Growth-promoting bacteria alleviates drought stress of *G. uralensis* through improving photosynthesis characteristics and water status

Wenjin Zhang\(^{a,b,}\), Zhicai Xie\(^{a,}\), Xiaojia Zhang\(^{a,}\), Duoyong Lang\(^{c}\) and Xinhui Zhang\(^{a,b}\)

\(^{a}\)College of Pharmacy, Ningxia Medical University, Yinchuan, People’s Republic of China; \(^{b}\)Ningxia Engineering and Technology Research Center of Hui Medicine Modernization, Ningxia Collaborative Innovation Center of Hui Medicine, Laboratory of Hui Ethnic Medicine Modernization, Ministry of Education, Ningxia Medical University, Yinchuan, People’s Republic of China; \(^{c}\)Laboratory Animal Center, Ningxia Medical University, Yinchuan, People’s Republic of China

**ABSTRACT**

Drought has been becoming increasingly serious abiotic stress that influences plant growth. Endophytes are non-pathogenic plant-associated bacteria that can play an important role in conferring plant resistance to drought stress. In this study, drought stress resulted in the evident breakdown of the chloroplast membrane system in leaf cells, whereas *Bacillus pumilus* inoculation improved the integrity of chloroplast and mitochondria cell structure. Thus chlorophyll content, photosynthetic parameters, and water use efficiency increased. The inoculation of endophytes alleviated the inhibitory effect of drought stress on *Glycyrrhiza uralensis* growth. We concluded that *B. pumilus* inoculation enhanced the growth and drought tolerance of *G. uralensis* through the protection of chloroplast submicroscopic structure, and thus increased chlorophyll content, efficient photosynthetic rate, and improved water state.

**Introduction**

*Glycyrrhiza uralensis* Fisch. is a popular medicinal plant generally used in Chinese medicine for its therapeutic actions of nourishing qi, alleviating pain, and tonifying spleen (Li et al. 2016). These effects can be attributed to the abundant pharmacologically active substances in its dried roots and rhizomes. *G. uralensis* is distributed in arid and semiarid areas of China and is always subjected to drought stress. Previous studies on *G. uralensis* have focused on its pharmacology, but the physiological adaptation of the species to drought stress has been rarely investigated (Liao and Wang 2014; Li et al. 2011). Therefore, studying the adaptability of this species to drought stress may be of economic relevance for successful cultivation.

Drought is a major adverse factor for plant growth under field conditions (Chen and Jiang 2010; Gong et al. 2013), and its effects will increase in the foreseeable future (Joetzjer et al. 2014). Drought affects plants at various levels of their organization by limiting photosynthesis, water, and nutrition uptake and damaging plasma membranes, thereby resulting in decreased growth (Yordanov et al. 2000; De Andrade et al. 2015). Severe drought disturbs mesophyll cell ultrastructure and causes broken membrane systems in chloroplasts, swollen grana, and degraded starch granules (Xu et al. 2008; Shao et al. 2016). Photosynthesis is a core function of plants and involves the capture of light energy to produce both chemical energy and reduce equivalents via photochemical processes (Calatayud et al. 2006). Yin et al. (2006) found that drought stress reduces *Populus przewalski* growth and productivity through decreased transpiration rate (E), net photosynthetic rate (*P*\(_{N}\)), and stomatal conductance (*g*\(_{s}\)). Stomata control temperature and water use efficiency (WUE) are vital to plants’ existence. Li et al. (2017) showed that under conditions of short-term water stress, plants increase their WUE by reducing stomatal aperture and transpiration rate. Moreover, drought stress also causes many morphological, physiological, and biochemical changes in plants (Anjum et al. 2011). Wu et al. (2008) reported that drought stress leads to the decreased seedling leaf area, photosynthetic pigment content, and photosynthetic efficiency of *Sophora davidii* seedlings. Zhang et al. (2007) also found that water deficiency decreases chlorophyll content and photosynthetic rate in soybeans, thereby resulting in decreased plant biomass, height, and seed yield. Although some plants have developed different ways to resist drought, including altered leaf structure, highly efficient photosynthesis or water utilization, and increased dry matter allocation, their ability is limited (Chaves et al. 2003; Liu et al. 2011; Ge et al. 2012). Therefore, many exogenous substances and beneficial microorganisms help improve the ability of plants to cope with drought stress. Among these microorganisms, bacterial endophytes are inoculated, and this process is an important method.

