

## MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE OF CITRUS ROOTSTOCKS TO SALINITY STRESS AT EARLY GROWTH STAGE

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A greenhouse study was conducted to determine the morphological, physiological and biochemical responses of four citrus rootstocks viz. Volkameriana (*Citrus volkameriana* Ten. and Pasq.), Rangpur Lime (*Citrus limonia* Osbeck), Trifoliolate Orange (*Pontius trifoliolate* (L.) Raf.) and Rough Lemon (*Citrus jambhiri* Lush.) to sodium chloride (NaCl) salinity applied at seedling stage. One year-old uniform citrus seedlings were grown in sand culture irrigated with modified half-strength Hoagland's solution and treated with different NaCl concentrations (0, 75, 100 and 150 mM) for 25 days. Parameters such as plant height, leaf drop and tip burning symptoms differed according to the ability of rootstocks type in coping with salt stress. Salinity significantly ( $P<0.01$ ) decreased leaf water potential, photosynthetic rate, transpiration rate and stomatal conductance values indicative of serious metabolic effects of salts on the citrus growth at 150 mM (NaCl). The ionic concentration of sodium and chloride was higher in concentration in Volkameriana ( $9.2\pm0.7$  and  $5.6\pm0.15$ ) and Trifoliolate Orange ( $10.2\pm0.10$  and  $6.3\pm0.18$ ) than Rangpur Lime ( $9.9\pm0.3$  and  $4.8\pm0.07$ ) and Rough Lemon ( $16.0\pm0.2$  and  $5.7\pm0.10$ ). Calcium and potassium concentrations in all rootstocks decreased with increasing salinity in the plant rhizosphere. Although salinity affected all four rootstock genotypes but Rangpur Lime ( $8.1\pm0.10$ ) followed by Rough Lemon ( $7.5\pm0.60$ ) performed better for potassium uptake as compared to Volkameriana ( $6.3\pm0.30$ ) and Trifoliolate Orange ( $7.4\pm0.20$ ). Rangpur Lime even showed new leaf sprouting at the highest level of sodium chloride (150 mM). The rootstock genotype Trifoliolate Orange proved to be highly sensitive to NaCl salinity in this study.

**Keywords:** Growth, ionic composition, physiology, sodium chloride

### INTRODUCTION

Citrus is one of the top ranked fruit crops of the globe and in Pakistan it is largely grown in the province Punjab (Chaudhary, 1994) where the presence of salts in the soil or irrigation water is a major threat to citriculture industry. Salinity not only impairs soil structure but also causes various physiological and metabolic dysfunctions and injuries leading to reduction in vegetative and reproductive growth and ultimately to the death of plants (Taiz and Zeiger, 2002). The presence of salt ions such as  $\text{Na}^+$  and  $\text{Cl}^-$  in the growth medium have been reported to adversely affect the plant growth through toxicity of specific ions due to excessive accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the leaves (Ali *et al.*, 2006) and nutritional imbalance by influencing uptake, transport and utilization of different nutrients (Grattan and Grieve, 1999; Zhu, 2003) which may result in reduction in water availability (Lloyd *et al.*, 1987), changes in hormonal balance (Camara-Zapata *et al.*, 2004) or combination of any of these factors. The reduction in plant growth and yield due to salinity; however, varies with plant species, salinity levels, and ionic composition of the salts that contribute to salinity.

In addition, salinity also increases the incident of *Phytophthora* (Sulistyowati, 1994; Al-Yassin, 2004) which is one of the most prevalent problems of citrus orchards in Pakistan and elsewhere.

Citrus is characterized as a salt-sensitive horticultural crop (Al-Yassin, 2004). However, rootstocks differ in their salinity tolerance as determined by the ability to restrict uptake and/or transport of the potentially toxic  $\text{Cl}^-$ ,  $\text{Na}^+$  or both ions from reaching the scions (Chen, 1992; Storey, 1995). In citrus, the accumulation of  $\text{Cl}^-$  ions in tissues have been linked to passive movement of water into the tissues (Storey and Walker, 1999; Moya *et al.*, 2003) thus causing a reduction in photosynthesis and transpiration (Moya *et al.*, 2002; Arbona *et al.*, 2006). Moreover, a particular threshold of leaf chloride concentration which is around 1.5% dry weight (Raveh and Levy, 2005) triggers leaf abscission through increased ethylene production (Arbona *et al.*, 2006). Accumulation of salts in chloroplast of sensitive species causes a reduction in chlorophyll contents thus affecting photosynthetic electron transport and PSII activity (Sudhir and Murthy, 2004) and this reduction in photosynthetic

activity is directly related to reduction in yield (Meloni *et al.*, 2003; Abbas *et al.*, 2013; Atiq-ur-Rahman *et al.*, 2014).

