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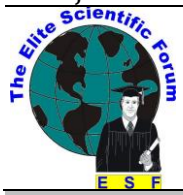

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RESEARCH ARTICLE

Salinity Induced Deleterious Effects on Biochemical and Physiological Processes of Tomato

Abdul Manan[1,*], Choudhary Muhammad Ayyub[2], Rashid Ahmad[3], Muhammad Adnan Bukhari[4] and Zaid Mustafa[5]
[1]Department of Horticulture, Ghazi University, Dera Ghazi Khan, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan
[2]Departmentof Agronomy, University of Agriculture, Faisalabad, Pakistan
[4]Department of Agronomy, UCAES, Islamia University of Bahawalpur, Bahawalpur, Pakistan
[5]Department of Horticulture, University of Sargodha, Sargodha, Pakistan

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***Corresponding Author:**
mananbukhari@gmail.com

ABSTRACT

This experiment was carried out to investigate the effect of salt stress (i.e. 0, 25, 50, 75, 100 and 125 mM NaCl) on two tomato genotypes Rio grande (tolerant) and Savera (sensitive). Salinity stress was applied on tomato plants after transplanting in sand culture in plastic pots. The data was collected on water relation, gaseous exchange, biochemical and ionic contents. Salinity predominantly decreased the water relation (water potential and osmotic potential), chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll) and gaseous exchange parameters (net photosynthetic rate, transpiration rate and stomatal conductance). On the other hand, biochemical attributes (total soluble protein, total free amino acids and proline contents) and antioxidant enzymes (catalase and peroxidase) were increased in both the genotypes. Sodium contents were increased in roots and leaves of both the varieties; whereas, potassium contents were decreased as salinity was applied. Results depicted that genotype Rio grande showed excellent performance under stress conditions while Savera showed poor performance under salinity stress.

INTRODUCTION

Agricultural production around the world is based on adequate supply of water, due to this farming is all the time subjected to various threats like drought and salt stress (Moshelion et al., 2015; Moghimi and Sepaskhah, 2015). About 50% losses to annual yield worldwide are mainly due to abiotic stresses (Rodziewicz et al., 2014). Salinity is a worldwide problem and threat to agriculture since decades. Increasing salt stress restricts plant growth and yield around the world and Pakistan (Ali et al., 2014, Mittelstet et al., 2015).

Root-zone salinity is built up by the presence of soluble salts (Levy and Syvertsen, 2004) in soil and irrigation water. Salt stress brings about osmotic stress and subsequently ionic toxicity and oxidative stress. Salt stress limits water available to plants hence causes osmotic stress. Osmotic stress leads to loss in turgor pressure of the plant especially in the leaves due to decreased water potential (Osakabe et al., 2014). Loss in turgor creates wilting that affects the plant morphology and biomass (Xu et al., 2010). At cellular level salinity brings about ionic toxicity by elevated Na^+ and Cl^- levels. Increased concentration of sodium affects the entry of K^+ ions (Flowers et al., 2015). In addition to this stoma are closed to safeguard water loss by decreased transpiration (*E*) (Orsini et al., 2012). It also limits the CO_2 intake consequently limits the net photosynthetic rate (*A*) (Carmo-Silva et al., 2012, Manan et al., 2016). As a consequence of salinity stress reactive oxygen species (ROS) are produced inducing oxidative stress in the crop plants (Choudhury et al., 2016). Plant produces antioxidants and osmoprotectants to bring about tolerance against oxidative stress and osmotic stress respectively (Garrido et al., 2014; Munns and Tester, 2008).

Tomato (*Solanum lycopersicum* L) belongs to family Solanaceae, is a very popular vegetable around the world. Tomato is consumed either fresh as salad or cooked or processed in the form of paste or tomato ketchup (Hanson and Yang 2016). Tomato contains essential nutrients like vitamin C and lycopene that

make it nutritionally very important as they help body fighting against gastro vascular diseases and even cancer (Ilahy et al., 2016, Smith et al., 2016). Tomato is moderately tolerant to saline environment. Salt stress also down regulates the physiological and biochemical processes going on in tomato (Manan et al., 2016, Al-Harbi et al., 2015, Rivero et al., 2014). Hence this research project was conducted to investigate the effects of salt stress on biochemical and physiological processes on tomato plants.