Endophytic bacteria are organisms inhabiting plants but do not cause any adverse effect on the host plant (Ryan et al. 2008; Gagné-Bourque et al. 2016). Plant growth-promoting bacteria (PGB) are common endophytic bacteria that can modulate physiological response for water shortage, thereby ensuring plant survival under such stressful conditions (Marasco et al. 2012; Marulanda et al. 2009, 2010).
Various PGB species can increase drought resistance in wheat (El-Afry 2012), maize (Vardharajula et al. 2011), bean (Sarma and Saikia 2014), and Brassica species (Saeed et al. 2016). Marulanda et al. (2007) also reported that PGB-inoculated Glomus species remarkably increases growth and yield and improves drought tolerance in arid and semiarid areas. The adaptation of plants inoculated with PGB has an important relationship with drought stress conditions (Kohler et al. 2008); such adaptation commonly involved enhanced water or nutrient uptake and transpiration (Smith et al. 2009; Yang et al. 2014), induction of plant growth hormone signaling (Gagné-Bourque et al. 2016), and increased antioxidase activity and photosynthetic rate (Gusain et al. 2014; Naveed et al. 2014). However, the precise mechanisms involved are up for debate and rely on the plant species involved (Zhang et al. 2015).

In this study, Bacillus pumilus, an important PGB, was isolated from G. uralensis. Khan et al. (2016) found that B. pumilus inoculation can enhance the combined stresses of NaCl and high boron tolerance in rice (Oryza sativa, L.). However, the effects of inoculating endophytic bacteria on the alleviation of drought stress on G. uralensis plants have been rarely investigated. In this study, we studied the effect of B. pumilus on G. uralensis plants under drought stress by analyzing changes in leaf ultrastructure, photosynthetic characteristics, and plant growth. The results may be useful for explaining the mechanism of B. pumilus inoculation on the drought tolerance of G. uralensis.

Materials and methods

Plant and endophytic bacteria material

One-year-old G. uralensis plants were collected from Yanchi county (the county in Ningxia Hui Autonomous Region of China) for pot experiment. The Bacillus pumilus strain was isolated from two-year-old G. uralensis plants grown by our lab and identified by 16 S rRNA gene sequencing. The bacteria suspension was cultured for 2 days at 28°C on a shaking gat 180 rpm. The cell density is 10^8 cfu mL^-1.

Experimental design and treatments

Pot experiment was conducted at the Ningxia Medical University during the growing season of 2017. The average temperatures for day and night were 28 and 16°C, respectively, and the light and dark periods were 14 and 10 h, respectively. The average temperatures for day and night were 28 and 16°C, respectively, and increased from plants and recovery (every afternoon rewatering) when transplanted seedlings normal growth on June 3, 2017. At the same time, inoculated plants (DB+) were watered with 400 mL of bacteria culture suspension, while non-inoculated plants (CK and DB−) were watered with 400 mL distilled water. After one week, plants were watered with bacteria culture suspension or distilled water again. The bacterial endophyte B. pumilus was inoculated twice during the growth period. There were five replications per treatment and each pot contains 8 plants. All pots were randomly arranged and periodically rotated to minimize the effects of environmental heterogeneity.

At 95 days after the last inoculation of the sample plants, photosynthetic parameters of G. uralensis plants were measured on typical cloudless days and three leaves were collected as the leaf material for anatomy. At 102 days, G. uralensis plants were collected as the sample for evaluation of data on various growth parameters, physiological and biochemical parameters respectively.

