

EFFECTS OF INOCULATION WITH DIFFERENT ARBUSCULAR MYCORRHIZA FUNGI ON ROOT GROWTH AND INFECTION OF *XANTHOCERAS SORBIFOLIUM* (BUNGE) SEEDLINGS UNDER DROUGHT STRESS

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Abstract

Arbuscular mycorrhizas (AM) fungi have been widely studied in physiology and photosynthesis, but the drought tolerance of *Xanthoceras sorbifolium* by AM fungi has not been fully explored. In order to explore the drought stress and drought tolerance of *X.sorbifolium* seedlings inoculated with different AM fungi. We conducted an indoor pot experiment with four levels of drought stress on *X. sorbifolium* without inoculation with *Funneliformis mosseae*, *Diversispora versiformis*, *Rhizophagus intraradices* and mixed fungi, respectively. The results showed the infection rate of each microbial agent can reach up to 88%. Under mild drought conditions, after 60 days of inoculation with *F. mosseae*, *D. versiformis*, *R. intraradices* and mixed microbes, the root surface area of *X. sorbifolium* seedlings increased by 47.63, 36.65, 10.35 and 54.42%, respectively, and the root volume increased by 59.97, 36.15, 58.83 and 60.72%. At different times and under different degrees of drought, inoculation with various microbial agents also resulted in varying degrees of improvement in biomass, root length, and other indicators, but had a weak effect on changing root configuration such as root tip and root bifurcation. Overall, the growth promoting effect of various microbial agents is the best under mild drought, with a microbial effect of mixed microbes > *Diversispora versiformis* > *F. mosseae* > *R. intraradices*.

Introduction

Drought is one of the key factors constraining agricultural, forestry and economic development (Aybuke *et al.* 2024). Improving the growth of plants in arid regions is of great significance for the development of agriculture and forestry in these areas. Root system is an important organ for providing water and nutrients to plants, while root morphology, such as root length, biomass, and root diameter, can change significantly under drought stress. Arbuscular mycorrhiza is a special structure formed by some fungi in the soil and the root system of higher plants, among which Arbuscular mycorrhizas (AM) are widely present, and arbuscular mycorrhizal fungi can form a symbiotic relationship with 70% of terrestrial plants (Kusakabe and Yamato 2023). Colonization by AM fungi can promote the accumulation of nutrients in the plant body through the improvement of plant photosynthesis and the alleviation of oxidative stress (Gupta *et al.* 2021). And additionally, the conclusion that AM fungi increase plant resistance to abiotic and biotic stresses has also been well recognized (Sophie *et al.* 2015). Research shows that inoculation of AM fungi in *Morus alba* under rocky desertified soil conditions can improve root morphology and thus promote plant growth (Zhang *et al.* 2023).

Xanthoceras sorbifolium is a small tree or shrub of the *Sapindaceae* family, mainly distributed in the Loess Plateau and Inner Mongolia Plateau (Wu *et al.* 2021). With its well-developed root system and superb resistance, it is an important soil and water conservation tree species in northern China, and due to the high nutritional and medicinal value of the oil extracted

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from its (Yao *et al.* 2013). Extensive research has been reported on the extraction and application of chemical components, seed sources, and genetics of *X. sorbifolium* (Liu *et al.* 2014, Wang and Li 2022, Miao *et al.* 2023). Currently, studies on drought resistance of *X. sorbifolium* are mainly focused on photosynthesis of the above-ground part, proteins and gene regulation, and few studies have been reported on the research resistance of AM fungi on *X. sorbifolium* (Du *et al.* 2021, Li *et al.* 2021, Li *et al.* 2022). Up to now, the changes in root morphology of *X. sorbifolium* seedlings inoculated with AM fungi under drought stress and the drought resistance of *X. sorbifolium* seedlings have not been clarified.

Therefore, this study takes *X. sorbifolium* as the research object, measures the root system indicators and growth physiological indicators of *X. sorbifolium* seedlings inoculated with four types of fungi under drought stress, explores the mechanism of AM fungi improving the drought resistance of *X. sorbifolium* seedlings, and provides theoretical basis for the subsequent application of AM fungi in *X. sorbifolium* seedling cultivation in arid areas.

Materials and Methods

The test seeds were provided by the planting base of *X. sorbifolium* in Otog Front Banner. Seeds with full grains and uniform size were selected, and germination was carried out by soaking seeds in warm water. The test strains were purchased from the Yangtze University, and selected from *F. mosseae*, *D. versiformis*, *R. intraradices*, and mixed microbes (*R. intraradices*: *F. mosseae*: *D. versiformis* =1:1:1), Dried whole cultures obtained from *Medicago* pot expansion with 120-100/10g active spores.

