Exogenous application of brassinolide can alter morphological and physiological traits of *Leymus chinensis* (Trin.) Tzvelev under room and high temperatures

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ABSTRACT

Plant growth regulating substances are involved in the physiological and metabolic processes of plants and enable them to cope with numerous environmental stresses. The effect of exogenously applied brassinolide (BR) with various concentrations (0.01, 0.1, and 1.0 mg L^{-1}) was studied on morphological and physiological traits of *Leymus chinensis* (Trin.) Tzvelev under room and high temperatures in pots. The experimental results revealed that high temperature stress substantially perturbed growth, photosynthetic pigments, and root activity of *L. chinensis*; however, the deleterious effects of high temperature were partially ameliorated by the foliar application of BR. Compared to room temperature, high temperature stress decreased the plant height, leaf area, plant fresh and dry weight, chlorophyll a and b content, chlorophyll a/b ratio as well as root activity, while exacerbated the membrane damage as indicated by enhanced production of malondialdehyde (MDA). Accumulation of proline content, soluble protein and sugar content in *L. chinensis* improved by heat stress, compared with normal temperature; application of BR further improved their production thus aiding in the attainment of tolerance against heat stress. Elevated levels of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) were observed under heat stress compared to room temperature, however, application of BR further proved beneficial in this regard. Our results indicated that BR could improve the growth and development of *L. chinensis* by enhancing the biosynthesis of photosynthetic pigments, osmolytes and antioxidant enzymes system in plants under both room and high temperature.

Key words: Antioxidants, brassinolide, growth, high temperature, *Leymus chinensis*.

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INTRODUCTION

The grassland of North China covers 41% of the total land area of China. It is widely distributed from southern Chinese loess plateau to northern Russian Baikal and from the Sanjiang plain of the eastern China to the Ulan Bator in Mongolia. *Leymus chinensis* (Trin.) Tzvelev is a perennial grass having high forage value and occupies the grasslands of China. It is commonly called as sheep grass and has good palatability and high nutritious value. Under good management practices, it yields 3000-7500 kg DM ha^{-1} and is considered one of the most important feed for livestock. However, it has been reported that about 90% of the grasslands in China have been degraded during the recent decades due to intensified land use, improper land reclamation, overgrazing and adverse climatic conditions (Bai et al., 2004). The increase in grazing pressure has shown its adverse effects on the canopy cover in grasslands. However, the abiotic stresses such as drought, salinity and high temperature have coupled this damage by making the plant survival difficult under such conditions.

Abiotic stresses induce alteration in morphological, physiological, biochemical, and molecular attributes of plants (Bajguz and Hayat, 2009). The carbon dioxide concentration has increased since past to day due to increased industrialization and burning of fossil fuels resulting in increase in atmospheric temperature. High temperature is an important abiotic stress which is becoming most limiting factor for normal plant growth and development, especially under climate change scenario which is projected to the average temperature be increased 1.1 °C in the last 50 yr (Ding et al., 2007). High temperature imparts its injurious effects on photosynthesis (Buchner et al., 2015), disrupts the overall balance of the metabolic processes, leading to the over production of reactive oxygen species (ROS) which ultimately causes oxidative damage to the plant cells (Larkindale et al., 2005). The enhanced synthesis of ROS causes lipid peroxidation of biological membranes, denaturation of proteins and damage to the nucleic acid eventually disturbing homeostasis (Mittler, 2002). These damages lead to the starvation, reduced ion flux, synthesis of toxic compounds and reduction in plant growth (Howarth, 2005).

Plants have devised with many mechanisms at physiological, biochemical and molecular level to combat with abiotic stresses to keep their growth in pace. Accumulation of some compatible solutes such as proline, soluble sugars and proteins, organic acids etc. is one of the most important trait of stress tolerant plants. Studies have reported enhanced production of ROS under high

temperature stress which causes biological damage to plants. Therefore, potential activity of antioxidant enzymes such as Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and ascorbate peroxidase (APX) is a key factor in attaining the heat stress tolerance (Shi et al., 2006). The activation of antioxidant system keeps the check and balance in the production and scavenging of the ROS for attaining some degree of tolerance.

