Foliar Application of 24-Epibrassinolide Improved Salt Stress Tolerance of Perennial Ryegrass

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Abstract. Perennial ryegrass (*Lolium perenne* L.) is a widely used turfgrass. In this study, the effect of exogenously applied 24-epibrassinolide (EBR) on salt stress tolerance of perennial ryegrass was investigated. The results indicated that pretreatment with four concentrations of EBR (0, 0.1, 10, 1000 nM) improved salt tolerance of perennial ryegrass. Exogenous EBR treatment decreased electrolyte leakage (EL), malondialdehyde (MDA), and H₂O₂ contents and enhanced the leaf relative water content (RWC), proline, soluble sugar, and soluble protein content under salt stress condition. Meanwhile, EBR reduced the accumulation of Na⁺ and increased K⁺, Ca²⁺, and Mg²⁺ contents in leaves after salt treatment. Moreover, EBR pretreatment also increased superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) activity, as well as ascorbic acid (AsA) and glutathione contents. These results suggested that EBR improved salt tolerance by enhancing osmotic adjustment and antioxidant defense systems in perennial ryegrass.

Salt stress is one of the most serious abiotic stresses affecting plant growth and productivity in many parts of the world (Yusuf et al., 2008). High salt content in plant tissues often leads to limited water uptake and ion imbalances due to excessive accumulation of Na⁺, Cl⁻, and limited amount of mineral nutrients (K⁺, Ca²⁺, and Mg²⁺) (Hasegawa et al., 2000). Salt stress may damage plants via membrane dysfunction, metabolic toxicity, and nutrient deficiencies (Essah et al., 2003). Salt stress also causes oxidative damage due to the overproduction of reactive oxygen species (ROS) such as OH⁻, O₂⁻ and H₂O₂ (Zhang and Huang, 2011). To scavenge these toxic ROS in cells, plants evolve complex antioxidant mechanism including antioxidant enzymes, such as SOD, peroxidase (POD), CAT, APX, and nonenzymatic antioxidants like AsA and reduced glutathione (GSH) (Fariduddin et al.,

2014; Wu et al., 2014). Activities and contents of these antioxidants were changed to detoxify overproduced ROS when plants were subjected to stress.

Brassinosteroids (BRs) are a new group of steroid hormones of plants, which is widespread in plant pollen, seeds, stems, leaves, and other organs. BRs exhibit high physiological activity even at low concentrations (Singh and Shono, 2005). Many studies have shown that BRs elicit a variety of physiological processes to enhance resistance of plants against abiotic stresses, such as drought, salt, low and high temperature, and heavy metal stress (Bajguz and Hayat, 2009; Vriet et al., 2012). EBR, a highly active synthetic analogue of BRs, is known to have a promotive impact on plant growth and metabolism. EBR shows high stability under field condition (Khripach et al., 2000). Recent reports have shown that EBR alleviates the adverse effects of salt stress and produces resistance in rice (*Oryza sativa* L.; Özdemir et al., 2004). Studies with tomato (*Lycopersicon esculentum* Mill.; Ogwen et al., 2008) and cucumber (*Cucumis sativus* L.; Liu et al., 2010) have also provided compelling evidence that BRs play essential role for plant growth. While most of the EBR studies mainly focused on crop growth and productivity, there are few studies that have been conducted on the regulatory mechanism under salt stress condition, especially for turfgrass.

Turfgrasses are increasingly suffered from salt stress due to the accelerated salinization of

agricultural lands and increasing demand on use of reclaimed or other secondary saline water for irrigation (Carrow and Duncan, 1998; Jiang et al., 2013). Perennial ryegrass (*Lolium perenne* L.), a cool-season grass, is widely used for home lawns, golf course, urban landscapes, and other sports fields due to its massive root system, strong regeneration ability, and resistance to trampling (Wang et al., 2013). It is also used for winter overseeding on sites where high quality is needed, like athletic fields, golf course fairways, and tee boxes (Marcum and Pessaraki, 2010). Salt stress caused a series of damages to the perennial ryegrass. Under salt stress condition, an increase in the lipid peroxidation was observed and antioxidant activities and gene expressions were significantly changed in perennial ryegrass (Hu et al., 2011, 2012). The objective of this study was to evaluate the protective effect of EBR treatment on perennial ryegrass under salt stress condition and detect changes of osmotic adjustment and antioxidant defense system in perennial ryegrass.

