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PROMOTIVE EFFECTS OF EPIBRASSINOLIDE ON PLANT GROWTH, FRUIT YIELD, ANTIOXIDANT, AND MINERAL NUTRITION OF SALINE STRESSED TOMATO PLANTS

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Abstract

An experiment was designed in a glasshouse to test the mitigation effects of exogenously applied 24-epibrassinolide (EBL) on tomato (*Solanum lycopersicum* L.cv. 'H2274 F1') plants grown at saline regime. The plants were subjected to 0 or 100 mMNaCl 10 days after germination and they were further grown for a week. At 17 d stage, the seedlings were sprayed with deionized water (control) or 0.5 or 1.0 µM EBL. Salinity resulted in significant decreases in dry matter, fruit yield, leaf water potential, leaf relative water content and maximum fluorescence yield (*F*v/*F*m), but increased proline content, electrolyte leakage (EL), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and activities of enzymes such as, catalase (CAT; EC. 1.11.1.6), superoxide dismutase (SOD; EC 1.15.1.1),and peroxidase (POD; EC. 1.11.1.7) in plants as compared to those in non-stressed plants. However, foliar application of EBL enhanced basic growth parameters, water relations and reduced the antioxidant enzymes, proline content, electrolyte leakage, and H_2O_2 and MDA contents. Salt stress enhanced root:shoot ratio, leaf sodium (Na^+) contents and Na^+:K^+ ratio, but reduced mineral nutrients such as, phosphorus (P), nitrogen (N), calcium (Ca^{2+}) and potassium (K^+) in the leaves and roots of both cultivars. Both doses of EBL resulted in increased N, P, K^+ and Ca^{2+}contents, whereas decreased Na^+ in salt stressed plants. The findings indicate that foliar application of EBL can mitigate damage caused by salinity stress on tomato plants by lowering the levels of Na, H_2O_2, MDA, electrolyte leakage and increasing activities of key antioxidant enzymes in the leaves.

Key words: Salinity, 24-epibrassinolide, Calcium, Nitrogen.

Introduction

Abiotic stresses minimize the growth and yield of plants (Shahid *et al.*, 2011a, Siringam *et al.*, 2012). It has been well documented that salinity restricts growth and productivity of most plants (Balal *et al.*, 2012; Hu *et al.*, 2012). Plants show considerable changes in biochemical and physiological processes to minimize detrimental effects induced by salinity (Leyva *et al.*, 2011; Shahid *et al.*, 2011a; Balal *et al.*, 2012; Shahid *et al.*, 2012). Salinity stress basically causes osmotic shock and ion-toxicity which ultimately induce lethal effects in plants (Balal *et al.*, 2012).

Plants adapt different strategies to cope with both osmotic and ionic shocks induced by salinity stress. Plants mitigate the salt-induced nutritional and osmotic stress effects by producing different organic compounds like proline, glycinebetaine, polyamines, amino acids and so on in the plants (Banu *et al.*, 2009; Hajlaoui *et al.*, 2010; Ashraf *et al.*, 2011; Balal *et al.*, 2012).

Water uptake is inhibited due to reduced osmotic potential in the soil solution caused by NaCl (Garcia-Sanchez *et al.*, 2002). Due to restriction of water uptake, water contents within the plant decrease and ultimately this leads to reduce growth rate. So it is needed to enhance the water content in the plants grown at salt stress regimes by supplying both organic (sugars, amino acids, proline and glycinebetaine) and inorganic calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in order to lower osmotic potential within the plant (Shahid *et al.*, 2015).

There are different types of mechanical and bio-chemical techniques to overcome the salinity-induced harmful effects of salinity stress on plants. The reclamation of soils is one of such approaches in order to make conditions of soils more suitable for plant growth but this method is time-consuming and highly costly. So, potential of salt tolerance may be improved by using various growth substances including gibberellins, auxins, ascorbic acid, proline, oxalic acid, polyamines, glycinebetaine, and brassinosteroids (Abbas *et al.*, 2010; Ashraf *et al.*, 2010; Roychoudhury *et al.*, 2011; Shahid *et al.*, 2015).

