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MODULATION OF MORPHO-PHYSIOLOGICAL TRAITS OF *Leymus chinensis* (TRIN.) THROUGH EXOGENOUS APPLICATION OF BRASSINOLIDE UNDER SALT STRESS

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ABSTRACT

Plant environmental responses involve dynamic changes in growth and signaling, yet little is understood as to how progress through these events is regulated. A pot experiment was conducted to assess whether exogenously applied brassinolide (BR) at various concentrations (0, 0.01, 0.1 and 1.0 mg/L) could alleviate the adverse effects of salt stress (0 and 150 mmol/L NaCl) on *Leymus chinensis*. The results revealed that salt stress substantially disrupted the growth, development and chlorophyll contents of *L. chinensis* which were improved by foliar application of BR. Moreover, as compared with the control, salt stress exacerbated the malondialdehyde content, and cell membrane permeability while decreased the leaf area, plant height, fresh and dry weight and root shoot ratio which ultimately hampered the seedlings growth. Applying BR elevated the contents of free proline, protein, and soluble sugars and thus, promoted the seedlings growth, being able to effectively alleviate the damage of NaCl stress. Although the activities of antioxidant enzymes, such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and glutathione reductase were increased due to salt stress in plant, however, exogenously applied BR further elevated the level of the antioxidant system. In conclusion, improvement of growth, development, osmolytes, chlorophyll contents and antioxidant enzymes in *L. chinensis* due to foliar applied BR was found to be associated with BR-induced positive role in stress amelioration.

Keywords: *Leymus chinensis*; Growth; Salt stress; Brassinolide (BR); Antioxidant enzymes.

INTRODUCTION

China is one of the countries with the most plentiful grassland resources in the world. Natural grassland is considered one of the most important renewable resources in arid and semi-arid regions of China. Moreover, grassland covers an area of approximately 400 million hectares, which is about 40% of the whole land area of China and 12% of the world’s grassland. During the last decades, the degradation of environment has rapidly aggravated and the total area of degraded steppe within the Xilin River Basin has increased up to 72% from 1985 to 1999 (Tong *et al*., 2002).

Leymus chinensis (Trin.) Tzvel. (sheep grass), is a key dominant species of typical grassland communities with significant economic and ecological value. Owing to its economical value, ecological importance and tolerant traits towards stress, it is regarded as one of the most promising grass species having great potential for grassland rehabilitation and restoration (Liu and Qi, 2004). It is a perennial plant with rhizomes, good palatability, and high forage value. Rhizomes of this dominant grass mainly lie horizontally under the soil surface, and are generally highly branched (Wang *et al*., 2004). Moreover, it is considered rich in proteins, carbohydrates, minerals with high potential to withstand against salt stress (Huang *et al*., 2002).

Soil salinity is generally regarded as one of the main environmental issues which is becoming dauntless challenge to the growth, development and productivity of plants and ultimately results in land degradation (Lin *et al*., 2014). Almost 7% of the world’s total land area is subjected to salts and the area is still exacerbating as a result of land degradation. There are approximately 2.56 million hectares of saline soils mainly distributed in north, northwest, northeast and other regions of China (Liu *et al*., 2001). The salinization-alkalization of soil is an increasing environmental issue and is considered a dominant limiting factor for declining grassland sustainability (Yang *et al*., 2007). In most salt-alkaline soil of northeast China, the main harmful salts are NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3, coming from neutral salts (Yang *et al*., 2008).