Plant growth regulators are the natural or synthetic substances when applied in small concentration induce some physiological response in the plants. It was further elucidated that plant growth substances when applied exogenously aside from enhancing the growth and development also mediate stress tolerance of plants (Hayat et al., 2008; Hasan et al., 2008). Brassinosteroids (BRs) are steroidal hormones that are essential for growth and development of plants. A large number of physiological processes and responses has been known to be altered by BRs such as cell division and differentiation, functioning of stomata, photosynthesis, respiration as well as ion transport (Divi and Krishna, 2009; Vriet et al., 2012). Reports have indicated that BR induces heat stress tolerance in plants. The thermotolerance attained by the plants in response to BR application is due to the synthesis of heat shock proteins (Dhaubhadel et al., 1999). Further, BRs are also involved in the regulation of ROS production and scavenging (Xia et al., 2009). The BR increase the activity of antioxidant enzymes which aid in detoxification of ROS produced under high temperature and reduce the membrane lipid peroxidation, determined by the accumulation of malondialdehyde (MDA) (Yun-ying and Hua, 2008). Therefore, keeping in view the above key factors the present study was carried out to evaluate the morphological, physiological and biochemical changes in *L. chinensis* in response to the exogenous application of BR under both room and high temperatures.

MATERIALS AND METHODS

Plant material and experimental design

Seeds of *L. chinensis* having 1000-seed weight about 2.5 g were collected in natural community of the Ecological Experimental Station of *L. chinensis* in Xilingole grassland. The experiment was carried out in the greenhouse incubator (10 h light/30 °C; dark 14 h/20 °C) at College of Agronomy and Biotechnology, Southwest University, Chongqing (29°49'32" N, 106°26'02" E; 220 m a.s.l.), China, from 26 March 2014 to 5 August 2014. The seeds were grown in incubator, after 1 wk the seedlings were transferred to sand culture and each pot (34 cm diameter and 24 cm depth) contained 40 seedlings. Adequate nutrient supply was ensured by applying the Hoagland's nutrient solution after 5 d interval. When seedlings attained the height of 16-18 cm, thinning was done to keep 25 seedlings per pot. After 2 d, foliar application of BR was practiced with three different concentrations viz. 0.01, 0.1, and 1.0 mg L^{-1}, while distilled water was applied as control. Pots containing seedlings were put in two same greenhouse incubators (Beijing Kingpeng greenhouse, JPK-005) and environmental conditions provided were: room temperature (20 °C/15 °C, day/night, light for 10 h) and high temperature treatment (38 °C/25 °C, day/night, light for 10 h). Second application of BR was applied after 4 d interval for better effect of BR. The experiment was conducted in randomized complete block design (RCBD) with factorial arrangement having eight treatments with six pots of each treatment and total of 48 pots (Table 1). Data pertaining to morphological traits, photosynthetic pigments, root activity, malondialdehyde (MDA), free proline, soluble protein and sugar contents, and antioxidant enzymes activity were recorded after 11 d of BR treatment.

Determination of morphological traits

The seedlings were uprooted carefully from pots and seedling height was measured. The seedlings were rinsed with tap water followed by rinsing with 2-3 times with distilled water. Filter paper was used to absorb the adhered water from the seedlings. Leaf area, leaf length, and leaf width were determined with MSD-971 analysis/scanner. The seedlings were weighed to determine the fresh weight. Seedling dry weight was determined by placing the seedlings in oven at 105 °C for 20 min to stop respiration, followed by drying at 70 °C for 48 h (Zhang et al., 2014).

