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Research Article

Effects of exogenous brassinolide and AM fungi on growth, photosynthetic characteristics and antioxidant enzyme system of *Leymus chinensis* under salt and alkali stress

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Abstract

Salinity and alkali stresses are a major abiotic stress negatively affecting crop productivity around the globe. Therefore, it is mandatory to develop the effective measures to mitigate the adverse impacts of these stresses for ensuring sustainable crop productivity and food security. Therefore, a pot experiment determined the effects of brassinolide application, inoculation with AM fungi (*Funneliformis mosseae*) and their combined use on the growth, photosynthesis and antioxidant system of *Leymus chinensis* under saline-alkali stress (0, 150 mmol/L). The mechanism of the two to alleviate the saline-alkali stress of *L. chinensis* was explored. The physiological and biochemical indexes of *Leymus chinensis* were significantly affected under saline-alkali stress (150 mmol/L). Inoculation of AM fungi and application of brassinolide effectively increased the biomass accumulation in the upper part (~ 25-40%) and root (15-35%) system of *L. chinensis* under saline-alkali stress. Further AMF also improved photosynthetic pigments (chlorophyll *a*, chlorophyll *b*), photosynthetic rate (Pn), Intercellular CO_2 concentration (Ci), stomata conductance (Gs), transpiration rate (Tr), chlorophyll fluorescence antioxidant enzymes (SOD: superoxide dismutase. CAT: catalase APX: ascorbate peroxidase, GR: Glutathione reductase) activity, and decreased malondialdehyde (MDA: ~ 40-50%) and hydrogen peroxide (H_2O_2: ~ 30-40%) accumulation. Therefore, under saline-alkali stress conditions, the combination of brassinolide and AM fungi proved better to mitigate their toxic effects.

Keywords: antioxidants; arbuscular mycorrhizal fungi; brassinolide; fluorescence; *Leymus chinensis*
Abbreviations: SOD: superoxide dismutase. CAT: catalase APX: ascorbate peroxidase, GR: Glutathione reductase, mmol: millimolar, ROS: reactive oxygen species, AMF: arbuscular mycorrhizal fungi, BR: brassinolide, +AM: AMF inoculation, -AM: no AMF inoculation, MDA: malondialdehyde, H_2O_2: hydrogen peroxide.

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Introduction

Soil salinity is a serious threat to crop productivity and its extent is continuously increasing due to anthropogenic activity. The extent of salt affected soils is increasing at the rate of 4×10^4 hectares per year which is a serious threat and needs dire attention (Rasheed *et al*., 2022a; Rasheed *et al*., 2022b). The excess of salts in soil rhizosphere induces the adverse impacts on plant growth, development and physiological and biochemical processes (Hussain *et al.,* 2019). Soil salinization in arid and semi-arid regions has become a key problem restricting economic plant yield and soil health (Liu *et al*., 2021; Khan *et al.,* 2023a). In China saline-alkali area exceeds 30% of the total cultivated land area due to the increase of land cultivation area and insufficient management measures (Liu *et al.,* 2021).

Saline-alkali stress induce adverse effects, such as osmotic stress, ion imbalance and pH stress which can reduce the plant growth and development (Jiao and Zhao, 2019). In green plants, saline-alkali stress adversely affects photochemical reactions, resulting in stomatal closure, increased intercellular CO_2 concentration, decreased activity of RuBisCo activity and increase accumulation of reactive oxygen species (ROS) (Dustgeer *et al.,* 2021). ROS damage membranes, proteins, lipids and DNA (Wang *et al*., 2017; Seleiman *et al.,* 2022). Plants possess a variety of antioxidants that can alleviate the adverse effects of excessive ROS accumulation. Among them, SOD is the initial enzyme to eliminate superoxide free radicals, and it can catalyze the disproportionation of superoxide anion into H_2O_2, which is then converted by CAT and APX into H_2O and O_2, thus greatly alleviating stress (Papalia *et al.,* 2018; Shaheen *et al.,* 2023). Besides these plants also accumulate various osmolytes (proline, glycine betaine, and sugars) which protect them from toxic effects of salinity and ensures the better plant growth under saline stress (Chattha *et al.,* 2022; Nawaz *et al.,* 2022).

Arbuscular mycorrhizal (AM) fungi belong to the subphylum brucella and it is an important functional microorganism widely found in soil. Most terrestrial plants can establish mutualistic and symbiotic relationships with them to better cope with environmental biological/abiotic stresses (Cao *et al*., 2021). Saline-alkali soil contained a large number of AM fungi, which could promote host nutrient absorption, improve root function and maintain leaf light Co-operative efficiency and free radical damage reduction (Cao *et al.,* 2021). Brassinolide (BR) is a kind of sterol hormone, which improves the plant growth and tolerance against different stresses (Yaqoob *et al.,* 2022). Studies have shown that BR can alleviate and improve the saline-alkali induced growth inhibition (Ghoname *et al.,* 2022). Photosynthetic characteristics and fluorescence parameters of *Medicago sativa* can enhance the stability of biofilm which can reduce malondialdehyde and H_2O_2 levels, and protect with chlorophyll (Ntoniou *et al*., 2017).

L. chinensis is a perennial rhizome asexual C_3 plant widely distributed in Eurasian grasses, Eastern China, Northeast China Plain and Mongolian Plateau. It contains relatively large amounts of trace minerals, vitamins, high-quality protein and carbohydrate, with rapid growth and high biomass, and has important economic and ecological value (Yu *et al.,* 2022). At present, the role of BR and AMF in alleviating abiotic stresses has been reported well. However, the role of combined use of BR and AMF in mitigating the toxic effects of saline-alkali stresses are not studied yet. Therefore, present study was conducted to determine the effect of combined use of BR and AMF on the growth, photosynthetic efficiency, gas exchange parameters, genes expression, and antioxidant activities of *L. chinensis* under saline-alkali stresses.

