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INFLUENCE OF SALT STRESS ON GROWTH AND BIOCHEMICAL PARAMETERS OF CITRUS ROOTSTOCKS

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Abstract

Soil salinity is a major abiotic stress in agriculture worldwide which affects plant morpho-physiology and ultimately leads to reduction in yield. To assess the effect of salinity on some physio-biochemical parameters in plants of ten different citrus rootstocks, an experiment was performed under controlled saline conditions. Plants were subjected to four salt treatments i.e. 0 (control), 30, 60 and 90 m*M* NaCl for 90 days in sand culture. In response to salt stress, the physiological responses were measured. Salinity affected all of the parameters under study. The high salt concentrations caused a great reduction in growth parameters such as fresh and dry weights of shoots and roots. These changes were associated with decrease in chlorophyll contents in leaves. With the increase in salinity level, the proline and sugar contents were increased, and it was concluded that these osmolytes play a key role in generating tolerance against salt stress.

Introduction

Plants bend toward many adaptive strategies in response to different abiotic stresses such as salinity, water stress, cold, heat etc. which adversely affect the plant growth and yield (McCue & Hanson, 1990). Among these stresses, salinity is the most detrimental which limits the plant growth and productivity (Raveh & Levy, 2005). Salinity is the buildup of soluble salts by which saline soils are formed (Levy & Syvertsen, 2004). Salinity may be due to many reasons but some of the adverse effects of salinity have been attributed to increase in chloride and sodium ions (Zekri, 2001; 2004) in different plant organs especially in citrus (Levitt, 1980) hence these ions create the critical conditions for plants survival by intercepting different plant mechanisms (Raveh, 2005; Grieve *et al.,* 2007). Sodium and chloride are the major ions, which cause many physiological disorders in citrus and limit plant growth and productivity (Kamal *et al*., 2003; Ashraf & Foolad., 2005). Excess of these salts also enhances the osmotic potential of soil matrix as a result of which water intake by plants is restricted (Garcia-Sanchez *et al*., 2002)

Citrus is a leading fruit crop of Pakistan (Ahmed *et al*., 2006). According to Swingle & Reece (1936), the genus citrus, member of family Rutaceae in plant kingdom, is a rich bounty of edible fruits of different species like mandarin (*C. reticulata* Blanco), oranges (*C. sinensis* L. Osbeck), grapefruit (*C. paradisi* Macf.), lemon (*C. limon* L. Burmf.) and lime (*C. aurantifolia* Swingle) with minor categories like tangerine, pummelos (*C. grandis* L.) and tangelos (Mandarin × Grapefruit). Citrus importance in agriculture and in world economy can be estimated by its wide distribution and large-scale production. It is economically very profitable fruit and is grown in more than 52 two countries of the world. In Pakistan, it is grown on an area of 192.3 thousand hectares with annual production of 2458.4 thousand tons (Anon., 2006-07). Despite having good climate for citrus production, the average yield of citrus in Pakistan is very low as compared to other citrus producing countries of the world. Excess of salts in our citrus growing areas is the major reason for low average yield. On the other hand, we are growing salt sensitive citrus rootstocks that lack salt tolerance potential (Boman, 2005). Pakistan's citrus is also subjected to decline because of utilization of underground brackish water for irrigation. Our canal system can not fulfill the farmers' irrigation requirements. So, to meet the irrigation needs, our farmers are pumping out the underground brackish water and using it for irrigating their fields and orchards (Rashid *et al.,* 2004). On the other hand, addition of effluents to canals makes it more salty and unfit for irrigation. Most of horticultural crops especially citrus (Greenway & Munns, 1980) can only grow under the conditions of low soil salinity and can not face higher concentrations of ions like Na^+ and Cl^- in soil solution that limits the nutrient availability to plant (Munns & James, 2003). Population of Pakistan is increasing day by day. So to feed this population, there is a dire need to utilize the saline area for the reasonable production of agronomic as well as horticultural crops.

To achieve optimal food production in saline regions, the most appropriate and logical choice is growing salt tolerant crops/genotypes which are best suited for these regions (Ashraf *et al.,* 2006; Khan, 2003). Rootstock in citrus play a key role for better growth and production (Garcia-Sanchez *et al*., 2003), and is helpful to stabilize the citrus industry and country's economy. It provides good root system to the plant and ultimately good vigor and satisfactory yield. It is only the rootstock that fights against all the dangerous and unfavorable factors of soil that limits the plant growth and development (Levy & Syvertsen, 2004). Unfortunately, our citrus grower does not know about salt tolerant citrus rootstocks. Most of them are growing old salt sensitive rootstocks that ultimately result in lesser growth and reduced citrus yield of the country. Therefore, to assess the salt tolerance of some citrus rootstock genotypes to be used in future, this study was conducted in sand culture under controlled conditions.

