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Impact of PGRs and Nutrients on CSI, NR activity, proline and SOD activity of tomato (*Solanum Lycopersicum)* genotypes under salinity condition

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Abstract: *An experiment was conducted to mitigate the salinity stress effect in tomato genotypes (PKM 1 and TNAU THCO 3) by using plant growth regulators and nutrients. Salinity was imposed by using NaCl at 100 mM concentration. Foliar application of plant growth regulators like brassinolide (0.5 ppm), salicylic acid (100 ppm), benzyl amino purine (50 ppm), ascorbic acid (100 ppm), glutathione (50 ppm), KNO_3 (0.5%) + $FeSO_4$ (0.3%) + Borax (0.2%) and nutrient PGR concoction (K_2SO_4 (0.5%) + $CaSO_4$ (0.5%) + Borax (0.2%) + NAA (20 ppm) were carried out at 20 and 40 DAT. The study revealed that, among the treatments, ascorbic acid recorded highest chlorophyll stability index (76.27%) followed by glutathione (75.63%). Salicylic acid registered superior nitrate reductase activity (148.96 µg NO_2 g^{-1} h^{-1}) followed by ascorbic acid (145.07) and brassinolide (143.90) compared to control in tomato genotypes. However, brassinolide performed better in enhancement of superoxide dismutase activity (299.17 Units mg^{-1} protein) and glutathione recorded highest proline content of (374.82 µg g^{-1}). Among the two genotypes used in this study, TNAU THCO 3 responded better for the application plant growth regulators and nutrients than PKM 1 under salinity.*

Keywords: *Tomato, salinity, PGRs, CSI, proline, NR and SOD activity*

INTRODUCTION

Agricultural production and soil health is agitated by accumulation of diverse substances to soil mainly high levels of salts. The main factor that contributes to this problem is the salt load in the water used for irrigation and the use of vast and versatile fertilizers. The soil salinity may cause several deleterious effects on growth and development of plants at physiological and biochemical level (Munns, 2002). These effects can be due to low osmotic potential of soil solution, specific ion effects, and nutritional imbalances or combined effect of all these factors (Marschner, 1995).

Higher level of salts in the soil builds up the high osmotic pressure of the soil solution which prevents the uptake of water which is necessary for seed germination. Hence, salinity affects seed germination by causing toxic effect to the embryo. Salinity decreased the germination percent, root length, callus size, coleoptile length and seedling growth (Bera *et al.,* 2006) in rice. Asha and Dhingra (2007) found that the plant height, stem diameter, dry weight of plant decreased with increasing levels of salinity in chickpea. The response of plants exposed to salinity stress is, decrease in plant water potential, which reduces plant water use efficiency (Cha-um *et al.,* 2004). Tomato is one of the most popular and widely grown vegetable crops in the world. As tomato is moderately sensitive to salinity, its fruit production was adversely affected by high salt concentration. The effects of salinity on the tomato are harmful, reducing the yield, increasing the incidence of blossom-end rot, and beneficial by increasing the concentration of soluble solids in fruits (Mizrahi and Pasternak, 1985).

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Applications of plant growth regulators (PGRs) are used to stress alleviation through enhance the plant growth and improve the yield (Mostafa and Abou Al-Hamd, 2011). Hence, the present investigation was carried out to mitigate the salinity stress effect by using various plant growth regulators and nutrients.

MATERIAL AND METHODS

The experiment was carried out with two tomato genotypes *viz.*, PKM 1 and TNAU THCO 3 with the salinity concentration of 100 mM NaCl in pot culture at glass house, Department of Crop Physiology, TNAU, Coimbatore. Red sandy soil was used for pot culture experiment by using red soil, sand and vermicompost in the ratio of 3:1:1. Uniform size pots (23 cm x 25 cm) were filled with 10 kg of soil. Twenty five days aged seedlings were transplanted and one plant was maintained in each pot. Salinity was imposed from transplanting onwards till the end of the harvest. Crop was applied with recommended dose of fertilizers (75:100:50 Kg NPK/ha, borax (10 Kg/ha) and $ZnSO_4$ (50 Kg/ha) as basal, 75 kg N/ha at 30 days after transplanting. Other operations like plant protection measures were carried out as per the recommended practice of Tamil Nadu Agricultural University, Coimbatore.

