*Pak. J. Bot.*, 43(6): 2707-2710, 2011.

# ENHANCED PROLINE SYNTHESIS MAY DETERMINE RESISTANCE TO SALT STRESS IN TOMATO CULTIVARS

SYED GHIAS ALI[1], ABDUR RAB[2], NAQIB ULLAH KHAN[2] AND KHALID NAWAB[2]

[1]*Center of Plant Diversity, University of Peshawar, Pakistan*
[2]*Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan*
*Corresponding author: abdurrabaup@gmail.com*

**Abstract**

The physiological and biochemical responses of tomato cultivars were studied at Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan during 2005-2006 for salt tolerance. Tomato cultivars were Roma Rio Super, Roma V.F., Chinese 87-5, Rio Grand and Super Blocky and subjected to salt stress (75 mM NaCl). Fresh weight, dry weight, and ions sodium and potassium accumulation, $Na^+/K^+$ ratio and proline content were determined after imposing the tomato cultivars to NaCl salt for 80 days. Salt stress significantly decreased the fresh and dry weight in Roma Rio Super, Roma V.F, Chinese 87-5 and Rio Grand, however, in Super Blocky the fresh and dry weight were enhanced under stress conditions. Salinity stress increased sodium uptake from 191.828 to 436.170 $\mu mg^{-1}$ D wt while potassium accumulation decreased from 1033.12 to 926.80 $\mu mg^{-1}$ D wt resulting in higher $Na^+/K^+$ ratio in stressed (0.48 g) as compared to unstressed control (0.19). The mean proline contents also increased from 28.95 to 40.96 µM Proline $g^{-1}$ F. wt with the maximum increase (57.378%) in Super Blocky followed by Rio Grand (49.325%).

## Introduction

Tomato (*Lycopersicon esculentum* L.) is the most widely grown commercially important vegetable through out the world. In Pakistan, tomato is grown on 31 thousand hectares with a total production of 306 thousand tons (Anon., 2008). Tomato is good source of minerals and vitamins (Colla *et al*., 2002). Seedlings of nursery of tomato plants are susceptible to various abiotic stresses including salinity (Alam *et al*., 1989; Amini and Ehsanpour, 2005; Chookhampaeng *et al*., 2007), which affects the plant growth and productivity. Salinity is a serious problem in Pakistan as some 6.3 million hectares (29%) of cultivated land is affected by salinity (Ijaz and Davidson, 1997). High salt concentration in the soil has been found to disrupt several physiological processes leading to reduction in growth, small fruit size and low yield (Munns, 2002; Shereen *et al.,* 2005; Alam *et al.,* 1989; Watad *et al*., 2003; Flowers, 2004). The survival of tomato plants in saline soils depends on their ability to synthesize organic compounds e.g. proline and sugars (Fariba and Ehsanpour, 2005; Flowers, 2004) and accumulation of $Na^+$ and $K^+$ (Borsani *et al.,* 2003) to decrease salt stress damage. Tomato cultivars may differ in their sensitivity to salinity stress (Alian *et al.,* 2000; Chookhampaeng *et al*., 2007), thus the selection of salt tolerant cultivars may help to improve the performance of tomato plants in saline conditions.

## Materials and Methods

Experiment on physiological and biochemical responses of tomato cultivars to NaCl stress was carried out at Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan during 2005-2006. Five tomato cultivars namely Roma Rio Super, Roma V.F., Chinese 87-5, Rio Grand and Super Blocky were screened for their salinity tolerance. Seedlings were raised in sand culture in trays and transplanted to earthen pots after one week of germination. All the plants were grown in the same media (well fertile garden soil) but irrigated with normal water and with salt (75 mM NaCl). The controls treatment was irrigated with tap water, while the salt stressed plants were irrigated with the tap water having NaCl solution. The salt stress treatment commenced after one week of transplantation and continued for 80 days. Irrigation with or without salt was applied with four days interval. The experiment was laid out in randomized complete block (RCB) design with two factors, having five cultivars and stress treatments {unstressed control and stressed (75 mM NaCl)} replicated five times. Shoots from each treatment was used for fresh and dry weight, $Na^+$, $K^+$ ions and Proline determination. The plants were harvested after 80 days and separated into roots and shoots. Fresh weights of shoots were determined and then were dried at 80°C for 48 hours for dry weight determination.

