

## Salinity Influence on *Leymus chinensis* Characteristics in a Temperate Meadow Ecosystem

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### Abstract

Salinity is an important restrictive factor for plant growth and ecosystem productivity. However, the endogenous mechanisms by which salinity constrains plant growth are not well understood. To determine the mechanism by which soil salinity suppresses plant growth under salt stress, the effect of soil salinity on hormones in the leaves of *Leymus chinensis* and the plant density, height and biomass were examined in Songnen meadow steppe. The plants with rhizosphere soil were collected in the growing season (May, June, July, September, October) from the field at different salt levels. The shoot density, height and biomass accumulation of *L. chinensis* highly decreased with the increase in the soil salinity. Salinity significantly reduced the synthesis of the hormones gibberellic acid (GA<sub>3</sub>) and indoleacetic acid (IAA), but it increased the concentration of abscisic acid (ABA). Significant negative correlations between the soil electrical conductivity and plant leaf hormones (GA<sub>3</sub>,  $r = -0.853$ ,  $P < 0.05$ ; IAA  $r = -0.971$ ;  $P < 0.01$ ) related to plant growth and positive correlation with ABA ( $r = 0.931$ ,  $P < 0.01$ ) were observed. Significant positive correlations between the plant hormones related to plant growth (GA<sub>3</sub> and IAA) were observed, but negative correlations were found between ABA and plant density ( $r = -0.872$ ,  $P < 0.05$ ) and height ( $r = -0.833$ ,  $P < 0.05$ ). The results suggest that soil salinity might restrict plant growth and biomass accumulation by reducing the synthesis of GA<sub>3</sub> and IAA and increasing the synthesis of ABA under salt stress.

**Keywords:** degradation, endogenous hormones, grassland, productivity, Songnen plain

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### Introduction

Salinity is one of most important factors that restricts agriculture and livestock husbandry. Over 800 million hectares of land are affected by salt all over the world (Munns, 2005). The poor soil conditions caused by salinity can hinder plant seed germination (Song *et al.*, 2005; Zhang *et al.*, 2010), seedling survival and growth (Song *et al.*, 2009). Numerous previous studies have demonstrated the mechanisms by which salinity affects plant growth using the three main approaches of osmotic effect, toxic effect, and nutritional stress (English and Colmer, 2013; Nublat *et al.*, 2001; Song *et al.*, 2006). It is also well known that changes in the concentrations of the endogenous hormones can affect plant growth (Nimir *et al.*, 2014). However, the effects of soil salinity on plant endogenous hormones and the endogenous mechanisms by which salinity suppresses the growth of plants are still not well understood.

*Leymus chinensis* (Trin.) is a typical perennial rhizomatous grass that is mainly distributed in the eastern portion of the Eurasian steppe zone. *L. chinensis* is the dominant species in the Songnen meadow steppe, with high economical and ecological value, and the proportion of *L. chinensis* is 85% in terms of the

total plot biomass (Zhu, 2004). However, the growth of *L. chinensis* is influenced by some biotic and abiotic factors. Over the past few years, many studies have researched the morphological and physiological adaptation of *L. chinensis* to environmental factors. For example, several studies have demonstrated that saline and alkaline stress the seed germination and production of *L. chinensis* (Chen *et al.*, 2013; Lin *et al.*, 2012), and warming, nitrogen addition and grazing influenced *L. chinensis* growth (Li *et al.*, 2014; Wang *et al.*, 2013; Zhong *et al.*, 2014). However, the endogenous mechanisms by which environmental factors suppress the growth of *L. chinensis* are not well understood. Previous studies have found that the hormones in the seeds of *L. chinensis* play a key role in their seed dormancy and germination (Ma *et al.*, 2010) and that exogenous hormones had little impact on enhancing the seed germination of *L. chinensis* (Ma *et al.*, 2008). Moreover, many studies have demonstrated that hormones can improve plant growth and biomass accumulation and enhance resistance against an adverse environment (Dayan *et al.*, 2010; Li *et al.*, 2013; Rastogi *et al.*, 2013; Wang *et al.*, 2014). Nevertheless, the effects of soil salinity on plant hormones and the relationship between plant