Several approaches are used to mitigate the adverse effects of soil and irrigation water salinity but a more permanent solution to this problem keeping in view the increasing food requirement of the world would be the use of salt tolerant crops. Annual crops can be replanted after soil amendments or leaching, but in case of fruit tree orchards like citrus; one way of improving the salt-tolerance of citrus is to graft scions on salt-tolerant rootstocks (Garcia-Sanchez *et al.*, 2002). Only one rootstock is supporting the citrus industry of Pakistan i.e. Rough Lemon (Ahmed *et al.*, 2006). This poses a threat to the whole industry as any devastating disease or climatic change which is unfavorable for Rough Lemon could wipe out whole citrus orchards. Thus, considering burgeoning salinity problem in Pakistan, the major objective in citrus breeding is to develop new salt-tolerant citrus rootstocks for future with an enhanced capacity of excluding potential toxic ions from leaves (Balal *et al.*, 2011). Although, rootstocks tolerance to salinity has been investigated for decades, the work on salinity tolerance of citrus rootstocks in Pakistan is very meager. The present study, therefore, was undertaken with the objectives to determine the relative tolerance of potential citrus rootstocks by exposing them to increasing levels of NaCl salinity and their salt tolerance was evaluated on the basis of various morphological and physiological parameters.

## MATERIALS AND METHODS

Present study was carried out at the Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan under green house conditions. One year old uniform size seedlings of four citrus rootstock genotypes viz. Volkameriana (*Citrus volkameriana*), Rangpur Lime (*Citrus limonia*), Trifoliate Orange (*Poncirus trifoliata*) and Rough Lemon (*Citrus jambhiri*) were grown in sand culture irrigated with modified half-strength Hoagland's solution (Hoagland and Arnon, 1950) for seven days as the acclimatization period. Four levels of NaCl (0, 75, 100 and 150 mM) were applied to the seedlings of various rootstocks along with the nutrient medium (½ strength Hoagland's solution) for 25 days before the collection of data. The desired salinity levels were developed by applying NaCl solutions with a 25% increment in salt concentration on every alternate day until the required levels of 75, 100 and 150 mM of NaCl were achieved. The experiment was laid out following completely randomized design with factorial arrangements and replicated five times.

The physical parameters studied included plant height, leaf drop and visual toxicity symptoms. Plant height was measured at the time of transplanting seedlings into sand and at the end of the experiment to compare the increase in height at different salt levels in different rootstock genotypes.

Number of leaves per plant was counted in the beginning and then at the end of the experiment to calculate leaf drop. Tip burning was evaluated on the basis of extent of damage i.e. mild, moderate or severe by observing visual toxicity symptoms in each salinity treatment and for each cuts rootstocks at the end of the experiment.

Leaf water potential and gas exchange parameters were measured at the end of the experiment using three leaves per plant. Leaf water potential was measured around 9-10 a.m. by placing freshly excised leaves from the middle portion of the seedlings in a pressure chamber (Scholander *et al.*, 1965). A portable infrared gas analyzer system LCA-4 (Analytical Development Company Ltd. Japan) was used to measure gas exchange parameters such as photosynthetic rate, stomata conductance and transpiration rate simultaneously by placing intact leaves to the plant in its chamber (Atkinson and Winner, 1990).

To estimate the ionic composition, all leaves from the citrus seedlings were harvested at the end of the experiment, washed and dried in an oven at 55°C for 48 hours. Then ground to a fine powder using electric grinder and stored at room temperature. Samples were wet digested as described by Yoshida *et al.* (1976) and Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>++</sup> were estimated by using a flame photometer (Sherwood Flame Photometer Model-410) while Cl<sup>-</sup> was estimated by silver ion titration method with a Sherwood Chloride Analyzer Model-926 (Gomez-Cadenas *et al.*, 1998). Leaf sap Cl<sup>-</sup> was also determined with the same method. Sap was extracted by freezing the leaf samples at -80°C followed by thawing and centrifugation (Raveh and Levy, 2005). Data collected were analyzed using analysis of variance procedures and significant treatment means were compared using Duncan's multiple range test (Steel *et al.*, 1997).

