration (A), leaf Na^+ concentration (B), root Cl^- concentration (C) and root Na^+ concentration (D) in different pea cultivars. **** indicate significant differences at $p<0.001$.

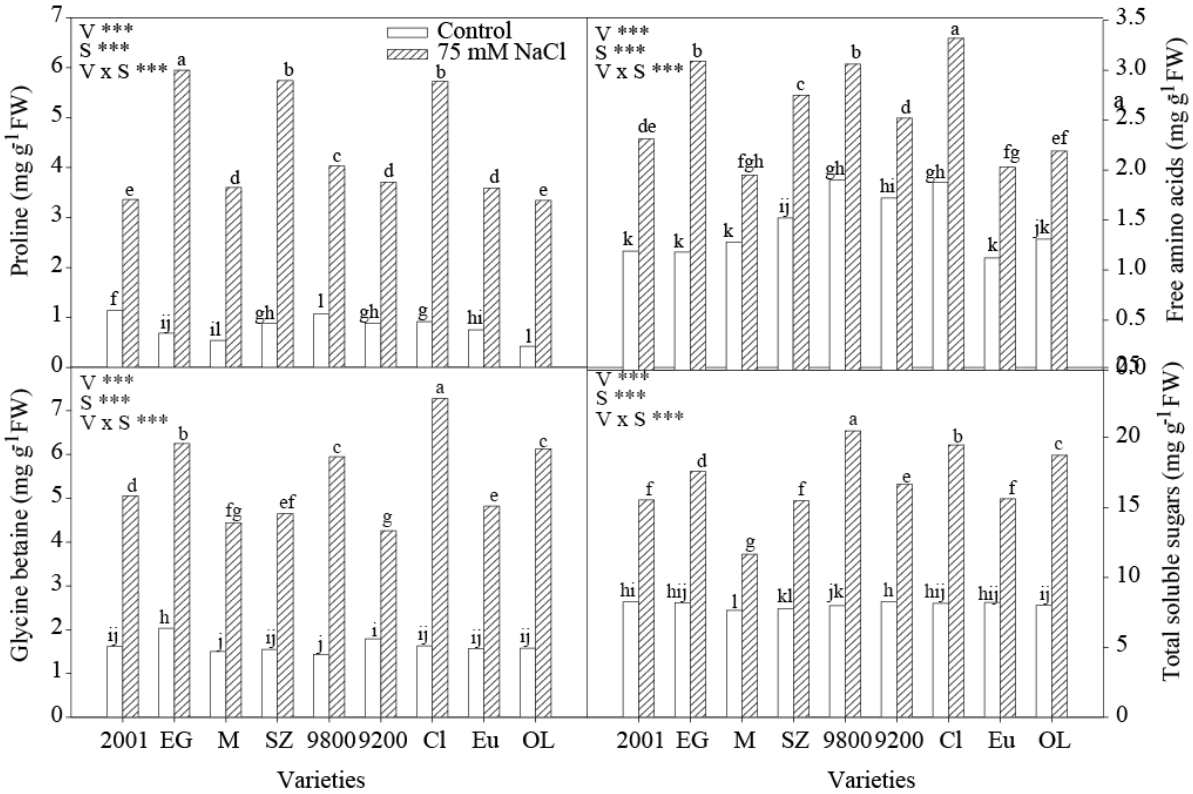

Fig. 3. Effects of salt stress on proline (A), total free amino acid (B), quaternarium ammonium compounds (QAC) (C) and total soluble sugar concentration (D) in different pea cultivars. Each value is the mean of three plants. **** indicate significant differences at $p<0.001$.

