



## Article

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## BIOMASS ACCUMULATION, PHOTOSYNTHETIC PIGMENTS, OSMOTIC ADJUSTMENTS AND ANTIOXIDANT ACTIVITIES OF *Leymus chinensis* IN RESPONSE TO BA, BR, AND GA

*Acúmulo de Biomassa, Pigmentos Fotossintéticos, Ajustes Osmóticos e Atividade Antioxidante de **Leymus chinensis** em Resposta a BA, BR e AG*

**ABSTRACT** - Abiotic stresses and poor biomass accumulation are chief constraints to accomplish potential yield in *Leymus chinensis*. An experiment was conducted to improve biomass and correlation of biomass accumulating attributes with photosynthetic pigments, osmotic substances and antioxidants under foliar application of different plant growth substances. The experiment was conducted at inner Mongolia Xilinguole, China, using a Randomized Complete Block Design and 5 replications. The treatments consisted of water (control); BA5 = application of BA (6-benzylaminopurine) at 5 mg L<sup>-1</sup>; BA25 = application of BA at 25 mg L<sup>-1</sup>; BA50 = application of BA at 50 mg L<sup>-1</sup>; BR0.02 = application of BR (brassinosteroid) at 0.02 g L<sup>-1</sup>; BR0.2 = application of BR at 0.2 mg L<sup>-1</sup>; BR2 = application of BR at 2 mg L<sup>-1</sup>; GA10 = application of GA (gibberellic acid) at 10 mg L<sup>-1</sup>; GA50 = application of GA at 50 mg L<sup>-1</sup> and GA100 = application of GA at 100 mg L<sup>-1</sup>. Application of all plant growth substances significantly improved biomass, osmotic adjustments, photosynthetic pigments and antioxidant activities compared to control. However, the most promising results were found with 0.2 mg L<sup>-1</sup> BR. The highest chlorophyll a/b, glutathione and ascorbate peroxidase activities were recorded with 25 mg L<sup>-1</sup> BA. Conclusively, 25 mg L<sup>-1</sup> BA, 0.2 mg L<sup>-1</sup> BR and 10 mg L<sup>-1</sup> GA exhibited more promising results than other concentrations for the evaluated attributes.

**Keywords:** antioxidants, reactive oxygen species, phyto regulators.

**RESUMO** - Estresses abióticos e baixo acúmulo de biomassa são as principais restrições para se atingir o potencial de rendimento de *Leymus chinensis*. Foi conduzido um experimento para melhorar a biomassa e a correlação de atributos de acúmulo de biomassa com pigmentos fotossintéticos, substâncias osmóticas e antioxidantes sob aplicação foliar de diferentes fitorreguladores. O experimento foi conduzido em Xilinguole, Mongólia Interior, China, usando um delineamento em blocos completamente casualizados com cinco repetições. Os tratamentos consistiram de água (controle); BA5 = aplicação de BA (6-benzilaminopurina) a 5 mg L<sup>-1</sup>; BA25 = aplicação de BA a 25 mg L<sup>-1</sup>; BA50 = aplicação de BA a 50 mg L<sup>-1</sup>; BR0,02 = aplicação de BR (brassinosteróide) a 0,02 mg L<sup>-1</sup>; BR0,2 = aplicação de BR a 0,2 mg L<sup>-1</sup>; BR2 = aplicação de BR a 2 mg L<sup>-1</sup>; AG10 = aplicação de AG (ácido giberélico) a 10 mg L<sup>-1</sup>; AG50 = aplicação de AG a 50 mg L<sup>-1</sup>; e AG100 = aplicação de AG a 100 mg L<sup>-1</sup>. A aplicação de todos os fitorreguladores melhorou significativamente a biomassa, os ajustes osmóticos, os pigmentos fotossintéticos e as atividades antioxidantes, em comparação com o controle. No entanto, os resultados mais promissores foram encontrados com a aplicação de BR a 0,2 mg L<sup>-1</sup>.

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Received: November 15, 2017

Approved: March 8, 2018

Planta Daninha 2019; v37:e019187872

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*As maiores atividades de clorofila a/b, glutationa e ascorbato peroxidase foram registradas com 25 mg L<sup>-1</sup> BA. Em conclusão, 25 mg L<sup>-1</sup> BA, 0,2 mg L<sup>-1</sup> BR e 10 mg L<sup>-1</sup> AG apresentaram resultados mais promissores que os de outras concentrações para os atributos avaliados.*

**Palavras-chave:** antioxidantes, espécies reativas de oxigênio, fitorreguladores.

## INTRODUCTION

*Leymus chinensis* has high value breeding traits, good palatability, nutritional, feed and economic value. Hence, it is one of the most important grassland species and source of livestock feed in China (Hong and Yunfei, 2004). However, numerous abiotic stresses hinder biomass accumulation of *L. chinensis* under the grassland ecosystem of China. Most importantly, drought, salinity and heat stress diminish productivity of *L. chinensis* and threatens sustainability of livestock feed in the country (Song et al., 2017).

