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CO-APPLICATION OF 24-EPIBRASSINOLIDE AND PUTRESCINE ENHANCES SALINITY TOLERANCE IN *SOLANUM LYCOPERSICUM* L. BY MODULATING STRESS INDICATORS AND ANTIOXIDANT SYSTEM

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ABSTRACT

In this study, we have evaluated the co-application of epibrassinolide (24-EBL) and putrescine (Put) on stress indicators and antioxidant system of *Solanum lycopersicum* L. grown under salinity stress. EBL and Put application along with NaCl stress showed differential responses in the activities of antioxidant enzymes (superoxide dismutase, catalase and guaiacol peroxidase). Significant increase in lipid peroxidation was observed in tomato plants treated with NaCl stress. A remarkable decrease in lipid peroxidation (LPO) was observed in plants treated with 24-EBL and Put supplemented with salt stress. Application of 24-EBL and Put was able to restore the ill effects on photosynthetic pigments (PPs). Moreover enhanced titers of ascorbic acid (ASA), total phenols (TPC), glutathione (GSH), proline and glycinebetains (GB) were also observed in tomato plants supplemented with 24-EBL and Put with/or without NaCl stress.

KEYWORDS : Solanum lycopersicum, salinity stress, 24-epibrassinolide, putrescine, stress indicators and antioxidants

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INTRODUCTION

For the last few decades, anthropogenic activities are deteriorating the health of ecosystems. The contamination of soil, water and the atmosphere due to the dispersal of industrial and urban waste generated by human activities has become a major environmental concern. Among various abiotic stresses salinity stress is one of the major agricultural constraint affecting plant growth and development in most parts of the world. Nearly 20% of the world's agricultural land is affected by salinity[1]. Naturally occurring salinisation is primarily caused by elevation of capillary water level and subsequent evaporation of saline ground water leaving the salts on the soil surface. Man made salinisation is more common in irrigated areas of arid regions which are more susceptible to salinisation[2]. High salinity causes hyperosmotic stress and ion disequilibrium that produces secondary effects or pathologies[3]. Salt stress causes adverse effects due to the production of reactive oxygen species (ROS)[4]. ROS such as superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$) and singlet oxygen ($^1O_2$) are produced under salt stress. ROS are well known to cause oxidative damages to lipids, carbohydrates, DNA which ultimately results in cell death[1]. Although ROS are produced under normal conditions, when O_2 comes in contact with metabolic systems it get converted to more reactive forms such as superoxide ion ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and singlet oxygen ($^1O_2$)[5]. These ROS are produced continuously as a byproduct of various metabolic reactions like photosynthetic evolution of O_2, photorespiration and energy transfer[6, 7 and 8]. Reactive oxygen species (ROS) produced under salt stress are known to attack polyunsaturated fatty acids giving rise to lipid hydroperoxides, increasing leakiness and causes secondary damage to membrane protein, DNA and RNA. To counteract the ill-effects of salinity stress, plants have unique
stress management strategies involving the action of antioxidants like ascorbic acid (ASA), glutathione (GSH), vitamin E, flavanoids (FLA), carotenoids (CAR)[4] and antioxidant enzymes *viz.* superoxide dismutase (SOD), catalase (CAT), guaiacol peroxide (GPOX), ascorbate peroxidase (APOX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate (DHAR)[9]. Besides this protection mechanism, plants also accumulate certain solutes called osmolytes such as proline, sorbitol and glycinebetains which are known to reduce salinity stress in plants[1]. Moreover the active involvement of plant growth regulators *viz*. auxins, jasmonates, brassinosteroids and polyamines in key physiological processes have been widely accepted[10, 11, 12 and 13].

Brassinosteroids (BRs) are polyhydroxylated derivatives of cholestane, which are widely distributed in the plant kingdom. Their pleiotropic involvement in plant cell elongation, cell division, vascular differentiation, growth and reproductive development[14, 15 and 16] demonstrated their importance as indispensable component of plant metabolism. BRs have been reported to protect plants from a number of stresses like heavy metal stress[17 and 13], water stress[18], chilling stress[19], heat stress[20] and salinity stress[21].

Polyamines (PAs) are the low molecular weight polycations found ubiquitously in all living organisms and functioning in a wide range of biological processes[22]. They are a group of natural compounds with aliphatic nitrogen structure, playing an important role in physiological processes[23]. PAs are involved in protecting plants against several types of stresses e.g heavy metal stress[24], chilling stress[25], water stress[26] and salinity stress[27].

