

Arbuscular Mycorrhizae and Water Relations in Citrus

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ABSTRACT

Citrus is one of the most important commercial fruit crops throughout the world, including China. However, it is grown in regions that are frequently subjected to water deficiency, which restricts the yield and quality of the crop. Citrus has very few and short root hairs and is highly dependent on arbuscular mycorrhizae, since the mutualistic symbiosis replaces some of the root hairs' functions. In this review, we describe arbuscular mycorrhizal symbiosis, mycorrhizal dependency of citrus, and the effect of drought stress on mycorrhizal development in citrus plants. We also describe advances in understanding how arbuscular mycorrhizal symbiosis improves water relations in citrus. These mechanisms include the direct water uptake and transport via external hyphae, the indirect effect of improved phosphorus nutrition, the improvement of osmotic adjustment and reactive oxygen metabolism, and the effect of glomalin produced by arbuscular mycorrhizae.

Keywords: arbuscular mycorrhizal fungi, rootstock, drought, symbiosis

Abbreviations: Ψ , leaf water potential; **AM**, arbuscular mycorrhizal; **APX**, ascorbate peroxidase; **ASC**, ascorbate; **BRSP**, Bradford-reactive soil protein; **CAT**, catalase; **G-POD**, guaiacol peroxidase; **GPX**, glutathione peroxidase; **GR**, glutathione reductase; **GRSP**, glomalin-related soil protein; **GSH**, glutathione; **H₂O₂**, hydrogen peroxide; **IRSP**, immunoreactive soil protein; **MD**, mycorrhizal dependency; **NSC**, total non-structural carbohydrate; **¹O₂**, singlet oxygen; **O₂⁻**, superoxide anion radical; **OH[•]**, hydroxyl radicals; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase. Abbreviations of *Citrus* and *Citrus* relatives names appear in **Table 4**

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INTRODUCTION

Of all the stresses that negatively affect plant growth, drought is among the most lethal from a global point of view (Zézé *et al.* 2007). This is because water is an integral component of the biochemical reactions in the plant that govern the numerous metabolic, physiological and biochemical processes affecting plant productivity (Alam 1999). Citrus is a major horticultural commodity worldwide, second only to bananas, in terms of volume of world trade. Most of citrus' global production depends on irrigation for economic production (Shalhevet and Levy 1990).

In citrus, drought stress strongly inhibits vegetative growth, reduces yield, and has a deleterious effect on fruit quality (Levy *et al.* 1978a, 1978b, 1979); it can even change the root-distribution pattern (Arbona *et al.* 2005; García-Sánchez *et al.* 2007). However, although the plant is mesomorphic, the leaves have many xeromorphic characteristics that help it survive drought (Levy and Syvertsen 2004). In

some regions, citrus plants are grown without irrigation, such as in hot, humid tropical areas with a monsoon-type rainfall distribution, despite four or five months of severe winter drought in most years. In addition, a number of soil microorganisms, including arbuscular mycorrhizal (AM) fungi, have been shown to alleviate symptoms of drought stress. Research on the relations between AM and water status of citrus began ca. 30 years ago, when Levy and Krikun (1980) reported the effect of AM fungi on water relations of citrus seedlings. Since then, a multitude of experiments have indicated that the AM symbiosis improves water-relations and plays an important role in citrus growth under conditions of drought stress (**Fig. 1**). The present review focuses on advances in our understanding of the mechanisms underlying AM's improvement of water relations in citrus.

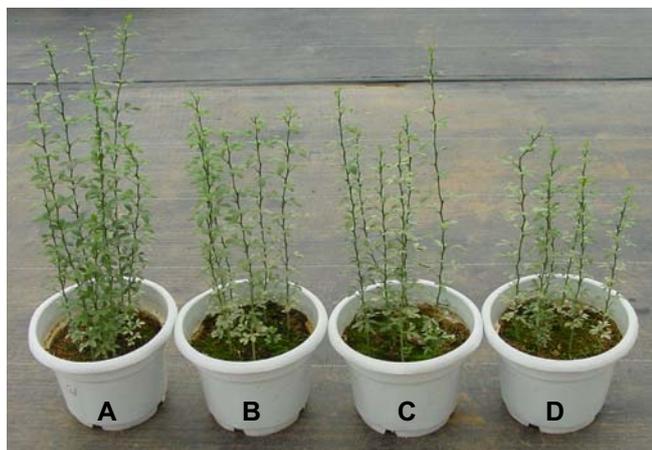


Fig. 1 Growth status of mycorrhizal and non-mycorrhizal TRIF seedlings grown in well-watered and drought stress conditions. (A) *G. versiforme*-inoculated TRIF seedlings grown in well-watered condition; (B) uninoculated TRIF seedlings grown in well-watered condition; (C) *G. versiforme*-inoculated TRIF seedlings grown in drought stress condition; (D) uninoculated TRIF seedlings grown in drought stress condition; (Figure by Q.S. Wu, unpublished results).