MATERIALS AND METHODS

This experiment was planned at the greenhouse of Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan to study the effects of salt stress (NaCl) on tomato, especially on physiological and biochemical processes. Seeds of 2 tomato genotypes one tolerant (Rio grande) and one sensitive (Savera) to salt stress selected from our previous study (Manan, 2016) were taken and surface sterilized with sodium hypochlorite 10% solution. The seeds were sown in plastic pots having sand as growth medium. Hoagland half strength solution was given to fulfill the nutritional requirements (Hoagland and Arnon 1950). Healthy seedlings were transplanted to other plastic pots of 9 L volume. The pots had the same growth conditions as for nursery. Salinity was induced after ten days of transplantation to the plants to that they may recover after the transplantation shock. Salinity treatments (0, 25, 50, 75, 100 and 125 mM NaCl) were applied in the intervals to safeguard plants from osmotic shock. All the treatments were laid out in CRD (Completely randomized design) two factor factorial. Data was collected after a week of salinity stress application.

Data was collected on the following parameters.

Water relations

Water potential (Ψw) was measured by cutting the leaf with razor and placed the leaf in the gasket of pressure chamber (Model, 615, USA). The readings were taken in the morning times. For osmotic potential (Ψo) same leaf was kept under -20ºC for a week then thawed to normal temperature and sap was extracted by disposable syringes. The sap was then used to check the osmotic potential by osmometer (Wescor Model-5500, USA).

Gaseous exchange

Net photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (gs) were measured by placing the fully expanded young leaf in the chamber of portable apparatus termed as Infra-Red Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, England). The gaseous exchange parameters readings were taken between 10:00 am to 12:00 pm.

Chlorophyll contents

The chlorophyll contents (chlorophyll a and chlorophyll b) were measured by the method of Arnon (1949) and Davies (1976). Fresh leaves were cut into small pieces and then 1 gram of these small pieces was put into the small bottles having 80% acetone solution. The extract obtained from the bottles was centrifuged at 14000 x g for 5 minutes and absorbance of the supernatant was taken at 645, 663 and 480 nm, using double beam Spectrophotometer (Hitachi-120, Japan). Chlorophyll a, b and total chlorophyll were calculated using the following formulae.

Chl a = [12.7 (OD 663) -2.69 (OD 645)] × V/1000×W

Chl b = [22.9 (OD 645) -4.68 (OD 663)] × V/1000×W

Total Chl = [20.2 (OD 645) + 8.02 (OD 663)] × V/1000×W

Determination of total free amino acids (TFA) and leaf proline contents

0.5 g fresh leaf sample was taken and TFA and leaf proline contents were determined by the methods described by Hamilton and Van Slyke (1973) and Bates et al. (1973), respectively.

Antioxidants enzymes activity assay

Peroxidase (POX) and catalase (CAT) activities were calculated by the method described by Chance and Maehly (1955) with some changes. The POX reaction solution (3 mL) was comprised of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 mL of enzyme extract. Differences in absorbance of reaction solution at 470 nm were calculated after every 20 seconds. One-unit POX activity was assigned as an absorbance change of 0.01 units per min. The POX activity was measured and expressed as unit $min^{-1}g^{-1}$FW basis. The CAT reaction solution (3 mL) was comprised of 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 mL of enzyme extract. Changes in absorbance of the reaction solution were recorded after every 20s at 240 nm. One-unit CAT activity was specified as an absorbance change of 0.01 units per min.

