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BRIEF COMMUNICATION

Amelioration of salt-induced oxidative stress in eggplant by application of 24-epibrassinolide

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Abstract

The effects of exogenous 24-epibrassinolide (EBR) on the growth, oxidative damage, antioxidant system and ion content in eggplant (*Solanum melongena* L.) seedlings under salt stress were investigated. Eggplant seedlings were exposed to 90 mM NaCl with 0, 0.025, 0.05, 0.10 and 0.20 mg dm^{-3} EBR for 10 d. EBR, especially at concentration 0.05 mg dm^{-3}, alleviated growth suppression caused by NaCl stress, decreased electrolyte leakage, superoxide production and content of malondialdehyde and H_2O_2 in NaCl-treated plants. EBR also increased activities of superoxide dismutase, guaiacol peroxidase, catalase and ascorbate peroxidase and the content of ascorbic acid and reduced glutathione. Furthermore, we also found that Na^+ and Cl^- contents were decreased, K^+ and Ca^{2+} contents and K^+/Na^+, Ca^{2+}/Na^+ ratios were increased in the presence of EBR under salt stress.

Additional key words: ascorbate peroxidase, ascorbic acid, catalase, brassinosteroids, electrolyte leakage, glutathione, guaiacol peroxidase, ion balance, reactive oxygen species, *Solanum melongena*, superoxide dismutase.

Salinity is one of the most important abiotic stresses limiting crop growth and yield (*e.g.* Eraslan *et al*. 2007). One of the biochemical changes occurring in plants subjected to salt stress is the accumulation of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (Ashraf and Foolad 2007, Daneshmand *et al*. 2010). Plants have also developed a complex defense system including enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and non-enzymatic constituents like ascorbic acid (AsA) and reduced glutathione (GSH) to protect plants against oxidative damage (Ashraf and Foolad 2007, Daneshmand *et al*. 2010, Maia *et al*. 2010). Furthermore, when plants are exposed to NaCl, cellular ion homeostasis may be impaired (Athar *et al*. 2008). Salinity was shown to increase the uptake of Na^+ or decrease the uptake of Ca^{2+} and K^+ which lead to nutritional imbalances. Low Na^+ accumulation and high shoot K^+/Na^+ and Ca^{2+}/Na^+ ratios may enhance salt tolerance in plants (Al-Karaki 2000, Liu *et al*. 2008). Therefore, enhancing antioxidant system and keeping ion balance in plant organs are necessary for improving tolerance to salinity.

Brassinosteroids (BRs) are a class of plant steroidal hormones essential for plant growth and development (Khripach *et al*. 2000, Janeczko and Swaczynová 2010, Kočová *et al*. 2010). Recent developments have shown

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Abbrevations: APX - ascorbate peroxidase; AsA - ascorbic acid; BRs - brassinosteroids; CAT - catalase; DTNB - 5-5'dithiobis (2-nitrobenzoic acid); EBR - 24-epibrassinolide; GSH - reduced glutathione; MDA - malondialdehyde; NBT - nitroblue tetrazolium; POD - guaiacol peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.
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that BRs can enhance plant tolerance to a variety of stresses, such as chilling stress (Liu *et al*. 2009b), salinity (Nunez *et al*. 2003, Ozdemir *et al*. 2004) or pathogen infection (Xia *et al*. 2009). BRs stimulate the antioxidant systems and increase the tolerance of rice to salinity (Nunez *et al*. 2003), tomato seedlings to heat stress (Ogweno *et al*. 2008), and cucumber to osmotic stress (Liu *et al*. 2009a). Eggplant (*Solanum melongena* L.), an important horticulture crop worldwide, is moderately sensitive to salinity (Akinci *et al.* 2004). Nonetheless, scarcely any studies have been conducted on the influence of BRs on eggplants under salt stress. Therefore, the aim of this study was to analyze the effects of 24-epibrassinolide (EBR) on the growth, lipid peroxidation, antioxidant system and ion balance in NaCl-treated eggplants to elucidate protective mechanism of exogenous EBR in alleviating salt injury.