Preparation of the leaf material for anatomy

Every treatment collected three leaves for examine the mitochondria and chloroplast ultrastructure of the mesophyll cells. A rectangular section of a leaf (3.0 × 1.5 mm) near the center vein of each leaf was removed with a blade. After fixation with 2.5% glutaraldehyde for 4 h, and washed with 0.1M phosphate buffer, pH 7.4, leaf cells were then post-fixed with 1% osmic acid at 4°C for 4 h, washed with 0.1 M phosphate buffer, and then dehydrated with different gradient acetone. The samples were exchanged in a series of acetone:resin mixtures at 3:1, 1:1, and 1:3 (v: v) and then placed in pure resin for 12 h at 37°C, 12 h at 45°C and 48 h at 60°C. The thin sections were cut from leaf samples with an LKB-V ultramicrotome and placed upon 250 mesh grids. Samples were double stained using, and lead citrate and then observed and randomly photographed using a Hitachi-600 transmission electron microscope (Xu et al. 2008).

Determination of chlorophyll

Chlorophyll was extracted with 0.3 g small pieces (2 × 5 mm) of fresh leaf tissue in 20 mL acetone solution (acetone and ethanol, 2:1, v/v). Each treatment was repeated for three times. After extraction at room temperature and dark conditions for 24 h, chlorophyll content in the supernatant was analyzed by measuring light absorbance at 663 and 645 nm, respectively, with an ultraviolet spectrophotometer (SP-752P, Shanghai Spectrum Instruments Inc., Shanghai, P. R. China), as described by Ming et al. (2007).

\[
\text{Chl a} = 12.72A_{663} - 2.59A_{645} \\
\text{Chl a} = 12.72A_{663} - 2.59A_{645} \\
\text{Chl b} = 22.88A_{665} - 4.67A_{663}
\]

The Chl a + b content = Chla + Chl b

\[
= 20.29A_{665} + 8.05A_{663}
\]

where (A_{663}) and (A_{645}) represent absorbance values read at 663 and 645 nm, respectively. Chl a, Chl b, and the Chl a+b contents were expressed as mg g^{-1} (FM).
Determination of photosynthetic parameters and water use efficiency

The net photosynthetic rate \( (P_N) \), stomatal conductance \( (g_s) \), transpiration rate \( (E) \) and intercellular CO\(_2\) concentration \( (C_i) \) of the leaves were measured using a portable photosynthesis system (LI-6400, LI-COR, Nebraska, USA). The measurements from the youngest fully expanded or flag leaves were taken on sunny days between 9:00 and 11:00 am to avoid potential stomatal closure during the middle of the day. The CO\(_2\) concentration in the leaf chamber was about 380 \( \mu \text{mol mol}^{-1} \) and the photosynthetic active radiation was about 1,100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). On the measurement date, five leaves from five individual plants in each of the five replicates of each treatment were analyzed. WUE is the leaf water use efficiency \( (\mu \text{mol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}) \) and calculated as follows (Xie and Su 2012): 
\[
\text{WUE} = \frac{P_N}{E};
\]
where \( P_N \) is the leaf photosynthetic rate, \( E \) is the leaf transpiration rate.

Determination of relative water content

Fully expanded third leaf from the top of the main stem of three plants from each treatment were selected to measure the relative water content (RWC). After measuring the fresh weights, leaves were placed in distilled water for 24 h at 4°C in darkness and the turgid weight was recorded. The samples were oven-dried at 60°C until constant weights were attained and leaf dry weight could be determined. RWC was calculated based on the formula suggested by Teulat et al. (2003) as follows: 
\[
\text{RWC} (\%) = \left( \frac{(F_W - D_W)}{(T_W - D_W)} \right) \times 100;
\]
where, \( F_W \) is the sample fresh weight, \( T_W \) is turgid weight and \( D_W \) is dry weight.

