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SHORT COMMUNICATION

IMPACT OF SEED TREATMENT WITH PLANT GROWTH REGULATORS AND NUTRIENTS ON ALLEVIATION OF SALINITY STRESS IN TOMATO UNDER *in vitro* CONDITIONS

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ABSTRACT

An *in vitro* experiment was conducted with two tomato (*Solanum lycopersicum)* genotypes (PKM 1 and TNAU THCO 3) to assess alleviation of salinity stress by seed treatment using plant growth regulators and nutrients under laboratory conditions. Salt stress was imposed at 60 mM concentration by using NaCl. Plant growth regulators like indole acetic acid (100 ppm), gibberellic acid (1 mM), benzyl aminopurine (50 ppm), salicylic acid (2 mM), ascorbic acid (100 ppm) and salts potassium chloride (1%) and calcium chloride (0.5%) were used to alleviate salinity effects. The study revealed that among ameliorants used, gibberellic acid showed significantly premier observations like highest germination percentage (76.7 and 86.7%), stress tolerance index (62 and 68.2%) and alpha amylase activity (11.02 and 11.55 µg maltose g^{-1} sample) in PKM 1 and TNAU THCO 3, respectively. Catalase activity was high (32.65 and 36.25 µg H_2O_2 g^{-1} min^{-1}) in salicylic acid treatment. Hybrid tomato TNAU THCO 3 responded well to PGRs and nutrients than variety PKM 1.

Key words: α-amylase, catalase, salinity, tomato, stress tolerant index, vigour index

INTRODUCTION

Salt stress considerably suppresses plant growth and development. Salts cause osmotic stress and ion toxicity which induces oxidative stress and nutritional imbalance in plants (Noreen *et al*., 2010). Soil salinity refers to the presence of high amount of soluble salts in soil. Most salt-affected soils possess high quantity of Na^+ and Cl^- ions derived from NaCl. These ions are highly toxic when accumulated in plant, and damage cells, inhibit growth and reduce yield (Lauchli and Grattan, 2007). Salt stress decreases germination and seedling growth (Ashraf *et al*., 2010) and suppresses leaf expansion so reduces photosynthetic area and dry matter production (Mansour and Salama, 2004).

Out of 13 billion hectares of land on earth, nearly one billion hectare is affected by salinity or sodicity (Asim and Biswas, 2014). Germination and seedling establishment are critical stages in plant life cycle especially under stress conditions. Various methods have been employed by plant physiologists in different crops to alleviate salt stress. Pre-soaking of seeds with low concentration of phytohormones is reported to be beneficial for growth and yield of crop species under saline conditions. Kabar (1987) suggested that endogenous hormone level is affected by environmental stress; however external applications of appropriate growth regulators optimize physical metabolic conditions for germination. Possibly under high salt concentrations naturally present hormones may be suppressed and that seed soaking with plant growth regulators supplies sufficient hormones for normal growth. Understanding the physiological basis of seed germination, usage of plant growth regulators and nutrients under saline conditions is important to alleviate the effects of salinity stress.

Tomato (*Solanum lycopersicum*), the most popular vegetable crop, provides a substantial quantity of vitamin C and A in human diet. India is one of the major tomato growing country and the areas either come under transitional tract, dry tract or facilitated with supplemental well irrigation. Seeds of tomato are highly sensitive to salinity and seed do not germinate at > 4 d $sm^{-1.}$ Tomato varieties PKM 1 and TNAU THCO 3 hybrid are widely cultivated in Tamil nadu (India) but they highly sensitive to salinity. No work on these genotypes under salinity conditions has been conducted. Hence, the present study was aimed to alleviate the salinity effect by various ameliorants like PGRs and salts.