Materials and Methods

Experimental details

The seeds of *L. chinensis* (Jisheng No.3) were collected from Luyuan Grassland Company, Changling County, Songyuan City, Jilin Province. The seeds were treated with 0.5% hypochlorous acid before sowing.

The surface of seeds disinfected with sodium for 5 min, then treated with 75% alcohol for 5 min, washed with sterile water for 5 times. The AM fungus *Funneliformis mosseae* was purchased from Plant Nutrition and Science Section of Beijing Academy of Agriculture and Forestry Sciences. The strains were propagated by *L. chinensis* and white clover, and the inoculants consisted of spores (30 /g), mycelia and roots and soil composition. Moreover, brassinosteroids (BR) was purchased from SIGMA-ALDRICH.

The tested soil was taken from (123°43 '52 "E, 44°44' 39" N) 0~20 cm from Songnen Grassland Ecological Station in Jilin Province the topsoil. The soil was contained organic matter content 22.6 g/kg, total nitrogen 1.27 g/kg, Total phosphorus 0.44 g/kg, alkali-hydrolyzed nitrogen 74.71 mg/kg, available phosphorus 5.63 mg/kg, available potassium 140.09 mg/kg, electrical conductivity 225.67 mu S/cm. After collecting the soil, the plant residues were removed, and soil was air-dried naturally, mixed through a 3 mm screen, and sterilized by high-pressure steam (121 °C, 1×10^5 kPa, 8 h).

Experimental treatments

The test was conducted from April to July 2021 in plastic pots in greenhouse sheltered from rain at the site of Baicheng Normal University, Jilin Province. A completely random design was used to set up with two inoculation modes: AMF inoculation (+AM), no AMF inoculation (-AM) and two brassinosteroid levels: 100 μg/L (BR), 0 μg/L (NB) (Song *et al*., 2015), and two saline-base levels: 0 mmol/L (NS), 150 mmol/L (SS). The experiment consisted of 8 treatment combinations, which were repeated 5 times. The saline-alkali stress treatments were chosen as ion composition of Northeast saline-alkali soil (Yang *et al*., 2020). To achieve saline-alkaline stress NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 were mixed into saline-alkali solution with molar mass ratio of 9:1:1:9.

The pots have 19 cm height, 14.5 cm diameter, 12 cm bottom diameter with filled with 4 kg soil. 20 germinated seeds of *L. chinensis* were sown in each pot. After 10 days of culture, the seedlings were thinned to 10 plants, and at the same time 100 mL saline-alkali solution were added into the pots for 3 consecutive days to achieve a total dosage of 300 mL. On the other hand, in NS treatment only distilled water was applied. After 1 week 450 mL brassinosteroids were added to the pots and experiments was done for 70 days.

Sample collection and index determination

Determination of biomass, root infection rate, spore density and mycelium length of Leymus chinensis

After the end of culture, all pots were harvested and above-ground and underground parts were taken and place in oven at 105 °C until the constant weight. The roots were cut into 1 cm long segments and stained with magenta solution. The mycorrhizal invasion was calculated by grid cross notation under the optical microscope for the staining rate. The spore number was determined by wet sieve and pour out method, and the length of mycelia was determined by grid line intercept method (Phillip and Ayman, 1970).

Determination of photosynthetic traits

Photosynthetic pigments were determined by mixed extraction method of acetone and ethanol. Moreover, portable photosynthesizing system (LI-6800) was used to measure Pn, Tr, Ci, and Gs (Wang et al., 2017). Chlorophyll fluorescence analyzer (WALZPAM-2500, Germany PAM-WALZ) was used to determine the leaves initial fluorescence (F_0), maximum fluorescence (F_m), initial fluorescence under normal light (F_0').

Determination of antioxidant enzymes

The content of malondialdehyde (MDA) ground part of *L. chinensis* was determined by thiobarbituric acid. The concentration of hydrogen peroxide (H_2O_2) was determined by trichloroacetic acid extraction – spectrophotometry. The activity of SOD, CAT, APX, and GR was determined standard procedures.

Extraction and determination of genes related to antioxidant enzymes

After sampling, the ground part was carefully and quickly washed with distilled water, then washed with PBS buffer several times, and stored in a dry ice incubator at -20 °C. The total RNA of the samples was extracted using an RNA separation kit (Qiagen, USA). The content and quality of the samples were determined by 1.0% agar-agar gel electrophoresis. cDNA was constructed by reverse transcriptase M-MLV(RNaseH) catalyzing the reverse transcription of Prime Script TMRT reagent Kit gDNAr (TaKaRa)

According to the transcriptome sequence information of *L. chinensis* published in GenBank database was searched to the enzyme genes associated with antioxidant enzymes (Cu/Zn-SOD, CAT, APX). Primer express was applied according to the sequences of the above genes 5.0 Software designed amplification primers (Table 1; Yang *et al.,* 2020). Moreover, SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan) and Bio-Rad CFX97 real-time detection system Quantitative PCR was performed in triplicate. Real-Time fluorescence quantitative PCR was performed by step one plus real-time PCR instrument. qRT-PCR reaction has following conditions: conditions: predenaturation at 95 °C for 5 min; melting denatured at 95 °C for 20 s, annealing at 60 °C for 60 s, extension at 72 °C for 30 s, 40 cycles. Real-time quantitative test results used $2^{-\triangle\triangle Ct}$ algorithm for relative expression analysis.