Materials and Methods

Plant materials and growth condition. The experiment was conducted in the greenhouse of Beijing Forestry University, China. Seeds of perennial ryegrass 'Evening shade' were obtained from Beijing Top Green, on 20 Mar. 2013. Healthy and uniform-sized seeds were selected and sown in plastic pots (15 cm diameter and 15 cm deep) filled with a mixture of sand and vermiculite (1:1). The grass was grown for 2 months under a 14-h photoperiod at 400 μmol·m⁻²·s⁻¹, relative humidity of 65 ± 5%, and average temperature of 22 ± 1 °C/17 ± 1 °C (day/night). All grasses were irrigated every 2 d and fertilized with 200 mL half strength Hoagland nutrient solution twice a week. The grasses were then transferred to a growth chamber under controlled environmental conditions: a 12-h photoperiod at 600 μmol·m⁻²·s⁻¹, relative humidity 70%, and 23 ± 1 °C/18 ± 1 °C (day/night) temperature.

Treatments. After 2 weeks in the growth chamber, the grass seedlings were treated with EBR (Sigma-Aldrich Company, St. Louis, MO) at 0, 0.1, 10, 1000 nM concentrations. Stock solutions of EBR were prepared by dissolving it in trace ethanol and stored at 4 °C. The required concentrations of EBR were prepared from the stock solutions and sprayed with a hand-held sprayer at 2.2 mL per pot 3 d before salt stress initiation and again 7 d after first application.

The EBR-treated grass was subjected to salt stress treatment at 250 mM sodium chloride (NaCl) 3 d after first EBR application. The salt treatment was increased stepwise at 50 mM every day until the concentration of 250 mM was attained. Salt concentration was monitored by measuring the electrical conductivity of the mixture in the pot, and the same volume of distilled water was added for the control. Therefore, there were five

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treatments including 1) control: only water, 2) salt stress (250 mM NaCl), 3) salt stress (250 mM NaCl) + 0.1 nM EBR, 4) salt stress (250 mM NaCl) + 10 nM EBR, and 5) salt stress (250 mM NaCl) + 1000 nM EBR.

Leaves were collected at 0, 7, 14, 21, and 28 d after the implementation of salt treatment. Relative water content and EL were measured immediately. Samples for the measurements of physiological parameters were frozen in liquid nitrogen and stored at -80°C until use.

Determination of RWC and EL. Fresh leaf tissues (0.1 g) were weighted (W_F) and then submerged in distilled water. After 24 h, the samples were weighed as turgid weight (W_T). The leaves were then dried at 80°C for 72 h and weighed as dry weight (W_D). Relative leaf water content was calculated as follows: $\text{RWC (\%)} = (W_F - W_D) / (W_T - W_D) \times 100\%$.

Fresh leaves (0.1 g) were placed in closed test tubes containing 10 mL deionized water and EL_1 was measured after shaken on a rotary shaker for 24 h at room temperature. Samples were then boiled in a water bath for 30 min and EL_2 was measured again. The leaf EL was calculated as follows: $\text{EL (\%)} = (\text{EL}_1 / \text{EL}_2) \times 100\%$.

Determination of proline and soluble sugar contents. Proline content was determined according to the method of Li and Feng (2011). Briefly, leaves (0.1 g) were homogenized with 5 mL 3% sulfosalicylic acid and boiled at 100°C for 10 min. Then 2 mL of the supernatant was mixed with 2 mL acetic acid and 3 mL acidic ninhydrin and heated at 100°C for 40 min. The reaction mixture was extracted with 3 mL toluene after cooling and absorbance at 520 nm was measured. Soluble sugar was measured according to the method of Buysse and Merckx (1993): samples (0.1 g) were homogenized with 10 mL distilled water and the mixture was boiled at 100°C for 1 h, the supernatant was used for measurements of soluble sugar and the absorbance was recorded at 390 nm, and the soluble sugar content was calculated from a standard plot.

Determination of ion concentrations. The contents of Na^+ , K^+ , Ca^{2+} , and Mg^{2+} in samples were measured according to the methods of Greweling (1976) and Jones et al. (1991). Briefly, the dried leaves (0.5 g) were weighed and ashed in the muffle furnace at 490°C for 8 h. The ashes were then dissolved with 10 mL 5N aqua regia mixed acid [$\text{HNO}_3\text{:HCl}$ (1:3v/v)] for the determination of the concentrations of ion elements, which were measured by flame atomic absorbance spectrometry (Shimadzu Corporation, Japan).