Diminished productivity of *L. chinensis* is a consequence of impairments in innumerable physiochemical processes that are adversely affected under stresses and ultimately decline biomass accumulation (Mustapha et al., 2009). Prominently, escalated biosynthesis of reactive oxygen species under stressed environments overcomes antioxidant defense mechanism. A plethora of singlet oxygen, hydrogen peroxide, hydroxyl and superoxide radicals overcome the biosynthesis of superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase. Consequently, imbalance in biosynthesis of reactive oxygen species and antioxidants causes oxidative stress (Sairam et al., 2011). Consequences of oxidative stress include decreased activities of antioxidants; aggravated degradation of chlorophyll, carotenoids and other photosynthetic pigments; enhanced membrane leakage and diminished photosynthetic and sink capacity. Impairments in these physio-chemical attributes often show a strong association with morphological parameters (Liu et al., 2016).

Among the numerous strategies that are used for alleviation of stresses, exogenous application of plant growth substances is an economical strategy to alleviate adverse implications of stress (Rodriguez-Furlán et al., 2016). Regarding plant growth substances, foliar application of brassinosteroids (BR) enhances the capability of plants to accumulate osmotic substances and decrease osmotic potential. Application of gibberellic acid (GA) and BR enhances the accumulation of soluble sugars, free amino acids and soluble proteins (Talaat et al., 2015). Thus, maintenance of water potential reduces sensitivity of plants towards stress. Moreover, application of BR boosts the biosynthesis of antioxidants and osmo-protectants and, thus, alleviates stress. Likewise, foliar applied GA enhances stem elongation and biomass accumulation while the application of 6-benzylaminopurine (BA) upregulates biosynthesis of photosynthetic pigments, cell division and growth (Mangieri et al., 2017). Ultimately, accumulation of biomass and productivity are enhanced under stress conditions. Application of GA remarkably improves biosynthesis of antioxidants, photosynthetic pigments and accumulation of osmotic substances (Leitão and Enguita, 2016). Likewise, foliar application of BR boosts the activities of chlorophyll synthesizing enzymes and stay-green trait. Consequently, sucrose partitioning to plant leaves and other parts is sustained for a longer time and, thus, dry matter accumulation of plants is enhanced (Yang et al., 2011).

Numerous strategies that are employed by plants under oxidative stress include boosting of antioxidants to escalate detoxification of reactive oxygen species; accumulation of osmotic substances to decrease osmotic potential and sustain a favorable gradient for inflow of water into cells and biosynthesis of photosynthetic pigment at rate higher than those of pigment degradation (Kamal et al., 2017). Therefore, these processes are extremely important for stress tolerance in *L. chinensis* and enhancement of biomass accumulation on a sustainable basis (Guo-Liang et al., 2009).

Moreover, physiochemical attributes have a strong correlation with morphological attributes. Hence, boosting of these mechanisms ultimately enhances biomass accumulation. Therefore, the present experiment was conducted to i) enhance biomass accumulation of *L. chinensis* on a sustainable basis through exogenous application of different plant growth substances ii) study

the physiochemical attributes as potential regulators of biomass accumulation in *L. chinensis* iii) determine which osmotic substances, photosynthetic pigments and antioxidants activities are correlated with biomass accumulation attributes of *L. chinensis*.

## MATERIALS AND METHODS

### Experimental site

The experiment was conducted in natural grassland at the Inner Mangolia Grassland Ecosystem Research Station, Chinese Academy of Sciences, in Inner Mongolia, Xilinguole, China, from July to August 2016. Longitude, latitude and altitude of the experimental site was 116°10.162', 43°50.671' and 105 m, respectively.

### Treatments

Different concentrations of 6-benzylaminopurine (BA), brassinosteroid (BR), gibberellic acid (GA) and water (control) were applied exogenously. The treatments consisted of water (control); BA5 = application of BA at 5 mg L<sup>-1</sup>; BA25 = application of BA at 25 mg L<sup>-1</sup>; BA50 = application of BA at 50 mg L<sup>-1</sup>; BR0.02 = application of BR at 0.02 mg L<sup>-1</sup>; BR0.2 = application of BR at 0.2 mg L<sup>-1</sup>; BR2 = application of BR at 2 mg L<sup>-1</sup>; GA10 = application of GA at 10 mg L<sup>-1</sup>; GA50 = application of GA at 50 mg L<sup>-1</sup> and GA100 = application of GA at 100 mg L<sup>-1</sup>. The treatments were applied thrice to each respective plot at seven-day intervals.

