



Full Length Article

Inoculation of *Funneliformis mosseae* to Enhance Desiccation Tolerance of *Populus euphratica* Seedlings in Hyper-arid Region

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Abstract

The influence of *Funneliformis mosseae* (Synonym: *Glomus mosseae*) on the physiology and growth of *Populus euphratica* seedlings was investigated under drought stress. To mimic natural conditions, plants were subjected to a gradual decrease of soil water content for a period of 21 d. Our results demonstrated that the fungus *F. mosseae* produced positive effects on plants. Colonization frequency and mycorrhizal infection intensity of the roots of *P. euphratica* seedlings in the inoculated group were higher than those in the non-inoculated group at 21 d of withholding irrigation. However, the leaf water potential and leaf gas exchange attributes of *P. euphratica* seedlings did not significantly affect either inoculated with *F. mosseae* or not under the optimum level of soil water content. In response to progressive decreases in soil water, remarkable changes were observed in predawn leaf water potential to the non-inoculated group compared to the inoculated group. Stomatal closure timing was 4 h delayed in the inoculated seedlings as compared to the non-inoculated seedlings when exposed to severe drought stress. However, a significant difference in the daily leaf stomatal conductance changes was found between the inoculated and the non-inoculated group at 15 d of withholding irrigation. The continuous increase in the soil water deficit resulted in a lower photochemical quenching coefficient in seedlings. Moreover, the initial seedling survival rate was 50% and 25% in the inoculated group and non-inoculated group, respectively. © 2017 Friends Science Publishers

Keywords: Arbuscular mycorrhizal fungi; *Populus euphratica*; Soil water deficit; Tolerance

Introduction

In arid regions, water constitutes the most important factor limiting plant survival, growth, and development. Water deficit seriously affects the morphology, structure, photosynthetic rate, growth, and metabolism of plants (Seki *et al.*, 2007). Approaches are required, which may alleviate drought stress on seedlings or trees in drought-affected areas. In arid and semi-arid regions, many plants have already formed a variety of acute responses and adaptive mechanisms to water deficit (Bacelar *et al.*, 2006). For example, the symbiosis of plant roots with arbuscular mycorrhizal fungi (AMF) is a widespread plant strategy to enhance nutrient acquisition, which copes with environmental stress (Brachmann and Parniske, 2006). AMF can act as a support system for seedling establishment (Van der Heijden *et al.*, 2004). It is generally believed that arbuscular mycorrhizal symbiosis can protect their host plants from adverse damage caused by drought stress (Heana *et al.*, 2008). However, AMF may have a positive, negative, or no influence on their host plants (García-Sánchez *et al.*, 2014; Ziane *et al.*, 2017), and

different AMF provide various benefits in different growing seasons (Van der Heijden *et al.*, 2006). The degree of response of mycorrhizal plants to drought stress depends on the specific fungal species, the interaction between the plant and the fungi, and the degree of drought stress (Heana *et al.*, 2008). Moreover, AMF have been used and studied in agronomic plants for its important influence on responses in plants, such as grape, soybean, rice, wheat, tomato, and lettuce subjected to abiotic stress (Ruiz-Sánchez *et al.*, 2010; Abdel and Chao, 2011; Baslam and Goicoechea, 2012), but has rarely been studied in trees in arid regions.

Populus euphratica Oliv., one of the first confirmed 388 rare and endangered plants in China, is a member of the Salicaceae family of trees found in the semiarid and hyper-arid regions of Midwestern Asia, North Africa, and southern Europe (30°–50°N) (Rajput *et al.*, 2016). In these regions, it is subjected to extreme environmental conditions, such as high solar radiation, extreme temperatures, high salinity, and high water stress (Chen *et al.*, 2006). China has the largest range and number of *P. euphratica* in the world, and the Tarim River, one of the longest arid inland rivers in the world, running between the Taklimakan Desert and the

Kuluke Desert, has the largest range and number of *P. euphratica* in China (Zhou *et al.*, 2010). *P. euphratica* plays an important role in maintaining the ecosystem function and protecting oases from sandstorms because it is well adapted to withstand local environmental conditions. However, up to more than half of the natural *P. euphratica* trees have disappeared due to water scarcity and the impact of man-made destructions in China since the 1950s. In order to protect and expand the use of *P. euphratica* in arid regions, it is necessary to improve its tolerance to environmental stresses, such as drought and salinity. Our recent research indicated that AMF played a critical role in improving the *P. euphratica* rhizospheric environment and therefore, was beneficial to arid riparian community construction (Yang *et al.*, 2008). However, there were low inoculum levels of AMF in the soil, and arbuscular mycorrhizal symbiont was established by *F. mosseae* associated with *P. euphratica* root in the lower reaches of the Tarim River (Yang *et al.*, 2013). If the native inoculum of AMF in the soil is inadequate to support vegetation recovery, it may be necessary to reinforce or replace it by utilizing the same AMF species with a non-native origin inoculation based on native AMF (Daniel *et al.*, 2009). It is not yet clear, however, whether the colonization of *F. mosseae* in the roots is beneficial or unfavorable to *P. euphratica*, especially under soil water deficit conditions. To the best of our knowledge, the present study is the first to investigate the interactive effects of *F. mosseae* and water deficit on *P. euphratica* in depth. Therefore, the objectives of this research were to test whether *F. mosseae* could enhance *P. euphratica* growth and improve water relations and photosynthesis, affecting the ability of the plant to adapt to soil water deficit, and examine the effects of inoculation of *P. euphratica* seedlings with *F. mosseae* in response to progressive soil water deficit in arid areas.

