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Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions

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Summary

The influence of arbuscular mycorrhizal (AM) fungus *Glomus versiforme* on plant growth, osmotic adjustment and photosynthesis of tangerine (*Citrus tangerine*) were studied in potted culture under well-watered and water stress conditions. Seven-day-old seedlings of tangerine were transferred to pots containing *Glomus versiforme* or non-AMF. After 97 days, half of the seedlings were subject to water stress and the rest were well-watered for 80 days. AM colonization significantly stimulated plant growth and biomass regardless of water status. The soluble sugar of leaves and roots, the soluble starch of leaves, the total non-structural carbohydrates (NSC) of leaves and roots, and the Mg^{2+} of leaves were higher in AM seedlings than those in corresponding non-AM seedlings. The levels of K^+ and Ca^{2+} in leaves and roots were higher in AM seedlings than those in non-AM seedlings, but differences were only significant under water stress conditions. Moreover, AM colonization increased the distributed proportions of soluble sugar and NSC to roots. However, the proline was lower in AM seedlings compared with that in non-AM seedlings. AM seedlings had higher leaf water potential (Ψ), transpiration rates (E), photosynthetic rates (Pn), stomatal conductance (g_s), relative water content (RWC), and lower leaf temperature (Lt) than corresponding non-AM seedlings. This research also suggested that AM colonization improved the osmotic adjustment originating not from proline but from NSC, K^+, Ca^{2+} and Mg^{2+}, resulting in the enhancement of drought tolerance.

Abbreviations: AM, arbuscular mycorrhiza; AMF, arbuscular mycorrhizal fungi; E, transpiration rates; g_s, stomatal conductance; Lt, leaf temperature; NSC, total non-structural carbohydrates; Pn, photosynthetic rates; RWC, relative water content; WS, water stress; WW, well-watered; Ψ, leaf water potential

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Introduction

Arbuscular mycorriza (AM) is a mutualistic symbiosis between AM fungi (AMF) and the roots of terrestrial plants. The ancient fungi colonize approximately 90% of the Earth's land plant species (Gadkar et al., 2001).

In many arid and semiarid regions, water stress has limited crop productivity (Maggio et al., 2000). AMF inoculation has been found to improve the water relations of many plants. Porcel and Ruiz-Lozano (2004) reported that leaf water potential was higher in stressed AM soybean than that in corresponding non-AM plants. Al-Karaki (1998) reported that shoot and root dry matters were higher for water stressed AM wheat than those for corresponding non-AM plants. However, the mechanism that AMF improve drought resistance and water flow through host plants still remains unclear. The potential mechanisms include: (1) extensive absorption of water by external hyphae (Auge et al., 2003; Faber et al., 1991; Ruiz-Lozano and Azcon, 1995), (2) stomatal regulation through hormonal signals (Goicoechea et al., 1997), (3) an indirect effect of improved P nutrition upon water relations (Fitter, 1988; Nelsen and Safir, 1982a), and (4) a greater osmotic adjustment in mycorrhizal plants (Auge et al., 1986; Porcel and Ruiz-Lozano, 2004; Ruiz-Lozano, 2003).

Most citrus species, such as sour orange, trifoliate orange, cleopatra mandarin, swingle citrumelo, and carrizo citrange, have rare and short root hairs, and are fairly dependent on AMF that are most *Glomus* species (Davies and Albrigo, 1994; Graham and Syvertsen, 1985). There are many reports on the effects on the growth and photosynthesis of citrus plants (Shrestha et al., 1995; Wu and Xia, 2004). The shoot size (dry weight and canopy area) of citrus seedlings was not affected by AM inoculation (Fidelibus et al., 2001). In contrast, AM inoculation stimulated the root growth (dry weight and length) of citrus seedlings. Syvertsen and Graham (1990) reported that AM colonization did not affect the net gas exchange of citrus seedlings. However, Shrestha et al. (1995) showed that the photosynthetic and transpiration rates of AM Satsuma mandarin on trifoliate orange rootstocks were faster than those of non-AM plants under high air temperature stress conditions; when the air temperature decreased, the photosynthetic and transpiration rates did not vary significantly between AM and non-AM trees. Moreover, most of the effects of mycorrhizal association were on stomatic regulation rather than on root resistance (Levy and Krikun, 1980).

When plants suffer from water stress, osmotic adjustment occurs in order to decrease their water potential to maintain a beneficial gradient for water flow from soil into plant roots. AM plants have a greater osmotic adjustment than non-AM plants (Porcel and Ruiz-Lozano, 2004). Whereas, the effect of AM inoculation on citrus osmotic adjustment is still unexplored.

