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Effect of the inoculation of plant growth-promoting rhizobacteria on the photosynthetic characteristics of *Sambucus williamsii* Hance container seedlings under drought stress

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that survive within the range of plant rhizosphere and can promote plant growth. The effects of PGPR in promoting plant growth, activating soil nutrients, reducing fertilizer application, and improving the resistance of plant inducible system have been widely investigated. However, few studies have investigated PGPR as elicitors of tolerance to abiotic stresses, especially drought stress. In this study, the effects of *Acinetobacter calcoaceticus* X128 on the photosynthetic rate (P_n), stomatal conductance (G_s), intracellular CO_2 concentration (C_i), and total chlorophyll content [Chl(a+b)] of *Sambucus williamsii* Hance seedling leaves under moderate drought stress and drought-rewatering conditions were determined. Compared with those of uninoculated seedlings, the average P_n values during the entire drought stress of inoculated seedlings increased by 12.99%. As the drought duration was lengthened, C_i of uninoculated leaves continued to increase after rapidly declining, whereas G_s continuously decreased. Furthermore, their photosynthetic properties were simultaneously restricted by stomatal and non-stomatal factors. After X128 inoculation, C_i and G_s of *S. williamsii* Hance leaves continued to decrease, and their photosynthetic properties were mainly restricted by stomatal factors. At the end of the drought stress, water stress reduced [Chl(a+b)] of *S. williamsii* Hance leaves by 13.49%. However, X128 inoculation decreased this deficit to only 7.39%. After water supply was recovered, P_n , G_s , and [Chl(a+b)] in uninoculated leaves were reduced by 14.23%, 12.02%, and 5.86%, respectively, relative to those under well-watered conditions. However, C_i increased by 6.48%. Compared with those of uninoculated seedlings, P_n , G_s , and [Chl(a+b)] in X128-inoculated seedlings were increased by 9.83%, 9.30%, and 6.85%, respectively. Therefore, the inoculation of X128 under arid environments can mitigate the reduction of chlorophyll, delay the restriction caused by non-stomatal factors to P_n in plant leaves under water stress, and can be more conducive to the recovery of photosynthetic functions of leaves after water supply is recovered.

Keywords: Plant growth-promoting rhizobacteria, *Acinetobacter calcoaceticus*, Photosynthetic, Drought stress, *Sambucus williamsii* Hance

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Introduction

Water stress is considered one important factor limiting worldwide agricultural productivity and efficiency (Vivien et al. 2017). Changes in global air temperature and climate are leading to longer drought periods and more extremely dry years (Cook et al. 2014; Miroslav et al. 2015). Climate change is predicted to decrease water availability and increase drought risk, which is one of the major agricultural problems reducing crop yield in an arid or semiarid area (Prudent et al. 2015; Ali et al. 2017). Under this context of climate change, plants will be more vulnerable to severe drought conditions (Kaushal and Wani 2016, Gaion et al. 2018). Because the leaf is the organ most responsive to environmental conditions (Nevo et al. 2000; Guerfel et al. 2009), its structure reflects the effects of water stress more clearly than the stem and roots. Drought triggers many leaf responses, especially stomatal closure that negatively impacts photosynthesis and crop yield under water-deficit conditions (Evans and Loreto 2000). The reduction of photosynthesis under water deficit can potentially be ascribed to a decrease in both stomatal and mesophyll conductance (Gaion et al. 2018). Furthermore, gas exchange in plants under drought stress is restricted by stomatal or non-stomatal factors with lowered photosynthetic rate, and plant growth and dry matter accumulation are severely impacted (Reed and Loik 2016). However, restricted by stomatal conductance under water stress, intracellular CO₂ concentration cannot satisfy the demand for photosynthesis. This condition is called the stomatal restriction of photosynthesis. A non-stomatal restriction of photosynthesis occurs when chloroplast activity and ribulose-1,5-bisphosphate carboxylase activity are reduced and photosynthetic function is weakened (Rakocevic et al. 2018; Staniak et al. 2017). Under mild and moderate water stress conditions, stomatal restrictive factors can greatly affect photosynthesis. However, under severe water stress condition, the effect of non-stomatal restrictive factors on photosynthesis plays a dominant role (Belasio et al. 2018).