Materials and Methods

Plant Material and Treatments

The experiment was conducted at the Ecology and Restoration Monitoring Test Station, located in the lower reaches of the Tarim River, from April to August 2010. Because the seeds of *P. euphratica* lose activity easily and their one year old seedlings are only approximately 5–10 cm in height with relatively few branches and leaves, they cannot meet the requirements for monitoring and analysis in this study. Thus, two year old *P. euphratica* seedlings, popularly used for local production in the lower reaches of the Tarim River, were selected for the experiments. The pot was a PVC tube, 30 cm in diameter and 50 cm high, seated on a plastic plate under a PVC tube bottom. The potting soil was obtained from local abandoned farmland, which was a *P. euphratica* forest. The basic nutrient content of the soil is shown in Table 1, and its bulk density was 1.024 g cm⁻³, and the field capacity of the soil was 29.47%. For the

convenience of irrigation, the soil was uniformly filled up about 7 cm from the top of the PVC tube. Soil was firstly mixed evenly with a disinfecting agent i.e., carbendazim, and then covered with plastic sheets for disinfection of the soil for 2 d prior to conducting the experiment. To minimize the toxic influence of carbendazim on *F. mosseae* activity, the disinfected soil was exposed to strong sunlight for 12 d (the half-life of carbendazim in the soil is generally 7–12 d) for the subsequent experiments.

Two year old *P. euphratica* seedlings that were uniform in canopy breadth, root spread range, root length, basal-stem diameter, and plant height were potted on April 7, 2010. The average height was approximately 51.9 cm. A single seedling was planted in each pot, and 64 pots were planted in total. At the time of transplantation, half of the seedlings were inoculated with 20 mL of *F. mosseae* inoculum (inoculated group, A), a mixture of spores, mycorrhizal root fragments, and rhizospheric soil (spore density was 50 spores mL⁻¹). The remaining seedlings were not inoculated (non-inoculated group, CK). The inoculum was evenly spread on the root of the *P. euphratica* seedlings at 8 cm below the topsoil. Seedlings were grown in well-watered conditions (watered by clean water until soil water drainage occurred from the bottom of the PVC tube one time per week) prior to imposing drought treatments. Considering that drought at a too early stage could inhibit the development of *F. mosseae* spores, and to mimic natural conditions in which drought stress develops gradually, 16 survived seedlings in the inoculated group and eight survived seedlings in the non-inoculated group were submitted to drought treatment during the three weeks (from July 18 to August 8, 2010) after transplantation. The experimental set-up considered two water conditions i.e., well-watered (W) and water deficient (D), as the main factors. Specifically, in the inoculated group, the well-watered treatment (AW) and water deficit treatment (AD), respectively, had eight pots which were randomly placed; in the non-inoculated group, the well-watered treatment (CKW) and water deficit treatment (CKD), respectively, had four pots. A single-plant per pot was considered to constitute a replicate. For the well-watered treatment, plants were watered after every 2 d to completely saturate the soil in the pot (until the water drained from the bottom of the PVC tube) in order to obtain non-limiting soil water conditions. The water deficit treatment was imposed by withholding irrigation for 21 d. At the beginning of the drought treatment, all pots were saturated with water and allowed to drain freely from the bottoms of the containers. During the period of the experiment, an automatic weather station (ICT, International, Sydney, Australia) was set up at the study site on a mast approximately at crown height. Temperature and photosynthetically-active radiation were recorded every 10 s.

The experiment was performed outdoors at a temperature between 25°C and 40°C from 08:00 to 20:00. The highest air temperature occurred between 16:00–18:00.

Table 1: Soil properties in the experiment (Mean \pm SD, n=3)

	Organic matter (g·kg ⁻¹)	Total nitrogen (g·kg ⁻¹)	Total phosphorus (g·kg ⁻¹)	Total potassium (g·kg ⁻¹)	Available nitrogen (mg·kg ⁻¹)	Available phosphorus (mg·kg ⁻¹)	Available potassium (mg·kg ⁻¹)
Soil sample	16.33 \pm 1.69	1.05 \pm 0.12	0.99 \pm 0.11	17.70 \pm 0.41	71.68 \pm 29.72	86.38 \pm 18.96	222.62 \pm 43.75

The photosynthetically-active radiation displayed a single peak profile, and the peak was approximately 1557 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 16:00. There was no rainfall during the experimental period, and no fertilizer was used.