Determination of growth parameters

At the end of the experiment, the plant height and the basal diameter were recorded from five replicates of each treatment. The plant height was measured from the base of the stem, at the soil level, to the terminal bud of the main stem. The basal diameter was measured with a digital micrometer (0.001 mm) at the soil surface. To determine total biomass, plants were divided into leaf, stem, and root. The tissues were oven-dried at 80°C to constant mass, and total dry mass of leaves, stems, and root was measured.

Statistical analysis

All data were present as mean \( \pm \) SE. The difference was analyzed by ANOVA through SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

Effects of inoculation with B. pumilus on ultrastructure of leaf mesophyll cell of G. uralensis under drought stress

Under non-stressed conditions (CK), the chloroplasts were normal and ellipsoidal in their shape. They were closely associated with the cell wall and a small number of plastoglobuli were found in the chloroplasts. Mitochondria were intact, with complete membrane structure and clear cristae (Figure 1 (A,D)).

Under drought stress (DB−), the damaged chloroplasts were observed inside mesophyll cells. They were more or less spherical in their shape, located far from the cell wall, and showed a disorganized membrane system of grana. However, starch grains were present and plastoglobuli were found in the chloroplasts. The mitochondria cell cytoplasm was very granulated and possessed electron-dense bodies (Figure 1(B,E)).

Under the inoculation with endophytic B. pumilus strain treatment (DB+), the chloroplasts were normal and ellipsoidal in their shape. They remained closely associated with the cell wall, with few exceptions, and showed an organized membrane system. The chloroplasts were intact, and the thylakoid membrane structure was clear and complete. They also contained numerous plastoglobulus and large starch grain.

Figure 1. Effect of B. pumilus on the leaf ultrastructure of G. uralensis grown under drought stress. CW: cell wall; Ch: chloroplast; GL: thylakoid; Go: golgi apparatus; Mi: mitochondria, N: nucleus; P: plastoglobulus; SG: starch granule; V: vacuole.
Most of the mitochondrial membrane structure was complete, with a few mitochondria membrane intact, but the ridge disappeared (Figure 1(C,F)).

The results showed that the chloroplast length was greater in CK and DB+ than in DB-, but the width exhibited opposite properties. DB- showed significantly increased width/length ratio compared with DB+ (Table 1).

**Effects of inoculation with B. pumilus on chlorophyll content of G. uralensis under drought stress**

In DB- treatment, drought stress remarkably reduced the contents of Chl a, Chl b, and the Chl a+b content (Figure 2). Samples inoculated with endophytic B. pumilus showed significantly increased Chl b and the Chl a+b content compared with DB- treatment (Figure 2).

Significant positive relations were observed between Chl a and Chl a+b (r = 0.579, p < 0.05); between Chl b and PN (r = 0.646, p < 0.05); E (r = 0.521, p < 0.05), and WUE (r = 0.693, p < 0.05); and between Chl a+b and E (r = 0.637, p < 0.05), gs (r = 0.654, p < 0.05), RWC (r = 0.614, p < 0.05; Table 2). Very significant positive relations were observed between Chl b and Chl a+b (r = 0.645, p < 0.01) (Table 2).

**Effects of inoculation with B. pumilus on the photosynthetic parameters of G. uralensis under drought stress**

Drought stress significantly decreased gs, Ci, and E compared with the control. Inoculation with endophytic B. pumilus significantly increased PN, gs, Ci, and E of leaves compared with non-inoculated plants. In DB+ treatment, PN, gs, Ci, and E were significantly increased by 60.4%, 58.7%, 7.24%, and 49.7% compared with DB- treatment, respectively (Figure 3).

Significant positive relations were observed between Ci and stem dry weight (r = 0.582, p < 0.05) and leaf dry weight (r = 0.659, p < 0.05). Very significant positive relations were observed between E and stem dry weight (r = 0.794, p < 0.01) (Table 2).