### Observations recorded

Plants were uprooted at 7 days after the last treatment, rinsed with distilled water, dried with tissue paper and processed to record numerous attributes. Plant height was measured from base to the tip of plant. Fresh weight was recorded with an electrical weighing balance, then oven-dried at 105 °C for 15 minutes, followed by drying at 65 °C to constant weight to determine plant dry weight. Photosynthetic pigments were determined with the method described by Wellburn (1994), and soluble sugars were quantified by using the anthrone colorimetric method (Li et al., 2008). Soluble proteins were determined with the Coomassie Brilliant Blue Method (Bradford, 1976). Free amino acids were determined with the ninhydrin colorimetric assay (Huang et al., 2010). The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were determined by the method of Parida (2004). The samples were accurately weighed, and 3mL pre-cooled extract and a little quartz sand were added. Grinding was performed in an ice bath at 4 °C using 2 mL extraction solution, in centrifuged tubes containing enzyme extracts and collected supernatant.

### Experimental design and statistical analysis

The experiment was conducted using a Randomized Complete Block Design (RCBD) and replicated 5 times. Each experimental plot area was 20 m × 20 m and an isolation distance of 1 m was maintained to reduce the volatilization mediated contamination of adjacent treatments.

Significance of treatments was determined through Multivariate Analysis of Variance, using the software DPS19.0. Microsoft excel-2016 was used for graphical presentation of data. Strength of association among recorded parameters was determined using the software STATISTIX-8.1 (Steel et al., 1997).

## RESULTS AND DISCUSSION

Application of plant growth regulators (PGRs) remarkably improved biomass accumulation, biosynthesis of photosynthetic pigments, accumulation of osmo-protectants and antioxidant defense mechanism of *L. chinensis* compared to control (water spray). Exogenous application of 0.2 mg L<sup>-1</sup> BR manifested a more promising response compared to other treatments, in general.

However, PGR-induced improvements in different biochemical and morphological processes were dissimilar with varying concentrations of different PGRs.

Significantly higher plant height (28.84 cm) and dry weight (20.18 mg) were recorded with application of BR at 0.2 mg L<sup>-1</sup> compared to other foliar sprays. Increases of 26% and 73% were recorded with 0.2 mg L<sup>-1</sup> BR compared to control for plant height and dry weight, respectively, while, application of BR at 0.2 mg L<sup>-1</sup> was closely followed by 25 mg L<sup>-1</sup> BA and 2 mg L<sup>-1</sup> BR, respectively, for plant height and dry weight. Different concentrations of BR and 25 mg L<sup>-1</sup> BA had statistically similar results and more fresh weight compared to the other treatments. However, there was relatively more improvement in fresh weight (69%) with 0.2 mg L<sup>-1</sup> BR compared to the water spray. Furthermore, comparatively smaller and statistically alike fresh, dry weight and plant height values were found with different concentrations of GA and water spray (Table 1).

**Table 1** - Effects of different plant growth regulators on the growth of *Leymus chinensis* plants under field conditions

Plant growth regulator	Treatment concentration (mg L <sup>-1</sup> )	Plant height (cm)	Fresh weight (mg)	Dry weight (mg)
BA	5	22.07 ±1.04 de	20.24 ±1.30 c	9.33 ±0.54 cd
	25	26.52 ±0.91 b	29.47 ±1.46 ab	15.06 ±1.20 b
	50	22.67 ±0.83 de	26.48 ±1.62 b	11.01 ±0.80 c
BR	0.02	23.19 ±0.74 de	33.86 ±2.32 a	15.52 ±1.21 b
	0.2	28.84 ±1.48 a	39.95 ±0.73 a	20.18 ±1.63 a
	2	25.86 ±1.21 bc	32.94 ±2.45 a	16.28 ±0.59 b
GA	10	22.93 ±1.38 de	13.06 ±0.52 d	5.94 ±0.45 de
	50	23.89 ±1.48 cd	16.89 ±0.80 cd	8.12 ±0.32 cde
	100	23.21 ±1.31 de	14.62 ±1.08 cd	5.42 ±0.43 e
Water (CK)	0	21.41 ±1.29 e	12.31 ±1.10 d	5.40 ±0.36 e

BA - 6-benzylaminopurine, BR - brassinosteroid, GA - gibberellic acid. Values in the Table are means of at least 5 replicates ± SE. Values followed by the same letter within columns are not significantly different according to the LSD test (P<0.05).