Soil Water, Growth and Physiological Parameters

Soil samples were collected by soil auger at 0–10, 10–20, 20–30 and 30–40 cm layers over the course of the experiment to evaluate soil water content at different soil depths. The soil water content (gravimetric) was measured with an oven-drying method. The soil water content at the 0–40 cm soil layer was calculated according to the soil water content at each layer.

Four seedlings were randomly selected from the eight seedlings in the AW and AD, respectively, and all of the seedlings in the CKW and CKD for the measurement of physiological parameters (predawn leaf water potential; leaf stomatal conductance; chlorophyll fluorescence) at 3, 6, 12, and 15 d of water withholding. Some parameters were not completely measured punctually due to bad weather. Growth of all seedlings was monitored by measuring stem height (from the substrate surface to the top of the seedling), basal stem diameter (at the base), and counting the number of lateral branches. The basal stem diameter and stem height per plant were measured with a flexible ruler and vernier caliper several times. Moreover, a mark was made on the basal-stem when the basal-stem diameter was measured for the first time; this position was used as the gauging point for each subsequent measurement. The increment in the stem height, basal stem diameter, and the number of lateral branches was calculated through the last measured value minus the first measured value, respectively. Each of these parameters was measured on July 18, before the pots were saturated with water and used as a reference. The initial survival rate of seedlings in the transplant (before conducting the drought treatment) was calculated as: survival rate = $N_0/N \times 100\%$, where N_0 is the survival plant number during the three weeks (from July 18 to August 8) after transplant; and N is the total plant number in the transplant.

Chlorophyll fluorescence has been utilized as a nondestructive and noninvasive means of quantifying damage to the leaf photosynthetic system of deciduous and evergreen trees. Chlorophyll fluorescence parameters were measured with a portable modulated chlorophyll fluorometer (Mini-Pam, WalzUlm, Germany). The measurements were conducted during the period of 08:00 – 20:00 at an interval of 2 h. Ten (10) leaves were measured at a time. Red light (intensity < 0.1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was taken as

the measure-light, and a saturation light pulse of 0.8 s in duration (intensity > 10,000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was supplied by an inner halogen lamp. Chlorophyll fluorescence of 10 randomly selected healthy and mature leaves per seedling (five from the top of the crown, five in the center), with three individuals, were measured per treatment on clear days from 08:00–20:00 at 2 h intervals to obtain the steady state fluorescence of specific time (F_s) and maximum fluorescence yield of the light-adapted state (F'_m). The initial fluorescence yield (F_o) and maximum fluorescence yield in the dark (F_m) were measured before dawn and at midday after shading with a black cloth for 20 min. The minimal fluorescence of light-adapted leaves (F_o'), actual photochemical efficiency of photosystem II (PSII) in light-adapted leaves (Φ PSII), and photochemical quenching coefficient (q_p) were calculated using the following formula, respectively (Zhu *et al.*, 2013):

$$F_o' = F_o / (F_v / F_m + F_o / F'_m) \quad (1)$$

$$F_v = F_m - F_o \quad (2)$$

$$\Phi \text{ PSII} = (F'_m - F_s) / F_m \quad (3)$$

$$q_p = (F'_m - F_s) / (F'_m - F_o') \quad (4)$$

On the same day, leaf stomatal conductance, one of the gas-exchange parameters measured on the same leaves used for chlorophyll fluorescence measurement and leaf stomatal conductance, was automatically recorded on 3–5 leaves of each individual plant tested from 08:00–20:00 at 2 h intervals. Three individuals measured per treatment were made each time using a porometer system (Porometer AP4, Delta-T Devices, Ltd., Cambridge, UK) every 3–6 d. Leaves in the plant were directly measured without harm, rather than being picked prior to measurement. Moreover, leaf stomatal conductance was measured three times for each leaf, and their mean value was used.

Leaf water potential (ψ) was measured with a dew point microvolt-meter (HR-33T, Wescor Inc., Logan, UT, U.S.A.), every 3–6 d. Two to three healthy and grown leaves from the well-lit portion of the canopy of each individual plant were picked, immediately sliced at the center, and placed in the C-52 sample chamber of the dew point microvolt-meter to measure ψ_s value, and their mean value was used for each individual plant tested. The ψ (MPa) was calculated using the following formula:

$$\psi = \psi_s / -7.5 \quad (5)$$

Since the local period of predawn was 06:00, predawn leaf water potential was the ψ at 06:00 in this study.

