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# Regular Article Impact of NaCl stress on the physiology of four cultivars of *S. lycopersicum*

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To evaluate the genotypic variation of salt stress response in tomato, some morphological/physiological analyses were conducted on four tomato genotypes Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM). Some predictive screening parameters were set and applied at an early stage of the growth of the tomato plants. Four tomato cultivars were grown in 0.5xMS with different concentration of NaCl (0, 50, 100, 150 and 200mM). 24-day period of salt stress was applied on 15-day old plants. Morphologic and physiologic changes were determined depending on increasing NaCl concentrations. The genotypes exhibited different responses in terms of plant growth, particularly root/shoot growth, FW/DW (Fresh-Weight / Dry-Weight), accumulation of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>. K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios were investigated. It was observed that, more K<sup>+</sup> or Ca<sup>2+</sup> absorbing plant with high K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios show better resistance to salt stress. As evidenced, PK appeared to be the most tolerant genotype while RM was the most sensitive one. AC and PR exhibited intermediary behaviours, suggesting the importance of making use of genetic variability. The research was conducted in a completely randomized design with three replications.

Keywords: Tomato, Genotypes, Salt stress, Salt tolerance, NaCl, Ions.

Salinity stress is recognized as one of the most lethal abiotic stresses interfering with the growth, development and biomass production of plants. Yield reductions induced by salinity may be due to both the osmotic stress that results from relatively high solute concentrations in the root growing medium, and specific toxicity due to the accumulation of high concentrations of Na and Cl in the plant, which provokes a of physiological wide variety and biochemical alterations that inhibit plant growth and production (Maggio et al., 2004; Munns 2005). Reduced photosynthesis, growth, and development are found in plants growing under high salinity are associated with ionic/osmotic effects, nutritional imbalance, or oxidative stress (Ashraf & Foolad 2007; Ahmad et al., 2008; Ashraf 2009; Lee et al., 2008; Munns & Tester 2008; Gill & Tuteja 2010). Due to sedentary nature plant cells evolve several mechanisms to achieve salt tolerance. When plants are allowed to grow in saline solution, it is the roots which are exposed at the first place to such stressful condition. Although there are opportunities to control salt entering leaves at various points along the transpiration stream, the root must perform a crucial function in the management of input and throughput. Root systems can exhibit enormous plasticity on the level of biomass, morphology, and/or physiology in response to different environmental parameters such as water and nutrient availability or excess ions (Rewald et al., 2011). It is a prerequisite to

understand how plants respond and adapt to this stress to prevent crop yield losses.

Increasing NaCl concentrations in nutrient solution adversely affect tomato shoots and roots, plant height, K<sup>+</sup> concentration, and K<sup>+</sup>/Na<sup>+</sup> ratio (Al-Karaki 2000). In different reports, salinity was shown to increase the uptake of Na<sup>+</sup> or decrease the uptake of Ca<sup>2+</sup> and K<sup>+</sup> (Neel et al. 2002). In general, Ca<sup>2+</sup> and K<sup>+</sup> concentrations decrease with salinisation but not in all genotypes of tomato as shown by Bolarin et al. (1995). Ability of plant genotypes to maintain higher levels of K<sup>+</sup> and Ca<sup>2+</sup> and low levels of Na<sup>+</sup> within tissue is one of the key mechanisms contributing to expression of high salt tolerance. In most cases, salt tolerant genotypes are capable of maintaining higher K<sup>+</sup>/Na<sup>+</sup> ratios in plant tissues (Mansour 2003, Zeng et al., 2003). Genotypes for high tolerance to salt stress the K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios and tissue Na<sup>+</sup> concentrations are, therefore, wisely used parameters for different crop species (Ashraf & Harris 2004; Santa-Cruz et al., 2002; Munns & James 2003).

Tomato is one of the most important horticultural crops in the world, and tomato plant growth was shown to be moderately sensitive or moderately tolerant to salinity depending on cultivar or growth stage (Santa-Cruz et al., 2002; Fernandez-Garcia et al., 2004; Estan et al., 2005). Study on the physiological responses of tomato seedlings to salt stress could give novel insight into the planting and modifying of tomato cultivars. The present report describes in vitro studies as an efficient method to study where four cultivars were compared with respect to their response to low, middle and high salinity in terms of plant growth, FW/DW and content of Ca2+, Mg2+, Na+ and K+.