Determination of physiological and biochemical traits

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content were measured using the method of Wellburn (1994). 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 malondialdehyde (MDA) content was assayed through thiobarbituric acid (TBA) assay (De-Vos et al.,

Table 1. Experimental design.

Treatments	
Temperature	Brassinolide concentration
	mg L^{-1}
Room temperature	0.00
Room temperature	0.01
Room temperature	0.10
Room temperature	1.00
High temperature	0.00
High temperature	0.01
High temperature	0.10
High temperature	1.00

1991). Leaf samples (0.5 g) were homogenized in 5 mL 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 root activity was measured using the triphenyltetrazolium chloride (TTC) method of Higa et al. (2010). Soluble protein content was determined using Coomassie brilliant blue method (Bradford, 1976).

Determination of soluble sugars was done by anthrone color method (Zhu et al., 2012). Plant material (0.5 g) was put into a centrifuge tube, where 80% ethanol was added. The mixture was incubated at 80 °C in a shaking water bath for 30 min, and then centrifuged at 5000 rpm for 5 min. The pellets were re-extracted twice with 80% ethanol. Extracted soluble sugars were determined spectrophotometrically using the anthrone method at 625 nm within 30 min.

Proline content was measured using the ninhydrin method (Bates et al., 1973). SOD, CAT, POD, APX, and GR activity was determined by the method of Parida et al. (2004).

Statistical analysis

Data were analyzed by ANOVA using the SPSS software (SPSS, version 19.0; IBM Corporation, Armonk, New York, USA) and the significant differences among the treatments were identified using the least significant difference (LSD) method at the 0.05 level.

RESULTS

Plant growth was severely hampered at high temperature. All the growth attributes viz. plant height, leaf area, leaf length and width and leaf fresh and dry weight of *L. chinensis* were lessened substantially when exposed to high temperature as compared to room temperature. However, plant growth was enhanced by the application of BR as compared to control at both room and high temperature. At room temperature, maximum plant height, plant fresh and dry weight was observed by the application of 1 mg L^{-1} BR. Leaf area and leaf width was promoted by 0.01 mg L^{-1} BR application. Leaf length was increased by 0.1 mg L^{-1} BR over other concentrations. At high temperature, application of 0.01 mg L^{-1} BR proved better in terms of plant height (12.94%), leaf area (125.47%) and leaf length (15.77%) as compared to control at high temperature. While, leaf width (35.83%), plant fresh weight (109.59%) and dry weight (89.64%) was improved by treatment with 0.1 mg L^{-1} BR as compared to control at high temperature (Table 2).

High temperature stress substantially affected the biosynthesis of photosynthetic pigments. However, the carotenoids were not impaired by high temperature stress, rather increased as compared to room temperature. At room temperature, chlorophyll a, b and total chlorophyll content were found at peak when *L. chinensis* plants were treated with 0.1 mg L^{-1} BR, which were 80.71%, 98.0%, and 85.57% higher than control. Maximum chlorophyll a/b ratio was noticed in control where BR was not applied. At high temperature, chlorophyll a and b content showed the similar pattern as at room temperature by showing the better results at 0.1 mg L^{-1} BR. Treatment of 1 mg L^{-1} BR considerably enhanced the carotenoids, total chlorophyll contents and chlorophyll a/b ratio up to 51.42%, 62.04%, and 41.61%, respectively, when compared with control (Table 3).

Root activity, production of MDA and accumulation of osmolytes in *L. chinensis* plants were affected significantly under high temperature; while, treatment with BR aided in the ameliorating the detrimental effects of the exposed stress. Under room temperature, treatment with 0.1 mg L^{-1} BR boosted the root activity, while minimum MDA production was noticed when 0.01 mg L^{-1} BR was applied. Accumulation of free proline and soluble protein content was the highest by 0.1 mg L^{-1} BR application. Soluble sugars considerably increased to maximum by 1.0 mg L^{-1} BR concentration. Under high temperature stress however, a decrease in root activity and an increase in MDA, free proline, soluble protein and sugars was observed. High temperature stress improved root activity (86.52%) and less MDA (27.73%) production was found as a consequence of treatment with 0.1 mg L^{-1} BR as compared to control. However, enhancement in free proline content (456.46%) was observed at 1.0 mg L^{-1} concentration of BR as compared to control. Soluble protein (45.93%) and sugars (41.31%) were increased at 0.1 mg L^{-1} BR concentration as compared to control under high temperature stress (Table 4).