Pearson´s correlation coefficients and linear regressions: When data at the end of the experimental period were pooled across cultivars for the 75 mM NaCl treatment, proline was positively correlated with AA and QAC but negatively correlated with Na^+. (Table 2, Fig. 4). QAC showed positive correlation with Na^+ while it was negative for sugar. There were significant positive correlations between QAC and Sugars, and between leaf Cl^- and leaf Na^+. Salt tolerance was not related to any parameter. Pooling Na^+ concentration versus leaf root concentration revealed that increasing Na^+ concentration in the roots decreased Na^+ concentration in the leaves, however, pooling leaf Cl^- versus root Cl^- revealed that increasing Cl^- concentration in the roots increased Cl^- concentration in the leaves.

Table 2. Pearson´s correlation coefficient between proline, amino acids (AA), quaternary ammonium compounds (QAC), chloride and sodium concentration in leaves of Samarina Zard (SZ), Olympia (OL), Early Green (EG), Climax (Cl), 2001-20 (2001), Meteor (M), Euro (E), 9200-1 (9200) and 9800-5 (9800) treated with 75 mM NaCl (n=27).

	Proline	AA	QAC	Sugars	Leaf Cl	Leaf Na
AA	0.77***†					
QAC	0.46*	0.66***				
Sugars	ns	0.68***	0.75***			
Cl^- leaf	ns	ns	ns	ns		
Na^+ leaf	-0.66***	-0.63***	ns	ns	0.58***	
Salt tolerance	ns	ns	ns	ns	ns	ns

Critical r of n =24 at $p<0.05 = \pm 0.40$

† 'n.s.', *, and *** indicate non-significant or significant differences at $p<0.05$, or 0.001, respectively