During recent decades, research pertaining to stress responses of halophytic plants has aided our understanding about the morphological, physiological, and biochemical processes of stress mitigation in plants. Thus, the halophyte *L. chinensis* has been regarded as a perfect model plant for investigating the stress adaptation mechanisms. The detrimental effects of salt stress on plant growth and productivity are due to its ionic and osmotic stress which severely hampers numerous physiological and biochemical mechanisms (Munns, 2005). Plants are supposed to sustain osmotic and ion balance by controlling the absorption, transportation, and sequestration of salt ions and accumulation of certain organic solutes (Flowers and Colmer, 2008). Numerous studies indicated that the photosynthesis and photosynthetic pigments are affected by a variety of stressful conditions (Li *et al*., 2011). Moreover, salt stress causes oxidative damage in plants by production of some reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). These ROS are generally produced in the cell and interact with vital cellular molecules and metabolites thereby causing a number of destructive processes and ultimately results in cellular damage (Ashraf, 2009). Malondialdehyde (MDA) is regarded as suitable marker for lipid peroxidation that has shown greater accumulation under stress conditions (Sofo *et al.,* 2004). Additionally, the accumulation of some compatible solutes and exudation of numerous organic acids are the regular defense responses among plants which have great potential to confer stress tolerance. The plant species are likely to evolve mechanisms to protect their cellular and subcellular systems from the deleterious effects of ROS by enhancing activities of some antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, and glutathione reductase (Agarwal and Pandey, 2004). Therefore, the activation of antioxidant system and keeping ion balance in plant organs are necessary for improving tolerance to salinity.

Plant growth regulators, both natural and synthetic, are extensively used for the improvement of plant growth and stress resistance (Anjum *et al.,* 2011a, b). Recently, brassinosteroids (BRs) are recognized as a new class of steroidal plant hormone known to elicit a wide array of physiological and biochemical processes in plants under stress conditions (Anjum *et al.,* 2011c). The capacity of BRs to enhance the activity of antioxidant system by elevating the activities and levels of enzymatic and non-enzymatic antioxidants has ranked them a favorite tool to improve resistance potential against numerous stresses including salinity (Slathia *et al*., 2012). Keeping in view the potential ameliorating role of BRs in diverse types of stresses, the current study was formulated with an aim to evaluate the alterations in the morphological, physiological and biochemical traits of *L. chinensis* under the conditions of BR application and to establish a relationship between the changes in these parameters and the degree of salinity tolerance.

MATERIALS AND METHODS

Experimental material and growth conditions: Healthy and uniform sized seeds of *L. chinensis* were obtained from Ecological Experimental Station of *Leymus chinensis* natural distribution of community in Xilingole grassland in December 2013. The collected seeds were dried at room temperature and put into air bags. The remaining seeds were put into refrigerator at 4 °C. Pot experiment was carried out in the greenhouse incubator (12 h light / 30°C; dark 12 h / 20°C; RH 65%-85%) at Southwest University, Chongqing, China (latitudes 29° 49′ 32″ N, longitudes 106° 26′ 02″ E and altitude 220 m) during February to July 2014. The seeds were grown in biochemical incubator, after one week the seedlings were transferred to sand culture and each pot (34 cm diameter and 24 cm depth) contains 28 seedlings. Hoagland nutrient solution was applied to ensure adequate nutrient supply after 5 days interval. When seedling attained 18 cm height, thinning was carried out to keep 25 seedlings per pot. Salt stress treatments were imposed at the concentration of 150 mmol/L NaCl and normal soil without NaCl salt as control. After one week of salt treatment BR was applied at three different concentrations viz 0.01 mg/L，0.1 mg/L，and 1.0 mg/L. Water spray was applied to *L. chinensis* plants as control treatment. Second spray of BR was applied again after 4 days interval to exploit full potential of BR application.

Data measurement: The sampling for morphological, physiological and biochemical attributes was done after 7 days of BR application. The seedlings were removed carefully from the pots, the above ground part and the underground part were separated, weighing the seedlings fresh weight and seedling height. The tap water was used to rinse all the seedlings, and then rinsed with distilled water 2-3 times and then filter paper was used to absorb the adhered water. The seedlings were placed in oven at 105 °C for 15 minutes then dried at 65 °C till constant weight which referred to as dry weight. Leaf area was determined with the help of leaf area meter (Model: MSD – 971).

The Chl a, Chl b, total Chl and Carotenoids were determined by the method as described by Arnon (1949). A leaf sample of 0.1 g was ground and placed in 15 mL centrifuge tube along with 10 mL of miscible liquids by 95.5% acetone and absolute ethyl alcohol in 1:1 ratio. Then covered with black plastic bag and kept at dark place until the sample changed into white. The absorbance of spectrophotometer was measured at 665, 649, 470 and 652 nm, respectively.

