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RESEARCH ARTICLE

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Effects of vesicular-arbuscular mycorrhizae on the drought resistance of wild jujube (*Zizyphs spinosus* Hu) seedlings

Abstract The current study explored the effects of vesicular-arbuscular mycorrhizae (VAM) inoculation on the growth and water requirement of pot-grown wild jujube (*Zizyphs spinosus* Hu). Three water regimes (20%, 40% and 60% of soil water content) were conducted. The VAM inoculation could significantly increase plant growth (including plant height, leaf area, and fresh and dry mass), enhance relative leaf water content, photosynthetic rates, transpiration rates and stomatal conductance, and improve plant drought tolerance. The water consumption of the mycorrhizal plants producing 1 g of dry matter was 18.7%–26.6% lower than the consumption of non-mycorrhizal plants grown under the same soil water content conditions.

Keywords VA mycorrhizae, wild jujube seedlings, drought resistance, growth

1 Introduction

Vesicular-arbuscular mycorrhizal fungi (VAM, *Gloums versiforme*) can be associated with the roots of most plants and can strongly impact water retention properties and the subsequent drought responses of its hosts. Previous work indicated that mycorrhizal-associated citrus rootstocks exhibited higher root hydraulic conductivity than non-mycorrhizal plants, resulting in enhanced drought resistance (Graham and Syvertsen, 1984). The effects of VAM on water absorption and utilization in apple, cherry and birch-leaf pear trees were studied under normal water status and drought-stressed conditions (Liu and Lou, 1988; Liu, 1989a; Liu, 1989b; Wu et al., 2006). Under normal water conditions, VAM enhanced the relative leaf water content and transpiration rates, and decreased stomatal resistance in apple and cherry trees. Under drought conditions, VAM also enhanced stomatal conductance, transpiration rate and relative leaf water content, and decreased leaf water potential and the permanent wilting point. When mycorrhizal and non-mycorrhizal plants were watered under continuous drought conditions, the pressure inflation of mycorrhizal plants recovered fast and the plants exhibited an enhanced growth compared with the non-mycorrhizal plants (Liu and Lou, 1988; Liu, 1989a; Liu, 1989b; Kaya et al., 2003; Pinior et al., 2005; Wu et al., 2006).

Jujube trees, also known as Chinese date trees, are fruit trees native to China that have been cultivated for over 4 000 years. Wild jujube (*Zizyphs spinosus* Hu) seedlings are currently used by Chinese jujube breeders as stock plants. Although jujubes are relatively drought-resistant, regular access to water sources ensures adequate fruit production. Previous research indicated that VAM could improve drought-resistance in fruit trees, in the current study, wild jujube seedlings were used to study the mechanism underlying drought tolerance and water retention in seedlings inoculated with VAM under various soil water content conditions. The findings herein provided a rationale for applying VA mycorrhizae to Chinese jujube trees.

2 Materials and methods

The mycorrhizal inoculum, *Glomus mosseae* (Nicol. Et Gerd.) Gerdemann et Trappe, was kindly provided by the College of Resources and Environment, China Agricultural University. The inoculum was obtained from pot cultures of corn inoculated with *Glomus mosseae*. Seeds of wild jujube (*Zizyphs spinosus* Hu) were surface-sterilized for 3 min in 0.1% $HgCl_2$ solution, then washed three times in water and soaked for 24 h in water at 40°C. Seeds were subsequently germinated at 28°C in an incubator.

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Water tests were conducted in plastic test pots, each containing 2 kg of sandy loam. The loam had an organic matter content of 1.013% at pH of 8.22. The available nitrogen content was 52.92 mg/kg, the available phosphorus content was 3.195 mg/kg and the level of effective kalium was 105.864 mg/kg. In this soil, the maximum water holding capacity was 15%. All soil samples were steam-sterilized at 121°C for 2 h.

Water control began after the seeds were sown. The six treatment groups included inoculated or non-inoculated seeds maintained in soil with a relative water content controlled at 20%, 40% or 60% ($n = 5$ per inoculation and water content group).

