

Five *Glomus* species affect water relations of *Citrus tangerine* during drought stress

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(Received June 12, 2006; Accepted November 22, 2006)

ABSTRACT. The efficacy of five *Glomus* species, *Glomus mosseae*, *G. geosporum*, *G. versiforme*, *G. etunicatum* and *G. diaphanum* was studied for the ability to improve water relations of *Citrus tangerine* Hort. ex Tanaka under well-watered and drought stress conditions in terms of growth, carbohydrate, photosynthetic characteristic and antioxidant enzymes activities. The ranking of five *Glomus* species for mycorrhizal dependency of *C. tangerine* was as follows: *G. mosseae* \approx *G. geosporum* > *G. versiforme* > *G. etunicatum* > *G. diaphanum*. In general, the arbuscular mycorrhizal fungi used in this study showed beneficial effects in these parameters. The colonization by *G. geosporum* showed the highest plant height, leaf number per plant, stem diameter, relative water content, soluble sugar, starch and total non-structural carbohydrates under well-watered and drought stress conditions and *G. etunicatum* colonization the least effects. *G. mosseae* seedlings showed the highest soluble protein concentration and catalase activity in leaves, *G. diaphanum* seedlings showed the highest superoxide dismutase activity, and *G. versiforme* seedlings showed the highest guaiacol peroxidase activity. Both *G. mosseae* and *G. geosporum* colonization showed greater transpiration rates and stomatal conductance. In addition, five *Glomus* species significantly decreased leaf temperature of mycorrhizal seedlings. The different arbuscular mycorrhizal fungal species differed in their ability to improve water relations of *C. tangerine*. Both *G. mosseae* and *G. geosporum* were more efficient fungi in improving water relations of *C. tangerine*, and *G. etunicatum* was less efficient fungi. Arbuscular mycorrhizal symbiosis improved water relations of *C. tangerine* in part due to increases of antioxidant enzymes.

Keywords: Arbuscular mycorrhizal fungi; Citrus; Drought; *Glomus*; Water relations.

Abbreviations: AM, arbuscular mycorrhizal; AMF, arbuscular mycorrhizal fungi; CAT, catalase; DS, drought stressed; *E*, transpiration rates; G-POD, guaiacol peroxidase; *g*_s, stomatal conductance; Lt, leaf temperature; NSC, total non-structural carbohydrates; Pn, photosynthetic rates; ROS, reactive oxygen species; RWC, leaf relative water content; SOD, superoxide dismutase; WW, well-watered.

INTRODUCTION

Arbuscular mycorrhiza symbiosis, a natural association between the roots of higher plants and arbuscular mycorrhizal fungi (AMF), are rather important in horticultural crops, because AMF are believed to improve host plants growth, water relations and acquisition of nutrients especially P from soil (Maronek et al., 1981). There is a role played by AMF in alleviating drought stress of higher plants as it appears that drought resistance is enhanced (Augé, 2001; 2004). Fidelibus et al. (2001) showed that four *Glomus* species isolated from arid, semiarid and mesic areas stimulated the root growth

(dry weight and length) of *Citrus volkameriana*, and leaf P concentration were 12-56% higher in arbuscular mycorrhizal (AM) plants than in non-AM plants under well-watered conditions. Mycorrhizal infection appeared to improve establishment of citrus into transplant situations by improving P uptake and reducing plant stress (Johnson and Hummel, 1985). Most effects of the mycorrhizal association were on stomatal regulation rather than on root resistance (Levy and Krikun, 1980). However, the precise mechanisms underpinning changes in water relations are still in doubt.

In higher plants, metabolism of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals is kept in dynamic balance under well-watered conditions. Drought stress often induces cellular damage and photo-oxidative damage, through the accumulations of ROS. As a consequence, higher plants evolve cellular responses like up-regulation of oxidative

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stress protectors (Reddy et al., 2004). Antioxidant defense enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase, guaiacol peroxidase (G-POD), and glutathione reductase, are designed to minimize concentrations of superoxide and hydrogen peroxide. The antioxidants containing ascorbate and glutathione are involved in scavenging ROS primarily via the Halliwell-Asada pathway (Apel and Hirt, 2004). Ruiz-Lozano (2003) reported that AM symbiosis might increase the drought resistance of higher plants by promoting antioxidant enzymes. However, the relationship between AMF and antioxidants is poorly known. Although antioxidants in bean (Lambais et al., 2003), red clover (Palam et al., 1993) and some shrub species (Alguacil et al., 2003) inoculated with AMF have been investigated, the differences in antioxidant enzymes due to drought and AMF have not been reported in citrus. We wanted to determine whether the presence of AMF would influence the antioxidant enzymes activities of citrus.