Table 1. Primer sequence information of qRT-PCR

Genes Name	Primer sequence	Melting point temperature (°C)
Actin	F-primer: 5′-GCCAACAGAGAGAAGATGACC-3′ R-prime: 5′-ATAGAGGGAAAGCACCGCCT-3′	58.37 60.98
Cu/Zn-SOD	F-primer: 5′-ACCACCAATGGCTGCATGT-3′ R-primer: 5′-ATCACCAGCATGGCGGATTT-3′	60.25 60.40
CAT	F-primer: 5′-GGCGCACCTTGTTAAGTTCC-3′ R-primer: 5′-TCAGTCAGGTCCTTTGTGGC-3′	59.76 59.89
APX	F-primer: 5′-TGAGTCATGGAGCGAATGCT-3′ R-primer: 5′-AATCCGCATAGGTGATACCTGG-3′	59.46 59.70

Data processing and statistical analysis

Microsoft Excel 2013 was used to collate the data, and SPSS 25.0 software was used for three-factor analysis of variance (ANOVA) and Duncan's multiple comparison ($P < 0.05$) was used to compare difference among treatments.

Results

Effects of BR and AM fungi on biomass (dry weight) and mycorrhizal development of Leymus chinensis under salt-alkali stress

Compared with NS, the aboveground parts, roots and total dry weight of *L. chinensis* under saline-alkali stress (SS) were significantly decreased (Figure 1). The exogenous BR significantly increased the growth of *L. chinensis*. The inoculation with AM fungi (+AM) also significantly increased the growth traits of *L. chinensis.* Under NS condition, brassinosteroids combined with AM fungus treatment produced highest root, aboveground and total dry matter weight respectively. Under SS condition, -BR-AM treatments had the lowest root, shoot and total dry matter weight of 0.94, 0.26 g/ basin and 1.20 g/ basin respectively.

Microscopic examination at harvest showed that mycorrhizal infection was not detected in the roots of plants treated without AM fungus inoculation. The infection rate, number of spores and length of mycelia in the root system were shown in Figure 1B, Figure 1C and Figure 1D. Compared with NS, root infection was observed under SS treatment (Figure 1B) and mycelium length (Figure 1D) were significantly inhibited, while spore number (Figure 1C) did not change significantly. Whether it's NS or under SS condition, root infection rate, spore number and mycelium length under +BR treatment were more as compared to –BR. Under the NS condition, compared with –BR; brassinosteroids (+BR) increased root infection rate, spore number and mycelial length by 28.57%, 8.33%, and 53.48% respectively.