Table 2. Effect of brassinolide on morphological traits of *Leymus chinensis* under room and high temperature.

Treatments							
Temperature	BR Conc.	Plant height	Leaf area	Leaf length	Leaf width	Fresh weight	Dry weight
	mg L^{-1}	cm	cm^2	cm		mg $plant^{-1}$	
Room temperature	0.00	24.5 ± 1.47bc	1.924 ± 0.074c	12.05 ± 0.2bc	0.274 ± 0.014ab	696.7 ± 15.3c	105.3 ± 12.9c
Room temperature	0.01	26.57 ± 0.57abc	2.35 ± 0.061a	11.55 ± 0.8cd	0.296 ± 0.007a	744.7 ± 5.0b	150.7 ± 10.1b
Room temperature	0.10	29.43 ± 5.4ab	2.202 ± 0.101b	13.78 ± 0.1a	0.282 ± 0.012ab	854.0 ± 12.2a	229.7 ± 5.5a
Room temperature	1.00	30.13 ± 2.93a	2.175 ± 0.069b	12.64 ± 0.16b	0.234 ± 0.025cd	875.0 ± 13.2a	235.0 ± 13.2a
High temperature	0.00	23.17 ± 3.37c	0.365 ± 0.02e	9.89 ± 0.17ef	0.187 ± 0.009e	178.3 ± 9.6f	47.3 ± 6.1f
High temperature	0.01	26.17 ± 3.19abc	0.823 ± 0.119d	11.45 ± 0.11cd	0.207 ± 0.011de	315.3 ± 14.0e	88.3 ± 4.0d
High temperature	0.10	25.73 ± 0.9abc	0.801 ± 0.059d	10.92 ± 0.2de	0.254 ± 0.01c	373.7 ± 7.2d	89.7 ± 6.0d
High temperature	1.00	23.37 ± 3.62c	0.484 ± 0.022e	10.31 ± 0.76e	0.214 ± 0.03de	306.3 ± 25.1e	66.0 ± 7.2e

Values are mean ± SE. Values followed by the same letter within columns are nonsignificantly different according to least significant difference test ($P < 0.05$)
BR Conc: Brassinolide concentration.

Table 3. Effect of brassinolide on photosynthetic pigments of *Leymus chinensis* under room and high temperature.

Treatments						
Temperature	BR Conc.	Chl a	Chl b	Carotenoid	Total chlorophyll	Chl a/b
	mg L^{-1}	mg g^{-1}				
Room temperature	0.00	1.374 ± 0.025c	0.501 ± 0.022c	0.017 ± 0.003e	1.892 ± 0.059c	2.744 ± 0.109a
Room temperature	0.01	2.074 ± 0.075b	0.813 ± 0.035b	0.044 ± 0.006bc	2.915 ± 0.118b	2.552 ± 0.019ab
Room temperature	0.10	2.483 ± 0.301a	0.992 ± 0.101a	0.035 ± 0.005d	3.511 ± 0.40a	2.500 ± 0.051ab
Room temperature	1.00	2.012 ± 0.039b	0.853 ± 0.05b	0.042 ± 0.004cd	2.9 ± 0.111b	2.364 ± 0.152ab
High temperature	0.00	0.642 ± 0.02d	0.315 ± 0.015e	0.035 ± 0.006d	1.03 ± 0.05d	2.038 ± 0.034b
High temperature	0.01	0.75 ± 0.01d	0.382 ± 0.011de	0.047 ± 0.003abc	1.204 ± 0.087d	1.965 ± 0.031b
High temperature	0.10	1.176 ± 0.157c	0.458 ± 0.092cd	0.050 ± 0.002ab	1.645 ± 0.074c	2.678 ± 0.828a
High temperature	1.00	1.23 ± 0.01c	0.427 ± 0.024cd	0.053 ± 0.003a	1.669 ± 0.044c	2.886 ± 0.143a