Brassinosteroids (BSs) are a specific group of low-quantity of steroidal hormones in plants (Bajguz & Hayat, 2009) which induce a broad range of responses such as, growth of pollen tube, synthesis of nucleic acids and proteins, thereby inducing growth by increasing both cell division and cell elongation (Clouse & Sasse, 1998; Hu *et al.*, 2000). A series of researches have also shown that BSs may enhance ability of the plant to improve stress tolerance, such as salinity (Ali *et al.*, 2007), chilling stress (Fariduddin *et al.*, 2011), water stress (Fariduddin *et al.*, 2009), aluminum (Ali *et al.*, 2008), nickel (Yusuf *et al.*, 2011), cadmium (Hayat *et al.*, 2010a), heat stress (Khan *et al.*, 2015) and low temperature and poor light (Cui *et al.*, 2017).

This study was aimed to test response of 24-epibrassinolide (EBL) as anti-stress compound in tomato plants grown at salinity stress and to study the antioxidant machinery systems and the oxidative stress. The hypothesis was that EBL would improve antioxidant enzymes' activities and decline oxidative stress which might lead to overcome the deleterious effects of salinity stress by enhancing water relations, plant growth and mineral nutrition.

Materials and Methods

Plant growth and treatments: An experiment was designed in a glasshouse conditions with tomato (*Solanum lycopersicum* L.) cv. 'H2274 F1'. Seeds of tomato were sterilized using (1% v/v of sodium hypochlorite solution before sowing and then seeds (3) were sown into 5.5 L of containers containing perlite. After germination, two plants were thinned and one plant was grown on in each container. The tomato plants were grown at 20-30◦C and greater than 10 ˚C temperatures day and night times, respectively by using a heater. To minimize water losing from surface of pots, all pots were covered with a black plastic. Nutrient solution contains (mg L^{-1}): N (270 NO_3 form) P, (31) K (234), Ca (200), S (64), Mg (48), Fe (2.8), Mn (0.5), B (0.5), Cu (0.02), Zn (0.05) and Mo (0.01). The pH of the nutrient solution was adjusted at 5.5 level using 10 mM of potassium hydroxide before using. The design of experiment was a RCBD and replicated three times. Each replicate contained five seedlings (eg. 15 seedlings for each treatment).

Ten days after germination, the plants were given the nutrient solution including 0 or 100 mMNaCl for a week. At 17 d stage, A solution (20 mL pot^{-1}) of deionized water (control) or 0.5 µM 24-epibrassinolide (EBL) prepared in 0.01% T-20, were sprayed foliarly to the plants of tomato once a week. Two plants per replicate were cut at fruit set stage to determine fresh weight and key growth parameters. The remaining three plants per replicate were grown on to determine both individual and total fruit weight per plant at the fruit ripening stage. To determine dry weight, the plants were cut, separated into shoots and roots and dried at 70◦C for 48 h.

Chlorophyll content: One gram leaf samples was taken from fully expanded leaves and ground in acetone (90%; v/v). The absorbance of filtrate was run on a UV-Visible Spectrophotometer (Shimadzu UV-120, Japan) and total amount of chlorophyll content was quantified according to method (Strain & Svec, 1966).

Leaf water potential: A fully youngest leaf from each plant at 8.00 a.m. was taken for measurement of water potential using a pressure chamber (PMS model 600, USA).

Leaf free proline contents: The leaf samples were extracted for proline content as details given by kaya *et al.* (2015). The readings recorded at 520 nm following the method of Bates *et al*. (1973).

Electrolyte leakage (EL): The EL was determined following the method by Kaya *et al*. (2015).The following formula was used for estimating electrolyte leakage (Dionisio-Sese & Tobita, 1998).

$$MP = EC_1 / EC_2 \times 100$$

Chlorophyll fluorescence measurements: Before measure Maximal quantum yield (*F*v/*F*m), leaves were adapted at dark for 30 min and then *F*v/*F*m was measured using a portable chlorophyll fluorometer (Photosynthesis Yield Analyzer Mini-PAM, Walz, Germany).

Antioxidant enzymes: The detail of procedure is given elsewhere (Kaya *et al*., 2015). For determination activities of CAT, SOD and POD, the methods of Kraus & Fletcher (1994), Beauchamp & Fridovich (1971) and (Chance & Maehly, 1955) were followed, respectively.