AM SYMBIOSIS

In 1885, A.B. Frank, a German scientist, coined the name “mycorrhiza” to describe the specific mutualistic association existing between higher plants and mycorrhizal soil fungi. The association is based on bidirectional movement of nutrients, in which carbon flows to the fungus and inorganic nutrients move into the plant (Sylvia 2004). There are seven types of mycorrhizae: arbuscular mycorrhizae, ectomycorrhizae, ectendomycorrhizae, arbutoid mycorrhizae, monotropoid mycorrhizae, ericoid mycorrhizae and orchid mycorrhizae. Citrus typically belongs to AM plants. AM symbiosis is the most widespread plant-root symbiosis, found in approximately 90% of the Earth’s land plant species (Gadkar *et al.* 2001; Gianinazzi *et al.* 2005). AM fungi have a broad host range, as evidenced by the fact that approximately 150 species of AM fungi colonize an estimated 225,000 species of plant hosts. Schüssler *et al.* (2001) classified AM fungi based on the morphological and molecular characteristics of their asexual spores into eight genera: *Glomus*, *Paraglomus*, *Sclerocystis*, *Scutellospora*, *Gigaspora*, *Acaulospora*, *Archaeospora* and *Entrophospora*. Most arbuscular mycorrhizae are characterized by the presence of intraradical hyphae (intercellular or intracellular in location), arbuscules (finely branched hyphae involved in nutrient exchange), extraradical mycelium (hyphae that connect the root to the soil), and spores formed in the extraradical mycelium (Peterson *et al.* 2004). With the exception of species in the genera *Gigaspora* and *Scutellospora* that produce auxiliary vesicles (sometimes called auxiliary bodies or cells) in the extraradical mycelium, all

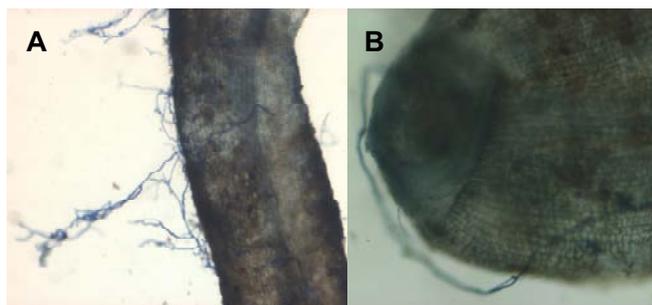


Fig. 2 Citrus (*Citrus unshiu* Marc. cv. ‘Guoqing No. 4’/*Poncirus trifoliata*) roots colonized by native AM fungi. (A) Extending extraradical mycelium. (B) Root cap and meristematic zone infected by native AM fungi. (Figure by Q.S. Wu, unpublished results).

other species form intraradical structures referred to as vesicles.

In the field, AM fungi can form structures in citrus roots, such as vesicles, arbuscules, entry points, extraradical mycelium, and intraradical hyphae (Fig. 2A). There are few vesicles in the AM roots of citrus, whereas the arbuscules are observed everywhere (Q.S. Wu, unpublished results). AM structures are found mainly in the root’s elongation and maturation zones. In addition, the root cap and meristematic zone are infected by native AM fungi (Fig. 2B). So far, the rhizosphere of citrus trees has found *Glomus*, *Sclerocystis*, *Gigaspora*, and *Acaulospora* species (Nemec *et al.* 1981; Vinayak and Bagyaraj 1990), and is fairly dependent on *Glomus* species (Davies and Albrigo 1994).

MYCORRHIZAL DEPENDENCY OF CITRUS

Gerdemann (1975) defined mycorrhizal dependency (MD) as the degree to which a plant is dependent on the mycorrhizal condition for maximum growth or yield at a given level of soil fertility. As a rule, MD is expressed as the ratio between the dry weights of AM and non-AM plants. Different citrus rootstocks show large differences in MD under controlled conditions. Rootstocks with long and abundant root hairs are less dependent than those with short or few root hairs. The order of MD of five rootstocks grown in a low-P sandy soil with *Glomus intraradices* was found to be: sour orange (SO) = Cleopatra mandarin (CLEO) > Swingle citrumelo (CTRM) > Carrizo citrange (CARZ) > trifoliolate orange (TRIF), when compared with non-inoculated rootstocks supplemented with P fertilization (Graham and Syvertsen 1985). Under well-watered conditions, rootstocks with lower MD, such as TRIF and its hybrid CARZ, generally exhibit greater hydraulic conductivity in their roots, as well as higher transpiration and CO₂ assimilation rates, implying that MD may be related to improved water relations of citrus rootstocks.