The co-application of BRs and PAs under salinity stress has not been done so far.

Therefore the aim of the present investigation was to study the exogenous coapplication of 24- epibrassinolide and putrescine in *Solanum lycopersicum* L. under salinity stress.

MATERIALS AND METHODS

(i)Plant material and treatments

The study material for the present investigation was *Solanum lycopersicum* L. Certified seeds of *Solanum lycopersicum* L. were procured from Punjab Agricultural University, Ludhiana, India. Seeds were surface sterilized with 0.01% sodium hypochlorite. Plantlets were raised from the surface sterilized seeds in the pots. Plantlets (10 cm in height) were transferred after 10 days to other pots with one plantlet in each pot, with each treatment performed in triplicate. These plants were then subjected to salinity stress with or without epibrassinolide and polyamine (Putrescine) alone or in various combinations by foliar spray method.

(ii)Treatments

The leaves of tomato plants were subjected to the following treatments:

(a)Salt Treatment

The salinity stress (NaCl) in the present investigation was generated by the application of NaCl at a concentration of 75 mM and 150 mM to 40-d-old tomato plants.

(b)Brassinosteroid Treatment

The brassinosteroid used in the present study was 24-epibrassinolide (24-EBL). Different concentrations of EBR were prepared from the stock solution of 10^{-5} M, previously prepared from 10^{-3} M EBR prepared in DMSO (Dimethyl sulphoxide). The concentration of 10^{-10} and 10^{-8} M EBR were prepared by serial dilution of the parent stock solution.

(c)Polyamine Treatment

The polyamine used in the present investigation was Putrescine dihydrochloride (Put). The Put concentration (1 mM) used in the present experiment was prepared by serial dilution of parent stock solution of 10 mM prepared in double distilled water.

(iii)Biochemical estimation of antioxidant enzymes

(a)Superoxide Dismutase (SOD, EC 1.15.1.1)

The SOD activity was determined as per method proposed by Kono (1978)[28]. About 1.8 ml sodium carbonate buffer, 750 µl nitroblue tetrazolium (NBT) and 150 µl Triton x-100 were taken in a cuvette. The reaction was initiated by the addition of 150 µl of hydroxylamine hydrochloride, followed by incubation for 2 mins and addition of 70 µl enzyme extract. This reaction mixture was taken in a cuvette and inhibition in the rate of reduction of NBT was recorded at 540 nm using UV/VIS absorption Spectrophotometer (Specord M-40, Jena, Germany).

(b)Guaiacol peroxidase (GPOX, EC1.11.1.7)

The GPOX activity was estimated as per the method of Putter (1974)[29]. In brief, reaction mixture consisted of 3 ml of phosphate buffer, 50 µl guaiacol solution, 100 µl enzyme extract and 30 µl of H_2O_2 solution. The rate of the formation of oxidized guaiacol product was followed spectrophotometrically at 436 nm using UV/VIS absorption Spectrophotometer (Specord M-40, Jena, Germany).

(c)Catalase (CAT, EC 1.11.1.6)

The activity of CAT was estimated according to the method developed by Aebi (1983)[30]. The reaction mixture consisted of 1.5 ml of potassium phosphate buffer (50 mM), 1.2 ml of H_2O_2 (150 mM) and 30 µl of enzyme extract. The change in the absorbance was read at 240 nm using UV/VIS absorption Spectrophotometer (Specord M-40, Jena, Germany).

(iv)Biochemical Estimation of antioxidants

(a)Glutathione content

The estimation of glutathione content was done by Sedlak and Lindsay (1968)[31]. About

0.5 g of treated and untreated leaves were homogenized in 5 ml of 0.2 M tris buffer. Then the extracts were centrifuged at 15,000 g at 10°C for 20 mins. About 0.2 ml of supernatant, 0.2 ml of distilled water, 0.2 ml 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) and 2 ml of methanol were taken in a test cuvette and readings were measured spectrophotometrically at 412 nm using UV/VIS (Specord M-40) absorption spectrophotometer. The concentration of GSH was determined by using reduced glutathione as standard at a concentration10 mg/ml.

(b)Determination of total phenol content

Total phenol content was determined by the method of Ragazzi and Veronese (1973)[32]. About 0.5 g F.W. of treated leaves were homogenized in 10 ml of distilled water. 1.5 ml of this extract was mixed with 3 ml of FC reagent and left undisturbed for 30 minutes in dark followed by addition of 3 ml of sodium carbonate solution. The readings of blue colored solution were taken spectrophotometrically at 680 nm.