Symbiotic Development

At the end of the experiment (21 d of water withholding) after measuring the growth, leaf stomatal conductance, predawn leaf water potential and chlorophyll fluorescence parameters, all seedling roots of different treatments were collected, respectively. After being rinsed with distilled water, the fresh fine roots were excised into 1.0–2.0 cm pieces, placed into a small glass bottle containing FAA solution (formaldehyde 5 mL, glacial acetic acid 5 mL and 70% ethanol 90 mL), and then stored at 4°C. The fixed root fragments were chosen for determination of the AMF colonization rate on the basis of the method described by Biermann and Linderman (1981). Thirty (30) fine roots of 1.0 cm in length were examined under an Olympus BX15 microscope. The number of root segments forming AM was counted with a crisscross lacing method and used to calculate the AMF colonization rate. The intensity of root colonization was determined by optical microscopic observations of 30 root fragments. The mycorrhizal colonization rate and root mycorrhizal colonization intensity were calculated using the following equation (Yang et al., 2008):

$$\text{Mycorrhizal colonization rate (\%)} = \frac{\text{infected root segments}}{\text{total root segments}} \times 100 \quad (6)$$

$$\text{Root mycorrhizal colonization intensity (\%)} = \frac{(95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1)}{\text{total root segments}} \quad (7)$$

Where, n_5 means the number of roots with infection level of 5 (colonization rate 90–100%); n_4 is the root number at level 4 infection (colonization rate 50–90%); n_3 is the root number at level 3 (colonization rate at 10–50%); n_2 is the root number at infection level 2 (colonization rate 1–10%); and n_1 is the root number at level 1 (colonization rate 0–1%).

Statistical Analyses

Statistical analyses were performed using SPSS 13.0 (SPSS, Inc., U.S.A.). Data were subjected to two-way analysis of variance (ANOVA) with inoculation treatment and water condition as sources of variation to determine significant differences among different treatments. Differences were discriminated by using the LSD test. Significant results were assumed for $p \leq 0.05$. The graphs were produced with SigmaPlot 9.0 (SPSS, Inc., USA).

Results

Soil Water Content and Symbiotic Development

The soil water content in the pot was gradually reduced along the time of withholding irrigation. At 6 d of withholding irrigation, the relative soil water content i.e., the ratio of soil water content to soil water holding capacity, at 0–40 cm soil layer in the inoculated group and the

non-inoculated group were 71.26 and 69.79%, respectively. At 15 d of withholding irrigation, the relative soil water content at 0–40 cm soil layer in the inoculated group and the non-inoculated group were 33.30% and 31.59%, respectively and there was no significant difference between the two groups. A criterion was used in which 60–70% and lower than 40% of relative soil water content were regarded as slight drought and severe drought, respectively (Hsiao, 1973). The relative soil water content was in slight drought or no drought in both groups at 6 d of withholding irrigation and in severe drought in both groups at 15 d of withholding irrigation.

The roots of *P. euphratica* seedlings inoculated with *F. mosseae* were infected. All mycorrhizal colonization rate and root mycorrhizal colonization intensity in the AD and the AW were higher than that in the CKD and the CKW at 21 d of withholding irrigation. The sequence of the mycorrhizal colonization rate was $AW > AD > CKW > CKD$, and the sequence of root mycorrhizal colonization intensity was $AW > AD > CKD > CKW$. ANOVA analysis indicated that the difference in the mycorrhizal colonization rate and the root mycorrhizal colonization intensity was significant ($p < 0.05$) among the four treatments. In particular, there was no significant difference in the mycorrhizal colonization rate between the AW and the AD. However, it was similar to the root mycorrhizal colonization intensity among the AD, AW and CKW, indicating that short-term soil water deficit had no influence on arbuscular mycorrhiza infection (Fig. 1).

Growth Parameters

The seedling initial survival rate was 50% and 25% in the inoculated group and the non-inoculated group, respectively. The growth rate of seedlings in the inoculated group was higher than that in the non-inoculated group in three months after transplant. All seedlings in the four treatments were still alive, with few leaves turning yellow, at the end of the drought treatment. However, there were differences in the growth rate of the seedlings among the treatments during the period of soil water deficit (Fig. 2). Specifically, the number of lateral branches that emerged in the AW was more than that in the CKW at 15 d of water withholding, and it was more in the AD than CKD (Fig. 2a). The increase of stem height and basal stem diameter of the seedlings in the AW were higher than those in the CKW after 15 d, but they increased slightly in the AD and exhibited no increase in the CKD at 15 d of water withholding (Fig. 2b, c). The increment of the number of lateral branches, stem height, and basal stem diameter of the seedlings (the last measured value minus the measurements for the first time) in the CKD and the CKW were smaller than AD and the AW, respectively (Fig. 2d). As expected, *F. mosseae* colonization stimulated plant growth and played an especially important role in plant growth while the plant suffered drought stress.