Drought stress also affects citrus MD. When red tangerine (REDT) was colonized separately by five different *Glomus* species - *G. mosseae*, *G. geosporum*, *G. versiforme*, *G. etunicatum* and *G. diaphanum*, drought stress notably reduced the MD of the plants (Wu *et al.* 2007b). However, the MD of TRIF seedlings was increased by drought stress when inoculated with *G. mosseae*, *G. geosporum* or *G. etunicatum* (Wu *et al.* 2006a). Therefore, the effect of drought stress on MD depends on the citrus rootstock, and within the same rootstock. MD differs with different fungal species. Under well-watered and drought-stress conditions, the MD ranking of five *Glomus* species was as follows: *G. mosseae* ≈ *G. geosporum* > *G. versiforme* > *G. etunicatum* > *G. diaphanum* (Wu *et al.* 2007b). It was concluded that the response of MD in citrus is affected by rootstock, drought stress and *Glomus* species, and that these effects are interdependent. In addition, the magnitude of MD of citrus may be accurately predicted by equations using soil P, Mn, Zn, and also Cu and pH as independent variables, because the MD of Troyer citrange (TROY) on *G. fasciculatus* in 26 California citrus soils was positively correlated with soil pH and inversely correlated with extractable soil P, Zn, Mn, Cu, percent organic matter, and cation-exchange capacity (Menge *et al.* 1982). In other words, citrus rootstocks exhibited the greatest MD with the least fertilization (Menge *et al.* 1978).

DROUGHT STRESS AFFECTS AM FUNGAL DEVELOPMENT OF CITRUS

The effects of drought stress on AM fungal development are summarized in Table 1. In all of these experiments, except those of Eissenstat *et al.* (1999) and Johnson and Hummel (1985), drought-stress decreased root colonization. The different results may be ascribed to both used species of AM fungi and citrus materials provided. Soil drying decreased the number of entry points in six experiments, whereas in one of them, entry points increased. When citrus plants

Table 1 Effect of drought stress on AM fungal development of citrus. Colonization was quantified as percent of roots colonized (col%).

Fungal species	Host species	Parameter	Length of drought (days)	Reference
<i>Glomus diaphanum</i>	<i>Citrus tangerine</i>	<col%, <entry points, <vesicles, <arbuscules	80	Wu <i>et al.</i> 2007b
	<i>Poncirus trifoliata</i>	<col%	80	Wu <i>et al.</i> 2006b
<i>G. etunicatum</i>	<i>Citrus tangerine</i>	<col%, >hyphal density	80	Wu <i>et al.</i> 2008
	<i>Citrus tangerine</i>	<col%, <entry points, <vesicles, <arbuscules	80	Wu <i>et al.</i> 2007b
<i>G. geosporum</i>	<i>Poncirus trifoliata</i>	<col%	80	Wu <i>et al.</i> 2006a
	<i>Citrus tangerine</i>	<col%, <entry points, <vesicles, <arbuscules	80	Wu <i>et al.</i> 2007b
<i>G. intraradices</i>	<i>Poncirus trifoliata</i>	<col%	80	Wu <i>et al.</i> 2006a
	<i>Poncirus trifoliata</i> × <i>Citrus sinensis</i>	>col%	~135	Johnson and Hummel 1985
<i>G. mosseae</i> mainly	<i>Citrus aurantium</i>	<col%	>40	Levy <i>et al.</i> 1983a
<i>G. mosseae</i>	<i>Poncirus trifoliata</i>	<col%	120, 113, 80	Wu <i>et al.</i> 2004, 2006a, 2008; Wu and Xia 2006a
	<i>Citrus tangerine</i>	>hyphal density	80	Wu <i>et al.</i> 2008
<i>G. sp.</i> FL904	<i>Citrus tangerine</i>	<col%, <entry points, <vesicles, <arbuscules	80	Wu <i>et al.</i> 2007b
	<i>Citrus aurantium</i>	=col%	42	Eissenstat <i>et al.</i> 1999
<i>G. versiforme</i>	<i>Citrus tangerine</i>	<col%, >entry points, <vesicles, <arbuscules	80	Wu <i>et al.</i> 2007b
	<i>Citrus tangerine</i>	<col%, <entry points, <vesicles, <arbuscules	97	Wu and Xia 2006a
<i>G. sp.</i> FL904	<i>Poncirus trifoliata</i>	<col%	80	Wu <i>et al.</i> 2006a
	<i>Poncirus trifoliata</i>	<col%, <entry points, <vesicles, <arbuscules	97	Wu <i>et al.</i> 2007a
<i>G. sp.</i> FL904	<i>Poncirus trifoliata</i>	>hyphal density	80	Wu <i>et al.</i> 2008

Symbols <, >, = indicate that drought stress decreased, increased, or did not affect the parameter, respectively.

were subjected to drought-stress, arbuscules and vesicles were clearly reduced in AM roots, whereas in an experiment performed by Wu *et al.* (2008), hyphal density in the soil increased. Based on an analysis of over 150 reports (Augé 2001), a relatively short drought duration was found to have no effect on AM colonization of potted plants, whereas longer drought decreased AM colonization. Levy *et al.* (1983a) observed an increase in AM infection of SO with decreasing soil moisture in the 0- to 60-cm soil layer in the field, but a very long interval between irrigations (40 days) reduced available water in the 0- to 30-cm layer and decreased AM colonization. Soil O₂ concentration decreases with depth, and frequent irrigation decreased O₂ concentration in the upper soil layers, thereby reducing AM infection. In a field experiment, AM colonization of adult pomelo (POML) cv. 'Shatianyou' trees increased with increasing of soil water content (Xue 2004) to an optimum of ~9 to 11%; further increases in water content had a negative effect on AM colonization.

