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Original Article

Drought adaptation of *Bauhinia faberi* var. *Microphylla* seedlings with dual inoculation of arbuscular mycorrhizal fungi

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Abstract: *Bauhinia faberi* via. *Microphylla* (BFM) is an important tree species for vegetation restoration in the dry valley of southwestern China. However, there were few studies on the application of arbuscular mycorrhizal fungi (AMF) in improving the drought adaptation of BFM. In order to investigate the response of BFM to water stress (WS), we tested four inoculation treatments ((no AMF, Control), *Glomus mosseae* (GM), *Glomus intraradices* (GI), *Glomus mosseae* + *Glomus intraradices* (GMI)) in pots, experimented under three field water holding capacity (WHC) of 70%, 50% and 30%. The changes of seedling survival rate (SR), AMF relative root length colonization rate (Col), growth, photosynthetic parameters, water status and leaf nutrients were examined. The results showed that under 30% WHC drought conditions, SR with dual inoculation of AMF was not higher than with single inoculation of GM, suggesting that increasing the diversity of AMF did not definitely improve plant SR, and that the species of inoculated AMF might have an important impact on SR. The sensitivity of dual inoculated Col to water stress was lower than that of single inoculation, which was more favorable for dual inoculated BFM seedlings adapting to drought environment. The overall drought resistance ability (*D*) also showed that dual inoculation of AMF improved plant drought adaptation compared with single inoculation, which was related to the higher Col of dual inoculated AMF. This study is of practical importance to promote vegetation restoration in arid areas in a cost-effective and environmentally friendly manner.

Keywords: *Glomus mosseae; Glomus intraradices*; *Bauhinia faberi* via. *Microphylla*; Water stress; Dual inoculation

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1 Introduction

Drought is one of the most important factors limiting plant growth and yield (Chaves et al. 2004). Drought can have many effects on plants, such as inhibiting photosynthesis, disrupting water and nutrient relations, and mechanically damaging protoplasts, all of which will lead to decreased growth (Talbi et al. 2015). It is often beneficial to apply targeted agricultural techniques that improve the resistance of plants to water stress in drought affected geographic areas, strategies such as change in land use (Wang et al. 2011a), use of chemical approaches to improve plant water use (eg., agrochemical water-banking with the aid of synthetic mimics of phytohormone abscisic acid (ABA), as well as the use of soil hydrogel) (Vaidya et al. 2022; Tomáková et al. 2020), conduct plant breeding and genetic engineering (Karthik et al. 2018), however, their application has been rather expensive and time consuming. Biological fertilization is one strategy that can be used to decline the effects of drought (Igiehon et al. 2021), which is relatively cost-effective and environmentally friendly. It is widely believed that plant drought resistance can be strengthened by inoculation with AMF (Azizi et al. 2021; Augé 2001).

Growth of plants inoculated with AMF will be improved and will have a positive effect on plant physiological and biochemical properties. For example, Yaghoubian et al. (2014) observed the plant-fungi symbiosis markedly improved the defense mechanisms, drought resistance, and growth of wheat plants. Nacoon et al. (2020) found that AMF could promote the growth of *Helianthus tuberosus* under drought conditions. Begum et al. (2019) reported that AMF inoculation and phosphorus supplementation alleviated drought-induced growth and photosynthetic decline in tobacco, by up-regulating the accumulation of antioxidant metabolites and osmolytes. Although, it has been reported that AMF plays an important role in mitigating the effects of drought stress in soybean, but the mixes of AMF species inoculation do not perform as well as the optimal single species inoculation, ruling out complementary effects, and suggesting a selection effect of AMF on the mitigation of drought (Grümberg et al. 2015). However, it has also been reported that the benefits of AMF species mixtures may be greater for plants than single AMF strain (Azizi et al. 2021). Whether increasing AMF diversity is beneficial for improving plant drought resistance remains controversial.

As a dominant perennial native shrub widely distributed in the arid valleys of southwest China, *Bauhinia faberi* via. *Microphylla* (BFM) has developed drought-adapted characteristics such as dwarf plant individuals, small leaf area and dorsal epidermal hairs, which not only reflect sunlight but also reduce the temperature of the leaf surface, and this also indirectly reduces transpiration (Klich 2000; Stenglein et al. 2005). Li et al. (2007) reported that seedling height, basal diameter, and leaf area of BFM under drought stress were reduced to different degrees, which directly reduced the evapotranspiration surface of the plant; this is an adaptive mechanism for plants to avoid drought stress. In addition, the leaves curl along the midrib when the solar radiation is strong at noon, which can reduce transpiration area and damage from strong light.

BFM not only adapted to drought in terms of morphological and structural changes, but also had physiological and biochemical characteristics in response to drought. As drought stress intensified, BFM net photosynthetic rate and stomatal conductance tended to decrease, while leaf temperature tended to increase, but changes in transpiration rate were not significant (Li et al. 2007), He & Zhang (2018) found that BFM can improve cellular osmoregulation and antioxidant capacity by accumulating proline and up-regulating protective enzyme activity. BFM has often been used for vegetation restoration in arid valley, for its drought adaptive performance and superior performance in soil conservation (Li et al. 2007; Zhang et al. 2016; Kong et al. 2012).

Wang et al. (2015) found that in the Minjiang river dry valley, southwest of China, AMF was significantly enriched under the shrub vegetation, AMF significantly affected soil nutrient content, soil aggregate stability, and soil enzyme activity (Wang et al. 2011b), *Glomus* accounts for more than 50% in the rhizosphere AMF community of BFM (Chen 2016). As the dominant genus of BFM rhizosphere, *Glomus* can boost drought resistance of plants by increasing plant nutrients uptake (such as phosphorus, silicon and calcium) (Song et al. 2013; Hassan et al. 2022; Sang et al. 2018). Moreover, the application of AMF was helpful for plants improving drought tolerance, which can reduce environmental pollution caused by the use of inorganic fertilizers (Begum et al. 2019). Nevertheless, knowledge on the mechanisms of *Glomus* inoculation improving drought tolerance in

BFM remains scarce, with only a few on the effects of single AMF, e.g., Song et al. (2013) found that single inoculations of *Glomus mosseae* and *Glomus coronatum,* promoted BMF growth and alleviated the limitation of soil phosphorus nutrient deficiency, and Zhang et al. (2016) reported that *Funnelrmis mosseae* had a significant effect on phosphorus uptake, biomass allocation and total nitrogen in BFM under drought conditions. However, the effect of dual *Glomus* species inoculation on BFM has not been reported.