Materials and Methods

Plant material and culture: To study the salt tolerance potential in citrus rootstock genotypes, an experiment was conducted in pots. Ten citrus rootstock genotypes (Rough lemon, Rubidux, Citrus Obvidea, Rangpur Lime, Sanchton Citrumillo, Citrumillo-1452, Carrizo Citrange, Yuma Citrange, Gada Dehi and Bitter Sweet Orange) were obtained from different research institutes i.e. Horticultural Research Station, Sahiwal (HRSS); Orange Research Institute Sargodha (ORI) and Experimental Fruit Garden, Institute of Horticultural Sciences of University of Agriculture, Faisalabad, Pakistan. Seeds were sown in nursery beds for getting the vigorous rootstock seedlings. Six months old seedlings of these citrus rootstocks were uprooted carefully by giving minimum damage to roots and transplanted into 30 × 25 cm black painted plastic pots filled with 6.5 kg (4.5 L volume basis) Astatula fine sand (hyperthermic, uncoated Typic Quartzipsamments) individually. The fine sand was having a pH of 6.0-6.5 with 7.2% and 1.2 % of field capacity and wilting percentage (Volume basis), respectively (Camara-Zapata *et al*., 2004). Pots were placed in greenhouse with a photoperiod of 16 h at the temperature of 26± 2°C, and 70 % relative humidity was maintained. The seedlings were watered with modified half strength Hoagland solution for 90 days under greenhouse conditions. (Hoagland & Arnon, 1950). After 90 days of growth in pots, four levels of NaCl salinity (0, 30, 60 and 90 m*M*) were applied to citrus rootstock plants along with half strength Hoagland solution for another 90 days. The plants were irrigated according to their requirement by checking the sand moisture in pots. The seedlings were adjusted to their final NaCl concentration by applying salinity in two-day intervals (30 m*M* salinity increment after two days interval until desired level reached) to avoid osmotic shock. The control was maintained with half strength Hoagland solution only. A completely randomized design was used for the experiment with three replicates.

Growth measurement: Fresh biomass of shoots and roots of each plant was recorded by weighing on electrical balance (Sartorious GC1603S-OCE, Germany). They were placed in an oven at 70°C until constant dry weight was recorded. Plant height was measured in centimeters from the base of stem to the top most leaf with the help of a meter rod. The average value for replicates was calculated.

Biochemical Analysis

Chlorophyll: Chlorophyll and carotinoids contents were estimated according to the method described by Davis *et al*., (1976). Fresh leaves (1.0 g) were cut into 0.5 cm segments and extracted overnight with 80 % acetone at -40°C and then incubated for half an hour at room temperature. The extract thus obtained was centrifuged at 14000 × g for 5 minutes and absorbance of the supernatant was measured at 645, 663 and 480 nm, using double beam Spectrophotometer (Hitachi-120, Japan).

Proline: The proline in citrus rootstocks was estimated according to the method used by Bates *et al*., (1973). A homogenized fresh leaf tissue (0.5 g) was added in 10 mL of 3% sulfo-salicylic acid. Homogenates of citrus rootstock fresh leaf samples were filtered through Whatman No. 2 filter paper. Two mL of the filterate was taken in a test tube containing 2 mL of acid ninhydrin solution (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 *M* orthophosphoric acid). Then, 2 mL of glacial acetic acid was added in a test tube containing filtrate and heated for 1 h at 100°C. Test tubes were then shifted in an ice bath to terminate the reaction. Reaction mixture was then extracted with 10 mL toluene and mixed vigorously by passing a continuous air stream for 1-2 minutes. Toluene was aspirated from chromophore. Aqueous phase was separated, warmed at room temperature and absorbance was noted at 520 nm, while toluene was used as a blank. Proline concentration was determined from a standard curve and calculated on fresh weight basis as follows:

$$(\text{Mole proline g}^{-1} \text{ fresh weight} = (\text{g proline mL}^{-1} \times \text{mL of toluene}/115.5) / (\text{g of sample}/5)$$

Sugar estimation: Total soluble sugars were determined according to Riazi *et al*., (1985). Fresh leaves (1 g) were ground in 10 mL of 80% ethanol (v/v). Centrifuged and filtered the extract and supernatant was used for the estimation of sugars. Plant extract (1mL) was taken in 25 mL test tubes and 6 mL anthrone reagent was added to each tube. The mixture was heated in boiling water bath for 10 minutes. The test tubes were ice-cooled for 10 minutes, and incubated for 20 minutes at room temperature (25°C). Optical density was recorded at 625 nm on a spectrophotometer. The concentration of soluble sugars was calculated from the standard curve developed with different concentrations of glucose using the above method.

Statistical analysis: All the statistical analyses were performed by using the STATISTICA statistical package. Completely Randomized Design (CRD) with three factor factorial arrangement was applied. Statistically significant differences were identified using analysis of variance (ANOVA); used Duncan's Multiple Range Test for determining significant mean differences; accepted the 0.05 probability as significant (Steel *et al*., 1997). Simple correlation coefficients between different variables were developed by using the same statistical package.

Results

Effect of NaCl on growth parameters: Sodium chloride caused a significant reduction in all the growth parameters considered (Fig. 1). The reduction was greater at higher NaCl concentrations (60 & 90 m*M*.) The fresh weight (FW) of roots and shoots, plant height, number of leaves and leaf area were gradually decreased with an increase in NaCl concentration (Fig. 1 & 2). The reduction was more pronounced at 90 m*M* in all the citrus rootstocks. The dry weight (DW) was also affected by NaCl treatment, with a greater reduction as NaCl concentration was increased in all the rootstocks studied. Maximum reduction in fresh and dry weights of shoots and roots was observed in genotype Carrizo Citrange while the genotype Rangpur Lime exhibited the minimum reduction in this regard (Fig. 1).

