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Interaction of drought and 5-aminolevulinic acid on growth and drought resistance of *Leymus chinensis* seedlings

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A B S T R A C T

Leymus chinensis is a dominant grass species in the Inner Mongolia steppes owing to its high vegetative productivity, good palatability for cattle, and abundant horizontally creeping rhizomes. Drought is generally regarded as one of the main environmental issues that is becoming a daunting challenge to the growth of plants, and ultimately results in land degradation. Drought stress influences plant growth and development by modulating physiological and biochemical events; however, some growth substances such as 5-aminolevulinic acid (5-ALA) show potential to ameliorate the damaging effects of drought. The objective of this study was to evaluate the response and mechanism of *L. chinensis* seedlings under drought stress, the effects of 5-ALA application, and the interaction of drought stress and 5-ALA application. Healthy and uniform-size seeds of *L. chinensis* were collected in a natural community of the Ecological Experimental Station in the Xilingole grassland. A potting experiment was carried out to determine the influence of exogenously applied 5-ALA at various concentrations (10 mg/L, 50 mg/L, and 100 mg/L) under different soil water regimes (50% and 80% soil relative water content) on the morphological and physiological attributes of *L. chinensis* plants from June to November 2014. The seeds were grown in a biochemical incubator, and then the seedlings were transferred to pots. Water and Hoagland nutrient solution was applied to ensure an adequate nutrient supply at 5-day intervals. When seedlings attained a height of 18–21 cm, 5-ALA was applied at different concentrations. Water spray was applied to *L. chinensis* plants as a control treatment. A second and third spray of 5-ALA was applied at 1-day intervals to exploit the full potential of 5-ALA application. Simultaneously, drought stress treatment was imposed using 50% soil relative water content; 80% soil relative water content was treated as the control. Therefore, there were eight treatments with three replications established in a random complete block design. The sampling for morphological, physiological, and biochemical attributes was conducted after 15 days of drought stress, respectively. The results showed that 5-ALA could promote the growth of *L. chinensis* seedlings, including plant height, leaf area, plant water content, biomass, root activity, and photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoid), under a soil relative water content of 80%. Among all concentrations, 10 mg/L 5-ALA proved to have the best effect on growth. Drought stress (50% soil relative water content) hampered plant growth. However, treatments of 5-ALA ameliorated the damaging effect of drought stress on seedlings, and improved the morphological index, biomass, plant water content, root activity, photosynthetic pigments, osmotic adjustments, and antioxidase activities viz. peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate (APX), but reduced malondialdehyde (MDA) content and electrical conductivity. Among all concentrations, 50 mg/L 5-ALA proved to have the best effect under drought stress. In summary, 5-ALA application improved the performance of *L. chinensis* by modulating growth and other morphological and physiological traits. However, the effect of 5-ALA on *L. chinensis* was concentration-dependent. Furthermore, significant interactions between drought stress and 5–ALA treatment (at a given spraying concentration) were observed with respect to leaf area, leaf width, fresh weight, root activity, chlorophyll b, carotenoids, MDA, proline, soluble protein, SOD, APX, and GR.

1. Introduction

As a worldwide problem, drought stress is one of the most important environmental stresses that limits plant growth and reduces grain yield by changing the morphological and physiological indices and gene expression [1,2].The negative effect of drought stress on plant is losing water and disequilibrating water balance. Also drought stress influence the normal activities of plant by damaging the photosynthetic organ and restraining the photosynthesis [3]. Drought stress can also inhibit the expansion of the blade, speed up the leaf aging and cause lipid

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membrane peroxidation, leading to the accumulation of active oxygen in plant, and followed by causing oxidative stress to plant [4,5]. Currently, grassland of China has been deteriorated along with the serious of drought stress and salinization, making the grassland productivity greatly reduced [6]. *Leymus chinensis* (Trin.) Tzvel is a perennial gramineous plant that widely spreads in Songnen plains, the west Liaohe plain and Hulunbuir and Xilingol steppe of Inner Mongolia. *L. chinensis* has the good palatability for cattle and forage value for its high quality of nutrition [7]. However, drought stress is threatening the improvement of yield and quality of *L. chinensis* plant, and then influences the development of local animal husbandry [8].

5-Aminolevulinic acid (5-ALA), also known as D-amino levulinic acid, D-amino ketone acid, is a kind of hydrocarbon that contains oxygen and nitrogen. It is a key precursor in the biosynthesis of all porphyrin compounds that can promote the synthesis of chlorophyll, regulate plant growth and development [9]. As biological metabolic intermediate, 5-ALA not only can improve the photosynthesis of plants and the quality of plant [10,11], but also have similar physiological function of plant hormones and can regulate physiological activity of protective enzyme system and osmotic regulation substance content to improve the performance of plants from adversity [11]. Studies have proved that low concentration of 5-ALA can improve the cold resistance [12,13] and salt resistance [14,15] of plant. However, the promotive effect of 5-ALA on different crops is crop-dependent [16]. Per our knowledge, no study has been carried out so far to increase drought resistance of *L. chinensis* through exogenous application of 5-ALA, and any research about the interaction effect of 5-ALA and drought stress. This study revealed the growth and resistance characteristics of *L. chinensis* under drought stress, different concentrations of 5-ALA and interaction of both treatments. This study will provide reference in relieving the damage of drought stress through application of 5-ALA and theoretical direction for high productivity of grassland.

