

EFFECTS OF NPK AND ALA ON ENDOGENOUS HORMONES OF *LEYMUS CHINENSIS* (TRIN.) TZVEL UNDER DROUGHT STRESS

XUEFENG ZONG, JUN LV, SHAKEEL AHMAD ANJUM¹, XIAO WU,
CHAO WU, YUNPENG LI AND SANGEN WANG*

College of Agronomy and Biotechnology, Southwest University, Chongqing 400716, China

Keywords: Leymus chinensis, 5-aminolevulinic acid (ALA), Growth regulation, Water stress

Abstract

An experiment was conducted to elucidate the negative impacts of drought on growth and hormones of *Leymus chinensis*. Results showed that plant height, dry weight and zeatin riboside contents declined under drought condition over the control. Whereas indole acetic acid, gibberellic acid and abscisic acid contents were augmented under stress environment over the normal conditions. Exogenous application of NPK and/or 5-aminolevulinic acid (ALA) depicted more pronouncing response to alleviate adversities of drought. Conclusively, foliar application of 'Urea (1%) + KH₂PO₄ (1%) + ALA (50 mg/l)' produced better results under drought stress on the evaluated parameters.

Introduction

Natural grassland ecosystem is vital regarding the sustainability of land, air and water (Xiliang *et al.* 2016). Water shortage often encumbers dry matter accumulation of *Leymus chinensis* and thus declines grassland productivity (Liu *et al.* 2016). Adverse implications of drought cause interference in physiological functions, growth and yield attributes of rice (Gao *et al.* 2017). However, the defense mechanisms are regulated by the production of endogenous growth regulators in plant (Hong *et al.* 2015). Various strategies are being explored to alleviate adversities of drought. Experimental findings suggested that foliar application of nutrients and plant growth regulators such as 5-aminolevulinic acid (ALA) proved helpful to alleviate adversities of stresses (Song *et al.* 2017, Li *et al.* 2019).

Foliar application of NPK and ALA might resolve adversities of drought. Hence the present study was conducted to evaluate the role of foliar application of NPK and ALA as potential regulators of hormones of *L. chinensis* and to alleviate adversities of drought and device suitable management strategies for drought affected *L. chinensis*.

Materials and Methods

Seed material of *Leymus chinensis* was procured from Ecosystem Positioning Station, Chinese Academy of Sciences from the inner Magnolia in 2015. The experiment was conducted in 2016 at Southwest University. Sowing was done in mid-April and seedlings were transplanted into pots; water losses were measured on daily basis and the lost water was replenished accordingly. Moisture content was maintained at 80 and 50% of relative soil water contents in control (normal) and drought treatments, respectively. Foliar application of water solution of ALA and nutrients was done at 18-20 cm plant height. The solutions were applied on plants three times following 3 days interval. Experiment was conducted in CRD with factorial treatment structure. Treatments were replicated three times.

*Author for correspondence: <wangsg@swu.edu.cn> ¹Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan.

Treatments were comprised of T1 = 80% of relative soil water contents + water; T2 = 80% of relative soil water contents + urea (1%) + potassium dihydrogen phosphate (1%); T3 = 80% of relative soil water contents + ALA (50mg/l); T4 = 80% of relative soil water contents + urea (1%) + potassium dihydrogen phosphate (1%) + ALA (50 mg/l); T5 = 50% of relative soil water contents + water; T6 = 50% of relative soil water contents + urea (1%) + potassium dihydrogen phosphate (1%); T7 = 50% of relative soil water contents + ALA (50 mg/l) and T8 = 50% of relative soil water contents + urea (1%) + potassium dihydrogen phosphate (1%) + ALA (50 mg/l).

Plant height was measured from collar to leaf tip and plant was put into an oven at 105°C for 15 min and then 65°C constant temperature till constant weight referred to as dry weight. The plant height and dry weight were assessed at 20 days after treatments (DAT). The endogenous hormones changes were assessed at 10, 15 and 20 days after treatments. The seedlings were first rinsed with tap water and then finally with distilled water for 2 - 3 times. Filter paper was used to absorb the adhered water to plants and then to determine abscisic acid (ABA), indole acetic acid (IAA), gibberellic acid (GA_3), zeatin riboside (ZR) using enzyme-linked immunosorbent assay (ELISA) (Teng *et al.* 2010). Data were analyzed using ANOVA with SPSS 19.0 statistical software and treatment's means were compared using LSD test ($p \leq 0.05$).