The experiment was laid out in completely randomized block design with three replications. The salinity was imposed with 100 mM NaCl water for irrigation from transplanting onwards. Nine treatments *viz.*, T_1 - Absolute control (Without salinity), T_2 - Control (Water spray), T_3 - Brassinolide (0.5 ppm), T_4 - Salicylic acid (100 ppm), T_5 - Benzyl amino purine (50 ppm), T_6 - Ascorbic acid (100 ppm), T_7 - Glutathione (50 ppm), T_8 - KNO_3 (0.5%) + $FeSO_4$ (0.3%) + Borax (0.2%), T_9 - Nutrient PGR Concoction K_2SO_4 (0.5%) + $CaSO_4$ (0.5%) + Borax (0.2%) + NAA (20 ppm) were given as foliar spray at 20 and 40 DAT.

CSI (Chlorophyll Stability Index) was estimated using the protocol described by Koleyoras (1958) and the CSI was calculated by using following formula and expressed in terms of per cent.

$$CSI = \frac{Total\,chlorophyll\,content\,(Treated)}{Total\,chlorophyll\,content\,(Control)} \times 100$$

The estimation of proline content was adopted from Bates *et al.* (1973) with slight modifications. The sample (1g) was homogenized with 10 mL of 3 per cent sulpho salicylic acid and centrifuged at 3000 rpm for 10 minutes. Two mL of the supernatant was taken in a test tube and 2 mL of glacial acetic acid, 2 mL of orthophosphoric acid and 2 mL of acid ninhydrin mixture were added. The contents were allowed to react at 100°C for 1 hour and then it was incubated on ice for 10 minutes to terminate the reaction. The reaction mixture was mixed vigorously with 4 mL of toluene for 15-20 seconds. The chromophore containing toluene was aspired from the aqueous phase, warmed at room temperature and optical density was read at 520 nm. The proline content was determined from the standard graph prepared using commercially available pure proline and expressed as $\mu g\ g^{-1}$.

Nitrate reductase activity was estimated in fully expanded functional leaves following the method of Nicholas *et al*. (1976) and the enzyme activity was expressed as $\mu g\ NO_2\ g^{-1}\ h^{-1}$. SOD activity was determined by using nitro blue tetrazolium (NBT) salt as described by Beau-champ and Fridovich (1971) and expressed in enzyme unit mg^{-1} protein. The data collected were subjected to statistical analysis in completely randomized block design following the method of Gomez and Gomez (1984).

RESULT AND DISCUSSION

CSI is an indicator of the stress tolerance capacity of the plants and is a measure of integrity of membrane (Murthy and Majumder, 1962). Measurement of CSI revealed that there was significant difference between the genotypes and treatments.

Due to salinity, CSI is declined up to 18.33 per cent in PKM 1 and 17.39 per cent in TNAU THCO 3 compared to absolute control. From the given treatments, the highest value of chlorophyll stability index was noticed in the ascorbic acid (76.27%) followed by glutathione (75.63%) and salicylic acid (75.48%), while the lowest value was observed in nutrient PGR concoction (72.93%). The positive effect of ascorbic acid is might be due to the stabilization and protection of the photosynthetic

pigments and the photosynthetic apparatus from oxidization (Hamada, 1998).

Higher CSI helps the plants to withstand under stress through better availability of chlorophyll, leading to increased photosynthetic rate, dry matter production and higher productivity. The primary effect of any stress at the cellular level is to affect the integrity of membrane which in turn leads to disruption of cellular compartment ultimately destruction chlorophyll contents (Fariduddin *et al*., 2009).