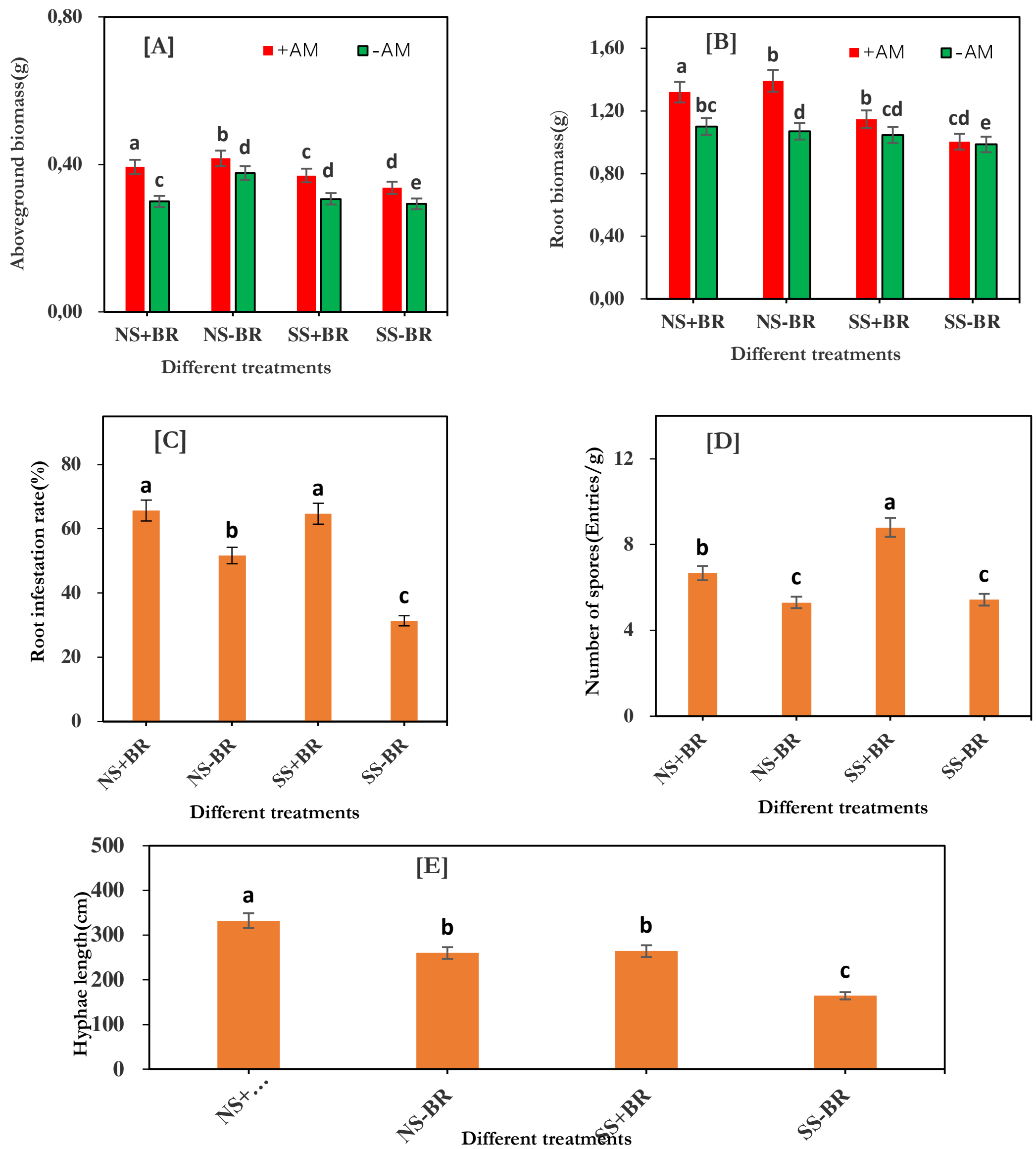


Figure 1. Effects of BR and AM fungi on biomass and mycorrhiza development of *Leymus chinensis* under salt and alkali stress. Different letters on the top of vertical bars are showing differences amid the treatments (P<0.05). NS: no saline-alkaline stress, BR: 100 µg/L BR, SS: 150 mmol/L saline-alkaline stress

Effects of BR and AM fungi on photosynthetic pigment content of Leymus chinensis under salt-alkali stress

According to Figure 2, the chlorophyll b content of *L. chinensis* under NS was higher than that of SS. Under NS condition, chlorophyll a content was higher in +BR than –BR. Except for chlorophyll a between -BR-AM and -BR+AM treatments, photosynthetic pigment content in other treatments was significantly higher with +AM than with -AM. Under SS condition, the contents of chlorophyll a and chlorophyll b in -BR-AM treatment were 7.95%-15.63% and 13.89%-36.73% lower than those in other treatments ($P<0.05$). Under NS condition, AM fungus inoculation reduced chlorophyll a and b value by 27.11% and 9.25%.

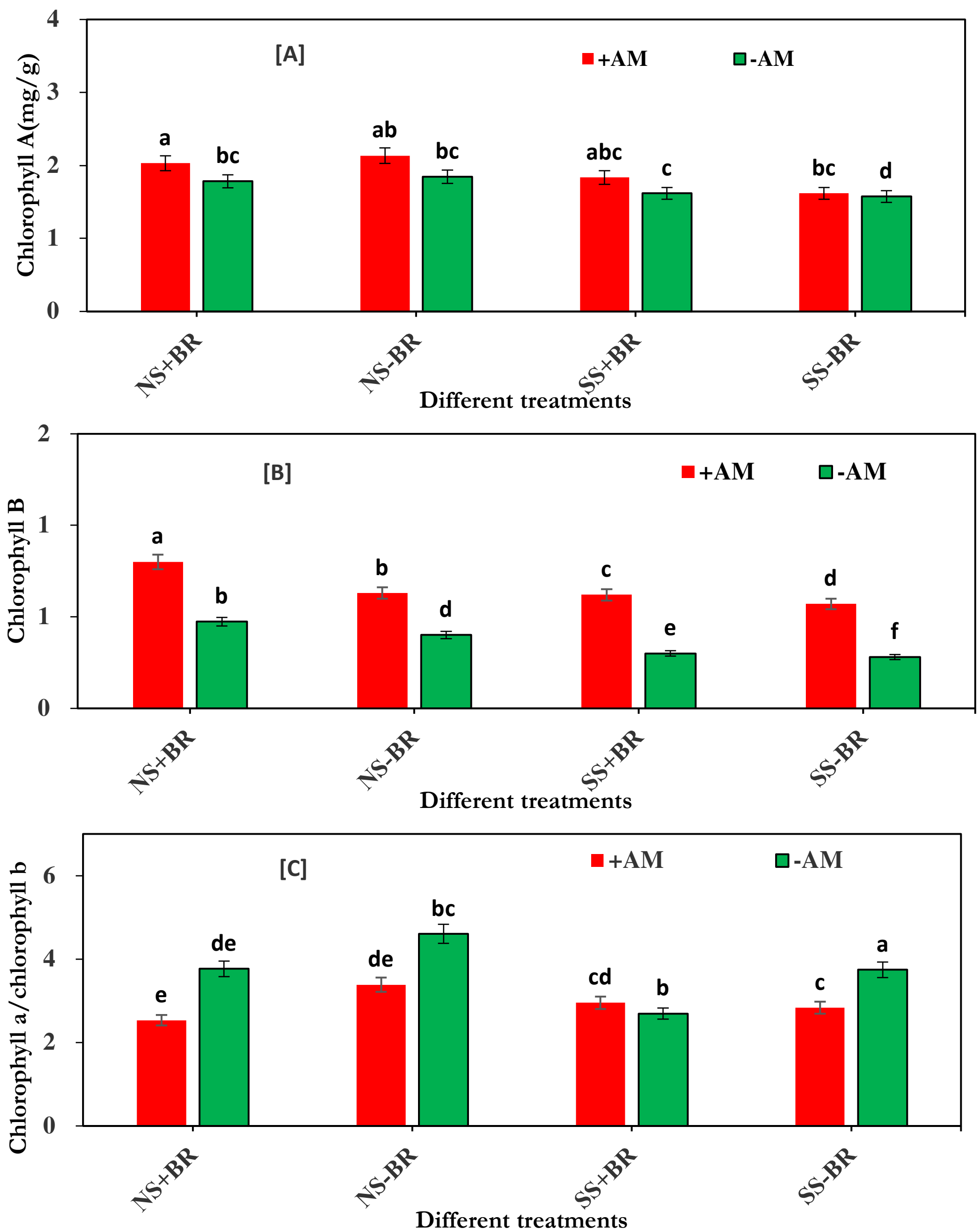


Figure 2. Effects of BR and AM fungi on photosynthetic pigment content of *Leymus chinensis* under salt and alkali stress. Different letters on the top of vertical bars are showing differences amid the treatments (P<0.05). NS: no saline-alkaline stress, BR: 100 μg/L BR, SS: 150 mmol/L saline-alkaline stress