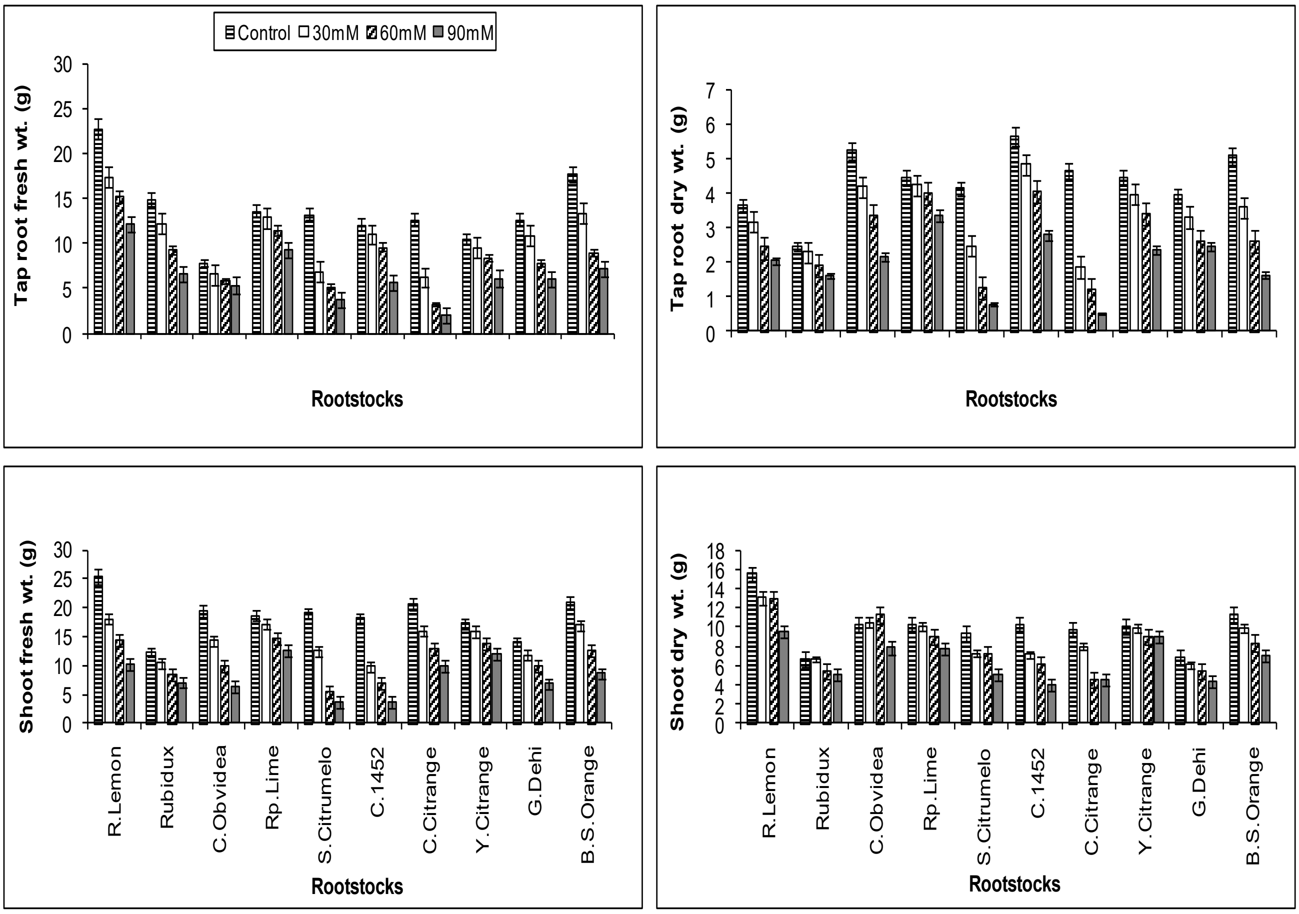

Fig. 1. Effect of salt stress on fresh and dry biomass of different citrus rootstocks.