Sodium and Potassium accumulation in dried shoots was determined using flame photometer (Watad *et al.*, 2003). The sodium and potassium ratio ($Na^+/K^+$) was calculated as follows:

$$\text{Ratio} = \frac{\text{Sodium content}}{\text{Potassium content}}$$

Proline contents in shoot tissues were determined according to Bates *et al*., (1973) using spectrophotometer by recording absorbance at 520 nanometer against toluene bank.

## Results and Discussion

**Fresh weight:** The tomato cultivar Chinese 87-5 excelled in fresh weight (59.734 g) followed by Rio Grand and Roma V.F, whereas Super Block VFN had revealed significantly lower fresh weight (41.063 g) (Table 1). Salinity stressed decreased the fresh weight from 55.43 to 45.99 g. While the fresh weight in four cultivars decreased due to salinity, with maximum decrease in Roma V.F. (32.3%) followed by Roma Rio Supper (22.4%), however, Super Blocky acquired higher (15.74%) fresh weight under salt stress condition. Salinity decreased the plant growth by osmotic effects which disrupt nutrients and water uptake due inhibition power. Excessive absorption of toxic ion ($Na^+$) may result in $Na^+$ toxicity resulting in enhanced senescence and decreased photosynthesis (Munns, 2002; Chookhampaeng *et al*., 2007). Thus, it is likely to observe the decreased fresh weight under salinity stress. The relatively higher fresh weight in Super Blocky indicates its tolerance to saline conditions.

**Table 1. Effect of salt stress on the fresh and dry weight of plant.**

| Cultivars | Fresh weight per plant (g) | | | Dry weight per plant (g) | | |
|---|---|---|---|---|---|---|
| | Control | NaCl stressed | Mean | Control | NaCl stressed | Means |
| Roma Rio Super | 46.62 | 34.64 | 40.63b | 5.70 | 5.13 | 5.42b |
| Roma V.F | 65.22 | 44.17 | 54.70a | 10.32 | 8.06 | 9.19a |
| Chinese 87-5 | 63.96 | 55.51 | 59.74a | 8.36 | 7.67 | 8.02a |
| Rio Grand | 63.81 | 51.06 | 57.43a | 8.60 | 7.87 | 8.23a |
| Super Blocky | 37.55 | 44.57 | 41.0 b | 5.45 | 5.63 | 5.54b |
| Means | 55.43a | 45.99b | | 7.69a | 6.687b | |

LSD Fresh wt. cultivars α 0.05=11.44, Prob. fresh wt. stress=0.015, Dry wt. cultivars α 0.05=1.72, Prob. dry wt. stress = 0.0043

**Dry weight:** The changes in dry weight were of comparable pattern to fresh weight (Fig. 1). There was significant decrease in dry weight of shoot due to salinity in all the tomato cultivars except Super Blocky. The percent decrease in dry weight relative to control was maximum in Roma V.F. (43.8%) followed by Roma Rio Supper (20.2%), while there was an increase of 6.228% in the dry weight of Super Blocky under salt stress conditions (Table 1 & Fig. 1). Since Blocky exhibited enhanced fresh weight and dry weight under saline conditions, it can be regarded as salt tolerant cultivar.

Salinity decreases leaf water potential, chlorophyll and photosynthesis (Elfeky *et al*., 2007) by decreasing stomatal conductance (Delfine *et al*., 2000), leaf area ratio and relative growth rate (Alarcon *et al*., 1994) that may contribute to decrease dry weight and fruit yield in tomato (Mahajan and Tuteja, 2005). Tomato cultivars showed significant variation in their response to salinity stress. Cultivars Roma Rio Super, Roma V.F, Chinese 87-5 and Rio Grand showed decrease in both fresh and dry weight due to salinity, however, both parameters were observed with increased mean values in Super Roma V.F.. Based on the severity of salt effect as measured by decrease in fresh and dry weight, Roma Rio Super, Roma V.F., Chinese 87-5 and Rio Grand seems salt susceptible cultivars while Super Blocky appears tolerant to applied salinity.