Therefore, in this study, we used pot experiments in a greenhouse to compare the effects of double and single inoculations of *Glomus* species (*Glomus mosseae* (GM) and *Glomus intraradices*) on the growth, physiological characteristics, water status, and leaf nutrients of BFM under WS. We sought to answer the following two questions: (1) whether the drought adaptation of dual inoculation of *Glomus* species is superior to single; and (2) what is the mechanism by which *Glomus* treatment affects the drought adaptation of BFM. The results will provide scientific basis for low-cost and environment-friendly vegetation restoration in the arid valley of Dadu River, China.

2 Materials and Methods

2.1 Experimental materials

Bauhinia faberi via. *Microphylla* (BFM) is a perennial deciduous shrub of the Leguminosae. It is one of the dominant native shrub species in the in the arid valley of Dadu River, located in the northeast edge of Hengduan mountains, southwest of China. It is mainly distributed on both sides of the river valley within the altitude of 1,500-2,000m, and the climatic conditions of the community distribution area are typical arid river valley climate. Owing to the foehn effect, the evaporation in the arid valley is much higher than the precipitation and these areas are characterized by severe drought and water deficiency (Li et al. 2007). One-year-old branches cuttings of BFM were selected on a sunny morning (7:30–11:30), take cuttings about 25 cm long from the tip, spray with 3‰ potassium permanganate solution to disinfect for further treatment.

G. mosseae (GM) and *G. intraradices* (GI) were both provided by the institute of plant nutrition and resources, Beijing Academy of Agricultural and Forestry Sciences. AMFs were inoculated with mixed media, including spores (spore density 353~545/100 g), mycelium and fully infested root segments (88% AMF infestation rate) and sand. The two AMF species of *Glomus* were selected because it is abundant and widely distributed in native and agricultural soils in the studied region (Chen et al. 2016).

The plant growth substrate was the nursery soil of the Alpine Ecosystem Observation and Experiment Station of Mt. Gongga, Chinese Academy of Sciences (hereinafter referred as Mt. Gongga Station), altitude is about 1600 m. Substrates has been passing through a 2 mm sieve and then autoclaved at 121°C for 2 hours to kill the soil microorganism, cooled and ready for use. The physicochemical properties of substrates are as follows, Organic matter (0.34%), pH 7.81, total nitrogen content (1.61 g/kg), total phosphorus content (0.77 g/kg), available phosphorus content (20.9 mg/kg), total potassium content (19.8 g/kg) and available potassium content (101 mg/kg).

2.2 Experimental design

A completely randomized block design was arranged in the study with two factors: (i) soil water treatments including normal irrigation (70% of field water holding capacity, WHC) , limited irrigation (50% WHC) and water deficit (30% WHC), the above three water stress levels were determined based on the results of earlier experiments (Zhang et al. 2017; Li et al. 2009); and (ii) AMF treatments including no AMF inoculations (Control) and inoculation with *G. mosseae* (GM), *G. intraradices* (GI), and the mixture of *G. mosseae* and *G. intraradices* (GMI). Each treatment has replicates.

The pot experiment was carried out in the greenhouse of Mt. Gongga Station, where sterilised BFM cuttings were transplanted into a pot (21 cm × Φ 19 cm) with 3 kg of sterilised substrate and 10 g of the respective AMF inoculant (about 35~45 spores plus colonised roots and mycelium) per plant. The "two-layer inoculation method" was adopted, firstly, plastic bowls were filled with 1/3 of the sterilised substrate, then 5 g of inoculant, then put the cuttings into the pot, followed by a 1/3 layer of sterilised substrate, then another 5 g of inoculant and last 1/3 of sterilised substrate were added in the plastic bowl to conducting WS experiment; as to treatments with 2 inoculants, mixed 5 g of each AMF inoculant in equal amounts and prepare in the same way as above; for treatments without AMF, we mixed inoculant of GM and GI provided by the Beijing Academy of Agricultural and Forestry Sciences in equal proportions, sterilized the mixture of inoculants at 120°C for 2 h, and applied to treatments without AMF in the same amount and way as AMF treatments, to create as uniform a background for plant growth as possible.

The inoculated plant cuttings were subjected to 2 months (2020.05.01~2020.6.30) AMF infestation period in greenhouse, which was covered with plastic film to prevent rainfall, the perimeter of the greenhouse was uncovered to ensure ventilation. The average monthly temperature during this period was 10.3°C, the average monthly relative humidity was 92% and the average monthly total net radiation was 214.7 MJ/m^2. The water treatment was started on 1 July 2020 and lasted for three months in greenhouse. Water control experiment was conducted based on the method described by Li & Bao (2009), using the weighing method to control soil water by watering once every other day, each time from 8:00 to 9:00 a.m., so that the actual mass water content of the potting soil under 70%, 50%, and 30% (WHC) conditions were maintained at (19.6±1.4)%, (15.4±1.4)%, and (9.8±1.4)%, and one plant-free potting soil per treated seedling was set as a control to estimate the evaporated water from the soil surface. Beside, to block the interaction between different treatments and to allow spatial separation between plants of each treatment, we arranged each treatment (containing 6 replicates) in a row in our experiment with about 20 cm separation between each row, and to prevent water loss, we sealed the bottom of the pots with ties and plastic membrane, and the average monthly temperature during this period was 12.1°C, the average monthly relative humidity was 96% and the average monthly total net radiation was 159.8 MJ/m^2.

2.3 Determination and analysis of experiment

2.3.1 Measurements of plant survival rate (SR) and plant growth

After 3 months of WS, SR is determined by manual counting; plants height, basal diameter (BD) and leaf area (LA) were determined by conventional method.

2.3.2 Leaf gas exchange

The net photosynthetic rate (P_n), transpiration rate (E) and stomatal conductance (g_s) were measured at last day of WS experiment, using a Li-6400 portable photosynthesizer (LI-COR, Li-6400, USA) with red and blue artificial light sources and CO_2-injected cylinders, the measurements were carried out between 9:30 and 11:00 a.m. on the same day, to avoid physiological disturbances caused by high light and heat at midday. The photosynthetic water use efficiency (WUE) was calculated from the ratio of P_n to E. The water potential was measured by Water potential measurement system (Wescor, Psypro, USA). Then all plants were harvested to analyze their growth, relative root colonization rate and nutrient characteristics.

2.3.3 Nutrient analysis of the leaves

All leaves were harvested after 3 months of WS, and parts of the leaves were taken and dried at 60°C in Mt. Gongga Station, then samples were brought to the public technology center of the Institute of Mountain Hazards and Environment, Chinese Academy of Sciences for leaf nutrient analysis. Air-dried leaf samples were weighed about 0.2000 g in a microwave digestion tank, added 8 ml of HNO_3 and digested in a microwave digester (CEM, MARS 6, USA), after the digestion, the elements (Ca, P) contents were determined by the Inductively coupled plasma atomic emission spectrometry (Optima 8300V, USA).