The soluble protein contents were determined by using the method of Bradford (1976). Soluble sugar contents were determined by following the procedure as described by Zhu *et al*. (2012). Proline contents were assessed by using the protocol of Bates *et al*. (1973).

The degree of lipid peroxidation was estimated by measuring Malondialdehyde (MDA) contents by following the method of Devos *et al*. (1991). Leaf samples (0.5 g) were homogenized in 5 ml of 5% trichloroacetic acid. The homogenate was centrifuged at 4000 r min^{-1} for 10 min at 25 °C. The supernatant was added 2-thiobarbituric acid (TBA), then the mixture was heated at 98 °C for 10 min and cooled. After centrifugation at 4000 r min^{-1} for 10 min, the absorbance was measured at 532 nm.

The seedlings root activity was measured through TTC method (Higa *et al*., 2010). Leaf electrical conductivity used to represent the plasma membrane permeability, this experiment adopts the conductivity meter measuring leaf conductivity (Nayyar *et al*., 2005), with relative electric conductivity to indicate the extent of the cell membrane stress injury.

The relative conductivity = conductivity before boiling/conductivity after boiling.

The antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) activities were determined by using the method of Parida *et al*. (2004).

Experimental design and statistical analysis: Pots were arranged in completely randomized design (CRD) in triplicate. The data set were statistically analyzed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA) using single factor analysis of variance technique, with least significant difference (LSD) test ($P \leq 0.05$) to compare the treatments means.

RESULTS

Plant growth attributes were severely affected by salinity stress. Salinity stress impaired the plant fresh and dry weight, root/shoot ratio and leaf area considerably ($p<0.05$). However, exogenous BR application enhanced plant growth even under salinity stress and increased plant biomass. Moreover, 0.1 mg/L BR concentration proved better than 0.01 or 1.0 mg/L when *L. chinensis* was grown under saline condition (Table 1). A maximum increment in plant leaf area (205%) and root/shoot ratio (80%) were observed when plants were exposed to BR foliar application at the concentration of 0.1 mg L^{-1} compared with salt stress.

Table 1. Influence of exogenous application of brassinolide (BR) on morphological traits of *L. chinensis* under salt stress.

Treatments	Plant height (cm)	Dry weight (mg $plant^{-1}$)	Fresh weight (mg $plant^{-1}$)	Root/ shoot ratio	Leaf area （cm^2）
Control (CK1)	24.71±1.92a	27.3±7.9c	197.7±68.9c	0.55±0.06ab	3.65±0.41bc
Salt stress (CK2)	24.41±2.96a	22.5±1.9c	178.5±30.8bc	0.45±0.01b	2.40±0.20c
Salt stress+0.01 mg/L BR	29.61±1.37a	59.0±1.9ab	352.2±85.4ab	0.76±0.01ab	6.46±0.28ab
Salt stress+0.10 mg/L BR	30.01±3.96a	77.3±3.3a	385.7±14.1a	0.81±0.02a	7.33±0.52a
Salt stress+1.0 mg/L BR	29.18±2.18a	44.3±2.9bc	351.7±10.5ab	0.69±0.04ab	4.41±0.29abc

Values in the table are means of at least three replicates ± SE. Values followed by the same letter within columns are not significantly different according to LSD test ($P < 0.05$).

Salinity stress led to considerable decline in soluble sugars and soluble protein contents in *L. chinensis*. However, exogenous application of BR especially at 0.1 mg/ L increased sugar noticeably ($p<0.05$). Similarly, inducing protein allocations were observed in BR foliar applications ($p<0.05$), 0.01 mg/L BR treatment > 0.1 mg/L BR treatment > 1.0 mg/L BR treatment > CK1 > CK2. Meanwhile, plant photosynthetic pigments like chlorophyll and carotenoids contents reduced under salt stress. Nonetheless, BR application at 0.01~0.1 mg/L enhanced chlorophyll and carotenoids contents in comparison with control and salt stress (Table 2).