Values are mean ± SE. Values followed by the same letter within columns are nonsignificantly different according to least significant difference test (P < 0.05) BR Conc: Brassinolide concentration, Chl a: chlorophyll a, Chl b: chlorophyll b.

Table 4. Effect of brassinolide on root activity, malondialdehyde (MDA), free proline and soluble protein and sugar contents of *Leymus chinensis* under room and high temperature.

Treatments						
Temperature	BR Conc.	Root activity	MDA	Free proline	Soluble protein	Soluble sugars
	Mg L^{-1}	µg g^{-1} h^{-1}	nmol g^{-1}	µg g^{-1}	mg g^{-1}	
Room temperature	0.00	155.46 ± 3.61d	15.82 ± 0.7d	35.72 ± 5.33d	11.93 ± 0.78e	6.91 ± 1.47e
Room temperature	0.01	182.46 ± 2.54b	14.6 ± 0.23e	37.81 ± 5.65d	11.95 ± 0.74e	8.25 ± 1.17de
Room temperature	0.10	260.59 ± 1.92a	14.89 ± 0.45de	52.89 ± 4.62d	15.11 ± 0.55d	9.14 ± 0.58d
Room temperature	1.00	165.23 ± 2.48c	15.37 ± 0.94de	52.54 ± 4.69d	14.22 ± 0.24d	12.51 ± 1.74c
High temperature	0.00	82.57 ± 1.84g	24.73 ± 0.55a	111.79 ± 13.27c	19.7 ± 0.29c	18.93 ± 0.64b
High temperature	0.01	150.34 ± 2.19e	19.86 ± 0.29b	151.03 ± 20.17c	22.98 ± 0.82b	25.62 ± 1.06a
High temperature	0.10	154.01 ± 1.6de	17.87 ± 0.51c	431.12 ± 20.66b	28.75 ± 1.33a	26.75 ± 0.73a
High temperature	1.00	96.58 ± 2.11f	18.3 ± 0.41c	622.07 ± 70.3a	22.07 ± 0.15b	17.81 ± 0.81b

Values are mean ± SE. Values followed by the same letter within columns are nonsignificantly different according to least significant difference test (P < 0.05). BR Conc: Brassinolide concentration.

A marked increase in the activity of antioxidant enzymes in *L. chinensis* was observed at high temperature in contrast to room temperature. Under room temperature, no effect of BR on SOD activity was noted as compared to control, rather its activity decreased with the application of BR than control. However, the activity of POD (66.58%) and CAT (296.62%) showed a quite considerable increase when 1.0 mg L^{-1} of BR was applied to the plants compared with control. The APX and GR activity was increased by 137.84% and 62.26%, respectively, by exogenous application of 0.1 mg L^{-1} BR on *L. chinensis* than control. Under high temperature stress SOD (28.71%), CAT (82.96%), and APX (180.52%) showed an obviously high activity by the treatment with 0.1 mg L^{-1} BR, while, POD (33.82%) and GR (335.81%) activity was highest when *L. chinensis* was applied with 0.01 and 1.0 mg L^{-1} BR, respectively, as compared to control (Table 5).

DISCUSSION

High temperature affects the plant growth by disrupting the photosynthetic system, enzyme activity, protein degradation and loss of membrane activity (Howarth, 2005). However, plants cope with stresses by modulating their growth pattern and alterations in phenological, physiological and metabolic processes taking place within plants. These changes are

Table 5. Effect of brassinolide on antioxidant enzymes activity of *Leymus chinensis* under room and high temperature.

