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Arbuscular mycorrhizal fungi induce sucrose cleavage for carbon supply of arbuscular mycorrhizas in citrus genotypes

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ABSTRACT

Arbuscular mycorrhizas (AMs) must gain carbohydrates (mainly glucose) through sucrose cleavage from their plant hosts. Using the AM fungus *Funneliformis mosseae* inoculated or non-inoculated citrus genotypes [red tangerine (*Citrus tangerina* Hort. ex Tanaka), kumquat (*Fortunella margarita* Lour. Swingle), and trifoliate orange (*Poncirus trifoliata* L. Raf.)], we tested the hypothesis that the sucrose cleavage would depend on sucrose-cleaving enzyme types and/or host genotypes for carbon supply to the AM development. Mycorrhizal inoculation significantly increased biomass production and glucose contents of leaf, root, and total plant, irrespectively of citrus genotypes. In contrast, under mycorrhization similar leaf sucrose contents were observed between these three citrus genotypes, whereas significantly higher sucrose contents were in roots of red tangerine and kumquat, and in total plant (leaf plus root) of red tangerine and trifoliate orange. Root AM colonization was significantly positively correlated with leaf and total plant glucose but not with root glucose or with sucrose in either leaf and/or root. Root AM colonization increased the activity of leaf acid invertase (AI) and sucrose synthase (SS) and root neutral invertase (NI) in trifoliate orange, leaf SS and root AI in kumquat, and root NI in red tangerine, but decreased leaf NI in all three citrus genotypes, leaf SS in red tangerine, root AI in red tangerine and trifoliate orange, and root SS in kumquat and trifoliate orange. These results support our hypothesis that the sucrose cleavage highly depended on both citrus genotypes and sucrose-cleaving enzyme types/activity, and thus may provide higher glucose as carbon source to support their fungal partners.

1. Introduction

Arbuscular mycorrhizal fungi (AMF), the phylum Glomeromycota, can form symbiotic associations with ~80% higher plants, where the fungi supply soil mineral nutrients and water to their plant hosts, in exchange of consuming ~20% host's photosynthetic carbohydrates for the fungal partners (Smith and Read, 2008). Generally sucrose in leaves constitutes the main form of carbohydrates for long-distance transport via the phloem to the sink organs such as roots and/or AMs (Doidy et al., 2012a). A higher accumulation of root sucrose occurred in tomato (*Lycopersicon esculentum*) colonized by an AM fungus *Funneliformis mosseae* (syn. *Glomus mosseae*) (Boldt et al., 2011). Nuclear magnetic resonance spectrometry showed that AM intraradical mycelia take up hexoses, primarily glucose, not the disaccharide sucrose and the hexose fructose (Bago et al., 2000; Solaiman and Saito, 1997). Root AMF colonization thereby increases the strength of root carbon (C) sink in plant–microbe–soil systems.

Prior to the transfer of hexoses at the plant–fungal interface sucrose is hydrolytically cleaved to glucose and fructose by sucrose synthase (SS) and/or invertase (Kühn and Grof, 2010). Plant invertases including acid invertase (AI) and neutral invertase (NI) cleave sucrose to both glucose and fructose, and SS cleaves sucrose to fructose and UDPG (Uridine diphosphate glucose) (Schubert et al., 2003; Sturm and Tang, 1999). As a result, the study of sucrose-cleaving enzymes is essential for understanding the sucrose cleavage in mycorrhizal plants.

In general, mycorrhizal inoculation did not affect AI activity in maize, bean, soybean, tobacco and mung bean (Blee and Anderson, 2002; Schellenbaum et al., 1998; Schubert et al., 1995, 2003). On the other hand, leaf SS activity was not affected by the AM fungus *F. mosseae* in cucumber (Black et al., 2000), but root SS activity was increased by *F. mosseae* and *G. caledonium* in maize and tobacco (Schubert et al., 1995). Interestingly, root SS activity between

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mycorrhizal and non-mycorrhizal soybeans was similar when they were 35-d-old, but was significantly higher under mycorrhization when they were 40-d-old (Schubert et al., 2003). These results indicated that the activity of sucrose-cleaving enzymes varied with plant species and/or growth status. However, all these tested plants are cereals and legumes, and studies of the roles of AMF on sucrose cleavage in woody or fruit plants are limited, let alone a comparison between plant genotypes.