MATERIALS AND METHODS

The *in vitro* study on tomato (*Solanum lycopersicum*) was conducted at the Department of Crop Physiology, TNAU, Coimbatore, Tamil Nadu (India) during the year 2015-2016. The seeds of two tomato genotypes i.e. PKM 1 and TNAU THCO 3 were soaked in plant growth regulators (PGR's) and nutrient solutions for 24 hrs. The treated seeds were dried under shade for 4 hr and placed on germination paper in each petridish separately, alongwith untreated and absolute control. The germination paper was moistened at regular intervals with 60 mM NaCl solution in case of salinity treatments and water in case of absolute control. The 60 mM NaCl solution was selected on the basis of preliminary evaluation of various salinity levels with respect to seed germination wherein no seed germinated in 100 mM NaCl and only 50% seeds germinated in 60 mM NaCl solution. The treatments comprised of absolute control (no saline or any other treatment), control (only saline water treatment), indole acetic acid (100 ppm), gibberellic acid (1 mM), benzyl aminopurine (50 ppm), salicylic acid (2 mM), ascorbic acid (100 ppm), KCl (1%) and $CaCl_2$ (0.5%). Twenty seeds were allowed to germinate in each petridish under ambient conditions. The experiment was laid out in a completely randomized block design with each treatment replicated three times.

The germination of seeds was recorded at 24 hr interval upto 15 days. Seeds were considered germinated when the radicle was at least 2 mm long. Shoot length was measured from collar region to the tip of longest. Seedling root length was measured from the base of stem to the tip of longest root. Vigour index was calculated using the formula designed by Abdul-Baki and Anderson (1973).

Vigor index = (Root length + Shoot length) x Germination percentage

Stress tolerance index was calculated using the formula proposed by Dhopte and Livera (1989).

Stress tolerance index = (Vigor index of treated seedling / Vigor index of absolute control seedling) x 100.

Catalase activity was assayed by $KMnO_4$ titration method (Gopalachari, 1963) and expressed as µg H_2O_2 g^{-1} min^{-1}. A leaf sample (500 mg) was macerated with 10 mL phosphate buffer and centrifuged at 3000 rpm for 10 min. The supernatant (1 mL) was taken in 5 beakers separately and 5 mL 1.5% sodium perborate and 1.5 mL phosphate buffer added. Later, 10 mL 2N H_2SO_4 was added after enzyme extract addition at 1, 2, 3 and 4 min. interval in 1^{st} four beakers, respectively. In last beaker, 10 mL H_2SO_4 was added before enzyme extract addition. The last beaker was maintained as blank. The contents in beaker were titrated against 0.05 N $KMnO_4$. Development of pink colour and its persistence for 30 sec. was considered as end point and the volume of $KMnO_4$ consumed measured. One millilitre $KMnO_4$ is equal to 0.85 µg H_2O_2. α-amylase activity was assayed by starch iodine method (Hawk *et al*., 1954) and expressed as µg maltose g^{-1} sample. The data were analyzed by analysis of variance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Germination is a complex phenomenon involving many physio-biochemical changes that lead to the activation of embryo. Salinity induces numerous disorders in seeds during germination. In present study, germination was reduced upto 60% by salinity. Among the PGRs and nutrients tested, gibberellic acid proved superior with 76.3 and 86.7% seed germination in varieties PKM 1 and TNAU THCO 3, respectively; followed by salicylic acid with 73.3 and 85.4% germination (Fig. 1). The reduction in germination may be attributed to the osmotic and ionic effect due salts in medium. Pre-treatment of seeds with plant growth regulators and nutrients alleviated salinity stress effects in early growth stages (Ashraf *et al*., 2010). Gibberellic acid treatment enhanced seed germination of linseed (Khan *et al*., 2010) and salicylic acid stimulated germination in wheat (Dolatabadian *et al*., 2009) and lentil (Misra and Saxena, 2009) under salt stress.