## RESULTS

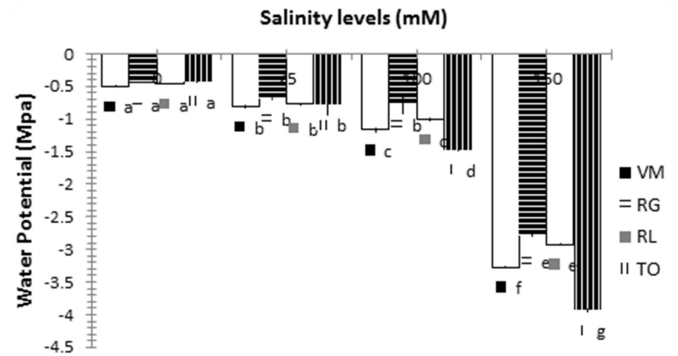
**Growth parameters:** Plant height decreased with increasing sodium chloride salinity in the growth medium in all citrus rootstocks but at different rates depending on the salinity level and the rootstock genotypes (Table 1). At 75 mM NaCl salinity level, reduction (% of control plants) in plant height was in the range of 13-55 % being Volkameriana and 55 % in Rough Lemon. However at 100 mM NaCl, Rangpur Lime and Volkameriana remained statistically similar with height increase of 1.04 cm and 1.00 cm, respectively. At 150 mM, salinity level, reduction in plant height in all rootstocks was statistically similar but significant from their respective controls.

Significant leaf drop and toxicity symptoms including leaf burning in all citrus rootstocks were only observed at maximum salinity levels (150 mM). Both Rangpur Lime and Rough Lemon showing less leaf drop 60 and 53%, respectively, as compared to Trifoliate Orange in which maximum leaf drop was observed at 150 mM (Table 1). No

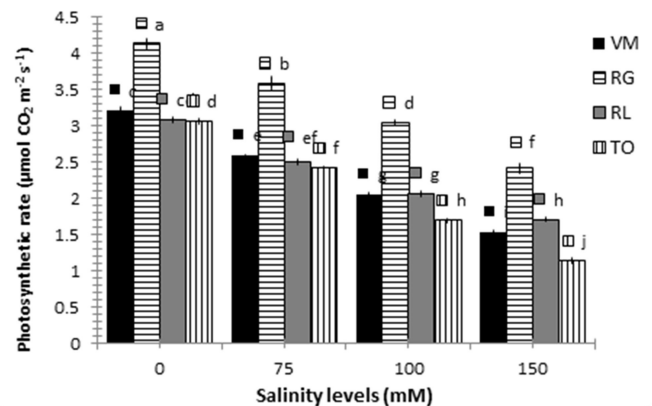
tip and leaf burning in Rangpur Lime seedlings at low (75

mM) level of NaCl were observed whereas the other three rootstock genotypes showed mild symptoms of burning at this salinity level. At 100 mM NaCl, Rangpur Lime and Rough Lemon showed mild burning symptoms as compared to Volkameriana and Trifoliate Orange which showed severe leaf tip burnings. Severe leaf tip and leaf burning symptoms were observed in seedlings of all citrus rootstocks at the highest (150 mM) level of NaCl salinity.

**Physiological parameters:** Significant decrease in leaf water potentials was recorded in all rootstocks with increasing salts in rhizosphere. At low salinity level (75 mM), all rootstocks behaved statistically similar with respect to leaf water potential. At 150 mM salinity level, citrus rootstocks Rangpur Lime showed minimum decrease in leaf water potential (-2.78 MPa) followed by Rough Lemon (-2.92 MPa). Both Rangpur Lime and Rough Lemon were statistically at par at 150 mM salinity level (Fig. 1).



**Figure 1.** Effect of increasing NaCl salinity on leaf water potential of four citrus rootstocks. Vertical bars show  $\pm$  standard deviation.



**Figure 2.** Effect of increasing NaCl salinity on photosynthetic rate of four citrus rootstocks. Vertical bars show  $\pm$  standard deviation.