Determination of H_2O_2 content and lipid peroxidation. The H_2O_2 content and lipid peroxidation was determined following the method of Patterson et al. (1984) and Li and Feng (2011). Leaf samples (0.1 g) were ground in 2 mL of 0.1% (m/v) trichloroacetic acid. The homogenate was centrifuged at $15,000\text{ g}$ for 15 min at 4°C , then 1 mL supernatant was added to 1 mL 10 mM potassium phosphate buffer (pH 7.0) and

2 mL 1M KI. The absorbance was read at 390 nm for H_2O_2 content measurement; lipid peroxidation was determined by measuring the content of MDA, which was extracted with 0.25% thiobarbituric acid (TBA) and the absorbance at 532 and 600 nm were measured.

Assays of enzyme activities and protein determination. Leaf samples (0.15 g) were used for enzyme extraction. Samples were ground in 2 mL of 50 mM phosphate buffer (pH 7.0). The phosphate buffer contained 1 mM ethylenediaminetetraacetic acid and 1% PVP-40. Then the extract was centrifuged at $15,000\text{ g}$ for 10 min at 4°C . The supernatant was used for measurements of enzyme activity and protein content. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction in nitroblue tetrazolium (NBT) at 560 nm (Li and Feng, 2011); POD activity was assayed by measuring the increase of absorption at 470 nm due to the oxidation of guaiacol; CAT activity was assayed by measuring the decrease of absorbance at 240 nm for 1 min, which reflect the ability to decompose H_2O_2 ; APX activity was measured as the rate of decrease in absorbance at 290 nm for 1 min (Wu et al., 2014); the soluble protein was determined following the method of Liu et al. (2013).

Determination of AsA and glutathione content. The determination of antioxidant metabolite contents were based on the methods of Li and Feng (2011). Leaves were homogenized and the supernatant was extracted at

4°C . The AsA content was determined by the absorbance at 530 nm after the addition of ascorbate oxidase, and the GSH content was measured by the absorbance at 412 nm.

Experimental design and statistical analysis. Grasses were arranged in a completely randomized block design with four replications. Results were analyzed with SPSS 18.0 (Gao et al., 2008), and the significance among different treatments were analyzed by one-way analysis of variance with significance set at $P \leq 0.05$ according to the least significant difference test.

Results

RWC and EL changes by salt and EBR treatments. As shown in Fig. 1, compared with the control, salt treatment (250 mM NaCl) decreased the RWC of perennial ryegrass by 11.3% and 14.1% at 14 d and 28 d, respectively. Application of EBR effectively improved the decline tendency when compared with salt stress treatment alone. Perennial ryegrass pretreated with 10 nM showed 6.7% and 8.3% higher RWC than those by salt stress alone at 14 d and 28 d, respectively. In contrast, the low concentration of 0.1 nM EBR had little effect on RWC (Fig. 1A).

Compared with the control, EL of perennial ryegrass was markedly increased after salt stress treatment. The highest EL was observed at 28 d after salt treatment, which increased 1.7-fold than the control. Pretreatment with three concentrations of EBR greatly decreased the EL under salt

Fig. 1. Effects of 24-epibrassinolide (EBR) on relative water content (A) and electrolyte leakage (B) of perennial ryegrass under salt stress. Different letters indicate significant difference between treatments ($P \leq 0.05$) according to the least significant difference test. Values are the mean of four replicates $\pm$ SD showed by vertical error bars.

condition. The lowest EL was obtained after 10 nM EBR treatment, which showed 38.1% and 42.0% decline at 14 and 28 d, respectively (Fig. 1B). These results indicated that exogenous EBR showed protective effects on perennial ryegrass under salt stress condition and 10 nM was the most effective concentration, so three treatments of control, salt stress, and salt + 10 nM EBR were selected for the rest of physiological data analysis.

Proline, soluble sugar, and protein content. As shown in Fig. 2, proline content exhibited a rapid increase in response to NaCl treatment and increased by 2.6-fold and 3.9-fold at 14 d or 21 d compared with the control, respectively. Application of EBR further increased the content of proline, when compared with salt treatment alone. The highest proline accumulation was found at 14 d after EBR treatment, which showed 68.0% increase than salt treatment alone (Fig. 2A).