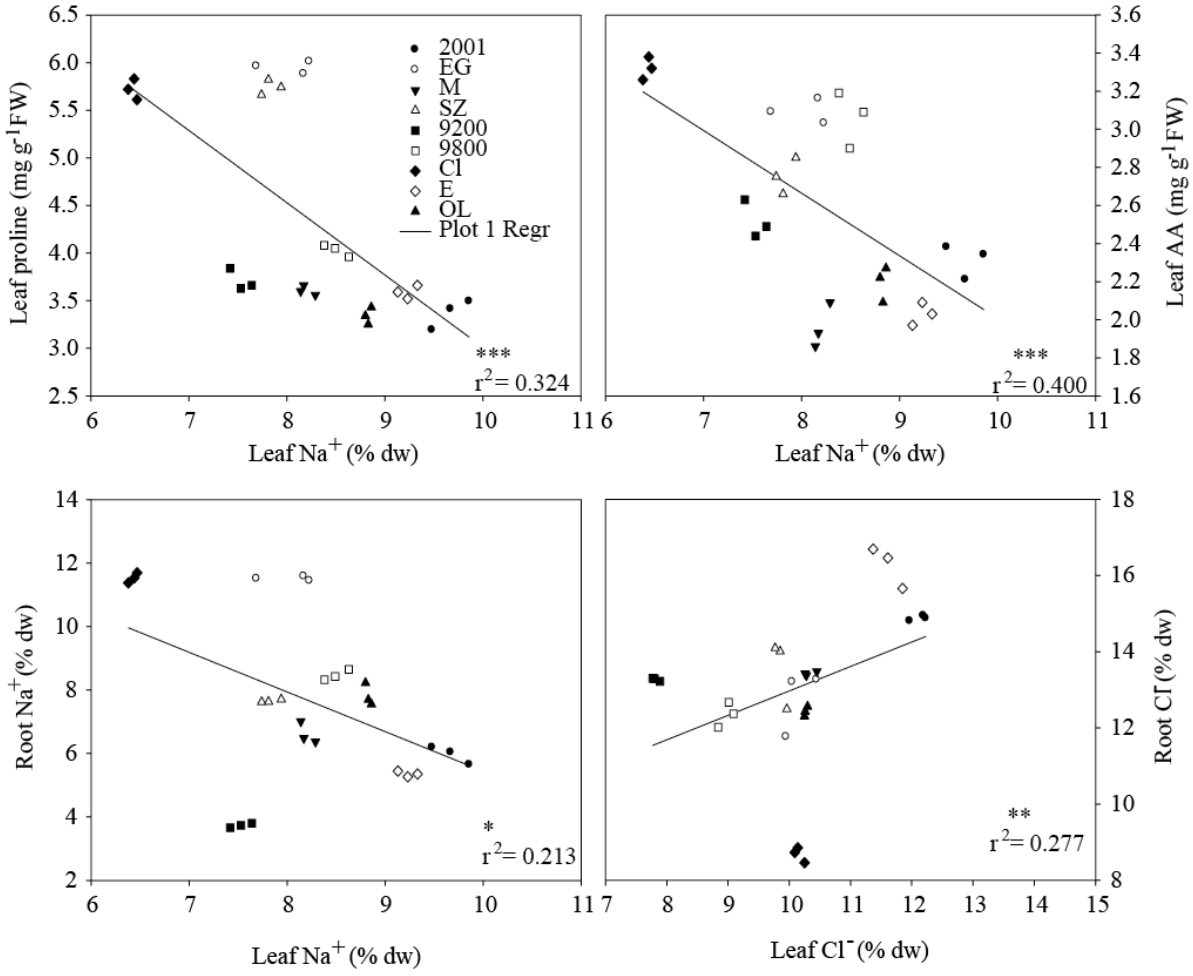

Fig. 4. Relationship between leaf proline, amino acid and root Na^+ concentrations *versus* leaf Na^+ concentrations (A, B and C, respectively), and between leaf Cl^- *versus* root Cl^- concentrations (D) in different pea cultivars treated with 75 mM NaCl. *, ** and *** indicate significant slope at $p<0.05$, 0.01 and 0.001, respectively.

Discussion

In previous studies it has been observed that pea cultivars possess a wide range of variation in salinity tolerance. For example, Cerda *et al.,* (1982) classified the pea cultivars SP-290 and Durana as moderately salt sensitive and moderately salt tolerant, respectively. Hernández *et al.,* (1995) also observed differences in the salt tolerance of two pea genotypes, designating to cv Granada as NaCl-tolerant and cv Challis as NaCl-sensitive. In present study, based on plant dry weight reduction, all the pea cultivars had similar salt tolerance as supported by non-significant interaction between cultivar x salt treatment. In addition, as total plant dry weight was decreased by 14% after 30 days of growth under saline conditions, relative to the control treatment (Fig. 1), it can be concluded that all these cultivars are moderately sensitive to salt stress (Maas & Hoffman, 1977). Nevertheless, these data analyzed in absolute terms (g dw per plant; Table 1) showed that the more vigorous cultivars were EG, SZ, 9200, 9800 and CL. Thus, from an agronomic point of view, these five cultivars could be the suitable for cultivation with irrigation water of both good and bad quality.

In addition to the total plant dry weight reduction by salt results our experiment also confirmed that the internodal distance and number of branches and leaves, could be decrease with the increase in the salinity of the root medium (Zhu *et al.*, 2001). The reduction in internodal distance and number of branches may be due to the reduction in turgor potential, necessary for cell elongation, sodium/chloride ion toxicity, and/or disturbances in metabolic pathways (Iqbal & Ashraf, 2005). Many reports also indicated that fresh and dry weight reduction under saline conditions may be due to the reduction in above ground biomass production (Sagi *et al.*, 1997).

Although salt tolerance was similar in all 9 cultivars and was not related with leaf Cl^- or Na^+ concentration (Table 2), differences in the leaf Cl^- and Na^+ concentrations among different cultivars were observed. As regards Na^+, the cultivar 2001 had the highest and CL had the lowest concentration. The fact that the relationship between leaf and root Na^+ concentration was negatively related indicates that the cultivars with ability to accumulate high Na^+ concentration in the roots avoided a high concentration in the leaves (Fig. 4). In other species such as citrus (García-Sanchez *et al.*, 2002), differences in the leaf Na^+ concentration among cultivars have also been observed. Although the mechanisms are not well known, these differences may arise in those cultivars able to withhold Na^+ in roots and stems, avoiding their transport to the leaves. As regards Cl^-, in our experiment, the leaf and root Cl^- concentration was positively correlated, whereas cultivars with high leaf Cl^- concentration, such as 2001 and SZ, also had the highest root Cl^- concentration (Figs. 2,4). This indicates that the roots of pea cultivars used in this experiment have not any ability to exclude Cl^- and so avoid the Cl^- transport from roots to leaves. Thus, different leaf Cl^- concentrations observed among the cultivars could be due to other factors like different leaf transpiration, water use efficiency and/or root to shoot ratio (Syvertsen *et al*., 2010; Akram *et al*., 2011).