The culture container was a 160 mm × 120 mm × 120 mm (caliber × ground diameter × pot height) pot, which was sterilized with potassium permanganate solution (1%) before use. The culture substrate was made from a mixture of charcoal soil, vermiculite and perlite at a ratio of 5:3:1 (v/v), and the substrate was autoclaved at 121°C for 2 hrs, cooled down and then loaded into pots at 1 kg per pot.

Table 1. Physicochemical characteristics of culture substrates.

Organic matter (g/k)	Total nitrogen (g/k)	Total phosphorus (g/k)	Potassium (g/kg)	pH
38.82	2.86	1.34	22.35	6.65

The experiment was a two-factor treatment of inoculation with AM fungi and drought stress, with factor I being the AM fungi inoculation treatment group, *Rhizophagus intraradices* (Ri), *Funneliformis mosseae* (Fm), *Diversispora versiformis* (Dv), and mixed strain mycorrhizal fungi (*Rhizophagus intraradices*+*Funneliformis mosseae*+*Diversispora versiformis*, abbreviated as RFD) and no inoculation control (CK); factor two was drought stress treatment group, normal water supply W1 (75-80% of water holding capacity in the field), mild drought W2 (55-60% of water holding capacity in the field), moderate drought W3 (40-45% of water holding capacity in the field), and severe drought W4 (25-30% of water holding capacity in the field), with a total of 20 treatments, and each treatment was replicated three times totaling 60 pots.

The experiment was conducted on April 13, 2023 in the greenhouse of College of Forestry, Inner Mongolia Agricultural University. Seeds that had been cracked were sown in pots and covered with 4-5 cm of soil, and 25 days after seedling emergence, lateral roots were observed to emerge from *X. sorbifolium* seedlings for the inoculation treatment. The inoculation was carried out using the root-dipping method, with 20 g of bacterial agent per pot, and to avoid errors, the

control group was inoculated with equal amounts of sterilized bacterial agent. After inoculation with the fungus agent, a short period of post-emergence routine management was carried out for 2 months, during which Holagland nutrient solution was watered once a week. After the mycorrhizal infestation of *X. sorbifolium* seedlings was observed, the drought stress was carried out by weighing method, and the relative water content of the soil was weighed every two days to ensure that the relative water content of the soil was constant, and the indexes were measured after 30 and 60 d of drought stress.

Five plants were randomly selected after the end of drought stress, and 10 sections of fine roots were randomly selected from each plant, cut into 1cm root segments. Using the trypan blue staining method for staining and sectioning, and the infestation was observed using a light microscope and counted (Giovannetti *et al.* 1980).

$$\text{Mycorrhizal infestation rates(\%)} = \frac{\text{Number of infested root segments}}{\text{Total number of root segments tested}} \times 100\%$$

The plants were left back to the laboratory, rinsed and cleaned, and then weighed the fresh weight of the aboveground (stem and leaves) and root system, respectively, after which they were put into the oven at 105°C for 10 min to kill the green, dried at 80°C to a constant weight, weighed the dry weight. The soil attached to the root system of *Xanthoceras sorbifolium* seedlings was removed and rinsed with distilled water, the surface water was absorbed with absorbent paper, the root images were scanned with Epson Expression 10000XL Root Scanner, and the root surface area, root volume, root length, and the number of root bifurcations were determined using WinRHIZO 2012 Root Analysis System software.

Results and Discussion

As shown in Fig. 1, it is a schematic diagram of the spore, hyphae, vesicle, and bundle branch structures of the Wenguan fruit root system after infection. In the early stage of the experiment (30d), compared with the normal water supply, the infection rates of Dv, Ri, Fm and RFD under mild drought increased by 75.00, 92.00, 40.54 and 38.89%, respectively. In the later stage of the experiment (60d), compared with the normal water supply, the infection rates of Dv, Ri and RFD under mild drought increased by 18.75, 60.87 and 37.72%, respectively, and the infection rate of Fm did not change (Fig.2). Compared with normal water supply, the mycorrhizal infestation rate of each fungus increased significantly under mild drought, this phenomenon also appeared in the flooding experiment about soybeans, and it was found that excess moisture reduced the colonization rate of AM fungi (Hattori *et al.* 2013, Rani *et al.* 2024), which may be related to the fact that AM fungi are unfavorable for mycelial invasion point generation at high soil moisture and that seedlings can obtain sufficient water without relying on mycorrhizae under sufficient moisture conditions. In the later stage of the experiment, the infection rate of each agent can reach 88%. And with the duration of drought, the infection rate of AM fungi to *X.sorbifolium* showed an increasing trend.