**Effects of inoculation with B. pumilus on RWC and WUE of G. uralensis under drought stress**

Drought stress caused evident reduction in RWC, and no difference in WUE compared with the control was observed. In DB+ treatment, WUE was significantly increased by 50% compared with DB- treatment (Figure 4).

Significant positive relations were observed between RWC and root dry weight (r = 0.616, p < 0.05), stem dry weight (r = 0.671, p < 0.05), and leaf dry weight (r = 0.681, p < 0.05; Table 2).

Drought stress remarkably reduced the root length, stem length, lateral root number, leaf area, and reduced root, stem, leaf dry weight compared with control (Table 3 and Figure 5).

Endophytic bacteria inoculation markedly changed the growth and biomass of G. uralensis compared with non-inoculated plants. Root length, stem length, lateral root number, root dry weight, stem dry weight, and leaf dry weight were significantly increased by 26.09%, 21.65%, 28.41%, 35.23%, 31.87%, and 43.97% compared with DB- treatment (Table 3 and Figure 5).

Very significant positive relations were observed between leaf area and root dry weight (r = 0.853, p < 0.01), stem dry weight (r = 0.880, p < 0.01), leaf dry weight (r = 0.871, p < 0.01) (Table 2). Very significant positive relations were

![Figure 2](image-url) **Figure 2.** Effect of B. pumilus on chlorophyll a content (panel A), chlorophyll b content (panel B), chlorophyll a/b (panel C) and the total chlorophyll content (panel D) of G. uralensis grown under drought stress. The different letters within the different treatments indicate the significant difference at 0.05 level.

### Table 1. Effect of B. pumilus on the changes of chloroplasts length and width in G. uralensis leaves under drought stress.

| Treatments | Length (cm)          | Width (cm) | Width/Length |
|------------|----------------------|------------|--------------|
| CK         | 42.94±2.21*          | 8.86±0.78b | 0.20±0.01b   |
| DB-        | 40.08±2.44*          | 1.88±1.39a | 0.48±0.03a   |
| DB+        | 44.32±3.26*          | 9.99±0.62b | 0.23±0.01b   |

Notes: Data are means ± SD (n = 5). Values in each column followed by different superscripted letters indicate significant differences (P < 0.05).
observed between dry weights of root and stem \( (r = 0.944, p < 0.01) \) and that of the leaf \( (r = 0.980, p < 0.01); \) Table 2).

**Effects of inoculated with B. pumilus on principal component analysis of total biomass and physiological traits under drought stress**

In this study, two principal components (PCs) extracted had an Eigenvalue of >1. The first component (PC I) accounted for 59.475% of the variance, whereas PC II accounted for 40.525% of the variance. These two PCs contributed 100% of the total variability amongst the *G. uralensis* inoculated with *B. pumilus* and assessed for drought stress-related traits (Table 4). The loading plot (Figure 6) showed that PC I gave Chl a/b, Chl b, and WUE as dominant variable, appearing as negative PC I values. PN, lateral root number, Ci, Chl a+b, and Chl a appeared as positive values for PC I and also appeared as positive values for PC II. Dominant variables for PC II were leaf area, total biomass, RWC, E, and gs, which displayed negative PC II values.

![Figure 3. Effect of *B. pumilus* on stomatal conductance (panel A), intercellular CO2 concentration (panel B), transpiration rate (panel C) and photosynthetic rate (panel D) of *G. uralensis* grown under drought stress. The different letters within the different treatments indicate the significant difference at 0.05 level.](image)

![Figure 4. Effect of *B. pumilus* on relative water content (RWC) (panel A), water use efficiency (WUE) (panel B) of *G. uralensis* grown under drought stress. The different letters within the different treatments indicate the significant difference at 0.05 level.](image)

**Table 2.** Linear correlations measured between biomass and physiological traits of *G. uralensis* inoculated with *B. pumilus* under drought stress.