ARBUSCULAR MYCORRHIZAE IMPROVE WATER RELATIONS IN CITRUS

Since Levy and Krikun (1980) first reported on the effect of AM fungi on water relations in rough lemon (RL) seedlings, studied citrus plants have included TRIF, REDT, SO, grapefruit/sour orange, 'Cara Cara' (*C. sinensis* L. cv. 'Cara Cara')/trifoliolate orange, citrange, CLEO, CTRM, CARZ, Alemow (MACR), POML, 'Bessie' Sweet orange (SwO), Troyer citrange (TROY) and Volkamer lemon (VOLK). The involved AM fungal species have included *G. mosseae*, *G. diaphanum*, *G. versiforme*, *G. intraradices*, *G. etunicatum*, *G. geosporum*, *G. sp.* FL904, *G. sp.* 25A, and *Gigaspora margarita*. Parameters studied have included osmotic adjustment, reactive oxygen metabolism, nutrition physiology, water physiology and AM fungal specificity. The research was conducted mainly in affiliation with the following academic organizations: (1) Citrus Research and Education Center, University of Florida; (2) Department of Plant Biology, Arizona State University; (3) Department of Ornamental Horticulture, University of Florida; (4) Department of Horticulture, College of Agricultural Sciences, Penn State University; (5) Lab of Pomology, Graduate School of Agriculture, Kyoto Prefectural University; (6)

Gilat Research Center, D.N. Negev; (7) College of Horticulture and Forestry, Huazhong Agricultural University; (8) College of Horticulture and Gardening, Yangtze University; (9) College of Horticulture and Landscape Architecture, Southwest University.

A large number of experiments have confirmed that AM citrus plants, both well-watered and drought-stressed, generally show higher stomatal conductance, transpiration and photosynthesis than their experimental non-AM counterparts (Fidelibus *et al.* 2001; Levy and Krikun 1980; Levy *et al.* 1983b; Wu and Xia 2006a; Wu *et al.* 2007b), implying that AM symbiosis allows leaves to maintain a more normal water balance and to fix more carbon during drought stress (Augé 2004). In addition, leaf water potential (Ψ) is usually increased by AM symbiosis, regardless of water limitations (Wu and Xia 2006a; Wu *et al.* 2007a). Leaves of AM and non-AM citrus plants might be expected to develop dissimilar symplastic solute pools, resulting in improved osmotic adjustment (Wu and Xia 2006a; Wu *et al.* 2007a). Leaf water content or relative water content are compared much less frequently in AM vs. non-AM citrus plants than leaf Ψ . AM symbiosis usually increases host growth during drought by affecting nutrient acquisition and possibly hydration. The contents of P, K, and Ca were found to be higher in AM citrus seedlings than in non-AM seedlings under both well-watered and drought-stress conditions, and the mycorrhizal contribution to seedlings was higher under drought stress than under well-watered conditions (Wu and Zou 2007). As a result, the effect of AM symbiosis on citrus nutrition is more pronounced in plants grown under drought stress than in those grown under well-watered conditions (Sánchez-Díaz and Honrubia 1994). Espeleta *et al.* (1999) in comparing different studies using citrus seedlings, concluded that, in general, AM effects on root responses to localized drought may be noticeably affected by tree developmental stage.

A field technique was designed to compare the AM roots of adult red grapefruit trees to VOLK exposed to dry surface soil (Espeleta *et al.* 1999). One wooden chamber (45×45×50 cm) was buried about 1 m from the bole of the tree and was covered with an insulated lid to exclude rainwater and sunlight. Each split-pot system consisted of two vertically arranged plastic pots with a transparent plastic window for root mapping. One split-pot contained AM

roots from an adult tree and the other non-AM roots from the same tree. This simple technique enabled the manipulation of AM fungal colonization on the trees. Unfortunately, this technique has not been taken advantage of by other mycorrhizal researchers.

Although AM fungal infection has been found to improve water relations in citrus plants, the mechanisms underlying the increase in drought resistance or improvement in water flow are uncertain. Potential mechanisms are described here.