Gas exchange parameters i.e. photosynthetic rate, transpiration rate and stomatal conductance were reduced in all four rootstocks depending on the specie. Rangpur Lime

all four rootstocks depending on the specie. Rangpur Lime showed superiority in performance regarding photosynthetic rate with  $4.14 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  at control and  $2.42 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  at the highest salt level (150 mM) (Fig. 2). The reduction in photosynthetic rate was more pronounced than transpiration rate. At 150 mM of NaCl, highest transpiration rate was measured in Rangpur Lime ( $5.09 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) followed by Rough Lemon with  $4.93 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  whereas Volkameriana and Trifoliate Orange both had comparatively lower transpiration rates (Fig. 3). Rangpur Lime maintained a fairly better stomatal conductance at all salinity levels and had the highest stomatal conductance ( $204 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) at the higher salinity level (150 mM) (Fig.4).

| Table 1. Effect of increasing NaCl salinity on increase in plant height, leaf area and transpiration rate in four citrus rootstocks. |                      |             |                              |             |   |              |
|--|----------------------|-------------|------------------------------|-------------|---|--------------|
| Rootstocks   | Plant Height (cm)    |             | Leaf Area (cm <sup>2</sup> ) |             | Transpiration rate (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> ) |              |
|  | Salinity levels (mM) |             | Salinity levels (mM)         |             | Salinity levels (mM)  |              |
|  | 0                    | 75          | 100                          | 150         | 0   | 75           |
| Volkameriana   | 1.84±0.07 c d        | 1.60±0.08 d | 1.00±0.11 e                  | 0.58±0.03 f | 0.20±0.02 f   | 15.40±0.67 b |
| Rangpur Lime   | 2.80±0.08 a          | 1.94±0.05 c | 1.04±0.09 e                  | 0.64±0.07 f | 0.00±0.00 f   | 7.00±0.44 d  |
| Rough lemon  | 2.26±0.12 b          | 1.00±0.07 e | 0.64±0.07 f                  | 0.56±0.06 f | 0.60±0.40 f   | 9.40±0.51 c  |
| Trifoliate Orange  | 1.90±0.04 c          | 1.18±0.17 e | 0.66±0.05 f                  | 0.40±0.05 f | 1.00±0.44 f   | 17.60±0.51 a |

| Table 2. Effect of increasing NaCl salinity on leaf ion status (Mean±SD) in four citrus rootstocks. |                                   |             |                                    |             |                      |              |
|---|-----------------------------------|-------------|------------------------------------|-------------|----------------------|--------------|
| Rootstocks  | Leaf Sodium (g kg <sup>-1</sup> ) |             | Leaf Calcium (g kg <sup>-1</sup> ) |             | Salinity levels (mM) |              |
|   | Salinity levels (mM)              |             | Salinity levels (mM)               |             | Salinity levels (mM) |              |
|   | 0                                 | 75          | 100                                | 150         | 0                    | 75           |
| Volkameriana  | 1.84±0.07 c d                     | 1.60±0.08 d | 1.00±0.11 e                        | 0.58±0.03 f | 0.20±0.02 f          | 15.40±0.67 b |
| Rangpur Lime  | 2.80±0.08 a                       | 1.94±0.05 c | 1.04±0.09 e                        | 0.64±0.07 f | 0.00±0.00 f          | 7.00±0.44 d  |
| Rough lemon   | 2.26±0.12 b                       | 1.00±0.07 e | 0.64±0.07 f                        | 0.56±0.06 f | 0.60±0.40 f          | 9.40±0.51 c  |
| Trifoliate Orange   | 1.90±0.04 c                       | 1.18±0.17 e | 0.66±0.05 f                        | 0.40±0.05 f | 1.00±0.44 f          | 17.60±0.51 a |