All experiments were conducted in a greenhouse in the specimen garden at Agricultural University of Hebei. For the VAM treatment, each pot was treated with 20 g of inoculated medium placed below the seeds. For the non-inoculated treatment, 20 g of inoculated sterilized medium was placed in each pot. Twenty wild jujube seeds were sown in each pot. After the seeds sprouted, ten plants were selected from each pot based on their growth level. During the experiment, the evaporated water per pot was measured daily, and the young seedlings were provided with a specific amount of water so that the relative water content in the soil was maintained at 20%, 40% or 60%, respectively. The extent of root colonization by mycorrhizal fungi was assessed by the straining method of acid fuchsine (Mao et al., 1999).

The plant height, the number of leaves, the fresh weight and dry weight of leaves, stems, and roots per pot were measured. Leaf water saturation deficits were calculated by the saturation water content method. Leaf photosynthetic rates, transpiration rates and stomatal conductance were measured by a photosynthesis surveying instrument. The content of proline in leaves was measured with a Daojin UV-120 spectrometer. Water requirement was calculated through measuring the water consumption yielding 1 g of dry matter (plants) per pot.

3 Results

3.1 Relationship between soil water content and VA mycorrhizal colonization

No colonization occurred in non-mycorrhizal wild jujube seedlings. In the VAM-inoculated plants, 100% of roots were colonized when the relative water content of the soil was at 60%. When the relative water content was decreased, the percentage of VAM colonization likewise decreased. Specifically, the VAM colonization of plants at 20% water content was less than that of plants at 40% and 60% water capacity (Table 1).

Table 1 Mean percentage of mycorrhizal colonization by *Glomus mosseae* of wild jujube seedlings under various treatment conditions

Inoculation	Relative water content of soil/%	Percentage of mycorrhizal colonization/%
+VAM	20	93.1b
+VAM	40	99.1a
+VAM	60	100a
−VAM	20	0
−VAM	40	0
−VAM	60	0

Note: +VAM = inoculated by VA mycorrhizal fungi, −VAM = non-inoculated by VA mycorrhizal fungi; mean separation of water treatments was analyzed by Duncan's multiple range test; means followed with different letters indicate significantly different ($P \leqslant 0.05$).

3.2 Effects of VA mycorrhizae wild jujube seedling growth under varying soil water content

The plant height, the number of leaves, and the fresh weight and dry weight of mycorrhizal-inoculated plants were greater than those of the non-mycorrhizal-inoculated plants grown under the same soil water content conditions (Tables 2 and 3). Thus, the VAM significantly promoted wild jujube seedling growth. The growth of mycorrhizal plants grown in 20% water content did not differ significantly from the growth of non-mycorrhizal plants grown at 60% soil water content. When compared with the non-mycorrhizal plants, the dry weight of mycorrhizal plants increased by 111%, 162% or 240% at soil water contents of 20%, 40% or 60%, respectively.

Table 2 Effect of VAM on the growth of wild jujube seedlings under various soil water contents

Inoculation	The relative water content of soil/%	Plant height/cm	Numbers of leaves
+VAM	20	9.73c	12.52c
+VAM	40	13.81b	19.34b
+VAM	60	16.91a	28.47a
−VAM	20	7.10d	8.64d
−VAM	40	9.59c	12.95c
−VAM	60	10.56c	14.05c

Note: +VAM = inoculated by VA mycorrhizal fungi, −VAM = non-inoculated by VA mycorrhizal fungi; mean separation of water treatments was analyzed by Duncan's multiple range test; means followed by unlike letters differ significantly ($P \leqslant 0.05$).

3.3 Effects of VAM on the water requirements of wild jujube seedlings

Mycorrhizal wild jujube seedlings required less water than the non-mycorrhizal plants to produce 1 g of dry matter. Specifically, when compared with the non-mycorrhizal plants, mycorrhizal plants required less water at 18.7%, 22.7% or 26.6% when grown in soil with a relative water content of 20%, 40% or 60%, respectively (Fig. 1). These findings indicate that the VA mycorrhizae enhanced the water utilization efficiency.