Significantly more and statistically similar biosynthesis of chlorophyll *a* and total photosynthetic pigments were recorded with 0.2 and 2 mg L<sup>-1</sup> BR whereas 0.2 mg L<sup>-1</sup> BR triggered/mediated augmentation in chlorophyll *a* and total photosynthetic pigments of 44% and 38% respectively, compared to control. By contrast, significantly higher chlorophyll *b* (0.51 mg g<sup>-1</sup>) and chlorophyll *a/b* (3.44) values were substantiated with 2 mg L<sup>-1</sup> BR and 50 mg L<sup>-1</sup> BA, respectively, compared to other foliar sprays. As regards carotenoid biosynthesis, relatively more and statistically alike carotenoid contents were recorded with 0.2 mg L<sup>-1</sup> BR and 10 mg L<sup>-1</sup> GA, compared to other sprays. Carotenoid biosynthesis was enhanced by 32% under 0.2 mg L<sup>-1</sup> BR as compared to control. Contrarily, application of water showed significantly lesser chlorophyll *a* (0.81 mg g<sup>-1</sup>) and carotenoid contents (0.163 mg g<sup>-1</sup>) than other PGRs. Comparatively lesser and statistically similar chlorophyll *b* and total photosynthetic pigments were recorded with water, 10 mg L<sup>-1</sup> GA and 50 mg L<sup>-1</sup> BA. However, significantly lesser and statistically alike chlorophyll *a/b* values were recorded with 2 mg L<sup>-1</sup> BR and 50 mg L<sup>-1</sup> GA (Table 2).

Exogenously applied BR at 0.2 mg L<sup>-1</sup> showed more amino acids (0.4147 mg g<sup>-1</sup>) than other PGRs. Comparatively, more and statistically alike soluble sugars were recorded under 0.02 and 0.2 mg L<sup>-1</sup> BR. Likewise, application of BR at 0.2 mg L<sup>-1</sup> resulted in more soluble proteins (14.96 mg<sup>-1</sup>), and it was statistically similar to 2 mg L<sup>-1</sup> BR and 25 mg L<sup>-1</sup> BA. Thus, 0.2 mg L<sup>-1</sup> foliar BR-induced increments in soluble sugars, proteins and free amino acids over control were 44%, 63% and 52%, respectively. Contrarily, significantly lesser and statistically identical soluble sugars were recorded with the application of 10, 100 mg L<sup>-1</sup> GA and water spray, while application of water spray, 10 and 100 mg L<sup>-1</sup> GA showed relatively lesser and statistically comparable soluble proteins and free amino acids (Table 3).

Application of BA at 25 mg L<sup>-1</sup> and BR at 0.2 mg L<sup>-1</sup> showed significantly more and statistically alike SOD activity. Moreover, SOD activity was boosted by 46% under the influence of 0.2 mg L<sup>-1</sup>

**Table 2** - Effects of different plant growth regulators on photosynthetic pigments of *Leymus chinensis* under field conditions

Plant growth regulator	Concentration (mg L <sup>-1</sup> )	Chlorophyll <i>a</i> (mg g <sup>-1</sup> )	Chlorophyll <i>b</i> (mg g <sup>-1</sup> )	Chlorophyll <i>a/b</i>	Carotenoid (mg g <sup>-1</sup> )	Total photosynthetic pigment (mg g <sup>-1</sup> )
BA	5	0.95 ± 0.01 d	0.333 ± 0.013 e	2.87 ± 0.20 c	0.187 ± 0.009 d	1.47 ± 0.10 d
	25	1.22 ± 0.05 b	0.388 ± 0.021 c	3.15 ± 0.11 b	0.194 ± 0.009 cd	1.85 ± 0.09 b
	50	1.00 ± 0.08 cd	0.292 ± 0.008 fg	3.44 ± 0.19 a	0.231 ± 0.005 ab	1.43 ± 0.08 de
BR	0.02	1.22 ± 0.07 b	0.385 ± 0.016 d	3.16 ± 0.14 b	0.232 ± 0.011 ab	1.90 ± 0.05 b
	0.2	1.44 ± 0.06 a	0.456 ± 0.018 b	3.15 ± 0.19 b	0.242 ± 0.019 a	2.13 ± 0.09 a
	2	1.36 ± 0.04 a	0.510 ± 0.012 a	2.67 ± 0.16 d	0.228 ± 0.008 ab	2.10 ± 0.11 a
GA	10	0.93 ± 0.02 d	0.312 ± 0.009 efg	2.98 ± 0.10 bc	0.238 ± 0.006 a	1.36 ± 0.06 de
	50	1.10 ± 0.02 c	0.409 ± 0.005 c	2.69 ± 0.22 d	0.212 ± 0.011 bc	1.84 ± 0.13 b
	100	0.99 ± 0.06 cd	0.317 ± 0.010 ef	3.12 ± 0.11 b	0.209 ± 0.013 bcd	1.62 ± 0.12 c
Water (CK)	0	0.81 ± 0.07 e	0.278 ± 0.025 g	2.91 ± 0.09 c	0.163 ± 0.015 e	1.32 ± 0.08 e

BA - 6-benzylaminopurine, BR - brassinosteroid, GA - gibberellic acid. Values in the Table are means of at least 5 replicates ± SE. Values followed by the same letter within columns are not significantly different according to the LSD test (P<0.05).