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that survive within the range of plant rhizosphere (Rubin et al. 2017; Shi et al. 2019) and can promote plant growth (Arkhipova et al. 2005), activate soil nutrients (Liu et al. 2017; Freitas et al. 2019), reduce fertilizer application (Vessey 2003), and improve the resistance of plant inducible systems (Yang et al. 2009). PGPR play an important role in enhancing plant growth through various mechanisms. The action mode of PGPR that promotes plant growth includes (I) nutrient fixation for easy uptake by plant abiotic stress tolerance in plants; (II) plant growth regulators; (III) siderophore production; (IV) volatile organic compound production; and (V) the

production of protection enzymes, such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases (Choudhary et al. 2011; Garcia-Fraile et al. 2015; Vejan et al. 2016). PGPR can produce phytohormones, especially cytokinin (CTK), which play an important role in PGPR-promoted plant growth (Ahmed and Hasnain 2010; Khan et al. 2014; Zhang et al. 2011). Liu et al. (2013) believed that PGPR can improve CTK synthesis, transportation, and redistribution to facilitate plant growth. CTK is also closely related to the stress resistance of plants (García de Salamone et al. 2001; Arkhipova et al. 2007). Wang and Huang (2004) found that synthesized 6-benzyl aminopurine can expedite the growth and physiological recovery of Kentucky bluegrass under arid environment. Research on grapes shows that zeatin and zeatin riboside are reduced by over 50% after drying treatment of their local root zones (Stoll et al. 2000). Following local drought stress treatment of tomato roots, zeatin riboside content in xylem is reduced (Kudoyarova et al. 2007). The concentrations of CTKs, such as zeatin and zeatin riboside, in binding form in the xylem juice of sunflower are reduced under drought stress (Hansen and Dörffling 2003). In addition, increasing CTK concentration can promote the opening of plant stomates (Vysotskaya et al. 2003). In view of relationships among PGPR, CTK, and plant drought resistance, the effect of CTK-producing PGPR on the photosynthetic properties of plants under drought stress must be explored.

Sambucus williamsii Hance is a deciduous tree or shrub that belongs to the *Caprifoliaceae* family. It is an important eco-functional plant integrating ornamental function and ecological restoration with extremely strong environmental adaptability, anti-drought and anti-barren properties, resistance against diseases and pests, stability, and high yield (Yang et al. 2016). Therefore, keeping in view the beneficial effects of PGPR, changes in the photosynthetic parameters of *S. williamsii* Hance leaves were determined under moderate drought stress to evaluate the effects of PGPR on plant photosynthetic characteristics in drought stress, with the aim to provide new insights into methods to improve the plant's drought resistance ability.

Materials and methods

Preparation of X128 and its bacterial solution

The bacterial strain X128 used in the present investigation was isolated and screened from walnut rhizosphere in drought stress using the serial dilutions method and bioassay for cytokinin production method described by Hussain and Hasnain (2011). Characteristics of physiology and biochemistry of the bacterial isolate are shown in Table 1. On the basis of 16S rRNA sequencing data, the bacterial isolate showed 99.2% similarity with

Table 1 Characteristics of physiology and biochemistry of *Acinetobacter calcoaceticus* X128

| | IAA produced ($\mu\text{g mL}^{-1}$) | Kinetin produced (ng mL^{-1}) | Siderophore production | P solubility | N-fixation | Trans-zeatin produced (ng mL^{-1}) | |
|--------------------------------|---|---|------------------------------|----------------------|-----------------|---|-----------------|
| Main ability | 26.71 (3.71 ^a) | 310.77(32.67) | – | – | – | 368.73 (31.26) | |
| | Glutamate transferase test | Citrate utilization test | Gas production of glucose | Gluten hydrolysis | Growth at 37 °C | Growth at 41 °C | Growth at 44 °C |
| Biochemical characteristics | + ^b | + | + | – | + | + | – |