Determination of ionic (Na^+ and K^+) contents

The ionic contents (Na^+ and K^+) were determined by digesting the plant material with concentrated sulfuric acid (5 mL) in the digestion tubes according to Wolf (1990). The digestion tubes were incubated at room temperature overnight. After this H_2O_2 (35%) @ 0.5mL was added in each tube and heated at 350 ºC until the fumes start coming out. When fumes were seen coming out the tubes were heated more half an hour. Then the tubes were allowed to cool, H_2O_2 (0.5 mL) was dropped while cooling. The digestion mixture was then filtered and the volume was increased to 50 mL in the volumetric flask. The digestion mixture was then used to analyze Na^+ and K^+ by Flame photometer (Jenway PFP-7, UK). A standard curve (SC) was drawn on the basis of graded series of standards (ranging from 10 to

100 mg L^{-1}) of Na^+ and K^+. The values of Na^+ and K^+ from flame photometer were compared with SC and actual ratios were calculated.

Statistical analysis

All the data collected were subjected to statistical analysis using analysis of variance technique by Statistix computer software. LSD test at 5% level of probability was employed to compare the individual means.

RESULTS

Water relations

Salinity decreased water potential in both the genotypes substantially but the decrease was more pronounced in Savera. Highest water potential (0.36 -MPa) was noted under control conditions as shown in table 1. The lowest water potential (0.93 -MPa) was observed under 125 mM NaCl stress. When we talk about genotypes response against salinity, Savera showed poor performance as compared to Rio grande. In case of osmotic potential, the trend was same but the difference between genotypes was not much pronounced.

Gaseous exchange

Net photosynthetic rate (*A*) was decreased markedly as salinity treatments were applied. By the application of 125 mM salinity the net photosynthetic rate was decreased 1.15 fold when compared to control. Savera showed poor performance to net photosynthetic rate as compared to Rio grande. Transpiration rate (*E*) also decreased with the application of salinity genotype Rio grande performed 1.12 fold higher than Savera. Same was the trend in stomatal conductance (*gs*) that was significantly decreased by the application of salinity in which maximum decrease 1.31 fold was observed under 125 mM NaCl application and maximum stomatal conductance was observed under control conditions figure 1.

Chlorophyll contents

Chlorophyll a contents were markedly reduced in both the genotypes under salinity stress however Savera was affected more. Chlorophyll a contents in Savera were reduced 1.10 fold more than Rio grande. Same trend was observed in chlorophyll b as salinity treatments were given the chlorophyll b contents started to decrease continuously as shown in figure 2. Maximum chlorophyll contents were reduced in Savera under 125 mM salinity that was more than 14% than control. While the reduction in Rio grande was 4.6 fold as compared to control.

Enzymatic antioxidants

Figure 3 clearly illustrates that both catalase (CAT) and peroxidase (POX) activity significantly increased with the application of NaCl stress on both the genotypes. However, with the application of 125 mM salinity CAT production was 1.08 fold more pronounced in Rio grande than Savera. Same were the results given by POX by the application of salt stress as overall peroxidase activity was noticed 1.11 fold higher in Rio grande than Savera.

Table 1: Effect of various NaCl stress levels on water potential (Ψw) andosmotic potential (Ψo) of two tomato genotypescvs. Rio grande and Savera

NaCl (mM)	Ψw (-MPa)	Ψo (-MPa)
Rio grande		
0	0.39 ± 0.02 g	0.96 ± 0.02 i
25	0.46 ± 0.02 f	1.27 ± 0.03 g
50	0.56 ± 0.01 e	1.43 ± 0.02 e
75	0.66 ± 0.02 d	1.48 ± 0.01 e
100	0.74 ± 0.02 c	1.65 ± 0.02 cd
125	0.77 ± 0.01 c	1.73 ± 0.01ab
Savera		
0	0.36 ± 0.03 g	1.08 ± 0.04 h
25	0.46 ± 0.01 f	1.35 ± 0.01 f
50	0.58 ± 0.01 e	1.48 ± 0.01 e
75	0.74 ± 0.02 c	1.60 ± 0.01 d
100	0.83 ± 0.01 b	1.67 ± 0.02bc
125	0.93 ± 0.01 a	1.79 ± 0.03 a

Values are mean of three replicates and fluctuation among the replicates is given by ± SE. Different alphabets indicate significant differences at P≤0.05.