**Table 3** - Effects of different plant growth regulators on soluble protein, soluble sugar and free amino acids in *Leymus chinensis* under field conditions

Plant growth regulator	Concentration (mg L <sup>-1</sup> )	Soluble sugar (mg g <sup>-1</sup> )	Soluble protein (mg g <sup>-1</sup> )	Free amino acid (mg g <sup>-1</sup> )
BA	5	15.39 ± 1.10 c	11.00 ± 0.92 bc	0.21 ± 0.013 e
	25	15.56 ± 0.87 c	13.90 ± 1.09 ab	0.28 ± 0.019 cd
	50	15.45 ± 0.98 c	11.86 ± 1.24 b	0.20 ± 0.0095 e
BR	0.02	17.81 ± 0.94 ab	11.65 ± 1.09 bc	0.32 ± 0.021 bc
	0.2	18.95 ± 0.75 a	14.96 ± 1.12 a	0.41 ± 0.026 a
	2	16.53 ± 1.21 bc	12.28 ± 0.92 ab	0.36 ± 0.032 b
GA	10	11.18 ± 0.61 d	6.76 ± 0.31 de	0.21 ± 0.012 e
	50	14.58 ± 1.08 c	8.78 ± 0.61 cd	0.23 ± 0.014 de
	100	12.16 ± 1.09 d	7.51 ± 0.63 de	0.21 ± 0.013 e
Water (CK)	0	10.60 ± 0.67 d	5.54 ± 0.22 e	0.20 ± 0.016 e

BA - 6-benzylaminopurine, BR - brassinosteroid, GA - gibberellic acid. Values in the Table are means of at least 5 replicates ± SE. Values followed by the same letter within columns are not significantly different according to the LSD test (P<0.05).

BR over control. Significantly higher activity of CAT and POD than of other treatments was found with application of 0.2 mg L<sup>-1</sup> BR. Moreover, significantly lower and statistically comparable CAT activity was recorded with 10 mg L<sup>-1</sup> GA and water spray, while comparatively lower SOD contents (294.95 U g<sup>-1</sup> FW) were recorded with water spray, and it was statistically at par with application of 10 and 100 mg L<sup>-1</sup> GA. Similarly, there was lesser POD activity with water spray than other treatments, and it was statistically similar to 10 mg L<sup>-1</sup> GA (Table 4).

There was significantly higher activity of GR (0.284 U g<sup>-1</sup> min<sup>-1</sup>) and APX (9.48 U g<sup>-1</sup> min<sup>-1</sup>), compared to other treatments, with 25 mg L<sup>-1</sup> BA. Thus, GR and APX activities were increased by 66% and 73%, respectively, under foliar application of 25 mg L<sup>-1</sup> BA over water spray while the least activities of GR (0.097 U g<sup>-1</sup> min<sup>-1</sup>) and APX (2.59 U g<sup>-1</sup> min<sup>-1</sup>) were found with the water spray. Moreover, statistically similar GR activities were recorded with the water spray and 100 mg L<sup>-1</sup> GA application. Similarly, statistically similar activities of APX were obvious with water, 0.02 and 0.2 mg L<sup>-1</sup> BR spray (Table 4).

There was a significantly positive relationship between biomass accumulation and photosynthetic pigments, biomass accumulation and osmotic adjustments, and biomass accumulation and antioxidant activities of *L. chinensis*. The contents of chlorophyll *a* and total photosynthetic pigments, and the value of chlorophyll *a/b* had a strong positive association with

**Table 4** - Effects of different plant growth regulators on antioxidant enzyme activities of *Leymus chinensis* plants under field conditions