Relative to the control, leaf soluble sugar contents of perennial ryegrass were increased because of salt stress treatment, and application of EBR further enhanced the content for 29.6% and 33.9% at 14 d and 21 d, respectively, when compared with salt stress alone (Fig. 2B).

Under salt stress condition, protein contents showed a slight increase at the beginning

and then decreased steadily, for 27.8% at 28 d after salt stress treatment. EBR pretreatment slowed down the decrease and the content increased by 37.2% when compared with salt treatment alone at 21 d (Fig. 2C).

Metal ion contents. After salt treatment, Na⁺ content in the leaves of perennial ryegrass increased up to 2.4-fold at 28 d. EBR pretreatment decreased Na⁺ accumulation in perennial ryegrass and the Na⁺ contents were 27.6% and 27.2% lower at 14 d and 21 d, respectively, than those by the salt treatment alone (Fig. 3A).

Contrary to the changes of Na⁺, a rapid decline of K⁺, Ca²⁺, and Mg²⁺ contents caused by NaCl treatment was observed. Compared with salt treatment alone, pretreatment with EBR alleviated the reduction of K⁺, Ca²⁺, and Mg²⁺ contents (Fig. 3B–D). At 21 d under stress condition, K⁺ content increased 51.6% in EBR pretreated plants, while the contents of Ca²⁺ and Mg²⁺ were also greatly increased due to exogenous EBR pretreatment (Fig. 3B–D).

H₂O₂ content and lipid peroxidation. Both H₂O₂ and MDA contents in the leaves of perennial ryegrass exhibited an increase in response to NaCl stress treatment. The maximum content of H₂O₂ appeared at 14 d after salt treatment was 71.9% higher than that in the control. MDA content also showed significant increase following salt treatment and markedly enhanced by 62.3% at 28 d after salt. EBR pretreatment decreased H₂O₂ and MDA contents compared with salt stress alone, and the largest reduction in H₂O₂ and MDA by EBR appeared at 14 d and 28 d, respectively, which were 29.6% and 21.9% lower than salt treatment alone (Fig. 4).

Enzyme activities. The activities of SOD, APX, and CAT in the leaves showed dramatically increases by 40.7%, 44.0%, and 32.3%,

respectively, at 28 d after salt treatment, while a decrease of POD activity appeared from 21 d after salt treatment. Application of EBR greatly elevated the activities of SOD, APX, and CAT when compared with salt stress alone by 18.3%, 26.4%, and 19.6%, respectively. However, pretreatment with EBR showed no effect on POD activity (Fig. 5).

AsA and glutathione contents. As compared with the control, salt stress significantly increased the content of AsA and GSH, especially for the AsA content that increased by 3.2-fold and 3.5-fold at 21 d and 28 d, respectively. EBR application elevated the content of AsA that reached the largest increasing by 94.1% at 14 d when compared with that by salt treatment alone (Fig. 6A). GSH contents were also highly enhanced by exogenous EBR treatment (Fig. 6B).

Discussion

In this study, the data showed that salt stress decreased the RWC and caused an increase of EL in the leaves of perennial ryegrass. However, application of EBR markedly enhanced the leaf water content, and reduced EL (Fig. 1), which has a positive effect to stability of membrane structure. These results were consistent with previous studies, which have reported that EBR pretreatment enhanced the RWC and decreased membrane permeability of *Chorispora bungeana* Fisch. et Mey under drought stress condition (Li et al., 2012) and improved the salt tolerance of two wheat (*Triticum aestivum* Linn.) varieties (Sairam, 1994). Similar results were also reported in *Brassica juncea* (L.) (Yusuf et al., 2008), which indicated that exogenous application of EBR-protected perennial ryegrass from salt stress-induced damages by decreasing the water loss and reducing damage of membrane.

Fig. 2. Effects of 24-epibrassinolide (EBR) on proline (A), soluble sugar (B) and protein content (C) of perennial ryegrass under salt stress. Different letters indicate significant difference between treatments ($P \leq 0.05$) according to the least significant difference test. Values are the mean of four replicates $\pm$ SD showed by vertical error bars.