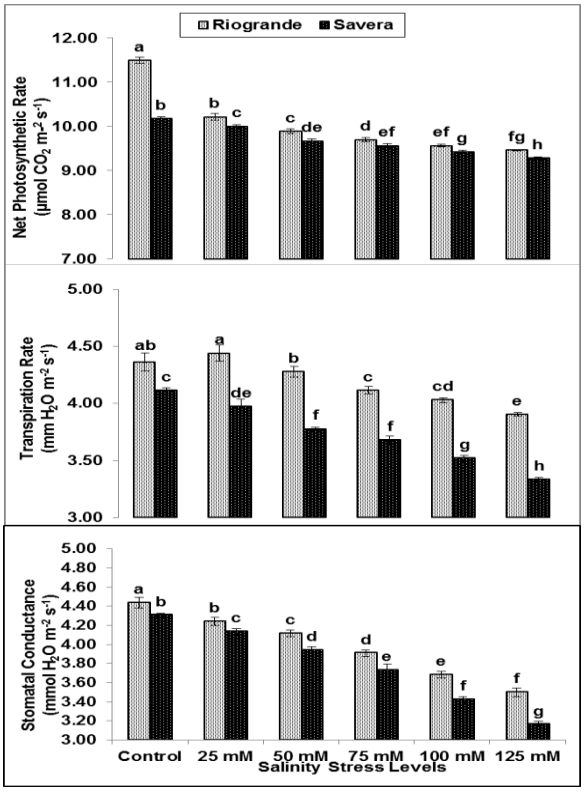

Fig. 1: Effect of various NaCl stress levels on gaseous exchange characteristics of tomato genotypes. Values are mean of three replicates, vertical bars are ± SE. Different alphabets indicate significant differences at P≤0.05.

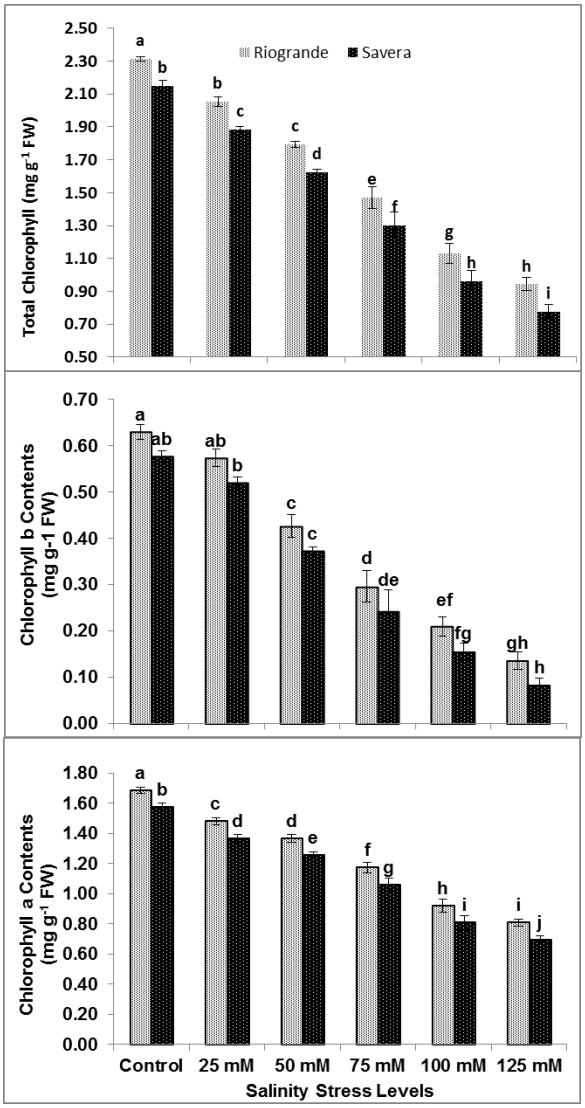

Fig. 2: Effect of various NaCl stress levels on chlorophyll contents of tomato genotypes. Values are mean of three replicates, vertical bars are ± SE. Different alphabets indicate significant differences at P≤0.05.