Root activity was obviously decreased ($p<0.05$), and membrane of *L. chinensis* damaged harshly under salinity due to enhanced MDA contents and electrical conductivity level. However, degree of severity was low in plants treated with lower concentration of BR. Salt stress increased proline contents and exogenously applied BR further elevated the level of the proline, 0.01 mg/L BR group > 0.1 mg/L BR group > CK2 > 1.0 mg/L BR group > CK1 treatment. Further perusal of data revealed that 0.01~0.1 mg/L BR application relieved salinity stress (Table 3).

Salt stress to a certain extent stimulated antioxidant activity and increased plant tolerance against salinity, however, exogenously applied BR further elevated the antioxidant enzymatic activities. SOD and GR activities were maximum at 0.01 mg/L BR, while decreased with further increase in the concentration of BR. However, APX activity in 1.0 mg/L BR was noticed highest. Similarly, POD enzyme activity increased with an increment in the concentration of BR. The CAT enzyme did not affect significantly ($p \leq 0.05$) in all treatments (Table 4). The dataset presented in table 4 revealed that 0.01 mg L^{-1} BR was better to modulate anti-oxidant defense of *L. chinensis* as compared to other concentrations of BR (0.01 mg L^{-1} and 1.0 mg L^{-1}) as well as over control.

Table 2. Influence of exogenous application of brassinolide (BR) on soluble sugar, soluble protein and photosynthetic pigments of *L. chinensis* under salt stress.

Treatments	Soluble sugar （mg g^{-1})	Soluble protein （mg g^{-1}）	Total chlorophyll contents （mg g^{-1}）	Chl a （mg g^{-1}）	Chl b （mg g^{-1}）	Carotenoids （mg g^{-1}）
Control (CK1)	16.04±0.03c	6.56±0.134ab	2.33±0.09a	1.08±0.04ab	1.08±0.07a	0.50±0.60ab
Salt stress (CK2)	13.64±0.02d	3.60±0.669b	2.21±0.08b	0.81±0.01b	0.98±0.04b	0.10±0.07b
Salt stress+0.01 mg/L BR	20.52±0.08b	15.01±0.360a	2.53±0.02a	1.27±0.02a	1.12±0.06a	0.66±0.03a
Salt stress+0.10 mg/L BR	24.48±0.07a	12.64±0.039a	2.50±0.03a	1.12±0.02ab	1.13±0.04a	0.67±0.07a
Salt stress+1.0 mg/L BR	19.36±0.06c	8.09±0.413ab	2.31±0.59a	0.95±0.04ab	1.04±0.03a	0.20±0.02b

Values in the table are means of at least three replicates ± SE. Values followed by the same letter within columns are not significantly different according to LSD test ($P < 0.05$).

Table 3. Influence of exogenous application of brassinolide (BR) on root activity, MDA contents, proline and electrical conductivity of *L. chinensis* under salt stress.

Treatments	Root activity （μg g^{-1}h^{-1}）	MDA （nmol g^{-1}）	Free proline （μg g^{-1}）	Electrical conductivity (%)
Control (CK1)	37.47±0.40c	21.2±0.34b	17.21±0.31b	10.8±0.028b
Salt stress (CK2)	29.09±0.36d	24.3±0.10a	21.67±0.73a	13.4±0.022b
Salt stress+0.01 mg/L BR	55.73±0.58a	20.1±0.83b	25.12±0.81a	11.8±0.013b
Salt stress+0.10 mg/L BR	45.71±0.11b	21.5±0.34b	23.67±0.32a	11.2±0.023b
Salt stress+1.0 mg/L BR	18.69±0.13e	24.4±0.12a	20.38±0.80a	18.1±0.011a

Values in the table are means of at least three replicates ± SE. Values followed by the same letter within columns are not significantly different according to LSD test ($P < 0.05$).

Table 4. Influence of exogenous application of brassinolide (BR) on antioxidant enzymatic activities of *L. chinensis* under salt stress.