(c)Determination of Ascorbic content

Ascorbic acid content was determined according to Cakmak and Marschner's (1992)[33] method.1g F.W. of leaf tissue was homogenized with 10 ml of methanol. Then 0.5 ml of this extract was mixed with 2.5 ml of 5% metaphosphoric acid and centrifuged at 5000 g for 20 mins. The reaction mixture consisted of 0.3 ml of centrifuged supernatant, 0.7 ml (150 mM) phosphate buffer containing 5 mM EDTA, 0.1 ml (10 mM) DTT. The excess of DTT was removed by the addition of 0.1 ml N-ethylmaleimide. After that, 0.5 ml of TCA, 2, 2'bipyridne, ethyl alcohol and 0.2 ml of ferric chloride were added, that resulted in the development of color in the reaction mixture. This reaction mixture was incubated at 45°C for 30 mins and the absorbance was measured at 525 nm. Ascorbic acid (0-100 µg/ml) was used as a positive control to determine the ascorbic acid content in the sample tissue.

(d)Proline estimation

Proline content was estimated by the method of Bates and others (1973)[34]. About 1g F.W. of tomato treated leaves were crushed in 10 ml of 3% sulphosalicylic acid and the homogenate was centrifuged at 16,000 g for 20 minutes at room temperature. After centrifugation, supernatants were mixed with 2.5% ninhydrin in glacial acetic acid. This reaction mixture was kept at 90°C in water bath for 1 h to develop the color. The reaction mixtures were then cooled immediately in ice bath followed by addition of toluene (6 ml) to separate the chromophore. The readings were taken spectrophotometrically at 520 nm and proline content was calculated by comparing the sample absorbance with the standard proline curve in a concentration range of 0-25 gm^{-3}.

(v)Biochemical Estimation of Stress Indicators

(a)Lipid peroxidation

Peroxidation of lipids was estimated according to the method of Heath and Packer (1968)[35]. Tomato leaves (0.5 g F.W.) supplied with EBL and Put with or without salinity stress were homogenized in 3 ml of 0.1% TCA. These samples were centrifuged at 10,000 g for 5 mins. Supernatants were treated with 3 ml of TBA (prepared in TCA). This solution was kept in water bath at 95°C. After 30 minutes these solutions were cooled immediately to stop the reaction. The readings were taken spectrophotometrically at 532 and 600 nm. MDA content was determined after subtracting the OD for non specific absorbance (600 nm) from the absorbance value at 532 nm.

(b)Glycinebetains

Glycinebetaine content was estimated by the method of Grieve and Grattan (1983)[36]. 1 g of oven dried leaf samples were homogenized in 10 ml of distilled water. Extracts were filtered and about 1 ml of filtered extract was mixed well with 1 ml of 2 N HCl. About 0.5 ml of this mixture was taken in a test tube and 0.2 ml of KI_3 (0.1M) was added to it and kept on ice

bath for 90 minutes with occasional shaking. To this reaction mixture, 2 ml of chilled distilled water and 20 ml of DCM were added. Two layers were formed of which the aqueous layer (upper) was discarded and lower organic layer was read spectrophotometrically at 365 nm using UV/VIS specord M-40 spectrophotometer. The concentration of betaine was calculated from a standard curve using betaine as standard at a concentration of (0 to 60 µg/ml).

(c)Determination of photosynthetic pigments

Tomato leaves (1 g DW) subjected to salinity stress with or without EBL and Put were homogenized with absolute methanol (10 ml) to make slurry. A pinch of $MgCO_3$ was added to reduce pigment decomposition. These mixtures were then kept overnight at 40°C in a refrigerator. The samples were centrifuged at 2500 g. To the resultant extract 10 ml of methanol was added. To minimize the photo-oxidation of pigments, all the procedure was performed in dim light. The readings were taken spectrophotometrically at 664.2, 648.6 and 470 nm, for the estimation of Chl a, Chl b and carotenoid contents respectively using the equations of Lichtenthaler (1987)37.

(vi)Statistical Analysis

The statistical significance of the experiments was analyzed by one way analysis of variance (ANOVA) with Tukey's test of significance. Statistical analyses were performed by using Sigmastat 3.5 software. The variance was analysed between the groups and within the groups. The mean values were expressed as mean + S.E.