This study is to evaluate the effects of *Glomus versiforme* on growth, osmotic active solutes and photosynthesis of tangerine (*Citrus tangerine*), a major using citrus rootstock in China, under well-watered and water stress conditions, and to examine the influence of *Glomus versiforme* on tangerine osmotic adjustment.

Materials and methods

Plant material and growth conditions

Seeds of tangerine were sterilized by immersion in 70% alcohol for 5 min, rinsed four times with distilled water, and germinated on wet filter paper in Petri dishes at 28 °C. Seven-day-old seedlings were transferred to plastic pots containing 3.371 kg of an autoclaved growing mixture (0.11 MPa, 121 °C, 2 h) of yellow soil, vermiculite and sphagnum (5:2:1, v/v/v), the characteristics of which were: pH 5.9, 1.3% organic matter, 29.97 mg kg^{-1} available phosphorus, 147.47 mg kg^{-1} alkali hydrolyzable nitrogen and 140.89 mg kg^{-1} available potassium. The soil was collected from the Fruit Sample Garden, Huazhong Agricultural University. The experimental pots were placed in a greenhouse under natural light from March to September, where no temperature controlling equipment was available. The photon flux density ranged from 550 to 900 μmol m^{-2} s^{-2}. The average day/night temperature was 25/18 °C; the relative humidity was 60–95%.

Mycorrhizal fungus inoculums

Mycorrhizal fungus inoculums, consisting of spores, soil, hyphae and infected jowar root fragment from a stock culture of *Glomus versiforme* (Karsten) Berch, were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. The inoculated dosage was 30 g of inoculums per pot containing approx. 2233 spores. Non-AMF treatments received the same weight of autoclaved growth mixture. Mycorrhizal inoculums were placed 5 cm below citrus seedlings at sowing time.

Experimental design

The experimental treatments made up of two soil water regimes (well-watered and water stress) and two mycorrhizal inoculations (*Glomus versiforme* and non-AMF) were arranged in a complete randomized blocks design. Each treatment (four seedlings per pot) was replicated eight times.

Water treatments began after 97 days of acclimation in greenhouse conditions, at which time well-watered (WW) pots were controlled with 75% of relative soil water content (−0.09 MPa) by weighing the substrate before and after drying at 105 °C for 24 h, and water stressed (WS) pots were watered with 55% of relative soil water content (−0.40 MPa). The water status in the substrate was daily determined and the amount of water loss was supplied to each pot to keep the designed soil water contents. The soil water potential was measured by a pressure plate apparatus.

Plant growth and mycorrhizal colonization measurement

After 80 days of water treatments, plants were harvested and plant height, stem diameter, leaf area and leaf number per plant were recorded. The shoot and root were separated and dried at 75 °C for 48 h. A fraction of roots were carefully washed, cut into 1 cm root pieces and fixed by FAA. Root samples were cleared with 10% KOH solution and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970), and microscopically observed for root colonization. At the same time, the number of AMF entry points, vesicules and arbuscules were calculated for the infected root. The AM infected percentage was counted by the following formula: AM infected percentage (%) = 100 × root length infected/root length observed.

Chlorophyll determination

Leaf chlorophyll content was assayed according to Li (2000). The extraction was made from a 100 mg-fresh sample in 25 mL acetone (80%) in the dark at the room temperature and was measured at 470, 646 and 663 nm with a UV/VIS spectrophotometer.

Relative water content and water potential determination

The fifth full leaf from the apices of these seedlings was used for relative leaf water content (RWC) and leaf water potential (Ψ) at 9:0 0 am on September 5, 2004. The equation RWC (%) = 100 × (FW−DW)/(SW−DW) was used for RWC calculation, where FW stood for fresh weight, DW for dry weight, and SW for saturated weight. SW was determined after floating the leaflet on distilled water for 24 h at the room temperature. Leaf water potential was measured using a pressure chamber (Li, 2000). The fully mature leaf was immediately wrapped in a plastic bag filled with breathing air and reading started in 3 min.

Inorganic ions analyses

Hundred milligrams of dry samples were used to extract inorganic ions with 20 mL distilled water at 100 °C for 2 h, refrigerated for 30 min, and then filtered through a piece of filter paper. K^+, Ca^{2+} and Mg^{2+} concentrations were determined by an Atomic Emission Spectrometer.