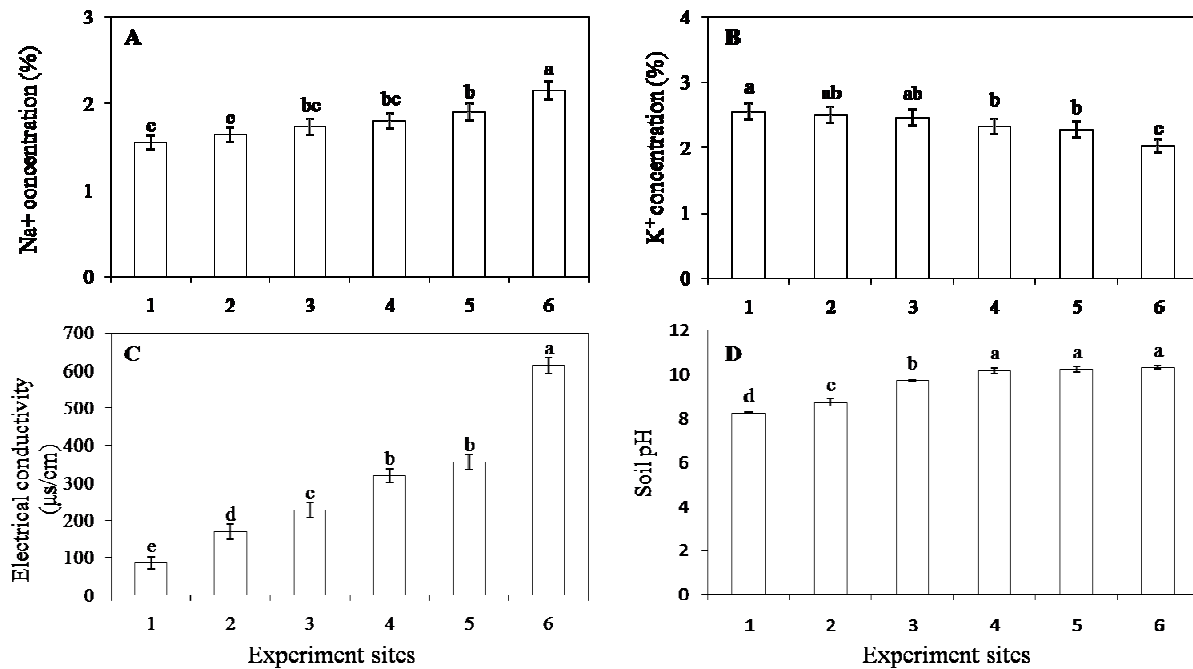


Fig. 1. Soil Na<sup>+</sup> (A) and K<sup>+</sup> concentrations (B), EC (C) and pH (D) of different soil plots according the soil salinity gradients. Different lowercase letters on each column represent significant differences at 0.05 level

hormones and plant growth in field conditions are not clear. The aims of this study were to understand the effects of soil salinity on leaf hormone content and the growth of *L. chinensis* and to elucidate the endogenous mechanisms by which salinity influences the growth of *L. chinensis* in field conditions.

## Materials and Methods

### Study site

Songnen meadow steppe, located in northeast China, is the most typical and largest meadow steppe and is the most important pasture, playing a key role in ensuring the development of animal husbandry. However, this meadow steppe is one of the three largest soda saline-alkali areas in the world. The salinized land of the Songnen meadow steppe forms approximately 60-70% of the total grassland, and the high salinity significantly decreases the forage grass productivity. Moreover, the extensive distribution of the saline-alkali soil has affected the plant distribution in this area (Zheng and Li, 1995). Determining the mechanism by which soil salinity limits plant growth can progress us toward designing an approach to reduce the negative impact of soil salinity on plant growth.

This research was conducted in the Songnen Grassland Ecological Research Station (44°45'N, 123°45'E), Northeast Normal University, Jilin Province, northeastern China. This grassland is a typical temperate meadow steppe. The area has a typical mesothermal monsoon climate, and the mean annual temperature is 4.6-6.4 °C. The annual accumulated temperature is 2545-3374 °C, and the frost-free period is approximately 140 days. The mean annual rainfall is approximately 400 mm, and more than 60% of the rainfall is concentrated from June to August. The annual evaporation capacity is 2-3 times more than the rainfall. Salinized soil is the main soil type in Songnen meadow steppe (Zhang *et al.*, 2015).

### Experiment design

The experiment was a completely randomized design with one factor: soil salinity, replicated 6 times. Six different salt levels according to the degrees of retrogressive succession and the soil salinity were included in our experiment. Before the experiment, the soils were measured, and the soil salinity increased gradually from experiment site 1 to site 6. At each experiment site, six plots were selected for sampling. Each plot area was 3×3 m. The distance between the sampling plots was more than 100 cm.