The root biomass and aboveground biomass of *X. sorbifolium* seedlings increased first and then decreased with the increase of drought stress, but the inoculation of AM fungi promoted the growth of aboveground biomass and root biomass under the same drought stress (Table 2). In the early stage of the experiment (30 d), under normal water supply, compared with CK, inoculation of Dv, Ri, Fm and RFD increased the aboveground biomass by 54.05, 40.54, 54.05 and 83.78%, respectively. Root biomass increased by 45.00, 38.33, 44.17 and 55.00%, respectively. In the late stage of the experiment (60 d), under normal water supply conditions, compared with CK,

inoculation of Dv, Ri, Fm and RFD increased aboveground biomass by 112.90, 35.48, 90.32 and 138.71%, respectively; under normal water supply, compared with CK, the root biomass increased by 108.41, 81.31, 87.85 and 114.95% after inoculation with Dv, Ri, Fm and RFD, respectively. The growth promoting effect of each bacterial agent under mild drought was the best, and the promoting effect of RFD was the most significant. The promotion effect of each fungus on root growth is superior to that on aboveground biomass, which is consistent with the research results of Tian *et al.* (2023), Duan *et al.* (2024).

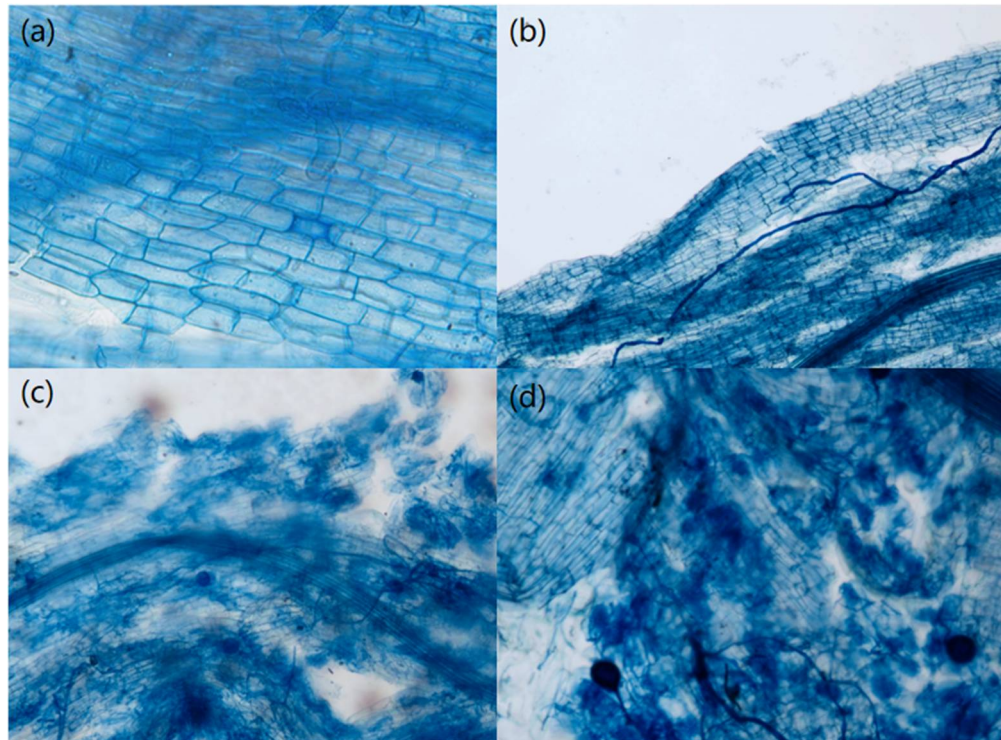


Fig. 1. Root system of AM fungi - invaded *X. sorbifolium* seedlings. (a) uninoculated *X. sorbifolium* seedling root system; (b) mycelium; (c) vesicles; (d) spores and clusters.

