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EFFECTS OF BRASSINOLIDE AND MACRO-NUTRIENTS FOLIAR APPLICATION ON GROWTH AND PHYSIOLOGICAL CHARACTERISTICS IN *LEYMUS CHINENSIS* (TRIN.)

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Abstract

A pot experiment was conducted to elucidate influence of foliar application of various combinations of BR and macro-nutrients (NPK) nutrition on morphological and physiological attributes of *Leymus chinensis* (Trin.). The results revealed that BR and NPK nutrient mixtures altered growth, photosynthetic pigments, and osmolytes of *L. chinensis* (Trin.). Furthermore, analysis of various factors revealed that most effective application was the treatment of urea 1%, potassium dihydrogen phosphate 1% and BR 0.1 mg/l which proved most advantageous pertaining to plant height, fresh and dry weight, biosynthesis of chlorophyll, carotenoids, soluble sugars and proteins. Results suggested that foliar applied BR and NPK mixtures on *L. chinensis* improves growth and alters plant physiology.

Introduction

Brassinolide (BR) has important function for promoting plant growth, but plant nutrition cannot keep up with growth when BR is used. *Leymus chinensis* (Trin.), also called the sheep grass, is a perennial rhizomatous grass enriched with high carbohydrates, protein and minerals having good palatability and forage value (Huang *et al*. 2002). Over the time the grasslands of China have been degraded because of changing climatic conditions, over grazing and land use practices and reduced soil fertility (Liang *et al*. 2006). *L. chinensis* has high adaptability to adverse climatic and edaphic conditions such as drought, low and high temperature, salinity and heavy metal stress and is supposed to possess the great potential of rehabilitation and renovation of the grasslands (Chen *et al*. 2013).

Brassinolide (BR) of novel plant hormones is involved in the improvement of growth and development of plants (Fukuta *et al.* 2002). Reports had revealed that exogenously applied BR promotes the plant growth and development and modulates various physiological processes (Kumar *et al.* 2012, Wang *et al.* 2016). BR promotes the plant growth and development but plant nutrition often cannot keep up with extolled growth and becomes the limiting factor. Foliar application of plant nutrients may enhance the plant growth, yield and nutrient use efficiency as well owing to the rapidity with which the most requiring nutrients are provided to the plants for better growth and yield (Habib 2012).

Plant growth regulating substances may be used for enhancing the plant growth and development to reap the benefits of the inputs and resources utilized. Nonetheless, plants require higher amounts of nutrients with exaggerated growth. Therefore, the present study was undertaken to balance the plant nutrition with plant growth by foliar application of BR with NPK nutrient mixtures and ascertain the growth, physiological and biochemical response of *L. chinensis* plants.

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Materials and Methods

Study was conducted to assess the response of *Leymus chinensis* to foliar applied BR and mixture of NPK nutrients at Southwest University, Chongqing, China, during October, 2014 to February, 2015. Healthy, pest free and same sized seeds were selected and spread evenly in Petri dishes lined with a double filter papers layer for germination. The Petri plates were kept in an incubator (25 ± 1°C incubation, 14 hrs/10 hrs (day/night) illumination time and 2500 lux light intensity). After one week of consistent growth, the *L. chinensis* seedlings were transplanted into pots (34 cm diameter and 24 cm depth). Prior to transplantation the pots were washed, disinfected and filled with humus and vermiculite (1 : 1 mix) soil containing total nitrogen 3.83 g/kg, total phosphorus 3.78 g/kg, total potassium 10.24 g/kg, available nitrogen 1007.43 mg/kg, available phosphorus 9.29 mg/kg, available potassium 132.5 mg/kg, organic matter 143.65 g/kg and with pH 6.4. Thirty seedlings were transplanted in each pot with the help of tweezers. Unified management practices were applied after the transplantation of seedlings up to 3 days. Seedlings were supplied with 25 ml Hoagland nutrient solution every 5 days to ensure suitable nutrient supply. Water (50 ml) was applied to each pot every 2 days. When the *L. chinensis* plants attained a height of 18 - 20 cm, thinning was done to keep 25 plants per pot.