Effects of BR and AM fungi on gas exchange traits of Leymus chinensis under salt-alkali stress

As can be seen from Table 2, Pn, Gs was all treated the rate (Tr) was lower than that under NS condition, but the Ci of AM fungi was higher under saline-alkali stress (SS). The Pn, Gs and Tr values of *L. chinensis* leaves was increased by 13.64%-24.34%, 12.86%-31.52% and 7.75%-21.64% with fungi and exogenous BR treatment. Under NS condition, BR + non-mycorrhizal treatment (-BR-AM) had the lowest value, which was 6.15%-11.31% lower than other treatments ($P<0.05$). In SS condition Ci of -BR-AM was the highest, which was increased by 9.92% ~ 22.80% compared with other treatments ($P<0.05$).

Table 2. Effects of BR and AM fungi on gas exchange traits of *Leymus chinensis* under salt-alkali stress

Dispose	Net photosynthetic rate Pn [μmol/(m^2·s)]	Intercellular CO_2 concentration Ci (mmol/mol)	Stomatal conductivity Gs[mmol/(m^2·s)]	Transpiration rate Tr [mmol/(m^2·s)]
NS -BR -AM	10.19±0.23c	124.88±5.99e	108.05±6.81c	4.39±0.30c
+AM	11.82±0.43b	135.96±4.51d	127.91±9.06b	4.90±0.18b
+BR -AM	11.58±0.26b	140.79±8.01cd	121.95±4.11b	4.73±0.17b
+AM	12.67±0.45a	133.06±5.53d	142.11±6.15a	5.34±0.31a
SS -BR -AM	8.21±0.25d	168.12±3.56a	88.82±±3.43d	3.78±0.18d
+AM	10.08±0.56c	152.95±6.21b	107.72±7.79c	4.29±0.25c
+BR -AM	10.52±0.40c	148.87±7.04bc	100.90±6.99c	4.25±0.21c
+AM	12.21±0.72ab	136.91±7.65d	126.08±9.6	4.88±0.20b
significance level				
SS	**	**	**	**
BR	**	**	**	**
AM	**	**	**	**
SS×BR	NS	**	NS	NS
SS×AM	**	**	NS	NS
BR×AM	*	*	NS	NS
SS×BR×AM	NS	**	NS	NS

Note: Different letters indicating differences amid treatments ($P< 0.05$); ** and * indicate three-factor ANOVA of 0.01 and 0.05 significant levels, and NS is not significant, the same below

Effects of BR and AM fungi on chlorophyll fluorescence parameters of Leymus chinensis under salt-alkali stress

The results indicate that compared with NS, saline-alkali stress (SS) resulted in maximum photochemical efficiency (Fv/Fm) and frustality of PSII in *L. chinensis*. Further, SS also decreased international photochemical efficiency (ϕPii) and photochemical quenching coefficient (qP) but increased non-photochemical quenching coefficient (NPQ). There ΦPSII and qP of AM (+AM) treatment was higher as compared to –AM treatment under saline-alkali stress. In terms of the maximum photochemical efficiency of PSII, under NS and SS conditions, no matter whether brassinosteroids were applied or not, +AM was compared with -AM The Fv/Fm values were the highest under brassinosteroids and AM fungal dual treatment (+BR+AM), respectively 8.56, 8.26. Under NS condition, ΦPSII (0.46) and qP (0.56) of +BR+AM treatment also had the maximum value. M Moreover, under SS NPQ value of -BR-AM treatment was the highest, which was 7.98% ~ 11.66% higher than that of other treatments (Table 3).

Table 3. Effects of BR and AM fungi on chlorophyll fluorescence parameters of *Leymus chinensis* under saline-alkali stress

Dispose	Maximum photochemical efficiency (Fv/Fm)	Actual photochemical efficiency (ϕPii)	Non-photochemical quenching coefficient (NPQ)	Photochemical quenching coefficient (qP)
NS -BR -AM	7.88±0.46bc	0.38±0.02c	6.97±0.35b	0.47±0.01d
+AM	8.22±0.25ab	0.46±0.04a	6.46±0.43c	0.56±0.03a
+BR -AM	7.99±0.48bc	70.41±0.02b	6.37±0.27c	0.52±0.02bc
+AM	8.56±0.21a	0.46±0.03a	6.23±0.25c	0.56±0.04a
SS -BR -AM	7.07±0.21d	0.29±0.02d	7.85±0.23a	0.35±0.03e
+AM	7.98±0.26bc	0.37±0.02c	7.03±0.34b	0.46±0.04d
+BR -AM	7.69±0.35c	0.38±0.03c	7.27±0.38b	0.46±0.02d
+AM	8.26±0.27ab	0.41±0.04b	7.14±0.35b	0.50±0.02c
significance level				
SS	**	**	**	**
BR	**	**	*	*
AM	**	**	**	**
SS×BR	NS	NS	NS	NS
SS×AM	NS	NS	NS	NS
BR×AM	NS	*	*	*
SS×BR×AM	NS	NS	NS	NS

Note: Different letters indicating differences amid treatments ($P< 0.05$); ** and * indicate three-factor ANOVA of 0.01 and 0.05 significant levels, and NS is not significant, the same below.