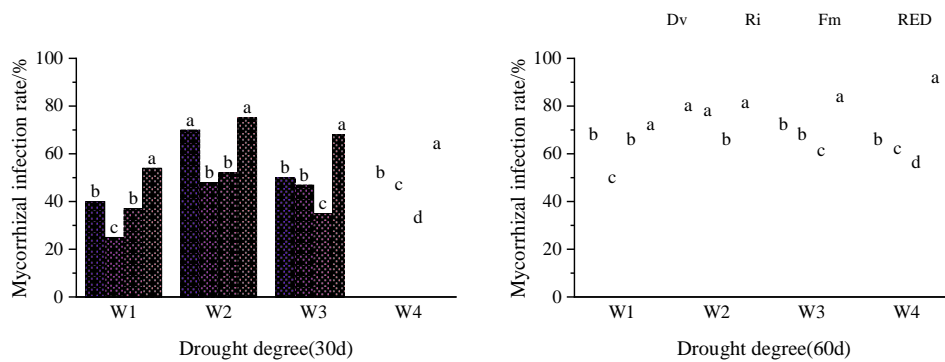


Fig. 2. Effects of drought stress on mycorrhizal infection rate of *X. sorbifolium* seedlings.

Table 2. Changing pattern of biomass of *X. sorbifolium* seedlings inoculated with AM fungi under drought stress.

Drought	Fungicide	aboveground biomass (30d)/g	aboveground biomass (60d)/g	root biomass (30d)/g	root biomass (60d)/g
W1	CK	0.37±0.04c	0.31±0.05d	1.20±0.25b	1.07±0.03b
	Ri	0.57±0.10b	0.66±0.00ab	1.74±0.35ab	2.23±0.35a
	Dv	0.52±0.04b	0.42±0.08c	1.66±0.02ab	1.94±0.13a
	Fm	0.57±0.04b	0.59±0.08b	1.73±0.52ab	2.01±0.47a
	RFD	0.68±0.01a	0.74±0.01a	1.86±0.10a	2.30±0.24a
W2	CK	0.39±0.04b	0.34±0.04b	1.00±0.16c	0.90±0.06b
	Ri	0.60±0.06ab	0.52±0.06ab	1.86±0.45ab	2.12±0.43a
	Dv	0.55±0.02ab	0.50±0.06ab	1.51±0.11b	1.98±0.54a
	Fm	0.60±0.20ab	0.50±0.17ab	1.56±0.17b	1.95±0.35a
	RFD	0.71±0.16a	0.61±0.13a	2.10±0.16a	2.25±0.44a
W3	CK	0.24±0.02c	0.28±0.03c	0.76±0.53b	0.64±0.04b
	Ri	0.49±0.03b	0.47±0.10b	1.33±0.17a	1.56±0.63a
	Dv	0.43±0.07b	0.44±0.04b	1.22±0.29a	1.49±0.27a
	Fm	0.38±0.07bc	0.35±0.05b	1.18±0.15a	1.47±0.05a
	RFD	0.62±0.10a	0.48±0.01a	1.43±0.03a	1.85±0.07a
W4	CK	0.22±0.02b	0.22±0.04c	0.50±0.04c	0.40±0.03b
	Ri	0.421±0.07a	0.43±0.06a	1.15±0.12ab	1.38±0.49a
	Dv	0.39±0.04a	0.40±0.02ab	1.06±0.15ab	1.26±0.04a
	Fm	0.39±0.04a	0.29±0.06bc	0.95±0.12b	1.00±0.18a
	RFD	0.47±0.09a	0.43±0.11a	1.32±0.31a	1.41±0.03a

Different lowercase letters indicate variability between treatments with the different bacterial agent at the same moisture level ($P < 0.05$).

The root length of *X. sorbifolium* seedlings increased first and then decreased with the increase of drought degree (Fig. 3). Inoculation of AM fungi promoted the increase of root length under the same drought stress. In the early stage of the experiment (30d), compared with CK, the total root length increased by 109.37, 34.82, 83.38 and 116.57%, respectively after inoculation with Dv, Ri, Fm and RFD under normal water supply. Under mild drought, Dv, Ri, Fm and RFD increased by 62.01, 27.19, 54.68 and 76.92%, respectively. At the late stage of the experiment (60d), compared with CK, the root length increased by 138.69, 79.94, 135.84 and 146.19%, respectively after inoculation with Dv, Ri, Fm and RFD under normal water supply. Under mild drought, Dv, Ri, Fm and RFD increased by 84.73, 56.04, 52.45 and 91.67%, respectively. On the whole, RFD had a better effect on the growth of root length of *X. sorbifolium*, and the effect of AM fungi on root growth was more obvious with the increase of stress time.

