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RESEARCH

Effects of brassinolide application on antioxidant characteristics and endogenous hormones of *Leymus chinensis* (Trin.) Tzvelev under different light intensity regimes

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ABSTRACT

Different light and shade intensities are the main constraints to achieve potential biomass accumulation in *Leymus chinensis* (Trin.) Tzvelev. An experiment was conducted to explain the physiochemical and morphological adversity triggered by various light intensities. Adverse impacts were lessened with exogenous brassinolide (BR). Treatments consisted of 100% natural light (T_0), 100% natural light + 0.1 mg L^{-1} BR (BT_0), light shade (70% natural light) (T_1), light shade + BR (70% natural light + 0.1 mg L^{-1} BR) (BT_1), moderate shade (50% natural light) (T_2), and moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR) (BT_2). These were completely randomized design treatments with five replicates. Results showed that dry biomass production and endogenous hormone levels (abscisic acid [ABA], zeatin riboside [ZR], gibberellic acid [GA], and indole acetic acid [IAA]) increased, while osmolyte accumulation (soluble sugars and proteins, free amino acids, and proline), antioxidant activity (peroxidase [POD], superoxide dismutase [SOD], and catalase [CAT]), and lipid peroxidation decreased under shade at T_0. Treatment T_1 depicted the most promising results for these attributes. However, biomass accumulation (33%-56%), osmotic substances (soluble sugars,13%-38%; soluble proteins, 9%-41%; free amino acids, 8%-16%; proline, 11%-41%), and antioxidant enzyme activities (POD, 11%-14%; SOD, 3%-33%; CAT, 4%-23%) increased. On the other hand, lipid peroxidation was reduced by BR application, as indicated by decreased malondialdehyde (MDA) content (7%-31%) and relative conductivity (7%-19%) under both natural and shade conditions. Likewise, the biosynthesis of ABA (2%-22%), ZR (11%-24%), and GA (30%-50%) improved under exogenous BR compared with the control. The IAA contents (22%-28%) decreased under foliar-applied BR compared with the control. Results suggested that light to moderate shade improved the biomass production and physiological performance of *L. chinensis*, while BR application exhibited further improvement.

Key words: Antioxidants, brassinolide, endogenous hormones, *Leymus chinensis*, shading.

INTRODUCTION

Leymus chinensis (Trin.) Tzvel. is a forage grass that is widely distributed throughout northern China, Siberia, and Mongolia (Huang et al., 2004). It is a perennial grass that has high productivity, nutritional value, and palatability (Wang et al., 2009). Moreover, extensive rhizomatous growth increases water uptake ability and enhances stress tolerance (Wang and Zhou, 2004). Owing to its adaptability to diverse environmental conditions, it grows in an area of 220 000 km^2 across different regions of China. *Leymus chinensis* is an important grassland species in China; it is a source of livestock feed and improves

soil aggregate stability and water conservation in northern China (Bai et al., 2010). The sustainability of grasslands in Inner Mongolia is highly unstable due to intensive grazing, imbalanced nutrient application, soil salinization, drought, heat, and erratic light intensities. The biosynthesis of photosynthetic pigments and osmotic substances therefore undergo adverse alterations that are triggered by different stresses. Finally, assimilate partitioning to stem and leaves is also reduced and this results in poor biomass accumulation (Katoh et al., 1998; Van der Wal et al., 2000; Augustine, 2003; Gassmann, 2004).

Among various environmental factors that adversely affect biomass accumulation, light intensity is of prime importance. Light provides energy for photosynthesis, which is a basic requirement for plant growth and development. Nevertheless, energy transfer from photosystem II (PS II) to the reaction center is much greater than electron transfer in the electron transport chain of light reactions under high light intensities. Consequences of this energy imbalance cause thylakoid unstacking, lipid peroxidation, oxygen evolving complex impairment, aggravated cyclic electron flow around photosystem I (PS I), and Mn-D1D2 complex disintegration, which ultimately result in photoinhibition (Mathur et al., 2014). On the other hand, light to moderate shade conditions lower air and leaf temperature, leaf transpiration, and reactive oxygen species (ROS) stress generation, which worsen at higher irradiance conditions and improve photosynthetic efficiency and plant growth (Valladares and Pearcy, 1997; Tang et al., 2015). Low levels of irradiance in leaves can sometimes result in decreased C gain and reduced plant growth (Aleric and Kirkman, 2005).