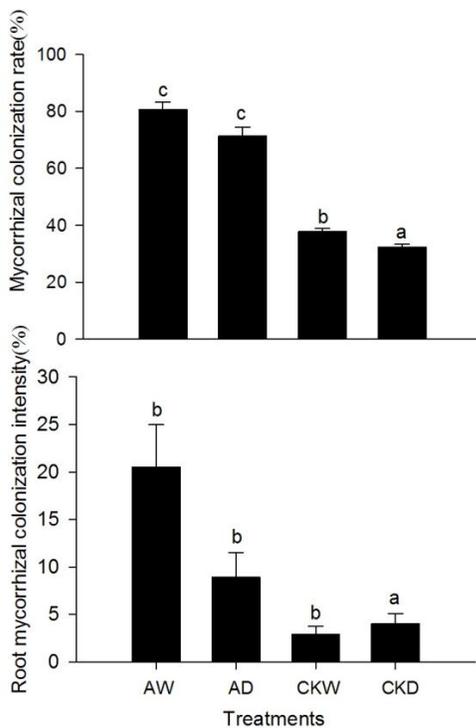


Fig. 1: Symbiotic development in the well-watered treatment in the inoculated group (AW), water deficit treatment in the inoculated group (AD), well-watered treatment in the non-inoculated group (CKW) and water deficit treatment in the non-inoculated group (CKD) at the end of the experiment. Histograms with different letters indicate that the values were significantly different ($p \leq .05$). Values are means. Errors bars are SD

Predawn Leaf Water Potential and Leaf Stomatal Conductance

The continuous increase in the soil water deficit resulted in decreasing predawn leaf water potential values for the seedlings of CKD and great fluctuation of predawn leaf water potential values (Fig. 3) for the seedlings of AD. The CKW seedlings' predawn leaf water potential had a slight fluctuation compared to the AW seedlings' predawn leaf water potential. Furthermore, the difference in predawn leaf water potential changed as the duration of water withholding increased. There was a difference of -0.26 MPa between the CKW and the CKD, and -1.45 MPa between the AW and the AD at 3 d of water withholding. At 15 d, the difference became -1.85 MPa between the CKW and the CKD, and -0.17 MPa between the AW and the AD. Progressive decreases in soil water content resulted in quite remarkable changes to predawn leaf water potential in the inoculated group as compared to the non-inoculated group.

In order to eliminate possible errors caused by differences in environmental factors e.g., wind and air temperature, leaf stomatal conductance of *P. euphratica*

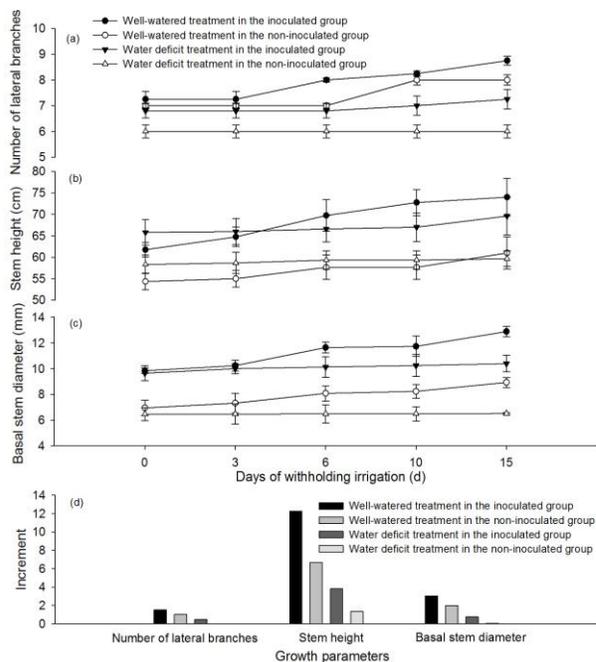


Fig. 2: Variations of growth parameters during the experimental period. Values are means. Errors bars are SD.

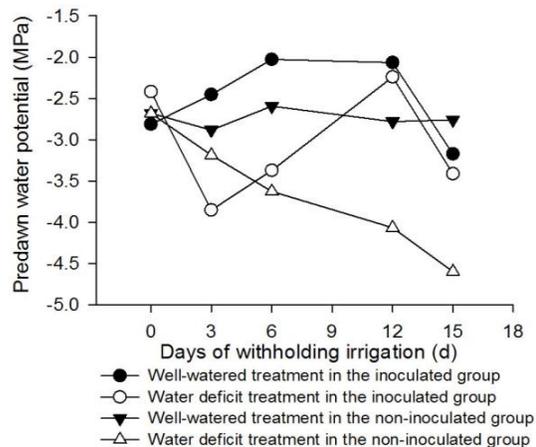


Fig. 3: Variations of predawn water potential of *P. euphratica* in different treatments during the experimental period

seedlings in all treatments were compared at the same times i.e., 3 d and 15 d of withholding irrigation. At 15 d of withholding irrigation, the daily changing trends of leaf stomatal conductance in all treatments were different, and the leaf stomatal conductance in the AW and the CKW were higher than those in the AD and the CKD (Fig. 4a).