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|---|-----------------------------------|--------------|-------------|------------------------------------|-------------|-------------|--|-------------|-------------|----------------------------|
| Rootstocks  | Leaf Sodium (g kg <sup>-1</sup> ) |              |             | Leaf Calcium (g kg <sup>-1</sup> ) |             |             | Stomatal conductance (mmol m <sup>-2</sup> s <sup>-1</sup> ) |             |             | Chlorophyll content (SPAD) |
|   | 0                                 | 75           | 150         | 0                                  | 75          | 150         | 0  | 75          | 150         |                            |
| Volkameriana  | 0.04±0.04 i                       | 1.80±0.03 f  | 4.50±0.10 d | 9.20±0.70 c                        | 31.7±0.20 f | 27.2±0.30 i | 21.5±0.20 k  | 17.8±0.00 m | 18.1±0.1 g  | 6.30±0.30 h                |
| Rangpur Lime  | 0.02±0.02 i                       | 1.10±0.10 gh | 4.30±0.10 d | 9.90±0.30 b                        | 35.6±0.50 e | 30.2±0.30 g | 23.0±0.30 j  | 18.4±0.0 m  | 19.9±0.10 a | 35.0±0.10 b                |
| Rough lemon   | 0.10±0.05 i                       | 1.60±0.10 fg | 4.00±0.10 d | 16.0±0.20 a                        | 46.9±0.50 a | 42.8±0.20 b | 38.3±0.20 c  | 28.4±0.0 h  | 17.4±0.10 b | 7.50±0.60 h                |
| Trifoliate Orange   | 0.10±0.06 i                       | 2.80±0.05 h  | 8.60±0.20 e | 10.2±0.10 b                        | 36.9±0.10 d | 28.9±0.20 h | 23.0±0.20 j  | 23.0±0.20 j | 18.4±0.10 b | 7.40±0.00 h                |

| Table 3. Effect of increasing NaCl salinity on sap chloride and leaf chloride build up (Mean±SD) in four citrus rootstocks. |                                    |             |             |             |                                     |             |             |             |  |
|---|------------------------------------|-------------|-------------|-------------|-------------------------------------|-------------|-------------|-------------|--|
| Rootstocks  | Sap Chloride (g kg <sup>-1</sup> ) |             |             |             | Leaf Chloride (g kg <sup>-1</sup> ) |             |             |             | Stomatal conductance (mmol m <sup>-2</sup> s <sup>-1</sup> ) |
|   | Salinity levels (mM)               |             |             |             | Salinity levels (mM)                |             |             |             |  |
|   | 0                                  | 75          | 100         | 150         | 0                                   | 75          | 100         | 150         |  |
| Volkameriana  | 0.20±0.30 g                        | 0.60±0.01 f | 1.00±0.03 e | 2.40±0.15 b | 0.60±0.11 i                         | 0.60±0.11 i | 0.60±0.11 i | 0.60±0.11 i | 5.60±0.15 b  |
| Rangpur Lime  | 0.20±0.02 g                        | 0.50±0.02 f | 0.90±0.02 e | 1.70±0.03 d | 0.70±0.00 i                         | 0.70±0.00 i | 0.70±0.00 i | 0.70±0.00 i | 4.80±0.07 c  |
| Rough lemon   | 0.30±0.20 g                        | 0.50±0.03 f | 1.10±0.11 e | 2.00±0.06 c | 0.70±0.11 i                         | 0.70±0.11 i | 0.70±0.11 i | 0.70±0.11 i | 5.70±0.10 b  |
| Trifoliate Orange   | 0.20±0.01 g                        | 1.10±0.10 e | 2.60±0.10 e | 3.40±0.07 a | 0.60±0.07 i                         | 0.60±0.07 i | 0.60±0.07 i | 0.60±0.07 i | 6.30±0.18 a  |

Figure 3. Effect of increasing NaCl salinity on transpiration rate of four citrus rootstocks. Vertical bars show ± standard deviation.

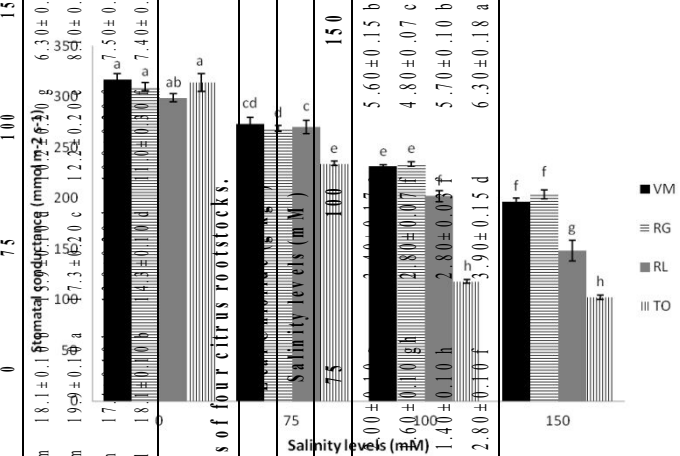
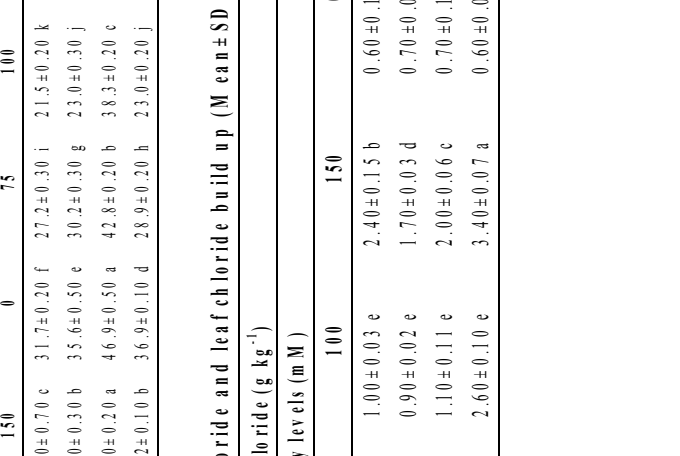