High light intensity mediated adversity in the defense mechanism can be boosted by strengthened antioxidant biosynthesis and enhance the stay green trait and ability to achieve osmotic adjustments to alleviate ROS. Plants have developed various defense systems such as increased carotenoid, xanthophyll cycle, and osmolyte synthesis, and improved antioxidant activity to reduce ROS production and lipid peroxidation (Hansen et al., 2002). Osmolytes, such as proline and soluble proteins and sugars, protect cellular constituents and macro molecules by physical quenching and chemical scavenging (Niu et al., 2016). Carotenoid-mediated quenching of ROS encompasses electron and proton donation to quench singlet oxygen (Siefermann-Harms, 1987). However, a plethora of ROS produced under intense radiations overcomes the antioxidant defense system and results in oxidative stress. Downregulation in antioxidant biosynthesis aggravates lipid peroxidation, photosynthetic pigment degradation, and diminishes the plant's ability to make osmotic adjustments under stress conditions (Shi et al., 2006).

Exogenously applied plant growth-regulating substances have the potential to alter growth, development, and physiological processes taking place in the plant (Anjum et al., 2011; 2013). Brassinosteroids (BRs) are polyhydroxysteroids that are mainly involved in plant growth and development (Jin-huan et al., 2015). Brassinosteroid-induced metabolic improvements include increased apoplast hydrolase activities, older cell wall degradation, and new cell wall biosynthesis. Therefore, BR enhances cell extension during growth and thus helps to accumulate biomass under a stress environment (Vriet et al., 2012). Plant growth regulators govern these processes by modulating endogenous hormone levels. Furthermore, BR improves plant growth by improving photosynthetic activity through enhanced synthesis and better protection of the photosynthetic apparatus attained by altering osmolyte accumulation and antioxidant activity, which reduces ROS production and lipid peroxidation (Mei-ru et al., 2015; Song et al., 2015). Likewise, the exogenous application of BR triggered rapid increases in the physiological processes that include electron flow maintenance in the electron transport chain and NADH biosynthesis enhancement during photosynthesis light reactions, thus improving C fixation. Sensitivity to photoinhibition declines because of improved accumulation of osmotic substances and antioxidants (Niu et al., 2016). The BR could regulate the photosynthesis of *L. chinensis* at varying light intensities (Yang et al., 2018). Information regarding high light intensity mediated adversity in the metabolic process of *L. chinensis* is indispensable to improve tolerance. Moreover, improvements in physiological attributes often result in increased morphological attributes and thus constitute an unexplained area of study to increase biomass accumulation in *L. chinensis* under stress environments. Therefore, the experiment was conducted to explore the adverse impacts of high light intensities on the physiochemical attributes of *L. chinensis* and evaluate BR foliar spraying as a potential regulator of antioxidants, osmotic substances, photosynthetic pigments, and eventually biomass accumulation.

MATERIALS AND METHODS

The experiment was conducted from May to August 2015 at the College of Agricultural Sciences and Biotechnology (29°49'32" N, 106°26'02" E; 220 m a.s.l.), Southwestern University, China. Local climate data is as follows: approximately

1200 annual sunshine hours, 18.2 °C average temperature, and 1200 mm annual precipitation. Seed material used in the experiment was obtained from the Inner Mongolia Ecological Experimental Station at the natural distribution community in November 2013. Collected seeds were dried at room temperature, put into cloth bags, and placed in a refrigerator at 4 °C. Seeds were then sown in germination trays in July 2014 and uniform-sized plants were transplanted into pots (diameter 24.5 cm, height 20 cm) in April 2015.