2.3.4 Observation of AMF colonization

The root soil was moved firstly, then, AMF colonization was observed by staining with acetate ink (Vierheilig et al. 1998). The relative root length colonization rate (Col) was determined by the root segment infestation weighting method according to Mc Gonigle & Miller (1990), using microscopic examination.

2.4 Data analysis

ANOVA was used to compare differences between treatments, means were compared with LSD fisher's test and differences were considered significant at $p<0.05$. All statistical analyses were performed by the SPSS 23.0 software for Windows (IBM SPSS Statistics, USA). Bar charts were drawn with Origin 11.7 software. Following the method of Zou et al. (2020), the measured values of physiological and ecological traits of the treatments were used to calculate the Composite Index (CI) value, D-value and ranking of the treatments, respectively.

AMOS 28.0 (IBM SPSS Statistics, USA) was used to construct a structural equation model (SEM), SEM was used to analysis the causality relationship among WS, biological or nonbiological indicators, plant growth and SR under AMF inoculation mode. When constructing the SEM, the plant height, basal diameter and leaf area obtained the common factor variance through the SPSS dimensionality reduction analysis, and the comprehensive factor “Growth” representing the plant height, BD and LA were calculated by weighting; g_s and E also used the same method to calculate the transpiration related comprehensive factor “Tra”; The same method was also used for leaf phosphorus (P) content and calcium (Ca) content to calculate the comprehensive nutrient-related factor "Nut".

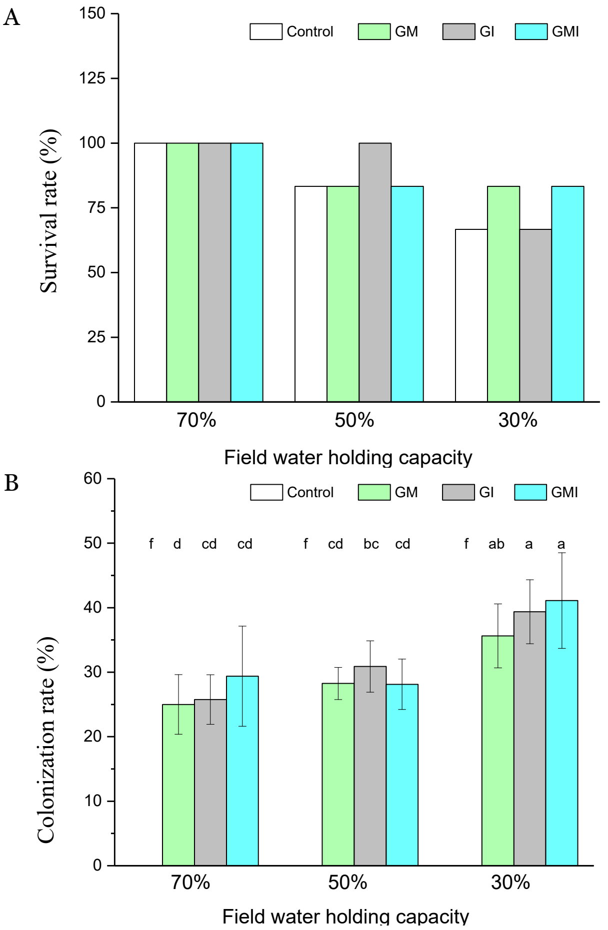


Fig. 1 Effects of water stress and arbuscular mycorrhizal fungi treatments on survival rate (A) and colonization rate (B) of *Bauhinia faberi* via. *Microphylla* seedlings. GM, *Glomus mosseae*; GI, *Glomus intraradices*; GMI, mixture of *Glomus mosseae* and *Glomus intraradices*. On the plot B, data represent average values (n = 10) and error bars are standard errors of the means. Different letters between columns of colonization indicating the significant differences (p < 0.05).

3 Results

3.1 AMF effect on SR and Col of *Bauhinia faberi* var. *Microphylla* under water stress

The results showed that uninoculated and GI inoculation BFM had the lowest survival rate after the 3 months of treatment under 30%WHC. Interestingly, GM and GMI groups under 30%WHC showed similar survival patterns as 50%WHC, and their SR under 30%WHC were also higher than the control group, suggesting that GM and GMI inoculation improved the SR of plant under water deficit condition.

Arbuscular mycorrhizal structure was not observed in the roots of plant without AMF inoculated. Under normal irrigation, limited irrigation and water deficit, Col was about 25.0%, 28.2% and 35.6% of GM were inoculated; 25.8%, 30.9% and 39.4% of GI were inoculated; 29.4%, 28.1% and 41.1% of GMI inoculation were inoculated, respectively (Fig. 1). Dual AMF inoculated plant have the highest Col in normal irrigation and water deficit group, however, there is not significant difference among GM, GI and GMI under the same stress level. Moreover, Col increased with the increasing of WS.

3.2 AMF effect on drought resistance of *Bauhinia faberi* var. *Microphylla* under water stress

3.2.1 Growth of seedlings

WS caused a decrease in the growth of plants. Under WS, the height, basal diameter (BD) and leaf area (LA) of non-inoculated plants were lower than those of normal irrigation to varying degrees. Especially, under the water deficit condition, the decreases of height and LA all reaching significant levels (p<0.05) (Figs. 2A, 2C). The greater the WS, the more harmful to the growth of plants. After GM inoculation, plant height, basal diameter and leaf area increased compared to the non-inoculated plants in the same WS level. The promoting of AMF treatment on growth were assessed as GMI>GM>GI by calculating the mean of increase ratio in plant height, BD and LA.

3.2.2 Photosynthetic variables

In non-inoculated plants, the P_n, E and g_s of plants showed a trend of firstly decreasing and then increasing with the increasing of WS (Fig. 3). However, after inoculation with GM and GMI, P_n was significantly higher than non-inoculated group (Fig. 3A) (p<0.05), where g_s of GM-inoculated plants was significantly higher in the limited irrigation group (p<0.05) (Fig. 3B), and E of GMI-inoculated plants was also significantly higher in the water deficit group (p<0.05) (Fig. 3C). However, there was no significant change (p<0.05) in P_n, g_s and E after GI inoculation (Figs. 3A, 3B, 3C). This result indicated that under water deficit conditions, GM inoculation alone was beneficial in reducing the effects of water stress on plant P_n and g_s, while dual inoculation of GM and GI helped plants to maintain P_n and promote E.

