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SALT STRESS-INDUCED PROLINE ACCUMULATION, CHANGES IN IONIC ADJUSTMENT (NA^+/K^+) AND CATALASE IN THE TOMATO (*SOLANUM LYCOPERSICUM*) LEAF

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ABSTRACT

Soil salinity is a major limitation to plant in many areas of the world. Present study has explored the effect of salinity on Proline content, mineral composition and antioxidant enzyme of tomato. Tomato plants were exposed to 0, 60, 90,120,150 mM NaCl.The Proline accumulation was studied by reading the absorption of chromophore at 520nm using spectrophotometer. The content of proline increase in tomato as the level of NaCl increase. Using atomic absorption spectrophotometer ion uptake for Na^+, K^+, Ca^+ and Mg^{2+} were determined. The Na content significantly increase but the content of K^+, Ca^+, K^+ and Mg^{2+} decrease significantly decrease as the salinity increase. The changes in the activity of antioxidant enzymes such as super oxide dismutase catalase (CAT: EC 1.11.1.6), in leaves of tomato cultivar (cv.) differing in salt tolerance were investigated. The activity of catalase increase as the level of salinity increase.

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INTRODUCTION

Vegetables are the good resource for overcoming micronutrient and macronutrient deficiencies and provide small holder farmers with much higher income and more jobs per hectare than staple crops. Tomato is cultivated for its fleshy fruits and it is called as protective food because of its special nutritive value and its wide spread production. Tomato is the richest source of nutrients, dietary fibres antioxidant like lycopene and beta-carotene, the compounds that protect cells from cancer (Hobson, 1993). But excessive salinity reduces the productivity of many agricultural crops including most of the vegetables like tomato. Stress factors based on their origins divided into 2 group's i.e. abiotic and biotic stress factors (Mahajan and Tuteja, 2005). Abiotic stress factors include cold and hot temperatures, drought, salinity, excessive water, radiation, various chemicals, oxidative stress, wind, and lack of soil nutrients. Saline soils are one of the major abiotic stresses that can adversely affect the overall metabolic activities and cause plant death (Roychoudury *et al*., 2008). About 20% of the world's cultivated land area and 50% of all irrigated land is affected by salinity (Moud and Maghsoudi, 2008). Salt stress affects some major processes such as germination, speed of germination, root/shoot dry weight and Na^+/K^+ ratio in root and shoot (Parida and Das, 2005). Early flowering reduced dry matter, increased root shoot ratio and leaf size caused by salinity which may be considered as possible ways of decreasing yield in plant under salt stress condition (Mengel *et al*., 2001). A great contributor to salinity stress is the accumulation of high concentrations of Na^+ in the leaf cell cytoplasm (Jha *et al*., 2010). Ionic imbalance occurs in cells due to excessive accumulation Na^+ and Cl^- ions that reduce uptake of K^+, $Ca2^+$, and Mn (Bayuelo-Jimenez *et al*., 2003). It inhibits the uptake and transport of different ions. Effects of salinity stress on plants have primarily focused on growth, proline accumulation, chlorophyll content, K/Na, Ca/Na ratio, Na^+ and Cl^-accumulation. The adaptation is generally associated with osmoregulation adjustment by using some osmotic regulators such as potassium, soluble sugar, proline and betaine (Munns, 2005; Hong-Bo *et al*., 2006). One of the mechanisms is proline accumulation into cell. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Kavi *et al*., 2005). However the significance of proline accumulation in osmotic adjustment is still debated and varies according to the species (Lutts *et al.*, 1996; Rodriguez *et al.*, 1997). Salinity exerts their effect by causing oxidative damage. This damage is caused by increased production of reactive oxygen species (ROS) (Smimoff, 1995). Excess production of ROS during stress results from impaired electron transport processes in chloroplast and mitochondria as well as from pathways such as photorespiration (Sanchez Rodriguez *et al.*, 2012). In the absence of a protective mechanism in plants ROS can cause serious damage to different aspects of cell structure and function such as initiating lipid per oxidation and damaging DNA, proteins and other small molecules (Arora *et al.*, 2002; Gill and Tuteja, 2010; Ahmad *et al.*, 2011)

Salt tolerance is generally attributed to up-regulated activities of antioxidant enzymes. In tomato salt-tolerance is attributed to the increased activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) (Mittova *et al.*, 2004). 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 synthesis or low activity of the proline oxidizing enzymes (Amini and Ehsanpour, 2005). Enhanced proline accumulation may regulate multiple processes required for survival in salt stress conditions (Maggio *et al.*, 2002). An important aspect of salinity stress is the interaction between Na and K ions (Greenway and Munns, 1980). Sodium provided in low amounts stimulates growth and development of plants and can improve the organoleptic characteristics of the edible parts (Satti *et al.*, 1996). However, high concentrations of Na in the soil inhibit plant growth and reduce commercial yield (Graifenberg *et al.*, 1993, 1996). Potassium regulates the osmotic potential of glycophyte tissues and therefore plays a role in water relations (Lauchli and Pfluger, 1978). Therefore, elevated levels of K could modulate the absorption and the transport of Na and limit the damages attributed to it.