Seeds of eggplant (*Solanum melongena* L. cv. Huqie 08-9) from Shanghai Academy of Agricultural Sciences, were germinated and seedlings were grown in greenhouse at natural irradiance, day/night temperature of 25/20 °C and relative humidity of 65 - 70 %. All plants were irrigated with half strength Hoagland nutrient solution every 2 d. When eggplant seedlings were at the fourth or fifth-true leaf stage, 90 mM NaCl and the 24-epibrassinolide (EBR, *Sigma*, St. Louis, USA) in different concentrations (0, 0.025, 0.05, 0.1 and 0.2 mg dm^{-3}) were added to the Hoagland nutrient solution. Control plants were grown without any additions. After 10 d, 30 plants were harvested and divided into shoots and roots. All materials were rinsed three times in distilled water after disinfecting with non-ionic detergent, then blotted on filter paper and weighed.

Electrolyte leakage was measured according to Shi and Sheng (2005). Malondialdehyde (MDA) content and superoxide production were measured as described by Jiang and Zhang (2001). H_2O_2 content was analyzed using the ferrithiocyanate method according to Sagisaka (1976). Total SOD was assayed by the nitroblue tetrazolium (NBT) method of Rao and Sresty (2000). POD was measured according to Hammerschmidt *et al*. (1982) by monitoring the rate of guaiacol oxidation at 470 nm. CAT was assayed as described by Durner and Klessing (1996) (coefficient of absorbance $\varepsilon = 39.4$ mM^{-1} cm^{-1}). APX was measured according to Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm ($\varepsilon = 2.8$ mM^{-1} cm^{-1}). The AsA concent was determined according to the method of Arakawa *et al*. (1981). The GSH content was assayed by following the change in A_{412} after the addition of 5-5'dithiobis (2-nitrobenzoic acid) (DTNB) according to the method of Griffiths (1980). Na^+, K^+ and Ca^{2+} in the leaves and roots were determined by the methods described by Allen *et al*. (1986). The Cl^- content was determined according to Chen *et al*. (2001). Differences between treatments were considered statistically significant when $P < 0.05$ according to the least significant difference (LSD) test.

The growth of eggplant was significantly reduced by NaCl (Table 1). Plant height, stem diameter, shoot fresh mass and root fresh mass decreased by 36.2, 39.5, 50.9 and 50.3 %, respectively, compared to the control. The large range of concentration of EBR (from 0.0005 to 0.5 mg dm^{-3}) has been used in previous studies (Nunez *et al*. 2003, Ogweno *et al*. 2008, Liu *et al*. 2009a). In this study, the application of 0.05 mg dm^{-3} EBR, the most appropriate concentration, enhances salt tolerance of eggplant seedlings, which was reflected in increase in plant height and fresh mass (Table 1). Amzallag (2002) emphasized that effectiveness of epibrassinolide on the growth of salt stressed plants depends on the species, plant developmental stage, concentration of epibrassinolide, and mode of application. Furthermore, since brassinosteroids have a role in cell elongation by promoting transverse orientation of microtubules (Khripach *et al*. 2000), growth improvement due to exogenous EBR application in this study might have been due to its accelerating effect on cell elongation and cell division.

Salt stress can increase ROS production, and in consequence plasma membrane leakage and the accumulation of MDA, an indicator of oxidative damage to the membranes (Athar *et al*. 2008, Hajlaoui *et al*. 2009). Treatment of leaves with 90 mM NaCl resulted in a marked increase in leakage of electrolytes, MDA content, superoxide production and H_2O_2 content (Table 1). However, exogenous EBR markedly ameliorated the oxidative damage and 0.05 mg dm^{-3} EBR caused a maximum effect. Our results are consistent with the observations made by Ozdemir *et al*. (2004), Zhang *et al*. (2007) and Arora *et al.* (2008), who observed the positive effects of BRs in salt-stressed *Oryza sativa*, *Medicago sativa* and *Zea mays*. The mechanisms that may explain EBR protective action against oxidative damage is the increasing cellular antioxidant system.