–: negative

^a Values are standard deviations ($n = 3$)^b +: positive

Acinetobacter calcoaceticus (AJ888983). X128 was identified as *A. calcoaceticus* and saved in the China General Microbiological Culture Collection Center as: CGMCC No.7071. The 16S RNA gene sequence was submitted to GenBank under Accession Number KC428748. *A. calcoaceticus* X128 was inoculated into LB culture medium and cultivated at 28 °C for 3 days with shaking at 180 r min⁻¹. As determined by High Performance Liquid Chromatography, this bacterial strain can produce 368.73 ng mL⁻¹ zeatin and 310.77 ng mL⁻¹ kinetin (Table 1). The OD₆₀₀ value of this bacterial suspension was determined via spectrophotometry. The bacterial suspension was centrifuged at 10,000 rpm for 10 min. The supernatant was obtained and added with isovolumetric Salkowski (50 mL of 35% HClO₄ + 1 mL of 0.5 M FeCl₃) color matching solution. The mixture was allowed to stand for 30 min in the dark and then its OD₅₃₀ value was determined. The IAA content in the fermentation liquor within unit volume was determined under OD₆₀₀=1. IAA content was calculated through the standard curve of IAA gradient dilution. X128 produced 26.71 $\mu\text{g (mL OD}_{600})^{-1}$ of IAA, thus proving that this bacterial strain had a certain potential of promoting plant growth.

Plot culture experiment

The experiment was performed in the greenhouse of Shandong Academy of Forestry. Pot culture soil comprised moistened soil with 34.64% field moisture capacity, 28.36 mg kg⁻¹ alkali-hydrolyzable nitrogen, 37.45 mg kg⁻¹ available phosphorous, 65.44 mg kg⁻¹ available potassium, and pH of 7.71. The pot was 25 cm in height and 30 cm in width. Each pot was filled with 12 kg of soil. A total of 108 pots were prepared, each of which was planted with one *S. williamsii* Hance container seedling with an average ground diameter of 3.82 ± 0.04 mm and average plant height of 23.55 ± 0.20 cm. After *S. williamsii* Hance container seedlings were planted, they

were uniformly tended, and soil moisture content was maintained at 70–75% of field moisture capacity.

A. calcoaceticus X128 was inoculated into beef extract-peptone medium and shook below 37 °C at a rate of 180 r min⁻¹ for 3 days. The culture was then transferred to fluid medium at 10% inoculum size and then cultivated below 30–35 °C for 12 h with shaking at 180 r min⁻¹. After viable bacterial count was determined, the culture was diluted to 2 × 10⁸ cfu mL⁻¹ using distilled water. On May 20, 2018, 72 pots were randomly selected for X128 inoculation. Approximately 25 mL of X128 bacterial solution was diluted to 250 mL, and then uniformly irrigated within the X128-treated *S. williamsii* Hance seedling rhizosphere. On June 24, 36 pots of X128-inoculated *S. williamsii* Hance seedlings were selected, and watering of these *S. williamsii* Hance seedlings together with 36 pots of non-X128-inoculated seedlings was stopped. On June 29 (40 days after X128 inoculation), the soil was naturally dried to 43.8% of field moisture capacity and then subjected to drought stress test. Soil moisture content in the well-watered treatment and that under drought stress was maintained at 70–75% and 40–45% of field moisture capacity, respectively. Soil moisture content was measured using a portable moisture meter (model HH2, Delta-t Devices LTD, Burwell, UK) with a wet sensor (Type Wet-2-K1, Delta-t Devices LTD, Burwell, UK) at 9:00 am each day. After soil moisture contents under drought stress treatment and X128 inoculation treatment reached the standard, water was occasionally supplemented, and soil moisture content was maintained relatively stable. On August 5, water supply was recovered for the two drought stress treatments until soil moisture content reached the control level.