Carbohydrate and proline determination

Soluble sugar was determined by the anthrone method (Li, 2000) using sucrose as the standard. Half a gram of fresh samples was placed in a 25 mL cuvette added with 10 mL distilled water, allowed to stand at 100 °C for 1 h, and filtered into 25 mL volumetric flasks. Reaction mixture of 7.5 mL contained 0.5 mL extracts, 0.5 mL mixed reagent (1 g anthrone+50 mL ethyl acetate), 5 mL H_2SO_4 (98%), 1.5 mL distilled water. The mixture was heated at 100 °C for 1 min and absorbance read at 630 nm. Soluble starch was determined by the anthrone method (Li, 2000) with sucrose as the standard. The remainder of measured soluble sugar was transferred to a 25 mL cuvette containing 10 mL distilled water and 1.0 mL $HClO_4$ (9.2 mol L^{-1}). The cuvette was placed in a boiling water bath for 30 min, cooled to 30 °C, and filtered into 25 mL volumetric flasks. Reaction mixture of 7.5 mL contained 0.5 mL extracts, 0.5 mL mixed reagent (1 g anthrone+50 mL ethyl acetate), 5 mL H_2SO_4 (98%), 1.5 mL distilled water. The mixture was heated at 100 °C for 1 min and absorbance read at 630 nm. Total non-structural carbohydrates (NSC) were the sum of soluble sugar and soluble starch.

For proline determination, fresh samples were extracted with 3% sulfosalicyclic acid, placed in a boiling water bath for 10 min and filtered through filter paper. Two milliliter of extract was added to 6 mL (final volume) assay media containing 2 mL ninhydrin solution and 2 mL acetic acid and incubated for 30 min at 100 °C and then cooled.

The colored product formed was extracted with 4 mL toluene by shaking and the absorbance of resultant organic layer was measured at 520 nm (Troll and Lindsley, 1955).

Photosynthesis measurement

Stomatal conductance (g_s), transpiration rates (E), photosynthetic rates (Pn) and leaf temperature (Lt) were measured by TPS-1 Photosynthesis System (USA) on eight replications per treatment from 9:00 to 10:00 am on August 30, 2004. The fifth full leaf from the apices of these seedlings was measured in the place where they grew. Reference CO_2 concentration ranged from 355 to 375 $\mu mol\,mol^{-1}$; cuvette air temperature ranged from 30.0 to 35.1 °C; photosynthetically active radiation ranged from 219 to 398 $\mu mol\,m^{-2}\,s^{-1}$. The values for photosynthesis were recorded after being stabilized and it took 54 min to measure all seedlings.

Statistical analysis

The experimental data were statistically analyzed by variance (ANOVA) with SAS 8.1software. Probabilities of significance were used to test the significance among treatments and interactions, and LSD ($P<0.05$) was used to compare the means.

Results

The roots of non-AM citrus seedlings were observed after water treatment, confirming the absence of mycorrhizae. The roots of seedlings inoculated with *Glomus versiforme* were infected (Table 1). Water stress significantly decreased AM colonization, entry points, vesicules and arbuscules.

Plant height, leaf area, leaf number per plant and stem diameter were lower for water stressed seedlings than those for well-watered seedlings (Table 2). However, AM seedlings under well-watered and water stress conditions had significantly higher shoot and root dry weights, plant height, leaf area, leaf number per plant and stem diameter than corresponding non-AM seedlings. The differences of plant height, shoot dry weight, root dry weight, leaf area, leaf number per plant and stem diameter were not significant between well-watered non-AM and water stressed AM seedlings.

The soluble sugar and soluble starch concentrations of leaves were increased by water stress (Table 3). AM seedlings had higher soluble sugar in leaves and roots, soluble starch in leaves, and NSC in leaves and roots than corresponding non-AM seedlings. In contrast, the proline of roots was significantly lower in AM seedlings than that in non-AM seedlings regardless of water treatments (Table 3). The proline of leaves was lower in AM seedlings than that in non-AM seedlings, but the differences were only significant under water stress conditions. The organic solutes distribution between leaves and roots showed that most organic solutes were located in leaves. The distributed proportions of soluble sugar and NSC to roots were increased by AM colonization. Forty six percent of soluble sugar were located in the roots of AM seedlings and 40% of soluble sugar in the roots of non-AM seedlings under well-watered conditions. Similarly, 43% of NSC were located in the AM seedlings roots and 38% of NSC in the non-AM seedlings roots under water stress conditions.