2. Materials and experimental design

2.1. Experimental material

Seeds of *L. chinensis* were collected for Chinese Inner Mongolia Ecological Experimental Station of *L. chinensis* natural distribution community in November 2013. Collected seed was dried at room temperature and sealed in bags and stored at 4 °C. Pot experiment was conducted in growth incubator during June to November 2014. The seeds were grown in incubator, after several days the seedlings were transferred to the pot that the diameter is 30 cm (each pot contains 40 seedlings). Each pot contains 10 kg sand culture (sand: humus = 3:1). Water was applied every five days according to the nature of treatment and each time adequate nutrient supply was ensured by applying 50 mL 1/4 Hoagland's nutrient solution. When seedlings attained the height of 18–21 cm, foliar applications of 5-ALA solution were carried out on *L. chinensis* seedlings with concentrations of 10, 50 and 100 mg/L and spray was applied continuous every day, in total 3 times. Distilled water was sprayed on seedlings as control treatment for comparison. Then drought stress was imposed. Reference Hsiao [17] hierarchies of plant water stress as well as other related researches on *L. chinensis* under drought stress [18–20]. In control soil relative water content was kept at 80% while at 50% of soil relative water content was used to impose drought stress. Soil was weighed daily to know the moisture consumed and water was applied as needed to ensure the adequate moisture content in each pot according to the nature of treatment [21]. There are 8 treatments and each treatment was replicated three times, in total 24 pots. Morphological and physiological attributes of *L. chinensis* were recorded 15 days after treatment (see Table 1).

Table 1
Experiment scheme for interaction of drought and 5-ALA.

Soil relative water content	5-ALA concentration (mg/L)			
	0	10	50	100
80% (normal water content, T)	T0(CK)	T1	T2	T3
50% (drought stress, HT)	HT0	HT1	HT2	HT3

2.2. Measuring method

2.2.1. Morphological indices, water content and dry matter content

The seedlings were removed carefully from the pot and were separated aboveground and underground parts for the determination of morphological indices, such as plant fresh weight, plant height. Seedlings were rinsed with water 2–3 times and then dried with filter paper. Fresh weight and seedling length were measured. Seedlings were dried in oven at 105 °C for 15 min followed by drying at 65 °C till constant weight to determine the seedling dry weight. Plant water content was determined based on the formula of Yang et al. [22].

$$\text{Watercontent} = \frac{\text{Freshweight}/\text{Dry weight}}{\text{Freshweigh}t}$$

The root shoot ratio is the ratio of fresh weight (dry weight) underground parts and aboveground parts. Leaf area, leaf length and leaf width were measured with MSD-971 scanner.

2.2.2. Determination of physiological and biochemical indices

The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were measured by the method of Arnon [23]. Select 0.1 g fresh leaves fully expanded and cut into filaments. Extract with 20 mL 95% ethanol to completely lose the green leaf and then measured using different wavelengths under 665, 649, 652 and 470 nm with an ultraviolet spectrophotometer. Selection of fresh leaves fully expanded 0.2 g and the soluble protein content was measured using Coomassie brilliant blue G-250 method [24]. Determination of soluble sugar was done by anthrone color method [25]. The malondialdehye (MDA) content was assayed using thiobarbituric acid (TBA) assay [26]. Selection of fresh leaves fully expanded 0.4 g and proline content was measured using the ninhydrin method [27]. Root samples (0.25 g) (including the root and rhizome) were weighted and root activity was measured by using the triphenyltetrazolium chloride (TTC) method of Higa et al. [28]. The leaf electrical conductivity was determined using a conductivity meter [29] which can represent the degree of cell membrane damage under stress, and the relative electric conductivity is the ratio of the electric conductivity boiled and not boiled. The method of extract of these antioxidant enzymes is refer to the method of Li et al. [30] and 0.5 g fresh leaves fully expanded were used to measure several antioxidant enzymes. Superoxide dismutase (SOD) was measured with the method of NBT-illumination method, catalase (CAT) was measured with ultraviolet absorption method, peroxidase (POD) was measured with Guaiacol oxidation method, ascorbate peroxidase (APX) was measured with ultraviolet absorption method and glutathione reductase (GR) activity was measured using the method of Khatuna [31].

2.3. Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA), analyses of drought stress, different concentrations of 5-ALA and the interaction of both treatments on *L. chinensis* seedling growth and physiological and biochemical characteristics. Average between multiple comparisons was measured using LSD (least significant difference) test. All the data were analyzed using SPSS 17.0 statistical software. Graphics are done using Excel.