Photosynthetic determination

Photosynthetic parameters were determined for the first time on May 20, 2018 at 10:00–10:30. A LI-6400 portable photosynthesis apparatus (LI-COR, USA) was used

to determine the photosynthetic rate, stomatal conductance, and intracellular CO₂ concentration of leaves. Three seedlings were randomly selected, and three healthy functional leaves of each seedling were selected with the same leaf position. Photosynthetic determination of each leaf was conducted for three times. After June 29, 2018, related photosynthetic indicators were determined every 6 days until the drought stress test ended on August 4, 2018. Leaves under different treatments were obtained at 1, 40, 50, 60, and 76 days after inoculation to measure chlorophyll content [Chl(a+b)] for three times. [Chl(a+b)] in the leaves was determined using the 96% ethanol immersion method (Ying et al. 2015). A total of 0.1 g of leaves from six seedlings was cut into small pieces (0.2 cm filaments) and extracted with 8 mL of 95% (v/v) alcohol in the dark for 24 h at 25 °C until the leaves were blanched. The absorbance of the supernatant was measured at 645 and 663 nm with a Shimadzu UV-2550 spectrophotometer (Kyoto, Japan). Chlorophyll concentrations were calculated by the standard method of Arnon (1949) and expressed as mg g⁻¹ fresh weight (FW). On August 11 (6 days after the recovery of water supply), the photosynthetic rate, stomatal conductance, intracellular CO₂ concentration, and [Chl(a+b)] of *S. williamsii* Hance leaves were determined.

Data processing

Data were analyzed using a completely randomized design. Analysis of variance (ANOVA) was carried out to evaluate the effects of different treatments on photosynthetic parameters and [Chl(a+b)] in *S. williamsii* Hance container seedlings. When significant differences were found among the treatments, least significant difference test was conducted to detect differences among individual treatment means. All statistical analyses were performed at a significance level of $p < 0.05$. ANOVA and multiple comparisons were performed using SPSS software (version 19.0; SPSS Inc., Chicago, Illinois, USA).

Results

Photosynthetic rate

During the entire drought stress process, regardless of *A. calcoaceticus* X128 inoculation, P_n of *S. williamsii* Hance leaves declined, as shown in Fig. 1. At 6 days after the drought stress, P_n of uninoculated seedlings significantly declined by 15.95% compared with that under well-watered treatment ($p < 0.05$). At 12 days, relative to well-watered leaves, P_n of leaves under drought stress was reduced by 10.31%. The reduction amplitude was decreased in comparison with that in the initial phase. This result may be due to *S. williamsii* Hance forming a certain adaptation mechanism to drought stress with time. At 36 days after the drought stress, P_n of *S.*

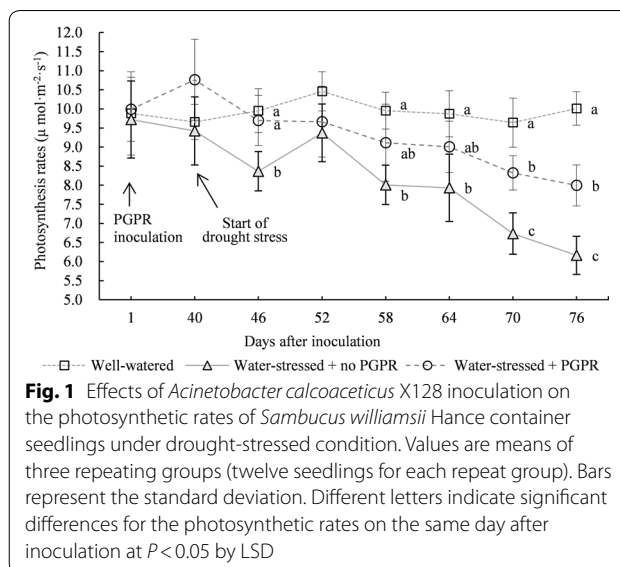


Fig. 1 Effects of *Acinetobacter calcoaceticus* X128 inoculation on the photosynthetic rates of *Sambucus williamsii* Hance container seedlings under drought-stressed condition. Values are means of three repeating groups (twelve seedlings for each repeat group). Bars represent the standard deviation. Different letters indicate significant differences for the photosynthetic rates on the same day after inoculation at $P < 0.05$ by LSD