Orthogonal experimental design was used with the choice of orthogonal table. The details of experimental design and treatments are presented in Table 1 and each was replicated thrice. Foliar solutions of BR and NPK nutrients in the form of urea (nitrogen 460.0 g/kg) and potassium dihydrogen phosphate (PDP) (pure potassium 340.2 g/kg, phosphorus 520.2 g/kg) were applied to the *L. chinensis* plants. A surfactant (plus Tween-80) was also added to the solution for improving its efficacy. Spray was done twice every five days after transplanting. The plants were covered with water droplets but not dripping appropriate. Ten days after treatment morphological, physiological and biochemical indices were measured.

Table 1. Experimental design and treatments.

Treatments coding scheme	Urea (%)	PDP (%)	BR (mg/l)
T_1	0	0	0
T_2	0	0.5	0.10
T_3	0	1	1.00
T_4	0	2	0.01
T_5	1	0	0.01
T_6	1	0.5	1.00
T_7	1	1	0.10
T_8	1	2	0
T_9	2	0	0.10
T_{10}	2	0.5	0
T_{11}	2	1	0.01
T_{12}	2	2	1.00
T_{13}	3	0	1.00
T_{14}	3	0.5	0.01
T_{15}	3	1	0
T_{16}	3	2	0.10

PDP: Potassium dihydrogen phosphate; BR: Brassinolide . The same as follows.

Plant height was measured from the tip of the plants to the base of stem. Afterwards, all seedlings were uprooted and rinsed with tap water, followed by rinsing with distilled water 2 - 3 times. Filter paper was used to absorb water adhered to the seedlings subsequently weighing to determine the fresh weight. Next seedlings were dried in oven at 105°C for 15 min and then at 65 °C till constant weight to determine the dry weight.

Photosynthetic pigments were measured by using Wellburn (1994) method. Soluble sugars were determined by anthrone colorimetric method (Li *et al.* 2008) and soluble proteins by Bradford (1976) method. The root activity was quantified by TTC method of Higa *et al*. (2010). Free amino acids were assessed by using ninhydrin colorimetric method of Huang *et al*. (2010). The data were analyzed using Microsoft Excel and analysis of variance technique with DPS. The treatments means were compared using the least significant difference test.

Results and Discussion

Foliar nutrition of *Leymus chinensis* plants with BR resulted in improved growth and development. Application of BR and nutrient mixtures enhanced the plant height, and fresh and dry weight of *L. chinensis* as compared to control. An increase of 25.9% in plant height was perceived by the application of T_7 (urea 1%, potassium dihydrogen phosphate (PDP) 1%, BR 0.1 mg/l). While, plant fresh and dry weight was enhanced by 175.6 and 137.6%, respectively, by T_6 (urea 1%, PDP 0.5%, BR 1 mg/l) when compared with control (Table 2). Analysis of various factors with range R indicated that plant height was in the order of BR > urea > PDP, and plant fresh as dry weights was ordered as urea > BR > PDP.

Table 2. Agronomic characters of *L. chinensis* treated with BR and different foliar fertilizers.

Treatments	Plant height (cm)	Fresh weight (g)	Dry weight (g)
T_1	28.64 bcd	0.996 ab	0.202 ab
T_2	30.52 abcd	1.819 ab	0.262 ab
T_3	26.64 d	2.686 a	0.400 ab
T_4	33.34 abc	2.140 ab	0.295 ab
T_5	34.58 ab	2.527 ab	0.314 ab
T_6	26.28 d	2.745 a	0.480 a
T_7	36.06 a	2.160 ab	0.340 ab
T_8	28.66 bcd	1.918 ab	0.342 ab
T_9	28.76 bcd	1.580 ab	0.156 b
T_{10}	26.74 cd	1.623 ab	0.288 ab
T_{11}	28.18 bcd	1.478 ab	0.215 ab
T_{12}	27.66 cd	1.274 ab	0.317 ab
T_{13}	27.10 cd	1.115 ab	0.171 ab
T_{14}	32.26 abcd	2.531 ab	0.352 ab
T_{15}	32.36 abcd	1.365 ab	0.293 ab
T_{16}	19.06 e	0.807 b	0.135 b
Coefficient of variation			
Urea	2.13^{NS}	2.19^{NS}	1.68^{NS}
PDP	0.11^{NS}	1.40^{NS}	0.51^{NS}
BR	5.20**	1.55^{NS}	0.87^{NS}

Values followed by the same letter are not significantly different according to least significant difference test ($p < 0.05$), NS = Non-significant, ** = Highly significant. The same as follows.