3.2.3 Water status

The leave WUE of non-inoculation plants increased by 24.8% and 37.8% in the limited irrigation and water deficit plants, respectively, compared to the normal irrigated plants (Fig. 4A). AMF inoculation treatments were effective in increasing plant WUE. Plants WUE significantly increased under all water conditions with inoculation of both GM and GMI (p<0.05), except for the water deficit group where GMI inoculation did not increase WUE to a significant level. GI inoculated plants had no significant effect on WUE.

Leaf Water potential (WP) of uninoculated plants was reduced to different degrees of WS conditions, resulting in the increased of plant water deficit (Fig. 4B). Leaf WP of AMF inoculated plants also decreased under normal irrigation, which was related to water consumption by mycorrhizal growth. However, as WS intensified, the WP of all plants inoculated of AMF was higher than that of non-inoculated in water deficit groups. It can be seen that AMF inoculation can alleviate the harm of drought on the plants, and the greater the drought, the more obvious the performance.

3.2.4 Leaf nutrients content

In the leaves of uninoculated plants, calcium (Ca) and phosphorus (P) contents decreased to different degrees in the limited irrigation group compared with the normal irrigation group, with P decreasing by 27.8%, reaching a significant level (p<0.05) (Figs. 5A, 5B). After inoculation with AMF, Ca and P contents

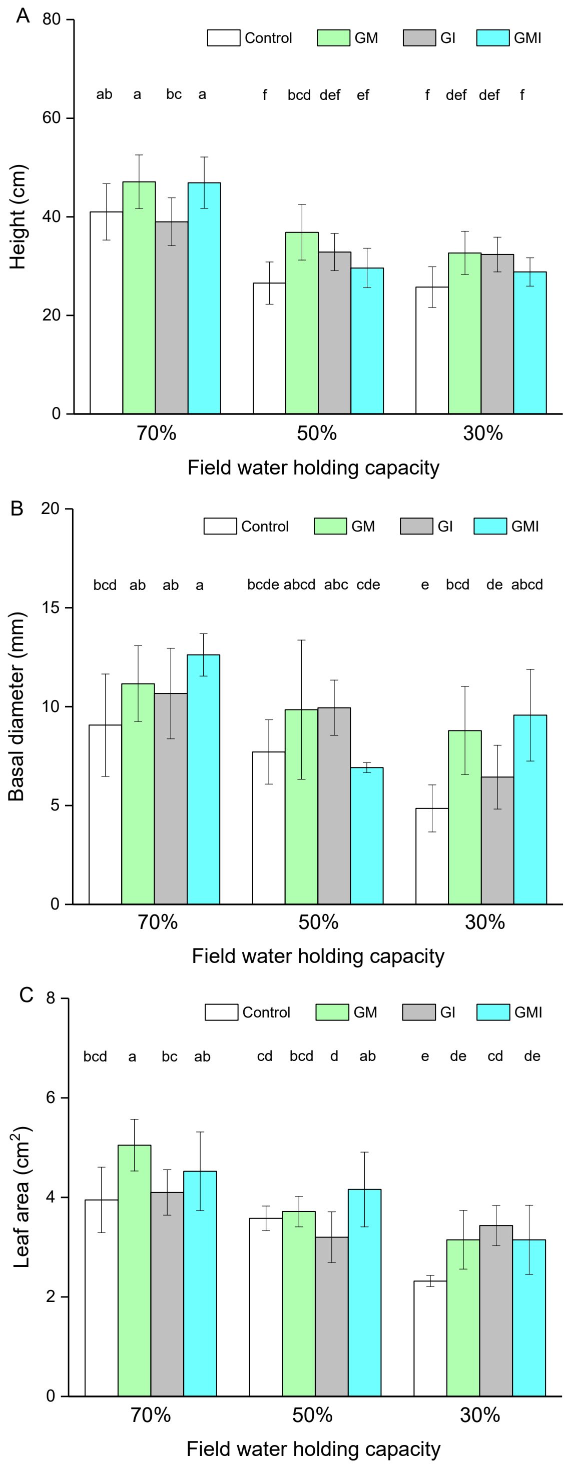


Fig. 2 Effects of water stress and arbuscular mycorrhizal fungi on (A) Height, (B) base diameter, and (C) leaf area of *Bauhinia faberi* via. *Microphylla* seedlings. GM, *Glomus mosseae*; GI, *Glomus intraradices*; GMI, mixture of *Glomus mosseae* and *Glomus intraradices*. Data represent average values (n = 6) and error bars are standard errors of the means. Different letters between columns indicate significant differences (p < 0.05).