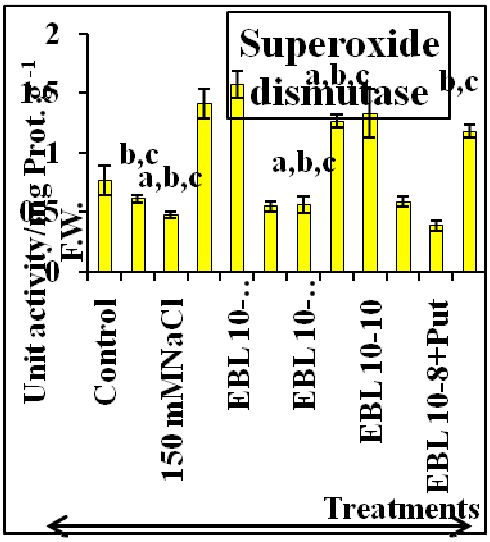
RESULTS

Effect of EBL and Put on antioxidant enzymes of tomato plants grown under salinity stress

Antioxidant enzymes are the key players of oxidative stress management. Therefore the present parameters will evaluate the effects of EBL and Put application on the enzyme activities in NaCl stressed plants, which will indicate the magnitude/type of EBL influence on the oxidative stress amelioration via modulation of antioxidant system.

Superoxide dismutase (SOD) Decrease in SOD activity was observed in plants treated with NaCl solution, in comparison with control. EBL and Put supplemented to NaCl solution enhanced SOD activity, with maximum increase noted for EBL 10^{-10} M, Put along with 75 mM NaCl solution (Fig. 1). Application of EBL alone enhanced SOD activity considerably, with significant increase in specific activity recorded in plants treated with 10^{-10} M EBL (Fig. 1).

Figure 1
Effect of EBL and Put on specific activity of SOD of 90 days old tomato plants grown under salinity stress.

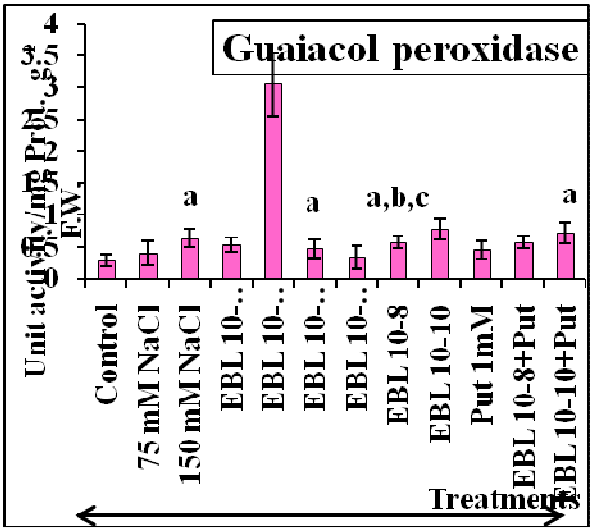


(^{a,b,c} indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at $p \leq 0.001$)

Guaiacol peroxidase (GPOX)

A small increase in GPOX activity was observed in plants treated with NaCl solution in comparison with control. A marked increase in GPOX activity was noted with EBL 10^{-10}, Put and 75 mM NaCl stress over salt stress alone (Fig. 2). No significant change in specific activity of GPOX was found in plants that underwent EBL and Put treatment alone over control (Fig. 2).

Figure 2
Effect of EBL and Put on specific activity of GPOX of 90 days old tomato plants grown under salinity stress.



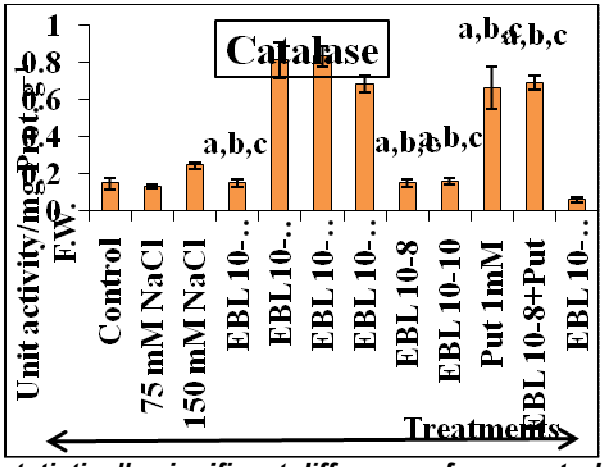
(^{a,b,c} indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at $p \leq 0.001$)

Catalase (CAT)

No significant change in CAT activity was noted for salt treated plants in comparison with untreated control. Addition of Put only enhanced CAT activity over control. A considerable increase in CAT activity was observed for salt treated plants when supplemented with EBL and Put (Fig. 3)

Figure 3

Effect of EBL and Put on specific activity of CAT of 60 days old tomato plants grown under salinity stress.

