Scientia Horticulturae 229 (2018) 132–136

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Quantitative estimation of water uptake by mycorrhizal extraradical hyphae in citrus under drought stress

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ARTICLE INFO

Keywords:
Hyphal length
Hyphal water absorption rate
Mycorrhization
Soil drought
Trifoliate orange

ABSTRACT

Mycorrhizal hyphae have the functioning on water absorption from soil, while the information regarding hyphal water absorption rate is not fully known. In this study, 37-μm nylon meshes were positioned in a pot bottom to allow mycorrhizal extraradical hyphae, but not roots, to pass through the mesh. The whole pot was placed in a beaker, where distilled water was supplied, reaching a 0.5-cm air gap between the pot bottom and water surface of the beaker. Citrus rootstock, trifoliate orange (*Poncirus trifoliata*) seedlings of pots were inoculated with *Funneliformis mosseae* and *Paraglomus occultum* and also exposed to well-watered (WW) and drought stress (DS). The 71-days soil DS significantly inhibited root mycorrhizal colonization and hyphal length in soil and mesh, regardless of mycorrhizal fungal species. The hyphal water absorption rate was 0.607–1.973 mg H_2O/h/mm for *F. mosseae* and 0.126–0.963 mg H_2O/h/mm for *P. occultum*, respectively. The DS treatment significantly elevated hyphal water absorption rate by 2.3–6.6 times. The increase of leaf water potential by mycorrhization was higher under DS than under WW. Our results provide a quantitative estimation of water absorption rate by mycorrhizal extraradical hyphae and also suggest more important water contribution of hyphae to the host plant under DS than under WW.

1. Introduction

Drought stress (DS), one of the important abiotic stresses, often causes the decrease of crop productivity. Moreover, under the present climate conditions, water is going to be a limiting resource for crop productivity (Sarwat and Tuteja, 2017). As a result, drought is chronic, random and unpredictable. Citrus trees are highly drought-sensitive, whose fruit quality and yield are reduced by DS, with low juice content in maturity (García-Sánchez et al., 2007).

Arbuscular mycorrhizal fungi (AMF), a kind of soil beneficial microbes, can establish a symbiotic association with roots of most land's plants, namely, arbuscular mycorrhiza (AM) (Smith and Read, 2008). AM symbiosis represents bidirectional roles between AMF and the host plant: AMF needs the photosynthates of the host plant for growth, and the host plant receives nutrients and water from mycorrhizal symbiosis (Smith and Smith, 2011). Studies in the past indicated that mycorrhizal association could help the host plant to enhance drought tolerance through (i). direct absorption of extraradical hyphae, (ii). biochemical mechanisms regarding osmotic adjustment, antioxidant protected system, and phytohormones, (iii). soil structure improvement by AMF-released glomalin, and (iv). molecular mechanisms regarding aquaporins, late embryogenesis, proteins, mRNA-binding proteins, etc. (Wu and Zou, 2017).

It is known that mycorrhizal hyphae can extend over 100 cm penetrating into soil particles from the root surface. In some cases, mycorrhizal hyphae can survive and even grow at extremely low soil water potential (Allen, 2006). Mycorrhizal hyphae themselves serve as a direct pathway for water flow in dry soils (Allen, 2009). As reported by Cosgrove et al. (1987), fungal hyphae of *Phycomyces blakesleeanus* potentially possessed 4.6×10^5 cm/s/MPa of hydraulic conductivity. Allen (2006) estimated that water transport of a 10-μm-diameter hypha was 0.14 nL/h, and the flux between the membranes was up to 131 nL/h. However, Tinker and Nye (2000) argued that mycorrhizal hyphae were too small, and such absorption and flux can be ignored, relative to total root water uptake. Despite the importance of water fluxes, the data regarding water fluxes within mycorrhizal hyphae are still scarce.

In the background, we utilized a special experimental equipment to quantitatively evaluate water absorption of mycorrhizal hyphae in trifoliate orange (a citrus rootstock used in the Southeast Asia) exposed to well-watered (WW) and DS. Such work will provide the direct evidence for hyphal contribution to water uptake.

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http://dx.doi.org/10.1016/j.scienta.2017.10.038
Received 18 August 2017; Received in revised form 18 October 2017; Accepted 27 October 2017

2. Materials and methods

2.1. Experimental equipment

The schematic diagram of the experimental equipment was presented in Fig. 1. A plastic pot having 11.5 cm upper diameter, 8.5 cm bottom diameter, and 14 cm height was used here. There were lots of holes at the bottom of the pot. Subsequently, 37-μm nylon meshes were positioned in the pot bottom. Such nylon mesh has the ability to allow mycorrhizal extraradical hyphae, but not roots, to pass through the mesh. The entire pot was placed in a glass beaker with 11 cm diameter and 13.5 height, whose function is water storage. A 2-mm bulge was established at the 3-cm place of the pot top, in order to have 2.5 cm air gaps between the plastic pot and the beaker.

2.2. Plant set-up

Two 4-leaf-old trifoliate orange seedlings without mycorrhization were transplanted into the plastic pot, in which 600 g autoclaved growth substrate (soil/sand = 4/1, v/v) were filled. The soil from a citrus orchard of the Yangtze University campus is taxonomically classified as Xanthi-Udic Ferralsols (FAO system), whose characteristics are pH 6.2, 8.5 g/kg organic carbon, 10.3 mg/kg available nitrogen, and 13.3 mg/kg Oslen-P.