Treatments						
Temperature	BR Conc.	SOD	POD	CAT	APX	GR
	mg L^{-1}	U g^{-1} FW	U g^{-1} min^{-1}			
Room temperature	0.00	509.96 ± 52.73c	230.07 ± 77.64d	25.49 ± 8.99d	5.1 ± 1.36d	0.0477 ± 0.0118c
Room temperature	0.01	453.87 ± 23.78cd	290.17 ± 31.67cd	38.46 ± 12.64d	8.5 ± 2.1cd	0.0695 ± 0.0319c
Room temperature	0.10	439.70 ± 28.36d	321.00 ± 32.50cd	65.89 ± 38.71cd	12.02 ± 1.34cd	0.0774 ± 0.0335c
Room temperature	1.00	460.25 ± 32.94cd	383.26 ± 60.08abc	101.10 ± 6.37bc	12.13 ± 3.31cd	0.0669 ± 0.0232c
High temperature	0.00	654.69 ± 18.29b	358.36 ± 13.22c	109.00 ± 39.19bc	11.76 ± 0.6cd	0.1399 ± 0.044c
High temperature	0.01	822.09 ± 26.88a	479.55 ± 79.33a	141.73 ± 32.88b	16.93 ± 2.46bc	0.3611 ± 0.0447b
High temperature	0.10	842.71 ± 46.02a	470.98 ± 28.66ab	199.43 ± 6.97a	32.99 ± 5.18a	0.3706 ± 0.0201b
High temperature	1.00	785.91 ± 16.57a	373.84 ± 63.53bc	143.41 ± 16.69b	26.49 ± 13.68ab	0.6097 ± 0.1738a

Values are mean ± SE. Values followed by the same letter within columns are nonsignificantly different according to least significant difference test (P < 0.05). BR Conc: Brassinolide concentration, SOD: superoxide dismutase, POD: peroxidase, CAT: catalase, APX: ascorbate peroxidase, GR: glutathione reductase, FW: fresh weight.

consequence of transcriptional and translational changes in stress related genes mediated by the hormonal stimulus (Wahid et al., 2007). Thus, the hormones are considerably important in this regard to induce stress tolerance. It is evident from our study that growth of *L. chinensis* was severely impaired due to heat stress, however, the exogenous application of BR aided in the mitigation of detrimental effects of heat stress on growth. Moreover, the growth was improved by BR application in both room as well as high temperature stressed condition and it was concentration dependent (Table 2). High temperature affects the assimilate partitioning by its negative effects on source and sink activities, resulting in reduced plant growth. Under high temperature, the transport and transfer processes in plants are severely affected, which are resulted from perturbed activities of assimilate partitioning (Taiz and Zeiger, 2006). These results are in accordance with Rivero et al. (2001) who reported that heat stress decreased the shoot dry weight of tomato and watermelon plants. Similarly Prasad et al. (2006) stated that exposure of sorghum plants to heat stress decreased the plant height due to reduction in shoot growth. There was an increase in plant height, leaf area and fresh and dry weight of *L. chinensis* plants by the treatment of BR (Table 2), which indicated that BR exerted positive effects on physiological processes and responses including cell division and differentiation and further improved the photosynthetic activity (Vriet et al., 2012). The results of our study are in agreement with Nassar (2004), who perceived the increase in plant height and fresh weight of banana by the treatment with BR.