Lipid peroxidation and hydrogen peroxide: The lipid peroxidation, as a product of malondialdehyde (MDA) content was determined according to method of Weisany *et al.* (2012). The hydrogen peroxide (H_2O_2) was measured following the procedure of Loreto & Velikova (2001). The detail of determination is provided by Kaya *et al.* (2015).

Chemical analyses: The details ofdetermining the concentrations of Sodium (Na), potassium (K) and calcium (Ca) were given elsewhere by Kaya *et al.* (2015) based on the method given by Chapman & Pratt (1982).Phosphorus and total nitrogen were determined by using the Vanadate-molybdate method as described in Jackson (1962) and the Kjeldahl apparatus, respectively.

Statistical analysis: The statistical package SAS version 9.1 (SAS Institute Inc., NC, USA) was used to obtain ANOVA of data for all parameters tested and significance was assessed at p≤5%.

Results

Some key growth and yield parameters: Salinity led to lower total chlorophyll, shoot and root dry weights of tomato as compared to these in non-stressed plants (Table 1). In salt stressed plants, the root/shoot ratio was increased due to markedly higher inhibition in shoot growth than that in root growth. However, foliar application of EBL significantly increased total chlorophyll, shoot and root growth and partly reduced root/shoot ratio in tomato crops grown under saline regime.

Salinity stress reduced both fruit yield and average fruit weight (Table 2). Both fruit yield and average fruit weight of tomato grown at saline regimes were enhanced by exogenously application of EBL, but EBL application did not significantly affect those in non-stressed plants except for 1 µM EBL treatment where it increased fruit yield.

Salinity stress reduced leaf water potential (Ψl) and maximum fluorescence yield (*F*v/*F*m) of tomato plants as compared to control tomato plants. However, salinity stress led to increase leaf osmolality (LO), leaf free proline contents and electrolyte leakage (EL) in the leaves (Tables 3 and 4). Exogenous applied EBL enhanced *F*v/*F*m and Ψl but reduced leaf proline, LO and EL.

Mineral nutrient contents: Salinity stress increased Na^+ concentrations in the leaves and roots of tomato plants grown at saline conditions, but it reduced significantly by foliar application of EBL (Table 5). Salinity stress reduced leaf K^+, Ca^{2+} P and N contents in the leaves, but exogenous application of EBL led to increase those elements in the leaves of plants grown at saline regime (Tables 5 and 6). Furthermore Na^+: K^+ ratio was increased by salinity stress, but this was lowered down by exogenous application of EBL (Table 5).

Table 1. Total chlorophyll (mg kg^{-1}), total dry weights of shoot, and root; root: shoot ratio of tomato grown in salt with or without epibrassinolide (EBL µM) applied via leaves.

Treatments	Total Chl	Total DM	RC	Root DM	Shoot DM	Root: Shoot ratio
		------------------------------ g / plant ------------------------------				
C	1254 ± 62b	159.6 ± 15.4a	100.0	9.4 ± 1.2a	150.2 ± 14.2[a]	0.063c
EBL 0.5	1295 ± 70a	161.0 ± 14.3a	100.8	9.6 ± 1.1a	151.4 ± 13.2a	0.063c
EBL 1.0	1316 ± 60a	164.0 ± 13.6a	102.8	9.7 ± 1.2a	154.3 ± 12.4a	0.063c
S	1066 ± 58e	57.7 ± 4.6c	36.2	5.6 ± 0.5c	52.1 ± 4.1c	0.107a
EBL 0.5	1116 ± 46d	85.0 ± 7.5b	53.2	6.8 ± 0.7b	78.2 ± 6.8b	0.087b
EBL 1.0	1204 ± 47c	89.3 ± 7.8b	56.0	7.0 ± 0.6b	82.3 ± 7.2b	0.085b

C: Control; S: 100 mMNaCl; Means followed by same letters in a same column show no significance among treatments at $p \leq 0.05$); ±: Standard error of means of three replicates

Table 2. Some yield attributes of tomato grown in salt with or without epibrassinolide (EBL µM) applied via leaves.