Furthermore, under different levels of soil water content, there were different responses in the stoma to soil water deficit between the inoculated group and the non-inoculated group. The daily changing trends of leaf

stomatal conductance in the AD and the CKD were different at 3 d of withholding irrigation. Analysis of ANOVA indicated that, except for the leaf stomatal conductance at 08:00 and 12:00, there was no significant difference in leaf stomatal conductance at other times between the AD and the CKD, which indicated that when the soil water content is at a good level either inoculation with *F. mosseae* or not does not significantly affect the leaf gas exchange of the seedlings. However, at 15 d of withholding irrigation, there was a difference in the daily changes of leaf stomatal conductance between the AD and the CKD. At 10:00, 12:00 and 14:00, the leaf stomatal conductance was obviously higher in the AD than that in the CKD which indicated that, during the period of 10:00–14:00, leaf gas exchanges in the AD are better than those in the CKD. The lowest leaf stomatal conductance in the AD occurred at 18:00 and 12:00 in the CKD. This indicated that, under the same environmental factors under severe drought stress, the timing of leaf stomatal closure in the non-inoculated group was delayed by 2 h as compared to the inoculated group (Fig. 4b).

Photochemical Quenching Index of PSII

Chlorophyll fluorescence parameters comprise a series of related multiple parameters. In this study, the photochemical quenching coefficient was selected as the representative for analysis. In order to eliminate possible errors caused by photosynthetically-active radiation and air temperature, the photochemical quenching coefficient in all treatments was compared at the same times i.e., 6 d and 15 d of withholding irrigation. The results indicated that whether inoculated or not inoculated with *F. mosseae*, the daily changing curves of photochemical quenching coefficient in the four treatments were similar at 15 d of withholding irrigation, and the ranking of photochemical quenching coefficient was AW > CKW > AD > CKD. There was no significant difference in photochemical quenching coefficient between the AW and the CKW, but it was significantly different between the AW and the CKD, the AD and the CKD, the AD and the AW, and the CKD and the CKW ($p \leq 0.05$). The daily changing trends (the difference between the maximal value and the minimal value) in the CKD and the CKW were higher than those in the AW and the AD (Fig. 5a). Moreover, the continuous increase in the soil water deficit resulted in lower photochemical quenching coefficient values. The photochemical quenching coefficient at 6 d of withholding irrigation was higher than those at 15 d in the AD and the CKD. Except for at 14:00, the difference in photochemical quenching coefficient at other times was not significantly different between the AD and the CKD. In contrast, there were significant differences in photochemical quenching coefficient at other times between the AD and the CKD at 15 d of withholding irrigation, except for those at 08:00 and 18:00, respectively.

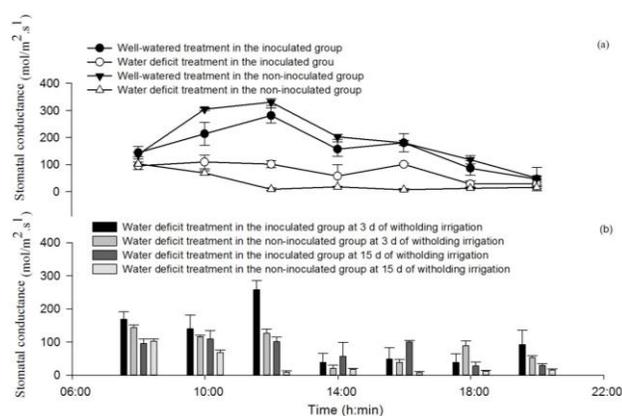


Fig. 4: Diurnal changes of *P. euphratica* leaf stomatal conductance. Values are means. Errors bars are SD.

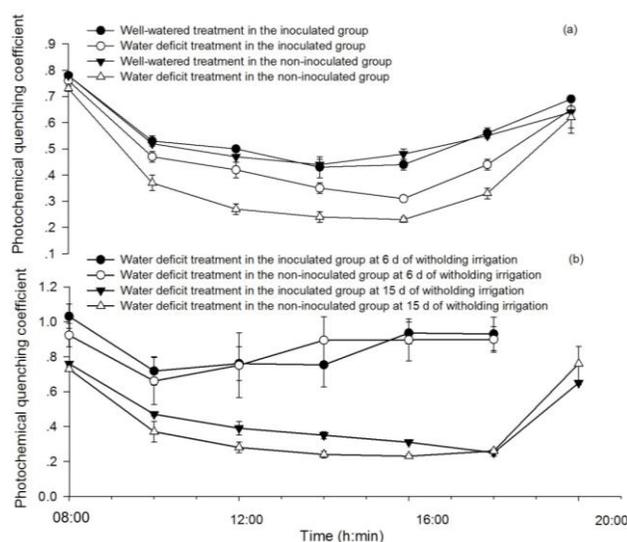


Fig. 5: Diurnal changes of leaf photochemical quenching coefficient. Values are means. Errors bars are SD

The results indicated that *F. mosseae* was favorable in improving photosynthesis for *P. euphratica* seedlings under soil water deficit instead of well-watered condition (Fig. 5b).

