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Water redistribution in mycorrhizosphere of trifoliate orange

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ABSTRACT

Mycorrhizal hyphae possess the ability of absorbing water directly from the surrounding matrix to root, whereas it is not clear whether water flow redistributes from hyphae to surrounding matrix. In this study, potted trifoliate orange (*Poncirus trifoliata*) seedlings were inoculated with an arbuscular mycorrhizal (AM) fungus *Funneliformis mosseae* and grown under well-watered (WW) and drought stress (DS) in Jingzhou, China. A 12.5-mL safety glass bottle with open mouth and growth substrate was placed into the pot, combining a root system *in-situ*. The bottle was sealed and collected after 6-week DS treatment. The soil DS-treatment strongly reduced root mycorrhizal colonization and soil hyphal length, relative to WW-treatment. A significantly higher soil water content of the bottle was found in AM-treated seedlings than in non-AM seedlings, irrespective of WW and DS, confirming the presence of hyphal water redistribution in AM-plants. Considerable reduction of hyphal water redistribution under DS only, but not under WW, implies that greater water status in AM-plants under DS would benefit plant biomass production uninterruptedly.

Key words: Citrus, Extraradical hyphae, Water flow, Water redistribution, Water stress

Arbuscular mycorrhiza (AM) is a symbiont between arbuscular mycorrhizal fungi (AMF) and roots of land's plants (Smith and Read 2008). AM assists the host plant for supply of immobile nutrients from soil to host plant, and in return, the host plant provides the photosynthates for AM development. In such process, AM has an important role facilitating nutrient and water uptake by the host plant and thereby the health of the host plant. Studies in the past showed that higher hydraulic conductivity of AM-plants under soil moisture deficit conditions, elevated the translocation of water within the mycorrhizal hyphal network spread within the mycorrhizosphere soil (Hardie and Leyton 1981) and aiding extraradical hyphal tips coupled with fewer wall layers, developing hydrophilic trait (Allen 2007). With the result, mycorrhizal hyphal tip can withdraw water from soil and translocate effectively within the hyphal framework of AMs, since few mycorrhizal hyphae with no septa act as highways of water transport in many of the arid soils (Allen 2007).

In the early days, Allen (2009) estimated the water flow of 90 nL/h in 10 µm diameter of mycorrhizal hypha between mycorrhizal hyphal network and plant roots. Graham and Syvertsen (1984) earlier reported water flow within roots via mycorrhizal hyphae was relatively lower than the water flow in plants, due to smaller diameter (Φ 10 µm) of the former. On the other hand, using a series of dyes and isotope analyses, Querejeta *et al*. (2003, 2007) found that in the night, water of hypha moved through the endodermis into intercellular hyphae, further flowing out the hyphae. As a result, the labeled water can move hydrophlic hyphal tips, in which it was exuded into the soil (Querejeta *et al*. 2007). These studies showed that water redistribution of hyphae could be a strong possibility, having key importance under arid ecosystem. The information about mycorrhizal hyphal water redistribution is very limited. The purpose of the present study was to validate the hypothesis about the enactment of hyphal water redistribution using trifoliate orange (*Poncirus trifoliata)*, a commercial citrus rootstock used in Southeast Asia for growing Satsuma mandarin under well-water (WW) and drought stress (DS) conditions.

MATERIALS AND METHODS

A five-leaf-old trifoliate orange seedling without mycorrhization was transplanted into a 4.7-L plastic pot, supplied with 2.8 kg of autoclaved (0.11 Mpa, 121°C, 2 h) soil and sand (2:1, v/v) on March 21, 2014. The experimental soil (the Xanthi-udic Ferralsol soil, FAO system) was collected from a citrus orchard on the Yangtze University compus (30°36′N, 112°14′E, 36 m above sea level), in which soil pH is 6.0, $KMnO_4$-N 12.1 mg/kg, Bray-P 15.7 mg/kg, and neutral NH_4OAc-K 22.3 mg/kg. An AM fungus, *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, was inoculated into the rhizosphere of the un-inoculated seedlings. The fungal strain was isolated from

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the rhizosphere of *Incarvillea younghusbandii* in Tibet, China, and further propagated in pot culture with spores and white clover. The inoculated dosage of *F. mosseae* was 1500 spores per pot. With regard to non-AMF treatment, same amount of autoclaved AM-inoculums was applied. The AM and non-AM seedlings were grown in a controlled condition in Jingzhou, China, characterized by photon flux density of 982 μmol/m^2/s, relative air humidity of 80% and night temperature of 27/20°C day. All the pots were weekly relocated at interval in order to eliminate environmental effects.