Water stress significantly decreased the K^+ concentration of roots and the Ca^{2+} concentration of leaves (Table 4). The K^+ and Ca^{2+} levels in leaves were higher in AM seedlings than those in non-AM seedlings, but the differences were only significant

Table 1. Root AM colonization, entry points, vesicules and arbuscules of *Citrus tangerine* subjected to four different treatments

Water status	AMF status	AM colonization (%)	Entry points (no. cm^{-1} root)	Vesicules (no. cm^{-1} root)	Arbuscules (no. cm^{-1} root)
WW	AMF	44.08a	6.7a	3.6a	5.5a
	Non-AMF	0c	0c	0c	0c
WS	AMF	32.96b	4.1b	1.8b	3.9b
	Non-AMF	0c	0c	0c	0c
Significance					
WS		*	*	*	NS
AMF		**	**	**	**
WS × AMF		*	*	*	NS

Note: The same letter within each column indicates no significant difference among treatments ($P<0.05$). NS-not significant. *$P<0.05$, **$P<0.01$.

Table 2. Root colonization, shoot dry weight, root dry weight, plant height, leaf number per plant and stem diameter of *Citrus tangerine* subjected to four different treatments

Water status	AMF status	Leaf area (cm^2)	Dry weight (g $plant^{-1}$)		Plant height (cm)	Leaf number per plant	Stem diameter (cm)
			Shoot	Root			
WW	AMF	10.68a	1.04a	0.67a	31.90a	28.4a	0.398a
	Non-AMF	8.80b	0.80b	0.37bc	21.23b	21.3b	0.327b
WS	AMF	8.40b	0.84ab	0.47b	20.71b	21.3b	0.327b
	Non-AMF	5.53c	0.49c	0.32c	14.24c	15.5c	0.274c
Significance							
WS		**	**	**	**	**	**
AMF		**	**	**	**	**	**
WS × AMF		NS	NS	NS	NS	NS	NS

Note: The same letter within each column indicates no significant difference among treatments ($P<0.05$). NS-not significant. *$P<0.05$, **$P<0.01$.

Table 3. Soluble sugar, soluble starch, total non-structural carbohydrate (NSC) and proline concentrations of *Citrus tangerine* subjected to four different treatments

Water status	AMF status	Soluble sugar (%fwt)		Soluble starch (%fwt)		Proline (mg g^{-1} fwt)		NSC (%fwt)	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
WW	AMF	7.89c	6.70a	7.01a	5.25a	0.29c	0.05c	14.48ab	11.96a
	Non-AMF	6.43d	4.29b	6.08b	5.00ab	0.59c	0.29b	12.52c	9.29b
WS	AMF	10.84a	7.87a	5.23c	4.23b	0.93b	0.08c	16.07a	12.10a
	Non-AMF	9.30b	5.30b	4.36d	3.23c	1.52a	0.69a	13.66bc	8.53b
Significance									
WS		**	*	**	**	**	**	*	NS
AMF		**	**	**	*	**	**	**	**
WS × AMF		NS	NS	NS	NS	**	**	NS	NS

Note: The same letter within each column indicates no significant difference among treatments ($P<0.05$). NS-not significant. *$P<0.05$, **$P<0.01$.

Table 4. K^+, Ca^{2+} and Mg^{2+} concentrations of *Citrus tangerine* subjected to four different treatments

Water status	AMF status	K^+ (mg g^{-1} dwt)		Ca^{2+} (mg g^{-1} dwt)		Mg^{2+} (mg g^{-1} dwt)	
		Leaf	Root	Leaf	Root	Leaf	Root
WW	AMF	28.36b	18.60a	6.98bc	6.24b	2.13ab	2.33a
	Non-AMF	26.66b	17.93a	6.68c	5.87b	1.87c	2.23a
WS	AMF	31.03a	15.66b	9.62a	7.70a	2.27a	2.39a
	Non-AMF	28.74b	13.73c	7.56b	6.23b	1.99bc	2.27a
Significance							
WS		**	**	**	*	NS	NS
AMF		*	*	**	*	**	NS
WS × AMF		NS	NS	*	NS	NS	NS

Note: The same letter within each column indicates no significant difference among treatments ($P<0.05$). NS-not significant. *$P<0.05$, **$P<0.01$.

under water stress condition. Although AM colonization did not affect the Mg^{2+} level in roots, the Mg^{2+} level in leaves had a significant difference between AM and non-AM seedlings under two water regimes conditions.