Discussion

Plants can make adjustments in their morphological and physiological properties to cope with unfavorable environmental conditions. Water potential constitutes one of the most important indicators of water status in plants. Our results show that the predawn leaf water potential in the CKD declined along with the reduction of soil water content, and it was equal to -5.11 MPa after 15 d of water deficit, which suggests that soil water deficit led to serious water deficit in *P. euphratica* seedlings. The predawn leaf water potential in the AD and the AW fluctuated along with the

reduction of soil water content as compared to CKW. Early research led to the belief that when the soil dehydrates, the leaf water potential decreases (Schulze, 1993). However, this is not so in the case of *Juglans regia* and *Diospyros* trees under severe water stress, which are still able to maintain higher leaf water potential. On the other hand, *Gleditsia sinensis* and *Diospyros lotus* are relatively low in leaf water potential under the same conditions (Shi *et al.*, 2009). The predawn leaf water potential of two year old olive trees (*Olea europaea* L., cv. Coratina) decreased rapidly after 7 d of water deficit by withholding water. Moreover, it was equal to -6.0 MPa after 15 d of water deficit (Bartolomeo *et al.*, 2009), but decreased to values below -1.5 Mpa after 15 d of withholding irrigation in two poplar clone plants (Grazia *et al.*, 2012). Our results reveal that the predawn leaf water potential of *P. euphratica* seedlings was affected by *F. mosseae* colonization, which is favorable to maintaining balanced water relations for *P. euphratica* seedlings when soil water deficit increased. However, these findings need to be confirmed by further study for low numbers of sample in this experiment.

Leaf stomata can effectively control water loss via both transpiration and CO₂ absorption. In this study, the leaf stomatal conductance in the inoculated group was higher than in the non-inoculated group at 15 d of withholding irrigation. Moreover, the leaf stomatal conductance in the inoculated group also exhibited an increasing trend during the period of 08:00–12:00. The photosynthetic rate and leaf stomatal conductance in mycorrhizal plants were higher than those in non-infected plants under the condition of soil water deficit (Sánchez-Blanco *et al.*, 2004). On the other hand, the leaf stomatal conductance in the non-inoculated group displayed a rapidly decreasing trend and reached the minimal value at 12:00. These results indicated that inoculated seedlings were beneficial for maintaining a good structure and function of their leaf stomata when the soil water content was in severe drought. In contrast, the non-inoculated seedlings were damaged due to the soil water deficit, leading to the inability of their leaves to open the stomata normally. The larger leaf stomatal conductance and the prolonged opening of leaf stomata are beneficial for plant gas exchanges, which can enhance the rate of CO₂ absorption and ultimately benefit photosynthesis. However, the larger leaf stomatal conductance can also increase water loss via transpiration. It is known that the stomatal diffusion rate of water is 1.56 times the rate of CO₂ absorption, and water loss depends more on stomatal conductance than photosynthesis (CO₂ absorption) (Zhao *et al.*, 2000). In this study, the leaf stomatal conductance in the inoculated group was higher under the same environment. This may be related to the higher water loss rate in the inoculated group as compared to that in the non-inoculated group, which leads to more rapid soil water consumption. However, there was no obvious significant difference in soil water content between the two groups. This may be due to the shorter duration of withholding irrigation and also that the

formation of arbuscular mycorrhizas in the root system reduces the temperature on the leaf surface, and thus reduces water loss (Wu and Xia, 2006). This also needs to be confirmed by further study.

In this study, the difference in photochemical quenching coefficient between the inoculated group and the non-inoculated group was not significant when the soil water content was in light drought stress; however, when the soil water content was in severe drought stress, the photochemical quenching coefficient of the inoculated seedlings was increased. This scenario indicated that the limiting effects of soil water deficit on the leaf photosynthetic activity in the inoculated seedlings is small, which is beneficial for seedling growth under drought. The amount of the absorbed light energy can surpass the amount of energy used for the photochemical reactions in PSII under stress. This extra amount of light energy is not dissipated, which can cause damage to the photosynthetic structure, and the damaged sites are primarily located within PSII (White and Critchley, 1999). The photochemical quenching coefficient reflects the proportion of light energy that is used in photochemical electron transfer, relative to the total light energy absorbed by the antenna (light harvesting) pigments within PSII. The reaction center within PSII must be in an opening state to maintain photochemical quenching coefficient at a higher level, which shows the higher electron transfer activity within PSII (Puertolas *et al.*, 2005). Changes in growth parameters e.g., plant height, are reflections of plant physiological activities (Miransari, 2010). Plants that have significant differences in their functions tend to also be quite different in their growth and development under the effects of AMF. Corn seedlings inoculated with AMF obviously stimulated growth in the seedling stage and significantly increased the colonization frequency of arbuscular mycorrhizal under drought stress (Li *et al.*, 2013), and inoculation with AMF stimulated *Macadamia ternifolia* seedling growth (Auge', 2001). In this study, during the period of drought treatment, the leaf stomatal conductance and photochemical quenching coefficient in the inoculated group were found to be higher than those in the non-inoculated group, indicating that under the conditions described in this experiment, *F. mosseae* had a beneficial effect on *P. euphratica* seedlings' water status, gas exchange, and increasing photosynthetic activity. These led to the stimulation of plant growth, as reflected mainly in the existence of more new branches and greater height in the inoculated seedlings compared to the non-inoculated seedlings. In this study, the seedling initial survival rate was 50 and 25% in the inoculated group and the non-inoculated group, respectively, although the seedlings were grown in well-watered conditions. This may be associated with quality of *P. euphratica* seedling or the other factors which need further study to confirm.