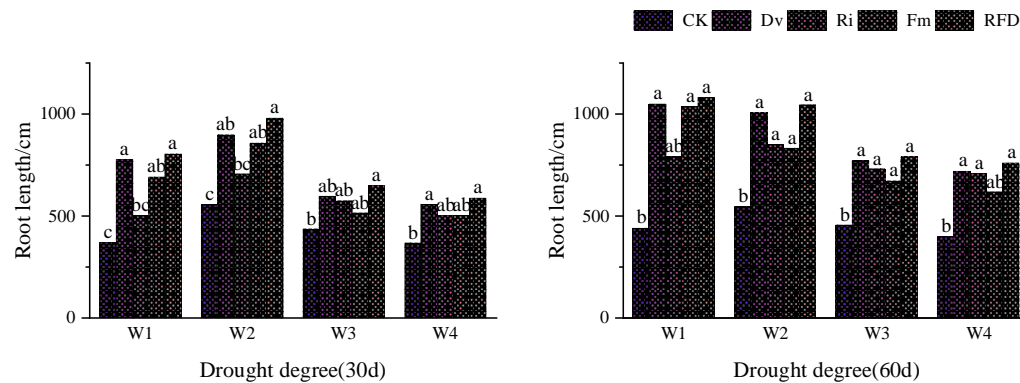


Fig. 3. Effects of AM fungi on root length of *X. sorbifolium* seedlings under drought stress.

The root surface area of *X. sorbifolium* seedlings increased first and then decreased with the increase of drought degree (Fig. 4). Inoculation of AM fungi promoted the increase of root surface area under the same drought stress. In the early stage of the experiment (30d), compared with CK, the root surface area of Dv, Ri, Fm and RFD increased by 70.03, 41.52, 73.14 and 91.74%, respectively under normal water supply. Under mild drought, inoculation Dv, Ri, Fm and RFD increased by 66.41, 28.02, 82.28 and 95.37%, respectively. In the later stage of the experiment, Dv, Ri, Fm and RFD significantly promoted the increase of root surface area under normal water supply. RFD had a better growth-promoting effect on the root surface area of *X. sorbifolium*, and the growth-promoting effect of AM fungi on the root system was more obvious with the increase of stress time.

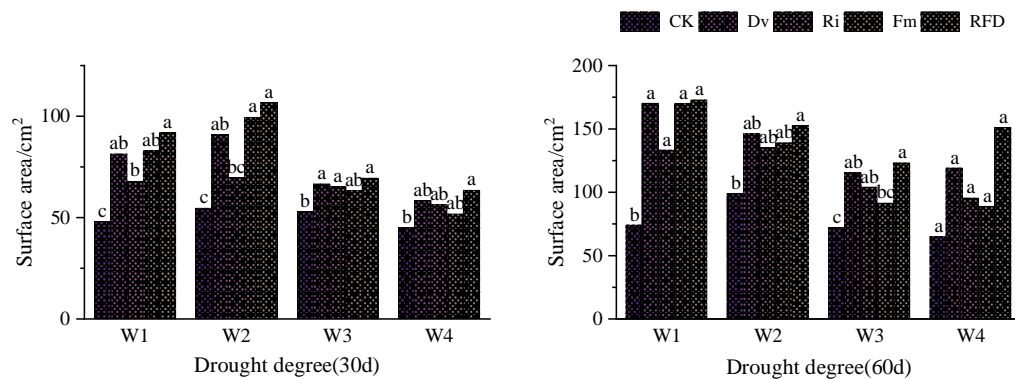


Fig.4. Effects of AM fungi on root surface area of *X. sorbifolium* seedlings under drought stress.

The root volume of *X. sorbifolium* seedlings increased first and then decreased with the increase of drought stress, and the inoculation of AM fungi promoted the increase of root volume under the same drought stress (Fig. 5). In the early stage of the experiment (30 d), Dv, Fm and RFD significantly promoted the increase of root volume under normal water supply, mild drought and moderate drought, and RFD had the best growth-promoting effect on root volume. At the late stage of the experiment (60 d), compared with CK, the root volume increased by 148.47, 97.18, 121.01 and 159.31% after inoculation with Dv, Ri, Fm and RFD under normal water supply.

