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Short communication

Changes in rhizosphere properties of trifoliate orange in response to mycorrhization and sod culture

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ABSTRACT

Sod culture with white clover is a common practice as part of soil management in citrus orchards. However, it is not clear whether such sod culture affects plant growth, soil properties, and the mycorrhizosphere of citrus. In this study, white clover was planted around trifoliate orange (a popular citrus rootstock) under mycorrhization with or without *Rhizoglomus intraradices*. After four months, the sod culture substantially stimulated root mycorrhizal colonization and soil hyphal growth. Plant growth performance of trifoliate orange was significantly increased by either mycorrhization under non-sod culture or sod culture under non-mycorrhization, whereas sod culture under mycorrhization significantly decreased the growth performance. Both mycorrhization and sod culture significantly increased the concentrations of easily extractable glomalin-related soil proteins (EE-GRSP), total GRSP (T-GRSP), and soil organic carbon (SOC), the distribution of water-stable aggregates in the size of 2–4, 1–2, and 0.5–1 mm, and the activity of soil peroxidase and phosphatase. The mean weight diameter was notably increased by mycorrhization, irrespective of sod or non-sod culture, but was higher with sod culture under mycorrhization than under non-mycorrhization. Root colonization, soil hyphal length, SOC, EE-GRSP, and T-GRSP were significantly and positively correlated with aggregate stability. These results suggested that sod culture stimulated mycorrhizal development and potentially improved soil properties in an AMF-inoculated citrus orchard.

1. Introduction

Arbuscular mycorrhizal fungi (AMF), a kind of root-inhabiting fungi, can form arbuscular mycorrhizal (AM) symbiosis with ~80% of land's plant, in which extraradical mycorrhizal hyphae in the soil may connect with the roots of different or the same plant species (Smith and Read, 2008). Such symbiosis allows underground communication of substances between the plants via a bridge called a common mycorrhizal network (Barto et al., 2012; Achatz and Rillig, 2014). Studies confirmed that AMs strongly accelerated plant growth, through improvements in soil structure, tolerance to abiotic/biotic stresses, and nutrient absorption, especially phosphorus (Wu et al., 2008; Ortas and Ustuner, 2014; Tauler and Baraza, 2015).

Glomalin, an important glycoprotein secreted by AMF, was first reported by Wright and Upadhyaya (1996). Glomalin is released into the soil, called the glomalin-related soil protein (GRSP), is a consortium of proteins of AMF and non-AMF origin (Rillig, 2004; Gillespie et al., 2011). GRSP is reported to protect soil aggregates against loss of water and nutrients, in addition to stabilizing soil aggregates (Nichols, 2008; Wu et al., 2014; Wang et al., 2015a). In the light of these functions, GRSP is considered as a sensitive indicator of soil quality (Lopez-Merino et al., 2015). It is difficult to anticipate whether the sod culture in perennial orchards stimulates GRSP production, resulting in any cascading effects on aggregate stability.

Soil enzymes are associated with biogeochemical cycling of nutrients and ecosystem responses in the soil (Wang et al., 2015b). Activities of soil enzymes are closely linked to nutrient

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transformation, soil stabilization, and soil fertility (Zhang et al., 2015). Our previous studies showed that AMF regulated changes in soil enzyme activities in the citrus rhizosphere under either P-stress or drought-stress conditions (Wu et al., 2008, 2015).

Citrus is one of the most important fruit crops globally, in terms of area and production. Citrus trees are considered highly dependent on the AM symbiosis as citrus roots are characterized by short and poorly distributed root hairs (Graham and Syvertsen, 1985). Citrus trees are predominantly grown in low fertility soil, such as kaolinite rich red and lateritic soils (Alfisols and Ultisols) (Srivastava et al., 2008). The sod culture system with white clover (*Trifolium repens* L.) is widely practiced in citrus orchards. Sod culture is reported to decrease the load of herbicide use, improve soil organic carbon (SOC), aid in proliferation of soil micro-organisms in forms of AMF, and inhibit soil-borne plant pathogens (Ishii et al., 2007; Wang et al., 2016). In citrus orchards with sod culture, there is scarce information about the changes of GRSP, soil enzyme activities, and aggregate stability under mycorrhization. Understanding the relationship between AMF, sod culture, and soil properties will provide important implications on the sustainability of soil fertility management vis-à-vis production consistency. In this context, the present study was carried out to assess the effects of AMF-inoculation and sod culture using white clover on plant growth, GRSP production, aggregate stability, and soil relevant enzyme activities in trifoliate orange [*Poncirus trifoliata* (L.) Raf., a popular citrus rootstock].

2. Materials and methods

2.1. Plant and inoculum culture

Seeds of trifoliate orange were sown in autoclaved (0.11 Mpa, 121 °C, 2 h) sands under the conditions of 27/20 °C day/night temperature, 740 μmol/m^2/s photon flux density, and 80% relative humidity. Subsequently, two four-leaf-old trifoliate orange seedlings of uniform size (nucellar seedlings) were transplanted into plastic pots (11.5 cm upper diameter × 8.5 cm bottom diameter × 14 cm height), and each was supplied with 2.0 kg of autoclaved (0.11 Mpa, 121 °C, 2 h) Xanthi-Udic Ferralsol soil (FAO system). The soil was collected from a citrus orchard on the Yangtze University campus (30°36′N and 112°14′E) and had a pH of 6.0, 121.0 mg/kg $KMnO_4$—N, 15.7 mg/kg Bray–P, and 122.3 mg/kg neutral NH_4OAc-K.

