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RESEARCH ARTICLE

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Effects of arbuscular mycorrhizal fungi on leaf solutes and root absorption areas of trifoliate orange seedlings under water stress conditions

Abstract The effects of the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* on plant growth, leaf solutes and root absorption area of trifoliate orange (*Poncirus trifoliata* (L.) Raf.) seedlings were studied in potted culture under water stress conditions. Inoculation with *G. mosseae* increased plant height, stem diameter, leaf area, shoot dry weight, root dry weight and plant dry weight, when the soil water content was 20%, 16% and 12%. AM inoculation also promoted the active and total absorption area of root system and absorption of phosphorus from the rhizosphere, enhanced the content of soluble sugar in leaves and roots, and reduced proline content in leaves. AM seedlings had higher plant water use efficiency and higher drought tolerance than non-AM seedlings. Effects of *G. mosseae* inoculation on trifoliate orange seedlings under 20% and 16% soil water content were more significant than under 12% soil water content. AM infection was severely restrained by 12% soil water content. Thus, effects of AM fungi on plants were probably positively related to the extent of root colonization by AM fungi. The mechanism of AM fungi in enhancing drought resistance of host plants ascribed to greater osmotic adjustment and greater absorption area of root system by AM colonization.

Keywords arbuscular mycorrhizal fungi, trifoliate orange, water stress, osmotic adjustment, absorption area

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1 Introduction

Arbuscular mycorrhizal (AM) symbiosis is the most common underground obligatory association between AM fungi and the roots of many plants and is found in approximately 90% of the Earth's plant species on land (Gadkar et al. 2001). Several studies have been conducted using AM fungi in the search for water relations of host plants (Augé, 2001). Colonization of the root system by AM fungi affords host plants greater resistance to water stress. *Glomus mosseae*-inoculated *Lactuca sativa* plants maintained higher shoot weight, root weight, nutritional levels (e.g. N, P, K, Ca, Mg), photosynthesis and water use efficiency (WUE) compared with non-AM ones under water stress conditions (Ruiz-Lozano et al., 1995). In addition, leaves of mycorrhizal *Ocimum basilicum* plants developed lower full-turgor osmotic potential than those of non-mycorrhizal plants during a lethal drought, which is indicative of greater active osmotic adjustment (Kubikova et al., 2001). However, lethal leaf water potential, lethal soil matric potential and length of the lethal drought episode were not affected by the AM inoculation (Kubikova et al., 2001). There is no doubt that AM colonization improves water relations of host plants, but the mechanisms are in debate, even though most of the effects have been attributed to nutritional changes of plants (Khalvati, 2005).

Citrus is one of the most important commercial fruit crops grown in South and Southwest regions of China. Most of the citrus trees are cultivated at the foothills, and its growth and output are often affected by water deficiency. *Poncirus trifoliata* is the main rootstock of citrus used in China and it is less drought resistant but exhibits better resistance to a cold environment. There are several studies of the effect of AM fungi on growth, photosynthesis and nutritional uptake of citrus trees (Ruiz-Sanchez et al., 1997; Arbona, 2005). However, osmotic adjustment of citrus exposure to water stress has not been examined in relation to AM symbiosis.

The objective of this experiment was to investigate whether AM colonization could modify osmotic adjustment matter and absorption area of root systems in *Poncirus trifoliata* seedlings under water stress conditions.

2 Materials and methods

2.1 Experimental design and statistical analysis

The experiment was laid out in a randomized complete block design. Experimental treatments consisted of factorial combinations of two mycorrhizal treatments (*Glomus mosseae* and non-AM fungus) with three water supply conditions (20%, 16% and 12% soil water content). Five replicates of each treatment (two pots per treatment) were done totalling 60 pots (four seedlings per pot).

All experimental data obtained were subjected to analysis of variance (ANOVA) using SAS (8.1), and means were evaluated by Fisher's protected least significant difference (LSD). Differences at $p<0.05$ were considered significant.

2.2 Soil and biological materials

Soil was collected from the Fruit Sample Garden, Huazhong Agricultural University (Wuhan, China). The mixture of the soil and clean sand (9:1, v/v) was regarded as substrate. The substrate was first air-tightly suffocated by 0.5% formaldehyde to kill native AM fungi of substrate and air-dried after 7 days. Substrate characteristics were as follows: pH 5.61; 8.4 g/kg organic matter; 1.26 g/kg total nitrogen; 11.85 mg/kg avail phosphorus; 31.85 mg/kg avail potassium; 26.3% maximum field capacity.