# Materials and methods

#### Plant material and growth conditions

Seeds of local cultivars like *Solanum lycopersicum* cv. Pusa Ruby, cv. Punjab Keshari and cv. Roma were purchased

from Amtala Seed Centre, Amtala, West Bengal, India and used for the research purpose. Seeds of *Solanum lycopersicum* cv. Ailsa Craig were obtained from Dr. D. Grierson (Nottingham University, UK).

Plants were grown in 0.25x Murashige & Skoog liquid medium (Sigma, Hi-media) and the experiments were done by treating them with or without salt (NaCl solution) for different time periods as described later. Then the plants were washed thoroughly with sterile de-ionized water and the roots and leaves were harvested. Seeds of different cultivars like Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC), Roma (RM) and were surface sterilized in 0.1% HgCl<sub>2</sub> for 10 min, and then rinsed with water. Selected seeds were then germinated aseptically on petriplates containing moistened filter paper. The seeds germinated after 3 days. The obtained seedlings were transferred to 0.25 x MS liquid media in aseptic condition and grown for 15 days (16 hrs dark and 8 hrs light period). After 15 days the plants were transferred to bottle with 50 ml of fresh 0.5 x MS (Sigma) liquid media containing increasing amount of NaCl like 0, 50, 100, 150, and 200 mM, containing 5 plants in each on Whatman blotting paper in three different sets. After 24 days, plants were washed thoroughly with sterile de-ionized water and the roots, stems and leaves were harvested and relative physiological indices were investigated.

# Measuring Root/Shoot length and FW/DW

The tomato plants of four different cultivars were grown in 0.25x MS for 15 days and then treated with 0, 50, 100, 150, and 200mM NaCl in 0.5x MS for 24 days. To observe the effect of salinity on plant growth in respect to shoot and root length, the measurements were recorded and then separated into leaf, stem and root parts. The parts first washed carefully with tap water to remove growing media and nutrient solutions and again washed with deionized water. Their surface water was completely dried by absorbent paper. Individual fresh weight of leaves, stems and roots per plant was taken. The same samples were then dried at 70°C for 48 h. Finally dry weights were recorded.

#### Endogenous level of Inorganic Ions

The tomato plants of four different cultivars were grown in 0.25x MS for 15 days and then treated with 0, 50 and 150mM NaCl in 0.5x MS for 24 days. Then the plants were washed with autoclaved deionized water thoroughly and samples (root, stem and leaves) were harvested. Plant materials were dried as described before. The dried plant materials were used for measurement of Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup>. After recording the DW, the dried parts were ashed in a muffle furnace at 500-600°C for 8 h by placing in a crucible. The ashes were dissolved in 10ml of 0.25 (N) HCl. According to necessity, each sample was diluted by deionized water and recorded in Varian Atomic Absorption Spectrophotometer. The total amount of magnesium, calcium, potassium and sodium ions were calculated from the respective standards, measured simultaneously.

#### Statistical Analysis

Treatments in the experiments were arranged in a Completely Randomized Design (CRD), with three replications. The collected data were presented with the respective standard errors of means and the least significant difference (LSD 0.05) between treatments, derived from an analysis of variance (ANOVA).

#### Results

#### Effects of salt stress on the growth

In the treatments of different saline concentration, the growth of tomato plants under study varied obviously and suppressed by salt stress, which was associated with salt content. Results showed that the growth of tomato seedlings was suppressed by salt treatment (Figure 1), but survived due to the water content and degree of succulence. In the treatment of 200 mM NaCl, leaves of tomato suffered great damage with all yellow leaves and growth was almost arrested.

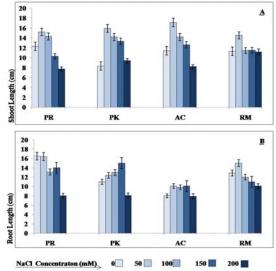


Figure 1. Effect of salinity stress on shoot length (A) and Root length (B) of four tomato cultivars Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM). Plants were treated with 0, 50, 100, 150 & 200 mM NaCl and used to take the measurement in cm. Data points and vertical bars represent means of triplicates and SE respectively.