Biosynthesis of photosynthetic pigments of *L. chinensis* plants was elevated by the impact of foliar applied BR and nutrient mixtures. Maximum increase in chlorophyll *a* (13.7%), chlorophyll *b* (27.2%) and total chlorophyll content (15.9%) occurred by application of T_7 (urea 1%, PDP 1%, BR 0.1 mg/l), while, chlorophyll *a*/*b* ratio (7.2%) increased by T_6 (urea 1%, PDP 0.5%, BR 1 mg/l), as compared to control. Carotenoids did not increase by application of nutrient mixtures instead a decrease was noticed by some treatment combinations and maximum decrease was observed by T_{16} (urea 3%, PDP 2%, BR 0.1 mg/l) when compared with control (Table 3). Analysis of factors with range R regarding photosynthetic pigments revealed that chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were in the order of urea > PDP > BR; while, chlorophyll *a*/*b* ratio was ordered as BR > PDP > urea.

Table 3. Photosynthetic pigment of *L. chinensis* treated with BR and different foliar fertilizers.

Treatments	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoid (mg/g)	Total chlorophyll (mg/g)	Chl *a*/*b*
T_1	1.90 abc	0.59 abcd	0.04 a	2.52 abcde	3.07abcd
T_2	1.54 bcde	0.51 bcd	0.04 a	2.08 bcde	2.93abcde
T_3	1.88 abcd	0.65 abc	0.04 a	2.59 abcd	3.03abcde
T_4	1.58 bcde	0.66 abc	0.04 a	2.71 abc	2.76bcde
T_5	2.00 ab	0.66 abc	0.04 a	2.84 ab	3.11abc
T_6	2.01 ab	0.54 bcd	0.04 a	2.16 abcde	3.29a
T_7	2.16 a	0.81 a	0.035 a	2.92 a	3.26ab
T_8	2.02 ab	0.48 bcd	0.035 a	1.74 cef	3.07abcd
T_9	1.21 e	0.65 abc	0.04 a	2.71 abc	3.26ab
T_{10}	1.68 abcde	0.62 abc	0.035 a	2.36 abcde	3.07abcd
T_{11}	1.63 abcde	0.54 bcd	0.04 a	2.22 abcde	2.94abcde
T_{12}	1.39 cde	0.43 cd	0.04 a	1.84 de	2.52ef
T_{13}	1.36 cde	0.50 bcd	0.04 a	1.92 cde	2.54def
T_{14}	1.34 de	0.46 bcd	0.04 a	1.83 de	2.72cde
T_{15}	1.44 cde	0.70 ab	0.025 b	2.26 abcde	2.03fg
T_{16}	0.54 f	0.34 d	0.01 c	0.945 f	1.58g
Coefficient of variation					
Urea	10.60**	1.58^{NS}	16.67**	5.97**	7.89**
PDP	7.56**	4.27*	10.00**	6.00**	9.24**
BR	2.48^{NS}	2.01^{NS}	4.67*	2.40*	10.96**

Different nutrient combinations had different expressions; some treatments enhanced the root activity and synthesis of soluble sugars, proteins and free amino acids. Elevated levels of root activity (47.1%) and soluble sugars (50.6%) were detected by T_9 (urea 2%, PDP 0%, BR 0.1 mg/l), and that of soluble proteins (4.2%) and free amino acids (669.3%) by application of T_7 (urea 1%, PDP 1%, BR 0.1 mg/l) (Table 4). Analysis of different factors with range R exhibited a differential order of various factors for these attributes *viz.* root activity (PDP > urea > BR), soluble sugars (urea > PDP > BR), soluble proteins (urea > BR > PDP) and free amino acids (BR > PDP > urea).

Growth and development can be modulated by altering the plant physiological and biochemical processes taking place within the plants. The changes in these vital plant processes can be induced by employing the plant growth regulating substances that act as stimulus for the alteration in transcription and translation of genes (Wahid *et al.* 2007). However, the overwhelming importance of plant nutrition cannot be overlooked as they are vital for better plant growth, yield and quality in concert with plant growth regulators (Sawan *et al.* 2009). In the present study, the foliar application of BR with NPK nutrients mixture substantially improved the

Table 4. Root activity, soluble sugar, soluble protein, free amino acids of *L. chinensis* treated with BR and different foliar fertilizers.