Seeds of trifoliate orange (*Poncirus trafoliata* (L.) Raf.) were surface sterilized in 75% alcohol for 5 min, and subsequently rinsed with sterilized water and sowed in autoclaved soil on December 7, 2002. Uniform seedlings, containing 3-4 cotyledons, were transferred to plastic pots on April 13, 2003. Each pot was filled with 3.870 kg of substrate.

AM inoculum, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, was obtained from the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences (Beijing, China). Twenty grams of inoculum contained 388 spores and were added to the appropriate pots at transplanting time just below the trifoliate orange seedlings.

2.3 Growth conditions

Trifoliate orange seedlings were grown in a plastic greenhouse, which had no controlling temperature equipments.

Soil water treatments began after 20 days of acclimatization. According to the method of Hsiao (1973), soil water treatments divide into 20% (ample water), 16% (slight water stress) and 12% (moderate water stress) soil water content. The water status in the substrate was daily gravimetrically determined and the amount of water loss was supplied to each pot to keep the designed soil water content.

2.4 Parameter analysis

Plant height and stem diameter were determined when seedlings were harvested on August 23, 2003. Leaf area was performed as described by Chen and Wang (2002). Plant shoots and roots were dried at 70°C for 48 h, and dry weights of shoots and roots were immediately recorded.

Root colonization was assessed on cleared and stained root samples (Phillips and Hayman, 1970). The AM infected percentage was counted by the following formula: AM colonization (%) = 100 × root length infected/root length observed. Mycorrhizal dependency was defined as the ratio of the dry weight of the AM seedlings and non-AM seedlings (Graham and Syvertsen, 1985).

Soluble sugars in leaves and roots were determined according to the anthrone method described by Wu and Xia (2006). Proline in leaves was estimated as recommended by Li (2000).

Absorption area of root system was determined by the procedure of Zhang (2000). WUE was accounted according to total water supply amount. WUE (g/kg) was defined as the ratio of the plant dry weight to the total water supply amount to the plant during water treatments.

The quantity of soil available phosphorus was determined by the method as described by Yan (1988).

3 Results

3.1 AM colonization

Mycorrhizal structure was not found in non-AM seedlings. AM inoculated percentage was 41.44%, 34.39% and 12.82% under conditions of 20%, 16% and 12% soil water content, respectively (Fig. 1). Accompanying the decrease of water content of soil, AM infected percentage was de-

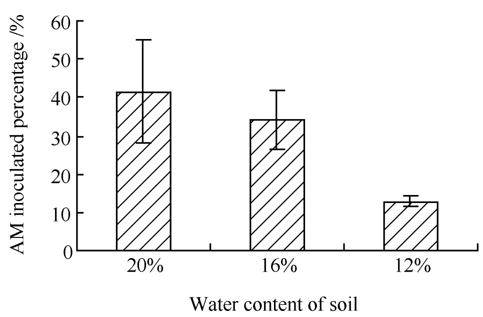

Fig. 1 Effect of AM inoculation and water stress on AM inoculated percentage of trifoliate orange seedlings

creased. No significant differences in AM infected percentage were observed between AM seedlings grown in 20% soil water content and AM seedlings grown in 16% soil water content, whereas AM infected percentage was notably higher in 20% or 16% soil water content than in 12% soil water content.

3.2 Effect of AM fungi on plant growth

Water stress significantly reduced stem diameter (Fig. 2A), plant height (Fig. 2B), leaf area (Fig. 2C), shoot dry weight (Fig. 2D), root dry weight (Fig. 2E) and plant dry weight (Fig. 2F), while AM infection significantly stimulated growth in trifoliate orange seedlings. Plant height, leaf area, stem diameter, shoot dry weight, root dry weight and plant dry weight were significantly higher in AM seedlings than in non-AM seedlings in 20% and 16% soil water content. AM inoculation notably increased shoot dry weight and stem diameter of seedlings grown in 12% soil water content, whereas plant height, leaf area, root dry weight and plant dry weight were similar in AM and non-AM seedlings under condition of 12% soil water content. AM colonization could alleviate plant growth depression by water stress. Mycorrhizal dependency of trifoliate orange seedlings was 141%, 130% and 128% under conditions of 20%, 16% and 12% soil water content, respectively.