Figure 4. Effect of increasing NaCl salinity on stomatal conductance of four citrus rootstocks. Vertical bars show ± standard deviation.



**Ionic composition:** The changes in ionic compositions in leaves of all treated citrus rootstocks were observed but the minimum effect was found in Rangpur Lime (Table 2). Sodium concentration at control was statistically similar in all rootstocks leaves but increased with the addition of salt in the root zone. Volkameriana absorbed least amount of sodium ( $9.2 \text{ g kg}^{-1}$ ) at the highest salt level while Rangpur Lime and Trifoliate Orange remained statistically same with  $9.9$  and  $10.2 \text{ g kg}^{-1} \text{ Na}^+$  in leaves, respectively.

Salinity caused a decrease in  $\text{Ca}^{++}$  and  $\text{K}^+$  concentration in all rootstock genotypes. A consistent decrease in  $\text{Ca}^{++}$  was found in all rootstock genotypes as salinity level increased. However, at  $100 \text{ mM}$  of  $\text{NaCl}$ , minimum decrease in was observed in Rough Lemon (18%) as compared to other three root stocks. In control, the highest amount of potassium was accumulated in Rangpur lime ( $19.9 \text{ g kg}^{-1}$ ) followed by Volkameriana and Trifoliate orange with  $18.1 \text{ g kg}^{-1}$ . All these genotypes were behaved statistically alike for potassium uptake. At  $75 \text{ mM}$  of  $\text{NaCl}$ , maximum decrease in  $\text{K}^+$  concentration was recorded in Volkameriana and Rough Lemon with 23 and 20% respectively. Potassium concentration decreased due to salt in all rootstocks studied but Rangpur Lime showed less decrease up to  $100 \text{ mM}$   $\text{NaCl}$ . At the highest salt level, it remained the same as Volkameriana and Rough Lemon (Table 2).

Leaf sap showed increased chloride concentration as the  $\text{NaCl}$  concentrations increased in medium in all plants. Analysis of dried leaves also showed increased accumulation of  $\text{Cl}^-$  in dry matter as the salt concentration increased. These changes occurred in all four rootstock genotypes but Rangpur Lime showed less  $\text{Cl}^-$  in leaf sap ( $1.7 \text{ g kg}^{-1}$ ) and also in leaf dry matter ( $4.8 \text{ g kg}^{-1}$ ) even at the highest level of salt provision (Table 3). The increase in  $\text{Cl}^-$  in leaf sap and dry matter was highest at  $150 \text{ mM}$   $\text{NaCl}$  in Trifoliate Orange being  $3.4$  and  $6.3 \text{ g kg}^{-1}$ , respectively.

## DISCUSSION

Height is an important variable of growth and development in plants. As the salt level provided to the citrus seedlings increased, their growth rate measured as increase in plant height, decreased. This is because salinity affects almost all physiological functions of a plant such as gas exchange affecting stomatal conductance, photosynthetic rate and transpiration rate contributing towards its development. Maas (1992) and Camara-Zapata *et al.* (2004) also observed decrease in growth rate in citrus rootstocks due to high osmolality in the external medium. Further high salts in the root zone compelled the plants to use more energy to extract water from the soil solution rendering a drought like effect which ultimately affected plant growth and development. Another change in the morphology of plants due to salinity was observed as the leaf and tip burning symptoms. These are the very first symptoms that appear on a plant under salt

stress. Rangpur Lime and Rough Lemon developed these symptoms later than the other two rootstocks and also showed less leaf drop as less leaves were damaged due to salinity. The results of this study are in conformity with the findings of Banuls and Primo-Millo (1995) and Lopez-CLiment *et al.* (2008) who reported leaf injury as the first step of salt toxicity in citrus followed by leaf abscission. Less leaf damage in Rangpur Lime is probably due to fewer uptakes of toxic  $\text{Cl}^-$  ions.