Citrus is one of the most important commercial fruit crops grown in wide areas of China. Most citrus species are fairly dependent on AMF as they have a positive growth response to AMF (Graham and Syversten, 1985) that are mostly *Glomus* species (Davies and Albrigo, 1994). Moreover, *C. tangerine* is one main citrus species cultivated in southwest regions of China and is a fine rootstock. Hence, basing on the positive effects of AMF on citrus, we also investigated the responses to different *Glomus* species on growth, carbohydrate, antioxidant enzymes activities and photosynthesis characteristics of *C. tangerine*, in order to select an efficient AM fungus from *Glomus*.

MATERIALS AND METHODS

Plant culture and biological materials

Seeds of *Citrus tangerine* Hort. ex Tanaka were surface sterilized in 70% alcohol for 5 min, subsequently rinsed with sterilized water and germinated on wet filter paper in Petri dishes in dark at 28°C. Seven-day-old uniform seedlings were transplanted into plastic pots (15×20 cm) containing 4.010 kg autoclaved (0.11 MPa, 121°C, 2 h) growing mixture of yellow soil (from Fruit Sample Garden, Huazhong Agricultural University), plus vermiculite and perlite (6:2:1, v/v/v), with pH 5.6, 0.9% organic matter, 9.99 mg kg⁻¹ available phosphorus, 84.53 mg kg⁻¹ alkali hydrolyzable nitrogen and 84.13 mg kg⁻¹ available potassium. The experimental pots were placed in a plastic greenhouse under natural light conditions from March to September, which had no controlling temperature equipment. The midday photosynthetically active photon flux density ranged from 550 to 900 μmol m⁻² s⁻². The average day/night temperature was 25/18°C and the relative humidity was 60-95%.

The AMF used in this study were *Glomus versiforme* (Karsten) Berch, *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *G. geosporum* (Nicol. & Gerd.) Walker, *G.*

diaphanum Morton & Walker, and *G. etunicatum* Becker & Gerdemann obtaining from Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. The inoculated dosage was approx. 1100 spores per pot. Non-AMF treatments (control seedlings) received the same weight of autoclaved growth mixture. The inocula were placed 5 cm below citrus at transplanting time.

Drought treatments began 90 days after transplant. Well-watered (WW) pots were maintained at 75% of the relative soil water content (corresponding to field capacity) by weighing the substrate before and after drying at 105°C for 24 h, and drought stressed (DS) pots were maintained at 55% of the relative soil water content (corresponding to 73% of field capacity). The water status in the substrate was determined daily and the amount of water loss was added to each pot in order to maintain the designed soil water content.

Experimental design

The experimental treatments were made up of two soil water regimes (WW and DS) and six AM inoculations (*G. versiforme*, *G. mosseae*, *G. geosporum*, *G. diaphanum*, *G. etunicatum* and non-AMF) and were arranged in a randomized complete block design. Three replicates of each AMF treatment were performed, totaling 36 pots with four seedlings per pot.

Parameter measurement

Eighty days after drought stress treatment were begun, plant height, stem diameter and leaf number per plant were recorded and the AM and non-AM seedlings were harvested. Half of seedlings were separated into roots and shoots, dried in hot-air oven at 75°C for 2 d, and dry weights of shoots and roots were recorded.

Part of fresh roots was carefully washed, cut into 1-cm long root segments and fixed by FAA at least 24 h. The root samples were cleared with 10% KOH solution, stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970), and examined microscopically for root colonization. At the same time, the number of entry points, vesicles and arbuscules were calculated in the infected root. The AMF infected percentage was calculated by the following formula: AM colonization (%) = 100× root length infected/root length observed. Mycorrhizal dependency was defined as the ratio of the dry weight (dry wt.) of the AM seedlings and non-AM seedlings (Graham and Syversten, 1985).