Treatments	Fruit yield g/plant	No. of fruit/plant	Average fruit weight (g/fruit)
C	6735b	43.01 a	156.6 a
EBL 0.5	6805ab	44.87 a	151.7 a
EBL 1.0	6830a	45.12 a	151.4 a
S	5715e	41.64 b	137.2 c
EBL 0.5	6041d	42.25 b	143.0 b
EBL 1.0	6250c	42.36 b	147.5 b

C: Control; S: 100 mMNaCl; Means followed by same letters in a same column show no significance among treatments at $p \leq 0.05$)

Table3. Maximum fluorescence yield (Fv/Fm), electrolyte leakage (EL) and proline (pro, µmol g^{-1}) tomato grown in salt with or without epibrassinolide (EBL µM) applied via leaves.

Treatments	Fv/Fm	EL (%)	Pro
C	0.807 ± 0.032a	14 ± 1.1c	1.10 ± 0.09d
EBL 0.5	0.802 ± 0.026a	13 ± 1.2c	1.07 ± 0.08d
EBL 1.0	0.809 ± 0.028a	13 ± 1.2c	1.06 ± 0.04d
S	0.677 ± 0.022c	25 ± 1.5a	2.97 ± 0.06a
EBL 0.5	0.702 ± 0.023b	21 ± 1.6b	2.35 ± 0.07b
EBL 1.0	0.714 ± 0.021b	19 ± 1.8b	2.12 ± 0.08c

C: Control; S: 100 mMNaCl; Means followed by same letters in a same column show no significance among treatments at $p \leq 0.05$); ±: Standard error of means of three replicates

Table 4. Leaf water potential (Ψl :MPa) and leaf osmolality (LO, Osmol/kg of tomato grown in salt with or without epibrassinolide (EBL µM) applied via leaves

Treatments	Ψl	LO
C	-0.32a	0.043d
EBL 0.5	-0.31a	0.038d
EBL 1.0	-0.31a	0.034d
S	-1.49c	0.124a
EBL 0.5	-1.32bc	0.103b
EBL 1.0	-1.18b	0.087c

C: Control; S: 100 mMNaCl; Means followed by same letters in a same column show no significance among treatments at $p \leq 0.05$)

Enzyme activities and ROS: Salinity stress resulted in an increase in the activities of enzymes such as, CAT, SOD, and POD in the leaves of plants. Exogenous applied EBL caused further increases in the activities of enzymes tested in tomato plants at saline condition (Table 7).

Moreover, concentrations of both MDA and H_2O_2 increased remarkably in the t plants subjected to saline conditions. Foliar spray of EBL lowered both H_2O_2 and MDA contents in tomato plants grown at saline regime (Table 7).

Discussion

In the present study, a significant decrease in growth attributes was observed in tomato plants grown at saline conditions. The similar results have been reported by Shahid *et al.* (2011a), Noreen & Ashraf (2009) and Shahid *et al.* (2015) in pea plants and by Kaya *et al.* (2015) in maize plant. However, the exogenous application of EBL enhanced plant growth of plants exposed to saline condition. Of the both doses of EBL, 1 µM EBL was more beneficial in most cases, by showing a significant increases in total chlorophyll, fruit yield and water relations parameters. These findings relating with the beneficial role of EBL are in agreement with earlier reports showing that foliar application of EBL improves salinity tolerance in crops, such as rice (Anuradha & Rao, 2001), (Hayat *et al.*, 2010b), bean (Rady, 2011), strawberry (Karlidag *et al.*, 2011), *Brassica juncea* (Hayat *et al.*, 2012), wheat (Talaat & Shawky, 2012), and pea (Shahid *et al.*, 2015).

The deleterious effects on fruit yield induced by salinity were mitigated by exogenous EBL, but 1 µM EBL was more effective. The results relating to the yield are consistent with the findings of Rady (2011) in bean and Shahid *et al.* (2011b, 2015) in pea. The clear role of EBL in improvement of yield is not known, but it may be suggested that its beneficial effect on yield might be due to assimilates and mineral elements being translocated to developing fruit during fruit stage and thus enhancing fruit size and weight. Different reportes have shown that EBL improve the translocation of various assimilate substances within the plant tissues (Fujii & Saka, 2001; Verma & Mishra, 2005). These reports indicate that alleviating effect of EBL could have been linked with its promoting to upregulate activities of antioxidant enzymes and improvement of assimilates within the plant body (Shahid *et al.*, 2015).