# Effect of salinity stress on Fresh and Dry weight

Plants from the hydroponic cultures of four genotypes PR, PK, AC and RM were compared for their fresh weight and dry weight from their control and treated plants (Figure 2, 3 & 4). In salt treated plants, fresh weight and dry weight of roots were mostly affected in all four cultivars. RM and AC showed less effect of salinity on shoots. PR and PK which showed sharp rise of FW/DW at low level and sharp reduction at high level of NaCl treatment in all the organs under study. When all the genotypes exhibited initially a moderate rise in FW/DW in leaves and reduction at higher concentration of salt, the only enhancement of FW/DW was found in RM and it was a gradual increase of 20-50%. In the cultivar AC no enhancement of FW/DW roots and shoots

were noticed under moderate stress like other three genotypes. In shoots it remained almost unaltered and in roots it only gradually got depleted with increasing salt concentration. From the histograms it appears, AC and RM behaved more or less in a similar fashion and similarity was also found in the behaviour PR and under of PK unfavourable environmental condition.

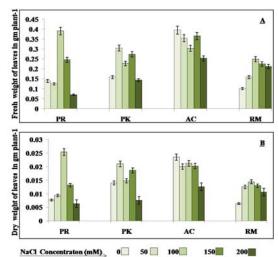


Figure 2. Effect of salinity stress on fresh weight (A) and dry weight (B) of the leaves of four tomato cultivars Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM). Plants were treated with 0, 50, 100, 150 & 200 mM NaCl and used to determine their fresh and dry weight (FW and DW). Data points and vertical bars represent means of triplicates and SE respectively.

# *Effect of salinity stress on mineral content* $(Na^+, K^+, Ca^{2+} and Mg^{2+})$

The four different cultivars of tomato were grown in 0.25x MS for 15 days and then treated with 0, 50 and 150mM NaCl in 0.5x MS for 24 days. Then the plants were washed with autoclaved double distilled water thoroughly and samples (root, stem and leaves) were collected, dried and powdered. The ash powder of plant materials were used for measuring the content of Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> (Figure 5). In normal condition (control) their level differed in roots, stems and leaves within 4 cultivars.

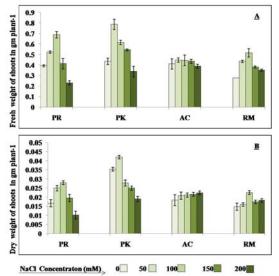


Figure 3. Effect of salinity stress on fresh weight (A) and dry weight (B) of the shoots of four tomato cultivars Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM). Plants were treated with 0, 50, 100, 150 & 200 mM NaCl and used to determine their fresh and dry weight (FW and DW).Data points and vertical bars represent means of triplicates and SE respectively.

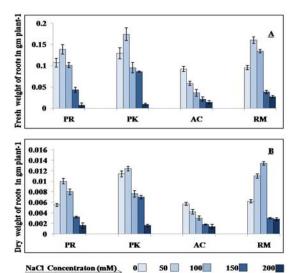


Figure 4. Effect of salinity stress on fresh weight (A) and dry weight (B) of the roots of four tomato cultivars Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM). Plants were treated with 0, 50, 100, 150 & 200 mM NaCl and used to determine their fresh and dry weight (FW and DW).Data points and vertical bars represent means of triplicates and SE respectively.

Ca<sup>2+</sup> ion level in leaves was found to be very high in PK and AC which is 3-4 fold higher than PR. In stem it was more or less same in four cultivars. Roots of AC, PK and PR exhibited its high accumulation. Overall, salinity stress reduced the Ca<sup>2+</sup> level in leaves with substantial enhancement in roots.