Varietal differences in leaf proline and amino acids accumulation were recorded for pea cultivars in present study, but in the nine cultivars studied there was an increase in the leaf proline, free amino acids, QAC compounds and total soluble sugars by increasing salinity in the root zone. Under salt stress conditions, high concentrations of proline and amino acids are produced to reduce the damages caused by salinity due to the toxic and/or osmotic effect (Ashraf & Haris 2004). Proline is a common cytoplasmic compatible solute, which is thought to have several roles including the stabilization of membranes and proteins that protect them against temperature extremes, toxic ions and oxidative damage (Samaras *et al.*, 1995; Khan *et al*., 2009). There was a negative significant relationship between leaf proline and amino acids concentration and leaf Na^+ concentration (Table 2). Climax (CL) was the cultivar which had the highest leaf Na^+ concentration and the least leaf proline concentration, indicates that the synthesis of proline and amino acids is linked with the osmotic process adjustment rather than Cl^- and Na^+ toxicity, so those pea cultivars with low leaf Na^+ concentration increased the synthesis of organic solutes to contribute to osmotic adjustment and so maintained an osmotic potential gradient to facilitate the inward water movement (Khan *et al.*, 2000). Under stress conditions, plants can accumulate other organic solutes, in addition to proline, like sugar, polyols, and quaternary ammonium compounds (Khan *et al.*, 1998; Balal *et al*., 2011). Glycinebetaine is one of the most abundant quaternary ammonium compounds under stress environment (Yang *et al*., 2003). Glycinebetaine is the osmoprotectant which stabilizes the structure and activities of enzymes, protein complexes and maintains the integrity of the membrane against the damaging effects of excessive salt. In present experiment, salt treatment increased the glycinebetaine and sugar accumulation, thus these results indicate that the high concentration of these organic solutes may also contribute towards their tolerance to salt.

In conclusion, the obvious effect of salinity on the nine pea cultivars was a reduction in the growth parameters. Based on the total plant dry weight reduction by salt treatment, all nine cultivars had similar salt tolerance as cultivar type x salt treatment interaction was not significant. However, in absolute terms, the cultivars EG, SZ, 9200, 9800 and CL showed more vigorous growth among the nine cultivars studied. The high concentration of organic solutes may be used as selection criteria for salt tolerance in pea because cultivars with higher proline, amino acid, sugar and QAC possessed higher biomass under saline conditions.

References

Akram, M., M.Y. Ashraf, M. Jamil, R.M. Iqbal, M. Nafees and M.A. Khan. 2011. Nitrogen application improves gas exchange characteristics and chlorophyll fluorescence in maize hybrids under salinity conditions. *Russian J. Plant Physiol*., 58(3): 394- 401.

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

Ashraf, M.Y., A.R. Awan and K. Mahmood. 2012. Rehabilitation of saline ecosystems through cultivation of salt tolerant plants, *Pak. J. Bot*., 44: 69-75.

Ashraf, M.Y., A.R. Azmi, A.H. Khan and S.S.M. Naqvi**.** 1994. Water relations in different wheat (*Triticum aestivum* L.) genotypes under water deficits. *Acta Physiol. Plant*., 16(3):231-240.