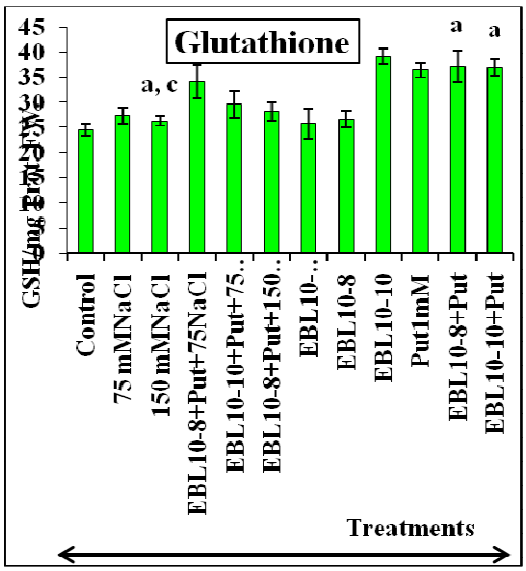


([a,b,c] indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

Effect of EBL and Put on antioxidant titers of tomato plants grown under salinity stress

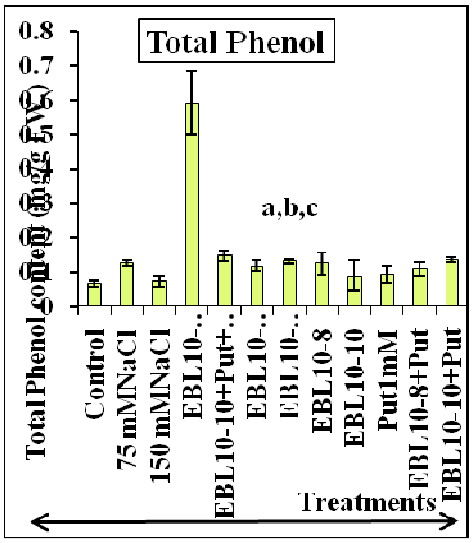
A number of antioxidants are actively implicated in stress amelioration. Assessment of EBL and Put with/without NaCl will reveal the change in antioxidant levels, which will further advance our understanding the role of EBL and Put in mitigating stress by modulating antioxidants.

Glutathione (GSH) An increase in GSH content was observed in plants growing under salinity stress than control (Fig. 4). A further increase in GSH content was found for plants underwent salinity stress and supplemented with EBL and Put as compared with control (Fig. 4). Application of EBL alone enhances titers of GSH considerably, with significant increase observed for 10^{-10} M EBL over control. Addition of Put also enhanced GSH content than untreated control (Fig. 4).



([a,b,c] indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

Total Phenol Content (TPC) TPC plays a significant role in oxidative stress mitigation. The concentration of TPC found in salt stress treated plants was significantly higher than control (Fig. 5). Plants treated with EBL and Put supplemented to NaCl solution showed increase in TPC than control, with maximum rise observed for EBL 10^{-8} M, Put and 75 mM NaCl solution (Fig. 5). No significant changes were found in plants treated with EBL and Put alone over control (Fig. 5).

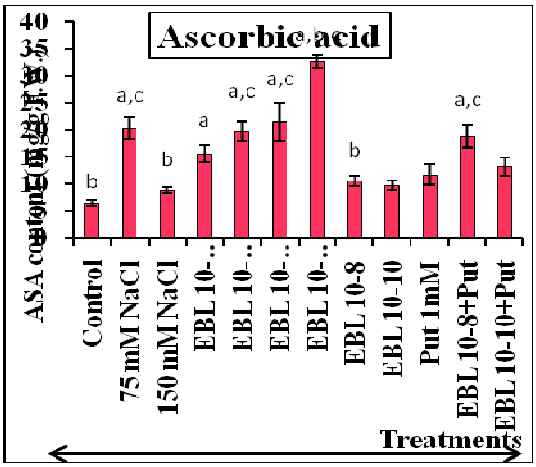


([a,b,c] indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001

Figure 5
Effect of EBL and Put total phenol content of 90 days old tomato plants grown under salinity stress.