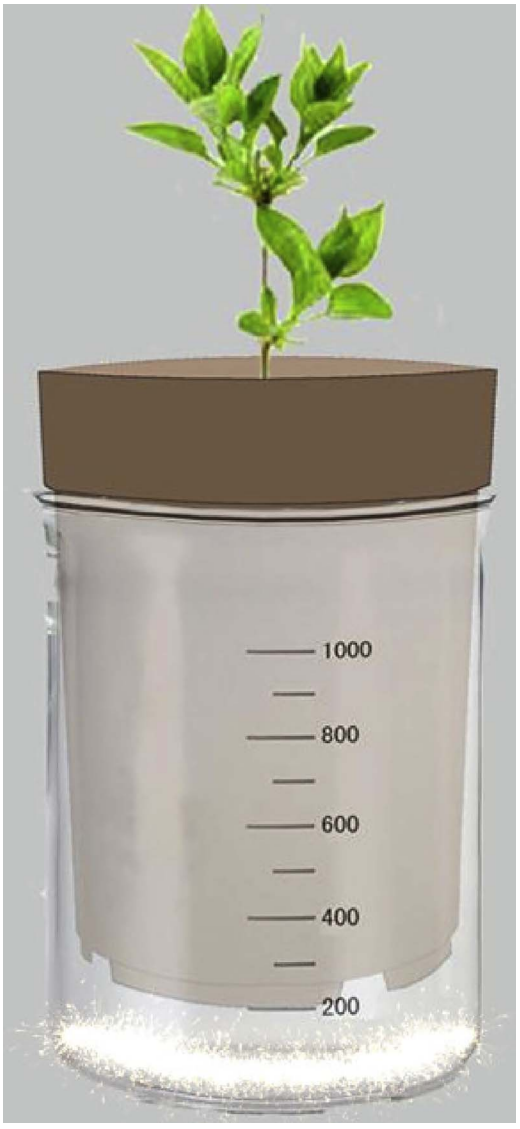

Fig. 1. Schematic diagram of the experimental equipment.

At the time of seedlings transplanting, 1000 spores of *Funneliformis mosseae* (Nicol. & Gerd.) Schüßler & Walker and *Paraglomus occultum* Walker Morton and Redecker were applied into the pots as AMF inoculation. For non-AMF treatment, the same quantity sterilized inoculants and 2 mL inocula filtrate (25 μm filter) were inoculated into the pots. The two AM fungal species were provided from the Bank of Glomeromycota in China and then were propagated through identified spores with *Trifolium repens* for 16 weeks. All the seedlings were grown in a greenhouse with 728–965 μmol/m^2/s photosynthetic photon flux density, 20–35/15–26 °C day/night temperature, and 70–95% relative humidity.

2.3. Experimental design

After seedlings transplanted, the plastic pots with the inoculated or non-inoculation seedlings were placed in the beaker, where distilled water was controlled in 2.0 cm positions of the beaker bottom. Such operation results in a 0.5-cm air gap between pots and beakers, in order to allow the extraradical hyphae of the inoculated plants passing through the nylon mesh and pot bottom to take up the water of beakers. Moreover, the 0.5-cm air gap would avoid the direct contact of both pot bottom and water.

Soil water content of the pots was controlled in well-watered (WW, 70% of the maximum field water holding capacity) for 87 days. Subsequently, half of the seedlings were expected to continue to maintain soil WW status for 71 days. The other seedlings were exposed to soil DS (50% of the maximum field water holding capacity of the substrate) for 71 days. Soil water content of each pot was kept daily by weighing, and any loss of water was resupplied to maintain the target soil relative water content. To reduce the potential water filtration from the pot bottom to the beaker at watering, the watered pot was placed on another beaker without water. After 30 min, the pot was replaced on the designed beaker supplied with water. The location of pots was shifted weekly to avoid different environmental effects. In addition, the beaker was packaged by a newspaper to reduce the evaporation of beaker waters.

The experiment consisted of a randomized complete block design with two soil water regimes (WW and DS) and three AMF (*F. mosseae*, *P. occultum*, and non-AMF) treatments, in a total of 30 pots with five replicates.

2.4. Estimation of hyphal water absorption rate

Water of the glass beakers was kept at the same weight in 6:00 am of 70th-day of DS treatment. Subsequently, water loss was determined every two hours, until 6:00 am of the 71th-day of DS treatment. The AM and non-AM seedlings were harvested. Nylon meshes of the pot bottom and rhizosphere soils were collected.

Water absorption rate of mycorrhizal extraradical hyphae was calculated by the following formula:

$$\text{Hyphal water absorption rate (mg } H_2O\text{/h/cm)} = (WL_{AM} - WL_{NAM})/24/L_h$$

where WL_{AM}, WL_{NAM}, 24, and L_h stand for water loss of beaker in AM treatment in same soil water regime (mg H_2O), water loss of beaker in non-AM treatment in same soil water regime (mg H_2O), 24 h (h), and total extraradical hyphal length in entire meshes of the pot bottom (mm) (see 2.5 Mycorrhizal and leaf water potential determination), respectively.

2.5. Mycorrhizal and leaf water potential determination

The 1-cm-long root subsamples were cleared by 10% (m/v) KOH at 95 °C for 1.5 h and stained with 0.05% trypan blue in lactophenol for