Total free amino acids (TFA) and leaf proline contents

Total free amino acids were progressively increased as salinity stress was given in both genotypes. However, increase TFA increase was 1.23 fold greater in Rio grande than Savera. Maximum TFA were recorded under 125 mM NaCl stress while minimum were observed under control as shown in figure 4. Leaf proline contents were also higher under saline conditions than control. Maximum proline contents 2.38 mmolProline g^{-1} FW were measured under 125 mM salinity in Rio grande genotype, minimum 1.32 mmolProline g^{-1} FWproline contents were recorded under control conditions in Savera genotype as depicted in figure 5.

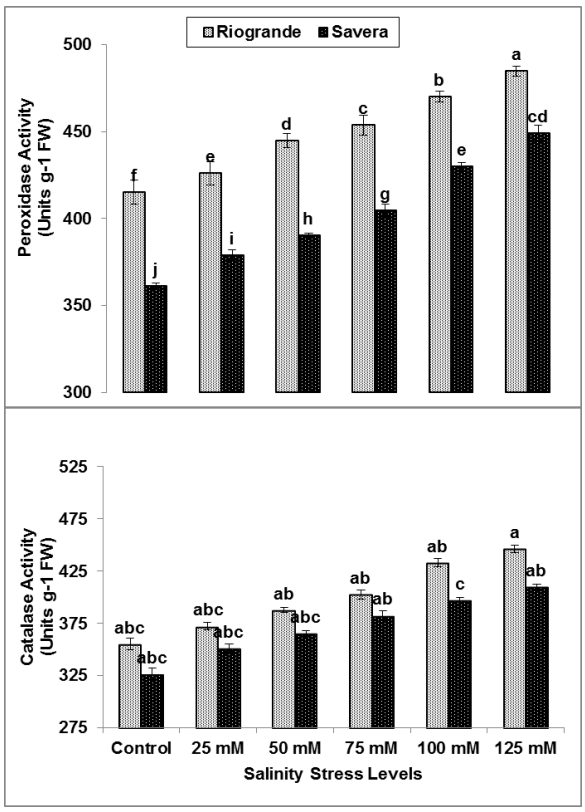

Fig. 3: Effect of various NaCl stress levels on antioxidant enzymes activity of tomato genotypes. Values are mean of three replicates, vertical bars are ± SE. Different alphabets indicate significant differences at P≤0.05.

Ionic (Na^+ and K^+) contents

Sodium contents in roots and leaves were augmented gradually by increasing salinity levels, however the tolerant genotype Rio Grande accumulated maximum (2.58 mg g^{-1} DW) Na in its roots but it was maximum (2.68 mg g-1 DW) in leaves of sensitive genotype Savera. Maximum reduction of potassium contents in leaves was noted in Savera at 125 mM salinity level, on the other hand in roots the maximum reduction was observed by Rio Grande. Maximum potassium contents were found in the leaves of Rio Grande and minimum potassium contents were found in the leaves of Savera at 125 mM salt stress level as shown in figure 6.

DISCUSSION

Plant water status is highly sensitive to drought and salinity stresses (Zhu, 2001). The analysis of tomato leaf water potential and osmotic potential in our studies under salt stress depicted a decrease in leaf water potential, which is also observed in other crop plants like in cucumber (Stępień and Kłbus, 2006), rice (Kang et al., 2005), peas (Shahid et al., 2012) and in maize