Treatments	SOD （Ug^{-1}FW^{-1}）	APX (U g^{-1}min^{-1})	GR (U g^{-1}min^{-1})	POD (U g^{-1}min^{-1})	CAT (U g^{-1}min^{-1})
Control (CK1)	743.03±1.75b	7.31±0.41c	0.057±0.040b	206.18±4.08c	95.04±4.11a
Salt stress (CK2)	811.91±8.80ab	9.52±0.03bc	0.069±0.027b	206.18±8.93c	99.12±1.14a
Salt stress+0.01 mg/L BR	857.91±1.45a	12.20±0.02ab	1.549±0.012a	638.02±1.06b	126.39±13.75a
Salt stress+0.10 mg/L BR	830.38±6.54ab	12.14±0.04ab	0.417±0.029b	697.42±7.06ab	127.28±2.47a
Salt stress+1.0 mg/L BR	824.31±3.88ab	13.95±0.06a	0.363±0.051b	763.10±11.98a	111.77±3.79a

Values in the table are means of at least three replicates ± SE. Values followed by the same letter within columns are not significantly different according to LSD test ($P < 0.05$).

DISCUSSION

Enhancing plant’s ability to tolerate abiotic stresses such as drought, salinity and heat is a challenging task for all plant scientists. Plant growth regulators and related compounds have shown positive effects pertaining to enhancement of plant growth performance and great potential to provide relieve to plants against stressful conditions. In our study, the growth related traits such as plant height, leaf area, fresh and dry mass declined considerably on being submitted to salinity stress. These results are in accordance with those of Cicek and Cakirlar (2008) who manifested that salinity resulted marked inhibition in the growth of different soybean cultivars. Moreover, similar results were found by Al-Maskril *et al.* (2010) who substantiated that the number of leaves, shoot fresh weight, shoot dry weight, shoot dry matter percentage, root fresh weight, root dry weight, root dry weight percentage, leaf area, and leaf area index were significantly affected by salinity levels (50 and 100 mM) in lettuce (*Lactuca sativa* L.). The reduction in growth related attributes might be credited to either decrease in cell elongation resulting from the inhibiting effect on growth promoting hormones which, in turn, led to a decrease in each of cell turgor, cell volume and eventually the cell growth (Banon *et al*., 2006), and due to blocking up of xylem and phloem vessels, thus, blocking any translocation through it. Moreover, reduction in the plant growth attributes might be ascribed to reduced water absorption due to osmotic effect, nutritional deficiency on account of the ionic imbalance and decreased numerous metabolic processes (Kumar *et al*., 2005). The *L. chinensis* plants exposed to NaCl and subsequently treated with BR, exhibited better growth, compared to those grown without BR treatments. In our study, the increased tolerance to the salinity stress was manifested in term of BR-induced improvement in growth. Foliar application of BR resulted in significant increases in growth parameters at high NaCl but the values obtained were the highest in 0.10 mg/L BR treatment than all other treatments. Increase in the leaf area induced by BR was further translated into improved growth of the plants as reflected in the enhancement in fresh and dry weights of the shoot. This is in agreement with earlier reports that BRs influence cell division and consequently leaf size (Yu *et al*., 2004; Anjum *et al.*, 2011 d). In addition, Vardhini and Rao (2001) found that different types of brassinosteroids increased shoot length, root length, and shoot fresh weight, the number of fruits per plant and total weight of tomato fruit per plant. Furthermore, this growth promoting effect of BRs under stress conditions is probably through their auxin like hormonal effect on cell division and cell enlargement (Morillon *et al*., 2001).

The present study also revealed that saline conditions caused a significant reduction in the photosynthetic pigment contents. This decrease in chlorophyll concentration may be ascribed to the increase in the activity of chlorophyll degrading enzyme chlorophylase under salinity conditions (Reddy and Vora, 1986). However, to some extent, foliar application of BR ameliorated the detrimental effects of salt stress on photosynthetic pigments in *L. chinensis*, which could be one of the reasons for growth stimulation by BR under saline conditions (Anuradha and Rao, 2003). These results are in agreement with Hasan *et al*. (2008) who corroborated that application of BR to stressed plants considerably enhanced pigment contents. The reason that sounds best in defending the said observation might be attributed to possibility of BR-induced impact on transcription and/or translation in the synthesis of pigments (Bajguz, 2000). Moreover, the improvement in chlorophyll contents by BR-treatment is similar with the earlier findings of Fariduddin *et al*. (2003) for *Vigna radiata*.