Plant growth regulator	Concentration (mg L <sup>-1</sup> )	SOD (U g <sup>-1</sup> ·FW)	POD (U g <sup>-1</sup> ·min <sup>-1</sup> )	CAT (U g <sup>-1</sup> ·min <sup>-1</sup> )	GR (U g <sup>-1</sup> ·min <sup>-1</sup> )	APX (U g <sup>-1</sup> ·min <sup>-1</sup> )
BA	5	327.12 ± 15.79 cd	5192.51 ± 92.51 d	38.65 ± 3.02 cd	0.219 ± 0.031 b	5.63 ± 0.19 bcd
	25	537.17 ± 6.70 a	5648.84 ± 75.93 d	48.24 ± 1.54 b	0.284 ± 0.012 a	9.48 ± 0.82 a
	50	432.72 ± 6.98 b	5302.28 ± 272.56 d	42.13 ± 3.31 c	0.221 ± 0.010 b	7.32 ± 0.60 b
BR	0.02	336.49 ± 18.11 c	6699.96 ± 220.69 c	42.45 ± 1.94 c	0.224 ± 0.016 b	4.41 ± 0.18 de
	0.2	549.83 ± 9.44 a	7528.84 ± 332.43 a	59.13 ± 2.47 a	0.237 ± 0.06 b	5.19 ± 0.13 bcd
	2	419.78 ± 5.74 b	6993.79 ± 60.20 ab	48.23 ± 2.19 b	0.216 ± 0.020 b	4.78 ± 0.23 cde
GA	10	295.63 ± 14.49 e	4181.22 ± 21.83 ef	21.68 ± 1.44 e	0.146 ± 0.007 cd	5.53 ± 0.53 bcd
	50	327.59 ± 9.56 cd	4503.28 ± 105.50 e	37.34 ± 2.78 cd	0.154 ± 0.019 c	6.94 ± 0.70 bc
	100	307.60 ± 18.48 de	4301.31 ± 41.17 e	34.18 ± 2.38 d	0.118 ± 0.004 de	5.07 ± 0.37 bcd
Water (CK)	0	294.95 ± 17.17 e	3656.37 ± 138.45 f	19.09 ± 1.37 e	0.097 ± 0.003 e	2.59 ± 0.17 e

BA - 6-benzylaminopurine, BR - brassinosteroid, GA - gibberellic acid. Values in the Table are means of at least 5 replicates ± SE. Values followed by the same letter within columns are not significantly different according to the LSD test (P<0.05).

fresh weight, dry weight and plant height. Also, the contents of soluble sugars, soluble proteins and free amino acids had a strong positive association with fresh weight, dry weight and plant height. The activities of antioxidants such as SOD, POD, CAT had a strong positive association with fresh weight, dry weight and plant height (Table 5).

Increments in plant height under foliar application of 0.2 mg L<sup>-1</sup> BR can be ascribed to BR mediated improvements in fresh and dry weight (Table 1). Foliar applied BR might have enhanced biosynthesis of assimilates and cell division. Consequently, more accumulation of assimilates accelerated the partitioning of carbohydrates to stem and plant architecture and, thus, enhanced plant height as compared to control. Moreover, BR triggered improvements in photosynthesis, and accumulation of carbohydrates was established from a strong positive association between plant height, chlorophyll *a* contents and total photosynthetic pigments (Table 5). Likewise, improvement in plant height compared to control under BR might also be a consequence of a boost in enzymatic activities. Increments in enzymatic activities might have detoxified reactive oxygen species and enhanced stress tolerance under field conditions. Ultimately, the plants maintained biosynthesis of carbohydrates for partitioning to different parts of plant architecture. Therefore, increments in plant height might be a consequence of improved plant defense mechanism. Furthermore, a strong, positive and highly significant correlation of superoxide dismutase, catalase and peroxidase with plant height accomplished the role of enzymes in plant height (Table 5). Application of BR improved activities of antioxidant enzymes and plant capability to achieve osmotic adjustments compared to control, and it ultimately enhanced biomass accumulation and plant height (Xuan et al., 2015).

Increments of biomass accumulation under foliar applied BR might be a consequence of enhanced biosynthesis of antioxidants and photosynthesis. Application of BR might have boosted the ability of *L. chinensis* to biosynthesize carbohydrates, which ultimately enhanced biomass accumulation. Carbohydrate partitioning to stem might have improved cell division and expansion and resulted in a boost in plant height. Moreover, a strong, positive and highly significant association of fresh and dry weight with chlorophyll *a*, chlorophyll *a/b*, total photosynthetic pigments and soluble sugars with fresh and dry weight accomplished the role of photosynthesis in biomass accumulation (Table 5). Likewise, increments in biomass accumulation can also be accredited to enhancement in antioxidant activities, free amino acids and total soluble proteins. Enhancement in antioxidant activities might have improved stress tolerance and plant capability to accumulate free amino acids and total soluble proteins. Ultimately, accumulation of solutes decreased water potential and cell expansion during growth. These processes might have resulted in enhancement of plant height and biomass accumulation. Furthermore, a strong, positive and highly significant correlation of antioxidants, soluble proteins and free amino acids with fresh and dry weight further accomplished antioxidant- and osmotic adjustment-mediated improvement in biomass accumulation (Table 5). Foliar application of BR enhanced plant height, vegetative growth, fresh and dry weight. Increments in biomass accumulation were a consequence of BR-induced improvements in osmotic substances and antioxidant enzymes (Mullet et al., 2017).