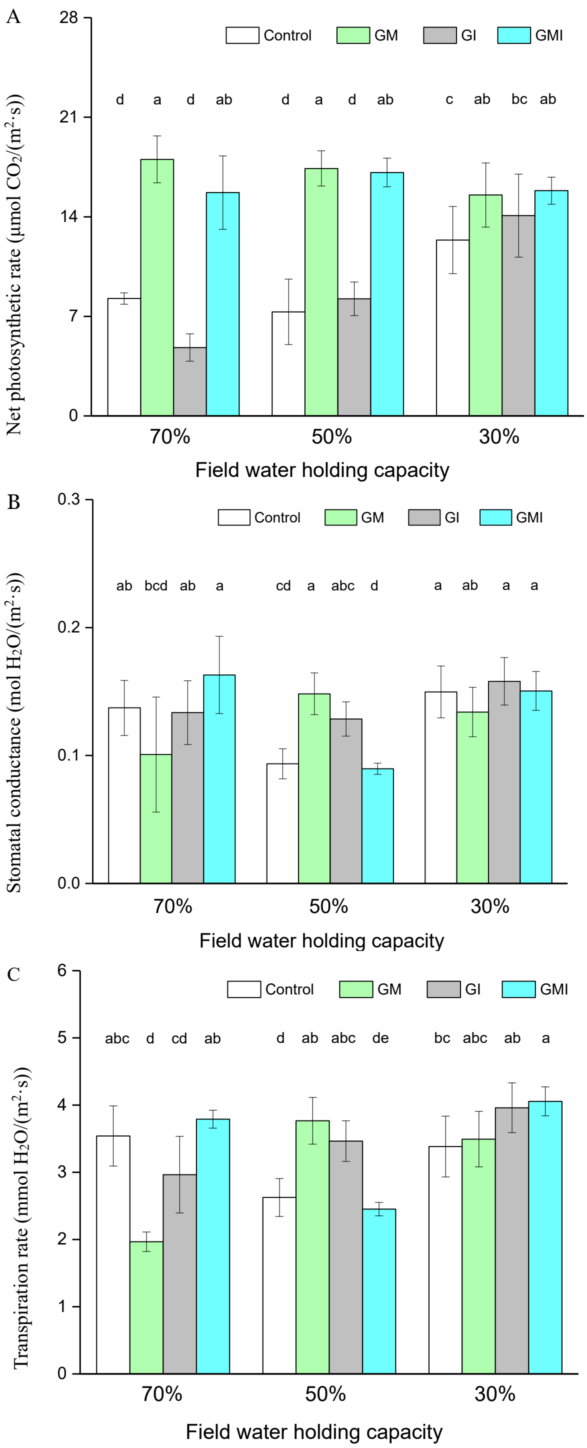


Fig. 3 Effects of water stress and arbuscular mycorrhizal fungi on (A) Net photosynthetic rate (P_n), (B) stomatal conductance (g_s) and (C) transpiration rate (E) of *Bauhinia faberi* via. *Microphylla* seedlings. GM, *Glomus mosseae*; GI, *Glomus intraradices*; GMI, mixture of *Glomus mosseae* and *Glomus intraradices*. Data represent average values (n = 6) and error bars are standard errors of the means. Different letters between columns indicate significant differences at $p < 0.05$ (Duncan's multiple range test).

increased to different degrees with the increasing of WS in both GM and GI inoculations. GMI inoculated plants had the highest leaf Ca content under normal irrigation and water deficit, especially under normal irrigation conditions, which was significantly higher than that of GI inoculated plants ($p<0.05$). It can be seen that dual inoculation with AMF was superior to single in terms of increasing the leaf Ca content of plants.

3.2.5 Drought resistance

The overall evaluation of drought resistance (D) of AMF treatments were determined by the affiliation

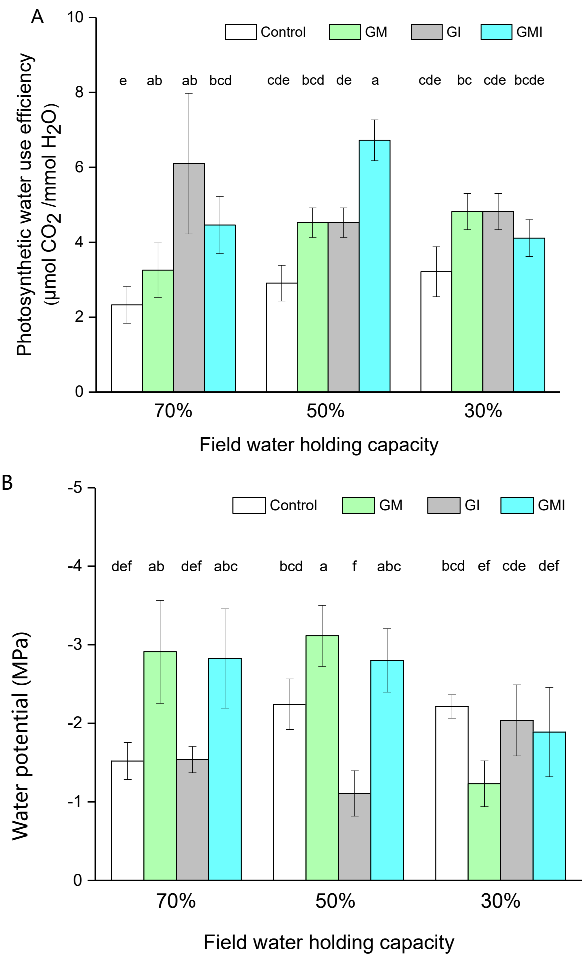


Fig. 4 Effects of water stress and arbuscular mycorrhizal fungi on (A) photosynthetic water use efficiency (WUE) and (B) water potential (WP) of *Bauhinia faberi* via. *Microphylla* seedlings. GM, *Glomus mosseae*; GI, *Glomus intraradices*; GMI, mixture of *Glomus mosseae* and *Glomus intraradices*. Data represent average values (WUE: n = 6; WP: n = 4) and error bars are standard errors of the means. Different letters between columns indicate significant differences at p < 0.05 (Duncan's multiple range test).

function for 11 indexes including plant height, BD, LA, P_n, g_s, E, WUE, WP, leaf Ca content, leaf P content and SR for each treatment (Table 1). The results showed that in the limited irrigation group, *D* values showing a trend of GMI>GI>GM. While in the water deficit group the *D* values has a trend of GM>GMI>GI. It can be seen that dual inoculation of AMF was better than that in single inoculation in improving drought adaptation of BFM, under limited irrigation condition. However, under water deficit condition, GM showed better in drought adaptation.

3.3 Causality between water stress and characteristic of plant with AMF inoculations

Structural equation models (SEM) were established to analysis causality relationship among WS, biological or nonbiological indicators and plant growth with AMF inoculations (Fig. 6). The fit parameters of structural equation model list on Fig. 6E. The overall effect of standardization showed that, for plants uninoculated AMF plants (Fig. 6A), WS directly affect transpiration related parameters (Tra) and leaf water potential (WP). Moreover, Tra was one of the important factors intensely affecting plant growth (p<0.05). It can be seen that plant growth was strongly influenced by WS directly, and WS also strongly influenced plant growth indirectly through Tra.

Plants inoculated with GM, WS had a strong direct effect on all factors including plant growth (Fig.6B), and also played an important indirect regulatory role on plant growth by strongly influencing Tra and Nut (p<0.01). Among the factors strongly affected by WS, WP influenced plant Tra and *Nut* (p<0.01) and was an important process factor for GM to improve plant adaptation to water stress. Similarly, in GI-inoculated plants, WP was an important factor affecting plant physiological and biochemical processes under WS conditions (Fig. 6C).

When double inoculated with GI and GM (Fig. 6D), WS did not have a strong direct effect on Col and P_n. Although Col and Tra were also both strongly affected by WP, just as the performance of GM inoculation (p<0.01), however, the effect of double inoculation with WP on Nut was still not significant. Meanwhile, Tra of double-inoculated AMF plants could intensely affect plant growth by its' significantly influence on Nut (p<0.05) and P_n (p<0.01), which was the most important process factor for WS to indirectly improve water stress adaptation in dual inoculated AMF plants (p<0.01).