Fresh leaf samples were homogenized in 5 mL of phosphate buffer (0.1 mol L⁻¹, pH 7.8), centrifuged at 4,200 ×g for 10 min at 4°C, and the supernatant was used for assays of soluble protein, SOD, G-POD and CAT. SOD activity was analyzed using the methods of Giannopolitis and Ries (1987) and was expressed as Unit g⁻¹ fresh weight (fwt.). One SOD unit was defined as the amount of enzyme that inhibited 50% nitro blue tetrazolium by light. CAT activity was measured according to Aebi (1984). G-POD

activity was determined using the method of Chance and Maehly (1955). Soluble protein was evaluated using bovine serum albumin as the standard (Bradford, 1976).

Soluble sugar and starch contents were determined by the anthrone method (Wu and Xia, 2006). Total non-structural carbohydrates (NSC) were the sum of soluble sugars and starch.

Stomatal conductance (g_s), transpiration rates (E), photosynthetic rates (P_n) and leaf temperature (L_t) were measured using TPS-1 Photosynthesis System (USA) on four replicated leaves randomized from three replicate pots of each treatment from 10:00 to 11:00 am. The fifth full leaf from the apices of these seedlings was measured in the place where they grew. The reference CO_2 concentration ranged from 354 to 368 $\mu\text{mol mol}^{-1}$; the cuvette air temperature ranged from 30 to 34°C; the natural photosynthetically active radiation ranged from 175 to 381 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The fifth full leaf from the apices of these seedlings was used for leaf relative water content (RWC) assays (Wu and Xia, 2006).

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) with Statistical Analysis System (SAS) 8.1 software (SAS Institute Inc., Cary, N.C.) and Fisher's

Protected least significant difference (LSD) ($p=0.05$) was used to compare treatment means. CORR program was used to analyze the correlation of two variables.

RESULTS

No mycorrhizal structure was found in any of the non-AM seedlings (Table 1). The roots of *C. tangerine* were infected by each *Glomus* species as shown by the presence of entry points, vesicles and arbuscules. Drought stress strongly reduced AM infection but the reduction in AM infection was the least pronounced with *G. versiforme*. Entry points, vesicles, arbuscules and AM colonization were highest in seedlings colonized by *G. mosseae* than in those colonized by other *Glomus* species under WW conditions. The mycorrhizal developments in the roots of AM seedlings inoculated with *G. mosseae* and *G. versiforme* were higher than with other *Glomus* species under DS conditions.

The highest shoot dry wt. was reached in seedlings inoculated with *G. mosseae* under WW conditions and *G. geosporum* under DS conditions (Table 2). The enhancement of root dry wt. and plant dry wt. growth under WW and DS conditions was highest by *G. mosseae* and lowest by *G. diaphanum*. The ranking of five *Glomus* species for the mycorrhizal dependency under WW conditions was as follows: *G. mosseae* > *G. geosporum*

Table 1. Effects of five *Glomus* species inoculation and drought stress on AM colonization, entry points, vesicles and arbuscules in *Citrus tangerine*.