Ascorbic acid (ASA)
ASA is a well known antioxidant, actively implicated in stress amelioration. Increase in ASA content was recorded in NaCl stressed plants when compared with controls, and supplementation with EBL

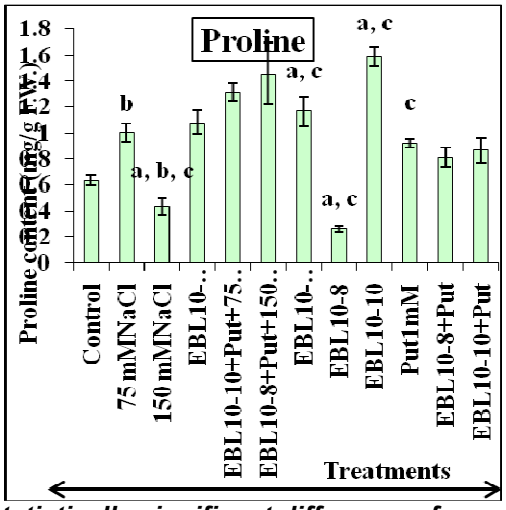
and Put was found to significantly enhanced ASA when compared with NaCl alone (Fig. 6). Elevated ASA levels were recorded for EBL and Put treatment alone over control (Fig. 6).



([a,b,c] indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

Figure 6
Effect of EBL and Put ASA content of 90 days old tomato plants grown under salinity stress.

Proline An increase in proline content was recorded in NaCl stress at a concentration of 75 mM over control, on the contrary a decrease in proline content was observed for 150 mM NaCl stress. The application of EBL and Put supplemented to NaCl further enhanced proline content when compared to salt stress treated plants alone (Fig. 7). A marked increase in proline content was noted for plants treated with EBL 10^{-10}M alone when compared with control as well as to salt treated plants alone (Fig. 7)



([a,b,c] indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

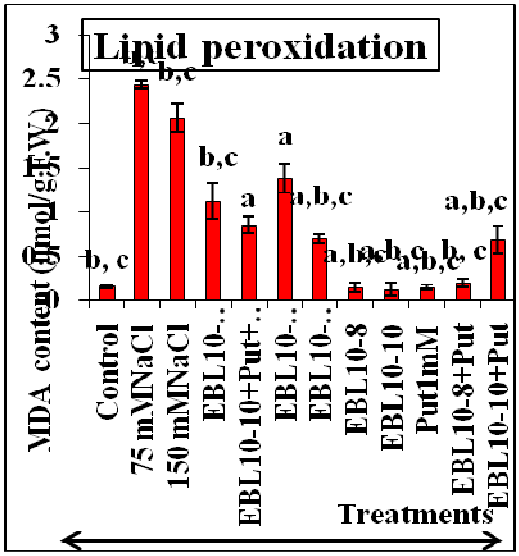
Figure 7
Effect of EBL and Put on proline content of 90 days old tomato plants grown under salinity stress.

Effect of EBL and Put on stress indicators of tomato plants grown under salinity stress

Stress indices reflect oxidative stress levels and the damages caused by stresses. Therefore, the evaluation of stress indicators against EBL and Put under salt stress may be used to learn the impact of BRs and PAs in improvement of salt stress tolerance in plants.

Lipid peroxidation

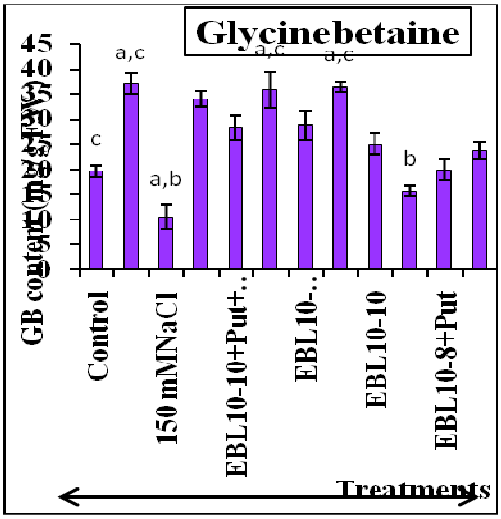
Membrane damage caused by salt stress was indicated with significant increase in MDA level as compared with control (Fig. 8). Coapplication of EBL and Put was able to reduce MDA content over salt stress alone. Individual application of EBL and Put was shown to reduce MDA content than salt treated plants alone (Fig. 8).



(^{a,b,c} indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at $p \leq 0.001$)

Figure 8
Effect of EBL and Put on MDA content of 90 days old tomato plants grown under salinity stress.