The g_s and Ψ in AM and non-AM seedlings were markedly decreased by water stress (Table 5). Moreover, AM seedlings had significantly higher *E*, Pn and g_s than non-AM seedlings regardless of water treatments. The *E* of AM seedlings was 30% higher

Table 5. Transpiration rates (E), photosynthetic rates (Pn), stomatal conductance (g_s), leaf temperature (Lt), water potential (Ψ), relative water content (RWC) and chlorophyll content of *Citrus tangerine* subjected to four different treatments

Water status	AMF status	E (mmol $m^{-2} s^{-1}$)	Pn (μmol $m^{-2} s^{-1}$)	g_s (mmol $m^{-2} s^{-1}$)	Lt (°C)	Ψ (MPa)	RWC (%)	Chlorophyll (mg g^{-1} fwt)
WW	AMF	2.95a	7.05a	153.75a	31.05d	−0.35a	95.11a	2.23a
	Non-AMF	2.27bc	5.73b	130.75b	31.85c	−0.54b	92.09bc	1.74b
WS	AMF	2.50b	6.19ab	129.38b	32.53b	−0.74c	92.49ab	1.68b
	Non-AMF	1.97c	4.14c	100.38c	34.35a	−0.94d	89.77c	1.63b
Significance								
WS		*	**	**	**	**	*	**
AMF		**	**	**	**	**	**	**
WS × AMF		NS	NS	NS	**	NS	NS	**

Note: The same letter within each column indicates no significant difference among treatments ($P < 0.05$). NS-not significant. *$P < 0.05$, **$P < 0.01$.

than that of non-AM seedlings under well-watered conditions and 27% under water stress conditions. Compared with the Pn of non-AM seedlings, the Pn of AM seedlings increased by 23% and 50% and the g_s by 18% and 29% under well-watered and water stress conditions, respectively.

AM colonization also affected Lt (Table 5). Compared with the Lt of non-AM seedlings under well-watered and water stress conditions, the Lt of AM seedlings decreased by 3% and 5%, respectively.

The Ψ and RWC were significantly increased by AM colonization under well-watered and water stress conditions (Table 5). The Ψ of well-watered AM seedlings was 35% higher than that of well-watered non-AM seedlings and 21% for water stressed seedlings.

Compared with that in non-AM seedlings, the chlorophyll content of leaves increased by 23% in AM seedlings under well-watered conditions and by 3% under water stress conditions, but the differences were only significant under well-watered conditions (Table 5).

Discussion

Water stress had a strong effect on AM development (Table 1). The AM colonization under well-watered conditions was 34% higher than that under water stressed conditions. The result was in accord with the finding of Kaya et al. (2003), who reported that water stress significantly decreased the AM colonization by *Glomus clarum* in watermelon (*Citrullus lanatus*). These suggested a negative effect of arid or semiarid environment for the AM development of host plants (Morte et al., 2000). However, as a whole, in viewing the literature, root colonization more often increased than decreased under water stress conditions (Auge, 2001).

We also observed the positive effects of AMF on the growth and the biomass of *Citrus tangerine* under well-watered and water stress conditions (Table 2). Similar results have been reported for other plant species (Al-Karaki, 1998; Fidelibus et al., 2001; Graham and Timmer, 1985; Johnson and Hummel, 1985; Kaya et al., 2003; Ruiz-Lozano and Azcon, 1996; Wu and Xia, 2004). The positive effect likely attributed to the improvement of phosphorus nutrition (Bethlenfalvay et al., 1988; Sweatt and Davies, 1984), the uptake of water by hyphae (Faber et al., 1991) and the increase of root length density (Bryla and Duniway, 1997).

Osmotic adjustment is considered to be an important component of drought tolerance mechanisms for high plants. Under water stress conditions, high plants accumulate some small molecules including organic solutes and inorganic ions to make higher osmotic adjustment. Organic solutes include soluble sugar, proline, etc.; inorganic ions include K^+, Ca^{2+}, Mg^{2+}, etc. Our results showed that soluble sugar, soluble starch, NSC, K^+ and Ca^{2+} levels were higher in water stressed AM seedlings than those in corresponding non-AM seedlings, and soluble sugar and NSC were higher in AM seedlings than those in non-AM seedlings under well-watered condition (Tables 3 and 4). The greater soluble sugar and NSC of AM seedlings roots may be due to the sink effect of the AM fungus demanding sugar from leaves (Porcel and Ruiz-Lozano, 2004). Osmotic adjustment involves the net accumulation of osmotically active solutes in cells in response to a fall in the water potential of their environment (Martinez-Ballesta et al., 2004). The net accumulations of NSC in leaves, K^+ in leaves, Ca^{2+} in leaves and roots, and Mg^{2+} in