AMF status	AM colonization (%)		Entry points (no. cm^{-1} root)		Vesicles (no. cm^{-1} root)		Arbuscules (no. cm^{-1} root)	
	WW	DS	WW	DS	WW	DS	WW	DS
<i>G. mosseae</i>	64.09 \pm 7.86a	34.60 \pm 3.61b	7.4 \pm 2.4a	4.2 \pm 1.1a	4.6 \pm 1.5a	2.0 \pm 0.7a	8.7 \pm 2.4a	3.1 \pm 0.6b
<i>G. versiforme</i>	47.80 \pm 5.16b	44.37 \pm 4.33a	4.3 \pm 1.3bc	4.6 \pm 1.1a	1.9 \pm 0.7b	1.7 \pm 0.7abc	4.6 \pm 0.7b	4.4 \pm 0.6a
<i>G. geosporum</i>	63.43 \pm 11.91a	29.91 \pm 3.49b	3.7 \pm 0.3bc	1.3 \pm 0.4bc	3.0 \pm 0.1ab	1.8 \pm 1.0ab	3.0 \pm 0.1bc	1.4 \pm 0.4c
<i>G. diaphanum</i>	25.76 \pm 0.92c	16.69 \pm 1.34c	6.3 \pm 2.1ab	2.2 \pm 0.4b	2.4 \pm 1.6b	0.8 \pm 0.3cd	5.5 \pm 3.2b	1.4 \pm 0.4c
<i>G. etunicatum</i>	27.07 \pm 4.55c	17.60 \pm 4.92c	3.6 \pm 0.3cb	1.4 \pm 0.4b	1.7 \pm 0.9bc	0.9 \pm 0.1bcd	2.5 \pm 1.2bc	1.1 \pm 0.4cd
Non-AMF	0 \pm 0d	0 \pm 0d	0 \pm 0d	0 \pm 0c	0 \pm 0c	0 \pm 0d	0 \pm 0c	0 \pm 0d

Mean \pm SD (n=3) followed by the same letter within a column shows non-significant difference at $p<0.05$ level.

Table 2. Effects of five *Glomus* species inoculation and drought stress on shoot dry wt., root dry wt., plant dry wt. and mycorrhizal dependency in *Citrus tangerine*.

AMF status	Shoot dry wt. (g plant $^{-1}$)		Root dry wt. (g plant $^{-1}$)		Plant dry wt. (g plant $^{-1}$)		Mycorrhizal dependency (%)	
	WW	DS	WW	DS	WW	DS	WW	DS
<i>G. mosseae</i>	0.87 \pm 0.27a	0.42 \pm 0.03b	0.65 \pm 0.24a	0.47 \pm 0.06a	1.45 \pm 0.51a	0.90 \pm 0.06a	309	265
<i>G. versiforme</i>	0.81 \pm 0.54ab	0.25 \pm 0.07c	0.50 \pm 0.20abc	0.30 \pm 0.11bc	1.33 \pm 0.74ab	0.54 \pm 0.17b	283	159
<i>G. geosporum</i>	0.83 \pm 0.35ab	0.57 \pm 0.19a	0.53 \pm 0.18ab	0.39 \pm 0.11ab	1.39 \pm 0.52a	0.96 \pm 0.28a	296	282
<i>G. diaphanum</i>	0.41 \pm 0.15bc	0.25 \pm 0.04c	0.28 \pm 0.10cd	0.20 \pm 0.06cd	0.69 \pm 0.25bc	0.45 \pm 0.06b	147	132
<i>G. etunicatum</i>	0.36 \pm 0.10c	0.23 \pm 0.05c	0.35 \pm 0.14bcd	0.26 \pm 0.12c	0.72 \pm 0.23bc	0.48 \pm 0.16b	153	141
Non-AMF	0.30 \pm 0.03c	0.22 \pm 0.02c	0.17 \pm 0.01d	0.13 \pm 0.02d	0.47 \pm 0.04c	0.34 \pm 0.01b	100	100

Mean \pm SD (n=6) followed by the same letter within a column shows non-significant difference at $p<0.05$ level.

Table 3. Effects of five *Glomus* species inoculation and drought stress on plant height, leaf number per plant, stem diameter and relative water content in *Citrus tangerine*.