|          | Root | Stem | Leaf | Chl a | Chl b | Chl a/b | Chl a+b | PN  | E   | g_s | Ci | RWC | WUE | LRN |
|----------|------|------|------|-------|-------|---------|---------|-----|-----|-----|----|-----|-----|-----|
| Stem     | .944** | .980** | .989** | .1    | .156  | .437    | .387    | .52  | .303 | .579*| .645**| .217 |
| Leaf     | .1    | .171 | .398 | .09    | -0.408 | -0.114  | -0.285  | 0.307|     |     |     |     |     |
| Chl a    |     |     |     |       |       |         |         |     |     |     |     |     |     |
| Chl b    |     |     |     |       |       |         |         |     |     |     |     |     |     |
| Chl a/b  |     |     |     |       |       |         |         |     |     |     |     |     |     |
| Chl a+b  |     |     |     |       |       |         |         |     |     |     |     |     |     |
| PN       | .255 | .261 | .563 | .305  | .646* | .391    | .395    |     |     |     |     |     |     |
| E        | .427 | .794**| .499 | .354  | .521* |          |         |     |     |     |     |     |     |
| g_s      | .477 | .580*| .659*| .42   | .459  | .133    | .654**  | .700*|     |     |     |     |     |
| Ci       | .018 | .149 | .076 | .067  | .467  | .22     | -0.266  | .02  | -0.147| .116 | .107|     |     |
| RWC      | .616*| .671*| .681*| .598  | .404  | .242    | .614*   | .406 | .266 | .283 | .283 | .283 | .283 |
| WUE      | -0.321| -0.075| .014 | .030  | .693* | .510*   | .625    | .907**| .540*| .472 | -0.599| .309 |
| LRN      | .164 | .134 | .103 | .182  | -0.079| -0.278  | -0.253  | -0.466| -0.297| -0.308| -0.158| .215 | -0.451|
| LA       | .853**| .880**| .871**| .132  | .491  | -0.182  | 0.29    | .334 | .247*| .464 | .143 | .720**| -0.159|

Notes: **Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
However, *B. pumilus* inoculation could alleviate this phenomenon caused by drought stress. These results indicated that *B. pumilus* inoculation could alleviate the damage caused by drought stress to chloroplast and mitochondrial structure.

**Discussion**

**Effects of inoculation with *B. pumilus* on ultrastructure of leaf mesophyll cell of *G. uralensis* under drought stress**

In general, drought induces lipid peroxidation and massively damages chloroplast ultrastructure and Chl content (Anjum et al. 2011). As the light reactions of photosynthesis occur in the grana, membrane integrity and photosynthetic activity may be closely related (Bonini et al. 2007; Matos et al. 2010). Vassileva et al. (2012) found that drought stress degraded granal thylakoids and increased the number and size of the plastoglobuli. In our study, drought stress caused the breakdown of the chloroplast membrane system, as evidenced by the barely distinguishable grana and numerous small plastoglobuli. Furthermore, the cytoplasm of mitochondria became granulated and vacuolar. *B. pumilus* inoculation treatment improved the integrity of chloroplast and mitochondrion structure. These observations were similar to those observed by Freeman et al. (1985), who found that endophytic bacterial inoculation can improve thylakoid formation in the chloroplasts in drought-stressed sunflower plants. Moreover, chloroplasts showed reduced length and abnormal shape, which may be a sign of enhanced water loss in leaves. Drought stress tended to decrease chloroplast length and increase chloroplast width and width/length ratio in the DB− treatment. However, *B. pumilus* inoculation could alleviate this phenomenon caused by drought stress. These results indicated that *B. pumilus* inoculation could alleviate the damage caused by drought stress to chloroplast and mitochondrial structure.