**Sodium accumulation:** Tomato cultivars showed significant variation in their sodium uptake (Table 2). NaCl stress increases $Na^+$ contents in dry tissues from 191.828 to 436.17 45 µM $Na^+$ $g^{-1}$ dry weight Among cultivars, significantly higher sodium absorption was recorded in cultivar Rio Grand (365.645 µM $Na^+$ $g^{-1}$ dry weight) followed by Super Blocky, Roma V.F. and Chinese 87-5 with 334.180, 305.970 and 290.780 µM $Na^+$ $g^{-1}$ dry weight, respectively with non-significant variation in these three cultivars. While the minimum was observed in Roma Rio Super (273.420 µM $Na^+$ $g^{-1}$ dry weight).

**Table 2. Effect of salt stress on sodium and potassium contents (µM $g^{-1}$ dry weight) on tomato cultivars under stress and control conditions.**

| Cultivars | Sodium (µM $Na^+$ $g^{-1}$ dry wt) | | | Potassium (µM $Na^+$ $g^{-1}$ dry wt) | | |
|---|---|---|---|---|---|---|
| | Control | Stressed | Mean | Control | Stressed | Means |
| Roma Rio Super | 180.11 | 366.73 | 273.42 b | 947.20 | 763.00 | 885 c |
| Roma V.F | 162.75 | 418.81 | 290.78 b | 906.00 | 896.00 | 901 c |
| Chinese 87-5 | 197.47 | 414.47 | 305.97 b | 1085.40 | 1039.40 | 1062 a |
| Rio Grand | 263.53 | 494.76 | 365.64 a | 1100.60 | 932.00 | 1016 b |
| Super Blocky | 182.28 | 486.08 | 334.18 a | 1124.0 | 1003.60 | 1065 a |
| Means | 191.828 b | 436.17 a | | 1033.12 a | 926.80 b | |

LSD $Na^+$ Content cultivars α 0.05 = 63.28, Prob. stress = 0.0024, LSD $K^+$ Content cultivars α 05 = 58.63, Prob. stress = 0.0039

**Potassium absorption:** Salt stress significantly decreased the potassium uptake in all tomato cultivars (Table 2). The minimum potassium content (885 µM $K^+$ $g^{-1}$ dry weight) was observed in Roma Rio Super followed by Roma V.F. and Rio Grand with 901 and 1016 µM $K^+$ $g^{-1}$ dry weight, respectively. The potassium accumulation in Super Blocky was significantly higher under both unstressed (1126.40 µM $Na^+$ $g^{-1}$ dry wt) and salt stress treatments (1003.6 µM $Na^+$ $g^{-1}$ dry wt). Overall effect of salt stress on cultivars was also significant (Table 2). Salt stress significantly decrease the potassium uptake in cultivars from 1033.12 µM $K^+$ $g^{-1}$ dry weight in control to 926.80 µM $K^+$ $g^{-1}$ dry weight in salt stress conditions. This observation is in accordance with the reports that excess Na+ uptake in salinity stress decreases the K+ absorption (Chookhampaeng *et al*., 2007).

**Sodium and potassium ratio:** Significant differences were observed in $Na^+/K^+$ ratio among various tomato cultivars and stress treatments. The $Na^+/K^+$ ratio in cultivars ranged from minimum 0.29 (Chinese 87-5) to the maximum of 0.37 (Rio Grand) (Fig. 1). Salinity stress caused significant increase from 0.19 to 0.48 in $Na^+/K^+$ ratio. The $Na^+/K^+$ ratio of the tissues was generally increased with salinity (Amini and Ehsanpour, 2005). The increase in $Na^+/K^+$ ratio due salt stress was maximum in Super Blocky (170%) followed by Roma V.F.(164.2%) while the minimum increase was observed in Chinese 87-5 (108.7%).