AMF status	Plant height (cm)		Leaf number per plant		Stem diameter (cm)		Relative water content (%)	
	WW	DS	WW	DS	WW	DS	WW	DS
<i>G. mosseae</i>	18.90±7.00ab	12.33±1.91b	22.3±3.3a	15.7±4.2ab	0.314±0.032ab	0.263±0.028a	94.54±3.10ab	92.08±2.72ab
<i>G. versiforme</i>	15.65±3.01bc	9.78±1.38bc	19.0±3.2a	13.8±2.9abc	0.269±0.038bc	0.217±0.023bc	94.84±4.27ab	94.25±3.01a
<i>G. geosporum</i>	21.40±4.27a	17.43±4.57a	22.2±3.9a	17.7±4.5a	0.323±0.040a	0.268±0.034a	96.04±1.97a	94.75±3.02a
<i>G. diaphanum</i>	17.25±5.49ab	9.62±2.06bc	20.8±7.4a	12.0±3.0bcd	0.315±0.067ab	0.231±0.031ab	92.74±1.47ab	90.26±3.13ab
<i>G. etunicatum</i>	11.27±2.42cd	8.22±1.46c	13.7±2.9b	10.7±2.5cd	0.250±0.042cd	0.205±0.046bc	90.97±0.32bc	88.18±4.19b
Non-AMF	8.70±1.88d	7.28±0.89c	10.8±1.7b	9.8±2.4d	0.201±0.028d	0.189±0.026c	88.25±1.86c	86.52±2.44b

Mean±SD followed by the same letter within a column shows non-significant difference at $p<0.05$ level. N=6 for plant height, leaf number per plant and stem diameter; n=3 for relative water content.

> *G. versiforme* > *G. etunicatum* > *G. diaphanum*. Under DS conditions the ranking of *Glomus* species for the mycorrhizal dependency was as follows: *G. geosporum* > *G. mosseae* > *G. versiforme* > *G. etunicatum* > *G. diaphanum*.

Plant height, leaf number per plant, stem diameter and RWC were stimulated by AM colonization (Table 3). *Glomus geosporum* inoculated seedlings had the highest values and *G. etunicatum* inoculated seedlings the lowest values.

The five *Glomus* species used in this experiment all significantly increased the soluble sugar and NSC levels in leaves of both WW and DS seedlings (Table 4). The highest soluble sugar and NSC content were observed in seedlings colonized by *G. geosporum* regardless of water status. The AMF colonization also stimulated starch accumulation in leaves of seedlings. *Glomus geosporum* seedlings had the highest starch contents, but *G. etunicatum* seedlings had the lowest starch contents.

The soluble protein, SOD, G-POD and CAT activities were increased by the five *Glomus* species colonization regardless of water status (Table 5). The soluble protein concentration was significant higher in *G. mosseae*, *G. versiforme* and *G. diaphanum* seedlings than in the non-inoculated control seedlings under WW and DS conditions. Except for *G. etunicatum* colonized treatment, other *Glomus* species colonized treatments

significantly increased the SOD activities of leaves under WW conditions, whereas the beneficial increments of AMF were found only in *G. mosseae* and *G. diaphanum* inoculated treatments under DS conditions. Except for *G. versiforme*, other *Glomus* species colonization did not affect the G-POD activities of leaves under WW conditions. However, under DS conditions, other *Glomus* species except for *G. diaphanum* significantly increased the G-POD activities of leaves. Although AMF colonization did not affect the CAT activities of leaves under WW conditions, *G. mosseae* and *G. versiforme* colonization notably increased the CAT activities of leaves under DS conditions. *Glomus mosseae* seedlings showed the highest soluble protein concentration and CAT activity in leaves, *G. diaphanum* seedlings showed the highest SOD activity, and *G. versiforme* seedlings showed the highest G-POD activity.

Glomus mosseae and *G. geosporum* inoculations significantly increased leaf *E* under two water regimes compared with control treatment (Table 6). Comparison of five *Glomus* species efficiency showed that *G. mosseae* under WW conditions and *G. versiforme* under DS conditions significantly increased Pn compared with non-inoculated control treatment. *Glomus mosseae*-seedlings showed the highest *g_s* and *G. etunicatum* seedlings the lowest value. The AM colonization usually decreased *L_t* regardless of water treatments, and *G. diaphanum* seedlings had the lowest *L_t* under WW and DS conditions.

Table 4. Effects of five *Glomus* species inoculation and drought stress on soluble sugar, starch, and total non-structural carbohydrates (NSC) of leaf in *Citrus tangerine*.