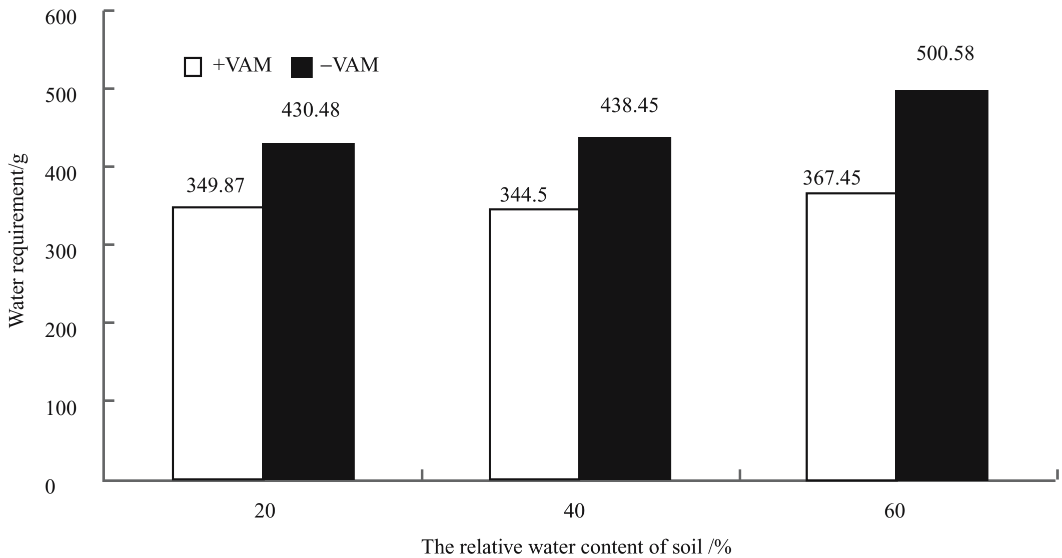
3.4 Effects of VAM on water physiology of wild jujube seedlings

Leaf water saturation deficits in the mycorrhizal plants were lower than in the non-mycorrhizal plants grown under identical water content conditions. When the relative soil

Table 3 Effect of VAM on the biomass of wild jujube (*Zizyphs spinosus* Hu) seedlings under various soil water contents (g/pot)

Inoculation	The relative water content of soil/%	Leaf		Shoot		Root		Plant DW
		FW	DW	FW	DW	FW	DW	
+VAM	20	3.67c	1.49bc	1.35c	0.82c	6.36c	2.88c	5.18c
+VAM	40	7.87b	2.94b	3.39b	2.11b	15.7b	7.17b	12.22b
+VAM	60	11.59a	4.65a	4.96a	3.22a	26.58a	10.44a	18.32a
−VAM	20	1.82d	0.8d	0.74d	0.45d	2.68c	1.2 d	2.45d
−VAM	40	3.19c	1.22cd	1.21c	0.75d	4.74c	2.69c	4.66c
−VAM	60	3.79c	1.39c	1.55c	0.91c	6.74c	3.08c	5.38c

Note: +VAM = inoculated by VA mycorrhizal fungi, −VAM = non-inoculated by VA mycorrhizal fungi; mean separation of water treatments was analyzed by Duncan's multiple range test; means followed by unlike letters differ significantly ($P \leqslant 0.05$).



Note: +VAM = inoculated by VA mycorrhizal fungi; −VAM = non-inoculated by VA mycorrhizal fungi.

Fig. 1 The amount of water required to produce 1 g of dry matter in VAM and non-VAM wild jujube seedlings grown in the relative soil water content of 20%, 40% and 60%, respectively

water content was at 20%, this difference was statistically significant. The concentration of proline in leaves increased as the soil water content decreased. Furthermore, proline concentrations in the mycorrhizal plant leaves were significantly lower than in the non-mycorrohizal plant leaves (Table 4). These results suggest that, under drought-stressed conditions, water processing in the leaves of the mycorrhizal plants was more efficient than in the leaves of non-mycorrhizal plants. Notably, when the relative soil water content was 40% or 60%, the stomatal conductance in the mycorrhizal plants was significantly higher than that in the non-mycorrhizal plants. When the relative soil water content was at 20%, the stomatal conductance in mycorrhizal plants was still higher than that in the non-mycorrhizal plants, but the difference did not reach significance. Furthermore, when compared with the non-mycorrhizal plants grown under the same water content conditions, the mycorrhizal plants exhibited higher net photosynthetic and transpiration rates (Table 4).