### Field sampling

On the 28<sup>th</sup> May, 19<sup>th</sup> June, 15<sup>th</sup> July, 5<sup>th</sup> September and 2<sup>nd</sup> October in 2012, the full expanded fresh leaves of *L. chinensis* in each plot were collected to measure their hormone content, and in each plot 3 subplots of 1×1 m were selected randomly. On each sampling, 50 leaves of each plot were collected and put into plastic bags and stored at -80 °C for later measurement of hormones. At the same time, the rhizosphere soil of *L. chinensis* was collected, and the pH and electrical conductivity (EC) were analyzed. The plant density of shoots per square meter were recorded, plant height (from petiole to the apex) was measured using a ruler, and the aboveground and belowground biomass of *L. chinensis* community per square meter were determined after drying 48 h at 65 °C in a stove. Moreover, the dryweights of those leaves used for measuring hormones were calculated according to the other aboveground biomass in the same experiment site and added into the total aboveground biomass.

### Soil properties analysis

The magnetic force agitator mixed every 10 min and rested for 30 min (Bao, 2005). The pH was measured using a HI98129 acidity meter, and the survey of electricity conductivity (EC) used a DDS-307 conductivity meter (Shanghai Thunder Magnetism Instrument Plant). The soil Na<sup>+</sup> and K<sup>+</sup> were analyzed using atomic absorption spectroscopy.

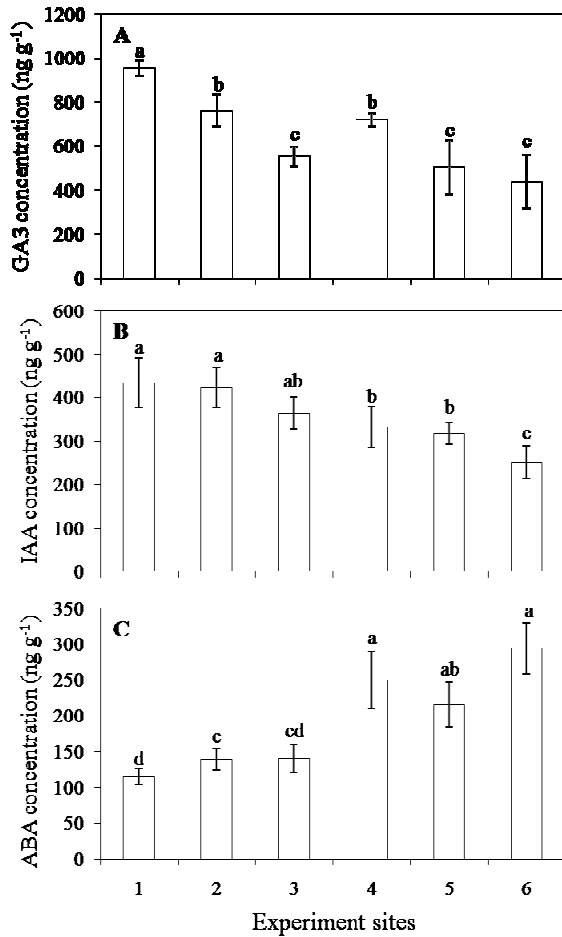


Fig. 2. Plant hormones GA<sub>3</sub> (A), IAA (B) and ABA (C) at different soil salinity gradients. Different lowercase letters on each column represent significant differences at 0.05 level

#### Measurement of endogenous hormone

The leaves of *L. chinensis* for hormones measurements were stored at -80 °C and weighed (1 g fresh weight). Four ml of extraction buffer consisting of 80% (v/v) methanol with 1 mM butylhydroxytoluene were added. The samples were ground using a mortar and pestle in an ice bath and then transferred into 10 ml tubes. The mortar was washed four times after each sample with 6 ml of extraction buffer and then added to the tubes. The samples were extracted at 4 °C for 4 h and were then centrifuged at 3500 r.p.m. for 10 min (Ma *et al.*, 2010). The supernatant of each extract was passed through a C18 solid-phase extraction column and then blown dry with N<sub>2</sub>. The endogenous GA<sub>3</sub>, IAA and ABA of the plant were quantified by enzyme-linked immunosorbent assays as described by Hess *et al.* (2002) and Zhang (2007).