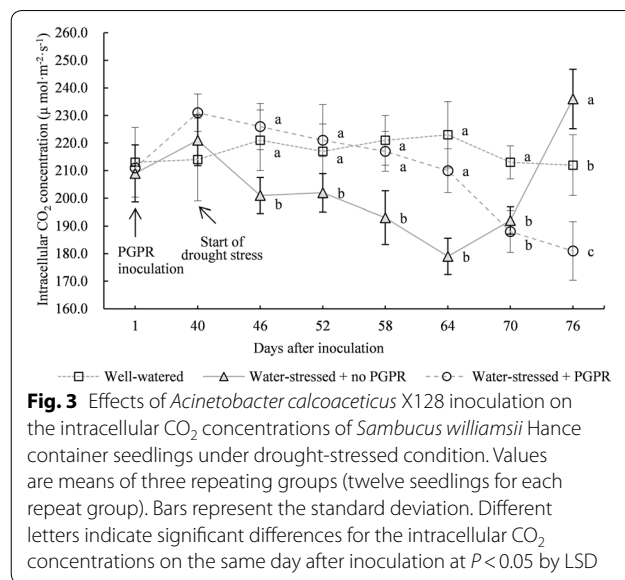
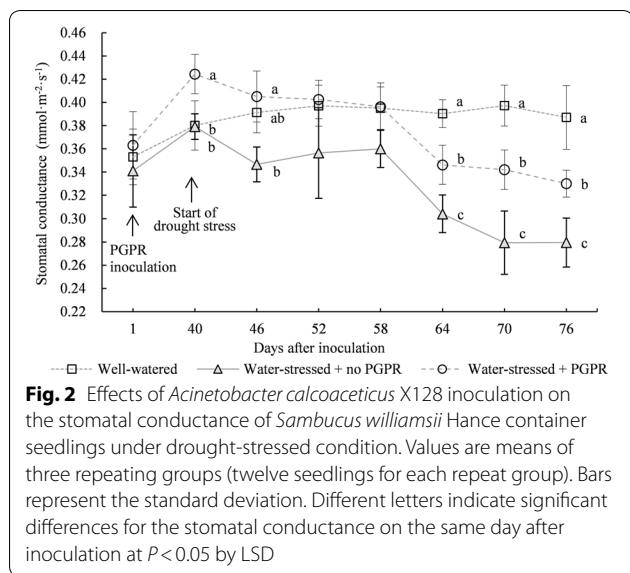
williamsii Hance leaves was reduced by 38.43% compared with that under well-watered treatment.

The average P_n values during the entire drought stress showed that X128 treatment increased P_n value by 12.99% relative to uninoculated treatment ($p < 0.05$). In the initial drought stress phase (at 6 days), X128 treatment increased P_n value by 15.90% in comparison with that under uninoculated treatment ($p < 0.05$). Thus, uninoculated *S. williamsii* Hance seedling could be easily impacted by drought stress. Subsequently, as the stress continued, P_n after X128 inoculation was elevated by 3.11%, 13.78%, 13.50%, 23.64%, and 29.73% compared with those not inoculated with X128. The difference between the two treatments gradually increased, indicating that as the drought stress duration was lengthened, the effect of X128 on inhibiting P_n reduction was gradually enhanced.

Stomatal conductance

In the initial drought stress phase (at 6 days), G_s value of uninoculated *S. williamsii* Hance leaves was reduced by 11.4% due to drought stress (Fig. 2). At 12 days after continuous drought stress, G_s value of uninoculated leaves slightly increased, possibly because *S. williamsii* Hance leaves gradually adapted to the arid environment. Afterward, G_s value continued to drop. Until the final stress phase, G_s value was remarkably reduced by 27.79% compared with that under well-watered treatment ($p < 0.05$).

At 40 days after X128 inoculation, G_s value was obviously increased by 11.6% compared with that of uninoculated leaves, indicating that X128 could promote the stomatal opening of *S. williamsii* Hance leaves. Under drought stress, G_s value under X128 inoculation treatment presented a continuously declining tendency. At 6 days after the drought stress, the difference between



X128 inoculation and well-watered treatment was insignificant in terms of G_s value ($p > 0.05$), but it was significantly increased by 16.8% compared with that of uninoculated drought stress treatment ($p < 0.05$). This finding indicates that X128 inoculation could relieve the inhibitory effect of drought stress on G_s value since the initial stress phase. During the entire stress period, G_s value under X128 inoculation was significantly elevated by 14.7% compared with that under uninoculated treatment ($p < 0.05$), indicating that X128 inoculation could inhibit the drought stress-induced reduction of G_s .