A shed with a 198 cm × 110 cm area and 1 m height was used to provide shade for the plants. The experiment was used a completely randomized design (CRD) and was replicated five times. The chemical BR reagent was 24-epibrassinolide (24-epiBR). Treatments consisted of 100% natural light (T_0), 100% natural light + 0.1 mg L^{-1} BR (BT_0), light shade (70% natural light) (T_1), light shade + BR (70% natural light + 0.1 mg L^{-1} BR) (BT_1), moderate shade (50% natural light) (T_2), and moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR) (BT_2). Treatment details are given in Table 1. Brassinosteroid was applied three times at 1 wk intervals (19 May 2015, 26 May 2015, and 2 June 2015) at 0.1 mg L^{-1} BR, while distilled water was used as the control. Spraying always occurred at 18:00 h to avoid the effect of light. A balanced amount of nutrients was supplied to the pots by applying 25 mL Hoagland's nutrient solution every 5 d. Pots were irrigated at 2-d intervals using drip irrigation. Dry biomass production, osmolyte accumulation, lipid peroxidation, endogenous hormone contents, and antioxidant activity were measured three times, that is, 20, 40, and 60 d after completing the BR sprayings.

Once uprooted, the plants were rinsed with water 2-3 times and dried with filter paper, followed by oven-drying at 105 °C for 30 min and then at 65 °C until reaching constant weight. Soluble sugars were assessed by the anthrone color method (Li et al., 2008). The Coomassie brilliant blue method was used to measure soluble protein content (Bradford, 1976). Free amino acids were quantified by the ninhydrin colorimetric method (Zhuang et al., 2016), while proline contents were evaluated using the process described by Bates et al. (1973). The malondialdehyde (MDA) content was assayed using the thiobarbituric acid (TBA) assay (De-Vos et al., 1991). Relative conductivity was measured by the method used by Nayyar et al. (2005). Leaf endogenous hormonal contents, that is, zeatin riboside (ZR), gibberellic acid (GA), abscisic acid (ABA), and indole acetic acid (IAA) were determined by an enzyme-linked immunosorbent assay (ELISA) (Bollmark et al., 1988; Teng et al., 2010). Peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) were measured by the method provided by Parida et al. (2004).

Data were statistically analyzed using the ANOVA technique with SPSS 19.0 statistical software (IBM, Armonk, New York, USA). Treatment means were compared by the least significant difference (LSD) test at the 5% probability level.

RESULTS

Dry matter production

Dry weight accumulation increased over time and higher dry weight was observed 60 d after treatment (DAT) than after 20 and 40 DAT. Dry weight increased more under shade than in natural light and more under exogenous BR than without BR spraying under all light intensities. Moreover, the highest dry weight was recorded with BT_1 at 20 DAT (262.0 mg per plant), 40 DAT (273.2 mg per plant), and 60 DAT (274.0 mg per plant). The BT_1 mediated improvements in dry weight were 56% (20 DAT), 33% (40 DAT), and 33% (60 DAT) compared with T_0. Minimum dry weight was observed for T_0 at 20, 40, and 60 DAT (Table 2).

Osmolyte accumulation

Shade stress decreased osmolyte accumulation in *L. chinensis* plants, while BR application promoted osmolyte accumulation. Soluble sugars, proteins, and free proline contents increased from 20 to 60 DAT. On the contrary, free amino biosynthesis increased over time up to 40 DAT and then decreased at 60 DAT. Shade stress decreased osmolyte

Table 1. Treatments details of brassinolide (BR) application under different light intensities.

	Light intensity		
Treatments	Control (100% natural light)	Light shade (70% natural light)	Moderate shade (50% natural light)
Control	T_0	T_1	T_2
0.1 mg L^{-1} BR	BT_0	BT_1	BT_2

Table 2. Effect of brassinolide (BR) on dry weight of *Leymus chinensis* under different light intensities.

Treatments	Dry weight		
	20 DAT	40 DAT	60 DAT
	mg plant^{-1}		
T_0	115.9 ± 2.8d	182.4 ± 3.9d	183.7 ± 3.1d
BT_0	133.4 ± 3.4d	201.2 ± 3.2cd	204.0 ± 1.7cd
T_1	224.7 ± 4.5b	262.8 ± 5.3ab	264.1 ± 3.1ab
BT_1	262.0 ± 2.7a	273.2 ± 1.9a	274.0 ± 4.2a
T_2	176.0 ± 3.8c	211.8 ± 3.6cd	212.7 ± 2.4cd
BT_2	190.2 ± 3.4bc	227.9 ± 4.0bc	230.6 ± 4.6bc