Salt stress is characterized by greater $Na^+$ uptake by the plants and its accumulation in the vacuole (Borsani *et al*., 2003). The excess of $Na^+$ could be extremely toxic to the plants. Thus, the salt tolerance of a genotype is determined by its ability to tolerate high $Na^+$ in shoots without serious effects or keeping high $Na^+$ in the roots (Chookhampaeng *et al*., 2007). Increased $Na^+$ uptake in saline conditions decreases the $K^+$ uptake due to inhibition power which upset the process of uptake (Table 1). Being a macronutrient, $K^+$ uptake is essential for the normal growth and functions of the plant. Thus, slat tolerance should also involve the ability to function despite a decrease in the absorption of $K^+$. The significantly lower sodium contents in Roma Rio Super, Roma V.F and Chinese 87-5 (susceptible cultivars) as compared to Rio Grande and Super Block V.F.N (tolerant cultivars) indicate that $Na^+$ toxicity may not explain the decrease in fresh and dry weight accumulation. Similarly, the decrease in $K^+$ uptake alone can not be responsible for toxic effects of salinity because the difference Roma V.F (susceptible) and Rio Grand (resistant) were non-significant. The $Na^+/K^+$ ratio strengthens this observation. The $Na^+/K^+$ ratio of stressed and unstressed tomato plants reveals that all cultivars showed increase $Na^+/K^+$ ratio in salt stress conditions. Super Blocky which could be considered tolerant cultivar in both fresh and dry weight changes (Table 1) showed the maximum (3.06 fold) increase in $Na^+/K^+$. By contrast, Chinese 87-5 which is salt susceptible cultivar had minimum (2.22 fold) increase in $Na^+/K^+$. Thus, it is clear that a cultivar may be tolerant despite high $Na^+/K^+$. This will require synthesis of protective compounds to minimize the toxic effects of increased $Na^+$ accumulation.

**Proline content:** The proline contents of the tested cultivars significantly differed under salt stress (Table 4 & Fig. 1). The maximum mean proline content was recorded in cultivar Super Blocky (43.888 µM Proline $g^{-1}$ F. wt) which was 2.33 fold higher than control. It was followed by 38.488 µM Proline $g^{-1}$ F. wt in Rio Grand that exhibited 1.97 fold increase proline accumulation. Thus, the salt tolerance of tomato cultivars is dependent on the ability to synthesize proline under salinity stress conditions (Maggio *et al.*, 2002).

**Table 3. Effect of salinity stress on $Na^+/K^+$ ratio of tomato cultivars.**

| Cultivars | Control | Stressed | Means | Change (Fold increase) |
|---|---|---|---|---|
| Roma Rio Super | 0.19 | 0.48 | 0.34b | 2.53 |
| Roma V.F. | 0.18 | 0.48 | 0.33b | 2.66 |
| Chinese 87-5 | 0.18 | 0.40 | 0.29c | 2.22 |
| Rio Grand | 0.22 | 0.53 | 0.37a | 2.41 |
| Super Blocky | 0.16 | 0.49 | 0.33b | 3.06 |
| Means | 0.19b | 0.48a | | |

LSD Sodium potassium ratio for cultivars at α 0.05 = 0.158, Probability = 0.0093

**Table 4. Proline contents (µM Proline $g^{-1}$F wt.) of various tomato cultivars of tomato under NaCl stress.**

| Cultivars | Control | NaCl | Means | Change (Fold increase) |
|---|---|---|---|---|
| Roma Rio Super | 28.370 | 29.392 | 28.876b | 1.03 |
| Roma V.F. | 31.992 | 32.084 | 32.038b | 1.01 |
| Chinese 87-5 | 29.080 | 29.184 | 29.132b | 1.01 |
| Rio Grand | 25.888 | 51.088 | 38.488ab | 1.97 |
| Super Blocky | 26.368 | 61.408 | 43.888a | 2.33 |
| Means | 28.95 b | 40.963 a | | |