The results regarding different physiological traits revealed that as the salt level increases, the plant lowers its water potential being in stress. Similarly significant variation was recorded in leaf water potential at different concentrations of salt and it was observed that leaf water potential is almost equal to the water potential of the whole plant. The decrease was less in Rangpur Lime indicating its resistance towards saline damage. This decrease in water potential due to salt stress was also observed by Al-Yassin (2004) and has been previously correlated with stomatal closure leading to various physiological disturbances (Gomes *et al.*, 2004). Whenever water retention is required by a plant under stress, it closes its stomata resulting in less transpiration. These changes lead to lowered photosynthetic rate which is the main energy providing source of the plant. This phenomenon occurred in all rootstocks but Rangpur Lime maintained these vital functions fairly. The results were found to be in agreement with Banuls and Primo-Millo (1995) and Perez-Perez *et al.* (2007) who also reported reduction in gas exchange parameters due to salt stress.

It has been previously reported by Camara-Zapata *et al.* (2004) and Anjum (2008) that tolerant rootstocks have a tendency to exclude  $\text{Na}^+$  and that sodium leaf concentration increases with the increase of  $\text{NaCl}$  in the external medium. A greater selectivity for potassium ion than sodium ion is also associated with salt tolerance Storey and Walker (1987). Potassium accumulation decreased with increasing salt but this was less in Rangpur Lime as compared to other rootstocks indicating its ability to tolerate  $\text{NaCl}$  salinity i.e. despite of the antagonism of the  $\text{Na}^+$  ions with  $\text{K}^+$  ions, this rootstocks was able to absorb more potassium from the soil as compared to other rootstocks. The same behavior was observed in case of calcium but the rootstocks which managed to absorb significantly high  $\text{Ca}^{++}$  from the medium as compared to other rootstocks was Rough Lemon. This decrease in  $\text{Ca}^{++}$  and  $\text{K}^+$  may be due to the antagonistic behavior of different ions ( $\text{Na}^+/\text{Ca}^{++}$  and  $\text{Na}^+/\text{K}^+$ ) present in the nutrient medium caused by high  $\text{Na}^+$  presence. The reduction in uptake of potassium due to high salts in the root zone was also observed by Garcia-Lidon *et al.* (1998) whereas reduced accumulation of foliar calcium in citrus rootstocks and more in the salt sensitive ones has been reported by Camara-Zapata *et al.* (2004).

High concentrations of  $\text{Cl}^-$  in the growth medium was also taken up by the plants and resulted in its accumulation which

has toxic effects and is reported to be the major cause of various dysfunctions including reduction in growth, injuries and disturbing of gas exchange parameters (Gomez-Cadenas *et al.*, 1998). All the rootstocks accumulated  $\text{Cl}^-$  in leaves with lowest in Rangpur Lime and highest in Trifoliate Orange. Same was the case for leaf sap chloride which is an indicator of how much  $\text{Cl}^-$  the roots are actually absorbing from the rhizosphere. Less the chloride is absorbed from the soil, less would be the damage to plant tissues and various vital plant processes. Rangpur Lime, which has been previously reported as a  $\text{Cl}^-$  excluder (Storey, 1995) showed least chloride in leaf sap followed by Rough Lemon, Volkameriana and Trifoliate Orange in increasing order of  $\text{Cl}^-$  absorption. This was also reported in other citrus rootstocks by Raveh and Levy (2005) and Lopez-CLiment *et al.* (2008).  $\text{Cl}^-$  accumulation in citrus rootstocks has also been considered as a parameter in ranking salt tolerance of citrus rootstocks (Zekri, 1993).

**Conclusion:** Rangpur Lime was found to be a better performer even at high salt concentration and coped better with the salt stress as compared to the other rootstocks species from the beginning i.e. low sodium accumulation and high potassium uptake and less effect on leaf water potential as it was able to extract more water from the saline sand solution which in turn lead to less stomatal closure, better photosynthesis and transpiration maintenance. These properties indicate its better survival to NaCl salinity and make it an important candidate to be considered for the production of citrus in salt affected soils of Pakistan.

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