Values are means of at least 5 replicates ± SE. Values followed by the same letter in the same column are not significantly different according to the LSD test ($P < 0.05$).
DAT: Days after treatment; T_0: 100% natural light; BT_0: 100% natural light + 0.1 mg L^{-1} BR; T_1: light shade (70% natural light); BT_1: light shade + BR (70% natural light + 0.1 mg L^{-1} BR); T_2: moderate shade (50% natural light); BT_2: moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR).

accumulation more than natural light. The highest accumulation of soluble sugars and free amino acids was recorded under natural light, while the minimum accumulation was observed under moderate shade. Soluble sugars and proline were higher under natural light and lower values for these attributes were observed under light shade than in other treatments. The BR mediated improvements increased under natural light, light shade, and moderate shade; values were 13%-38%, 0%, and 14%-20%, in soluble sugars, 9%-41%, 3%-64%, and 5%-41% in soluble proteins, 1%-2%, 8%-16%, and 3%-19% in free amino acids, and 11%-41%, 14%-39%, and 14%-37% in proline contents, respectively, compared with the corresponding controls (Tables 3 and 4).

Table 3. Effect of brassinolide (BR) on water soluble sugars, proteins, and free amino acids of *Leymus chinensis* under different light intensities.

Treatments	Soluble sugars			Soluble proteins			Free amino acids		
	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT
	mg g^{-1}			mg g^{-1}			µg g^{-1}		
T_0	24.39±1.42b	26.40±0.52b	29.35±1.66b	7.96±0.13b	10.15±0.25bc	39.39±0.44c	145.8±20.5a	298.0±21.4bc	218.1±13.1a
BT_0	33.70±0.60a	31.26±0.06a	33.12±1.00a	9.22±0.48a	14.29±0.33a	42.81±0.06a	147.6±11.2a	250.2±13.9d	223.2±3.6a
T_1	19.81±2.09c	23.61±0.17c	20.09±0.64d	5.18±0.3d	6.66±0.73e	39.4±0.07c	111.6±13.0a	264.8±6.6bcd	192.7±3.9ab
BT_1	18.14±0.9cd	19.26±0.74e	18.68±0.70d	8.52±0.1ab	9.09±0.06cd	40.64±0.09b	120.0±2.1a	308.2±19.2ab	223.9±1.3a
T_2	15.94±0.43d	20.83±0.36d	23.25±0.20c	6.22±0.12c	8.63±0.1d	37.11±0.13d	129.4±18.9a	344.6±11.2a	166.0±12.9b
BT_2	19.08±0.54cd	23.72±0.3c	27.23±0.76b	8.77±0.2a	10.65±0.2b	39.13±0.19c	133.1±16.1a	255.6±7.0cd	197.2±13.3a

Values are means of at least 5 replicates ± SE. Values followed by the same letter in the same column are not significantly different according to the LSD test ($P < 0.05$).
DAT: Days after treatment; T_0: 100% natural light; BT_0: 100% natural light + 0.1 mg L^{-1} BR; T_1: light shade (70% natural light); BT_1: light shade + BR (70% natural light + 0.1 mg L^{-1} BR); T_2: moderate shade (50% natural light); BT_2: moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR).

Table 4. Effect of brassinolide (BR) on proline, malondialdehyde (MDA), and relative conductivity of *Leymus chinensis* under different light intensities.

Treatments	Proline			MDA			Relative conductivity		
	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT
	µg g^{-1}			µmol g^{-1}			%		
T_0	31.94±0.62a	55.54±1.36c	132.78±6.08bc	31.23±0.6a	34.66±1.04a	45.74±0.76a	2.8±0.21a	4.65±0.34a	27.63±0.23a
BT_0	35.61±1.83a	70.11±1.12a	187.35±4.99a	22.77±1.49bcd	23.88±0.66b	42.75±0.12a	2.26±0.12b	4.31±0.10a	23.71±0.89bc
T_1	17.00±0.28d	34.45±1.49e	96.85±2.54e	26.11±1.24b	21.79±3.28b	28.91±1.01bc	1.58±0.14d	2.96±0.11c	17.43±0.47d
BT_1	20.39±1.74cd	47.73±1.31d	110.29±4.58de	20.17±1.96d	19.41±0.72b	24.73±2.18d	1.47±0.13d	2.42±0.56c	16.11±0.54d
T_2	21.47±0.61bc	45.72±0.32d	125.23±4.36cd	25.52±0.38bc	29.93±0.6a	31.32±1.16b	2.17±0.09bc	3.96±0.14ab	21.84±0.47bc
BT_2	24.43±0.31b	62.76±1.83b	147.99±9.54b	21.61±1.24cd	21.12±1.21b	26.8±0.8cd	1.74±0.21cd	3.16±0.31bc	20.06±1.5cd