MATERIALS AND METHODS

The plant material which was included in our study was a tomato variety (C-21) purchased from Division of Vegetable Science, IARI, and New Delhi. The seeds were surface sterilized with dilute solution of sodium hypo chlorite (NaOCl) to prevent any fungal contamination and then rinsed three times with distilled water. Then seeds were grown in Petri dishes containing double layered wet filter paper with tap water in order to check the viability of seeds. The seeds were sown in five sets in an earthen pot containing equal quantities (4kg) of loamy sand soil. Salt treatment of NaCl was prepared using sodium chloride salt in concentrations of 60, 90, 120 and 150mM in soil, leaving one set as a control. The samples were taken from two weeks old seedlings for physiological analysis.

Proline determination in tomato

The proline content in was determined by the method of Bates *et al.*, 1973 using D-Proline as standard.

Method

Frozen plant material was homogenized in 3% aq. Sulphosalicyclic acid (0.01g/0.5ml) and the residue was removed by centrifugation at 12,000 rpm for 10 mi.Then added 1 ml of the homogenized tissue in 1 ml of acid-ninhydrin and 1 ml of glacial acetic acid in a test tube for 1 hr at 100° C and the reaction is terminated in an ice bath. Reaction mixture was extracted with 2ml of toluene mixed vigorously and left at room temperature for 30 min until the separation of two phases .The chromophore containing toluene(1ml,upper phase) was warmed to room temperature an its optical density was measured at 520nm using toluene as a blank. The proline concentration was determined by preparing a standard curve using D-Proline.

Mineral and Ionic Composition

These were obtained from the oven dried material.

Sodium and Potassium

200 mg of oven dried and well grinded material was taken in a 50 ml conical flask to which 50 ml of diacid mixture was added (H_2SO_4& $HClO_4$, 4:1).When fumes were heated gentallyon a hot plate till the formation of dense white fumes. When fumes reduced & subsided, heating was increased & digestion was continued for another 25-30 minutes to obtain a colourless digest. The digest was obtained, cooled and diluted to 25 ml distilled water. This acid digest was used further for the estimation of Na & K contents were determined using Flame photometer and expressed as mg g^{-3} tissue dry weight. Prior to determination of Na and K contents of tissue digested. It was calibrated using graded concentration of 0-100 ppm solution of Na and K respectively.

Na & K content = O.D. × Dilution factor/Wt. of tissue taken = x µg.

For Quantitative estimation of Catalase in tomato

Tomato sample were taken and crushed in methanol and heated till 80% of methanol evaporates. 10% of extract in 50 ml of buffer (0.067M, pH 7.0 was prepared by mixing 8 ml of A and 42 ml of B in 16:84 ratio, where A contains 1.36g of monobasic sodium sulphate in 50 ml of distilled water and B contains 2.68g of Dibasic sodium phosphate in 50ml of distilled water) followed by addition of H_2O_2 phosphate buffer which is prepared by adding 0.75 ml of 15% H_2O_2 to 50 ml of phosphate buffer (pH 7.0). After 10 min. Read the absorbance was taken at 240 nm (Cakmak and Marschner, 1992).

RESULTS AND DISCUSSION

Proline determination in tomato

The proline concentration was increased with increase in NaCl concentration. Also when compared to standard the proline conc. was decreased in sample. The maximum proline concentration was shown in 150mM of NaCl concentration and minimum conc. was shown in control set.

Table 1a. Analysis of variance table for proline content

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	20888.72	2	10444.36	9.319677	0.003609	3.885294
With in groups	13448.14	12	1120.678			
Total	34336.862	14				

Since $p \leq 0.05$. We can reject the null hypothesis and say that there is an effect of NaCl on proline.

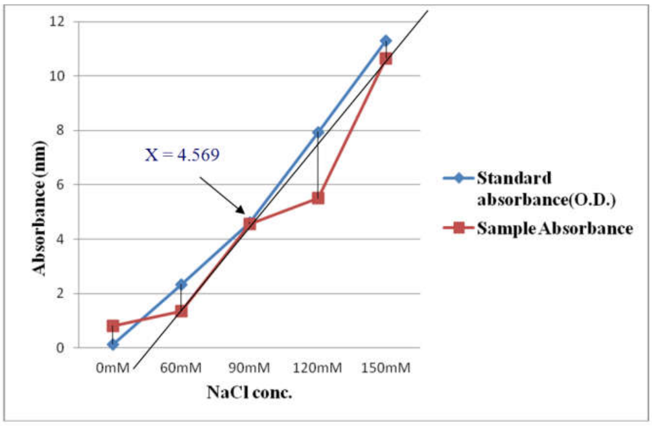

Figure 1. Effect of different concentrations of NaCl on proline content

Mineral and ionic composition

Sodium and Potassium

The sodium content is more than the potassium content with increase in salt concentration. Maximum sodium content and also ratio between two was at 150mM concentration and minimum content was shown in control set or 0mM concentration.