Ashraf, M.Y., K. Akhtar, F. Hussain and J. Iqbal. 2006. Screening of different accessions of three potential grass species fron Cholistan desert for salt tolerance. *Pak. J. Bot*., 38(5):1589-1597.

Ashraf, M.Y., M. Ashraf, K. Mahmood, J. Akhter, F. Hussain and M. Arshad. 2010. Phytoremediation of saline soils for sustainable agricultural productivity, *In: Plant Adaptation and Phytoremediation* (Eds.): M. Ashraf, M. Oztruck and M.S.A. Ahmad Published by Springer, the Netherlands. pringer Science+Business Media B.V., pp. 335-356.

Athar, H.U.R., M. Ashraf, A. Wahid and A. Jamil. 2009. Inducing salt tolerance in canola (*Brassica napus* L.) by exogenous application of glycinebetaine and proline: response at the initial growth stages. *Pak. J. Bot.*, 41: 1311-1319.

Awan, A.R., M.I. Chughtai, M.Y. Ashraf, K. Mahmood, M. Rizwan, M. Akhtar, M.A.A. Qureshi, M.T. Siddiqui and R.A. Khan. 2012. Comparison for physico-mechanical properties of farm-grown *Eucalyptus camaldulensis* Dehn. with conventional timbers. *Pak. J. Bot.*, 44(6): 2067-2070.

Balal, R.M., M.Y. Ashraf, M.M. Khan, M.J. Jaskani and M. Ashfaq. 2011. Influence of salt stress on growth and biochemical parameters of citrus rootstocks. *Pak. J. Bot*., 43(4): 2135-2141.

Bates, L.S., R.P. Waldron and I.W. Teaxe. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.

Bonilla, I., A. EL-Hamdaoui and L. Bolaños. 2005. Boron and calcium increase *Pisum sativum* seed germination and seedling development under salt stress. *Plant Soil*, 267: 97-107.

Cerda, A., M. Caro and F.G. Fernandez. 1982. Salt tolerance of two pea cultivars. *Agron. J*., 74: 796-798.

Cicerk, N. and H. Cakirlar. 2002. The effect of salinity on some physiological parameters in two maize cultivars. *Bulg. J. Plant. Physiol*., 28: 66-74.

Flowers T,J. 2004. Improving crop salt tolerance. Journal of *Experimental Botany*, 55: 307- 319.

García-Sanchez, F.J., J. Jifon, M. Carvajal and J.P. Syvertsen. 2002. Gas exchange, chylorophyll and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstocks. *Plant Science*, 162: 705-712.

Grieve, C.M. and S.R. Gratan. 1983. Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70: 303-307.

Hamilton, P.B. and D.D. Van Slyke. 1943. Amino acid determination with ninhydrin. *J. Biol. Chem*., 150: 231-233.

Hernández, J.A., E. Olmos, E.J. Corpas, F. Sevilla and L.A. del Río. 1995. Salt-induced oxidative stress in chloroplast of pea plants. *Plant Sci*., 105:151-167.

Iqbal, M. and M. Ashraf. 2005. Changes in growth photosynthetic activity and ionic relations in spring wheat. *Plant Growth Regul*., 60: 41-52.

Karajol, K. and G.R. Naik. 2011. Seed germination rate as a phenotypical marker for the selectionof NaCl tolerant cultivars in pigeon pea (*Cajanus cajan* L.; MILLSP.). *World J. Sci. Technol.*, 1(2): 1-8.

Kausar, A., M.Y. Ashraf, I. Ali, M. Niaz and Q. Abbass. 2012. Evaluation of sorghum varieties/lines for salt tolerance using physiological indices as screening tool. *Pak. J. Bot*., 44(1): 47-52.

Khan, M., I.A. Ajmal and A.M. Showalter. 2000. Effect of salinity on growth, water relations and ion accumulation of subtropical perennial halophytes, *Atriplex griffithii* var. stocksii. *Ann. Bot.*, 85: 225-232.