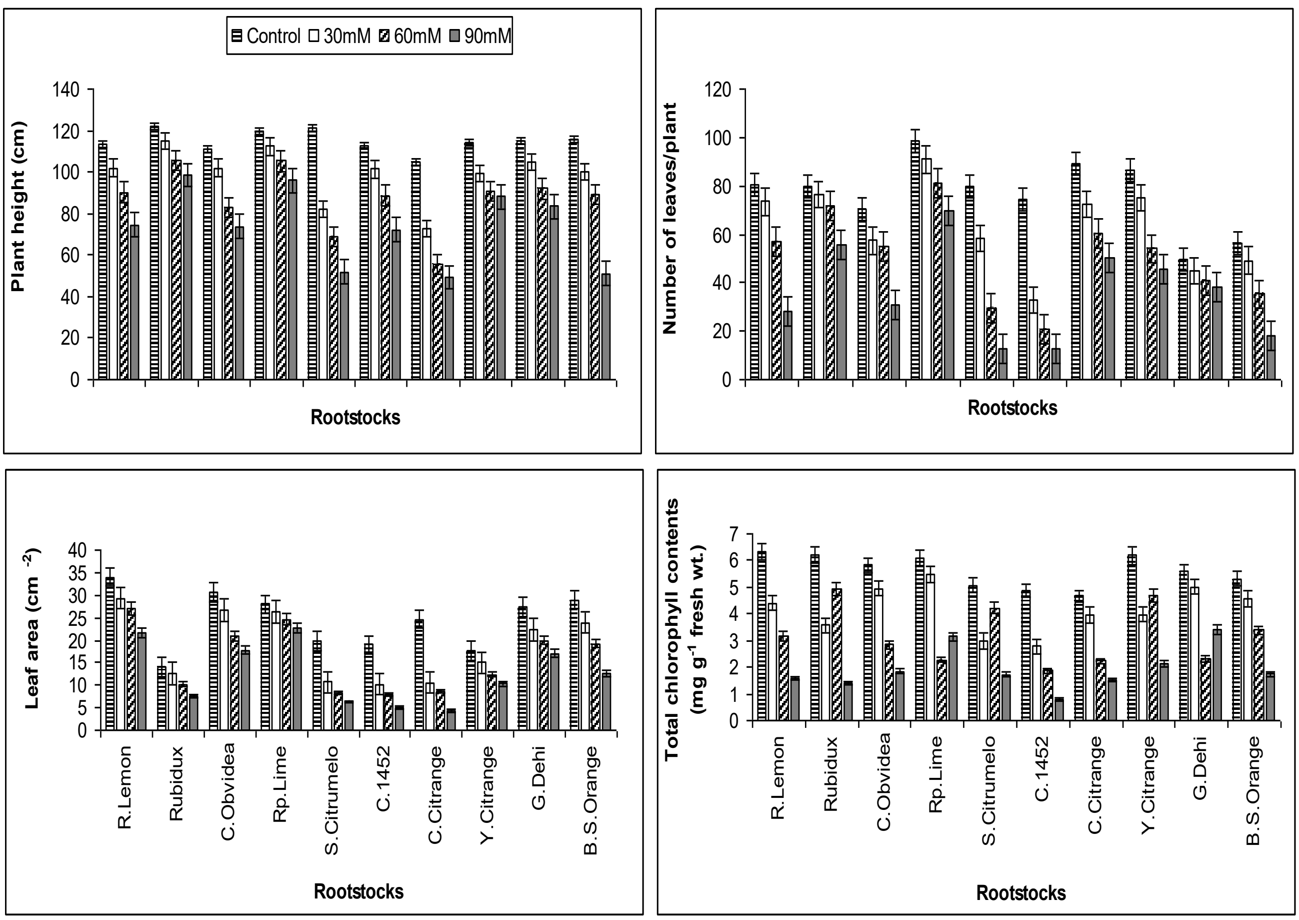

Fig. 2. Effect of salt stress on plant height, number of leaves plant^{-1}, leaf area and total chlorophyll contents of different citrus rootstocks.

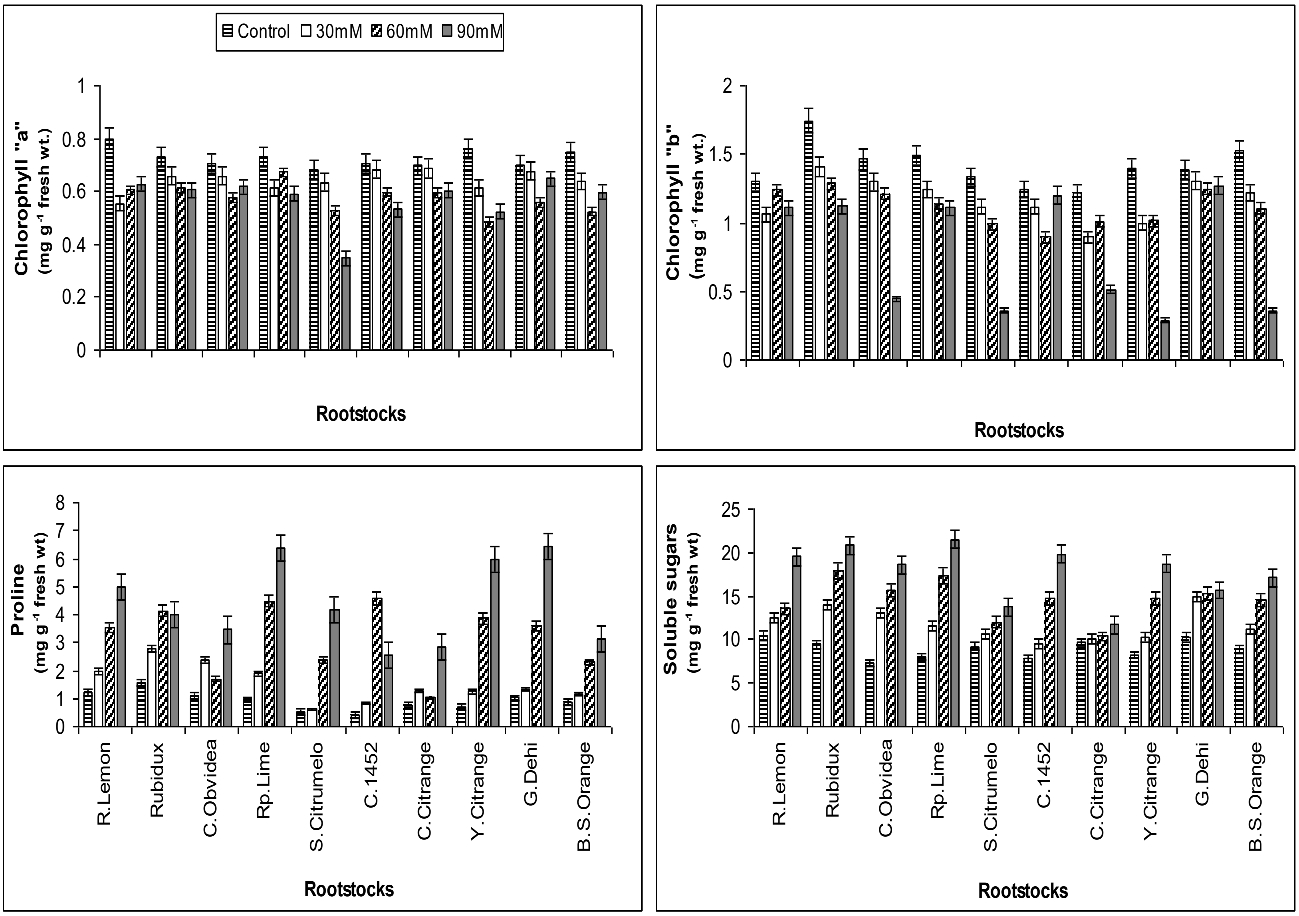

Fig.3. Effect of salt stress on chlorophyll, proline and soluble sugars of different citrus rootstocks.