Under mild drought, Dv, Ri, Fm and RFD increased by 59.97, 36.15, 58.83 and 60.72%, respectively. At the later stage of the experiment, Dv, Ri, Fm and RFD significantly promoted the growth of root volume under normal water supply and severe drought, among which RFD had the best growth-promoting effect, followed by Dv. Compared with the early stage of the experiment, the root volume of *X. sorbifolium* seedlings increased in the later stage of the experiment. Under severe drought, the root volume of CK treatment increased by 66.67 % in the late stage of the experiment compared with the early stage of the experiment, and the inoculation of Dv, Ri, Fm and RFD increased by 121.13, 119.18, 141.42 and 98.79%, respectively.

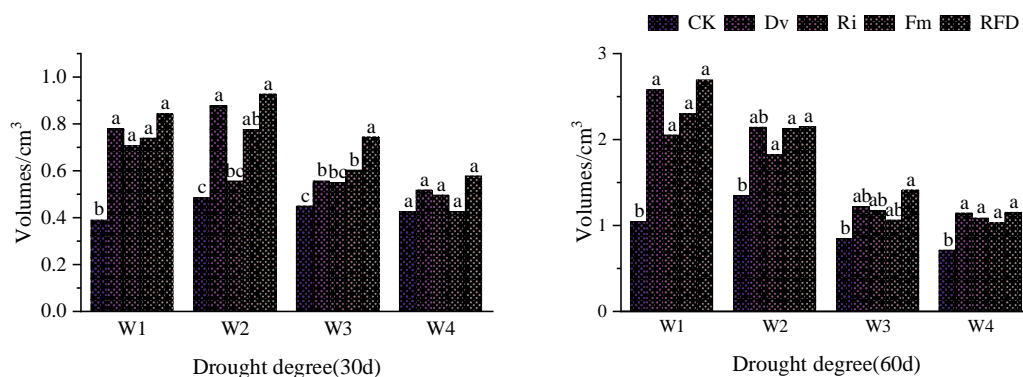


Fig. 5. Effects of AM fungi on root volume of *X. sorbifolium* seedlings under drought stress.

The root tip of *X. sorbifolium* seedlings increased first and then decreased with the increase of drought stress, and the inoculation of AM fungi promoted the increase of root tip number under the same drought stress (Fig. 6). In the early stage of the experiment (30d), Under mild drought, Dv, Ri, Fm and RFD increased by 34.17, 18.94, 19.21 and 38.07%, respectively. Dv, Fm and RFD significantly increased the number of root tips under normal water supply, mild drought and moderate drought, and RFD had the best growth-promoting effect on root tips. In the late stage of the experiment (60d), under normal water supply, compared with CK, the number of root tips increased by 14.14, 24.96, 25.42 and 66.37%, respectively after inoculation with Dv, Ri, Fm and RFD, respectively. Under mild drought, Dv, Ri, Fm and RFD increased by 24.01, 20.25, 17.88 and 72.39%, respectively. RFD significantly increased the number of root tips. Compared with the early stage of the experiment, the number of root tips of *X. sorbifolium* seedlings increased in the later stage of the experiment. Under severe drought, the number of root tips of CK treatment increased by 37.45 % in the late stage of the experiment compared with the early stage of the experiment, and the inoculation Dv, Ri, Fm and RFD increased by 50.79, 75.54, 51.71 and 65.56%, respectively. Overall, AM fungi had no significant effect on the increase of root tip number of *X. sorbifolium* seedlings under drought stress, while RFD had a better effect on the growth of root tip number of *X. sorbifolium* seedlings.

The root bifurcation of *X. sorbifolium* seedlings increased first and then decreased with the increase of drought stress (Fig. 7). Under the same drought stress, inoculation with AM fungi promoted the increase of root bifurcation number. In the early stage of the experiment (30 d), compared with CK, the number of root bifurcations increased by 56.47, 22.40, 43.90 and 85.49%, respectively after inoculation with Dv, Ri, Fm and RFD under normal water supply. Fm and RFD significantly increased the number of root bifurcations under normal water supply and mild

drought, and RFD had the best effect on the number of root bifurcations. In the late stage of the experiment (60 d), under normal water supply, compared with CK, the number of root bifurcations increased by 74.06, 65.41, 38.50 and 94.99%, respectively after inoculation with Dv, Ri, Fm and RFD, respectively. Compared with the early stage of the experiment, the effect of AM fungi on increasing the root bifurcation number of *X. sorbifolium* seedlings was enhanced in the later stage of the experiment, but on the whole, the change of root bifurcation number was small.