3.3 Effect of AM fungi on absorption area of root system

Effects of *G. mosseae* on active and total absorption areas of the root system of trifoliate orange seedlings are shown in Figs. 3A and 3B. Total and active absorption areas of root systems were greater in AM seedlings than in non-AM ones under condition of 20% soil water content. Active absorption area of the root system in seedlings grown in 16% soil water content was increased by AM colonization, while total absorption area of the root system in AM and non-AM seedlings was similar. Total and active absorption areas of the root system were not affected by AM colonization under condition of 12% soil water content. In addition, water stress and *G. mosseae* inoculation did not affect the ratio of active absorption area and total absorption area of the root system.

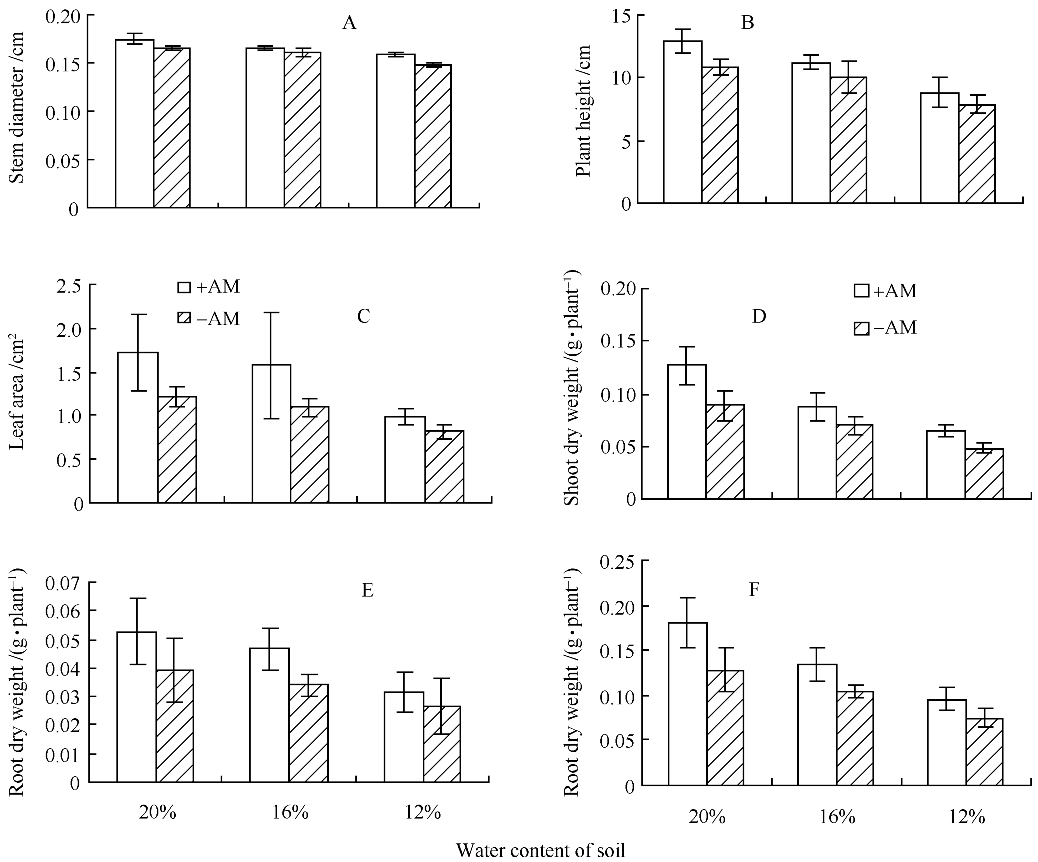

Fig. 2 Effects of AM fungi on stem diameter (A), plant height (B), leaf area (C), shoot dry weight (D), root dry weight (E) and plant dry weight (F) of trifoliate orange seedlings under water stress conditions

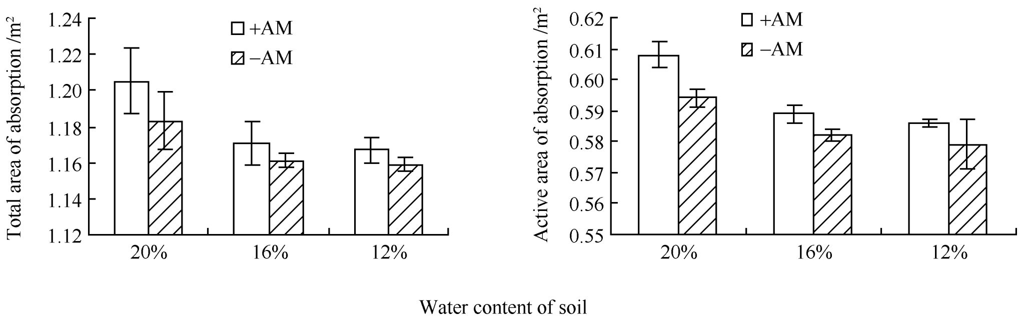

Fig. 3 Effect of AM fungi on the absorption area in the root of trifoliate orange seedlings under water stress conditions