**Table 5** - Correlation analysis showing strength of association among recorded attributes under exogenous application of plant growth regulators in *Leymus chinensis*

Parameter	FW	DW	PH	CHL <i>a</i>	CHL <i>b</i>	CHL <i>a/b</i>	CAR	TPP	SS	SP	FAA	SOD	POD	CAT	GR
DW	0.99**														
PH	0.75*	0.81**													
CHL <i>a</i>	0.71*	0.77**	0.78**												
CHL <i>b</i>	0.27 <sup>NS</sup>	0.17 <sup>NS</sup>	0.04 <sup>NS</sup>	-0.41 <sup>NS</sup>											
CHL <i>a/b</i>	0.90**	0.93**	0.90**	0.91**	-0.01 <sup>NS</sup>										
CAR	0.52 <sup>NS</sup>	0.47 <sup>NS</sup>	0.44 <sup>NS</sup>	0.43 <sup>NS</sup>	0.27 <sup>NS</sup>	0.57 <sup>NS</sup>									
TPP	0.80**	0.83**	0.84**	0.94**	-0.18 <sup>NS</sup>	0.96**	0.45 <sup>NS</sup>								
SS	0.92**	0.92**	0.66*	0.70*	0.18 <sup>NS</sup>	0.85**	0.50 <sup>NS</sup>	0.79**							
SP	0.92**	0.92**	0.76*	0.63*	0.30 <sup>NS</sup>	0.63*	0.40 <sup>NS</sup>	0.71*	0.92**						
FAA	0.89**	0.92**	0.85**	0.86**	-0.04 <sup>NS</sup>	0.95**	0.49 <sup>NS</sup>	0.90**	0.80**	0.74*					
SOD	0.79**	0.82**	0.86**	0.53 <sup>NS</sup>	0.36 <sup>NS</sup>	0.74*	0.30 <sup>NS</sup>	0.61 <sup>NS</sup>	0.68*	0.88**	0.66*				
POD	0.97**	0.97**	0.74*	0.79**	0.12 <sup>NS</sup>	0.92**	0.57 <sup>NS</sup>	0.83**	0.93**	0.88**	0.93**	0.69*			
CAT	0.91**	0.90**	0.82**	0.73*	0.21 <sup>NS</sup>	0.89**	0.45 <sup>NS</sup>	0.83**	0.92**	0.95**	0.80**	0.84**	0.89**		
GR	0.82**	0.82**	0.60 <sup>NS</sup>	0.81**	0.31 <sup>NS</sup>	0.67*	0.31 <sup>NS</sup>	0.54 <sup>NS</sup>	0.81**	0.94**	0.56 <sup>NS</sup>	0.79**	0.75**	0.81**	
APX	0.19 <sup>NS</sup>	0.21 <sup>NS</sup>	0.33 <sup>NS</sup>	0.10 <sup>NS</sup>	0.28 <sup>NS</sup>	0.22 <sup>NS</sup>	0.14 <sup>NS</sup>	0.15 <sup>NS</sup>	0.24 <sup>NS</sup>	0.48 <sup>NS</sup>	-0.06 <sup>NS</sup>	0.54 <sup>NS</sup>	0.08 <sup>NS</sup>	0.41 <sup>NS</sup>	0.61 <sup>NS</sup>

FW - Fresh weight; DW - Dry weight; PH - Plant height; CHL *a* - Chlorophyll *a*; CHL *b* - Chlorophyll *b*; CHL *a/b* - Chlorophyll *a/b*; CAR - Carotenoids; TPP - Total photosynthetic pigments; SS - Soluble sugars; SP - Soluble proteins; FAA - Free amino acids; SOD - Superoxide dismutase; POD - Peroxidase; CAT - Catalase; GR - Glutathione reductase; APX - Ascorbate reductase.

Increased biosynthesis of photosynthetic pigments under BR and BA over the water spray can be attributed to improvements in antioxidant enzymes and accumulation of osmotic substances. The increase in enzyme activity might have detoxified free oxygen radicals, diminished lipid peroxidation of biomembranes and ultimately enhanced the activity of chlorophyll biosynthesizing enzymes. Moreover, improvements in antioxidants might have enhanced the stay-green trait and escalated biosynthesis of photosynthetic pigments over degradation. Consequently, chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b*, carotenoids and total photosynthetic pigments were improved under foliar-applied BR and BA. Furthermore, a highly significant and positive association of POD, CAT and GR with chlorophyll *a*; SOD, POD, CAT and GR with chlorophyll *a/b*; SOD and POD with total photosynthetic pigments accomplished the role of antioxidants in synthesis of photosynthetic pigments (Table 5). Likewise, increments in photosynthetic pigments under BR and BA can also be ascribed to an increase in fresh and dry weight. Hence, greater photosynthetic area and biomass accumulation might be an outcome of greater biosynthesis of photosynthetic pigments rather than degradation. A highly significant and positive association of photosynthetic pigments with biomass accumulation attributes for most of the instances further accomplished biomass-mediated improvements in photosynthetic pigments (Table 5). Application of BR under stressed conditions improved the activity of the antioxidant system by increasing the activities and levels of enzymatic and non-enzymatic antioxidants (Slathia et al., 2012). Similarly, application of BA enhanced shoot length, root density, chlorophyll contents, quantum yield of photosynthesis and net photosynthesis (Mangieri et al., 2017).