Direct water uptake and transport via external hyphae

Increasing evidence has confirmed that external hyphae can directly take up water from soils. The external mycelium has several functions, the most important of which is the uptake and translocation of water and mineral nutrients, such as, P from the soil solution to the roots. The highly branched nature of the absorbing hyphae increases the surface area for water uptake. Hyphal growth away from the depletion zone at the root surface extends the region from which water can be absorbed (Peterson *et al.* 2004). Hyphae are able to extend into soil pores that are too small for roots to enter (Morgan *et al.* 2005). Hyphal water transport is involved in changes in transpiration and hydraulic conductivity. Under well-watered conditions, AM RL usually exhibits higher transpiration than non-mycorrhizal plants (Levy and Krikun 1980). Under non-limited soil moisture conditions, AM CARZ and SO seedlings exhibit better root hydraulic conductivity and higher transpiration (Graham and Syvertsen 1984). Wu and Xia (2006b) reported greater active and total absorption areas of root systems in AM vs. non-AM TRIF seedlings, especially with soil water contents of 20% and 16%. Root length of VOLK was stimulated by inoculation with geographic isolates of *Glomus* (*G. sp.* 25A, *G. mosseae* 51C, *G. mosseae* 114C, and *G. intraradices* FL208) from arid, semi-arid and mesic areas (Fidelibus *et al.* 2001). Abundant water in mycorrhizal citrus roots, due to direct water uptake, ensures normal physiological metabolism. Therefore, the maintenance of AM activity through direct water translocation could potentially improve the drought resistance of plants during periods of dry soils. Even after prolonged drought (70-80 days), AM hyphae persisted in soil with a water potential as low as -20 MPa (Querejeta *et al.* 2003). Therefore, adequate management of the AM symbiosis is vital to enhancing plants' drought resistance. Moreover, enhanced symplastic water transport via expression of the plasma membrane aquaporin gene (*NtAQP1*) is important for the efficiency of AM symbiosis, at least under drought conditions (Porcel *et al.* 2005b).

The hyphal contribution to water transport is rather large. When the hydraulic conductivity of an entire hypha was measured at 1 cm/(s MPa), with a hyphal diameter of 10 mm, water transport under a 0.5 MPa gradient is 5.4 nl/h; considering the hydraulic conductivity of an entire hypha of 10 cm/(s MPa), transport can reach 54 nl/h (Allen 2006). Rapid channels of water transport and rapid water-potential equilibration can occur within hyphal cells, because the cells of fungal hyphae have no membrane (Wu and Xia 2004). Owing to the bidirectional flow of water in hyphae, the reverse water flow into the soil is much smaller than the flow rates associated with transpiration.

Indirect effect of improved P nutrition

P is considered the most important plant-growth-limiting factor supplied by the AM association, because its restricted mobility in soils causes depletion zones around the roots (Brundrett and Abbott 2002). Hyphae of AM fungi are primarily responsible for helping plants acquire P. The P-acquisition process involves the transportation of P from the soil solution, across the membrane of the fungal hypha with the help of phosphate transporters in the external mycelium, movement of P along the hyphae to the arbuscule, un-

loading of the P from the fungal arbuscules at the arbuscule-cortical cell interface with the help of phosphate transporters on plant and fungal membranes, and uptake of this P by the plant cortical cells via transporters (Garg *et al.* 2006).

The increased drought resistance of host plants due to AM symbiosis may be a secondary response to better host nutrition – particularly P (Henderson and Davies 1990). Nelsen and Safir (1982b) found that the ability of AM onions to maintain P uptake under conditions of low soil moisture was the basis for the observed improved drought tolerance. Yano-Melo *et al.* (1999) reported that *G. clarum* and *G. etunicatum* enhance growth, photosynthesis and transpiration of *Musa*, but that these effects are probably caused by improved host P nutrition. A potted experiment showed that *G. versiforme* colonization of TRIF seedlings significantly increases the P content of well-watered and drought-stressed leaves by 9% and 16%, respectively (Wu and Zou 2007). Although drought stress reduced P content in leaf tissues of CARZ seedlings inoculated with *G. intraradices* after transplanting into large containers, P levels of AM seedlings were greater than those of non-AM seedlings, implying that AM infection improves the establishment of transplanted citrus by improving P uptake (Johnson and Hummel 1985). In citrus plants, transpiration and hydraulic conductivity were not significantly influenced by AM colonization under conditions of adequate P nutrition and soil moisture (Graham *et al.* 1987). However, AM plants grown under low soil P had higher stomatal conductivity than their non-AM counterparts. Addition of P fertilizer to non-AM plants essentially eliminated the differences in resistance to water transport (Nelsen and Safir 1982a, 1982b), and the increased water transport in AM roots was correlated with improved P nutrition (Hardie and Leyton 1981). It was concluded that improved P status of citrus due to AM symbiosis strongly affects water relations in citrus – and that in and of itself, the flow of water through the hyphae is not the primary factor. Therefore, in dry soil, mycorrhizal enhancement of P acquisition becomes much more important. However, no difference was observed in leaf P contents of AM vs. non-AM *Citrus jambhiri* seedlings (Dixon *et al.* 1988).