Effect of NaCl on proline contents: Saline conditions caused a significant (p<0.05) increase in proline accumulation in all the citrus rootstocks (Fig. 2). Maximum proline accumulation was recorded at higher salinity level i.e. 90 m*M* followed by 60 m*M* and 30 m*M* respectively. On the basis of maximum proline accumulation, the highest salt tolerance was recorded in citrus rootstocks i.e. Rangpur Lime followed by Rubidux and Gada Dehi. Similarly Carrizo Citrange and Bitter Sweet Orange showed the minimum salt tolerance by accumulating the lowest amounts of proline. The rootstock × treatment interaction was significant (p<0.05). Rubidux had the highest value for proline contents showing its maximum accumulation under controlled conditions as compared to others under the same conditions. Thus, Rubidux was the best one by the virtue of the highest proline accumulation while Sanchton Citrumello represented the poorest performance due to the lowest proline accumulation under 30 m*M* salinity level. However, citrus rootstock Rough Lemon showed better performance by accumulating maximum proline contents under 60 m*M* salt stress but Carrizo Citrange and Citrus Obvidea showed the poorest performance by accumulating lower amounts of proline as compared to the others under the same treatment. Under 90 m*M* NaCl salinity, the rootstocks Rangpur Lime and Gada Dehi represented an efficient performance due to the maximum proline accumulation while Citrumello-1452 occupied the lowest position because of lower proline contents under higher salt stress.

Effect of NaCl on total soluble sugars: Salt stress caused an increase in total soluble sugars in all the citrus rootstocks (Fig. 2). The maximum total soluble sugars concentration was recorded under higher salinity treatment (90 m*M*) as compared to control (Fig. 2). The comparison among the citrus rootstocks for total soluble sugars indicated that they differed significantly. The citrus rootstocks, Rubidux and Rangpur Lime, showed the maximum sugar accumulation under salt stress. The lowest total soluble sugar contents were recorded in Sanchton Citrumelo and Carrizo Citrange while moderate amounts of total soluble sugars were estimated in Rough Lemon, Gada Dehi and Citrus Obvidea. Non-significant differences were found between Rough Lemon and Gada Dehi for total soluble sugars. The lowest quantities of total soluble sugars were estimated under control conditions for Citrus Obvidea while citrus rootstock Rough Lemon and Gada Dehi had the maximum quantities of this attribute. Under 30 m*M* salt stress, maximum total soluble sugar contents were observed in Gada Dehi while Citrumello-1452 gave the lowest values of total soluble sugar contents under the same level of salt stress. Similarly under 60 m*M* NaCl level, the best performance was observed for Rough Lemon while Citrumelo-1452, Yuma Citrange and Gada Dehi behaved in a similar fashion by giving the similar values under this treatment. Citrus rootstock Bitter Sweet Orange had the lowest value for total soluble sugars followed by Sanchton Citrumilo. Under 90 m*M* NaCl salinity, the maximum total soluble sugar contents were observed in Rangpur Lime while this attribute was minimum in Carrizo Citrange.

Discussion

Growth like fresh and dry weights of root and leaves markedly decreased with salinity (Fig. 1). It is well established fact that plants growing under saline condition remain stunted due to reduction in cell elongation and cell division, which are under the control of different auxins, whose synthesis is retarded by the salinity (Loreto *et al.,* 2003; Ndayiragije & Lutts, 2006). Plant growth is important character in determining the salt tolerance ability of different citrus rootstocks. The citrus rootstocks, Rubidux, Gada Dehi and Rangpur Lime, maintained higher root and leaf dry as well as fresh weights under saline conditions. Salt stress significantly reduced the biomass yield in all the tested rootstocks but rootstocks like Rangpur Lime, Rubidux and Gada Dehi were least affected at all salinity levels (Fig. 1 & 2). The reduction in biomass increased with the increase in salinity which is obvious because of disturbances in physiological and biochemical activities under saline conditions (Craine, 2005; Munns *et al*., 2006) that may be due to the reduction in leaf area and number of leaves (Romero-Aranda *et al*., 1998; Dong *et al*., 2007). Decrease in growth parameters like plant height and number of leaves were also decreased with salinity. Although plant height is genetically controlled but environmental factors also have strong influence in the expression of genes as it is very clear from the present study that different citrus rootstocks maintained different plant heights under normal condition as well as under saline conditions (Fig. 1). Since the citrus rootstocks, Rubidux, Gada Dehi and Rangpur Lime, maintained higher plant height and other growth parameters under saline conditions, so they can be categorized as tolerant one.

Chlorophyll is membrane bound and depends upon the membrane stability thus under saline conditions it seldom remains intact (Ashraf *et al*., 1992; Ashraf & Khan, 1993; Shah, 2007). Decrease in chlorophyll contents due to salinity has also been reported elsewhere (e.g. Gu *et al*., 2004). However, some studies (Evain *et al*., 2004; Paranychianakis & Chartzoulakis, 2005) have also reported an increase in chlorophyll contents in some cultivars of different plant species. Accordingly, different workers from these studies gave different reasons for increase or decrease in chlorophyll contents. Salinity may also be responsible for lower values of stomatal conductance, photosynthesis and relative water content (Naumann *et al*., 2007; 2008). However, researchers summarized the results by showing that reduction in chlorophyll may be due to variation in its synthesis between the plant species due to variation in specific enzymes under saline conditions (Kreps *et al*., 2002; Keutgen & Pawelzik, 2007 a). Furthermore, in the present study, another interesting feature was the shift in chlorophyll a/b ratio which showed that reduction in chlorophyll b was more severe than chlorophyll a which affected the chlorophyll a/b ratio. The difference between salt tolerant and salt sensitive citrus rootstocks can be determined based on this criterion.