Fig. 3. Effects of 24-epibrassinolide (EBR) on the content of Na⁺ (A), K⁺ (B), Ca²⁺ (C), and Mg²⁺ (D) in perennial ryegrass under salt stress. Different letters in each column indicate significant difference between treatments ($P \leq 0.05$) according to the least significant difference test. Values are the mean of four replicates $\pm$ SD showed by vertical error bars.

Salt treatment increased the contents of proline and soluble sugar, but decreased soluble protein content in leaves of perennial ryegrass (Fig. 2), Dalio et al. (2011) have reported that proline and soluble sugar show more active roles to protecting plants from salt stress in *Cajanus cajan* (L.). In this study, the decrease of soluble protein was probably due to the protein catabolism induced by NaCl treatment, since salt stress reduce the synthesis of proteins and promote protein degradation. A similar decrease of protein content in wheat under

salt stress has been reported by Shahbaz and Ashraf (2008). As osmotic adjustment substances, proline, soluble sugar, and protein played essential roles for plants to adapt to stress (Dalio et al., 2011). Proline, under stress conditions, acts not only as osmotic substances, but also as a source of carbon and nitrogen for recovery from stress (Jain et al., 2001) and ROS scavenger protecting the plant under stress conditions, in addition, soluble sugar acts as the carbon frame to synthetic organic and provide energy for metabolism, proline shows protective effects on protein degradation (Li et al., 2012). Under stress conditions, plants evolved a high capacity to accumulate osmoprotectants for intracellular osmotic homeostasis. In our study, compare with salt condition, a further drastic increase in content of proline, soluble sugar, and protein were observed after the treatment with EBR, indicating that the induction of osmoprotectants is tightly related to the improved salt tolerance in perennial ryegrass. These observations were consisted with the result obtained in snap beans (*Phaseolus vulgaris* L.) that osmotic adjustment can be considered to be an important process in the EBR-induced protective reaction of plants to salt stress (El-Bassiony et al., 2012).

As described above, salt stress adversely affected the ion homeostasis, which caused an increase of Na^+ and drastically decrease in K^+ , Ca^{2+} , and Mg^{2+} contents. Exogenous application of EBR mitigated the accumulation of Na^+ and enhanced K^+ content as well as Ca^{2+} and Mg^{2+} (Fig. 3). The results were consistent with the observations in eggplant (*Solanum melongena*) and wheat by Ding et al. (2012) and Ali et al. (2006). Accumulation of excess Na^+ perturbs metabolic processes, and the high Na^+ concentration results

in the decreased K^+ , Ca^{2+} , and Mg^{2+} influx and increased Na^+ influx, which lead to ion toxicity and osmotic stress that seriously affect the normal growth of plants (Fariduddin et al., 2013b). Therefore, improved Na^+ exclusion and K^+ , Ca^{2+} , and Mg^{2+} absorption showed protective effect for salt tolerance in plants (Ali et al., 2006). Thus, exogenously applied EBR could ameliorate ion toxicity and nutritional imbalance caused by salt stress, and promoted the process of osmotic adjustment.

In accordance with the results of Athar et al. (2008), treatments with NaCl resulted in a marked increase in MDA and H_2O_2 contents. However, foliar application of EBR effectively reduced MDA and H_2O_2 content, and particularly treatment at 10 nM caused maximum effects (Fig. 4). The similar reduction in MDA and H_2O_2 contents due to BRs application were also observed in rice (Özdemir et al., 2004) and *Medicago sativa* (L.) (Zhang et al., 2007) under salt stress. Under normal condition, the generation and degradation of ROS in plants were in dynamic equilibrium. When subjected to salt stress, plants produce large amounts of ROS such as OH^- , O_2^- , and H_2O_2 (Fariduddin et al., 2013a; Mittler, 2002) which cause a series of oxidative damage and lead to the accumulation of MDA. MDA as the indicator of lipid peroxidation directly reflects the degree of injury suffered in the plant by salt stress (Athar et al., 2008). The levels of MDA and H_2O_2 content were lower in EBR-treated plants than those by salt treatment alone, which indicated that exogenous EBR reduced the lipid damage and protected the structural integrity of the membranes, thereby, alleviating oxidative damage induced by salt stress.