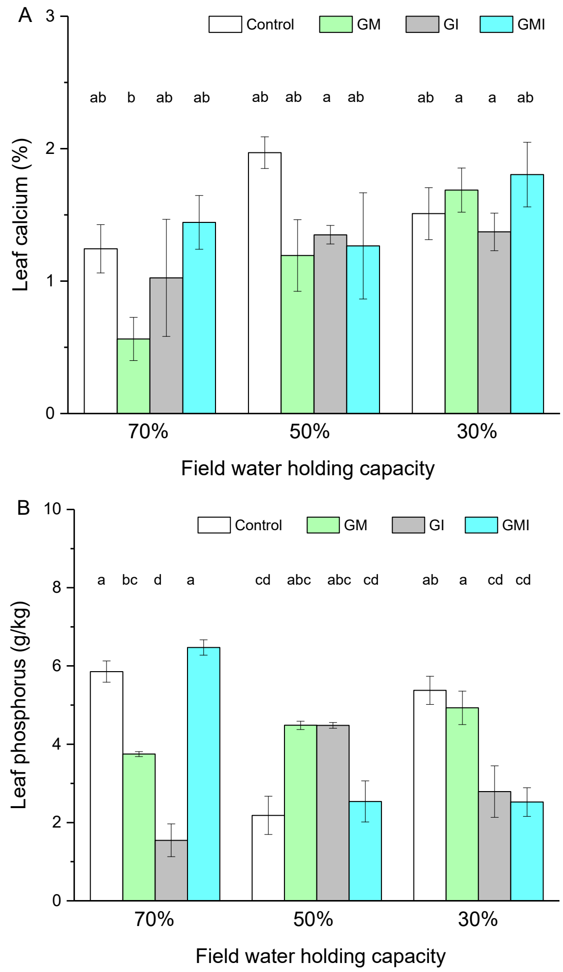


Fig. 5 Effects of water stress and arbuscular mycorrhizal fungi on concentration of (A) leaf calcium (Ca), (B) leaf phosphorus (P) of *Bauhinia faberi* via. *Microphylla* seedlings. GM, *Glomus mosseae*; GI, *Glomus intraradices*; GMI, mixture of *Glomus mosseae* and *Glomus intraradices*. Data represent average values (n = 3) and error bars are standard errors of the means. Different letters between columns indicate significant differences at p < 0.05 (Duncan's multiple range test).

4 Discussion

4.1 Responses of uninoculated plant under water stress

Under WS, the plant height, BD and LA were found to be lower than those of the normal irrigated to different degrees. plant height was reduced by

Table 1 Value of each treatment's comprehensive index (CI) and *D* value (comprehensive evaluation of drought resistance)

Treatment	CI_1	CI_2	CI_3	CI_4	CI_5	CI_6	CI_7	CI_8	CI_9	CI_{10}	CI_{11}	*D*	Rank
70%WHC-Contrl	3.950	41.000	10.068	8.257	0.137	3.540	2.330	-1.520	1.244	5.857	0.010	1.144	10
50%WHC-Contrl	3.245	17.193	6.605	6.490	0.064	1.947	3.630	-3.308	3.120	0.815	0.010	3.781	9
30%WHC-Contrl	1.363	16.172	4.008	18.498	0.163	3.234	4.428	-3.228	1.831	4.939	0.004	4.646	8
70%WHC-GM	6.456	54.108	11.645	39.416	0.074	1.093	4.550	-5.571	0.255	2.401	0.007	10.802	2
50%WHC-GM	3.497	33.138	9.629	36.669	0.160	4.010	8.784	-6.377	1.146	3.434	0.007	1.015	11
30%WHC-GM	2.512	26.048	10.557	29.223	0.131	3.449	9.963	-0.995	2.290	4.151	0.007	8.115	4
70%WHC-GI	4.256	37.098	11.301	2.802	0.130	2.483	1.793	-1.554	0.844	0.409	0.010	5.729	6
50%WHC-GI	2.592	26.320	9.823	8.207	0.120	3.390	3.128	-0.806	1.466	3.431	0.010	5.366	7
30%WHC-GI	2.984	25.525	6.424	24.005	0.182	4.430	6.806	-2.729	1.513	1.330	0.004	0.877	12
70%WHC-GMI	5.184	53.695	15.827	29.853	0.194	4.058	8.544	-5.250	1.676	7.152	0.010	11.222	1
50%WHC-GMI	4.381	21.406	7.744	35.474	0.059	1.699	19.406	-5.158	1.289	1.101	0.004	8.270	3
30%WHC-GMI	2.512	20.265	9.092	30.363	0.165	4.645	7.253	-2.344	2.618	1.088	0.004	7.129	5

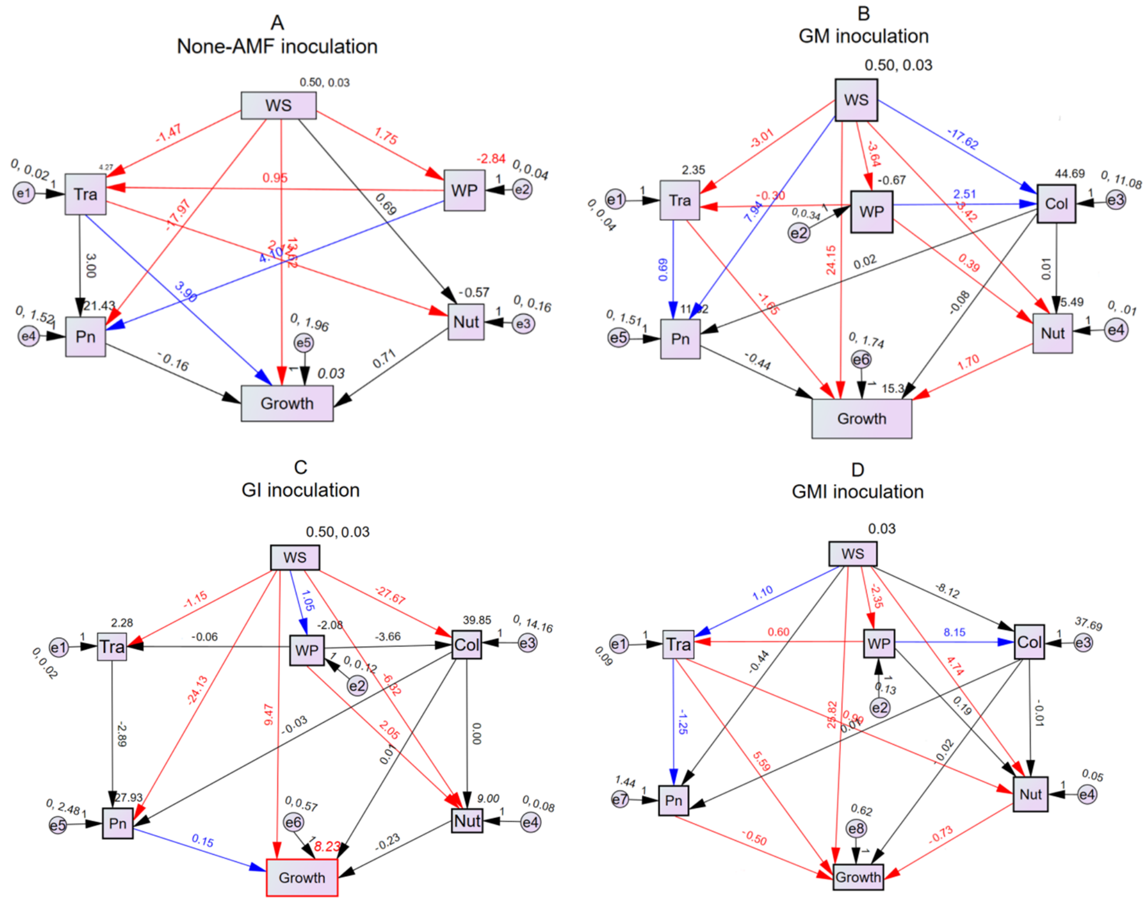
Notes: GM, *Glomus mosseae*; GI, *Glomus intraradices*; GMI, mixture of *Glomus mosseae* and *Glomus intraradices;* WHC, Field water holding capacity.