**Effects of inoculation with *B. pumilus* on the chlorophyll content and photosynthetic parameters of *G. uralensis* under drought stress**

Photosynthesis is an important physico-chemical process that occurs in higher plants and is directly linked to plant biomass production; it is very susceptible to drought stress (Yang et al. 2014). At the whole-plant level, the influence of drought stress is generally perceived as a decline in photosynthesis and directly affects the photosynthetic characteristics of plants, which in turn affects physiological and biochemical processes. In the present study, drought stress (DB−) caused evident reductions in gs, Ci, and E compared with the control. The decline in photosynthetic parameters suggested that the photosynthetic efficiency may be strongly affected by water stress. Given that drought stress caused stomatal closure in the *G. uralensis* plants, it may result in the reduction of Ci and reduce CO2 absorbability, thereby limiting leaf photosynthesis, decreasing carbon assimilation capacity and photosynthetic matter accumulation, and ultimately limiting *G. uralensis* growth. These results were similar to those of Yin et al. (2006). In inoculated plants, *B. pumilus* inoculation (DB+) significantly improved gs, E, and Pn in *G. uralensis* leaves (Figure 3(A−D)), and this result was consistent with previous studies (Huang et al. 2011; Heidari and Golpayegani 2012). Our results suggested that *B. pumilus* inoculation can alleviate the negative effect of drought stress to the photosynthetic characteristic of *G. uralensis*.

The mechanism of drought stress suppression of photosynthesis is generally attributed to stomatal limitation and/or non-stomatal/metabolic limitation (Zhang et al. 2013). Under drought stress, the first response of plants is to close their stomata to reduce water loss while decreasing their time stomatal conductance (ggs) and transpiration rate (E). Consequently, photosynthesis is limited by stomata (De Mezer et al. 2014; Flexas and Medrano 2002). ggs is an important parameter that reflects the gas exchange capacity of leaves, and high ggs facilitates the entry of external CO2 into the leaves, which can maintain a high Pn. In this study, a very significant or significant positive correlation was observed between ggs and E, Pn, stem dry weight, and leaf dry weight (Table 2), thereby indicating that these parameters have synergistic effect on each other. The difference in
photosynthetic efficiency between DB+ and DB− was likely caused by stomatal limitation rather than by non-stomatal limitation, thereby contributing and ultimately leading to increased biomass.

Photosynthetic pigments are important to plants for harvesting light and producing reducing powers (Farooq et al. 2009). A plant’s response to drought stress is to change its photosynthetic pigment content. A decline in chlorophyll content under water deprivation regimes is the primary cause of photosynthesis inactivation (Shukla et al. 2012). Chlorophyll content is decreased under drought stress, which affects the photosynthesis of soybean (Zhang et al. 2007) and oriental lily (Zhang et al. 2011). In the present study, significant positive relations were observed between Chl b and PN, E, and WUE. Significant positive relations were observed between Chl a+b and E, gs, and RWC. These results indicated that chlorophyll content may adjust water relation, thereby further affecting photosynthesis and improving the resistance of G. uralensis under drought stress (Table 2). Endophyte inoculation has a positive effect on the maintenance of chlorophyll when subjected to drought stress; however, the underlying mechanism remains unknown (Guler et al. 2016; Mo et al. 2016). Our study found that the Chl a, Chl b, and the Chl a+b contents decrease under drought stress. Inoculated plants under drought stress showed significantly increased Chl b and Chl a+b content compared with non-inoculated plants (Figure 2), thereby supporting the results of Yan et al. (2013). They found that inoculated plants have higher Chl a, Chl b, and Chl a+b contents compared with non-inoculated plants. Hence, the current study showed that low chlorophyll pigment content, photosynthetic parameters, and structure of damaged chloroplasts and mitochondria are due to drought stress, which damages chloroplast structure, reduces chlorophyll synthesis efficiency, and ultimately leads to photosynthesis suppression. The normal ultrastructure of chloroplasts and increased PN and plant chlorophyll pigment (Figures 1-3) in the inoculated treatments indicated that B. pumilus inoculation could alleviate the damage to the photosynthetic apparatus of G. uralensis under drought stress.