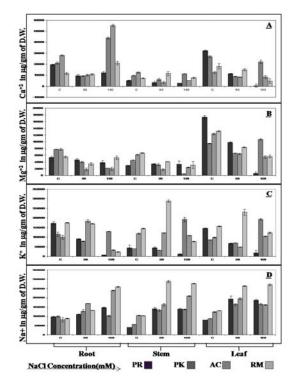


Figure 5. Effect of salinity stress on the level of Calcium Ca<sup>+2</sup>(A), Magnesium Mg<sup>+2</sup>(B), Potassium K<sup>+</sup>(C) and Sodium Na<sup>+</sup>(D) in four different tomato cultivars Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM) by variance spectrophotometer. Ash sample from root, stem and leaf tissue of 4 tomato cultivars, exposed to different concentration of NaCl (0, 50 and 150mM) for 24 days. Data points and vertical bars represent means of triplicates and SE respectively.

Untreated leaf contained higher amount of Mg<sup>2+</sup> in comparison to stem and root. Salinity stress initially did not show any major difference in stems and roots of all the treated and untreated cultivars. But stress with 150mM NaCl, the Mg<sup>2+</sup> level in leaves of PK goes up which showed initial depletion in 50mM salt stress. Though in AC and RM its concentration consistently came down with increasing level of stress bur PR exhibited it got depleted in much higher amount.

The K<sup>+</sup> level in normal plants varied within the cultivars in roots, stem, and leaves. Roots contained higher level of K+ in all 4 cultivars. Untreated stem-part of AC and RM contained higher level of K<sup>+</sup> than PR and PK. In leaves surprisingly PK accumulated very high level of K+ even in 150mM of high salt stress. Salinity stress of 150mM also could enhance the K<sup>+</sup> level in other parts under study particularly in PK. AC showed initial enhancement in root, stem and leaf under 50mM stress. RM also showed enhancement of K<sup>+</sup> under 50mM stress in stem but higher than 50 mM salt enough to resist K<sup>+</sup> was stress accumulation in other three cultivars.

The Na<sup>+</sup> level in control plants showed low level in roots and stems in all 4 cultivars. In leaves of AC and RM it was 2 fold higher than in PR and PK. After salinity stress the level of Na<sup>+</sup> ion was found to be at high level in RM and enhancement was detected in stems and leaves of other cultivars also. Basically it appeared that Na+ ion was transported from the roots to the leaves via stems, as the Na<sup>+</sup> level was lower in roots than shoots and leaves in all 4 cultivars. The Na<sup>+</sup> ion level in PR and PK was enhanced 2 fold or less by salinity stress in roots, stems and leaves. The cv PK maintained higher level of K<sup>+</sup> in leaves of plants exposed to salinity stress whereas in PR, the level of K<sup>+</sup> was found to be always low in the leaves whereas the Na<sup>+</sup> ion is high in PR. In contrast the K<sup>+</sup> level in leaves of PK was higher than the Na<sup>+</sup> level in leaves.

Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> ratios (Figure 6 and 7) differed in four cultivars. In PR both the ratios decreased with increasing salt concentration in root, stem and leaves it reduced gradually whereas in AC root, stem, leaves though show a decline but in root the decreasing degree was more pronounced. Na<sup>+</sup>/Ca<sup>2+</sup> ratios in AC also decreased but in root it initially decreased substantially after that in 150mM NaCl concentration it again increased a little.

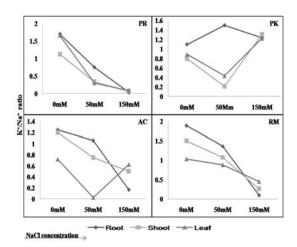


Figure 6. Effect of salinity stress (0, 50 and 150mM NaCl for 24 days) on the K<sup>+</sup> / Na<sup>+</sup> ratio of root, stem and leaves of four different tomato cultivars Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM).

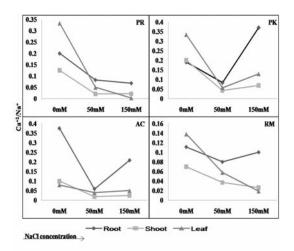


Figure 7. Effect of salinity stress (0, 50 and 150mM NaCl for 24 days) on the Ca<sup>+2</sup>/ Na<sup>+</sup> ratio of root, stem and leaves of four different tomato cultivars Pusa Ruby (PR), Punjab Keshari (PK), Ailsa Craig (AC) and Roma (RM).