Salinity stress caused noticeable accumulation of free proline in the *L. chinensis* plants. However, the BR application as foliar spray further elevated the osmolyte level. In the meantime, the soluble protein and soluble sugar contents also increased noticeably. Nilovskaya *et al*. (2001) indicated that application of epibrassinolide increased protein content in barley plants. The promoting effects of BRs on the protein content could be ascribed to activation of transcription and translational mechanisms of specific stress tolerance genes (Kagale *et al*., 2007). These results are in accordance with Arora *et al*. (2008) who corroborated that enhanced levels of proteins were observed under the influence of BRs indicating the synthesis of stress-protective proteins in the maize seedlings. Brassinolide application as foliar spray increased the levels of free proline in *L. chinensis* plants grown under saline condition as compared to the control plants. The results obtained from the present study are similar with those of Ozdemir *et al*. (2004) who indicated that 24-epibrassinolide increased proline content in rice under salinity stress. Similarly, the soluble sugar contents of stressed *L. chinensis* further enhanced by different concentrations of BR. Brassinolide at 0.01~0.10 mg/L were most effective in increasing the levels of these osmolytes in *L. chinensis.*

Phytotoxicity from salt stress is closely related to the production of reactive oxygen species (ROS) in plants. An imbalance between ROS generation and ROS scavenging leads to oxidative damage. ROS can react with lipids, DNA and proteins, and causes membrane damage and enzyme inactivation resulting in inhibition of plant growth (Mittler, 2002). Lipid peroxidation is used as index of membrane damage caused by various abiotic stresses in plants. Salt stress was observed to damage cell membrane as indicated by higher concentration of MDA content observed in salt stressed plants when compared with untreated control. Whereas, Koca *et al*. (2007) observed increased lipid peroxidation in sesame cultivars subjected to salinity stress. The present observations are consistence with those obtained by Ozdemir *et al*. (2004), Arora *et al*. (2008), who observed that brassinosteroids treatment lowered the lipid peroxidation in the seedlings by reducing the contents of MDA in *Oryza sativa* and *Zea mays*. However, BR increased to 1.0 mg L^{-1} concentration observed to be detrimental, as the root activity of the 1.0 mg L^{-1} BR treatment was lower, MDA concentration and electrical conductivity were higher, which implied the membrane of *L. chinensis* suffered a damage.

It has been emphasized that the induction of antioxidant enzymes, like superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) is to protect the plant tissues from salt stress damage (Mittova *et al*., 2002; Bor *et al*., 2003). In our study, salinity conditions caused significant accumulation of antioxidant enzymes. It was further recorded that foliar application of BR at varying concentrations elevated antioxidant enzymes activity such as superoxide dismutase, catalase, glutathione peroxidase and ascorbate peroxidase in plants under stress conditions thereby conferring NaCl stress tolerance in *L. chinensis* plants. Similarly, improved activities of antioxidant enzymes such as SOD, POD, CAT, GR and APX and reduced lipid peroxidation had been observed upon BR application to *Zea mays* seedlings under NaCl stress (Arora *et al*., 2008). The higher level of these antioxidant enzymes suggested a possible role of BR in mitigation of oxidative burst generated by salt stress.

Conclusion: Salt stress affects all the major processes such as growth, yields, photosynthetic pigments, protein synthesis, and lipid metabolism. The stress resistance due to application of BR was reflected through the improvement of *L. chinensis* growth. The effective dose in improving growth and other attributes was 0.01~ 0.10 mg/L BR spray under salinity. Hence, BR is a component of the stress response and exogenous BR application can improve the salt tolerance by stimulating plant growth and increasing the activities of antioxidant enzymes.

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REFERENCES

Agarwal, S., and V. Pandey (2004). Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol. Plant. 48: 555-560.

Al-Maskril, A., L. Al-Kharusi, H. Al-Miqbali, and M. Khan (2010). Effects of salinity stress on growth of lettuce (*Lactuca sativa*) under closed recycle nutrient film technique. Inter. J. Agric. Bio. 12: 377-380.