In this study, the activities of SOD, APX, CAT, and POD increased in response to salt stress. However, EBR pretreatment at 10 nM markedly stimulated the activities of SOD, APX, and CAT, and a slight increase in the POD activity compared with salt stress treatment alone (Fig. 5). Previous reports have showed that application of BRs had a positive effect on antioxidant enzymes to protect plants under stress condition (Alscher et al., 2002; Ogwenio et al., 2008). Bajguz and Hayat (2009) found that treatment with EBR increased CAT and APX activities in *Chlorella vulgaris*. Eggplant pretreated by EBR showed great increase in SOD, POD, CAT, and APX activities and increased tolerance to high temperature stress (Wu et al., 2014). To scavenging the overproduction of ROS in cells caused by stress, plants evolve complex antioxidant mechanism including antioxidant enzymes, such as SOD, POD, CAT, APX, and non-enzymatic antioxidants like AsA and GSH (Fariduddin et al., 2014). The efficiency of ROS scavenging system in plants play an important role in protecting plants from oxidative stress: SOD is the first line of defense to counter the superoxide (O_2^-) radical and catalyzes the conversion of O_2^- to H_2O_2 , then H_2O_2 can be eliminated by

Fig. 4. Effects of 24-epibrassinolide (EBR) on H_2O_2 (A) and malondialdehyde (MDA) (B) content of perennial ryegrass under salt stress. Different letters indicate significant difference between treatments ($P \leq 0.05$) according to the least significant difference test. Values are the mean of four replicates $\pm$ SD showed by vertical error bars.

Fig. 5. Effects of 24-epibrassinolide (EBR) on superoxide dismutase (SOD) (A), ascorbate peroxidase (APX) (B), catalase (CAT) (C), and peroxidase (POD) (D) activities of perennial ryegrass under salt stress. Different letters in each column indicate significant difference between treatments ($P \leq 0.05$) according to the least significant difference test. Values are the mean of four replicates $\pm$ SD showed by vertical error bars.

Fig. 6. Effects of 24-epibrassinolide on ascorbic acid (AsA) (A) and reduced glutathione (GSH) (B) content of perennial ryegrass under salt stress. Different letters indicate significant difference between treatments ($P \leq 0.05$) according to the least significant difference test. Values are the mean of four replicates $\pm$ SD showed by vertical error bars.

CAT, POD, and APX, and broken into H_2O and O_2^- (Alscher et al., 2002; Behnamnia et al., 2009). APX is crucial for the removal of H_2O_2 in the cytosol and chloroplast (Li and Feng, 2011). So, the increase in the activities of antioxidant enzymes and reduction of ROS resulted in alleviating oxidative injury and concurrently improving the grasses' resistance to salt stress. These results suggest that EBR-induced improvement in growth of perennial ryegrass under saline condition may due to improved SOD-APX-CAT antioxidant system.

As nonenzymatic antioxidants, AsA and GSH have also been shown to play an essential role in protecting plants from salt stress (Yuan et al., 2013). In our study, the enhancement of AsA and GSH content were observed in the plant subjected to salt stress alone and by foliar spray of EBR under salt conditions (Fig. 6). Similar results have also been observed in *Xanthoceras sorbifolia* Bunge seedlings under water stress (Li and Feng, 2011). The increase in AsA is consistent with the higher level of APX activity after treatment with EBR under salt stress. As the substrate of synthetic APX, the higher content AsA stimulated the increase of APX activity, while GSH also accelerated the metabolism of antioxidant enzyme and participated in the regeneration of the entire cycle of the antioxidant systems (Bartwal et al., 2013). These results suggested that EBR treatment elevated the level of antioxidant system and enhance the salt tolerance of perennial ryegrass.

In conclusion, application of EBR (the optimum concentration was 10 nM) regulated a variety of physiological processes to overcome the inhibitory effect caused by salt

stress, and then alleviated the salt stress-induced membrane injury. Accumulation of osmotic regulation substances after EBR pretreatment showed positive effect on intracellular osmotic homeostasis, which protects the perennial ryegrass from ion toxicity and nutrition imbalance caused by salt stress. Concurrently, the antioxidant defense systems also played important roles to alleviate the adverse effects caused by salt stress in perennial ryegrass. In this study, effects of EBR and salt stress on most physiological parameters were found as early as 7 d after salt stress initiation under growth chamber conditions. Therefore, 4 weeks of salt stress exposure can be considered long enough to test EBR effects on salt tolerance in perennial ryegrass. The results of this study suggest EBR may have a great potential of use for turfgrass management in the soils with high salt content. However, further investigation of the EBR effects on salt stress tolerance in turfgrasses is needed under field conditions.

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