AMF status	Soluble sugar (% fwt.)		Starch (% fwt.)		NSC (% fwt.)	
	WW	DS	WW	DS	WW	DS
<i>G. mosseae</i>	9.65±0.32a	10.30±0.17a	8.44±1.33b	8.60±0.40bc	18.09±1.08b	18.90±0.37b
<i>G. versiforme</i>	10.01±1.53a	10.25±1.37a	7.95±1.24b	8.97±0.23bc	17.96±2.30b	19.22±1.17b
<i>G. geosporum</i>	10.13±0.70a	11.18±0.85a	13.50±0.46a	12.69±0.96a	23.62±1.02a	23.87±0.28a
<i>G. diaphanum</i>	9.85±0.64a	10.20±0.34a	9.26±0.56b	9.80±0.07b	19.11±0.89b	20.00±0.28b
<i>G. etunicatum</i>	9.75±0.69a	9.97±1.42a	7.79±1.40b	8.16±1.66cd	17.54±1.02b	18.13±1.91b
Non-AMF	7.49±0.49b	7.95±1.11b	5.60±0.79c	6.79±0.55d	13.10±1.07c	14.74±1.56c

Mean±SD (n=3) followed by the same letter within a column shows non-significant difference at $p<0.05$ level.

Table 5. Effects of five *Glomus* species inoculation and drought stress on soluble protein, superoxide dismutase (SOD), guaiacol peroxidase (G-POD) and catalase (CAT) activities of leaf in *Citrus tangerine*.

AMF status	Soluble protein (% fwt)		SOD (U g ⁻¹ fwt.)		G-POD (U g ⁻¹ fwt.)		CAT (U g ⁻¹ fwt.)	
	WW	DS	WW	DS	WW	DS	WW	DS
<i>G. mosseae</i>	25.40±2.14a	24.12±1.19a	381.07±36.59b	668.41±14.62ab	275.47±4.25ab	362.50±25.00b	828.3±199.28a	916.7±72.17a
<i>G. versiforme</i>	23.39±1.18ab	22.54±1.22ab	384.06±22.79b	606.06±48.46bc	377.78±155.36a	480.05±41.80a	812.5±225.35a	905.9±67.85ab
<i>G. geosporum</i>	20.53±1.44bc	20.73±3.15bc	407.19±38.52b	618.72±25.14bc	264.33±14.15ab	305.66±19.81bc	785.1±31.94a	867.4±51.49abc
<i>G. diaphanum</i>	25.78±2.40a	21.24±1.60abc	646.19±45.19a	731.48±74.67a	267.17±4.95ab	286.96±48.97cd	804.4±151.55a	785.1±31.94bcd
<i>G. etunicatum</i>	23.04±2.96ab	19.00±1.42cd	367.00±52.47bc	589.96±54.07bc	133.33±16.67c	332.02±49.33bc	722.2±48.11a	740.1±127.27d
Non-AMF	19.51±0.77c	16.19±1.09d	285.18±73.81c	541.30±67.50c	184.53±26.83bc	228.43±40.02d	559.0±52.36a	741.9±13.97cd

Mean±SD (n=3) followed by the same letter within a column shows non-significant difference at $p<0.05$ level.**Table 6.** Effects of five *Glomus* species inoculation and drought stress on transpiration rates (E), photosynthetic rates (Pn), stomatal conductance (gs) and leaf temperature (Lt) in *Citrus tangerine*.

AMF status	E (mmol m ⁻² s ⁻¹)		Pn (μmol m ⁻² s ⁻¹)		g _s (mmol m ⁻² s ⁻¹)		Lt (°C)	
	WW	DS	WW	DS	WW	DS	WW	DS
<i>G. mosseae</i>	2.35±0.38a	2.63±0.41a	6.35±1.07a	4.93±1.85bc	152.75±37.47a	146.50±20.11a	33.2±0.3c	33.7±0.3b
<i>G. versiforme</i>	1.95±0.39abc	1.82±0.33bc	5.35±1.25bc	5.28±1.76a	104.75±13.74b	103.00±6.38b	32.5±0.2d	32.7±0.1c
<i>G. geosporum</i>	2.38±0.19a	2.29±0.26ab	4.25±0.47bc	4.08±0.56abc	142.25±7.59a	131.75±19.14a	33.7±0.2b	33.6±0.1b
<i>G. diaphanum</i>	2.25±0.28ab	1.40±0.43c	4.10±1.82bc	3.08±1.23bc	137.00±12.73a	103.50±12.15b	30.8±0.3e	31.9±0.2d
<i>G. etunicatum</i>	1.67±0.16c	1.79±0.36bc	3.15±1.08c	2.90±0.30bc	101.00±8.52b	96.75±9.60bc	33.4±0.1bc	33.7±0.2b
Non-AMF	1.88±0.31bc	1.60±0.39c	3.08±0.43c	2.20±1.68c	107.00±9.91b	79.75±16.84c	34.1±0.2a	35.1±0.3a