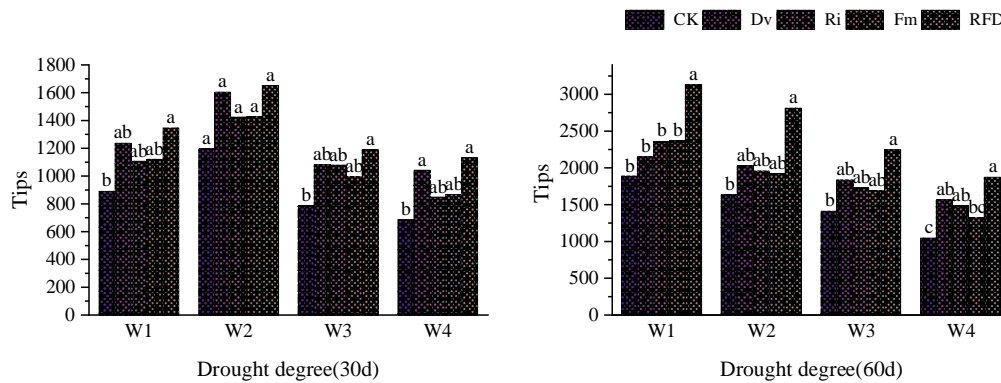


Fig. 6. Effects of AM fungi on root tip number of *X. sorbifolium* seedlings under drought stress.

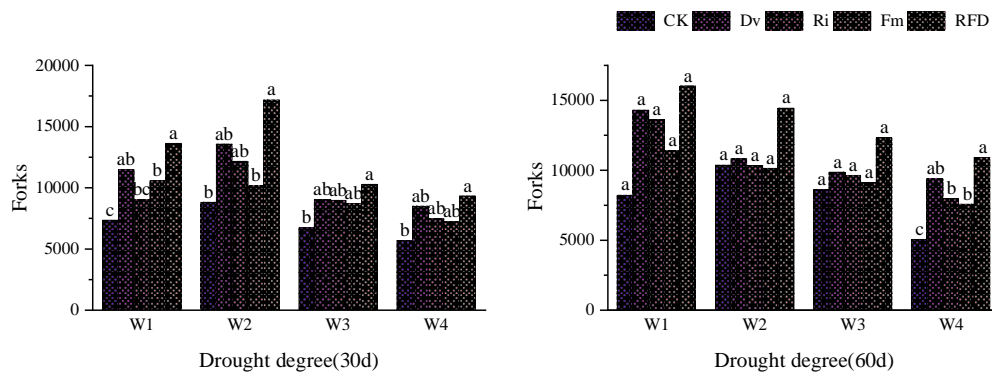


Fig. 7. Effects of AM fungi on root bifurcation number of *X. sorbifolium* seedlings under drought stress.

The root density of *X. sorbifolium* seedlings increased first and then decreased with the increase of drought stress, and the inoculation of AM fungi promoted the increase of root density under the same drought stress (Fig. 8). In the early stage of the experiment (30 d), Under mild drought, Dv, Ri, Fm and RFD increased by 39.69, 35.04, 38.78 and 81.86%, respectively. Dv, Fm and RFD significantly promoted the increase of root density under normal water supply, mild drought and moderate drought, and RFD had the best effect on root density. At the late stage of the experiment (60 d), compared with CK, the root density increased by 72.96, 11.77, 54.01 and 79.68%, respectively after inoculation with Dv, Ri, Fm and RFD under normal water supply. Under mild drought, inoculation with Dv, Ri, Fm and RFD increased by 90.63, 49.67, 82.00 and 105.27%, respectively. With the increase of drought stress, AM fungi promoted the increase of

root density of *X. sorbifolium* seedlings, but on the whole, the increase of root density of *X. sorbifolium* seedlings was small. Under severe drought, the root volume of CK treatment increased by 0.18% in the later stage of the experiment compared with that in the early stage of the experiment, and the inoculation of Dv, Ri, Fm and RFD increased by 8.09, 0.93, 22.99 and 21.62%, respectively.

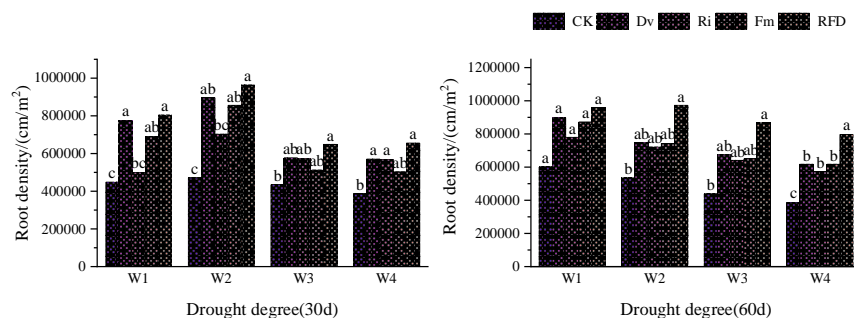


Fig. 8. Effects of AM fungi on root density of *X. sorbifolium* seedlings under drought stress.