The photosynthetic activity of plants is dependent on the stability of photosynthetic system and enzyme activity, which may be affected by high temperature stress. The results of present study revealed that there was a drastic reduction in the chlorophyll content and chlorophyll a/b ratio under high temperature stress (Table 3). These results are in line with Prasad et al. (2011), who exhibited a decrease in chlorophyll content and rate of photosynthesis by high night temperature up to 8% and 22%, respectively, than optimum night temperature. It was observed, however, that a marked increase occurred in the carotenoid content under high temperature stress (Table 3), which is indicative of some degree of tolerance acquired by *L. chinensis* plants under high temperature stress, as the carotenoids have some role in heat stress tolerance. Under both room as well as high temperature stressed conditions, treatment with 0.1 and/or 1.0 mg L^{-1} BR proved beneficial regarding enhancement of the chlorophyll as well as carotenoid content (Table 3). The increment in biosynthesis of photosynthetic pigments might be attributed to changes induced in transcription and translation by BR (Bajguz, 2000). Yun-ying and Hua (2008) reported similar results and narrated the protective role of brassinolide in improvement of chlorophyll content in rice seedlings under heat stress. It was observed that root activity was severely affected by heat stress as compared to room temperature, however, the BR showed the ameliorative effect on root activity at high temperature. Similar results were obtained by Yun-Ying et al. (2009), who indicated that the root activity of *indica* rice was reduced in response to heat stress, while MDA level was increased as compared to normal temperature.

It is generally understood that heat injuries to the plants are physiologically based on the over-production of reactive oxygen species, reduction of antioxidant-enzyme activities and membrane damage (Zhu et al., 2005). An increased level of MDA was observed in *L. chinensis* plants by the effect of high temperature stress, which is an index of lipid peroxidation and damage of biological membranes but the treatment with BR lowered the MDA content under normal and stressed conditions (Table 4). The said observations are in accordance with Ogweno et al. (2008) who reported that there was an enhanced production of MDA by the heat treatments of tomato; however, 24-epibrassinolide application reduced the MDA level in plants. There was excessive production of proline, soluble protein and sugar content in *L. chinensis* plants under the high temperature stress which was further improved by the application of BR with most beneficial results obtained at 0.1 mg L^{-1} concentration (Table 4). Under stressed conditions plants tend to accumulate osmolytes in their cells in response to abiotic stresses (Kumar et al., 2012). The accumulation of osmolytes in plants in response to BR is correlated to higher levels of the heat shock proteins and corresponding mRNA during the heat stress (Dhaubhadel et al., 1999; Ozdemir et al., 2004). Additionally, Geetika et al. (2014) reported an increase in proline and protein content of *Brassica juncea* by the application of 28-homobrassinolide.

The present study revealed that an enhanced production of antioxidant enzymes viz. SOD, POD, CAT, APX, and GR were observed when the *L. chinensis* plants were exposed to heat stress; which was further improved by the exogenous application of BR to the plants particularly at 0.1 and/or 1.0 mg L^{-1} concentration (Table 5). The activity of the antioxidant enzymes is very crucial for scavenging the reactive oxygen species, whose production is increased under the stressed conditions (Gapper and Dolan, 2006). Reactive oxygen species are quenched by antioxidant enzymes and BR induces the activity of antioxidant enzymes (Ogweno et al., 2008). The enhanced biosynthesis and activity of antioxidant enzymes is attributed to increased transcription of genes encoding mRNA for antioxidant enzymes (Bajguz and Hayat, 2009). Our findings are similar to Mazorra et al. (2002) used tomato and Kumar et al. (2014) used mustard who reported an elevated production of antioxidant enzymes by the application of BR under high temperature stress.

CONCLUSIONS

A reduction in growth, synthesis of photosynthetic pigments, soluble protein and sugar content was observed under high temperature stress. However, exogenous application of brassinolide (BR) to *Leymus chinensis* improved the high temperature stress tolerance reflected by enhanced antioxidant activity and reduced malondialdehyde activity.

Exogenous application of 0.1 mg L^{-1} BR proved better in improving stress tolerance capacity, the leaf area, plant fresh weight and dry weight were improved as compared to control at high temperature. On the basis of our results, it can be stated that exogenous application of BR can improve the stress tolerance in plants by improving the plant growth, synthesis of photosynthetic pigments and antioxidant enzymes activity.

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