The experiment was conducted in a randomized block design using two factors, first factor as inoculated treatments with or without *F. mosseae* and second factor as soil water regimes, WW and DS. After the seedlings were inoculated with the AM fungus, soil water content of pots under WW status was regulated in such a way that corresponded to gravimetric 75% of the maximum field soil water holding capacity. All the WW treatments were kept for 16 weeks of AM-inoculation. Subsequently, half of the pots were subjected to six-week DS, corresponding to gravimetric 55% of the maximum field soil water holding capacity. Each treatment had five replicates, using as many as 20 pots.

A 12.5-ml safety glass bottle with open mouth was used. The bottle was supplied with 14.0 g of above same growth substrate. Before a day of soil DS treatment, we randomly selected a root system *in-situ* from the substrate of the pots, and then placed them into the bottle. A rubber plug was used to seal the mouth of the bottle. To further prevent water flow between the bottle and outer substrates, paraffins were used to seal the rubber plug. And, the bottle with the roots was buried into the pots *in-situ*. There were two bottles per pot. After soil water was controlled for 6-week DS treatment, the seedlings were harvested. The bottle was taken out from the pots after cutting the root system.

Soil water content of the bottle after harvesting was determined by weighing after over-drying at 100°C for 48 h. Hyphal water redistribution (H_{wr}) was calculated according to the following formula: $H_{wr} = (W_1 – W_2) / A$, where W_1 stands for the soil weight of the bottle before loading into the bottle, W_2 for the soil weight of the bottle after plants harvested, and A as the surface area of the root from the bottle. With regard to the determination of root surface area, an EPSON Flatbed Scanner, Epson Perfection V700 was used to scan the root systems of the bottle collected, and the root surface area was determined after analyzing the root images with the WinRHIZO 2007d.

Root mycorrhiza was stained by the protocol of Phillips and Hayman (1970) with 0.05% trypan blue in lactophenol, and root colonization was expressed as the percentage of colonized root lengths versus observed total root lengths. Soil hyphal length was determined according to the protocols as suggested by Bethlenfalvay and Ames (1987).

Data (means ± SD, n = 5) were analyzed by variance (ANOVA) with SAS software. The Duncan's Multiple Range Tests at 0.05 levels were used to compare significant differences between the treatments.

RESULTS AND DISCUSSION

Changes in mycorrhizal colonization and soil hyphal length

Our study showed the presence of no mycorrhizal structure either in the root or in the soil in non-AM seedlings. The inoculated seedlings exhibited 38.68%–53.52% of root AMF colonization and 0.34–0.58 m/g soil hyphal length (Table 1, Fig 1). The 6-week soil DS treatment heavily inhibited the intensity of root colonization and soil hyphal length by 27.7% and 41.4%, respectively, over soil WW treatment. This is consistent in earlier studies in *Pelargonium graveolens* (Amiri *et al*. 2015) and *Trifolium repens* (Tuo *et*

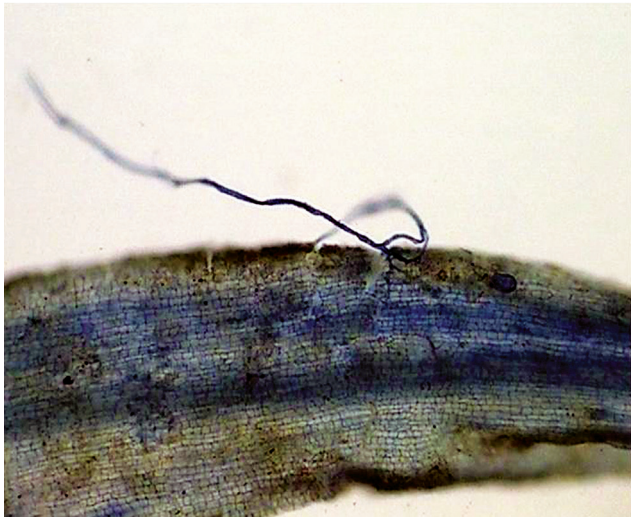

Fig 1 Root mycorrhizal colonization and developed extraradical hyphae in *Funneliformis mosseae*-colonized trifoliate orange seedlings.

Table 1 Mycorrhizal developed status in root and soil and total plant biomass of trifoliate orange exposed to well-watered (WW) and drought stress (DS) conditions

Water regimes	AMF status	Root colonization (%)	Soil hyphal length (m/g soil)	Total plant biomass (g FW/ plant)
WW	-AMF			2.29±0.21c
	+AMF	53.52±4.88a	0.58±0.04a	5.93±0.93a
DS	-AMF			1.49±0.31d
	+AMF	38.68±5.21b	0.34±0.04b	3.15±0.33b

Data (means ± SD, n = 5) followed by different letters among treatments represent significant differences at the 5% level. '–'.