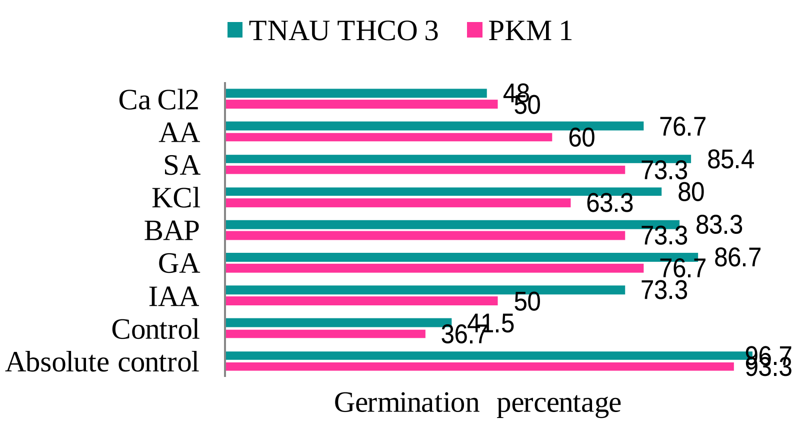

Fig. 1: Effect of plant growth regulators and nutrients on germination percentage of tomato genotypes under salinity condition; IAA - indole acetic acid (100 ppm), GA - gibberellic acid (1 mM), BAP - benzyl aminopurine (50 ppm), KCl - potassium chloride (1%), SA - salicylic acid (2 mM), AA - ascorbic acid (100 ppm) and $CaCl_2$ - calcium chloride (0.5%)

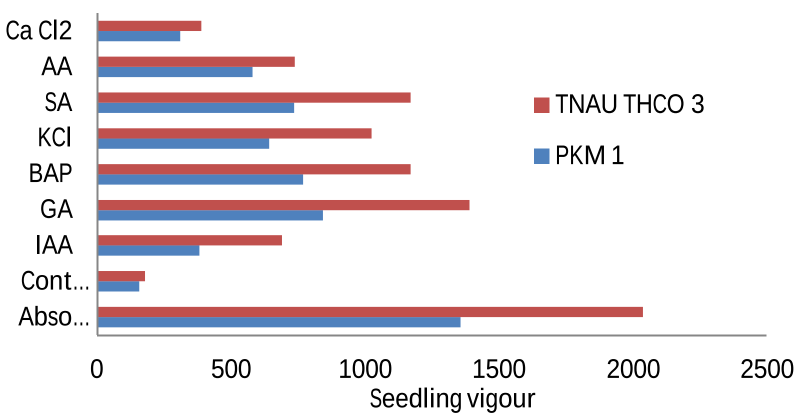

Fig. 2: Effect of plant growth regulators and nutrients on seedling vigour of tomato genotypes under salinity condition (For legends see Fig. 1)

Vigour index is a well established parameter that reveals how a seed can produce better seedling under stress conditions. Vigour index in tomato varieties PKM 1 and TNAU THCO 3 was best in gibberellic acid (842.2 and 1388.9, respectively), followed by benzyl amino-purine (768.2 and 1170.4) [Fig. 2]. Patel and Mankad (2014) attributed increase in vigour index of *Tithonia rotundifolia* seedling by gibberellic acid to the enhanced seed germination.

Stress tolerance index (STI) is a more stable character and useful tool to screen abiotic tolerant genotypes (Dutta and Bera, 2008). In present study, the tomato seeds treated with PGRs and nutrients showed higher STI than control seeds. Gibberellic acid gave highest STI of 62.0 and 68.2%; followed by benzyl aminopurine (56.6 & 57.5%). High STI revealed that higher seed germination with elevated root and shoot length leads to superior vigour index (Fig. 3). Stress tolerance due to seed pre-treatment with PGRs and nutrients suggested that these molecules trigger the expression of tolerant stress genes rather than have any direct effect as protectant (Senaratna *et al*., 2000).