Results and Discussion

Inclusively drought imposition depicted negative impacts on endogenous hormones and growth attributes. However, specific response of treatments was distinct for evaluated parameters. Foliar applied NPK and/or ALA depicted statistically alike and significantly different plant height under normal conditions. Under drought, significantly better plant height was observed in 50% of relative soil water contents with 1% urea, potassium dihydrogen phosphate and 50 mg/l ALA solution. Similarly, NPK and/or ALA treatments produced more dry weight (Fig. 1). The similar results had been reported by Li *et al.* (2019).

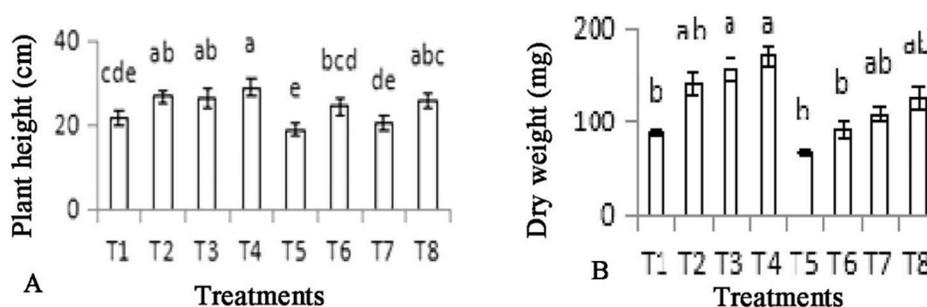


Fig. 1. Effects of NPK and ALA application on the plant height (A) and dry weight (B) of *L. chinensis*.

ABA contents were amplified under drought stress over the control for all treatments. Under drought, boost in ABA was observed till 15 DAT with urea + KH_2PO_4 + ALA which diminished at 20 DAT. Contrarily, ALA exhibited decline in ABA up to 15 DAT and augmented at 20 DAT under drought. Nevertheless, highest ABA was observed with water and lowest with urea + KH_2PO_4 + ALA throughout under stressed environment (Table 1). Comparatively, more IAA contents were recorded with urea + KH_2PO_4 + ALA than other treatments under control and drought. Whereas, water depicted less IAA than other treatments under stress and non-stress conditions. Likewise, constant increment in IAA was recorded till 15 DAT for all treatments under

drought. However, IAA contents were lesser at 20 DAT than 15 DAT under drought (Table 1). Foliar application of NPK and ALA was found to ameliorate growth and development under drought stress in *L. chinensis* (Song *et al.* 2017), however, little information is available about endogenous hormones changes. Endogenous hormones regulate the plant growth and physiological functions (Mohammadi *et al.* 2017). The defense mechanism is regulated by the production of endogenous plant growth hormones such as auxins, abscisic acid, jasmonic acid, brassinosteroids and indole etc. (Hong *et al.* 2015, Radhakrishnan *et al.* 2016).

Table 1. Effects of NPK and ALA application on endogenous hormones of *L. chinensis*. (ng/g).