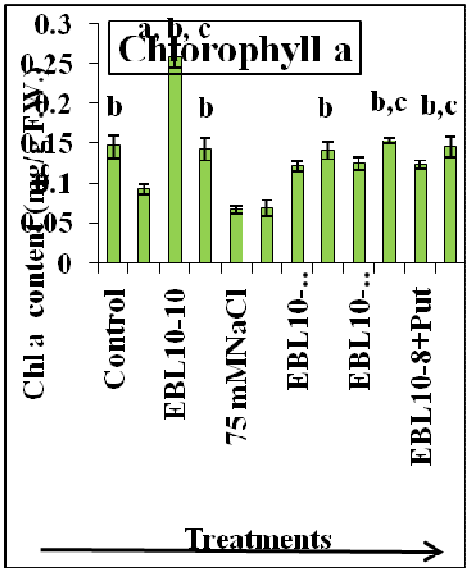
Glycinebetaine (GB) Differential responses in GB content was observed for salt treated plants (Fig. 9). Significant increase in GB content was found in plants given EBL and Put alone and in their respective combinations without salt stress as compared to control. Supplementation of EBL and Put in combination to salt stress further enhances titers of GB in comparison to salt stress alone (Fig. 9)



(^{a,b,c} indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

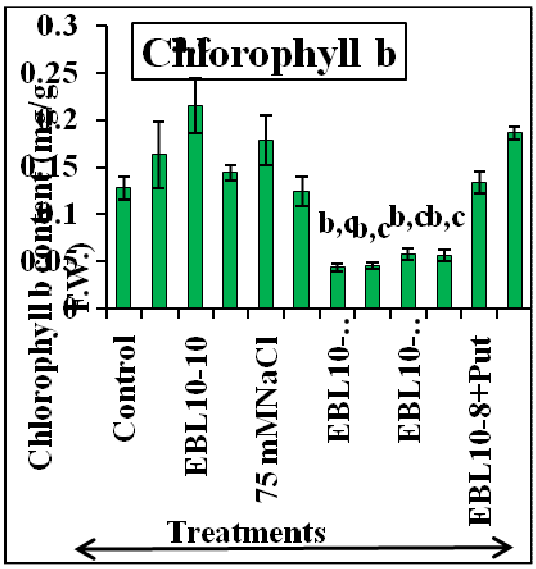
Figure 9
Effect of EBL and Put on GB content of 90 days old tomato plants grown under salinity stress.

Photosynthetic pigments (PP) PPs are known to decline under salt stress. Plants grown under NaCl stress showed reduced levels of Chl.a when compared with control, on the contrary an increase in Chl.b and CAR content was observed under salinity stress (Fig. 10, 11 and 12). However application of EBL and Put showed an improvement in PPs when compared with plants treated with NaCl stress alone. EBL and Put application alone and their respective combinations also enhanced PPs as compared with control (Fig. 10, 11 and 12).



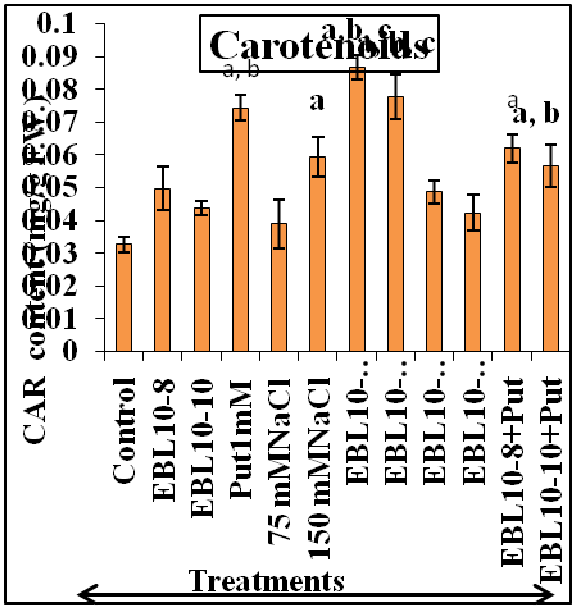
(^{a,b,c} indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

Figure 10
Effect of EBL and Put on Chl a content of 90 days old tomato plants grown under salinity stress.



(^{a,b,c} indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

Figure 11
Effect of EBL and Put on Chl b content of 90 days old tomato plants grown under salinity stress.



(^{a,b,c} indicate statistically significant differences from control, 75 mM NaCl and 150 mM NaCl stress respectively at p ≤ 0.001)

Figure 12
Effect of EBL and Put on carotenoid content of 90 days old tomato plants grown under salinity stress.