Anjum, S.A., L. Wang, M. Farooq, L. Xue, and S. Ali (2011a). Fulvic acid application improves the maize performance under well-watered and drought conditions. J. Agron. Crop Sci. 197: 409-417.

Anjum, S.A., L. Wang, M. Farooq, I. Khan, and L. Xue (2011b). Methyl jasmonate-induced alteration in lipid peroxidation, antioxidative defence system and yield in soybean under drought. J. Agron. Crop Sci. 197: 296-301.

Anjum, S.A., L.C. Wang, M. Farooq, M. Hussain, L.L. Xue, and C.M. Zou (2011c). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J. Agron. Crop Sci. 197: 177-185.

Anjum, S.A., M. Farooq, L.C. Wang, L.L. Xue, S.G. Wang, L. Wang, S. Zhang, and M. Chen (2011d). Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. Plant Soil Environ. 57(7): 326-331.

Anuradha, S., and S.S.R. Rao (2003). Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigment loss and increased nitrate reductase activity. Plant Growth Regul. 40: 29-32.

Arnon, D.L. (1949). Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24(1): 1-15.

Arora, N., P. Sharma, R. Bhardwaj, and H.K. Arora (2008). Effect of 28-homobrassinolide on growth, lipid peroxidation and antioxidative enzyme activities in seedlings of *Zea mays* L. under salinity stress. Acta Physiol. Plantarum, 30(6): 833-839.

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotech. Adva. 27: 84-93.

Bajguz, A. (2000). Effect of brassinosteroids on nucleic acid and protein content in cultured cell of *Chlorella vulgaris*. Plant Physiol and Biochem. 38: 209-215.

Banon, S.J., J. Ochoa, J.A. Franco, J.J. Alarcon, and M.J. Sanchez-Blanco (2006). Hardening of *oleander* seedlings by deficit irrigation and low air humidity. Environ. Exp. Bot. 56: 36-43.

Bates, L.S., R.P. Waldren, and I.D. Teare (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205-207.

Bor, M., F. Ozdemir, and Turkan I. (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. Plant Sci. 164: 77-84.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Cicek, N., and H. Cakirlar (2008). Effect of salt stress on some physiological and photosynthetic parameters at three different temperature in six soyabean (*Glycine max* L. Merr.) cultivars. J. Agron. Crop Sci. 194(1):34-46.

Devos, C., H. Schat, M. Dewaal, R. Vooijs, and W.H.O. Ernst (1991). Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silenecucubalus*. Physiol. Plantarum. 82: 523-528.

Fariduddin, Q., A. Ahmad, and S. Hayat (2003). Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide. Photosynthetica, 41: 307-310.

Flowers, T.J., and T.D. Colmer (2008). Salinity tolerance in halophytes. New Phytologist. 179(4): 945-963.

Hasan, S.A., S. Hayat, B. Ali, and A. Ahmad (2008). 28-homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidants. Environ. Pollut. 151: 60-66.

Higa, A., Y. Mori, and Y. Kitamura (2010). Iron deficiency induces changes in riboflavin secretion and the mitochondrial electron transport chain in hairy roots of *Hyoscyamus albus*. J. Plant Physiol. 167(11): 870-878.

Huang, Z.H., J.M. Zhu, X.J. Mu, and J.X. Lin (2002). Advances on the mechanism of low sexual reproductivity of *Leymus chinensis*. Grassland China, 24: 55-60.

Kagale, S., U.K. Divi, J.E. Krochko, W.A. Keller, and P. Krishna (2007). Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. Planta, 225(2): 353-364.

Koca, H., M. Bor, F. Ozdemir, and I. Turkan (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ. Exp. Bot. 60(3): 344-351.

Kumar, R., V. Goyal, and M.S. Kuhad (2005). Influence of fertility-salinity interactions on growth, water status and yield of Indian mustard (*Brassica juncea*). Indian J. Plant Physiol. 10: 139-144.

Li, Q., X. Liu, M. Yue, X.F. Zhang, and R.C. Zhang (2011). Effects of physiological integration on photosynthetic efficiency of *Trifolium repens* in response to heterogeneous UV-B radiation. Photosynthetica, 49: 539-545.