Treatments	ABA			IAA		
	DAT 10 d	DAT 15 d	DAT 20 d	DAT 10 d	DAT 15 d	DAT 20 d
T1	129.08±2.76c	87.80±0.61d	73.43±4.84f	68.11±1.07e	63.95±2.85h	135.28±4.63d
T2	99.78±4.04f	68.56±3.43e	97.16±2.31e	85.77±1.09d	103.57±2.62g	173.47±5.84c
T3	115.84±0.89d	111.70±0.41c	92.09±3.69e	121.99±1.11c	167.76±2.05f	113.69±4.95d
T4	99.82±1.36ef	93.78±1.34cd	78.16±4.38f	186.17±6.44a	190.45±1.50e	208.04±4.38b
T5	213.84±5.29a	258.76±3.97a	272.90±9.44a	87.93±0.94d	204.00±0.81d	225.35±12.13b
T6	123.74±2.39c	155.45±1.96b	173.07±3.82b	67.35±3.44e	244.44±0.05b	209.72±7.81b
T7	157.50±.20b	143.19±2.08b	152.90±0.38c	176.09±8.07b	234.34±1.32c	153.82±3.33c
T8	110.28±5.74de	141.08±4.43b	135.47±0.32d	125.97±4.46c	307.41±5.12a	290.86±4.89a
	GA			ZR		
T1	5.28±0.19c	2.22±0.04de	2.86±0.56d	43.73±0.32a	38.11±0.28c	46.51±1.75c
T2	11.79±0.96a	1.81±0.40e	3.63±0.13d	43.76±1.59a	46.39±1.51b	52.32±2.97b
T3	9.05±0.29ab	2.10±0.13de	3.26±0.06d	44.90±0.84a	50.90±1.35a	53.85±0.08b
T4	11.11±1.03a	4.25±0.38bc	1.98±0.15e	41.97±0.79a	55.39±0.02a	58.47±0.94a
T5	11.37±0.57a	3.45±0.17cd	5.54±0.18c	33.60±0.86b	20.73±0.48f	24.64±0.73e
T6	6.50±0.84bc	4.90±0.04ab	6.75±0.09b	34.81±1.09b	29.93±1.42e	25.07±0.29e
T7	8.62±0.37ab	5.56±0.38ab	4.78±2.34c	35.26±1.23b	35.79±1.04c	30.96±0.31de
T8	11.51±1.07a	5.80±0.17a	8.61±0.07a	42.58±1.33a	33.96±0.16d	31.20±1.35d

Table 2. Effects of NPK and ALA on hormones proportion of *L. chinensis*.

Hormones	T1	T2	T3	T4	T5	T7	T8
IAA/ABA	1.84	1.79	1.23	2.66	0.83	1.01	0.13
ZR/ABA	0.63	0.54	0.58	0.75	0.09	0.20	0.23
GA/ABA	0.04	0.04	0.04	0.03	0.02	0.03	0.06

GA₃ contents were more than other treatments due to application of urea + KH₂PO₄ at 10 and 20 DAT under control. Whereas, urea + KH₂PO₄ + ALA depicted higher GA than other treatments at 15 DAT. Likewise, incessant decline in GA was recorded for ALA under drought. Whereas, all other treatments depicted decline in GA up to 15 DAT and enhanced slightly at 20 DAT under control and drought (Table 1). Urea + KH₂PO₄ + ALA manifested higher ZR than other treatments under drought and control. Whereas less ZR contents were observed under both water treatment conditions. Enhancement in ZR was obvious with the time span for all treatments except water under control (Table 1). Auxin and GA contents were augmented under stress as upregulation of

antioxidants enhanced detoxification of ROS (Ostrowski *et al.* 2016). Therefore, higher assimilates might have increased hormonal regulation under stress (Leitao and Enguita 2016). Escalated ABA under stress over control might have declined ZR activity. Ultimately, ZR mediated cell division might have impaired and hence dry weight and plant height was declined under drought (Rubio-Wilhelmi *et al.* 2011). Nonetheless, NPK and ALA sustained ZR contents to some extents and thus dry weight and plant height was enhanced with Urea + KH_2PO_4 + ALA over water (Gupta and Rashotte 2014).

Ratio of IAA/ABA, ZR/ABA and GA/ABA declined under drought over control (Table 2). Water and urea + KH_2PO_4 depicted more IAA/ABA than other treatments under control and drought conditions, respectively. Minimum IAA/ABA was observed for ALA and urea + KH_2PO_4 + ALA under normal and drought conditions, respectively. Ratios of ZR/ABA and GA/ABA were higher with urea + KH_2PO_4 + ALA than other treatments (Table 2). Yang *et al.* (2011) pointed out that for regulation of plant growth, the ratio of endogenous hormones was more important to their concentrations in some way.

The results of present study revealed that drought negatively impacted growth and endogenous hormones. Dry weight, plant height and ZR declined under drought over control (Fig. 1). Whereas, IAA, GA and ABA contents were augmented under stressed environment over normal conditions. Foliar applied nutrients and ALA significantly changed endogenous hormones and their ratio. Urea (1%) + KH_2PO_4 (1%) + ALA (50 mg/l) depicted more promising results than other treatments.