Values are means of at least 5 replicates ± SE. Values followed by the same letter in the same column are not significantly different according to the LSD test ($P < 0.05$).
DAT: Days after treatment; T_0: 100% natural light; BT_0: 100% natural light + 0.1 mg L^{-1} BR; T_1: light shade (70% natural light); BT_1: light shade + BR (70% natural light + 0.1 mg L^{-1} BR); T_2: moderate shade (50% natural light); BT_2: moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR).

Lipid peroxidation

Lipid peroxidation in *L. chinensis* plants decreased when exposed to shade stress, which was further lowered by BR application. The MDA content and relative conductivity increased from 20 to 60 DAT. Shade decreased lipid peroxidation and the order of MDA content and relative conductivity under the influence of light was natural light > moderate shade > light shade. Exogenous BR triggered a decrease in MDA contents of 7%-31%, 11%-23%, and 14%-29%, and 7%-19%, 7%-18%, and 8%-20% in relative conductivity under natural light, light shade, and moderate shade, respectively, when compared with their respective controls (Table 4).

Endogenous hormone levels

The exposure of *L. chinensis* plants to shade resulted in increased endogenous hormone levels. A rapid increase in the endogenous levels of all the hormones was recorded under exogenous BR more than in their respective controls at each shade level. On the contrary, the IAA contents decreased under foliar BR compared with the control at each shade level. The hormone endogenous levels increased over time after treatment up to 40 DAT and decreased again at 60 DAT. However, hormone endogenous levels were not affected in the same way under different levels of shade for different durations. Abscisic acid contents at 20, 40, and 60 DAT were higher under light shade than in other shade treatments. The lowest ABA contents were quantified under natural light at 20 and 60 DAT and under moderate shade at 40 DAT. Zeatin riboside depicted a highly erratic response under different BR and shade treatments over time after applying the treatments. The highest and lowest ZR contents were recorded under light shade and natural light, respectively, at 20 DAT, moderate and light shade, respectively, at 40 DAT, and natural light and moderate shade, respectively, at 60 DAT. Likewise, more GA contents were recorded under light shade than in other treatments at 20 and 60 DAT, while moderate shade showed the highest GA at 40 DAT. The lowest GA was recorded under natural light at 20 and 40 DAT and moderate shade at 60 DAT. Exogenous BR mediated improvements under natural light and light shade, and moderate shade had content values of 2%-22%, 9%, and 5%-9% in ABA, 11%-24%, 16%, and 15% in ZR, and 30%-50%, 0%, and 18%-55% in GA, respectively. Meanwhile, IAA decreased by 22%-28% and 10%-26% under natural light and light shade, respectively, and increased by 4% under moderate shade compared with their respective controls (Tables 5 and 6).

Antioxidant profile

Antioxidant activity decreased under shade conditions and increased with BR application at each shade level. The POD activity increased up to 40 DAT and then decreased at 60 DAT, while SOD and CAT contents continued to improve over time from 20 to 60 DAT. Antioxidant activity under shade decreased and was ordered as natural light > moderate shade > light shade. The treatment of *L. chinensis* plants with BR improved antioxidant enzyme activity at each shade level The BR mediated improvements triggered under natural light, light shade, and moderate shade values of 11%-14%, 2%-28%, and 9 in POD %, 3%-33%, 1%-20%, and 2%-3% in SOD, and 4%-23%, 2%, and 3%-11% in CAT, respectively (Table 7).

Table 5. Effect of brassinolide (BR) on endogenous abscisic acid (ABA) and indole acetic acid (IAA) contents of *Leymus chinensis* under different light intensities.