LSD for cultivars at α 0.05 = 10.15, Probability for stress treatments = 0.00193

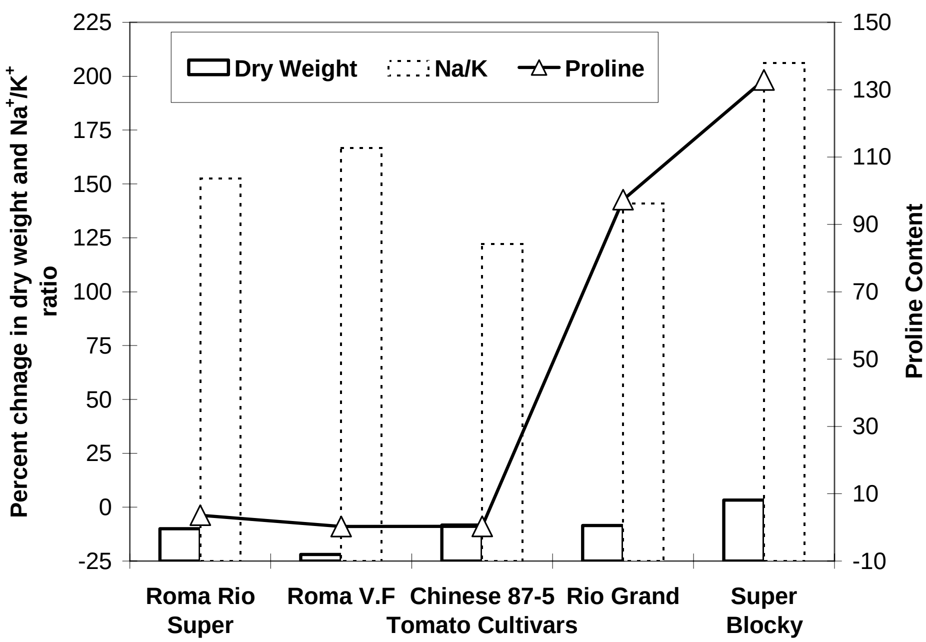


Fig. 1. Changes in dry weight, $Na^+/K^+$ ratio and proline content in tomato cultivars under salinity stress.

We observed 2.33 and 1.97 fold increase in proline accumulation in shoots of Super Blocky and Rio Grand, the salt tolerant tomato cultivars.

Earlier it has been reported that the increased $Na^+$ and decreased $K^+$ levels leads to $Na^+$ toxicity (Borsani *et al*., 2003) and proline accumulation may be due to expression of genes encoding key enzymes of proline syntheis or low activity of the proline oxidizing enzymes (Amini and Ehsanpour, 2005). Our findings suggested that the ability of enhanced proline synthesis in salt stress condition may determine the tolerance levels of plants to salinity. It is possible that, enhanced proline accumulation may regulate multiple processes required for survival in salt stress conditions (Maggio *et al*., 2002).

It can be concluded that salinity stress results in increased $Na^+$ and decreased $K^+$ uptake leading to increased $Na^+$ accumulation and $Na^+$ toxicity (Borsoni *et al*., 2003). It may also decrease the $Na^+/K^+$ balance and, thus, invoke the toxic effects on increased $Na^+$ and or decreased $K^+$ uptake. Since Super Blocky and Rio Grand could tolerate salinity stress despite high $Na^+/K^+$ ratio indicating that $Na^+$ alone or $Na^+/K^+$ imbalance may not explain salt tolerance in these cultivars and enhanced proline synthesis and accumulation may the primary mechanism in salt tolerance (Maggio *et al*., 2002). Proline may either stimulate other responses or act as protective compounds to minimize the salt induce damage.

## References

Alam, S.M., S.S.M. Naqvi and A.R. Azmi. 1989. Effect of salt stress on growth of tomato. *Pak. J. Sci. & Ind. Res*., 32(2): 110-113.