**Effects of inoculation with B. pumilus on RWC and WUE of G. uralensis under drought stress**

RWC and WUE can reflect the physiological state of plants (Lei et al. 2007; Baslam and Goicocheca 2012) and are decreased significantly by drought stress (Boughaileb et al. 2016). In this study, drought stress limited the RWC compared with CK treatment. Plants inoculated with endophyte showed higher leaf RWC and WUE compared with non-inoculated plants under water stress (Agami et al. 2016). In inoculated plants, B. pumilus inoculation could ensure that the plant had higher WUE by up to 50% compared with non-inoculated plants. WUE is an important index used to evaluate the ability of a plant to maintain water equilibrium by adjusting water income and expenditure; increased WUE is an indicator of a plant’s adaptation to water deficiency to conserve water (Chen et al. 2005; Zhang et al. 2011). High WUE combined with high gs, Ci, and E showed that B. pumilus inoculation can be beneficial for water transportation through plants and can help plants keep their stomata open. Therefore, the response may be an important mechanism for G. uralensis plants to adapt to drought stress.

**Table 4. Eigenvectors and percentages of accumulated contribution of principal components in G. uralensis inoculated with B. pumilus under drought stress.**

| Variable            | PC I          | PC II         |
|---------------------|---------------|---------------|
| Total biomass       | 0.996         | −0.092        |
| Chl a               | 1.000         | 0.026         |
| Chl b               | −0.057        | 0.998         |
| Chl ab              | −0.191        | 0.982         |
| PN                  | 0.881         | 0.472         |
| E                   | 0.368         | 0.930         |
| g_s                 | 0.798         | −0.602        |
| g_i                 | 0.835         | −0.550        |
| Ci                  | 0.870         | 0.494         |
| RWC                 | 0.989         | −0.151        |
| WUE                 | −0.231        | 0.973         |
| Lateral root number | 0.818         | 0.576         |
| Leaf area           | 0.999         | −0.035        |
| Eigen value         | 7.732         | 5.268         |
| Contribution rate (%) | 59.475       | 40.525        |
| Cumulative percentage (%) | 59.475 | 100          |
the total variance into different factors. The PCA partitioned the total variance into two PCs that contributed the maximum percentage (100%, Table 4). The higher eigenvector of PC I comprises Chl a, total biomass, and leaf area, some of which had very significant positive correlation. The higher and positive eigenvector of PC II includes PN, Chl b, and WUE (Figure 6). Inoculation with B. pumilus under drought stress affected chlorophyll content, and photosynthetic parameters and water status further affected biomass. We hypothesized that the complete chloroplast and mitochondrial structure provides energy or place for the synthesis of chlorophyll and that high chlorophyll content provides the raw materials for photosynthesis. Furthermore, efficient photosynthesis may have provided energy for plant growth and maintains high WUE, ultimately allowing the inoculated G. uralensis plants to resist drought stress.

Conclusion
Drought stress affects the growth, dry matter, and harvestable yield in many plant species. However, the tolerance of any species to this menace varies remarkably. Inoculation with B. pumilus can enhance the drought tolerance of G. uralensis, as reflected by the improved plant growth and physiological status. This treatment is associated with the following physiological aspects. First, it leads to the improved maintenance of leaf ultrastructure to sustain physiological metabolism, as evidenced by the intact chloroplasts and clear and complete thylakoid membrane structure in the inoculated treatment. Second, it improves the plant’s ability to ensure high photosynthetic capacity and chlorophyll content. Finally, it improves plant growth and WUE. All these cumulative effects of B. pumilus inoculation ultimately increased the drought tolerance of the G. uralensis. Therefore, this biological strategy of endophyte inoculation should be encouraged for wide application in cultivated G. uralensis, especially in arid and semi-arid regions.

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Notes on contributors
Wenjin Zhang is a teacher in Ningxia Medical University.
Zhical Xie is a master in Ningxia Medical University.
Xiaojia Zhang is a master in Ningxia Medical University.
Duoyong Lang is a teacher in Ningxia Medical University.
Xinhui Zhang is a teacher in Ningxia Medical University.

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