Plants have the ability to increase their water suction force when they are subjected to various stresses. Since salinity creates osmotic stress as well as ion toxicity resulting in decrease in soil metric potential, as a result of which plants also lower their osmotic potential for osmotic adjustment. Lowering of osmotic potential results due to an increase in intracellular solutes which is an adopted mechanism of plants to external stress (Rahman *et al*., 2002; Meloni *et al*., 2004). It allows the maintenance of turgor under salinity stress, as a result of which plant becomes able to maintain the vital processes. In plants, accumulation of different solutes due to salt stress has been reported by many workers. The commonly reported solutes are proline, glycinebetaine, sugar, amino acid and polyols etc. So, leaf proline concentrations were also increased due to salinity in all the citrus rootstock (Table 2). It has been extensively reported that proline content increases due to salinity stress in different plants (James *et al*., 2002; Ashraf & Bashir, 2003; Roussos & Pontikis, 2007). The rootstock, Rangpur Lime was, successful in maintaining the highest proline levels as compared to others. Reports indicate that proline content provides also rapid mechanism for maintaining the turgor and affects the solubility of various proteins (Abraham *et al*., 2003) and protects them against denaturation under saline condition (Jantaro *et al*., 2003; Tonon *et al*., 2004). It is also associated with protein hydrolysis induced by the salinity. However, there are some contrasting reports available in literature which indicates that proline accumulation is not due to protein hydrolysis (Wimmer *et al*., 2003). It is also evidenced that osmotic adjustment under saline conditions is due to accumulation of solutes and proline has a great role in this process (Ueda *et al*., 2004). Inherited difference in the accumulation of proline in various genotypes of plant species has also been reported and this osmolyte is widely accumulated in higher plants, than other amino acids under salt stress conditions (Abraham *et al*., 2003). In contrast, Tal *et al*., (1979) reported more proline accumulation in salt sensitive species of tomato than in tolerant wild relatives. Reports indicated that accumulation of proline contributes towards membrane stability (Hanson & Burnet, 1994; Dondini *et al*., 2001) and mitigates the effect of NaCl on cell membrane disruption (Mansour, 1998). The present study also confirms that proline accumulation varies in different citrus rootstocks. It seems that tolerant citrus rootstock like Rangpur Lime maintained higher proline accumulation under various levels of salinity. However, the present findings did not agree with the inherited content of proline. From the present findings, it seems that proline along with other osmolytes had a role in osmotic adjustment because the citrus rootstock with higher proline content showed higher biomass yield.

Accumulation of total soluble sugars is a common phenomenon under stress condition (William *et al*., 2000; Murakeozy *et al*., 2003). Garg *et al*., (2002) and Rolland *et al*., (2002) reported an increase in total soluble sugars with progressive increase in salinity, which have an important role in osmoregulation (Mohanty *et al*., 2002; Martino *et al*., 2003). Increase in total soluble sugars increased the osmotic concentration (solutes concentration) under saline conditions in citrus rootstocks which reduced the osmotic potential. The salt tolerant citrus rootstocks accumulated more sugar which is effective in maintaining turgor by decreasing the osmotic potential and shows higher osmotic adjustment. In the present study, total soluble sugars increased with the increase in salinity irrespective of citrus rootstock. However, accumulation of total soluble sugar was greater in Rubidux and Rangpur

Lime rootstocks than others. The results of this study also indicated that amount of total soluble sugars was higher than other solutes like proline, glycinebetaine, total free amino acids; many reports confirm these findings (Fougere *et al*., 1991; Hsu *et al*., 2003). Accumulation of sugars contributed more towards the osmotic adjustment than other solutes under saline conditions (Popp & Smirnoff, 1999; Atienza *et al*., 2004).

Conclusion

The assessment of the effects of salinity on the growth and biochemical attributes in citrus rootstock genotypes lead us to conclude that all the considered parameters were significantly affected by the salt stress. The results of this study are in accordance with the earlier reports which show that in response to osmotic stress, the synthesis of compatible organic solutes occurs and favor the hypothesis that proline acts as protective compound during salt stress. Thus, these organic solutes could be used as a biochemical marker for assessing increased salt tolerance in citrus rootstocks.

References

Abraham, E., G. Rigo, G. Szekely, R. Vagy, C. Koncz and L. Szabados. 2003. Light dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteriod in *Arabidopsis*, *Plant Mol. Biol.*, 51:363-372.

Ahmed, W., M. A. Pervez, M. Amjad, M. Khalid, C. M. Ayub and M. A. Nawaz.2006. Effects of stionic combination of growth and yield of kinnow (*C. reticulate* Blanco). *Pak. J. Bot.*, 38: 603-612.

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

Ashraf, M. and M. R. Foolad. 2005. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Exp. Environ. Bot.*, 59:206-216.

Ashraf, M., M. H. Bokhari and S. N. Chishti. 1992. Variation in osmotic adjustment of accessions of lentil (*Lens culinaris* Medic.) in response to drought stress. *Acta Bot. Netherlands.*, 41:51-62.

Ashraf, M. Y. and A. H. Khan. 1993. Effect of NaCl on nitrogen status of sorghum, *In: Current Developments in Salinity and Drought Tolerance of Plants* (Eds.): S.S.M., Naqvi, R. Ansari, T.J. Flowers and A.R. Azmi. Publisher: Plant Physiology Division, AEARC, Tandojam, Pakistan, pp. 84-88.

Ashraf, M. and A. Bashir. 2003. Salt stress induced changes in some organic metabolites and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. *Flora*, 198:486-498.

Atienza, S. G., P. Faccioli, G. Perrotta, G. Dalfino, W. Zschiesche, K. Humbeck, A. M. Stanca and L. Cattivelli. 2004. Large-scale analysis of transcripts abundance in barley subjected to several single and combined abiotic stress conditions. *Plant Sci.*, 167: 1359-1365.

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

Boman, J. B. 2005. Salinity effects on Florida Grapefruit in the Indian River Region. University of Florida, *Indian River Research and Education Center*, Ft. Pierce, FL.