#### Data analysis

A one-way analysis of variance (ANOVA) using SPSS 16.0 software (SPSS 16.0 for windows, USA) was applied to compare the variation in the soil pH, EC, Na<sup>+</sup>, K<sup>+</sup>, hormones GA<sub>3</sub>, IAA, and ABA, and plant biomass among different soil salinity degrees

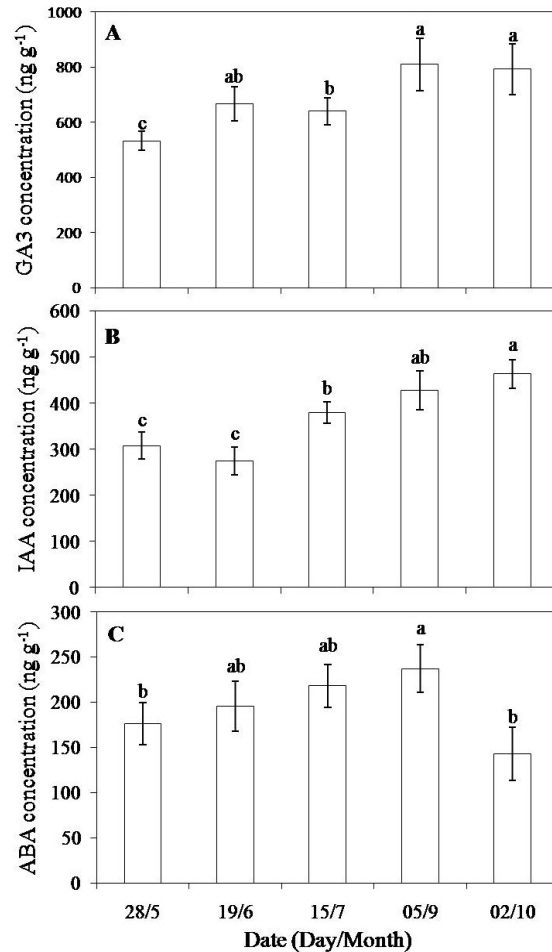


Fig. 3. Effects of soil salinity on plant hormones GA<sub>3</sub> (A), IAA (B) and ABA (C) at different times. Different lowercase letters on each column represent significant differences at 0.05 level

as well as the leaf differences in GA<sub>3</sub>, IAA, ABA content at different times. The statistical significances were compared using a least significant difference (LSD) at the 0.05 level. The correlations between the soil condition, plant biomass and hormone concentrations in the plant leaves were calculated. All of these values are expressed as mean ± SE.

## Results

#### Soil characteristics

The soil Na<sup>+</sup> and K<sup>+</sup> differed significantly among the six study sites (all  $P < 0.001$ ). Along the salinity gradients, the soil Na<sup>+</sup> significantly increased from 1.55% to 2.2%. The soil Na<sup>+</sup> concentration of experiment site 6 was significantly higher by 38.7% compared to site 1 (Fig. 1A). The soil K<sup>+</sup> concentration significantly decreased ( $P < 0.001$ ) from plot site 1 to site 6 (Fig. 1B). The soil EC and pH significantly increased along the salinity gradient, and the soil EC and pH in sites 4, 5 and 6 were significant higher than in sites 1, 2 and 3; in site 6, the soil EC and pH were higher by 621% ( $P < 0.001$ ; Fig. 1C) and 26% ( $P < 0.05$ ; Fig. 1D) compared to site 1, respectively.

Table 1. The effects of soil salinity on plant growth of *L. chinensis* in the field

Experiment sites	Plant density (Plant shoot m <sup>-2</sup> )	Plant height (cm)	Aboveground biomass (g dw m <sup>-2</sup> )	Underground biomass (g dw m <sup>-2</sup> )
1	1106 ± 105a	62.8 ± 7.6a	312.7 ± 22.6a	272.3 ± 23.6a
2	973 ± 88a	57.6 ± 8.5ab	329.3 ± 22.5a	240.4 ± 15.2a
3	728 ± 86b	51.2 ± 6.2ab	261.8 ± 23.3b	195.9 ± 13.3ab
4	610 ± 65b	48.3 ± 5.6b	227.2 ± 18.3b	176.7 ± 18.3b
5	396 ± 29c	43.3 ± 6.3b	206.6 ± 24.2b	85.3 ± 8.4c
6	325 ± 22d	40.1 ± 5.0b	158.9 ± 13.9c	78.3 ± 7.8c

Different lowercase letters on each column represent significant difference among different soil salinity gradients at 0.05 level