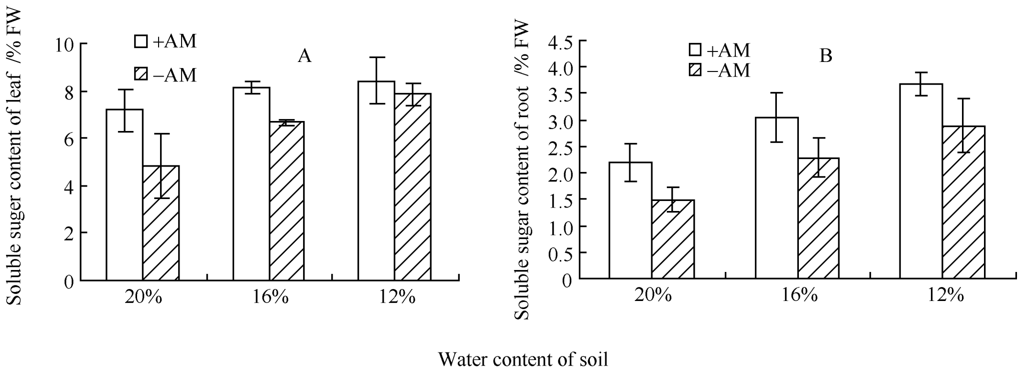

Fig. 4 Effect of AM fungi on soluble sugar content of leaves and root in trifoliate orange seedlings under water stress conditions

3.4 Effects of AM fungi on soluble sugars of leaves and roots

Figures 4A and 4B show that both water stress and AM inoculation increased the content of soluble sugars in leaves and roots of both AM and non-AM seedlings. AM leaves had significantly higher soluble sugars than non-AM leaves in 20% and 16% soil water content, while no significant differences were observed in 12% soil water content (Fig. 4A). Soluble sugars of roots were notably greater in AM seedlings than in non-AM seedlings regardless of water treatments (Fig. 4B). Thus, sensitivity to water stress was greater in roots than in leaves.

3.5 Effect of AM fungi on proline of leaves

Effect of *G. mosseae* on the proline content of leaves in trifoliate orange seedlings is shown in Fig. 5. In general, the content of proline in leaves of AM and non-AM seedlings were increased by water stress, and AM colonization decreased the content of proline in leaves. Significant differences were observed between AM and non-AM seedlings grown in 20% and 16% soil water content, whereas no significant differences were found in 12% water content soil. In addition, the proline content of leaves was similar between AM seedlings grown in 16% water content soil and non-AM seedlings grown in 20% water content soil. This result obviously indicated that AM inoculation could improve the water relations of trifoliate orange seedlings.

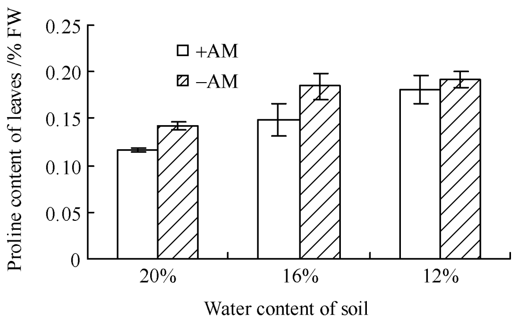

Fig. 5 Effect of AM fungi on proline content of leaves in trifoliate orange seedlings under water stress conditions

3.6 Effect of AM fungi on the phosphorus available from the rhizosphere

Water stress reduced the absorption of the available phosphorus from the rhizosphere in the roots of trifoliate orange seedlings (Fig. 6). However, available phosphorus was lower in the rhizosphere of AM roots than in the rhizosphere of non-AM roots regardless of soil water status. This meant that *G. mosseae* inoculation increased the absorption of available phosphorus from rhizosphere resulting in greater use efficiency of soil phosphorus in AM seedlings.