Table 4 Effect of VAM on water physiology and photosynthetic rates of wild jujube seedlings

Inoculation	Relative water content of soil/%	Leaf water saturation deficit/%	Concentration of proline in leaves/%	Stomatalconductance /($mmol \cdot m^{-2} \cdot s^{-1}$)	Transpiration rates/($mmol \cdot m^{-2} \cdot s^{-1}$)	Net photosynthetic rates/($\mu mol \cdot m^{-2} \cdot s^{-1}$)
+VAM	20	11.03b	0.0386b	22.10b	1.88b	1.36b
+VAM	40	8.10b	0.0230c	40.32a	3.16a	4.93ab
+VAM	60	5.34b	0.0155c	46.04a	3.62a	8.97a
−VAM	20	29.96a	0.1521a	10.60b	1.17c	−2.46c
−VAM	40	14.40b	0.0630b	18.50b	1.98b	−1.92c
−VAM	60	7.37b	0.0571b	20.22b	2.33b	1.36b

Note: +VAM = inoculated by VA mycorrhizal fungi, −VAM = non-inoculated by VA mycorrhizal fungi; mean separation of water treatments was analyzed by Duncan's multiple range test; means followed by unlike letters differ significantly ($P \leqslant 0.05$).

4 Discussion

Under suitable water conditions, 100% of wild jujube (*Zizyphs spinosus* Hu) seedlings were colonized by *Glomus mosseae*. Even when grown under serious drought conditions, the percentage of colonized seedlings remained above 93.1%. These results indicate that when wild jujube seedlings are inoculated with *Glomus mosseae*, they are highly susceptible to colonization. These findings are consistent with the previous conclusion from field studies reporting a high natural occurrence of VA mycorrhizal fungi in Chinese jujube trees (Liu, 1989). The ease by which wild jujubes and Chinese dates are colonized by VAM fungi may explain, in part, the ability of these trees to resist the damage from stressed conditions.

The VAM can significantly increase wild jujube seedling growth. When the relative soil water content was at 20%, the mycorrhizal plant dry weight was 111% of the weight of non-mycorrhizal plants. Moreover, when the relative soil water content was 60%, the mycorrhizal plant dry weight was 240% of the weight of non-mycorrhizal plants. This effect of mycorrhizae on the growth of wild jujube seedlings is more striking than its impact on the growth of other plants, such as trifoliate orange, peach and *Avena sativa* (Barea and Azcón-Aguliar, 1982; Dutra et al., 1996; Khan et al., 2003).

The fresh weight and the dry weight of mycorrhizal plants grown in the condition of 20% water content were not significantly different from the weights of non-mycorrhizal plants grown in that of 40% water content. Thus, the effects of VAM inoculation were equivalent to increasing the water content of the soil by 20%. It is clear that the VA mycorrhizal fungi inoculation is an effective measure to enhance drought resistance in Chinese dates.

Proline is a key osmoregulatory element in plants experiencing conditions of water stress. Under some conditions, various plants produce a large amount of proline to enhance osmosis and prevent dehydration. Thus, the quantity of proline produced can reflect the degree of water stress. Our results suggested that the quantity of proline in wild jujube leaves increased as the water content of the soil decreased. When grown under the same water conditions, mycorrhizal plant leaves contained less proline than the non-mycorrhizal plant leaves. Thus, when under the same degree of water deprivation, the mycorrhizal plants are less physiologically stressed than the non-mycorrhizal plants.

Stress from dehydration can cause stomata close, and a decrease in stomatal conductance and transpiration rates in leaves, which may prevent the roots from absorbing and transporting water. This may eventually result in a decrease in net photosynthetic rates. The findings from the current study indicated that VA mycorrhizae may improve stomatal conductance and transpiration rates in leaves, increase net photosynthetic rates and biomass, and significantly enhance drought tolerance in wild jujube seedlings.

Findings in the current literature suggest three mechanisms by which VA mycorrhizae enhances drought tolerance in plants. First, hyphae can absorb soil water directly. Under drought-stressed conditions, hyphae can utilize the soil water that is not accessible by the roots. Thus, the water supply to the plant is improved, effectively enhancing drought tolerance. Second, hyphae can absorb nutrients including phosphorus, zinc and many other elements. In this way, plant nutrition can be improved, and plant growth can be likewise increased. Ultimately, the VA mycorrhizal plants have more roots capable of absorbing water. Finally, VA mycorrhizae can regulate the balance of internal hormones in plants to indirectly influence water metabolism in the affected plant (Jos é Mbarea and Concepci ó n Azc ó n-Aguliar, 1982; Geddeda et al., 1984; Murakami et al., 1991; Dutra et al., 1996).

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