Nitrate reductase (NR) is an important enzyme for nitrogen assimilation ultimately protein synthesis in plant cell which is highly sensitive to any abiotic stress condition. NR activity is vital for the metabolic and physiological status of plants and can be used as a biomarker for abiotic stress assessment since, nitrate reductase activity decreases in plants exposed to abiotic stress (Azcon *et al*., 1996). The nitrate reductase activity was high in salicylic acid (148.96 µg NO_2 g^{-1} h^{-1}) followed by ascorbic acid (145.07) and brassinolide (143.90) and the least value for nitrate reductase activity was recorded in KNO_3 (0.5%) + $FeSO_4$ (0.3%) + Borax (0.2%) (135.50 µg NO_2 g^{-1} h^{-1}) (**Table. 1**).

This shows that the enzyme activity is accelerated by the foliar spray of growth regulators and nutrients. According to the results obtained by Bhupinder and Usha (2003), foliar spray of salicylic acid protects NR activity and maintains protein and nitrogen content under abiotic stress in wheat seedlings. Brassinosteroid alleviates the adverse effects of salt-stress on growth, pigmentation, and nitrate reductase activity in rice (Anuradha and Rao, 2003).

Accumulation of **proline** in plants is a mechanism by which plants tolerate salt stress and develop anti-stress ability. Proline acts as an osmoprotectant, membrane stabilizer and reactive oxygen species (ROS) scavenger, protection of sub-cellular structures, enzymes and in increasing cellular osmolarity (turgor pressure) that provide the turgor necessary for cell expansion under stress conditions (Reddy *et al*., 2004). Present findings also revealed that, proline content was increased under salt stress compared to absolute control indicated that the adoptive mechanism of crop plant under stress condition. However, the increment is not sufficient to protect the plant under salt stress. All the treatments showed higher proline content in

Table 1
Effect of plant growth regulators and nutrients on chlorophyll stability index (%) and Nitrate reductase activity (µg NO_2 g^{-1} h^{-1}) of tomato genotypes under salinity

Treatments	*Chlorophyll stability index (%)*			*Nitrate reducates activity (µg NO_2 g^{-1} h^{-1})*		
	PKM 1	*TNAU THCO 3*	*Mean*	*PKM 1*	*TNAU THCO 3*	*Mean*
Absolute control	79.55	85.19	82.37	177.28	188.48	182.88
Control (Water spray)	67.23	72.57	69.90	133.40	99.50	116.45
Brassinolide (0.5 ppm)	71.63	77.79	74.71	161.29	126.50	143.90
Salicylic acid (100 ppm)	72.08	78.87	75.47	163.46	134.46	148.96
Benzyl amino purine (50 ppm)	71.29	76.72	74.00	149.30	129.37	139.34
Ascorbic acid (100 ppm)	73.01	79.52	76.26	158.31	131.82	145.07
Glutathione (50 ppm)	72.52	78.73	75.62	154.50	125.02	139.76
KNO_3 (0.5%) + $FeSO_4$ (0.3%) + Borax (0.2%)	71.27	76.31	73.77	150.49	120.50	135.50
Nutrient PGR Concoction [K_2SO_4 (0.5 %) + $CaSO_4$ (0.5 %) + Borax (0.2 %) + NAA (20 ppm)]	69.74	76.12	72.93	153.40	126.50	139.95
Mean	**72.03**	**78.87**	**75.45**	**155.71**	**131.35**	**143.52**
	V	T	**VxT**	V	T	**VxT**
SE (d)	0.39	0.83	1.17	0.68	1.45	2.05
CD (P=0.05)	0.79	1.68	2.38	1.38	2.94	4.16

both genotypes compared to control and absolute control. Glutathione registered the highest proline content of 374.82 µg g^{-1} followed by brassinolide of 370.76 µg g^{-1}. Least proline content was observed in benzyl amino purine (358.13 µg g^{-1}) (**Figure 1**).