35.2% and 37.2% in the limited irrigation and water deficit treatment groups, respectively, and both BD and LA were reduced by 46.5% and 41.3% in the water deficit group, all at significant levels (p<0.05). As well as the plant root development, stress tolerance, water carrying capacity, and number of viable leaves were reduced to different degrees, resulting in lower biomass accumulation (Leventis et al. 2021). It was largely attributed to the effect of WS on photosynthesis. Plant photosynthesis is particularly sensitive to water deficit (Da Matta et al. 2002). We found that P_n, g_s and E showed a trend of firstly decrease and then increase with the increasing of water deficit, it may due to stomata which could quickly sense the changes in atmospheric humidity or root water potential, by lowering leaf g_s to prevent water dissipation and maintain its P_n, leading to the decrease of E (Leventis et al. 2021).

However, as the WS increased, intracellular CO_2 concentration (*Ci*) and CO_2 concentration reach equilibrium, leaf g_s showed a tendency to decrease firstly and then increase (Lawlor et al. 2009), Under water stress conditions, photosynthesis is reduced by stomatal limitation due to stomatal conductance limitation, resulting in *Ci* concentrations that do not cover photosynthetic requirements (Farquhar & Shrkey 1982), P_n and E also have the same performance, and WUE increased by 24.8% and 37.9% in the limited irrigation and water deficit plants, respectively. It can be seen that within a certain WHC, limited irrigation reduces photosynthesis and transpiration mainly by inducing stomatal closure, thus reducing plant growth. Nevertheless, the effect of stomatal restriction on photosynthesis was weakened by the increasing of plant water deficit. Tezara et al. (1999) also indicated that under moderate as well as severe water deficit, non-stomatal limitation was the main cause of changes in plant physiological processes (Grassi et al. 2005; Flexas et al. 2004), that reduced photosynthesis was mainly due to reduced chloroplast activity and ribulose 1,5-diphosphate carboxylase (Rubisco) activity. A combination of stomatal and non-stomatal factors limited photosynthesis in plants under water deficit conditions (Flexas et al. 2002).

4.2 Responses of Dual and single AMF inoculation plant under water stress

Under severe WS, compared with uninoculated seedlings, the Col in dual AMF inoculation was higher than that of GM and GI, and both GM and GMI inoculations improved plant SR. Some previous studies supported our results (Azizi et al. 2021; Amiri et al. 2017), they reported that regardless of the water regime, *F. mosseae* and *R. intraradices*, and in particular, their combined inoculation, resulted in high Col. Especially, under severe WS, all inoculations treatments increased SR compared to uninoculated seedlings. Our results showed that dual inoculation of AMF improves Col, which was more conducive to increase plant water and nutrients uptake, thereby increasing plant survival rate. However, SR of plants inoculated with dual AMF was not higher than that inoculated with GM alone, suggesting that increasing the diversity of AMF does not necessarily improve SR of plants under WS, van der Heijden et al. (2003) also reported that increased mycorrhizal diversity did not result in a greater biomass of two naturally coexisting plant grass species. It is likely that the species of AMF



E

Structural equation model	Chi-square	df	Chi/df	GFI	CFI	RMESA
None-AMF	8.3	3	2.77	0.922	0.970	0.248
GM	4.8	5	0.96	0.907	0.982	0.165
GI	12.8	6	2.13	0.906	0.968	0.198
GMI	6.1	4	1.53	0.946	0.990	0.134

Fig. 6 Structural equation model simulating the relationship among water stress (WS), plant physiological indices and growth under arbuscular mycorrhizal fungi inoculation treatments. (A) Structural equation model without arbuscular mycorrhizal fungi inoculation treatment; (B) Structural equation model with *Glomus mosseae* (GM) inoculation treatment; (C) Structural equation model with *Glomus intraradices* (GI) inoculation treatment; (D) Structural equation model with mixture of *Glomus mosseae* and *Glomus intraradices* (GMI) inoculation treatment. The comprehensive factor calculated by weighting, Growth representing the plant height, base diameter and leaf area; Tra representing stomatal conductance (g_s) and transpiration rate (E); Nut representing leaf Phosphorus (P) and Calcium (Ca) content. The path coefficients are located above the arrows. A red line indicates a significant effect at $p < 0.01$, and a blue line indicates a nonsignificant effect at $p < 0.05$. The R^2 values above each variable indicate the proportion of the variance explained. (E) The fit parameters of each structural equation model.

inoculated will have a greater impact on the survival rate of BFM.

Compared with uninoculated seedlings, AMF inoculated plants showed an overall increase in P_n, E and WUE, where E was higher in dual AMF inoculation plants than single in the water deficit group, and leaf Ca content was also highest in the dual AMF inoculated plants, but g_s and P_n did not differ significantly between single and dual AMF inoculation. In addition, the overall evaluation value of plant drought tolerance (*D)* in GMI was more than 7 times higher than GM under limited irrigation, however, in the water deficit group, it was only 13.8% lower than GM. In general, the benefits of dual inoculation AMF on plant drought tolerance were better than those of single inoculation, the contribution of GM to dual inoculation drought adaptation was greater than that of GI. Hoeksema et al. (2010) also believes that the benefit of mixed AMF inoculation was generally higher than that of single inoculation, such an effect was even observed when only isolates of the same phylogenetic clade were studied (Gustafson et al. 2006; Wagg et al. 2011), excluding complementarity effects (Grümberg et al. 2015), dual AMF inoculation on plant growth, photosynthesis and other parameters measured were not a simple equivalent addition of the effect of single AMF.