leaves and roots of AM seedlings were 11.0%, 9.4%, 37.8%, 23.4%, 6.6% and 2.6%, respectively; the corresponding accumulations of non-AM seedlings were only 9.1%, 7.8%, 13.2%, 6.1%, 6.4% and 1.8%, respectively (Tables 3 and 4). These resulted in a greater osmotic adjustment in AM seedlings and allowed AM seedlings to accumulate more carbohydrates and increase plant biomass.

It seems likely that a high-level proline accumulation may play a role in drought tolerance and make plants survive short drought, and recover from stress (Delauney and Verma, 1993; Ruiz-Lozano, 2003; Sanchez et al., 1998). Our study indicated that AM colonization decreased the proline accumulation of AM seedlings leaves and roots (Table 3). The results suggested that AM colonization enhanced host plant drought tolerance, which did not correlate with proline but with NSC, K^+, Ca^{2+} and Mg^{2+}.

AMF provide water and nutrition to their host plants, and in return host plants transfer their carbohydrate to AMF for the energy source. We observed that the distributed proportions of soluble sugar and NSC to roots were increased by AM inoculation (Table 3). Forty-two percent of soluble sugar were located in AM seedlings roots and 36% in non-AM seedlings roots under water stress conditions. Forty-five percent of NSC were located in the roots of AM seedlings and 43% of NSC in the roots of non-AM seedlings under well-water conditions. The results suggested that AM symbiosis mainly demanded soluble sugar provided by host plant. A ^{14}C-labeling experiment showed that mycorrhizal roots, respectively, accumulated 66% and 68% of the ^{14}C-labeled photosynthates translocated to the roots of sour orange and carrizo citrange (Koch and Johnson, 1984). The distribution was independent of the status of phosphorus in leaves. Nemec and Guy (1982) also reported the increases in total soluble sugar, starch and NSC of citrus roots colonized by *Glomus macrocarpus*. However, the transferring processes of carbohydrate from host plant to AMF are unclear. Moreover, AMF are capable of converting absorbed soluble sugar into storage compounds that are not readily available to the plant, such as glycogen, mannitol or trehalose (Maronek et al., 1981).

AM and non-AM plants often display different photosynthetic characters. AM onion had higher Ψ and E than non-AM onion grown in low soil phosphorus conditions. The E and g_s in AM lettuce were higher than those in non-AM plants under well-watered and water stress conditions (Ruiz-Lozano et al., 1995). Our study confirmed that AM citrus seedlings had higher Pn, E, and g_s than corresponding non-AM ones (Table 5). However, the g_s and E of citrus taxa usually were not changed by AM colonization (Auge, 2001), though Graham and Syvertsen (1984) observed that Mycorrhizal colonization tended to have higher E of carrizo citrange in a low phosphorus soil. Stomatal physiology was affected by mycorrhizal infection, as shown by decreased stomatal resistances to water and by increased transpirational fluxes and rates of photosynthesis. The effects could be mediated by increased stomatal opening, thus increasing the K^+ concentrations in mycorrhizal plants (Harley and Smith, 1983). We first reported that AM colonization significantly decreased the Lt of *Citrus tangerine* regardless of water status (Table 5). Some studies indicated that the internal hormone (e.g. cytolinin, abscisic acid) levels in mycorrhizal plants correlated with their gas exchanges (e.g. Pn and g_s) (Druge and Schonbeck, 1992; Goicoechea et al., 1997). The mycorrhizal effect on g_s did not correlated with phosphorus nutrition (Ebel et al., 1996). The greater E and g_s of AM plants, compared with those of non-AM plants, implied a lower resistance to vapor transfer from inside the leaves to the atmosphere when exposed to the same water conditions. Similarly, the higher Ψ and RWC, and the lower Lt of AM plants, compared with those of non-AM plants, were propitious to moving liquid water through the plants to the evaporating surfaces in the leaves (Nelsen and Safir, 1982b).

In short, our results showed that AM colonization changed the plant growth, osmotic adjustment and photosynthesis characters of *Citrus tangerine*. The results suggested that the benefit of AM colonization under water stress conditions was due to the enhancement of osmotic adjustment.

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