Treatments	ABA content			IAA content		
	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT
	ng g^{-1}			ng g^{-1}		
T_0	57.33 ± 4.01b	151.48 ± 2.54bc	62.57 ± 4.25bc	182.17±8.48b	517.16 ± 10.86a	221.52 ± 8.78b
BT_0	69.86 ± 0.01a	153.77 ± 2.29b	60.25 ± 0.46c	134.2±6.42d	400.98 ± 8.47c	159.71 ± 8.11d
T_1	72.45 ± 1.05a	154.29 ± 4.25bc	77.73 ± 0.57a	144.73±10.96cd	468.42 ± 3.31b	194.91 ± 2.83c
BT_1	67.90 ± 0.52a	168.25 ± 1.99a	62.38 ± 0.67bc	130.44±5.47d	389.06 ± 2.49cd	144.04 ± 2.53d
T_2	66.56 ± 0.18a	142.62 ± 1.65cd	66.73 ± 0.05b	274.15±11.27a	391.31 ± 9.07cd	241.8 ± 11.61ab
BT_2	60.15 ± 0.10b	149.16 ± 1.99bc	72.66 ± 0.79a	174.67±9.24bc	371.76 ± 6.3d	250.93 ± 7.02a

Values are means of at least 5 replicates ± SE. Values followed by the same letter in the same column are not significantly different according to the LSD test ($P < 0.05$).

DAT: Days after treatment; T_0: 100% natural light; BT_0: 100% natural light + 0.1 mg L^{-1} BR; T_1: light shade (70% natural light); BT_1: light shade + BR (70% natural light + 0.1 mg L^{-1} BR); T_2: moderate shade (50% natural light); BT_2: moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR).

Table 6. Effect of brassinolide (BR) on endogenous zeatin riboside (ZR) and gibberellic acid (GA) contents of *Leymus chinensis* under different light intensities.

Treatments	ZR content			GA content		
	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT
	ng g^{-1}			ng g^{-1}		
T_0	29.2 ± 1.84bcd	56.14 ± 1.25b	39.25 ± 0.22a	1.75 ± 0.08e	7.69 ± 0.06c	2.23 ± 0.06d
BT_0	32.45 ± 1.46bc	69.5 ± 2.18a	35.86 ± 1.81a	2.36 ± 0.12d	11.5 ± 0.08b	2.90 ± 0.02c
T_1	42.03 ± 1.66a	55.3 ± 3.10b	26.94 ± 2.64b	4.95 ± 0.12a	10.85 ± 0.62b	3.91 ± 0.09a
BT_1	25.4 ± 2.20d	64.13 ± 1.93a	21.08 ± 0.17c	3.24 ± 0.09b	10.45 ± 0.00b	2.68 ± 0.07c
T_2	34.89 ± 1.52b	65.68 ± 2.19a	25.79 ± 0.86bc	2.81 ± 0.09c	14.21 ± 0.17a	2.11 ± 0.02d
BT_2	26.86 ± 2.29cd	65.12 ± 2.24a	29.75 ± 1.26b	3.31 ± 0.07b	13.29 ± 0.41a	3.26 ± 0.10b

Values are means of at least 5 replicates ± SE. Values followed by the same letter in the same column are not significantly different according to the LSD test ($P < 0.05$).
DAT: Days after treatment; T_0: 100% natural light; BT_0: 100% natural light + 0.1 mg L^{-1} BR; T_1: light shade (70% natural light); BT_1: light shade + BR (70% natural light + 0.1 mg L^{-1} BR); T_2: moderate shade (50% natural light); BT_2: moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR).

Table 7. Effect of brassinolide (BR) on antioxidant enzymes of *Leymus chinensis* under different light intensities.

Treatments	POD			SOD			CAT		
	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT	20 DAT	40 DAT	60 DAT
	U g^{-1} min^{-1}			U g^{-1} FW			U g^{-1} min^{-1}		
T_0	9374.7±315.72b	9473±196.02b	3877.91±283.61b	68.74±0.49b	48.09±1.55b	238.92±11.1a	145.88±4.71ab	170.35±12.53b	580.99±10.69a
BT_0	10580.81±154.3a	10506.29±136.18a	4433.22±240.37a	70.51±0.05a	64.16±2.25a	257.68±5.65a	151.8±5.4a	208.7±10.67a	624.24±31.52a
T_1	6640±103.35d	5270.81±337.67c	1165.37±45.56d	57.68±0.14d	40.46±1.75c	126.6±10.42c	111.69±3.9c	123.4±11.08c	374.7±17.79cd
BT_1	6751.21±336.32d	6011.07±135.48c	1492.57±25.58d	58.12±0.22d	42.26±0.96bc	151.53±5.28bc	113.48±3.91c	114.94±4.6c	346.11±17.96d
T_2	7246.19±221.33cd	8776.01±619.14b	2604.31±142.64c	65.25±0.08c	45.95±0.3bc	166.7±12.76b	131.53±2.96b	167.93±7.77b	438.85±5.73bc
BT_2	7884.51±289.51c	8801.05±105.68b	2550.06±56.18c	65.57±0.26c	47.48±2.91b	170.23±10.88b	134.85±3.03b	156.8±8.88b	486.08±22.78b