DISCUSSION

Among various abiotic stresses, salinity stress is one of the major agricultural constraint affecting plant growth and development in most parts of the world. Ubiquitously, no toxic substance restricts plant growth more than salt. Salt stress causes accelerated development, growth inhibition and senescence and ultimately leads to death after prolonged exposure to salt stress[38]. Moreover ROS are produced under stress conditions. Plants defend themselves against ROS by the actions of enzymatic and non enzymatic scavengers, which scavenge or neutralize the ROS[39]. Among phytohormones, BRs and PAs have been widely used to confer salt stress tolerance in plants[40] and 41. Keeping this in mind, the present study was therefore aimed to understand the possible ameliorative action of BRs and PAs in NaCl stress mitigation.

In the present investigation, we noted enhanced activities of GPOX, SOD and CAT in tomato plants under salinity stress which further got improved by the application of EBL and Put with or without salinity stress. Enhanced activities of these enzymes suggest their effective role in removal of H_2O_2[39]. Our results are supported by the findings of Choudhary et al. 2011[42] where they found that application of EBL and Put significantly enhanced the activities of GPOX, SOD and CAT.

The Present study revealed significant production of ASA, TPC, proline and GSH and their enhancement upon EBL and Put application alone or in combinations revealed the role of EBL and Put in ameliorating salt stress (Fig.). Phenolics may inhibit the production of ROS; act as radical scavengers or radical-chain breakers thus quenching strongly oxidative free radicals such as the hydroxyl radical[43]. The various combinations of EBL and Put with NaCl showed significant improvement in all antioxidant parameters thereby indicating positive and complementary impact of EBL and PAs interactions on the antioxidant system of tomato plants. Certain osmolytes like proline, glycinebetaine, mannitol and sorbitol get accumulated to overcome the ill-effects of salt stress in plants[1]. Enhanced titers of proline in spinach leaves treated with putrescine and ethephon under salt stress were observed by Ozturk and Demir, 2003[44]. Similarly Choudhary et al. 2010[45] observed an enhanced amount of ASA in *Raphanus sativus* seedlings supplemented with EBL and Put along with heavy metal stress.

The present study revealed the ameliorative role of 24-EBL and Put in tomato plants growing under salinity stress. Salt stress was observed to damage cell membrane as indicated by higher concentration of MDA content observed in salt stressed plants. However reduced MDA content was observed in leaves treated with EBL and Put with/or without salinity stress (Fig. 1). Decline in MDA content brought by EBL, Put and Spd can be attributed to its ability to enhance the activity of CAT, GPOX and SOD enzymes which in turn scavenge ROS and hereby reducing the impact of ROS on lipids. The present results are consistent with the results of Ali et al. 2008[46] such that EBL application could significantly reduce membrane damage in Brassica juncea L. plants subjected to salinity stress. Similarly application of 24-EBL reduce MDA content in Oryza sativa under salinity stress[47]. Phytohormonal implication in ameliorating oxidative damage under salt stress is still in an area least explored. NaCl stress was observed to increase GB content of tomato plants as compared to control. The glycinebetaine concentrations were stimulated under salinity stress suggesting their role as an effective osmoprotectant during stressful conditions[48]. No significant changes were observed in tomato plants with different combinations of EBL, Put and salt stress.

Application of NaCl stress at various concentrations results in decline in PPs. EBL treatment at various concentrations enhanced the chlorophyll values. EBL and Put at various concentrations when applied to tomato plants under salt stress, improved chlorophyll values as compared to salinity stressed plants alone

were observed. The results were consistent with the findings of Choudhary et al. 2010[45]. Increase in chlorophyll content in turn suggests improved photosynthesis and help the plant to combat the ill effects of salinity stress. Along with chlorophyll, CAR also plays an important role in oxidative stress tolerance. CARs are lipid soluble antioxidant playing an important role in oxidative stress tolerance[4]. They protect photosynthetic apparatus against various harmful stresses[49]. There was an enhancement in the CAR content in tomato plants treated with NaCl stress at various concentrations as compared to control.

The present findings, therefore indicated synergistic interactions of EBL and Put at the physiological level. The various combinations of EBL and Put supplemented to NaCl stress revealed significant improvement in the antioxidants (ASA, GSH and TPC) and modulating the activities of antioxidant enzymes (CAT, SOD and GPOX). The findings disclosed how EBL and Put reduce salt stress by significantly lowering the MDA content. Their co-application also negates the ill effects on photosynthetic pigments. Therefore, results obtained in the present investigation suggest synergistic interactions of EBL and Put in ameliorating the NaCl stress, via enhancing the titers of antioxidants and activities of antioxidant enzymes and reducing lipid peroxidation.

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