The positive role of glutathione on proline content might be due to involvement in the new synthesis of proline, hence glutamic acid is a precursor for proline bio-synthesis. The accumulation of proline under salt stress conditions has been correlated with increased stress tolerance in plants (Misra and Gupta, 2005). The positive effect of brassinolide on proline synthesis in pepper was already reported by Houimli *et al*. (2010) under saline condition. In addition, the increased content of chlorophylls could result from protection by proline of thylakoid membranes against the attack of ROS as reported by Kavi-Kishor *et al*. (2005).

Superoxide dismutase (SOD) is a key enzyme to nullify the effect of super oxide which is produced by Haber-Weiss reaction. The antioxidant enzymes and metabolites are reported to increase under various environmental stresses reported in tolerant cultivars than in the susceptible ones (Sreenivasulu *et al*., 2000).

Maximum SOD activity was recorded in brassinolide (299.17 Units mg^{-1} protein) followed by salicylic acid (290.35 Units mg^{-1} protein) and ascorbic acid (286.50 Units mg^{-1} protein) (**Figure 2**). The

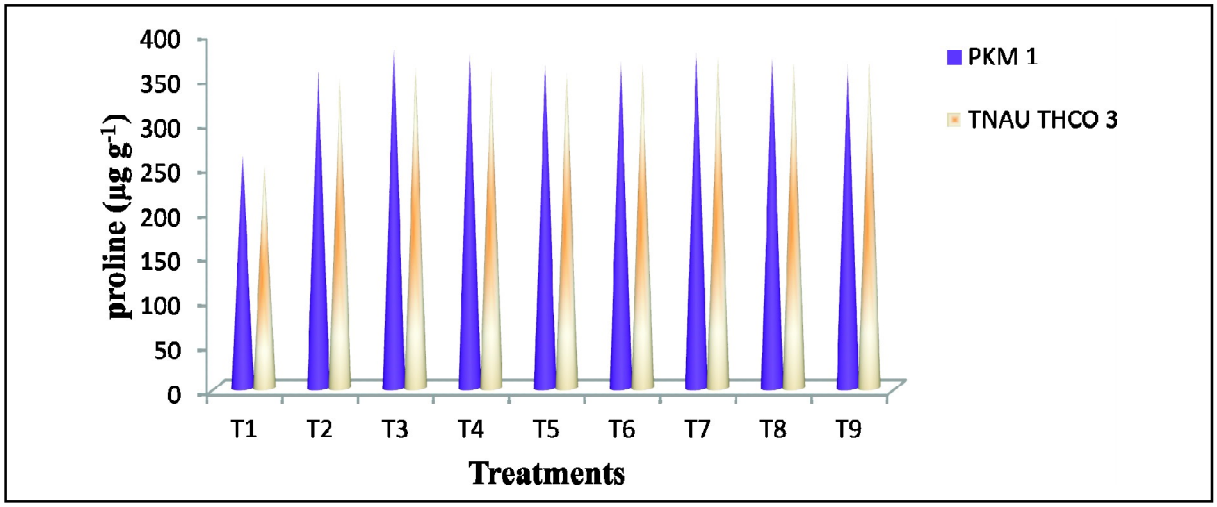

Figure 1: Effect of plant growth regulators and nutrients on proline content (µg g^{-1}) of tomato genotypes under salinity