Intracellular CO₂ concentration

The effects of different treatments on C_i value at different drought stress times are shown in Fig. 3. In the initial drought stress phase (at 6 days after the drought stress treatment), in comparison with that under well-watered treatment, C_i value of uninoculated *S. williamsii* Hance leaves significantly declined by 9.05% ($p < 0.05$), indicating that the short-time drought stress could lead to reduction of the C_i value of *S. williamsii* Hance leaves. With continued stress, C_i value continued to decline, but it started increasing at 30 days. Up to the final stress phase, C_i was even significantly higher than that under well-watered treatment. At 36 days after the drought stress treatment, C_i value of walnut leaves under uninoculated drought stress treatment was elevated by 9.81% compared with that under well-watered treatment ($p < 0.05$).

During the entire stress process, the change of C_i value under X128 inoculation was not consistent with that under uninoculated treatment. In the initial drought stress phase, C_i value of leaves under X128 inoculation did not show any significant change

relative to that under well-watered treatment ($p > 0.05$). However, it presented a continuously declining tendency with drought stress time. As the drought stress was continued for 36 days, C_i value under X128 treatment was significantly decreased by 15.42 ($p < 0.05$) compared with that under well-watered treatment. The level was equivalent to that at 24 days under DR drought stress treatment.

Chlorophyll content

As drought stress was continued, the effects of different treatments on [Chl(a+b)] were different (Table 2). Before the drought stress and during the initial drought stress phase (at 10 days after the drought stress), the differences among the three treatments in terms of [Chl(a+b)] of *S. williamsii* Hance leaves were insignificant ($p > 0.05$). This finding indicates that X128 inoculation had a minor influence on [Chl(a+b)] of *S. williamsii* Hance leaves under well-watered condition and that *S. williamsii* Hance could strongly resist drought stress. With continued stress, [Chl(a+b)] under uninoculated drought stress treatment started declining. At 76 days after the inoculation (36 days after the drought stress), [Chl(a+b)] under uninoculated drought stress treatment greatly declined by 13.49% ($p < 0.05$) compared with that under well-watered treatment. Thus, drought stress could result in the decomposition or loss of chlorophyll in *S. williamsii* Hance leaves. However, X128 inoculation decreased this deficit to only 7.39%. X128 inoculation could remarkably inhibit the degradation of total chlorophyll in the final drought stress phase so that it could be basically maintained at the control level.

Table 2 [Chl(a+b)] (mg g⁻¹) change with different experimental treatments under drought stress

| Treatments | Days after inoculation | | | | |
|-------------------------|--|--------------|--------------|---------------|---------------|
| | 1 | 40 | 50 | 60 | 76 |
| Well-watered | 5.31 ^a (0.23 ^b)a ^c | 5.27 (0.31)a | 5.35 (0.17)a | 5.26 (0.13)a | 5.41 (0.16)a |
| Water-stressed +no PGPR | 5.19 (0.16)a | 5.33 (0.24)a | 5.24 (0.17)a | 4.82 (0.10)b | 4.68 (0.18)b |
| Water-stressed +PGPR | 5.22 (0.21)a | 5.29 (0.28)a | 5.24 (0.22)a | 5.15 (0.17)ab | 5.14 (0.22)ab |

^a Values are means of three repeating groups (twelve seedlings for each repeat group)

^b Numbers are standard deviations

^c Different letters indicate significant differences among treatments at $P < 0.05$ by LSD

Photosynthetic parameters after the recovery of water supply

The photosynthetic parameters of *S. williamsii* Hance seedlings bearing drought stress after their water supply was recovered for 6 days are shown in Table 3. Compared with those under well-watered conditions, the seedlings under uninoculated drought stress showed reduced P_n , G_s , and [Chl(a+b)] by 14.23%, 12.02%, and 5.86%, respectively. However, C_i was increased by 6.48%. In the final drought stress phase (at 36 days after the drought stress), P_n , G_s , and [Chl(a+b)] were reduced by 38.43%, 27.29%, and 8.37%, respectively, and C_i was increased by 11.32% relative to those under well-watered treatment. After the water supply was recovered for *S. williamsii* Hance seedlings, photosynthetic functions were partially recovered. Compared with seedlings under uninoculated drought stress treatment, seedlings under X128 inoculation showed increased P_n , G_s , and [Chl(a+b)] by 9.83%, 9.30%, and 6.85%, respectively, basically recovering to the control levels, except that C_i was slightly reduced. Therefore, under the drought stress intensity in this study, the drought stress borne by X128-inoculated *S. williamsii* Hance seedlings was irreversible.