Recently, BRs have been reported to regulate the activities of antioxidant enzymes. In our study, application of EBR, particularly 0.05 mg dm^{-3} EBR, enhanced activities of SOD, POD and CAT and content of AsA and GSH, compared to the salt stress alone, suggesting that EBR play an important role in amelioration of salt-induced oxidative stress through increasing antioxidant defense system. Similarly, Arora *et al*. (2008) reported that 28-homobrassinolide increased activities of SOD, POD, CAT and APX in NaCl-stressd maize seedlings. Ozdemir *et al*. (2004) found that in rice under salinity stress EBR did not affect the SOD and CAT activities, but increased the activity of POD. The conflicted results could be related to the type of BRs, experimental material and treatments.

Previous studies have shown that salt stress may cause imbalance of cellular ions resulting in ion toxicity and osmotic stress in plants (Athar *et al*. 2008).

Table 1. Plant height [cm], stem diameter [mm], shoot fresh mass (SFM) [g], root fresh mass (RFM) [g], electrolyte leakage [%], MDA content [nmol g^{-1}(DM)], $O_2^{\cdot-}$ producing rate [nmol g^{-1}(DM) min^{-1}], H_2O_2 content [nmol g^{-1}(DM)], activities of SOD [U g^{-1}(DM)], POD [U g^{-1}(DM)], CAT [mmol g^{-1}(DM) min^{-1}] and APX [μmol g^{-1}(DM) min^{-1}], content of AsA [μmol g^{-1}(DM)], GSH [μmol g^{-1} (DM)], Na^+, Cl^-, K^+ and Ca^{2+} [mmol g^{-1}(DM)], the ratios of K^+/Na^+ and Ca^{2+}/Na^+ in eggplant seedlings subjected to salinity stress (90 mM NaCl) and 0.025, 0.05, 0,10 and 0.20 mg dm^{-3} EBR applied along with 90 mM NaCl. Means ± SD, n = 3. Different letters within each row indicate significant differences ($P < 0.05$) according to the least significant difference (LSD) test.