Effects of BR and AM fungi on antioxidant enzyme activity, MDA content and H_2O_2 concentration of Leymus chinensis under salt-alkali stress

The activities all the antioxidants were significant increased. Saline-alkali stress and AM fungus inoculation and BR (+BR) also increased SOD, CAT, APX and GR activities in shoots of *L. chinensis*, and most of them reached significant levels. Figure 3E and Figure 3F showed that salt and alkali stress (SS) significantly increased H_2O_2 concentration and MDA content in *L. chinensis* compared with NS. In terms of AM (+AM) inoculation, the H_2O_2 concentration and MDA content of brassinosteroids (+BR) under SS condition were lower than those under -BR, but there was no significant difference under NS condition. As far as brassinosteroids (+BR) was concerned, the MDA content and H_2O_2 concentration of *Leymus chinensis* were significantly decreased by AM fungi treatment under SS condition compared with -AM treatment, and the latter was significantly different, but there was no significant effect under NS condition. Under SS condition, H_2O_2 and MDA contents in +BR treatment were significantly lower than those in -BR treatment.

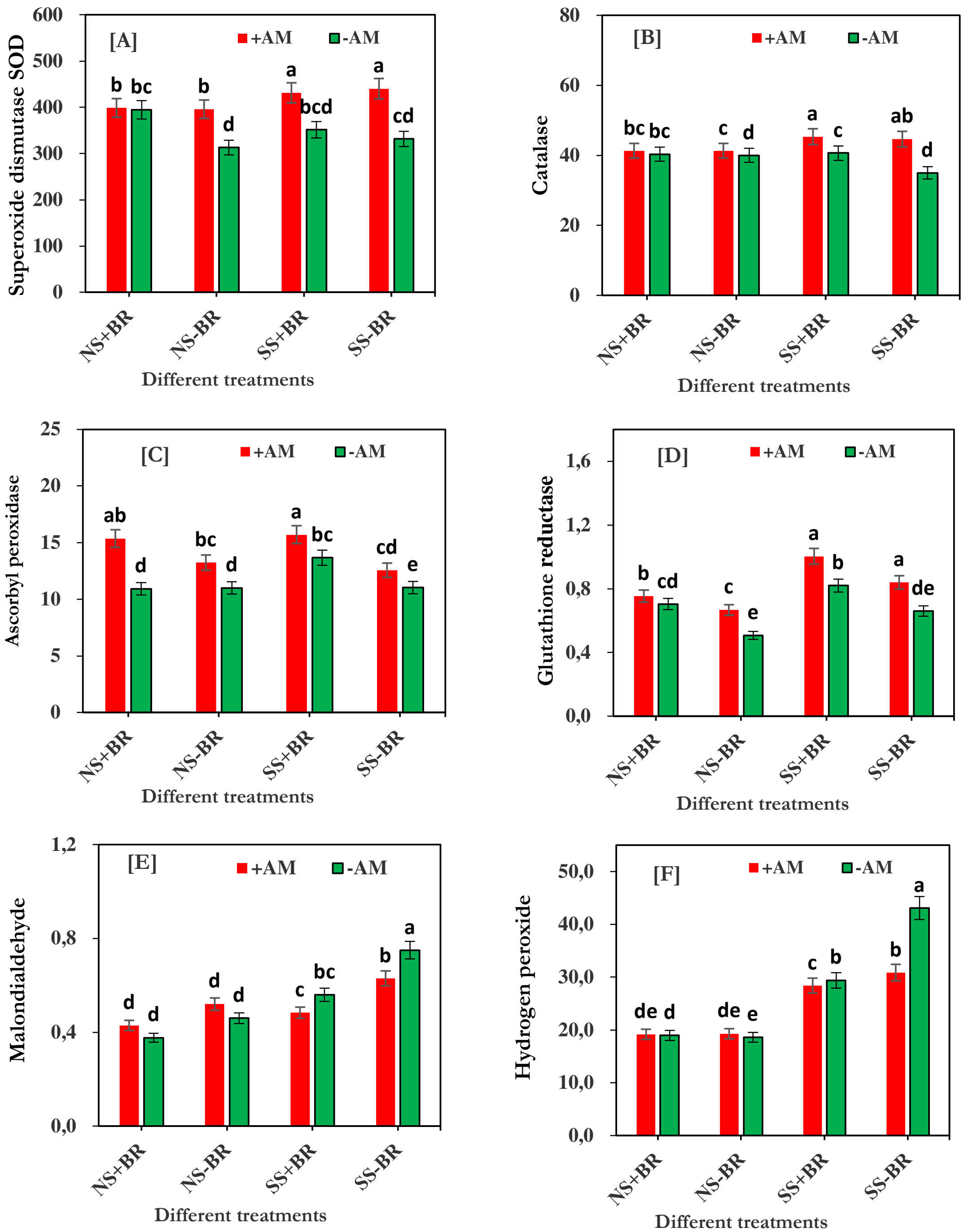
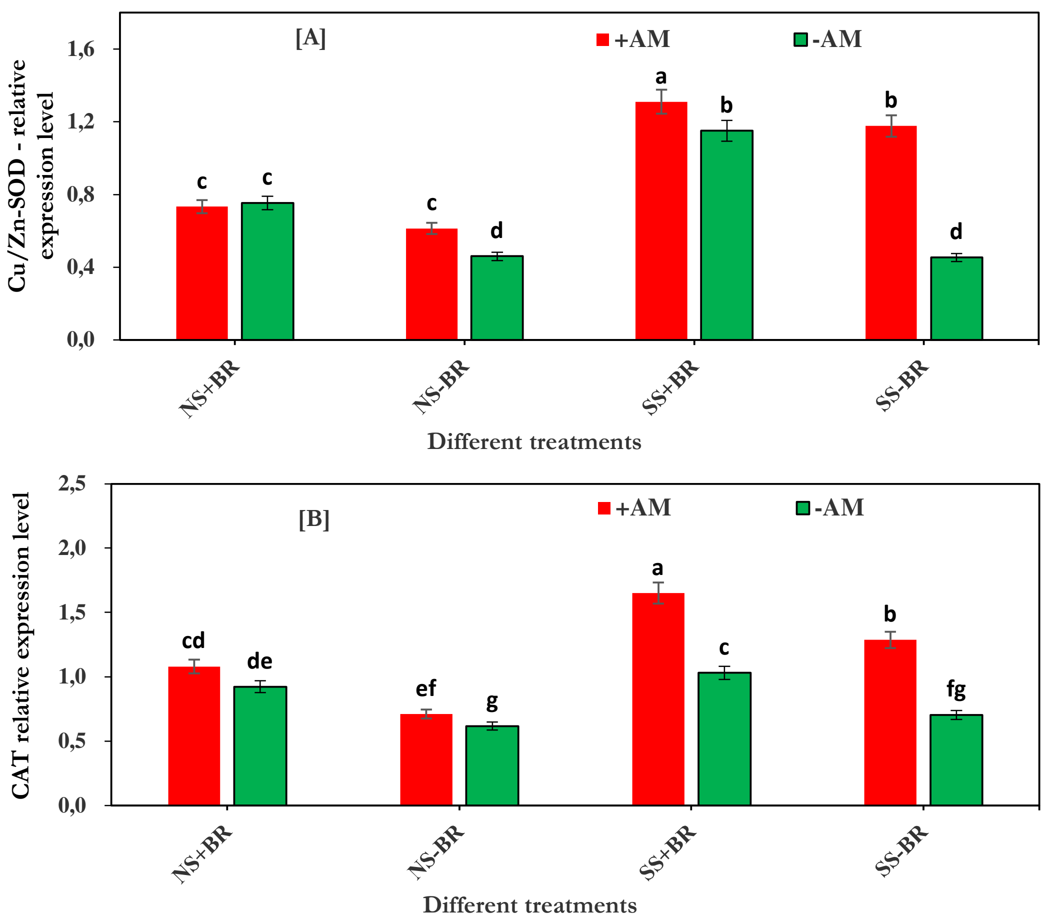