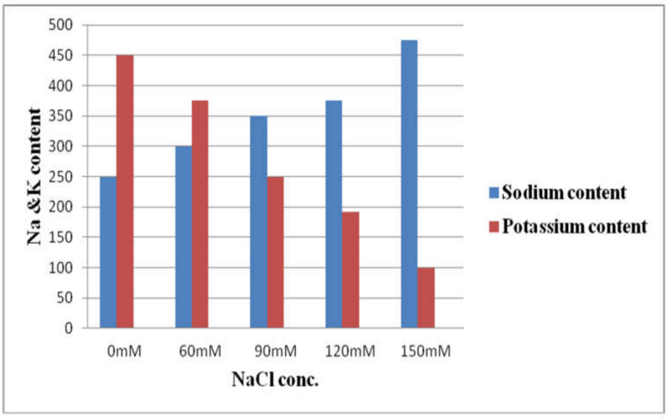

Figure 2a. Effect of different concentration of salt on sodium and potassium content

Table 2a. Analysis of variance table for sodium content

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	236922.1	2	118461.1	16.2556	0.000384	3.885294
With in groups	87448.8	12	7287.4			
Total	324370.9	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on sodium content.

Table 2b. Analysis of variance table for Potassium content.

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	100778.5	2	50389.27	3.630865	0.055468	3.88529
With in groups	166536.4	12	13878.03			
Total	267314.9	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on potassium content

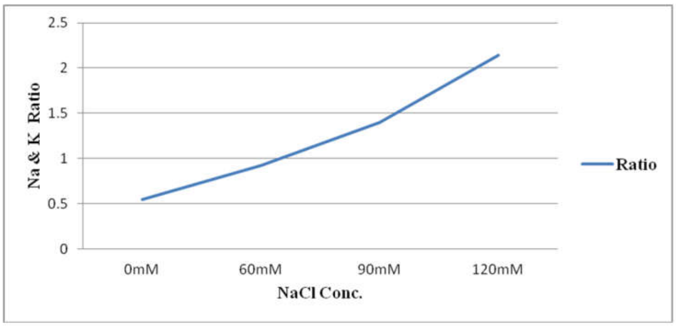

Figure 2b. Effect of different concentration of salt on sodium and potassium ratio

For Quantitative estimation of Catalase in tomato

It was clearly shown from table that the catalase activity was increased with increase in salt conc. The maximum catalase activity was shown at 150mM conc. while minimum was shown ain control set.

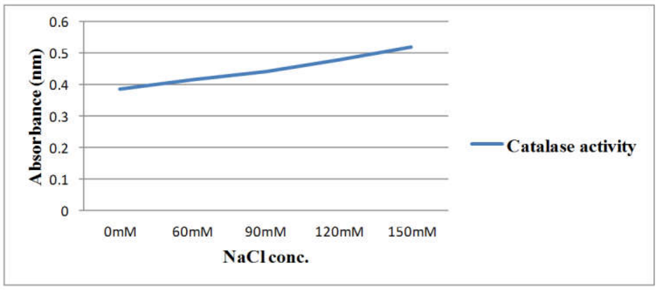

Figure 3. Effect of different concentration of salt on catalase activity

Table 3. Analysis of variance table for catalase activity

Source of Variation	SS	df	MS	F	P-value	F-crit
Between groups	23261.71	2	11630.86	10.47824	0.00233	3.885294
With in groups	13320.02	12	1110.001			
Total	36581.73	14				

Since p≤0.05. We can reject the null hypothesis and say that there is an effect of NaCl on catalase activity.

The extent of plant injury by elevated concentration is specific and strongly depends on the environmental conditions and on the availability of salt concentration. In the present study we took the one variety of tomato (C 21). This variety was grown under NaCl salt stress. Different studies on salt stress indicate that salinity have an adverse effect on the growth of tomato plant. In this study we have taken five concentrations (0mM, 60mM, 90mM, 120mM, 150mM) of NaCl were used in order to evaluate its effect on proline, sodium potassium ratio, catalase activity and antioxidant activity. Proline content was also observed after applying NaCl stress. As shown in Table 1 the proline content is increased with NaCl concentration and at 150mM conc. tomato variety show maximum amount of proline content. Significant differences were observed in Na^+/K^+ ratio among tomato variety under stress treatments. The Na^+/K^+ ratio in taken tomato variety ranged from minimum 0.55 (0mM) to the maximum of 3.80 (150mM) (Fig. 2 a). Salinity stress caused significant increase in Na^+/K^+ ratio. The Na^+/K^+ ratio of the tissues was generally increased with salinity (Amini and Ehsanpour, 2005). 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. There is an increase in catalase activity with increase in NaCl conc. as shown in Table – 3 & Fig. – 4. Maximum catalase activity was observed at 150mM NaCl conc. (0.521) which is reduced with decrease in NaCl conc. (0.385) is observed in 0mM conc. of NaCl stress (Control).

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