Parameters	Control	NaCl	NaCl+0.025 EBR	NaCl+0.05 EBR	NaCl+0.10 EBR	NaCl+0.20 EBR
Plant height	12.43±0.86a	7.93±0.31c	9.63±0.36b	11.09±0.56ab	9.89±0.84b	9.37±0.61b
Stem diameter	3.52±0.32a	2.13±0.05c	2.69±0.09b	2.79±0.26b	2.71±0.09b	2.27±0.10b
SFM	5.01±0.37a	2.46±0.09d	3.29±0.10bc	3.72±0.24b	2.96±0.26cd	2.79±0.22cd
RFM	1.67±0.10a	0.84±0.02d	1.11±0.06bc	1.48±0.17a	1.26±0.10b	1.03±0.10cd
Electrolyte leakage	13.73±1.67d	27.10±1.58a	22.71±0.97b	17.32±0.99c	18.76±1.08c	23.88±0.97b
MDA	38.85±4.19e	105.93±2.67a	68.74±7.47cd	57.80±6.11d	76.55±2.88bc	86.55±8.50b
$O_2^{\cdot-}$	4.37±0.28d	11.10±0.17a	5.41±0.19bc	4.98±0.14c	5.41±0.28bc	5.85±0.40b
H_2O_2	48.03±8.46e	215.57±9.67a	162.53±9.24c	125.27±10.1d	182.43±2.54b	195.53±5.90b
SOD	284.70±3.30d	341.60±10.5c	378.40±15.4b	403.89±8.50a	397.12±8.70a	379.09±6.00c
POD	21.14±0.80d	21.75±1.32d	33.02±0.97bc	43.86±3.75a	35.98±4.07b	29.35±1.64c
CAT	2.22±0.06e	2.50±0.05c	2.80±0.07b	3.30±0.10a	2.44±0.08c	2.35±0.04d
APX	0.75±0.02c	1.05±0.04a	1.09±0.04a	1.11±0.02a	1.06±0.04a	0.96±0.08b
AsA	3.81±0.20e	4.68±0.19d	5.30±0.14cd	7.64±0.71a	6.47±0.33b	5.85±0.26bc
GSH	2.88±0.17d	3.10±0.15d	3.63±0.13c	4.59±0.17a	4.25±0.28b	3.68±0.10c
Na^+ in roots	0.31±0.01e	2.37±0.20a	1.79±0.04b	1.19±0.06d	1.51±0.06c	1.79±0.04b
Na^+ in leaves	0.33±0.02f	1.71±0.10a	1.00±0.07d	0.65±0.03e	1.21±0.07c	1.42±0.09b
K^+ in roots	0.65±0.03a	0.30±0.02d	0.38±0.02c	0.59±0.05a	0.50±0.02b	0.38±0.02c
K^+ in leaves	1.71±0.03a	0.85±0.05d	1.45±0.02b	1.52±0.07a	1.10±0.03c	0.93±0.13d
Ca^{2+} in roots	0.20±0.01a	0.10±0.00d	0.11±0.00c	0.16±0.00b	0.12±0.012c	0.11±0.00c
Ca^{2+}in leaves	0.43±0.01a	0.25±0.02d	0.31±0.03c	0.41±0.02a	0.37±0.01b	0.30±0.01c
Cl^- in roots	0.38±0.01f	1.39±0.15a	0.88±0.06c	0.52±0.03e	0.70±0.03d	1.07±0.03b
Cl^- in leaves	0.32±0.01d	0.91±0.08a	0.62±0.04b	0.37±0.00d	0.49±0.02c	0.66±0.05b
K^+/Na^+ in roots	2.13±0.08a	0.13±0.01d	0.21±0.02d	0.49±0.02b	0.33±0.02c	0.21±0.02d
K^+/Na^+ in leaves	5.11±0.23a	0.50±0.02e	1.46±0.10c	2.36±0.17b	0.92±0.08d	0.66±0.11de
Ca^{2+}/Na^+ in roots	0.64±0.04a	0.04±0.00d	0.06±0.00cd	0.13±0.009b	0.08±0.00c	0.06±0.00cd
Ca^{2+}/Na^+ in leaves	1.30±0.08a	0.14±0.01e	0.32±0.03c	0.64±0.04b	0.31±0.02cd	0.21±0.01d

Therefore, to enhance Na^+ and Cl^- exclusion and K^+ and Ca^{2+} absorption and thereby maintain K^+/Na^+ and Ca^{2+}/Na^+ ratios is crucial for salt tolerance in high plants (Liu *et al*. 2008). In the present investigation, exogenous EBR significantly decreased the accumulation of Na^+ and Cl^-, while enhanced that of K^+ and Ca^{2+} as well as K^+/Na^+ and Ca^{2+}/Na^+ ratios (Table 1). An increase in cytosolic Ca^{2+}, as a second messenger, might induce further processes including expression of osmotic response genes and antioxidant enzymes (Athar *et al*. 2008). From these reports it is suggested that EBR-induced ionic changes might have triggered the antioxidant system. Thus, EBR-induced salt tolerance in eggplant was due to better antioxidant system for the effective removal of ROS and maintenance of ion homeostasis.

To conclude, our results suggested that a suitable concentration of EBR (0.05 mg dm^{-3}) had a protective effect on growth, antioxidant system and ion content of eggplant against salt stress. However, the detailed mechanisms need to be further elucidated.

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