Figure 3. Effects of BR and AM fungi on antioxidant enzyme activity (A~D), malondialdehyde (E) and H_2O_2 concentration (F) under saline-alkali stress. Different letters on the top of vertical bars are showing differences amid the treatments ($P<0.05$). NS: no saline-alkaline stress, BR: 100 μg/L BR, SS: 150 mmol/L saline-alkaline stress.

Effects of BR and AM fungi on the expression of antioxidase-related genes in Leymus chinensis under salt-alkali stress

The expression levels of Cu/Zn-SOD, CAT and APX genes in *L. chinensis* were significantly increased under SS Especially the Cu/Zn-SOD gene (Figure 4). Among them, the expression level of Cu/Zn-SOD gene under NS condition (Figure 4A) was treated with -BR-AM was lower than other treatments by 26.98% to 33.33% ($P<0.05$). Under NS condition, the expression level of CAT gene (Figure 4B) was different between BR treatment (-BR and +BR). There was no significant difference between -AM+BR and +AM+BR among AM treatments (-AM and +AM). Under SS condition, BR treatment (-BR, +BR) and AM treatment (-AM, +AM) were significantly different, and the expression level of -BR-AM treatment was the lowest, compared with other treatments and it showed a reduction of 36.36% to 57.57% (P<0.05). The expression level of Cu/Zn-SOD gene under NS condition (Figure 4A) was treated with -BR-AM was lower than other treatments by 26.98% to 33.33% ($P<0.05$). Under NS condition, the expression level of CAT gene (Figure 4B) was different between BR treatment (-BR and +BR). There was no significant difference between -AM+BR and +AM+BR among AM treatments (-AM and +AM). Under SS condition, BR treatment (-BR, +BR) and AM treatment (-AM, +AM) were significantly different, and the expression level of -BR-AM treatment was the lowest, compared with other treatments and it showed a reduction of 36.36% to 57.57%($P<0.05$).



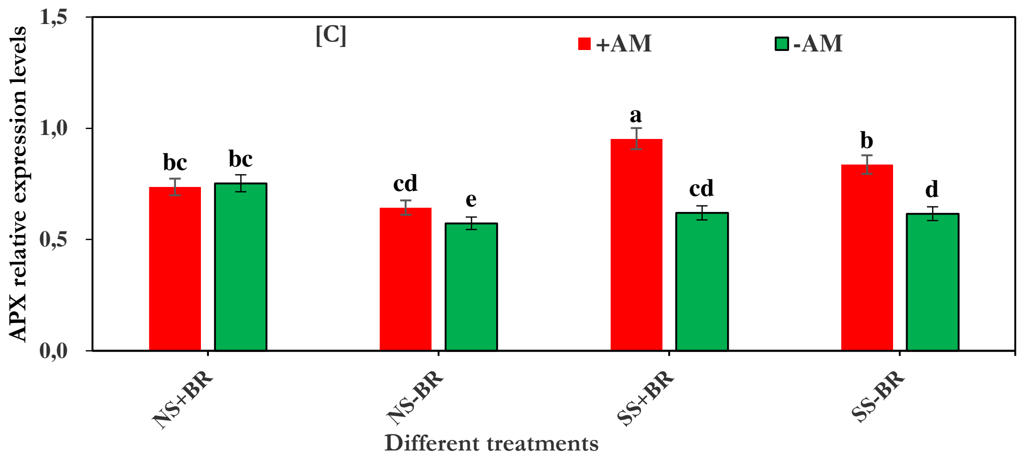


Figure 4. Effects of BR and AM fungi on the expression levels of antioxidant enzymes Cu/Zn-SOD, CAT and APX genes in *Leymus chinensis* under salt and alkali stress. Different letters on the top of vertical bars are showing differences amid the treatments (P<0.05). NS: no saline-alkaline stress, BR: 100 μg/L BR, SS: 150 mmol/L saline-alkaline stress.