Lin, J., Z. Li, S. Shao, Y. Wang, and C. Mu (2014). Effects of various mixed salt-alkaline stress conditions on seed germination and early seedling growth of *Leymus chinensis* from Songnen Grassland of China. Not. Bot. Horti. Agrobo. 42(1): 154-159.

Liu, G.S., and D.M. Qi (2004). Research progress on the biology of *Leymus chinensis*. Acta Prataculturae Sinica, 13: 6-11.

Liu, X., Shi J. and Zhang X.Y. (2001). Screening of salt tolerance germplasms and tagging the tolerance gene using microsatellite markers in wheat. Acta Botanica Sinica, 43(9): 948-954.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7(9): 405-410.

Mittova, V., M. Tal, M. Volokita, and M. Guy (2002). Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. Physiol. Plantarum, 115: 393-400.

Morillon, R., M. Catterou, R.S. Sangwan, B.S. Sangwan, and J.P. Lassalles (2001). Brassinolide may control aquaporin activities in *Arabidopsis thaliana*. Planta, 212: 199–204.

Munns, R. (2005). Genes and salt tolerance: bringing them together. New Phytol. 167: 645–663.

Nayyar, H., T.S. Bains, and S. Kumar (2005). Chilling stressed chickpea seedlings: effect of cold acclimation, calcium and abscisic acid on cryoprotective solutes and oxidative damage. Environ. Exp. Bot. 54: 275-285.

Nilovskaya, N.T., N.V. Ostapenko, and I.I. Seregina (2001). Effect of epibrassinolide on the yield and drought resistance of spring wheat. Agrokhimiya, 2: 46-50.

Ozdemir, F., M. Bor, T. Demiral, and I. Turkan (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. Plant Growth Regul. 42: 203-211.

Parida, A.K., A.B. Das, and P. Mohanty (2004). Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidative enzymes. J. Plant Physiol. 161: 531-542.

Reddy, M.P., and A.B. Vora (1986). Changes in pigment composition, hill reaction activity and saccharide metabolism in bajra (*Pennisetum typhoides* S&H) leaves under NaCl salinity. Photosynthetica, 20: 50-55.

Slathia, S., A. Sharma, and S.P. Choudhary (2012). Influence of exogenously applied epibrassinolide and putrescine on protein content, antioxidant enzymes and lipid peroxidation in *Lycopersicon esculentum* under salinity Stress. Am. J. Plant Sci. 3: 714-720.

Sofo, A., B. Dichio, C. Xiloyannis, and A. Masia (2004). Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. Physiol. Plantarum 121: 58-65.

Tong, C., J.R. Yang, W.Y. Yong, and S.P. Yong (2002). Spatial pattern of steppe degradation in Xilin River Basin of Inner Mongolia. J. Natural Res. 17(5): 571-578.

Vardhini, B.V., and S.S.R. Rao (2001). Effect of brassinosteroids on growth metabolite content and yield of *Arachis hypogaea*. Indian J. Phytochem. 6(3): 326-328.

Wang, Z.W., L.H. Li, X.G. Han, and M. Dong (2004). Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level? Acta Oecol. 26: 255-260.

Yang, C.W., J.N. Chong, C.Y. Li, C.M. Kim, D.C. Shi, and D.L. Wang (2007). Osmotic adjustment and ion balance traits of an alkali resistant halophyte

Kochia sieversiana during adaptation to salt and alkali conditions. Plant and Soil, 294: 263-276.

Yang, C.W., D.C. Shi, and D.L. Wang (2008). Comparative effects of salt stress and alkali stress on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *Suaeda glauca* (Bge.). Plant Growth Regul. 56: 179-190.

Yu, J.Q., L.F. Huang, W.H. Hu, Y.H. Zhou, W.H. Mao, S.F. Ye, and S. Nogues (2004). A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J. Exp. Bot. 55: 1135-1143.

Zhu, W.Z., M. Cao, S.G. Wang, W.F. Xiao, and M.H. Li (2012). Seasonal dynamics of mobile carbon supply in *Quercus aquifolioides* at the upper elevational limit. PLOS ONE. 7(3): e34213.