Values are means of at least 5 replicates ± SE. Values followed by the same letter in the same column are not significantly different according to the LSD test ($P < 0.05$).
DAT: Days after treatment; T_0: 100% natural light; BT_0: 100% natural light + 0.1 mg L^{-1} BR; T_1: light shade (70% natural light); BT_1: light shade + BR (70% natural light + 0.1 mg L^{-1} BR); T_2: moderate shade (50% natural light); BT_2: moderate shade + BR (50% natural light + 0.1 mg L^{-1} BR).
POD: Peroxidase; SOD: superoxide dismutase; CAT: catalase.

DISCUSSION

Various environmental factors affect grassland productivity in China. *Leymus chinensis* is highly adaptable to various environmental conditions and can survive under different abiotic stresses (Ma and Liang, 2007; Wang et al., 2008). However, the scarce availability of information regarding different light intensity mediated adversity (Yang et al., 2018) and associated perturbations in the *L. chinensis* metabolism needs extensive exploration. Moreover, adverse impacts of different light intensities might also influence biomass and other morphological traits and can thus decrease potential benefits to be had by growing *L. chinensis* (Chen et al., 2013).

Light is generally considered as the most important factor controlling growth and development as well as for plant physiology and biochemistry. However, light intensity below the threshold often impairs growth, while it causes photoinhibition above the threshold (Valladares and Niinemets, 2008). Low intensity light induces poor synchronization of phytochrome red and far red and thus hampers cell division and growth under low light intensity (Aleric and Kirkman, 2005). Carbon fixation in photosynthesis also decreases, which ultimately downregulates. On the contrary, high light intensity disrupts the PS II structure and introduces a negative feedback mechanism for photosynthesis (Jordan et al., 2005). Therefore, under varying light intensities, it is a prerequisite to regulate numerous metabolic plant processes to enhance tolerance against erratic changes in light radiations. Among the innumerable management strategies, exogenous application of plant growth regulators and nutrients constitutes an important and economically feasible strategy to reach the biomass potential (Wahid et al., 2007). In this perspective, light shade enhanced biomass production, which was boosted by BR spraying (Table 2). The increase in biomass production under shade and BR application might be due to improved photosynthetic activity due to better synthesis (Yang et al., 2018) and osmotic mediated adjustments to

protect photosynthetic pigments (Chaves et al., 2008; Hayat et al., 2011). Similarly, Perrin and Mitchell (2013) reported that increased shade levels improved biomass production and growth of the European yew. Hayat et al. (2010) reported enhanced plant height, leaf area, and plant biomass production of *Vigna radiata* by applying 28-homobrassinolide.

Antioxidant enzyme activity, as well as other defense mechanisms, is regulated by the production of endogenous plant growth hormone levels. A study revealed that light regulated the genes in chrysanthemum plants that encode various plant hormones such as auxins, abscisic acid, jasmonic acid, BRs, and indole (Hong et al., 2015). Nonetheless, studies are required to determine the regulatory mechanisms of osmolyte accumulation, antioxidant activity, and endogenous hormone levels under various light regimes. Although low light intensity impairs cell division, it reduces availability for C fixation in photosynthesis and nutrient uptake from the soil medium. Finally, cofactor availability for enzyme activation and osmotic substance accumulation decrease and ROS biosynthesis worsens. Afterward, the plethora of ROS overcomes the scavenging mechanism and exposes the plant to oxidative stress (Hansen et al., 2002). Plants cope with ROS by producing osmolytes and antioxidant defense systems (Niu et al., 2016). High osmolyte accumulation levels in *L. chinensis* plants were observed under natural light, decreased under light shade, and increased again under moderate light shade conditions. Similarly, lipid peroxidation was higher in natural light, decreased in light shade, and started to increase again in moderate shade. The BR application enhanced osmolyte accumulation and decreased lipid peroxidation at each light level (Tables 3 and 4). Light shade (75% natural light) decreased osmolyte accumulation and MDA contents in *Torreya grandis* seedlings compared with full sunlight, 50% light, and lower shade levels (Tang et al., 2015). Furthermore, Kumar et al. (2014) reported that applications of 28-homobrassinolide increased soluble sugar accumulation and reduced non-reducing sugars and soluble proteins in *Brassica juncea* seedlings. Liu et al. (2011) reported decreased MDA content in *Chorispora bungeana* plants with BR application compared with the control.