Based on the above-described experiments, P insufficiency and small shoots of non-AM plants are usually confounding factors, and thus controlling for AM effects on host P nutrition is a challenge (Augé 2001). Young AM and non-AM *C. volkameriana* had similar shoot sizes following the addition of P fertilizer to non-AM citrus plants (Fidelibus *et al.* 2001). Even with different allotments of P fertilizer, treatment with four geographic isolates of *Glomus* stimulated root growth compared with non-AM citrus plants after the recovery period following a soil-drying episode. Leaf P was lowest in non-AM plants. There was a positive correlation between leaf P content and root dry weight and length ($r = 0.71$ and 0.83 , respectively). Thus the improved P nutrition of plants due to AM association may be an indirect mechanism, a consequence of the improved root system. However, an opposite view was brought forward by Graham *et al.* (1987). These authors reported that when 5-month-old AM (*G. intraradices*) and non-AM (fertilized with soluble P) CARZ and SO seedlings were comparable in size and P sufficiency, AM symbiosis did not significantly enhance water relations of citrus under two drought-stress cycles of short duration. Under such conditions, the stress affected primarily the roots, rather than hyphal nutrient uptake (Hartmond *et al.* 1987). Therefore, further studies on the relationship between P nutrition and AM citrus water relations are needed.

Improvement of osmotic adjustment

Osmotic adjustment is an important adaptive response to drought stress in higher plants. The term refers to the lowering of osmotic potential due to a net accumulation of solutes in response to water deficit, and is distinct from the change in osmotic potential due to increased solute concentration associated with reductions in cell water content

Table 2 Effects of the AM fungus, *Glomus versiforme*, on the levels of the solutes participating in osmotic adjustment in TRIF seedlings under well-watered and drought-stress conditions

Parameter	Well-watered				Drought stress			
	Leaf	Significance	Root	Significance	Leaf	Significance	Root	Significance
K ⁺	>	*	>	NS	>	*	>	*
Ca ²⁺	>	NS	>	*	>	*	>	*
Mg ²⁺	<	NS	<	NS	=	NS	<	NS
Proline	<	*	<	NS	<	*	<	NS
Soluble sugar	>	*	>	*	>	*	>	NS
Soluble starch	>	*	>	NS	>	*	>	*
NSC	>	*	>	*	>	*	>	*
Glucose	<	*	>	*	>	NS	>	*
Fructose	<	*	>	*	>	NS	>	NS
Sucrose	<	NS	>	*	>	*	>	*

Symbols <, > indicate that AM symbiosis decreased or increased the parameter, respectively. Symbol = indicates that the parameter was similar in AM and non-AM plants.

*, NS: significant or non-significant differences ($p < 0.05$) in the parameter, respectively.

Source: Reconsolidation from *Acta Physiologiae Plantarum* (Wu *et al.* 2007a).

under drought (Chimenti *et al.* 2006). In the earliest work on the subject, Augé *et al.* (1986) concluded that AM fungi decrease the osmotic potential of leaves at full turgor and at the turgor loss points, with a corresponding increase in pressure potential at full turgor. AM association enabled plants to maintain leaf turgor and conductance under greater tissue water deficits, and at lower leaf and soil water potentials. Similarly, drought stress notably decreased leaf Ψ in TRIF seedlings, but the decrease was larger in non-AM seedlings (-0.53 MPa) than in their AM counterparts (-0.44 MPa) (Wu *et al.* 2007a). AM symbiosis significantly increased seedlings' leaf Ψ and relative water content, regardless of soil-water status.

The solutes participating in the osmotic adjustment are inorganic ions (K⁺, Ca²⁺, Mg²⁺) or uncharged organic compounds (proline, sucrose). AM symbiosis alters these solute levels in citrus plants regardless of soil water status. For example, AM citrus plants grown under drought-stress conditions exhibited higher soluble sugar content in their leaves, higher soluble starch and total non-structural carbohydrate (NSC) contents in leaves and roots, and higher glucose content in roots, sucrose content in leaves and roots, and K⁺ and Ca²⁺ levels in leaves and roots, relative to drought-stressed non-AM plants (Table 2; Wu *et al.* 2007a). These beneficial responses of solutes to AM fungal colonization enhance citrus plant drought tolerance by osmotic adjustment, thus protecting and stabilizing macromolecules and structures from damage induced by drought stress (Martinez *et al.* 2004).

The accumulation of proline is a commonly observed metabolic response of higher plants, including citrus, to water deficit (Levy 1980). In Wu and Xia (2006a) and Wu *et al.* (2007a), AM citrus plants generally showed lower proline levels than non-AM plants, when exposed to well-watered and drought-stress conditions. It is well-known that a high proline level may help plants survive short droughts and recover from stress (Sanchez *et al.* 1998). The decrease of proline in AM plants may be attributed to either their greater drought resistance or decreased injury in these plants grown under drought conditions, implying that the plants are more successful at avoiding drought stress (Augé 2004; Porcel and Ruiz-Lozano 2004). Further experiments confirmed that AM infection decreases the gene expression of Δ^1 -pyrroline-5-carboxylate synthetase (a key proline synthetase) under drought-stress conditions (Porcel *et al.* 2004).