In several short experiments, drought did not have a significant effect on mycorrhizal colonization involving potted plants (Yooyongwech *et al.*, 2013)

However, water deficit can influence mycorrhizal colonization in pepper colonized by *G. fasciculatum* and subjected to water deficit (Davies *et al.*, 2002). In this study, inoculation of *F. mosseae* can significantly improve the arbuscular mycorrhiza symbiotic state in the roots of *P. euphratica* seedlings, and the short-term soil water deficit did not affect the mycorrhizal colonization rate and the root mycorrhizal colonization intensity in the non-inoculated seedlings. However, the mycorrhizal colonization rate and the root mycorrhizal colonization intensity in the non-inoculated seedlings were not zero, which could be due to the roots of the seedlings having already been infected by the AMF before they were transplanted, or due to the presence of native AMF in the soil that were not completely inactivated by the disinfection treatment. It is also possible that the root systems were infected by the AMF that were transmitted from the atmosphere or water to the seedlings in the opening pot during the four month period from the start of inoculation with *F. mosseae* to the harvest of the *P. euphratica* seedlings. Regardless of whether they were infected by AMF before or after transplantation, the mycorrhizal colonization rate and the root mycorrhizal colonization intensity in the non-inoculated seedlings were obviously lower than those in the inoculated seedlings, demonstrating that *F. mosseae* can improve the arbuscular mycorrhiza symbiotic state in the root of the seedlings. Furthermore, even in the presence of arbuscular mycorrhiza in the non-inoculated group, the inoculated seedlings growth was still better than that of the non-inoculated seedlings and showed that the colonization of *F. mosseae* is beneficial for the seedlings' physiological activities. In this study, the mycorrhizal colonization rate and the root mycorrhizal colonization intensity in the seedlings root were lower than those in the *P. euphratica* growing in the middle- and lower-reaches of the Tarim River (Yang *et al.*, 2013). This disparity may be related to the individual difference of *P. euphratica* seedlings or may be due to the incomplete disinfection with carbendazim. Although the toxicity of carbendazim was minimized through exposing disinfected soil to strong sunlight for 12 d, there may still be certain amounts of toxicity residue remaining in the soil, which can inhibit the activity of *F. mosseae* and thus reduce the mycorrhizal colonization rate and the root mycorrhizal colonization intensity in the *P. euphratica* seedlings' root. It is also conceivable that the drought stress inhibited the colonization of arbuscular mycorrhiza, because water stress can seriously inhibit colonization frequency in the root system of *Artemisia ordosica* (He *et al.*, 2008).

Results of this study shed light on the mechanisms of stress-resistance of plants in arid regions, but it is difficult to elucidate the precise mechanisms responsible for the effects of AMF because numerous factors e.g., plant nutrition and root system size, are simultaneously affected by AMF. Many experiments indicate that AMF facilitate phosphorus uptake, enhance nitrogen acquisition, and cause hormonal and transcriptional profiles to change (Biermann and

Linderman, 2000; López-Ráez *et al.*, 2010; Pedranzani *et al.*, 2016) because extra radical AM hyphae spread in the soil around the root and provide a surface area by which the AMF absorbs nutritional elements. We believe that there are multiple processes in the mechanisms of AMF-enhanced plant drought tolerance only under certain conditions, and a process may exist that is more important than the other processes.

Conclusion

Colonization of *F. mosseae* stimulated plant growth and ultimately alleviated the deleterious effect of drought stress on *P. euphratica* seedlings through mediating the many physiological mechanisms under the condition of severe water scarcity. Seedling survival rate was 50% and 25% in the inoculated group and non-inoculated group, respectively. Quite remarkable changes were found in the predawn leaf water potential in the non-inoculated group compared to the inoculated group in response to progressive decreases in soil water. There was also a difference in the daily changes in the leaf stomatal conductance between the inoculated group and the non-inoculated group at 15 d of withholding irrigation. Moreover, the timing of leaf stomatal closure in the inoculated seedlings was delayed by 2 h as compared to the non-inoculated seedlings under severe drought stress. The daily changing curves of the photochemical quenching coefficient in the *P. euphratica* seedlings were similar regardless of inoculation with *F. mosseae*, but the daily changing trend differences varied under the same soil water content, and continuous increase in the soil water deficit resulted in lower photochemical quenching coefficient.

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