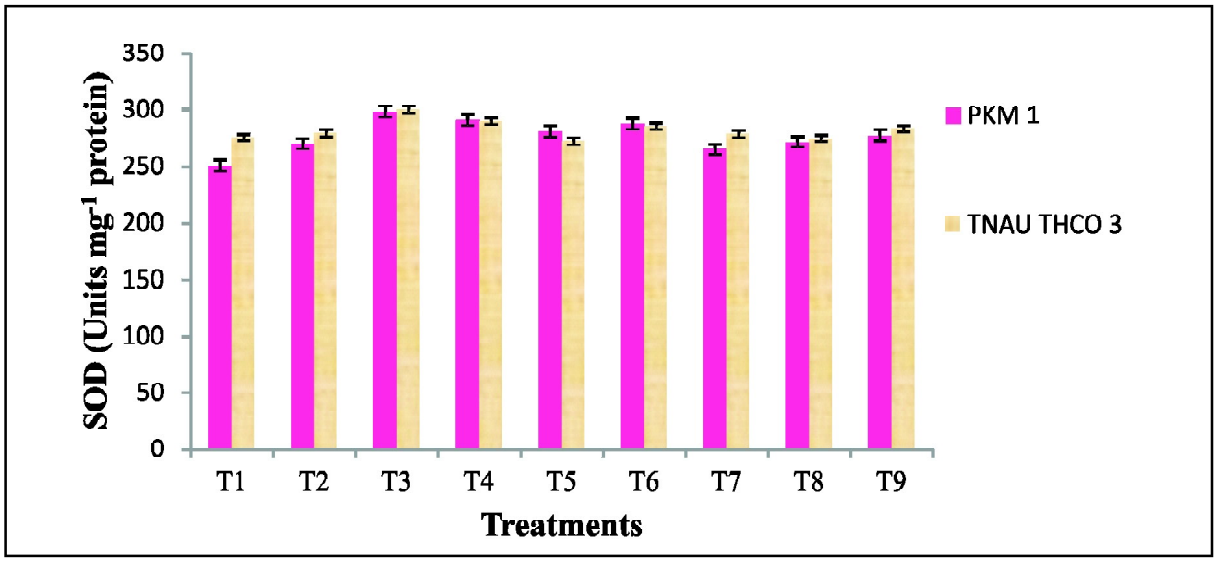

Figure 2: Effect of plant growth regulators and nutrients on SOD activity (Units mg^{-1} protein) of tomato genotypes under salinity

minimum SOD activity was registered in glutathione (272.19 Units mg^{-1} protein). This findings could be supported by the observations of El-Mashad and Mohamed (2012) furnished that the treatment with 0.05 ppm brassinolide as foliar spray mitigated salt stress by inducing superoxide dismutase, peroxidase, polyphenol oxidase activities responsible for antioxidation and detoxification as well as by elevating contents of ascorbic acid, tocopherol, and glutathione.

In other findings, salicylic acid pretreatment of young tomato plants exposed to 100 mM NaCl increased the non-enzymatic antioxidant defense system and detoxifying capacity of the plant tissue (Szepesi *et al.*, 2008). In tomato plants grown under salt stress, foliar application with salicylic acid decreased lipid peroxidation and increased activities of the antioxidant enzymes SOD, CAT, GPX and DHAR as well as the contents of ascorbate and glutathione (He and Zhu, 2008).

References

Anuradha, S and Rao, S.S.R. (2003), Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth and improved photosynthetic pigment levels and nitrate reductase activity. Plant Growth Regulation, **40**: 29-32.

Asha, S., and Dhingra, H.R., (2007), Salinity mediated changes in yield and nutritive value of chickpea seeds. Indian Journal of Plant Physiology, **12(3)**: 271-275.

Azcon, R., Gomez, M., and Tobar, R.M., (1996), Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. Biology and Fertility of Soils, **22**:156-16.

Bates, L.S., Waldren, R.P., and Teare, I.D., (1973), Rapid determination of proline for water stress studies. Plant Soil, **39**: 205-207.

Bera, A., Pati, M.K., and Bera, A., (2006), Brassionolide ameliorates adverse effect on salt stress on germination and seedling growth of rice. Indian Journal of Plant Physiology, **11(2)**: 182-189.

Bhupinder, S., and Usha, K. (2003), Salicylic acid induced physiological and biochemicalchanges in wheat seedlings under water stress. Plant Growth Regulation, **39**: 137-141.