Khan, M.A., M.U. Shirazi, M.A. Khan, S.M. Mujtaba, E. Islam, S. Mumtaz, A. Shereen, R.U. Ansari and M.Y. Ashraf. 2009. Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pak. J. Bot.,* 41(2): 633-638.

Khan, M.A., A.U. Irwin, A.M. Showalter and H.D. Dewald. 1998. NaCl induced accumulation of glycinebetaine in four subtropical halophytes from Pakistan. *Physiol. Plant*., 102: 487-492.

Mass, E.V. and G.J. Hoffman. 1977. Crop salt tolerance–Current assessment. *J. Irrig*., Drain. Div., ASCE 103 (IR2): 115-134.

Noreen, Z., M. Ashraf and Mahmood-ul-Hassan. 2007. Inter-accessional variation for salt tolerance in pea (*Pisum sativum* L.) at germination and screening stage. *Pak. J. Bot*., 39: 2075-2085.

Okcu, G., M.D. Kaya and M. Atak. 2005. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). *Turk. J. Agric. For*., 29: 237-242.

Riazi, A., K. Matruda and A. Arslam. 1985. Water stress induce changes in concentration of proline and other solutes in growing regions. *J. Exp. Bot*., 36: 1716-1725.

Sagi, M., N.A. Savidov, N.P.L. Vov and S.H. Lips. 1997. Nitrate reductase and molybedenum cofactor in annual rye grass as affected by salinity and nitrogen source. *Physiol. Plant*., 9: 546-553.

Sakamoto, A. and N. Murata. 2002. Role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell Environ*., 25: 163-171.

Samaras, Y., R. Bressan, M. Csonka, M. Garcia Rios, P. D'Urzo and D. Rhodes. 1995. Proline accumulation during water deficit. *In: Environment and plant metabolism Flexibility and acclimation* (Ed.): N. Smirnoff. Oxford Bios Scientific Publishers, USA.

Saneoka, H., K. Shiota, H. Kurban, M.I. Chaudhary, G.S. Premachandra and K. Fujita. 1999. Effect of salinity on growth and solute accumulation in two wheat lines differing in salt tolerance. *Soil Sci. Plant Nutrition*, 45: 873-880.

Serraj, R. and T.R. Sinclair. 2002. Osmolyte accumulation: can it really help increase crop yield under drought condition: *Plant Cell Environ*., 25: 333-341.

Silberbush, M., and J. Ben –Asher. 2001. Simulation study of nutrients by plants from soilless cultures as affected by salinity build up and transpiration. *Plant and Soil*, 233:59-69.

Syvertsen, J.P., J.C. Melgar and F. García-Sanchez. 2010. Salinity tolerance and leaf water use efficiency in citrus. *J. Amer. Soc. Hort. Sci*., 135(1): 33-39.

Tester, M. and R. Davenport. 2003. Na+ tolerance and Na+ transport in higher plants. *Ann. Bot*., 91: 503-527.

Waheed, A., I.A. Hafiz., G. Qadir, G. Murtaza, T. Mahmood and M. Ashraf. 2007. Effect of salinity on germination, growth, yield, ionic balance and solute composition of pigeon pea (*Cajanus cajan* (L.) Mill.). *Pak. J. Bot*., 39: 312-312.

Waraich, E.A., A. Rashid, M.Y. Ashraf, Saifullah and A. Mahmood. 2011. Improving agricultural water use efficiency by nutrient management in crop plants. *Acta Agriculturae Scandinavica Section B _ Soil and Plant Science*, 1-14

Yang, W.J, P.J. Rich, J.D. Aztell, K.V. Wood and C.C. Bonham. 2003. Genotypic variation for glycinebetaine in sorghum. *Crop Sci*., 43: 162-169.

Zhu, G.Y., J.M. Kinet and Lutts. 2001. Characterization of rice (*Oryza sativa* L.) F-3 populations selected for salt resistantance. I. Physiological behavior during vegetative growth. *Euphytica*, 121: 251-263.

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