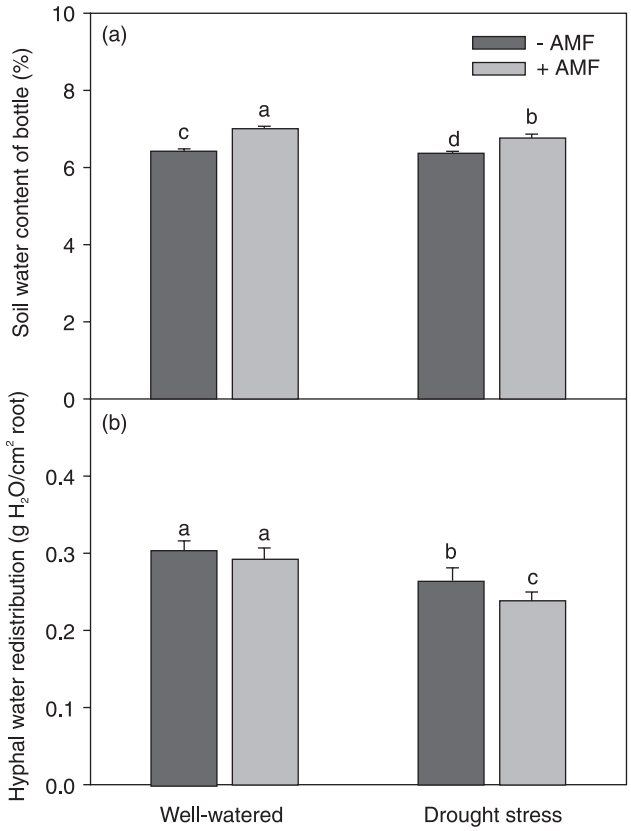

Fig 2 Soil water content of bottle (a) and hyphal water redistribution (b) in *Funneliformis mosseae*-colonized trifoliate orange seedlings exposed to well-watered (WW) and drought stress (DS) conditions. Data (means ± SD, n = 5) followed by different letters above the bars among treatments represent significant differences at the 5% level.

al. 2017). The inhibition of mycorrhizal colonization by soil DS was attributable to the reduction in hyphal length, plant photosynthates, and spore germination (Wu *et al*. 2013).

Response on plant biomass

The total plant biomass of trifoliate orange seedlings was significantly reduced under DS compared to under WW, irrespective of AM- or non-AM plants (Table 1). The AMF-inoculated seedlings represented 159.0% and 111.4% significantly higher total plant biomass than non-AMF ones under WW and DS, respectively. Similar results were earlier reported in white clover (Tuo *et al*. 2017) and mungbean (Habibzadeh *et al*. 2013). Such plant biomass responses under mycorrhization could be seen on account of mycorrhiza-enhanced nutrient absorption by extraradical hyphae (Habibzadeh *et al*. 2013).

Hyphal water redistribution

The soil water content of the bottle was not significantly different in non-AM seedlings between WW and DS, whereas significantly lower in AM-seedlings under DS than under WW (Fig 2a). On the other hand, the inoculated seedlings had 9.2% and 6.8% significantly higher soil water content than the non-inoculated seedlings exposed to WW and DS, respectively. The increase in soil water content of the bottle by mycorrhization possibly derived from a hyphal water redistribution from plant roots to soils by hyphae. As proposed by Querejeta *et al*. (2003), during the night, water can flow back roots, where water of root further moved out of extraradical hyphal tips into the surrounding matrix, even neighboring plants.

Analysis of water change per cm^2 root in this study showed that H_{wr} between AM and non-AM seedlings was not significantly different under WW, while was 9.5% significantly lower in AM over non-AM seedlings under DS (Fig 2b). It shows that mycorrhizas induced a lower H_{wr} under DS only. Possibly, under WW, roots are strong enough to absorb soil moisture and redistribute the root water. While the water flowed from hypha into soil is of lower magnitude compared to transpired rate of water flow (Allen 2006). Tinker and Nye (2000) argued that mycorrhizal hyphae were too small, and such changes on account of H_{wr} can be ignored, relative to total root water uptake in a given day. However, under DS, to maintain the normal water relations of AM-plants, lower H_{wr} in AM-plants meant more water retaining in roots of the host plant through reduced water redistribution from roots to surrounding matrixes. The water redistribution might play a critical role for AM-plants that maintained better water relations of the host plant (Allen 2009). As a result, AM-plants represented greater plant biomass production than non-AM plants under DS (Table 1). In fact, studies in the past indicated greater leaf water potential in AM than in non-AM plants in *Alnus glutinosa*, *Onobrychis viciifolia*, *Plukenetia volubillis*, and *Poncirus trifoliata* (Orfanoudakis *et al*. 2010, Tian *et al*. 2013, Kong *et al*. 2014, Zou *et al*. 2017). However more incisive work is needed for further confirmatory studies relating H_{wr}.

Conclusion

Our study confirmed the H_{wr} from the AM roots to the surrounding matrixes, besides H_{wr} was significantly inhibited by soil DS. Mycorrhizal inoculation formed a lower H_{wr} under DS, but not under WW, which played a critical role in maintaining better water relations of the host plant exposed to DS, at least in China.

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