However, the comprehensive effect of dual AMF inoculation on plants may lead to highly variable of plant adaptation in symbiosis, because AMF has a selective effect in alleviating water deficit impact (Grümberg et al. 2015). The interspecific competition among multiple AMF species usually has a negative impact on the Col of each species (Wagg et al. 2011; Jansa et al. 2008), competition between AMF species within the host is particularly intense during inoculation of multiple AMF species for in-root colonization (Engelmoer et al. 2014), probably because the space for in-root growth is more limited and there is competition between different AMF species for mineral nutrients and survival space (Medina et al. 2003), resulting to the fact of the Col by mixed inoculation was not the result of the additive colonization by individual isolates (Jansa et al. 2008), Moreover, the environmental background may also be important in determining the results of inoculation of AMF mixes (Powell et al. 2018), and the synergy between AMF mixtures in a specific environment cannot be completely ruled out.

4.3 Drought resistance mechanisms in single vs dual AMF inoculation plant

The SEM analysis showed that WS directly and significantly affected WP, Tra and Col in the single inoculated AMF plants, among which, where WP of GM inoculated plants could indirectly inhibit plant growth decline through significant effects on Tra and Nut. In GI inoculated plants, WP only had a significant direct effect on Nut, but not on Tra and Col,

and their overall *D* values for drought tolerance were lower than those of GM inoculated plants. It can be seen that the strong and direct effect of WP on a series of responses of plant physiological processes by improving plant WP is an important process for AMF inoculation alone to resist the negative effects of drought stress.

The symbiosis with AMF was beneficial in maintaining WP in BFM leaves, which was related to the enhanced water uptake in the rhizosphere of the plant by the extra-root mycelium, estimated by Ruth et al. (2011) to be about 20 % of the total water uptake of the plant. In addition, AMF promotes plant Ca, Si, and p uptake, increasing the osmotic material in the plant, causing a decrease in osmotic potential, which also facilitates sustained water uptake from outside. AMF also helped the enhances of intercellular water flow (Ruiz-Lozano et al. 1995; Barzana et al. 2012), in mycorrhizas, symplastic movement within the hyphae themselves may provide an additional important pathway for water movement in the root cortex of the colonized roots (Reid 1979). Besides, mycorrhizas can also affect the cell-to-cell pathway through effects on plant aquaporin expression and/or activity (Ruiz-Lozano & Aroca 2010), may allow AM plants to respond better to water demands from the shoot, especially under water deficit conditions. In addition, AMF also helps to reduced water loss from leaves (Barzana et al. 2014; Wu et al. 2013). The indirect impact of WP on P_n, through Tra, they were beneficial in alleviating drought- induced plant stomatal closure (Seki et al. 2007), increased leaf stomatal conductance and increasing transpiration flux (Wang et al. 2017), allowing mycorrhizal plants under drought stress to typically have higher biomass than non-mycorrhizal plants (Sebastiana et al. 2018).

In dual AMF inoculation plants, Tra regulated plant growth by strongly and directly affecting P_n and Nut, while nutrient acquisition showed a stronger response to plant transpiration (Leventis et al. 2021; Flexas et al. 2006). The performance was different from single inoculated AMF plants, where WP is the most important process factor in plant physiological and biochemical processes. The indirect effect of Tra on P_n in dual inoculated AMF plants was beneficial in alleviating drought-induced plant stomatal closure (Seki et al. 2007), increasing leaf stomatal conductance and increasing transpiration flux (Wang et al. 2017), allowing mycorrhizal plants under drought stress to typically have higher biomass than non-mycorrhizal plants (Sebastiana et al. 2018). In addition, plants of dual AMF inoculation did not significantly affect Col under WS, while the Col of single AMF inoculation plants all strongly and directly affected by WS (Figs. 6B, 6C, 6D), suggesting that dual AMF colonization plants were less sensitive to drought stress than single one.

The improved drought adaptation and growth benefits of dual AMF inoculation plants were superior to those of single inoculation, which was associated with a higher Col of dual AMF inoculation plants compare to single inoculation. The increase of Col was more conducive to improving the effective spatial allocation of the root system, optimizing root architecture, and promoting root accessibility to soil water (Liu et al. 2018; Hashem et al. 2019), thus facilitating the promotion of plant water uptake, nutrient uptake, and P_n increasing. These symbiotic advantages feed back to the plant, which enable mycorrhizal plants to have better hydrodynamic characteristics and water relations (Bitterlich et al. 2018), improving plant adaptation to drought stress, and delay the emergence of drought response in plants (Ren et al. 2019). It would causing cascade of morphological, physiological, biochemical and molecular responses affected plant metabolism (Osakabe et al. 2014), which improved plant growth. Azizi et al. (2021) also reported that dual inoculation with AMF significantly improved common myrtle leaves physiologically, reducing electrolyte leakage, malondialdehyde and proline concentrations, making it the most effective and cost-effective method for optimising resistance to WS; and this effect was observed even when only AMF from the same phylogenetic branch studied. And the AMF complementation effect was excluded.

5 Conclusion

In this study, we found that inoculation with *Glomus* genus effectively mitigated the negative effects of water stress on BFM growth, photosynthetic parameters, and water status, and dual AMF inoculation was superior to single inoculation in terms of plant growth benefits and improved drought adaptation. However, SR of dual AMF inoculation plants was not higher than that of single inoculated GM, suggesting that the increasing of AMF diversity did not necessarily improving SR of plants under water stress condition. It is likely that the species of AMF inoculated will have a greater impact on the survival rate of BFM. The effect of dual AMF inoculation on plant SR, growth, photosynthesis and other measured parameters was not the simple equivalent accumulation of single AMF species, which we believe is due to the fact that Col of dual AMF inoculation was greater than that of single inoculation, but still not equal to the simple equivalent accumulation of single inoculated AMF species. By constructing SEM models, we further verified that WP was an important factor for single inoculation of AMF to improve plant drought stress, while dual AMF inoculation plants was Tra. The sensitivity of AMF Col to water stress was less for dual AMF inoculation than for single inoculation, and dual AMF inoculation was more beneficial to improve the drought adaptation of BFM seedlings.

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Author contributions

ZHU Wan-ze and LI Xia contributed to the study conception and design. Material preparation, data collection and analysis were performed by LI Xia, WANG Wen-wu, MA Sheng-lan, SHENG Zhe-liang and SHU Shu-miao. The first draft of the manuscript was written by LI Xia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics Declaration

Data Availability: Dada is available on reasonable request from the corresponding author.

Conflict of Interest: ZHU Wan-ze, the corresponding author of this article is the scientific editor of Journal of Mountain Science. He was not involved in the journal's review of, or decisions related to this manuscript.

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