Cha-um, S., Kirdmanee, C., and Supaibulwatana, S., (2004), Biochemical and physiological responces of thai jasmine rice (*Oryza sativa* L. sp. Indica cv.) to salt stress. Science Asia, **30**: 247-253.

El-Mashad, A.A.A., and Mohamed, H.I. (2012), Brassinolide alleviates salt stress and increases antioxidant activity of cowpea plants (*Vigna sinensis*). Protoplasma, **249:** 625-635.

Fariduddin, Q., Khanam, S., Hasan, S.A., Ali, B., Hayat, S., and Ahmad, A., (2009), Effect of 28- homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of *Brassica juncea* L. Acta Physiologiae Plantarum, **33**: 889-897.

Fridovich, I., (1976), In: Free radicals in biology, ed. W. A. Pryor. New York: Academic Press. 1: 247.

Gomez, K.A., and Gomez, A.A. (1984), Statistical procedures for agricultural research. (2nd Ed.) John Wiley and sons, NewYork, USA Pp680

Hamada, A.M. (1998), Effect of exogenously added ascorbic acid, thiamine or aspirin on photosynthesis and some related activities of drought stress wheat plants. In Garab G (eds.): Photosynthesis: Mechanism and effects. Kluwer Academic Publishers, Dordrecht, pp. 2581-2584.

He, Y., and Zhu, Z.J. (2008), Exogenous salicylic acid alleviates NaCl toxicity and increases antioxidative enzyme activity in *Lycopersicon esculentum*. Biology Plantarum, **52**: 792-795.

Houimli, S.I.M., Denden, M., and Mouhandes, B.D., (2010), Effects of 24-epibrassinolide on growth, chlorophyll, electrolyte leakage and proline by pepper plants under NaCl stress. Eurasian Journal of Bioscience, **4**: 96-104.

Kavi-Kishor, P.B., Sangam, S., Amrutha, R.N., Sri-laxmi, P., Naidu, K.R., Rao, K.R.S.S., Rao, S., Reddy, K. J., Theriappan, P., and Sreenivasulu, N., (2005), Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: ITS implications in plant growth and abiotic stress tolerance. Current Science, **88 (3)**:424-438.

Koleyoras, A.S., (1958), A new method of determining drought resistance. Plant Physiology, **33**: 232-233.

Marschner, H., (1995), Mineral Nutrition of Higher Plants,. 2nd Edition Academic Press, San Diego, CA., pp 889.

Misra, N and Gupta, A.K., (2005), Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. Plant Science, **169**:331-339.

Mizrahi, Y., and Pasternak, D., (1985), Effect of salinity on quality of various agricultural crop. Plant and Soil, **89**: 301-307.

Mostafa, G.G., and Abou Al-Hamed, M.F., (2011), Effect of gibberellic acid and indole-3 acetic acid on improving growth and accumulation of phytochemical composition in *Balanites aegyptica* plants. American Journal of Plant physiology, **6**: 36-43.

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

Murthy, K.S. and Majumder, S.K., (1962), Modifications of the technique for determination of chlorophyll stability index in relation to studies of drought resistance in rice. Current Science, **31**: 470-471.

Nicholas, J.C., Harper, J.E., and Hageman, R.H., (1976), Nitrate reductase activity in soybeans. Effect of light and temperature. Plant Physiology, **58**: 731-735.

Reddy, A.R., Chaitanya, K.V., and Vivekanandan, M., (2004), Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology*, **161**:1189-1202.

Sreenivasulu, N., Grimm, B., Wobus, U., and Weschke, W., (2000), Differential response of antioxidant com-pounds to salinity stress in salt-tolerant and salt sensitive seedlings of foxtail millet (*Setaria italica*). Physiologiae Plantarum, **109**: 435-442.

Szepesi, A., Poor, P., Katalin, G., Horvath, E., and Tari, I., (2008), Influence of exogenous salicylic acid on antioxidant enzyme activities in the roots of salt stressed tomato plants. Acta Biology Szegediensis, **52**: 199-200.