Treatments	Root activity (μg/g*h)	Soluble sugars (mg/g)	Soluble proteins (mg/g)	Free amino acid (mg/100g)
T_1	307.77 a	15.86cde	25.27 ab	20.81 k
T_2	170.65 a	19.40 b	25.78 a	63.59 g
T_3	215.79 a	13.72 efg	21.16 de	34.99 j
T_4	220.80 a	14.30 ef	20.96 def	85.17 e
T_5	284.92 a	14.83 ef	21.10 de	61.16 g
T_6	288.99 a	12.85 fg	19.48efg	39.84 i
T_7	163.75 a	25.44 a	26.33 a	160.10 a
T_8	188.08 a	18.43 bc	21.39 de	56.04 h
T_9	425.72 a	23.88 a	25.61 a	104.67 d
T_{10}	350.03 a	18.07 bcd	25.88 a	100.56 d
T_{11}	225.53 a	20.08 b	21.51 cd	43.84 i
T_{12}	137.87 a	15.65 de	23.42bc	73.16 f
T_{13}	239.71 a	9.32 h	19.09 fg	75.26 f
T_{14}	221.94 a	12.29 fg	18.92 g	114.14 c
T_{15}	360.55 a	20.21 b	20.23 defg	138.25 b
T_{16}	111.88 a	11.13 gh	11.53 h	17.12 k
Coefficient of variation				
Urea	0.31^{NS}	15.22**	33.51**	197.35**
PDP	1.67^{NS}	10.54**	9.82**	206.18**
BR	0.07^{NS}	2.65^{NS}	9.84**	795.83**

plant height, and fresh and dry weight of *L. chinensis*, when compared with control. Increase in growth of *L. chinensis* plants by BR and NPK application indicates that balanced nutrition is required along with the use of growth regulators to keep the uplift in plant growth in pace (Table 2). Similar results were reported by Hayat *et al*. (2010) who observed an increase in root and shoot length, plant fresh and dry weight, and leaf area of *Vigna radiata* by treatment with 28-homobrassinolide. Similarly, Xiong *et al*. (2016) reported an increase in plant height, leaf area, and above and below growth biomass production by the application of 24-epibrassinolide. Growth of *L. chinensis* plants was exalted by the application of NPK application (Table 2). Khalid and Shedeed (2015) reported increased plant height and branches per plant, enhanced leaf growth and plant biomass accumulation of *Nigella sativa* by NPK foliar application.

Appropriate biosynthesis and functioning of the photosynthetic machinery is the basic requirement for improved plant growth and development. Photosynthetic pigments reflect the photosynthetic capacity of plants, and ultimately growth and production. Foliar application of BR and NPK nutrient mixtures exhilarated the biosynthesis of chlorophyll and carotenoids of *L. chinensis* plants, when compared with control (Table 3). Increased synthesis of photosynthetic pigments by the application of BR might be due to transcriptional and translational alterations in genes (Bajguz 2000). The present results are in agreement with the report of Fariduddin *et al*. (2014), who reported an elevation in the biosynthesis of chlorophyll content in Indian mustard when BR was used. Application of NPK nutrients resulted in the improved chlorophyll content of *L. chinensis* plants might be due to balanced nutrition (Table 3). Similar results were indicated by Landgren *et al*. (2013) who observed that application of NPK solution enhanced the chlorophyll and carotenoids content in *Abies nordmanniana*.

The results revealed that treatment of *L. chinensis* plants with BR in combination with NPK improved the soluble proteins and sugars, and free amino acids in some way, as compared to control (Table 4). The soluble sugars, soluble proteins and free amino acids can be used as osmolytes in plants and might scavenge free radicals. These results are more or less similar to previous results by Kumar *et al*. (2014), who reported that treatment of *Brassica juncea* seedlings with 28-homobrassinolide increased the synthesis of soluble sugars, reducing and non-reducing sugars, and soluble proteins as well. In the present study, enhanced synthesis of proteins and sugars was observed by the application of foliar NPK (Table 4). Khalid and Shedeed (2015) reported elevated levels of protein and sugar contents in *N. sativa* by foliar application of NPK fertilizers.

The results of the experiment revealed that foliar application of BR in combination with NPK mixtures enhanced the growth, photosynthetic pigments, soluble sugar and proteins of *L. chinensis* plants and hence can be employed to balance the plant nutrition with exaggerated plant growth and development by altering the physiology and biochemistry of plants.

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