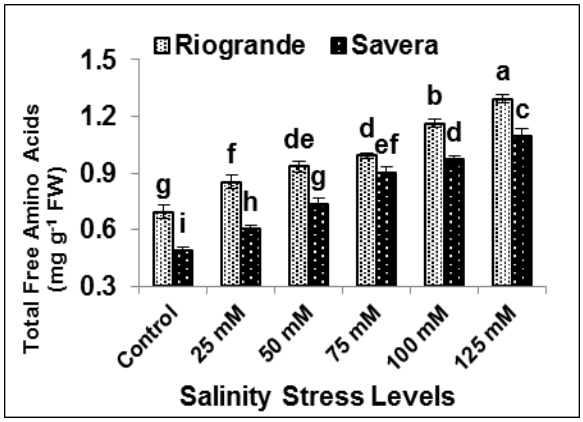

Fig. 4: Effect of various NaCl stress levels on total free amino acids of tomato genotypes. Values are mean of three replicates, vertical bars are ± SE. Different alphabets indicate significant differences at P≤0.05.

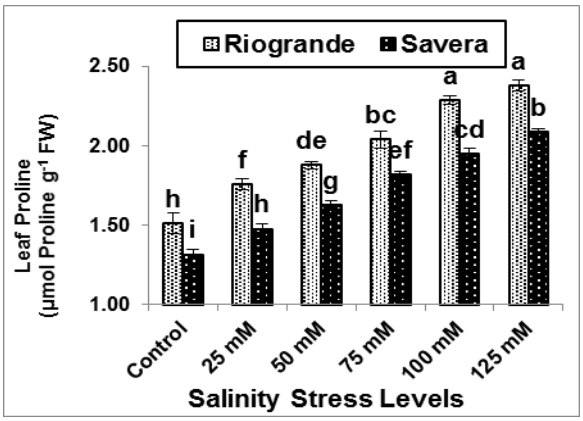

Fig. 5: Effect of various NaCl stress levels on leaf proline contents of tomato genotypes.Values are mean of three replicates, vertical bars are ± SE. Different alphabets indicate significant differences at P≤0.05.