Camara-Zapata, J. M., F. Garcia-Sanchez, V. Martinez, M. Nieves and A. Cerda. 2004. Effect of NaCl on citrus cultivars. *Agronomie*, 24:155-60.

Craine, J. M. 2005. Reconciling plant strategy theories of Grime and Tilman. *J. Ecol.*, 93:1041-1052.

Davis, T. D., B. E. Haissig and N. Sankhla. 1998. Adventitious root formation by cuttings. Advances in Plant Sciences Series, Vol. 2. Dioscorides Press, Portland. pp. 315.

Dondini, L., S. Bonazzi, S. Del-Duca, A. M. Bregoli and D. Serafni-Fracassini. 2001. Acclimation of chloroplast transglutaminase to high NaCl concentration in a polyamine deficient variant strain in Dunaliella salina and in its wild type. *J. Plant Physiol.*, 158:185-97.

Dong, Y., T. Ji and S. Dong. 2007. Stress responses to rapid temperature changes of the juvenile sea cucumber (*Apostichopus japonicus* Selenka). *J. Ocean Uni. Chin.*, 6:275-280.

Evain, S., J. Flexas and I. Moya. 2004. A new instrument for passive remote sensing: 2. Measurement of leaf and canopy reflectance changes at 531 nm and their relationship with photosynthesis and chlorophyll fluorescence. *Remote Sens. Environmen.*, 91: 175-185.

Anonymous. 2001. FAOSTAT Agricultural Data. Agricultural production, crops. Available at http://faostat.fao.org/faostat/collections?subset agriculture Accessed on 10February 2005; verified on 17 March 2005. *United Nations Food and Agriculture Organization*.

Fougere, F., D. Le-Rudulier and J. G. Streeter. 1991. Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids and cytosol of alfalfa (*Medicago sativa* L.). *Plant Physiol.*, 96:1228-1236.

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

Garcia-Sanchez, F., M. Carvajal, A. Cerda and V. Martinez. 2003. Response of Star Ruby grapefruit on two rootstocks to NaCl salinity. *Hort. Sci. Biotechnol.*, 78:859-865.

Garg, A. K., J. K. Ownes and R. J. Wu. 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stress. *Proceeding of the National Academy of Sciences of the United States of America*, 99:15898-15903.

Greenway, H. and R. Munns. 1980. Mechanism of salt tolerance in non halophytes. *Annu. Rev. Plant Physiol.*, 31: 149-190.

Grieve, A. M., D. L. Prior and B. K. Bevington. 2007. Long-term effect of saline irrigation water on growth, yield, and fruit quality of Valencia orange trees. *Aus. J. Agri. Res.*, 58:342-348.

Gu, R., S. Fonseca, L. Puskas, L. Hackler, A. Zvara, D. Dudits and M. Pais. 2004. Transcript identification and profiling during salt stress and recovery of *Populus euphratica*. *Tree Physiol.*, 24:265-276.

Hanson, A. D. and M. Burnet. 1994. Evolution and metabolic engineering of osmoprotectant accumulation in higher plants, In: *Biochemical and Cellular Mechanisms of Stress Tolerance in Plants*, (Ed.): J.H. Cherry. Springer-Verlag, *Berlin*, 291-301.

Hoagland, D. R. and D. J. Arnon. 1950. The water culture method for growing plants without soil .*California Agricultural Experiment Station Circular*, 347: 1-32.

Hsu, S. Y., Y. T. Hsu and C. H. Kao. 2003. The effect of polyethylene glycol on proline accumulation in rice leaves. *Biol. Plant.*, 46:73-78.

James, R. A., A. R. Rivelli, R. Munns and S. V. Caemmerer. 2002. Factors affecting CO_2 assimilation, leaf injury and growth in salt stressed durum wheat. *Funct. Plant Biol.*, 29:1393-1403.

Jantaro, S., P. Maenpaa, P. Mulo and A. Incharoensakdi. 2003. Content and biosynthesis of polyamines in salt and osmotically stressed cell of Synechocystis sp. PCC 6803. *FEMS Microbiol Lett*., 228:129-35.

Kamal, A., M. S. Qureshi, M.Y. Ashraf and M. Hussain. 2003. Salinity induced changes in some growth and physio-chemical aspects of two soybeans [*Glycine max* (L.) Merr.] genotypes. *Pak. J. Bot.*, 35:93-97.

Keutgen, A. J. and E. Pawelzik. 2007a. Modifications of strawberry fruit antioxidant pools and fruit quality under NaCl stress. *J. Agri. Food Chem.*, 55:4066-4072.

Khan, M. A. 2003. NaCl inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. *Biol. Plant.*, 47:437-440.

Kreps, J. A., Y. Wu, H. S. Chang, T. Zhu, X. Wang and J. F. Harper. 2002. Transcriptome changes for *Arabidopsis* in response to salt, osmotic and cold stress. *Plant Physiol.*, 130:2129-2141.

Levitt, J. 1980. *Responses of plants to environmental stresses*. Academic Press, New York.

Levy, Y. and J. P. Syvertsen. 2004. Irrigation water quality and salinity effects in citrus trees. *Hortic. Rev.*, 30:37-82.

Loreto, F., M. Centritto and K. Chartzoulakis. 2003. Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. *Plant Cell Environ.*, 26:595-601.

Mansour, M. M. F. 1998. Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiol Biochem.*, 36:767-772.

Martino, C. D., S. Delfine, R. Pizzuto, F. Loreto and A. Fuggi. 2003. Free amino acids and glycinebetaine in leaf osmoregulation of spinach responding to increasing salt stress. *New Phytol.*, 158:455-463.