Mean±SD (n=4) followed by the same letter within a column shows non-significant difference at $p<0.05$ level.

DISCUSSION

Different *Glomus* species inoculated on *C. tangerine* showed different mycorrhizal development (Table 1). *Glomus* species apparently responded differently to soil conditions and host plant affected mycorrhizal development. Moreover, plant species of low mycorrhizal dependency tended to limit mycorrhizal colonization (Graham et al., 1991). Under WW conditions, AM colonization was significantly positive correlated ($r=0.9664$, $p<0.01$) with corresponding mycorrhizal dependency, whereas there was no correlated ($r=0.3891$, $p>0.05$) between AM colonization and corresponding mycorrhizal dependency under DS conditions. Thus, variations of the correlation in this experiment were due to different soil water status, which affected the responses of AMF. The correlation between AM colonization and mycorrhizal dependency in low P soil could represent a functional response of the fungus to host carbon availability (Graham et al., 1991).

In general, AM fungal efficiency is measured in terms of growth status of host plant under different environmental conditions (Ruiz-Lozano et al., 1995). In this study, we observed that *G. mosseae* and *G. geosporum* colonization resulted in higher plant biomass (shoot dry wt., root dry wt. and plant dry wt.) and plant growth responses (plant height, leaf number per plant, and stem diameter) than other three *Glomus* species regardless of water status (Tables 2, 3). Moreover, for mycorrhizal dependence, *C. tangerine* highly depended on *G. mosseae* and *G. geosporum* under two water regimes. Thus, the two more efficient AMF used in this study were *G. mosseae* and *G. geosporum* under WW and DS conditions. Our study showed that AM seedlings had the higher levels of soluble starch, soluble sugar and NSC in leaves than non-AM seedlings regardless of water status (Table 4). These results are supported by higher rates of Pn perhaps in response to great carbon demand by its allocation to roots. The result was in accord with the finding of Nemec and Guy (1982), who reported that *G. macrocarpus* inoculation increased the levels of total soluble sugars, starch and NSC in leaves of *Cleopatra mandarin*.

It is well documented that AM symbiosis causes the increases of antioxidant enzymes activities of host plants. In an early study, SOD activity was higher in the roots of mycorrhizal *Lactuca sativa* plants than those of non-AM plants (Ruiz-Lozano et al., 1996). Lambais et al. (2003) reported that, under low P soil conditions, CAT activity was induced in *Phaseolus vulgaris* roots colonized by *G. clarum*. Inoculation with *G. claroideum* increased the activities of SOD and CAT in *Rhamnus lycioides*. We also observed that inoculation with five *Glomus* species usually increased the SOD, G-POD and CAT activities in leaves of *C. tangerine* (Table 5). Moreover, *Glomus* species had different responses to SOD, G-POD and CAT. For example, *G. diaphanum* colonization significantly increased SOD activity, *G. versiforme* colonization G-POD activity, and *G. mosseae* colonization CAT

activity. It is known that SOD converts superoxide into hydrogen peroxide, which is then eliminated by CAT. When mycorrhizal plants had higher antioxidant enzymes activities, cellular and photo-oxidative damages would be decreased by AMF colonization. Thus, mycorrhizal plants may have important ecological implications for adaptation to adverse environmental conditions. Nodules from stressed mycorrhizal *Anthyllis cytisoides* exhibited higher antioxidant enzyme activities, which were related to the removal of ROS (Goicoechea et al., 2005). Although external hyphae, stomatal regulation and indirect P nutrition can enhance water uptake by mycorrhizal roots (Nelsen and Safir, 1982; Faber et al., 1991; Ruiz-Lozano and Azcon, 1995; Goicoechea et al., 1997), it seems that AM symbiosis may lead to a lower drought-induced oxidative stress in mycorrhizal plants due to higher antioxidant enzymes. Enzymatic polymorphism in different strains of AMF might be related to their efficiency in root colonization and their influence on plant growth (Garcia-Garrido et al., 2000). In addition, our results indicated that the level of AM colonization was negative correlated with SOD activities ($r=-0.6579$, $p<0.05$).