Discussion

Soil salinization is one of the serious threats to the crop productivity and global food security. It limits different physiological and biochemical mechanism of plants and lead to serious reduction in growth and development (Jiao and Zhao, 2019; Yang *et al*., 2020; Shao *et al.,* 2023). The accumulation and allocation of plant biomass is an important function for assessing resource acquisition and environmental adaptation strategies. The results of this study showed that saline-alkali stress significantly reduce the aboveground root system and total dry weight of *L. chinensis*. However, inoculation with AM significant offset the negative effects of saline-alkaline stress and appreciably improved the growth and biomass production of *L. chinensis* (Tang *et al.,* 2022). Brassinosteroids (BR) is a new growth promoter and it play a crucial role against biotic and abiotic stresses (Ntoniou *et al.,* 2017; Manghwar *et al.,* 2022). In this study, under NS and SS (saline-alkali stress) conditions; the application of BR significantly increased the biomass accumulation of *L. chinensis* as compared to no BR application. In addition, the aboveground parts, roots and total dry weight of *L. chinensis* treated with combined BR and AM fungal inoculation (+BR+AM) were also higher than those treated with -BR or -AM alone. The results showed that BR promoted the function of AM fungi. Under saline-alkali stress (SS), root infection rate, spore density and mycelium length were significantly decreased under BR treatment this resulted in better uptakes of both water and nutrients and resulting in considerable increase in growth and biomass production. Previous studies have shown that plant hormones are important factors affecting the development of AM fungi in the process of symbiosis (Liao *et al.,* 2016; Cao *et al.,* 2021; Gao *et al.,* 2023).

Chlorophyll fluorescence indication the assembly and functions of photosynthesis subcellular and leaf development levels (Orcar-castell *et al.,* 2014; Hassan *et al.,* 2020). Photosynthetic pigment content and photosynthetic gas exchange parameters are important factors that determine the intensity and process of photosynthesis. This study reported that contents of chlorophyll a+b in *L. chinensis* was significantly decreased under salt-alkali stress. At the same time inoculated AM fungus inoculation significantly increased the synthesis of both chlorophyll contents possibly by increasing the uptake of important nutrients (iron and magnesium) that are considered to play a fundamental role in chlorophyll synthesis (Cao *et al.,* 2021; Aouz *et al.,* 2023; Khan *et al.,* 2023b). This study further showed that under saline-alkali stress, gas exchange parameters (Pn, Gs, Tr) and chlorophyll fluorescence parameters (Fv/Fm, ΦPSII, qP), were significantly decreased. However, nonchemical quenching coefficient (NPQ) were significantly increased. These results indicate that saline-alkali stress leads to stomatal closure which increased intracellular CO_2 concentration which is considered as an adverse impact in plants. However, exogenous supply of BR and AMF regulate the stomata opening, improve efficiency of PS-II and alleviate the inhabitation in photosynthesis and ensures the better photosynthetic efficiency, chlorophyll fluoresce and subsequently plant growth and development (Song *et al*., 2015).

Under normal conditions, a dynamic balance is maintained between the production and clearance of intracellular ROS in plants (Wang *et al*., 2017; Hassan *et al.,* 2023). However, ROS accumulate excessively under biotic/abiotic stress, leading to oxidase stress and lipid peroxidation. In this study, it was found that saline-alkali stress significantly increased H_2O_2 concentration, and the inoculation of AM fungi reduce the accumulation of H_2O_2 by increasing antioxidant activities and genes expression (Cao *et al.,* 2020; Song *et al*., 2015). In this study, the H_2O_2 concentration and MDA content of *L. chinensis* were significantly reduced under saline-alkali stress with BR and AMF application. This study showed that combined AMF can reduce the membrane damage caused by excessive accumulation of reactive oxygen species in mycorrhizal plants.

Brassinosteroids are natural sterols and it has much higher physiological activity than auxin, gibberellin and cytokinin. The application of BR has been found to eliminate the excessive ROS that can reduce the cell damage caused by oxidative stress (Hasanuzzaman *et al.,* 2020; Yaqoob *et al.,* 2022). This study showed that combined AMF fungus inoculation and BR application significantly increased all antioxidant activities and genes expression. The increase in antioxidant activities and expression levels of antioxidant genes countered the toxic effects salt-alkaline stresses by decreasing the H_2O_2 and MDA production which ensured the better growth and biomass of production in *L. chinensis*.

Conclusions

In conclusion, both AMF fungus inoculation and brassinosteroids effectively improved the growth of *Leymus chinensis*, by increasing photosynthetic performance and alleviating the damages caused saline-alkaline stress. Brassinosteroids also promoted the growth and development of AM fungi, which in turn improved the plant performance by protecting photosynthetic pigments, maintaining photosynthetic physiology, activating antioxidant enzymes, and reducing MDA and H_2O_2 production.

Authors' Contributions

ZWG conducted the research and prepared the manuscript. JL, QL, JL, JL, MB, XL, QZ, and YC reviewed and edited the manuscript. AR reviewed the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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