In RM Na<sup>+</sup>/K<sup>+</sup>, Na<sup>+</sup>/Ca<sup>2+</sup> ratios decreased steadily. In PK both the ratios found to be increased. In root Na<sup>+</sup>/K<sup>+</sup> increased significantly in low salt concentration but in higher salt concentration it started decreasing moderately. Na<sup>+</sup>/Ca<sup>2+</sup> initially decreased highly in all the plant parts whereas in 150mM NaCl sharp rise was noticeable in root.

#### Discussion

Several authors reported the use of NaCl for in vitro salinity screening in different plants (Vijayan et al., 2003; Zhao et al., 2009). Our study showed that NaCl treatment caused reduction in the overall growth of four tomato cultivars (PR, PK, AC and RM) as compared to their control plants. The plants though could survive in 200 mM NaCl but became succulent, with stunt growth and yellowing of leaves due to chlorosis. PK showed least chlorosis along with better survival rate in severe salt stress (data not shown). Dogan (2010) reported that chlorophyll concentration was lesser in salt-sensitive cultivars than in salt-resistant cultivars of tomato. It is presumably true that for PK, better vegetative growth (Figure 1) and survival rate may have contributed to salt tolerance to some extent. Since plants are sessile, they have developed mechanisms to adjust various stresses. Osmotic adjustment might play a critical role in the growth of tomato seedling under the condition of salt stress. So far FW and DW of leaves and shoots (Figure 2 and 3) are concerned the impact was lesser in AC whereas PR and PK showed sharp rise at low level and sharp reduction at high level of NaCl treatment. Moreover in RM, biomass was found to be with increasing increase in salt concentration. Consistent reduction in root biomass has been noticed in all the four cultivars (Figure 4). Such variability between tomato cultivars for biomass reduction after salt stress has been stated previously (Alian et al., 2000). Other researchers reported (Maggio et al., 2007; Mohammad et al., 1998; Tıpırdamaz & Karakullukçu 1993; Hajer et al., 2006) similar results. As reported by Al-Rwahy (1989), the reduction of the dry weights due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl and Na. Excessive accumulation of Na ions in root, stem and

leaf, leads to plant depression by preventing K<sup>+</sup> and Ca<sup>2+</sup> accumulation, which also triggers the reduction in dry weights at high NaCl concentrations (Caines & Shannon 1999). On the other hand, some researchers have reported no such relation between dry weights and Na<sup>+</sup> concentrations (Al-Karaki 2000; Dasgan et al., 2002). Previous studies also reported that there may not any such relationship between biomass production and salt tolerance at the early stage of growth of the tomato genotypes (Dasgan et al., 2002), underlying the necessity to add other criteria to evaluate tomato tolerance to salt stress.

One of the harmful effects of salinity on plant growth is the excessive accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in the leaves (Zhang and Blumwald 2001; Munns et al., 2002; Ashraf and Harris 2004). Plants have improved complex mechanisms for adaptation to osmotic and ionic stress caused by high against Protection various salinity. environmental stresses has been well documented (Khan and Singh 2008; Gill et al., 2011). This accumulation under saline conditions depends on the plant's capacity to limit the uptake of these elements (Koval and Koval 1996). In our ionomic analysis, the level of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> showed great variation in the accumulation after 24 days of exposure to salinity stress (Figure 5). This reflects a differential NaCl-induced imbalance between uptake and translocation to the shoot of these nutrients and plant growth. Na<sup>+</sup> level was found increasing gradually with increasing level of salt concentration in all the cultivars. Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of high concentrations of either inorganic ions or low molecular weight organic solutes. Although both of these play a crucial role in plants grown under higher saline conditions, their relative contribution varies among species, among cultivars and even between different compartments within the same plant (Ashraf 1994; Greenway and Munns 1980). Here 150mM of NaCl