Discussion

Drought stress, X128, and photosynthetic characteristics of leaves

Photosynthesis is the physiological foundation for plant growth and reflects plant growth vigor and drought resistance (Yang et al. 2014). Drought stress causes

stomatal closing and a reduction of G_s and P_n (Liang et al. 2018). During the initial drought stress phase in this study, P_n value of leaves declined to a certain degree. However, as *S. williamsii* Hance seedlings adapted to the arid environment, P_n somehow increased. With continued drought stress, P_n value decreased again in the final stress phase. In their research on soybeans, Prudent et al. (2015) found that as drought stress duration is lengthened, photosynthetic rate continues to decline, which is greatly different from the conclusion drawn in the present study. This difference may be caused by the varied response modes of different plants to drought stress.

X128 inoculation under arid environment could notably repress the reduction amplitude of P_n and G_s values of *S. williamsii* Hance seedling and leaves. Except for the initial drought stress phase (at 6 days), this inhibitory effect of X128 was gradually enhanced with continued drought stress. Thus, the higher the drought stress intensity borne by the plant, the stronger the promoting effect of X128 on P_n value. Under well-watered conditions, PGPR can elevate the P_n value of plant leaves and promote dry matter accumulation (Freitas et al. 2019). However, there are few similar research reports under arid environment because different environmental conditions, such as weather, soil property, and moisture, affect microbial growth and reproduction and only PGPR are competitive and can be planted within the rhizosphere so as to exert their growth-promoting effect. The adaptation of PGPR to soil environment is a decisive factor of their functional

Table 3 Photosynthetic parameters of *S. williamsii* Hance leaves on the 6th day after the recovery of water supply

| Treatments | P_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | G_s ($\text{mmol m}^{-2} \text{s}^{-1}$) | C_i ($\mu\text{mol mol}^{-1}$) | [Chl(a + b)] (mg g ⁻¹) |
|--------------------------|--|--|------------------------------------|------------------------------------|
| Well-watered | 9.84 ^a (0.36 ^b)a ^c | 0.391 (0.017)a | 212.31 (10.17)b | 5.44 (0.31)a |
| Water-stressed + no PGPR | 8.44 (0.27)a | 0.344 (0.020)b | 226.06 (8.59)a | 4.09 (0.22)b |
| Water-stressed + PGPR | 9.27 (0.44)ab | 0.376 (0.014)a | 199.34 (11.03)c | 4.37 (0.14)a |

^a Data are the means of three repeating groups (twelve seedlings for each repeat group)

^b Numbers are standard deviations

^c Different letters indicate significant differences among treatments at $P < 0.05$ by LSD

effect (Vejan et al. 2016). X128 selected in this study was screened out from arid environment. Thus, X128 may be adaptative to arid ecological environment and influence the photosynthetic properties of *S. williamsii* Hance leaves under arid environment.