Alarcon, J.J., M.J.S. Blanco, M.C. Bolarin and A. Torrecillas. 1994. Growth and osmotic adjustment of two tomato cultivars during and after saline stress. *Plant & Soil*, 166: 43-47.

Alian, A.A., A. Altman and B. Heuer. 2000. Genotypic differences in salinity and water stress tolerance of fresh market tomato cultivars. *Plant Sci.*, 152: 59-65.

Amini, F. and A.A. Ehsanpour 2005. Soluble proteins, carbohydrates and $Na^+/K^+$ changes in two tomato (*Lycopersicon esculentim* Mill.) cultivars under *in vitro* salt stress. *Am. J. Biochem. & Biotechnol*., 1: 212-216.

Anonymous. 2010. Federal Bureau of Statistics (FBS). Year Book. Federal Bureau of Statistics, Ministry of Economic Affairs and Statistics, Govt. of Pakistan.

Bates, L.S., R.P, Waldren and I.D. Tears. 1973. Rapid determination of free proline for water-stress studies. *Plant and Cell*, 39: 205-207.

Borsani, O., V. Valpuesta and M.A. Botella. 2003. Developing salt tolerant plants in a new century: A molecular biology approach. *Plant Cell Tissue and Organ Culture*, 73: 101-115.

Chookhampaeng, S., W. Pattanagul and P. Theerakulpisut. 2007. Screening some tomato commercial cultivars from Thailand for salinity tolerance. *Asian J. Plant Sci*., 6: 788-794.

Colla, G., J.P. Mitchell, D.D. Poudel and S.R Temple. 2002. Changes of tomato yield and fruit elemental composition in conventional, low input and organic systems. *J. Sustainable Agric*., 20: 53-67.

Delfine, S., A. Alvino, M.C. Villani, G. Santarelli, F. Loreto, M. Centritto, M.I. Ferreira and H.G. Jones. 2000. Agronomic and physiological aspect of salinity stress on a field-grown tomato crop. *Acta Hort.*, 537: 647-654.

Elfeky, S.S., M.E.H. Osman, S.M. Hamada and A.M. Hasan. 2007. Effect of salinity and drought on growth criteria and biochemical analysis of *Catharanthus roseus* shoot. *Intl. J. Bot*., 3: 202-207.

Fariba A. and A.A. Ehsanpour. 2005. Soluble proteins, proline, carbohydrates and $Na^+/K^+$ changes in two tomato (*Lycopersicon esculentum* Mill.) cultivars under *in vitro* salt Stress. *Am. J. Biochem. & Biotechnol*., 1: 212-216.

Flowers, T.J. 2004. Improving salt tolerance. *J. Exp. Bot*., 55: 307-329.

Ijaz, K. and A.P. Davidson. 1997. Baseline Socio-economic survey, Joint Satina Project. IWASRI, Lahore, 77p.

Maggio, A., S. Miyazaki, P. Veronese, T. Fujita, J.I. Ibeas, B. Damsz, M.L. Narasiman, P.M. Hasegawa, R.J. Joly and R.A. Bressan. 2002. Does praline accumulation play an active role in stress induced growth reduction? *Plant J*., 31: 699-712.

Mahajan, S. and N. Tuteja. 2005. Cold, salinity and drought stresses: An Overview. *Arch. Biochem. Biophys*., 444: 139-158.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell and Environ*., 25: 239-250.

Shereen, A., S. Mumtaz, S. Raza, M.A. Khan and S. Soloangi. 2005. Salinity effects on seedling growth and yield component of different rice inbred lines. *Pak. J. Bot*., 37: 131-139

Watad, A.E.A., P.A. Pesci, L. Reinhold and H.R. Lerner. 2003. Proton fluxes as a response to external salinity in wild type and NaCl adapted Nicotiana cell lines. *Plant Physiol*., 81: 454-459.

(Received for publication April 22, 2009)