Glomus mosseae and *G. versiforme* had no significant effect on gs of *Vigna unguiculata* under WW and DS conditions (Diallo et al., 2001). Mycorrhizal infection improved gs in *Rosmarinus officinalis* plants under DS conditions (Sanchez-Blanco et al., 2004). The gs of citrus taxa was usually not changed by AM colonization (Augé, 2001). Our results found that *C. tangerine* inoculated with *G. mosseae*, *G. diaphanum* and *G. geosporum* had significant higher gs than non-inoculated seedlings regardless of water status (Table 6), whereas the inoculation of *G. etunicatum* used in this study did not affect gs (Table 6). Mycorrhizal effects on gs were attributable to mycorrhizal roots and mycorrhizal soils, as well as to mycorrhizal infection of roots alone (Augé et al., 2004). Ruiz-Lozano et al. (1995) reported that the effect on gs was related to growth promotion. In addition, we observed in this study that five *Glomus* species significantly decreased Lt of mycorrhizal seedlings compared with non-inoculated control treatment (Table 6). This was likely due to the greater evaporative cooling of higher *E* in mycorrhizal seedlings (Wu and Xia, 2006).

In conclusion, all the data suggested that five *Glomus* species used in the present study had an ability to improve the water relations of *C. tangerine* and higher antioxidant enzymes activities of leaves. The more efficient fungus in *C. tangerine* was *G. mosseae* or *G. geosporum* and the least was *G. etunicatum* under both WW and DS conditions.

Acknowledgements. We are grateful to Y.-S. Wang for providing the AM fungal inocula. This work was supported by Science and Technology Exploitation Special Item (2003EP090018; 2004EP090019) for Three-Gorge migrant, Ministry of Science and Technology Department of the People's Republic of China.

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5種球囊黴屬真菌影響了乾旱脅迫下紅橘的水分代謝

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以 5 個球囊黴屬真菌 (*Glomus mosseae*, *G. geosporum*, *G. versiforme*, *G. etunicatum* 和 *G. diaphanum*) 為供試菌種，接種于正常水分和乾旱條件下的紅橘實生苗上，從植株生長、碳水化合物、光合特性和抗氧化酶等方面比較各個菌種改善紅橘水分代謝的能力。結果表明，紅橘對以上 5 種菌種的依賴性大小順序是：*G. mosseae* \approx *G. geosporum* $>$ *G. versiforme* $>$ *G. etunicatum* $>$ *G. diaphanum*。5 種菌種的接種對紅橘的生長、碳水化合物、光合特性和抗氧化酶均顯示出有利的影響。在正常水分和乾旱條件下，接種 *G. geosporum* 對紅橘株高、葉片數、莖粗、葉片相對含水量、可溶性糖、澱粉和總的非結構性碳水化合物顯示出最好的效果，接種 *G. etunicatum* 表現最小的效果。接種 *G. mosseae* 的紅橘葉片顯示最高的可溶性蛋白含量和過氧化氫酶活性，接種 *G. diaphanum* 的紅橘葉片有最高的超氧化物歧化酶活性，接種 *G. versiforme* 的紅橘葉片觀察到最高的愈創木酚過氧化物酶活性。此外，5 個球囊黴屬真菌顯著降低菌根化植株的葉片溫度。不同的菌種顯示出不同的效果去改善紅橘的水分代謝，其中 *G. mosseae* 和 *G. geosporum* 是較高效的，*G. etunicatum* 是較低效的。叢枝菌根共生體改善紅橘水分代謝可能部分歸結於抗氧化酶活性的升高。

關鍵詞：叢枝菌根真菌；柑橘；乾旱；*Glomus*；水分代謝。