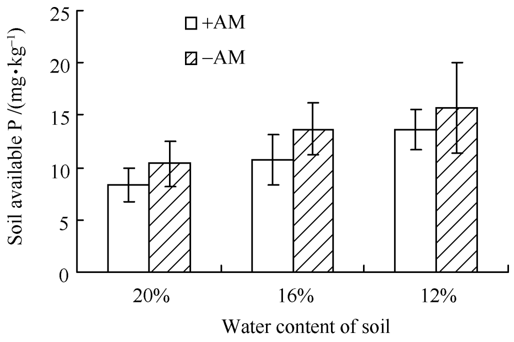

Fig. 6 Effect of AM fungi on soil phosphorus available in rhizosphere of trifoliate orange seedlings under water stress conditions

3.7 Effect of AM fungi on WUE

WUE was not significantly affected by water treatments (Fig. 7). Compared with that in non-AM seedlings, WUE increased by 40% in AM seedlings under condition of 20% soil water content, by 29% under condition of 16% soil water content and by 20% under condition of 12% soil water content, but the differences were only significant under conditions of 20% and 16% soil water content. The WUE increments by AM colonization were gradually decreased, accompanied with the decrease in the soil water content. The phenomenon was probably related to the extent of root colonization by AM fungi.

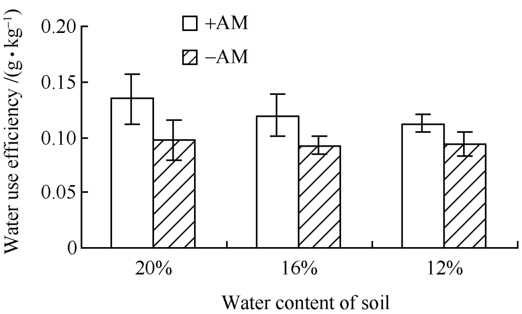

Fig. 7 Effect of AM fungi on the WUE of trifoliate orange seedlings under water stress conditions

4 Discussion

Osmotic adjustment, defined as lowering of the osmotic potential due to net solute accumulation in response to water stress, may help to preserve the metabolic processes and is regarded as a beneficial drought-tolerance mechanism (Martinez et al., 2004). Soluble sugars and proline are important organic solutes in osmotic adjustment. It was evident from the present study that AM seedlings had higher soluble sugars than non-AM seedlings regardless of soil water status, especially in 20% and 16% soil water content. The results indicated that photosynthetic carbohydrate, especially soluble sugars, were conducive to be retained in AM seedlings by AM colonization. Similarly, Wu et al. (2006) found that soluble sugars were greatly increased in the leaves of trifoliate orange seedlings inoculated with *G. mosseae* exposed to water stress and rewatering. These accumulations of soluble sugars in AM plants may result in greater osmotic adjustment, which is an important adaption of plants to water stress. Augé et al. (1987) also confirmed that regardless of phosphorus fertilization, osmotic and bulk water potential in the leaves of *Rosa hybrida* were 0.5–1.1 MPa higher in AM and in non-AM plants. These suggested that AM colonization helped host plants to sustain water stress by osmotic adjustment and to rapidly recover plant growth after rewatering (Subramanian et al., 1997).

In contrast to soluble sugars, proline in leaves was lower in AM seedlings than in non-AM seedlings. This is consistent with previous findings (Ruiz-Lozano and Azcon, 1997). The lower accumulation of proline may indicate that the stressed intensity is lower in AM than in non-AM plants. Thus, AM plants do not need more proline to protect them by osmotic adjustment and more successfully avoid water stress. Wild type plants colonized by AM fungi accumulated more proline, while AM-modified plants accumulated less proline (Vazquez et al., 2001), indicating that genetic characteristics of host plants affect proline synthesis.

Our results indicated that active and total absorption areas of root systems were greater in AM than in non-AM trifoliate orange seedlings, especially in 20% and 16% soil water content. Fidelibus et al. (2001) reported that geographic isolates of *Glomus* inoculation stimulated the root length of *Citrus volkameriana*. Greater root lengths and diameters would form higher surface absorption areas of roots mainly due to external hyphal growth in the soil (Hardie and Leyton, 1981). External hyphae took part directly in water uptake and transport (Faber et al., 1991; Wu and Xia, 2004). Plentiful water in AM plants would ensure normal process of physiology metabolisms, resulting in greater WUE. The result is in concurrence with that of Lu et al. (2003), Qi et al. (2000) and Wu et al. (2004). The aforementioned results indicated that higher drought resistance of AM plants might attribute to the increments of absorption areas of root systems by AM colonization. However, water transfer by external hyphae was rather low (Graham and Syvertsen, 1984). Therefore, the mechanism of AM fungi in enhancing drought resistance of host plants is still to be elucidated.

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