Table 2. Correlation between soil salinity and plant leaf hormones (GA<sub>3</sub>, IAA and ABA) and shoot density, height and biomass of *L. chinensis*

Correlations	Na <sup>+</sup> concentration (%)	K <sup>+</sup> concentration (%)	pH	EC ( $\mu$ scm <sup>-1</sup> )	GA <sub>3</sub> concentration (ngg <sup>-1</sup> )	IAA concentration (ngg <sup>-1</sup> )	ABA concentration (ngg <sup>-1</sup> )	Plant density (Plant shootm <sup>-2</sup> )	Plant height (cm)	Aboveground biomass (gdw m <sup>-2</sup> )
Na <sup>+</sup> concentration (%)	1									
K <sup>+</sup> concentration (%)	-0.989**	1								
pH	0.841*	-0.808	1							
EC ( $\mu$ scm <sup>-1</sup> )	0.995**	-0.994**	0.821*	1						
GA <sub>3</sub> (ngg <sup>-1</sup> )	-0.891*	0.776	-0.859*	-0.853*	1					
IAA (ngg <sup>-1</sup> )	-0.978**	0.968**	-0.918**	-0.971**	0.856*	1				
ABA (ngg <sup>-1</sup> )	0.911*	-0.949**	0.839*	0.931**	-0.654	-0.93**	1			
Plant density (Plant shoot m <sup>-2</sup> )	-0.932**	0.902*	-0.960**	-0.905*	0.900*	0.963**	-0.872*	1		
Plant height (cm)	-0.954**	0.910*	-0.919**	-0.923**	0.949**	0.957**	-0.833*	0.985**	1	
Aboveground biomass (gdw m <sup>-2</sup> )	-0.588	0.519	-0.763	-0.579	0.773	0.601	-0.551	0.683	0.696	1
Belowground biomass (gdw m <sup>-2</sup> )	-0.897*	0.909*	-0.803	-0.876*	0.717	0.887*	-0.891*	0.918**	0.894*	0.486

Note: \* represent significant difference at 0.05 level; \*\* represent significant difference at 0.01 level

### Hormones of leaves

The endogenous hormones of *L. chinensis* leaves showed a sharp change along the soil salinity gradient. As shown in Fig. 2A and 2B, the GA<sub>3</sub> and IAA concentrations of *L. chinensis* exhibited significant variation (all  $P < 0.05$ ) along the soil salinity gradient. The GA<sub>3</sub> concentration decreased from 952 ng g<sup>-1</sup> at site 1 to 437 ng g<sup>-1</sup> at site 6 ( $P < 0.05$ ; Fig. 2A), and the IAA concentration decreased from 434 ng g<sup>-1</sup> at site 1 to 251 ng g<sup>-1</sup> at site 6 ( $P < 0.05$ ; Fig. 2B). In contrast, the concentration of ABA increased with increasing salinity: the ABA concentration at site 6 increased by 156% ( $P < 0.05$ ; Fig. 2C) compared with that at site 1. In addition, the concentrations of GA<sub>3</sub> (Fig. 3A), IAA (Fig. 3B) and ABA (Fig. 3C) all increased ( $P < 0.05$ ) across all the sampling sites as the growing season progressed.

### Plant growth

With the increase of soil salinity, the growth of *L. chinensis* was inhibited significantly (Table 1): the plant density, height, and aboveground and belowground biomass were highly reduced. The plant density and shoot height of *L. chinensis* in higher salinity sites (site 5 and 6) were reduced by 65% ( $P < 0.05$ ) and 31% ( $P < 0.05$ ) compared to those in lower salinity sites (site 1 and 2), respectively. The aboveground and belowground biomass of *L. chinensis* in lower soil salinity sites (site 1 and 2) were higher by 43.2% ( $P < 0.05$ ) and 49% ( $P < 0.05$ ) compared with those in higher salinity sites (site 5 and 6).

### Correlation among soil salinity, hormones and plant density, height and aboveground biomass

Significant negative correlations were observed between the soil Na<sup>+</sup>, EC (exclusive K<sup>+</sup>) and the hormones GA<sub>3</sub> (all  $P < 0.05$ ) and IAA (all  $P < 0.01$ ) in the leaves of *L. chinensis*, but there was a positive correlation with ABA (all  $P < 0.01$ ). Significant negative effects of the soil Na<sup>+</sup> on the plant shoot density ( $r = -$

0.932,  $P < 0.01$ ), height ( $r = -0.954$ ,  $P < 0.01$ ) and belowground biomass ( $r = -0.897$ ,  $P < 0.05$ ) were detected (Table 2), and significant negative correlations between the soil EC and plant shoot density, height and belowground biomass were also found (all  $P < 0.05$ , Table 2).