Salinity results in the reduction in leaf water potential (Ψl) as a result of reduction in water status and cell turgidity of the plants (Chapin, 1991; Hayat *et al.*, 2010b). EBL induced increases in growth of plants grown at saline regime and might have been also because of increases in leaf Ψl consequently the improvement of moisture level in leaf tissues.

Table 5. Sodium (Na^+), K^+ and Na^+:K^+ ratios in the leaves and roots of tomato grown in salt with or without epibrassinolide (EBL µM) applied via leaves.

Treatments	Leaf			Root	
	Na^+	K^+	Na^+:K^+	Na^+	K^+
	mmol/kg DW				
C	32 ± 2c	364 ± 22a	0.09d	67 ± 3c	97 ± 5a
EBL 0.5	31 ± 2c	366 ± 19a	0.09d	63 ± 3c	95 ± 5a
EBL 1.0	30 ± 3c	372 ± 21a	0.09d	64 ± 2c	96 ± 6a
S	347 ± 18a	265 ± 17d	1.31a	289 ± 18a	69 ± 4b
EBL 0.5	253 ± 13b	295 ± 17c	0.86b	192 ± 10b	74 ± 4b
EBL 1.0	230 ± 14b	338 ± 14b	0.68c	190 ± 10b	76 ± 4b

C: Control; S: 100 mMNaCl; Means followed by same letters in a same column show no significance among treatments at $p \leq 0.05$); ±: Standard error of means of three replicates

Table 6. Nitrogen (N), P and Ca^{2+}contents in the leaves and roots of tomato grown in salt with or without epibrassinolide (EBL µM) applied via leaves.

Treatments	Leaf			Root		
	N	P	Ca^{2+}	N	P	Ca^{2+}
	mmol/kg DW					
C	1132 ± 59b	66 ± 4a	172 ± 9a	659 ± 31a	16 ± 0.9c	166 ± 11a
EBL 0.5	1137 ± 53ab	65 ± 3a	174 ± 9a	656 ± 32a	17 ± 0.9c	174 ± 10a
EBL 1.0	1148 ± 51a	67 ± 4a	179 ± 10a	656 ± 33a	15 ± 0.7c	172 ± 10a
S	835 ± 36e	32 ± 2d	102 ± 6c	457 ± 26c	24 ± 1.4b	72 ± 3c
EBL 0.5	962 ± 42d	39 ± 2c	149 ± 8b	478 ± 24bc	34 ± 1.1a	110 ± 6b
EBL 1.0	1120 ± 45c	57 ± 2b	172 ± 9a	496 ± 29b	38 ± 1.2a	110 ± 8b

C: Control; S: 100 mMNaCl; Means followed by same letters in a same column show no significance among treatments at $p \leq 0.05$); ±: Standard error of means of three replicates

Table 7. Activities/concentrations of superoxide dismutase (SOD: Unit/mg protein/min), catalase (CAT: Unit x100/mg protein), peroxidase (POX: ΔA_{470}/min/mg protein), hydrogen peroxide (H_2O_2: µmol g-1 Fw) and malondialdehyde (MDA: nmol g−1 FW) in the leaves of tomato grown in salt with or without epibrassinolide (EBL µM) applied via leaves.

Treatments	SOD	CAT	POX	H_2O_2	MDA
C	46c	1.24c	8.25c	1.19d	1.42d
EBL 0.5	41c	1.22c	8.29c	1.17d	1.45d
EBL 1.0	41c	1.21c	8.22c	1.12d	1.41d
S	125b	2.24b	24.12b	6.42a	10.36 a
EBL 0.5	172a	2.92a	34.12a	4.22b	7.39b
EBL 1.0	182a	3.01a	36.14a	3.54c	6.22c

C: Control; S: 100 mMNaCl; Means followed by same letters in a same column show no significance among treatments at $p \leq 0.05$)

The maximum fluorescence yield (Fv/Fm) was significantly lowered in leaves of tomato grown at saline condition. These results agree with those reported by Shahbaz *et al.* (2008) who reported that salinity stress lowered Fv/Fm of wheat plants. The present results indicate that foliar spray of EBL to the leaves of plants grown at saline medium increased the Fv/Fm which suggest that this compound improved the protection of PS II shown as Fv/Fm. This harmful effect of salinity stress might have been due to the loss of integrity in the thylakoid membrane which are so sensitive to stress (Hayat *et al.*, 2010b; Haldimann & Feller, 2005).