An analysis of the net accumulation of solutes in citrus plants in response to drought stress indicated that better osmotic adjustment in AM plants originates not from proline, but from K⁺, Ca²⁺, Mg²⁺, glucose, fructose and sucrose (Wu *et al.* 2007a).

Improvement of reactive oxygen metabolism

In higher plants, reactive oxygen species (ROS) are continuously produced as byproducts of various metabolic path-

ways in different cellular compartments (Apel and Hirt 2004). Under steady-state physiological conditions, these molecules are scavenged by different components of the antioxidative-defense system. However, the equilibrium between production and scavenging of ROS may be perturbed by a number of adverse environmental factors, including drought stress. A variety of ROS, such as superoxide anion radical (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH·) and singlet oxygen (¹O₂), are then induced (Jung 2004). Higher plants rapidly detoxify the poisonous ROS by various cellular enzymatic and non-enzymatic mechanisms, thus protecting them from oxidative injury (Asada 1999). Non-enzymatic antioxidants include the major cellular redox buffers ascorbate (ASC) and glutathione (GSH), as well as tocopherol, flavonoids, alkaloids, and carotenoids. Enzymatic ROS-scavenging compounds in plants include superoxide dismutase (SOD), guaiacol peroxidase (G-POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), and catalase (CAT).

In an experiment performed by Wu *et al.* (2006b), *G. versiforme* inoculation notably decreased malondialdehyde (MDA), H₂O₂ and O₂⁻ in roots and drought-stressed leaves of TRIF seedlings (Table 3), indicating a lower accumulation of ROS in AM seedlings. H₂O₂ accumulated less in branched arbuscules and around hyphal tips penetrating the host cell (Salzer *et al.* 1999). Moreover, H₂O₂ accumulation was not observed in the fungal hyphal tips growing along the middle lamella, or in appressorium or vesicles. The intracellular accumulation of H₂O₂ was found in the cytoplasm of AM plants close to intact and collapsing fungal structures, whereas intercellular H₂O₂ was located on the surface of the fungal hyphae (Fester and Hause 2005). It was concluded that in the cells of AM roots, locally induced accumulation of H₂O₂ is limited, both near the intracellular AM hyphae and at the intercellular hyphal surface. It is now clear that H₂O₂ may also function as a molecular signal in plant cells, triggering tolerance against various abiotic stresses (Neto *et al.* 2005).

In both well-watered and drought-stressed REDT roots, SOD activity was significantly higher in AM than in non-AM plants (Wu *et al.* 2006c), indicating the induction of some new SOD isozyme (Palma *et al.* 1993) or expression of the Mn-SOD II gene (Ruiz-Lozano *et al.* 2001). AM infection generally increased the activities of G-POD, CAT, APX and GR in citrus plants exposed to well-watered conditions, drought stress or drought recovery (Table 3; Wu and Xia 2005; Wu *et al.* 2006a, 2006b, 2006c, 2007b; Yu *et al.* 2007). Moreover, the levels of non-enzymatic antioxidants, such as GSH and ASC, were often higher in AM than in non-AM citrus plants (Table 3; Wu *et al.* 2006b, 2006c). Therefore, when AM plants are subjected to drought stress, enzymatic and non-enzymatic ROS-scavenging mechanisms are rapidly induced and enhanced, thus partly explaining the lower ROS levels in these plants. Finally, AM symbiosis protects the host plant against oxidative damage, in turn

Table 3 Effects of the AM fungus, *Glomus versiforme*, on ROS and components of ROS-scavenging mechanisms in TRIF seedlings under well-watered and drought-stress conditions.

Parameter	Well-watered				Drought stress			
	Leaf	Significance	Root	Significance	Leaf	Significance	Root	Significance
MDA	<	*	<	*	<	*	<	*
H ₂ O ₂	<	NS	<	*	<	*	<	*
O ₂ ⁻	<	NS	<	*	<	*	<	*
SOD	>	NS	>	NS	>	NS	<	*
G-POD	>	*	>	NS	>	*	<	NS
CAT	>	NS	>	NS	>	*	>	NS
APX	<	NS	>	*	>	*	>	*
GR	>	*	>	NS	>	*	<	NS
Soluble protein	>	*	>	*	>	NS	>	*
ASC	>	*	>	NS	>	*	>	*
GSH	>	*	>	*	>	*	>	*

Symbols <, > indicate that AM symbiosis decreased or increased the parameter, respectively.

*, NS: significant or non-significant differences ($p < 0.05$) in the parameter, respectively.

Source: Wu QS, Xia RX, Zou YN (2006b) Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. *Journal of Plant Physiology* 163, 1101-1110, ©2006, with kind permission from Journal of Plant Physiology.

enhancing drought tolerance. That is to say, AM protection against the oxidative stress caused by drought is perhaps one of the most important mechanisms by which the AM symbiosis increases the tolerance of host plants to drought (Ruiz-Lozano 2003).