Table 1: Impact of PGRs and salts on enzyme activities in tomato under salinity condition

Treatments	α-amylase activity (μg maltose g^{-1} sample)		Catalase activity (μg H_2O_2 g^{-1} min^{-1})	
	PKM 1	TNAU THCO3	PKM 1	TNAU THCO3
Absolute control (without saline)	13.97	15.36	52.39	58.55
Control (with saline)	6.27	6.78	44.08	47.70
Indole acetic acid (100 ppm)	6.32	6.75	39.20	42.80
Gibberellic acid (1 mM)	11.02	11.55	36.96	40.56
Benzyl amino purine (50 ppm)	9.31	9.86	38.55	42.15
Salicylic acid (2 mM)	10.87	11.40	32.65	36.50
Ascorbic acid (100 ppm)	9.82	10.10	34.82	38.40
Potassium chloride (1%)	8.05	8.45	40.14	43.74
Calcium chloride (0.5 %)	6.76	7.07	36.46	40.06
SE	0.098	0.124	0.522	0.598
$CD_{0.05}$	0.210	0.252	1.205	1.264

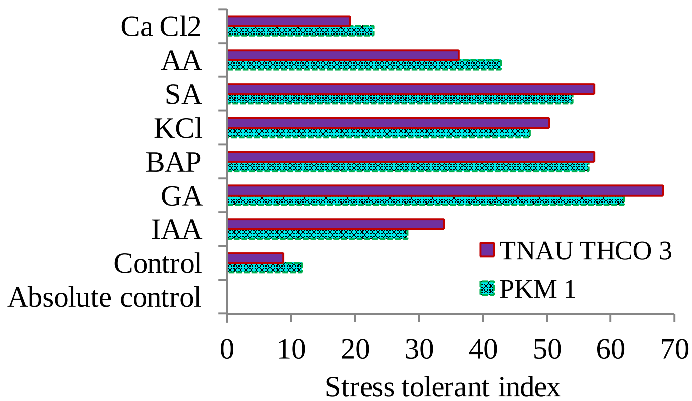

Fig. 3: Effect of plant growth regulators and nutrients on stress tolerant index of tomato genotypes under salinity conditions (For legends see Fig. 1)

Amylase activity was high in gibberellic acid (11.0 & 11.6 μg maltose g^{-1}) followed by salicylic acid (10.9 & 11.4 μg maltose g^{-1}) in PKM-1 and TNAU THCO 3, respectively (Table 1). GA reportedly is responsible for the synthesis of specific metabolite desired for seed germination in rice grown under saline conditions (Acharya *et al.*, 2008). Salicylic acid stimulates germination in wheat under stress conditions (Sakhabutdinova *et al.*, 2001).

Antioxidant enzyme activity dramatically increased under salinity stress. The seed treatments under salinity stress revealed that ameliorants like salicylic acid have highest catalase activity (32.6 & 36.5 μg H_2O_2 g^{-1} min^{-1}) followed by ascorbic acid (34.8 & 38.4 μg H_2O_2 g^{-1} min^{-1}) in PKM 1 and TNAU THCO 3 (Table 1). The lower activity was seen in absolute control than control (60 mM NaCl). It indicated that CAT activity was enhanced in salinity than in absolute control owing to adoptive mechanism in seedling against salinity. Salicylic acid and ascorbic acid act as non-enzymatic anti-oxidants which protect seedlings against salinity- induced oxidative stress by enhanced catalase activity. Catalase is the most active enzyme to offset H_2O_2 effect produced under abiotic stresses. The present study supports the findings of Kang and Saltveit (2002) that pretreatment of banana with salicylic acid before stress exposure leads to the activation of superoxide dismutase, catalase and ascorbate peroxidase. Elnaz and Ahmad (2012) demonstrated that ascorbic acid treatment significantly increased anti-oxidant enzymes like catalase, polyphenol oxidase and superoxide dismutase in sunflower plants treated with 300 mM NaCl. The study revealed that gibberellic acid and salicylic acid perform better in ameliorating stress effect. Tomato genotypes TNAU THCO 3 responded well to PGRs and nutrients under salinity than PKM 1.

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