stress was found to enhance the Mg<sup>2+</sup>, K<sup>+</sup> and Ca2+ level markedly in stems and leaves of PK compared to the other three cultivars (PR, AC and RM). Increasing trend of Ca<sup>2+</sup> in root was noticed in PK and AC. Enhancement of K<sup>+</sup> was found only in the root sample of PK. The level of Mg<sup>2+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> varied significantly within the cultivars. Their level was found to increase gradually with increasing level of salt concentration in the leaves of PK. Despite stressful condition PK showed enhancement in the amount of K<sup>+</sup> and Ca<sup>2+</sup>. Since leaf is the site of photosynthesis, the controlled regulation of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> level in PK even after salinity stress can be considered as significant. The cv.RM also showed enhancement of K<sup>+</sup> level in roots and stems in response to moderate salt stress but it rapidly diminished under higher stress of 150mM. Ca2+ ions can control salt tolerance in different ways. First of all, they maintain Na<sup>+</sup> accumulation in tissues (Rengel 1992), and prevents Na<sup>+</sup> ions entering into the cell (Maathius et al., 1996).

Preservation of or increase in Ca2+ concentration could induce maintenance of K<sup>+</sup>, because the presence of Ca<sup>2+</sup> seems to be necessary for K<sup>+</sup>-Na<sup>+</sup> selectivity and for the maintenance of an appropriate amount of K<sup>+</sup> concentration in plant cells. Rengel (1992), Neel et al. (2002) and Rubio et al. (2003) also reported that low values of Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> ratios in roots being a better indicators of salt stress than the Na<sup>+</sup> concentration alone. Plant ability to maintain higher levels of K<sup>+</sup> and Ca<sup>2+</sup> and low levels of Na<sup>+</sup> is one of the keystones to express high salt tolerance. At 150mM salt stress cv. PK showed elevation in Ca2+ accumulation in aerial parts like stem and leaves (Figure 5), it is possible that increased accumulation might help cv. PK fight against salt stress. The results of numerous earlier studies have indicated that in response to salt or dehydration stress, small molecules such as abscisic acid (ABA) and calcium are utilized by the plant to induce various signalling cascades. These

pathways use various proteins such as phospholipases, kinases, calmodulin, calcium-binding proteins and transcription factors to activate genes necessary for water-related stress tolerance (Xiong et al., 2002; Chinnusamy et al., 2004; Munns 2005; Yamaguchi-Shinozaki and Shinozaki 2006). Root, stem and leaf K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios of 4 cultivars against the control treatment (Figure 6 & 7) show that there was large variations in K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios among 4 cultivars under study. Root K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios of PK cultivar was less affected by NaCl treatment than the other three cultivars. The controlled accumulation of Na<sup>+</sup> and high K<sup>+</sup>/Na<sup>+</sup> ratios might have enhanced such tolerance. The K<sup>+</sup>/Na<sup>+</sup> ratio has been used as a nutritional indicator by a number of authors to select salt tolerant varieties in tomato crops (Asch et al., 2000; Al-Karaki 2000; Dasgan et al., 2002; Juan et al., 2005). Santa-Cruz et al. (2002) observed that the K<sup>+</sup>/Na<sup>+</sup> ratio in leaves of tomato plants submitted to salt stress is a better overall indicator of the ability to combat salinisation. The maintenance of high K<sup>+</sup>/Na<sup>+</sup> ratio is also important for tomato salt tolerance. In our study, we have noticed that PK could tolerate high concentration of NaCl in terms of their growth and survival. The result for the K<sup>+</sup>/Na<sup>+</sup> ratio value also supports PK's better resistance capability to salt stress and PK also came out with highest Ca2+/Na+ ratio which is also supportive for PK's adaptability to better unfavourable environmental condition compared to the other three cultivars. Studies of other researchers indicate that an increase in concentrations of Ca2+ and K+ in plant under salt stress could improve the harmful effects of salinity on growth and yield (Grattan and Grieve 1999; Sivritepe et al., 2003; Kaya et al., 2003) of crops like melon, cucumber, pepper. Reduction of K<sup>+</sup> and Ca<sup>2+</sup> ions in plant tissues at high level of NaCl treatments is also a very known fact for some plants like melon and eggplant (Savvas and Lenz 2000), spinach (Wilson et

*al.*, 2000), pepper (Aktas *et al.*, 2006), squash plant (Yıldırım *et al.*, 2006).