Present results showed that salinity stress increased the concentration of Na^+, but reduced K^+ and Ca^{2+} in tomato plants. Similarly, it has been reported that salinity stress resulted in imbalance and deficiencies of nutrient as well as specific ion toxicity in the plants (Ashraf, 2004; Munns, 2002; 2005; Tuna *et al*., 2007). Foliar spray of EBL lowered Na^+ and increased K^+ contents in the leaf of plants exposed to saline regime. Present findings do not support the results of Hayat *et al.* (2000) who reported that EBL did not change K^+ level of mustard plants grown at saline conditions.

Furthermore, Na^+/ K^+ ratios increased in tomato plants grown at salt stress. This may be due to that high Na^+ inhibiting high affinity K^+ transporters (Gassman *et al.*, 1996; Amtmann & Sanders, 1999).

When the plants are exposed to stress, they generate huge amount of reactive oxygen species (ROS) which may oxidize proteins, nucleic acids and lipids (Schutzendubel & Polle, 2002) and this process causes abnormalities at cell levels (di Toppi & Gabbrielli, 1999). Accumulation of H_2O_2 could increased membrane leakage which might rapid the Haber-Weiss reaction, leading to the production of hydroxyl radical and thus lipid peroxidation (Mittler, 2002; Del Rio *et al.*, 2003; Karabal *et al.*, 2003; Molassiotis *et al.*, 2006; Cervilla *et al.*, 2007; Ardic *et al.*, 2009). Plants can overcome the deleterious effects of ROS successfully by inducing antioxidant metabolites such as proline and enzymes (Schutzendubel & Polle, 2002; Noreen & Ashraf, 2009). Furthermore, the activities of the antioxidant enzymes not only increased in plants grown at NaCl stress and treated with EBL compared to control plants, but also reduced malondialdehyde (MDA) and the hydrogen peroxide (H_2O_2) content. The present results are in agreement with those of Ogweno *et al.* (2008) who reported that EBL lowered both MDA and H_2O_2 content in the leaves of salinity-stressed plants. It has been suggested that the high activities of antioxidative enzymes by EBL is due to a gene regulated phenomenon (Goda *et al.*, 2002). So, EBL may regulate the detoxification of ROS incorporating it as a part of mechanism (Hayat *et al.*, 2010b).

Increase in both the activities of enzymes and accumulation of proline led to improve tolerance to NaCl stress in the present experiment. It has also been reported that EBL enhanced antioxidant enzyme activities in the plants subjected to saline condition (Ali *et al.*, 2007). The role of EBL in the controlling of ROS has been proved that they have ability to lead to regulation of special antioxidant genes and so enhance the activities of antioxidative enzyme such as CAT, SOD and POD (Nunez *et al.*, 2003; Cao *et al.*, 2005; Ogweno *et al.*, 2008). Furthermore, under stress condition, proline has a role in stabilizing cell membranes (Bandurska, 2001) and countering ROS (Matysik *et al.*, 2002) as well as act as osmoprotectant (Hartzendorf & Rolletschek, 2001) elevating the activities of antioxidant enzymes and enhanced proline improved tolerance to salinity stress in our study by enhancing plant growth, photosynthetic pigment and water relations.

Conclusions

In conclusion, salinity stress led to a considerable reduction in both dry matter and fruit yield as well as key nutrients tested in tomato plants compared to that in control plants. However, increases in leaf Na, proline, electrolyte leakage, H_2O_2 and MDA led to down-regulation of PS II activity. Exogenous applied EBL partly overcame the adverse effects of salinity stress on key physiological attributes which were positively influenced by reducing leaf Na, electrolyte leakage, MDA and H_2O_2 contents and by further increasing antioxidant enzymes and key nutrient elements.

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