Acknowledgement

The authors are grateful to the authorities of National Key Basic Research Program of China (2014CB138806) along with Crop Germplasm Resources Utilization and Innovation Base Program of the 111 Project of China (104510-205001).

References

- Gao Huanye, Zong Xuefeng, Lu Jun, Zhang Yan, Dong Yufeng, He Xiujuan, Xu Yu and Wang Sangen 2017. Resistant physiological response of rice to combined stress of high temperature and drought at grain-filling stage. *Ecology and Environmental Monitoring of Three Gorges* **2**: 11-20.
- Gupta S and AM Rashotte 2014. Expression patterns and regulation of SICRF3 and SICRF5 in response to cytokinin and abiotic stresses in tomato (*Solanum lycopersicum*). *Journal of Plant Physiology* **171**: 349-358.
- Hong Y, X Tang, H Huang, Y Zhang and S Dai 2015. Transcriptomic analyses reveal species-specific light-induced anthocyanin biosynthesis in chrysanthemum. *BMC Genomics* **16**: 202-220.
- Leitao AL and FJ Enguita 2016. Gibberellins in penicillium strains: challenges for endophyte-plant host interactions under salinity stress. *Microbiological Research* **183**: 8-18.
- Li Najia, Muhammad Shahid, Xuefeng Zong, Jun Lv, Daibin Wang, Amna Saleem, Shakeel Ahmad Anjum and Sangen Wang 2019. Exogenously applied 5-aminolevulinic acid mediated physiochemical regulations ameliorate weak light stress in tobacco seedlings. *Bangladesh Journal of Botany* **48**: 353-358.
- Mohammadi MHS, N Etemadi, MM Arab, M Aalifar, M Arab and M Pessarakli 2017. Molecular and physiological responses of Iranian perennial ryegrass as affected by trinexapac ethyl, paclobutrazol and abscisic acid under drought stress. *Plant Physiology and Biochemistry* **111**: 129-143.
- Ostrowski M, A Ciarkowska and A Jakubowska 2016. The auxin conjugate indole-3-acetyl-aspartate affects responses to cadmium and salt stress in *Pisum sativum* L. *Journal of Plant Physiology* **191**: 63-72.

- Radhakrishnan R, JM Park and IJ Lee 2016. *Enterobacter* sp. I-3, a bio-herbicide inhibits gibberellins biosynthetic pathway and regulates abscisic acid and amino acids synthesis to control plant growth. *Microbiological Research* **193**: 132-139.
- Rubio-Wilhelmi MM, E Sanchez-Rodriguez, MA Rosales, Blasco Begona, JJ Rios, L Romero, E Blumwald and JM Ruiz 2011. Effect of cytokinins on oxidative stress in tobacco plants under nitrogen deficiency. *Environmental and Experimental Botany* **72**: 167-173.
- Song Jixuan, Shakeel Ahmad Anjum, Xuefeng Zong, Rong Yan, Ling Wang, Aijie Yang, Umair Ashraf, Ali Zohaib, Jun Lv, Yan Zhang, Yufeng Dong, and Sangen Wang 2017. Combined foliar application of nutrients and 5-aminolevulinic acid (ALA) improved drought tolerance in *Leymus chinensis* by modulating its morpho-physiological characteristics. *Crop & Pasture Science* **68**: 474-482.
- Teng Zhonghua, Zhi Li, Lv Jun, Zong Xuefeng, Wang Sangen and He Guanghai 2010. Effects of high temperature on photosynthesis characteristics, phytohormones and grain quality during filling-periods in rice. *Acta Ecologica Sinica* **30**: 6504-6511.
- Xiliang L, L Zhiying, R Weibo, D Yong, J Lei, G Fenghui and H Xiangyang 2016. Linking nutrient strategies with plant size along a grazing gradient: Evidence from *Leymus chinensis* in a natural pasture. *Journal of Integrative Agriculture* **15**: 1132-1144.
- Yang WB, Wang ZL, Yin YP, Li WY, Li Y, Chen XG, Wang P, Chen EY, Guo JX, Cai T and Ni YL 2011. Effects of spraying exogenous ABA or GA on the endogenous hormones concentration and filling of wheat grains. *Scientia Agricultura Sinica* **44**: 2673-2682.

(Manuscript received on 16 July, 2019; revised on 25 July, 2020)