### Discussion

The soil Na<sup>+</sup>, K<sup>+</sup> and EC reflected the ion concentration in the soil, e.g., its salinity; our results suggest that there is a significant salinity gradient from site 1 to 6, and the soil alkalinity increased, too. A previous study reported that a higher ratio of Na<sup>+</sup>/K<sup>+</sup> suppressed plant growth (Li *et al.*, 2008). In the current study, the concentration of Na<sup>+</sup> increased and K<sup>+</sup> decreased in the soil, which might induce more Na<sup>+</sup> uptake and less K<sup>+</sup> uptake and then suppress the growth of *L. chinensis*. A previous study demonstrated that a higher pH leads to a decline in the plant leaf area of *Vicia faba* (Pitann *et al.*, 2011). In the present study, the soil pH significantly increased with increasing salinity. The results suggest that a high soil pH might inhibit the growth of *L. chinensis*, which was proved in our later results.

Many studies have demonstrated that the content of GA<sub>3</sub> and IAA in plants can regulate plant development and improve plant growth (Egamberdieva, 2009; Jamil and Rha, 2007; Liu *et al.*, 2013). In the present result, we found that the concentration of GA<sub>3</sub> and IAA significantly decreased with increases in soil salinity, which might suggest that soil salinity can inhibit the synthesis of plant hormones. Despite of the concentrations of GA<sub>3</sub> and IAA in the leaves of *L. chinensis* being reduced significantly due to the increase of salinity, the synthesis of GA<sub>3</sub> and IAA in the plant roots are not clear from this study and should be considered in further studies. Previous studies have found that ABA can suppress the growth of plants, but some others have found

that ABA can modify the subsequent responses of the plant and improve plant growth under salt stress (Etehadnia *et al.*, 2008; Veselov *et al.*, 2008). Moreover, the concentration of ABA increased with increasing soil salinity, indicating that a large amount of Na<sup>+</sup> can stimulate the synthesis of ABA under salt stress (Veselov *et al.*, 2008).

Our results found that the soil salinity highly reduced the plant shoot density, height and aboveground biomass of *L. chinensis* in the saline-alkali Songnen meadow ecosystem, which will restrict the development of animal husbandry. Moreover, the decline of *L. chinensis* density will accelerate grassland degradation. However, changing the GA<sub>3</sub> and IAA contents might improve the density and productivity of *L. chinensis* and then affect the process of community succession. This path to reduce the soil salinity and prevent secondary salinization should be considered when utilizing Songnen meadow steppe.

In the present study, a significant negative correlation between the soil salinity and the plant shoot density, height and belowground biomass of *L. chinensis* were also detected, consistent with many previous studies (Liu *et al.*, 2011; Yang *et al.*, 2008). Although negative effects of the soil salinity on plant hormones and plant growth were observed, a significant positive correlation between plant hormones (GA<sub>3</sub> and IAA) and plant height, density, and aboveground and belowground biomass were also detected. These results suggest that the soil salinity might suppress plant growth by inhibiting the synthesis of GA<sub>3</sub> and IAA and stimulating synthesis of ABA in Songnen meadow steppe. However, the relationships among the soil salinity, hormone contents and growth rate of *L. chinensis* are not very clear and require further study. Several previous studies have demonstrated that an appropriate amount of hormone treatment can improve plant seedling survival and productivity (Gurmani *et al.*, 2006; Jamil and Rha, 2007). In future grassland management, GA<sub>3</sub> and IAA might be used to reduce the negative effects of soil salinity on plant growth and obtain higher forage grass productivity in saline grassland.

## Conclusions

With increasing soil salinity, the contents of GA<sub>3</sub> and IAA, plant density, height and biomass significantly decreased, whereas the content of ABA increased. A significant positive correlation among the hormones GA<sub>3</sub> and IAA and the plant shoot density, height and biomass of *L. chinensis* and negative correlation between ABA and the plant shoot density, height and biomass were observed. These results highlight that the soil salinity might suppress plant growth by regulating the synthesis of hormones under salt stress. Despite being preliminary, this study has improved our understanding of the physiological mechanisms by which salt suppresses plant growth under salt stress.

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