As per previous reports, salt tolerant cultivars are capable of maintaining higher K<sup>+</sup>/Na<sup>+</sup> ratios in plant tissues (Mansour 2003; Zeng *et al.*, 2003) like rice. K<sup>+</sup>/Na<sup>+</sup> and  $Ca^{2+}/Na^{+}$ ratios and tissue Na<sup>+</sup> concentration are, wisely used parameters to determine high salt tolerance of different crops (Ashraf and Harris, 2004; Santa-Cruz et al., 2002; Dasgan et al., 2002; Munns and James 2003). Rajasekaran (2000) showed that there were significant differences in K<sup>+</sup>/Na<sup>+</sup> ratios even among different organs of Lycopersicum spp. due to the possible differences in vegetative growth.

Though in our present in vitro study, biochemical changes were not determined but previously we performed biochemical experiments with leaves of 2 month old three tomato cultivars only (PR, PK and AC) which was an *in vivo* study (unpublished data). That study indicated that 6hrs salt shock treatment with 200 mM NaCl was enough to cause significant in tomato. Among several changes biochemical changes we showed, the changes in the level of Proline, Polyamines (PAs), H<sub>2</sub>O<sub>2</sub>, MDA (malondialdehyde) can be mentioned here to correlate our present study. Proline is one of the most important osmoprotectants in plants. Under salt stress most plant species exhibit a remarkable increase in their proline content (Patel and Pandey 2008; Dasgan et al., 2009). Huge amount of proline accumulation we noticed in cv.PK. Proline accumulates in plants under a broad range of stress conditions such as water shortage, salinity, extreme temperatures, and high light intensity (Aspinall and Paleg 1981; Mansour 2000) and is believed to play a major role in plants osmotic adjustment. Our earlier study also showed that PK produced higher level of polyamines (putrescine and spermidine) when treated with 200 mM NaCl. MDA content, a product of lipid peroxidation, has been considered an indicator of oxidative damage (Shalata et al., 2001). As per our previous finding, MDA

produced during peroxidation was found almost unaltered in PK. It is often used as an indicator of oxidative damage. The controlled lipid peroxidation in PK must have given it a better protection against oxidative damage under salt stress. Previously we also interpreted that cultivar PK has better adaptive mechanism in scavenging H<sub>2</sub>O<sub>2</sub>. According to several earlier reports, under stressful condition H<sub>2</sub>O<sub>2</sub> accumulation and lipid peroxidation in sensitive cultivars is higher. Plants under salt stress displayed an increase in the generation of H<sub>2</sub>O<sub>2</sub> (Gueta-Dahan et al., 1997; Roxas et al., 2000). Excessive amounts of highly reactive ROS can damage proteins, lipids and nucleic acids by oxidation (Halliwell 1985). Therefore, it is critical that the plant counteract the production of reactive oxygen species with mechanisms for neutralizing them. Taken together, our result show that the salt tolerance in tomato depend greatly on the osmotic adjustment (proline, PAs), keeping reactive oxygen species and MDA under control. Non enzymatic antioxidants also played constantly in encountering adverse condition.

Together with the previous findings and the data (physiological and ionomic) presented in this study revealed complex interactions between NaCl uptake and growth responses. In response to NaCl treatment in the four genotypes, changes were greater in the more tolerant ones. It was possible to conclude that PK is a salttolerant genotype according to a series of physiological indices.

# Conclusion

The genetic differences present a good basis to provide information about genotypes that could be grown in salt-affected areas to chance crop productivity. In this comparative study, exogenous application of NaCl affected uptake and distribution of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>+2</sup> and Mg<sup>2+</sup> in four tomato genotypes which characterized different levels of sensitivity among the tomato genotypes and in different organs.

Along with this according to other physiological parameters like vegetative growth, FW, DW It is proposed that PK efficiently outperformed other three cultivars PR, AC and RM. Further study should allow the key regulators of salttolerance to be elucidated and thus facilitate attempts either to breed or to engineer these traits into the salt-sensitive cultivars.

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