The AM fungus *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl (Sieverding et al., 2014) purchased from the Bank of Glomeromycota in China (BGC) was used. At the time of transplanting, ~1300 spores per pot were inoculated into the surroundings of the plant roots. The non-AMF treated pots also received the same amount of autoclaved AMF inoculum with the addition of 2 mL filtrate (25 μm) of the inoculum for similar microflora except the mycorrhizal fungus (Neumann and George, 2005). The AMF strain was propagated by pot culture on the basis of the identified fungal spores and white clover as the host plant grew in the mixture of sand and soil (1:1, v/v) for 12 weeks.

Fifteen seeds of white clover per pot were sown into the surroundings of the trifoliate orange 3 weeks after trifoliate orange transplanting. Each pot was thinned to 10 white clover plants one week after sowing. The experiment was conducted during April 2–August 13, 2014 in a glass house (Photosynthetic photon flux density is 880 μmol/m^2/s, day/night temperature 28/21 °C, and relative humidity 85%) of the Yangtze University campus.

2.2. Experimental design

The experiment was arranged in a completely randomized block design, combining inoculation with or without *R. intraradices* and trifoliate orange with or without white clover. The four treatments are simplified as: +AMF + WC (Pots treated with *R. intraradices* and white clover), −AMF + WC (Pots treated with white clover), +AMF − WC (Pots treated with *R. intraradices*), and −AMF − WC (Pots treated without both *R. intraradices* and white clover), with each treatment replicated four times.

2.3. Measurement of variables

Plant growth parameters such as plant height, stem diameter, and number of leaves per plant were recorded at the harvest stage. The trifoliate orange seedlings were divided into the shoot and root, whose dry weight was determined after oven-drying at 75 °C for 48 h.

The 1-cm-long fresh root segments (40 root segments per treatment) were stained by tryblue according to Phillips and Hayman (1970), and root AMF colonization was expressed as the percentage of AMF colonized root lengths against total observed root lengths. Soil hyphal length was measured according to the protocol described by Bethlenfalvay and Ames (1987).

The percentage of water-stable aggregates (WSAs) at different sizes of 0.25–0.5, 0.5–1, 1–2, and 2–4 mm was determined by the wet-sieve method (Kemper and Rosenau, 1986). Aggregate stability was calculated using the mean weight diameter (MWD (an indicator of aggregate stability)) of 0.25–4 mm WSAs (Kemper and Rosenau, 1986) following the formula: $MWD = \sum_{i=1}^{n} XiWi$, where *Xi*, *Wi*, and *n* are for average diameter of the *i* sieve opening (mm), proportion of the *i* size fraction in the total sample mass, and number of size fractions, respectively.

Determination of easily extractable glomalin-related soil protein (EE-GRSP) and difficultly extractable glomalin-related soil protein (DE-GRSP) was carried out following the procedure as described by Wu et al. (2015). Procedurally, a 1 g dry soil sample was incubated in 8 mL 20 mM citrate (pH 7.0) at 121 °C and 0.11 Mpa for 30 min and centrifuged at 10,000*g* for 3 min. The supernatant was utilized for the assay of the EE-GRSP

Table 1
Mycorrhizal status and plant growth performance of *Rhizoglomus intraradices*-colonized trifoliate orange seedlings in response to different treatments.

Treatments	Mycorrhizal status		Plant growth performance				
	Root colonization (%)	Soil hyphal length (cm/g DW soil)	Plant height (cm)	Stem diameter (mm)	Leaf number (#/plant)	Shoot biomass (g DW/plant)	Root biomass (g DW/plant)
+AMF + WC	64.3 ± 7.4a	8.6 ± 0.8a	15.19 ± 2.01bc	2.42 ± 0.03c	17 ± 2b	0.69 ± 0.04c	0.35 ± 0.03b
+AMF − WC	0c	0c	30.74 ± 3.48a	3.29 ± 0.01a	32 ± 4a	1.76 ± 0.13a	0.55 ± 0.07a
−AMF + WC	45.0 ± 5.5b	6.5 ± 0.4b	19.18 ± 5.21b	2.82 ± 0.02b	19 ± 3b	0.84 ± 0.06b	0.52 ± 0.03a
−AMF − WC	0c	0c	13.48 ± 2.14c	2.29 ± 0.02c	17 ± 2b	0.61 ± 0.03c	0.39 ± 0.05b

Data (means ± SD, $n = 4$) followed by different letters indicate significant differences among treatments after using the Duncan's multiple range test ($P < 0.05$). Abbreviation: +AMF: inoculation with *Rhizoglomus intraradices*; −AMF: inoculation without *Rhizoglomus intraradices*; +WC, sod culture by white clover; −WC: without sod culture by white clover.