Improvements in accumulation of osmotic substances (soluble sugars, proteins and free amino acids) under BR over control can be attributed to a boost in enzymatic activities. Increment in activities of antioxidant enzymes might have augmented scavenging of reactive oxygen species (ROS). Ultimately, damage caused by ROS to membranes was alleviated and plant capability to gain osmotic adjustments was enhanced. A strong, positive and highly significant association of antioxidant enzymes with osmotic substances further confirmed the antioxidant-mediated accumulation of osmotic substances (Table 5). Likewise, biomass accumulation might have enhanced photosynthetic area and photosynthate accumulation. Ultimately, more photosynthate

augmented plant capability to accumulate soluble sugars, proteins and amino acids. Relatively, more expansion of cell during growth ultimately caused more biomass and greater plant height. Application of BR imparted stress tolerance by enhancing accumulation of osmotic substances. Accumulation of free amino acids and soluble sugars decreased osmotic potential and water potential. Hence, water movement from the apoplast to cells was enhanced and cellular turgidity was maintained owing to BR application (Bajguz and Hayat, 2009).

Boom in activities of enzymatic antioxidants can be attributed to the role of BR and BA in osmotic adjustments. Application of plant growth substances might have improved the ability of plants to achieve osmotic adjustments and, therefore, water retaining ability was also enhanced at subcellular level. Consequently, higher cell water contents declined sensitivity to reactive oxygen species. Moreover, higher osmotic substances under BR and BA application might accelerate SOD-mediated conversion of superoxide radicals ( $O_2^{\bullet-}$ ) to hydrogen peroxide ( $H_2O_2$ ) in the chloroplast. Accelerated generation of  $H_2O_2$  was accomplished by increased activities of POD, APX and CAT. Afterwards,  $H_2O_2$  detoxification to water might have been enhanced. Enhancement in scavenging of  $H_2O_2$  to water was further established by an increase in GR activity. Furthermore, a strong, positive and highly significant correlation between antioxidants and osmotic substances accomplished the role of cellular water retention in decreasing sensitivity to oxidative stress (Table 5). Moreover, improvements in activities of antioxidants can also be related to boost in biosynthesis of photosynthetic pigments under BR and BA over control. Thus, accelerated detoxification of  $O_2^{\bullet-}$  and improvements in SOD activity further established the role of BR in biosynthesis of photosynthetic pigments. Thus, biosynthesis of photosynthetic pigments was greater than degradation, which resulted in significant improvements in biosynthesis of photosynthetic pigments. Furthermore, a strong, positive and highly significant association of SOD, POD and CAT with chlorophyll *a* and *a/b* established the antioxidant detoxification of ROS and biosynthesis of chlorophyll (Table 5). Foliar application of BR enhanced plant height, number of leaves per plant, shoot and dry weight per plant, SOD, POD, CAT, APX, GR and mono-dehydro ascorbate reductase. Improvements in morphological attributes were linked with a boost in antioxidant activities (Talaat et al., 2015). Foliar-applied BA declined the generation of  $O_2^{\bullet-}$  and  $H_2O_2$  but enhanced the biosynthesis of SOD, POD and glutathione peroxidase (Rubio-Wilhelmi et al., 2011).

Overall, exogenous application of different plant growth substances significantly improved biomass accumulation, photosynthetic pigments, accumulation of osmotic substances and antioxidant defense mechanism compared to control (water spray). However, application of BR at rate of  $0.2 \text{ mg L}^{-1}$  showed more promising results than the other treatments. Thus, remarkably more plant height, fresh and dry weight, chlorophyll *a*, *b*, carotenoids, total photosynthetic pigments, soluble sugars, proteins, free amino acids, SOD, POD and CAT activities were recorded with the application of  $0.2 \text{ mg L}^{-1}$  BR, whereas highest chlorophyll *a/b*, GR and APX activities were found with  $25 \text{ mg L}^{-1}$  BA. Conclusively, foliar application of  $25 \text{ mg L}^{-1}$  BA,  $0.2 \text{ mg L}^{-1}$  BR and  $10 \text{ mg L}^{-1}$  GA significantly enhanced accumulation of biomass and osmotic substances, biosynthesis of photosynthetic pigments and antioxidant activities.

## ACKNOWLEDGEMENTS

The authors are grateful to the National Basic Research Program of China (2014CB138806).

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