As analyzed (Fig. 9), with the deepening of drought, the proportion of 0~0.2 mm in the root system of *X. sorbifolium* seedlings in each fungicide treatment group showed an overall trend of increasing and then decreasing, and reached the maximum value under W2 conditions. Under different drought stresses, the 0-0.2 mm root system of CK treatment was smaller than that of other treatments, and the percentage of 0-0.2 mm root system of CK treatment was higher than that of other treatments in W2 treatment. The 0.2~0.5, 0.5~1 and >1 mm root systems of *X. sorbifolium* under each fungicide treatment showed an increasing trend with the deepening of drought. Under the same drought stress, the proportion of 0-0.2 mm roots in W2 and W3 of AM fungi inoculated *X. sorbifolium* was smaller than that in CK treatment, and the proportion of 0.2-0.5 mm and 0.5-1 mm roots was higher than that in CK treatment. This is consistent with the research results on *Gleditsia sinensis* Lam, and fully proved that AM fungi could improve plant drought resistance by adjusting the root structure (Ma *et al.* 2022).

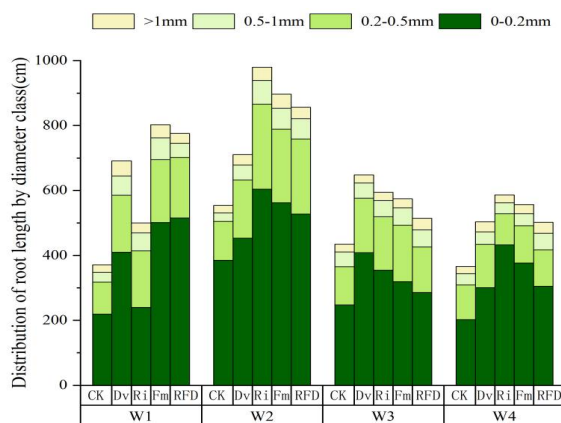


Fig. 9. Effects of AM fungi on root length of different diameter classes of *X. sorbifolium* seedlings under drought stress.

Table 3. Affiliation function values of various indexes of *X. sorbifolium* seedlings under drought stress.

Treatment	Ab	Rb	RL	RSA	RV	RT	RF	RD	Total
W1	CK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Dv	0.65	0.82	0.94	0.76	0.86	0.76	0.66	0.92
	Ri	0.48	0.70	0.30	0.45	0.70	0.47	0.26	0.15
	Fm	0.65	0.80	0.74	0.80	0.77	0.50	0.51	0.68
	RFD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
W2	CK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Dv	0.66	0.78	0.81	0.70	0.89	0.90	0.57	0.86
	Ri	0.50	0.46	0.35	0.29	0.16	0.50	0.40	0.47
	Fm	0.66	0.51	0.71	0.86	0.66	0.50	0.16	0.78
W3	RFD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	8.00
	CK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Dv	0.66	0.85	0.75	0.83	0.37	0.73	0.64	0.67
	Ri	0.51	0.69	0.66	0.76	0.34	0.73	0.63	0.66
	Fm	0.37	0.63	0.37	0.63	0.52	0.51	0.56	0.36
W4	RFD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	8.00
	CK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Dv	0.80	0.79	0.86	0.72	0.60	0.79	0.77	0.69
	Ri	0.68	0.68	0.62	0.63	0.46	0.36	0.49	0.68
	Fm	0.68	0.55	0.62	0.37	0.00	0.41	0.43	0.43
RFD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	

Select eight indexes of aboveground biomass (Ab), root biomass (Rb), root length (RL), root surface area (RSA), root volume (RV), root tip (RT), root bifurcation (RF) and root density (RD) to evaluate the comprehensive drought resistance of *X. sorbifolium* seedlings after inoculation with different AM fungi (Table 3). The drought resistance of *X. sorbifolium* seedlings inoculated with each agent under different drought stress was slightly different, but it was significantly higher than that of CK treatment. In general, the membership function value under RFD inoculation was 8, and the drought resistance of *X. sorbifolium* seedlings was better, followed by Dv inoculation.

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