McCue, A. M. and A. D. Hanson. 1990. Drought and salt tolerance: toward understanding and application. *Trends Biotechnol.*, 8: 358-362.

Meloni, D. A., M. A. Oliva, C. A. Martinez and Cambraia. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.*, 49:69-76.

Mohanty, A., H. Kathuria, A. Ferjani, A. Sakamoto, P. Mohanty, N. Murata and A. K. Tyagi. 2002. Transgenics of an elite indica rice variety Pusa Basmati 1 harbouring the *codA* gene are highly tolerant to salt stress. *Theor. Appl Genet.*, 106:51-57.

Munns, R. and R. A. James. 2003. Screening methods for salt tolerance: a case study with tetraploid wheat. *Plant and Soil*, 253: 201-218.

Murakeozy, E. P., Z. Nagy, C. Duhaze, A. Bouchereau and Z. Tuba. 2003. Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *J. Plant Physiol.*, 160:395-401.

Naumann, J. C., D. R. Young and J. E. Anderson. 2008. Leaf chlorophyll fluorescence, reflectance and physiological response to fresh water and salt water flooding in the evergreen shrub, *Myricacerifera. Environ. Exp. Bot.*, 63:402-409.

Ndayiragije A. and S. D. Lutts. 2006. Do exogenous polyamines have an impact on the response of a salt sensitive rice cultivar to NaCl? *J. Plant Physiol.*, 163:506-16.

Naumann, J. C., D. R. Young and J. E. Anderson. 2007. Linking leaf chlorophyll fluorescence properties to physiological responses for detection of salt and drought stress in coastal plant species. *Physiol. Plant.*, 131:422-433.

Paranychianakis, N. V. and K. Chartzoulakis. 2005. Irrigation of Mediterranean crops with saline water: from physiology to management practices. *Agri. Ecosys. Environmen.,* 106:171-187.

Popp, M. and N. Smirnoff. 1999. Polyol accumulation and metabolism during water deficit. In: Smirnoff, N. (ed.): Environment and plant metabolism: Flexibility and acclimation. *Bios Scientific*, *Oxford*, 199-215.

Raveh, E. and Y. Levy. 2005. Analysis of xylem water as an indicator of current chloride uptake status in citrus trees. *Sci. Hortic.*, 103: 317-327.

Raveh, E. 2005. Methods to assess potential chloride stress in citrus: analysis of leaves, fruit, stem-xylem sap and roots. *Hort. Technol.*, 15: 104-108.

Rashid, P., J. L. Karmoker, S. Chakrabotty and B.C. Sakel. 2004. The effect of salinity on ion accumulation and anatomical attributes in mungbean (*Phaseolus radiatus*) seedlings. *Int. J. Agri. Biol.*, 6: 495-498.

Rahman, M. S., H. Miyake and Y. Takeoka. 2002. Effects of exogenous glycinebetaine on growth and ultrastructure of salt-stressed rice seedlings (*Oryza sativa* L.). *Plant Prod. Sci.*, 5:33-44.

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

Romero-Aranda, R., J. L. Moya, F. R. Tadeo, F. Legaz and E. Primo-Millo.1998. Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. *Plant Cell Environ.*, 21:1243-1253.

Roussos, P. A. and C. A. Pontikis. 2007. Changes of free, soluble conjugated and bound polyamine titers of jajuba explants under sodium chloride salinity *In vitro*. *J. Plant Physiol.*, 64:895-903.

Shah, S. T., R. Zamir, J. Ahmad, H. Ali and G. Lutfullah. 2007. *In vitro* regeneration of plantlets from seedlings explants of guava (*Psidium guajava* L.) cv. Safeda. *Pak. J. Bot.*, 40:1195-1200.

Steel, R. G. D., J. H. Torrie and D. A. Dicky. 1997. *Principles and procedures of statistics-a biometrical approach* 3rd edn. McGraw Hill Book international Co., Singapore: 204-227.

Swingle, W. T. and P. C. Reece. 1936. The botany of citrus and its wild relatives. In: *The citrus Industry.* (Eds.): W. Reuther, H. J. Weber and L. D. Batchelor.vol.1, History, world distributions, botany and varities, *University of California press,Berkeley, California, USA*: 190-243.

Tal, M. and I. Gardi. 1976. Physiology of polyploid plants: Water balance in autotetraploid and diploid tomato under low and high salinity. *Physiol Plant.*, 38:257-61.

Tonon, G., C. Kevers, O. Faivre-Rampant, M. Graziani and T. Gaspar. 2004. Effect of NaCl and mannitol iso-osmotic stresses on proline and free polyamine levels in embryogenic Fraxinus angustifolia callus. *J. Plant Physiol.*, 161:701-8.

Ueda, A., A. Kathiresan, M. Inada, Y. Narita, T. Nakamura, W. Shi, T. Takab and J. Bennett. 2004. Osmotic stress in barley regulates expression of a different set of genes than salt stress does. *J. Exp. Bot.*, 55:2213-2218.

Williams, L.E., R. Lemonie and N. Saucer. 2000. Sugars transporters in higher plants: a diversity of roles and complex regulation trends. *Plant Sci*, 5:283-290.

Wimmer, K. H., A. Muhling, P. H. Lauchli, H. E. Brown and Goldbach. 2003. The interaction between salinity and boron toxicity affects the subcellular distribution of ions and proteins in wheat leaves. *Plant Cell Environ.*, 26:1267-1274.

Zekri, M. 2001. Salinity effect on seedling emergence, nitrogen and chloride concentrations, and growth of citrus rootstocks. *Proceedings of the Florida State Horticultural Society*, 114:79-82.

Zekri, M. 2004. Strategies to manage salinity problem in citrus. *Proceedings of International Society of Citriculture*, 639-643.

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