Effect of glomalin produced by AM fungi

AM hyphae have been shown to bind soil particles into water-stable aggregates (Tisdall and Oades 1979). This binding was found to be due to glomalin, an immunoreactive glycoprotein produced by the hyphae of AM fungi (Wright and Upadhyaya 1996). This protein is abundant in a wide range of soil environments at concentrations of 2 to 15 mg/g, and even up to >60 mg/g (Wright and Upadhyaya 1998; Wright *et al.* 1999). Glomalin appears to be as ubiquitous as the AM fungi themselves (Wright and Upadhyaya 1998). Large glomalin pools also result from its high persistence in the soil (Rillig *et al.* 2001). In soils, glomalin is quantified as glomalin-related soil protein (GRSP), an alkaline-soluble protein material linked to AM fungi that is defined by the extraction conditions (Rillig 2004; Nichols and Wright 2006; Vodnik *et al.* 2008). There are currently two detection methods used to quantify GRSP: the Bradford protein assay, yielding Bradford-reactive soil protein (BRSP), and an ELISA yielding immunoreactive soil protein (IRSP) (Rosier *et al.* 2006).

The concentration of BRSP in soils of well-watered and drought-stressed TRIF seedlings colonized by *G. versiforme*, *G. mosseae*, and *G. diaphanum* varied from 1.64 to 1.93 mg/g (Wu *et al.* 2008). Drought stress reduced the BRSP concentration slightly, albeit not significantly. The trend of BRSP in plants subjected to drought stress paralleled that of hyphal density, indicating a connection between the two ($r = 0.7169$, $p < 0.0001$). BRSP was positively correlated with water-stable soil aggregates (>2 mm, $r = 0.6217$, $p < 0.01$; 1-2 mm, $r = 0.6153$, $p < 0.01$; > 0.25 mm, $r = 0.6481$, $p < 0.001$). Thus, AM soils maintained more water-stable aggregates and better soil structure, and consequently held more moisture (Augé *et al.* 2001). An indirect mechanism enabling water uptake by roots of AM plants consists of preventing the development of significant gaps between the root and soil, thereby maintaining liquid continuity across the soil-root interface (Reid 1984). Taken together, the increased water-stable aggregates and higher BRSP due to AM colonization lead to better soil structure and alter the soil's moisture-retention properties which, in turn, lead to better plant drought resistance.

CONCLUSIONS

The statement that AM symbiosis can improve water relations and drought responses in citrus invariably holds true. Most of the above-described studies have focused on how

AM fungi affect citrus plants. In fact, research has often neglected the "mycorrhizal soil", even though mycorrhizal colonization of soil can affect that soil's moisture-retention properties, and thereby have marked direct and total effects on plant dehydration tolerance (Augé 2004).

Plants can adapt to drought stress by the induction of specific genes, whose products are thought to function in stress tolerance and responses (Yamaguchi-Shinozaki and Shinozaki 2005). Recently, some molecular studies have been conducted on AM symbiosis with lettuce (Ruiz-Lozano *et al.* 2001; Porcel *et al.* 2004, 2005a, 2006, 2007), soybean (Porcel *et al.* 2004, 2005a, 2006, 2007), tobacco (Porcel *et al.* 2005b, 2007), and maize (Porcel *et al.* 2007) under drought-stress conditions. These studies have revealed that AM symbiosis in combination with drought stress might induce a variety of specific genes (e.g. *Mn-sod II* gene, *G. intraradices* 14-3-3 gene, and a gene from *G. intraradices* encoding a binding protein) expression to enhance drought tolerance of host plants. However, there is little information on AM citrus plants at the molecular level. Despite the many advances described herein, the molecular basis of drought-stress tolerance in AM plants remains far from being understood (Porcel *et al.* 2004) because it is important to analyze functions of stress-inducible genes for further understanding molecular mechanisms of stress tolerance and response of drought-stressed plants after inoculated with AM fungi.

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Table 4 Abbreviations of Citrus and Citrus relatives' names.

Abbreviation	Name
CARZ	Carrizo citrange (<i>C. sinensis</i> [L.] Osbeck × <i>Poncirus trifoliata</i> L.)
CLEO	Cleopatra mandarin (<i>C. resnii</i> Hort ex Tan.)
CTRM	Swingle citrumelo (<i>C. paradisi</i> × <i>P. trifoliata</i>)
MACR	Alemow (<i>C. macrophylla</i> Westr.)
POML	Pomelo (<i>C. grandis</i> Osbeck.)
REDT	Red tangerine (<i>C. tangerine</i> Hort. ex Tan.)
RL	rough lemon (<i>C. jambhiri</i> Lush.)
SO	sour orange (<i>C. aurantium</i> L.)
SwO	sweet orange (<i>C. sinensis</i> [L.] Osbeck.)
TRIF	trifoliolate orange (<i>Poncirus trifoliata</i> L.)
TROY	Troyer citrange (<i>C. sinensis</i> × <i>P. trifoliata</i>)
VOLK	Volkamer lemon (<i>C. volkameriana</i> Chapot.)

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