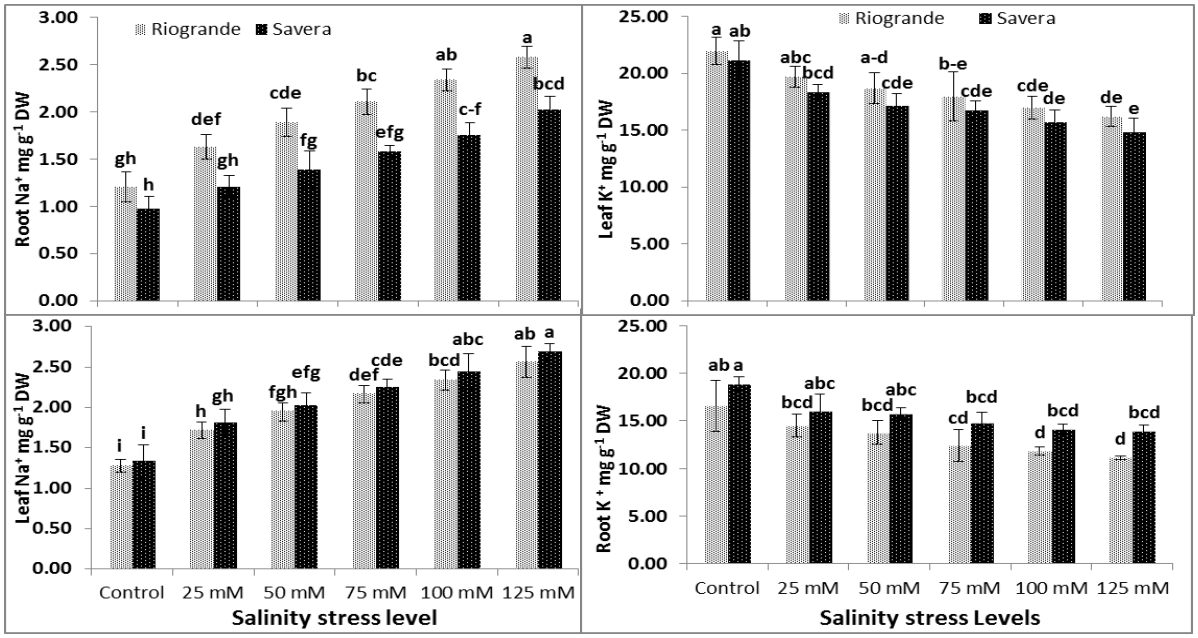

Fig. 6: Effect of various NaCl stress levels on leaf and root ionic ($Na^+$and K^+) contents of tomato genotypes. Values are mean of three replicates, vertical bars are ± SE. Different alphabets indicate significant differences at P≤0.05.

(Giaveno et al., 2007). Decrease in water potential is due to salts accumulated in the outer environment that causes osmotic stress (Munns, 1993). In our findings response of water potential was different at different salinity levels from control to 125 mM NaCl level. The severity of 100 to 125 mM salinity decreased maximum water potential. Reduced water contents lead to the stomatal closure to safeguard further loss of water by transpiration (Manan et al., 2016, Everard et al., 1994, Perera et al., 1994). In addition to reduced transpiration and stomatal closure, net photosynthesis also reduced under salt stress by the production of ROS, not proper functioning and decrease in chlorophyll contents and rubisco (Lutts et al., 1996, Zhang et al., 2009, Zribi et al., 2009). Net photosynthetic rate (*A*) decreased significantly in both the cultivars in our studies. Various researchers reported the reduction of net photosynthetic rate under salinity stress (García-Mata and Lamattina, 2002, Jia et al., 2002, Munns and Tester, 2008). The ROS production under salinity stress is obvious (Hasanuzzaman et al., 2011). The ROS being a cause of oxidative stress also needed for signaling for the production of enzymatic antioxidants like catalase and peroxidase and other enzymes (Mittler, 2002, Apel and Hirt, 2004, Miller et al., 2010). So it can be solid reason of increase of CAT and POX under saline stress

in our results. In addition to enzymatic antioxidants the TFA, proline and TSP also increased under salt stress. Free amino acids are likely to be produced under stress conditions (Pérez-Alfocea et al., 1993, Hsu et al., 2003, Shahid et al., 2012). Salinity causes accumulation of sodium ions in tissue that imbalances the nutritional status of plant, thus disturbs the potassium status. It is well documented that sodium concentration increases in plants under salt stress and suppresses the potassium concentration (Dasgan et al., 2002, Akram et al., 2010). Potassium ion is very important and necessarily required for different physiological mechanisms but under salt stress Na^+ ions replace K^+ ions, consequently causing the nutritional imbalance. In present study, both tested tomato genotypes revealed an increase in the leaf Na^+ while decreased leaf K^+ contents. However, genotype Rio Grande exhibited the minimum concentration of Na^+ and increased K^+ in leaves. On the other hand, Savera showed the elevated leaf Na^+ and minimum leaf K^+ contents. This difference in sodium and potassium in both the genotypes may be due to their genetic potential or root permeability to these ions. The salt tolerant genotypes transport very small amount of toxic ions (Na^+) to the upper areas like leaf, they store them in their roots, that's why the phenomenon of photosynthesis proceeds normally in tolerant genotypes. This is an adaptation of tolerant plant species to withstand the adverse conditions that sensitive species substantially lack. Similar observations were found by (Maggio et al., 2007) in tomato. It is concluded that lower concentration of sodium in leaves and higher concentration of sodium in roots while lower concentration of potassium in roots and higher concentration of potassium in leaves can be essential for an adaptive mechanism for tomato plants under salt stress. Present results are in agreement with Akram et al. (2010).

Conclusion

NaCl stress substantially affected the growth, physiological and biochemical attributes of tomato in both cultivars. But the comparison within varieties depicted that Rio Grande remained tolerant as compared to Savera. So it is recommended that Rio Grande should be grown in salt affected areas.

Authors' contributions

All authors contributed equally in designing the research, conducting the experiments and writing this manuscript.

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