Drought stress, X128, stomatal restriction, and non-stomatal restriction

The factors of the plant under drought stress, which caused reduced P_n value in leaves, could be mainly divided into stomatal restriction and non-stomatal restriction (Erel et al. 2015). When the drought stress was mild, P_n reduction was mainly due to the closure of some stomates (Tombesi et al. 2015). Under severe drought stress, the activities of some enzymes participating in carbon fixation in the chloroplast were inhibited due to moisture loss of chloroplast and cells. Furthermore, P_n value was reduced mainly due to non-stomatal factors (Signarbieux and Feller 2011). C_i and G_s are criteria for stomatal or non-stomatal factors causing P_n reduction. C_i value was reduced because of stomatal restriction, whereas it was elevated due to non-stomatal restriction. When both stomatal and non-stomatal restrictions existed, the change law of C_i value depended on the dominant factor (Reed and Loik 2016; Bellasio et al. 2018). The present study showed that in the initial drought stress phase, P_n reduction of *S. williamsii* Hance leaves was accompanied by a reduction of G_s and C_i , indicating that P_n reduction was mainly restricted by stomatal factors. However, as the drought stress duration was lengthened, G_s continued to decline, whereas C_i gradually increased after declining initially. This finding indicates that P_n reduction started being restricted by non-stomatal factors besides stomatal factors, photosynthetic activity of mesophyll cells was degraded, and photosynthetic organ structure was injured. Furthermore, after the water supply was recovered, P_n failed to recover to the level under well-watered condition. However, after X128 inoculation, C_i value continuously decreased during the entire drought stress period. Therefore, under the drought stress intensity in this study, P_n reduction of *S. williamsii* Hance leaves was closely associated with stomatal factors and was always restricted by stomatal factors but not non-stomatal factors, even during the final stress phase. This finding indicates that under the stress intensity in this study, X128 inoculation could reduce the structural injury of photosynthetic organs of leaves, mitigate the restrictions caused by non-stomatal factors to P_n value of leaves under water stress, and contribute to the rapid recovery of photosynthetic functions after the recovery of water supply treatment.

Drought stress, X128, and [Chl(a+b)]

Leaf photosynthesis is generally correlated with chlorophyll content (Kanwal et al. 2017). Results of several studies indicate that water stress adversely affects the chlorophyll content, and the higher the drought intensity is, the greater the chlorophyll content decreases. (Nageswara Rao et al. 2001; Fotovat et al. 2007). The results of this study indicate that the leaf chlorophyll content significantly decreased under drought stress compared with that under well-watered conditions; this result confirmed the findings of previous studies. In the initial and middle drought stress phases in this study, [Chl(a+b)] in *S. williamsii* Hance leaves did not significantly change regardless of X128 inoculation. This finding indicates the strong adaptation ability of *S. williamsii* Hance to drought stress to a certain degree. Nevertheless, as the drought stress duration was lengthened, [Chl(a+b)] in uninoculated leaves was reduced to a certain degree, which might be related to the destructive effect of water stress-induced membrane lipid peroxidation of chloroplast. After the water supply was recovered, [Chl(a+b)] of uninoculated *S. williamsii* Hance leaves failed to recover to the well-watered level, indicating that *S. williamsii* Hance experienced irreversible drought stress-induced injury. Ying et al. (2015) believed that as the drought stress intensity increases, [Chl(a+b)] in *Camptotheca acuminata* seedlings continues to decrease, similar to the study result regarding elderberries. If the stability of [Chl(a+b)] can be guaranteed under moderate drought stress, it will be conducive to inhibiting P_n reduction and enhancing plant stress resistance (Nageswara Rao et al. 2001). After X128 inoculation, [Chl(a+b)] of *S. williamsii* Hance leaves did not significantly change under moderate drought stress, proving that X128 inoculation improved the adaptation ability of *S. williamsii* Hance to arid environment to a certain degree.

The X128 selected in this study could generate CTKs of certain concentrations. CTKs can promote the opening of stomates and elevate photosynthetic rate (Arkhipova et al. 2007; Vysotskaya et al. 2003). CTKs can inhibit the loss of total chlorophyll to a certain extent (Abbasi et al. 2011). In the present study, [Chl(a+b)] of *S. williamsii* Hance leaves was somehow reduced during the final drought stress phase but was suppressed by X128 inoculation. Therefore, reducing the loss of total chlorophyll and regulating the opening and closure of stomates by elevating CTK content in plant leaves may be a mechanism of X128 in relieving the inhibitory effect of drought stress on P_n value of plant leaves. PGPR inoculation under arid environment can effectively inhibit the reduction of P_n and improve the plant's adaptation ability to arid environment. These findings will be of outstanding

significance to forestation and forest tending on arid mountains.

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Authors' contributions

FL, HM designed the study. FL, XL, ZD BM, and LP performed research. FL analyzed data and wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The authors declare that all the data and materials used in this study are available.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All authors agree with the contents of the manuscript and its submission to the journal.

Competing interests

The authors declare that they have no competing interests.

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