Endogenous hormones regulate plant growth and physiological functions such as photosynthesis, enzyme activity, and gene expression. It has been known that shade significantly affects endogenous hormones (Zhang et al., 2011). In the present study, the endogenous hormone levels (ABA, IAA, ZR, and GA) in *L. chinensis* plants were enhanced under different shade regimes (Tables 5 and 6). Wang et al. (2014) found that leaf ABA and IAA increased, while leaf and root ZR content decreased in sweet potato (*Ipomoea batatas*) under 40% and 70% shading. In the present study, the ABA, ZR, and GA contents increased, while the IAA content decreased in *L. chinensis* plants under exogenous BR compared with their respective controls (Tables 5 and 6). Plant growth increased under different exogenous treatments of GA compared with the control, which ultimately upregulated the biosynthetic genes of GA (Tong et al., 2014). Gaudinová et al. (1995) noticed that the application of two synthetic BRs, 24-epibrassinolide (24-epiBR) and 2α,3α,17β-trihydroxy-5α-androstan-6-one (THA-BR), enhanced cytokinin and IAA contents in tobacco callus tissues. However, the exogenous BR application in Arabidopsis did not increase IAA levels in BR-deficient mutants and wild type plants (Nakamura et al., 2003). Bajguz (2009) observed an increased ABA level in *Chlorella vulgaris* with BR application, which is similar to our results.

Plants enhance antioxidant activity to keep ROS activity balanced and maintain plant growth (Wang et al., 2012). Song et al. (2015) found that BR application adjusted osmolytes and antioxidant enzymes in *L. chinensis* under drought stress. Hu et al. (2013) indicated that a combination of ABA and BR can enhance *L. chinensis* growth and the net photosynthetic rate. Yang et al. (2018) point out that foliar application of BR increased photosynthesis of *L. chinensis* under different light intensity levels. Further antioxidant enzymes, namely, POD, SOD, and CAT were higher under excessive light conditions (100% natural light), decreased at 70% natural light, and were enhanced once again at 50% natural light; however, BR application on *L. chinensis* plants enhanced the antioxidant activity at each shade level (Table 7). Results indicate that the enhanced antioxidant activity at 100% and 50% natural light was well correlated with ROS production, as indicated by enhanced MDA content and relative conductivity (Table 4). The BR mediated improvements in antioxidant enzymes might be a consequence of improved biosynthesis of osmotic substances, endogenous hormones, photosynthetic pigments, and decreased lipid peroxidation under different radiation levels (Ali et al., 2008). Activities of SOD, APX, and glutathione peroxidase (GSH-Px) in *Torreya grandis* seedlings were enhanced under full sunlight and 50% natural light and the lowest antioxidant activity was exhibited at 75% natural light (Tang et al., 2015). Similarly, Wu et al. (2014) reported enhanced antioxidant activity in *Solanum melongena* with BR application under cold stress. Kumar et al. (2014) reported exaggerated antioxidant activity with BR application on *B. juncea* under high temperature.

CONCLUSIONS

Shade enhanced biomass production and endogenous hormone levels and reduced osmolyte accumulation, antioxidant activity, and lipid peroxidation. However, brassinolide (BR) application increased biomass production, osmolyte accumulation, antioxidant activity, and endogenous hormone levels, and lowered lipid peroxidation under all light regimes. It was concluded that BR application improves biomass production and the physiological performance of *Leymus chinensis* plants under both natural and shade conditions.

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