With an annual 3% increase in recent five years citrus plantation has reached to ~2.3 million hectares in 2011 in China. Citrus plants mainly consist of three genera, including *Citrus*, *Fortunella* and *Poncirus*. Among them, most *Citrus* and *Fortunella* species are used for field cultivar grafts whereas *Poncirus* species are used for rootstocks in the nursery. In general, almost all citrus plants are strongly AM dependent due to no or little root hairs in fields (Wu and Zou, 2009). Because of the requirement of photosynthetic carbohydrates for plant and fungus recognition, Graham and Eissenstat (1994) considered the host genotype as an important factor controlling the rate and extent of AM formation in citrus plants, thereby resulting in differentiation of AM dependency in citrus cultivars (Menge et al., 1978). Since sucrose-cleaving enzyme activities determine the cleavage of sucrose into glucose and fructose, we hypothesize that the sucrose cleavage for carbon supply of AMs relies on the sucrose-cleaving enzyme types and genotypes of the host plant. To test this hypothesis in a pot study with three citrus genotypes [red tangerine (*Citrus tangerina* Hort. ex Tanaka), kumquat (*Fortunella margarita* Lour. Swingle), and trifoliate orange (*Poncirus trifoliata* L. Raf.)] widely used in China, our objectives were to compare the impacts of an ubiquitous AM fungus *F. mosseae* on plant biomass production, plant sucrose and glucose contents, relevant sucrose-cleaving enzyme activities, and then related these variables to root mycorrhization.

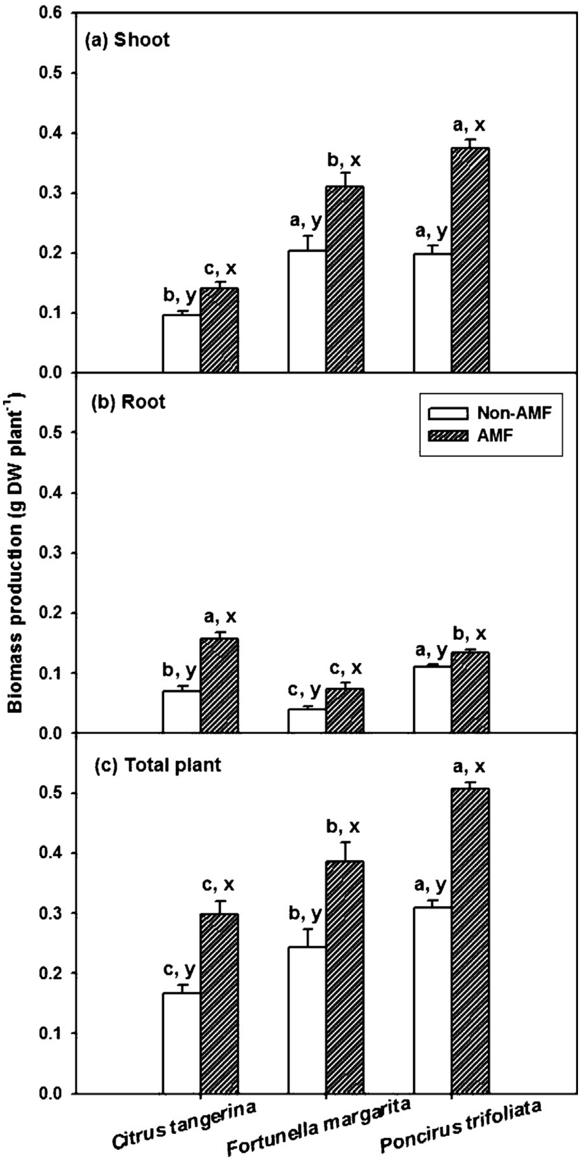

Fig. 1. Effects of *Funneliformis mosseae* colonization on shoot (a), root (b), and total plant (c) biomass production in three 5-month-old citrus genotype plants, *Citrus tangerina* (red tangerine), *Fortunella margarita* (kumquat), and *Poncirus trifoliata* (trifoliate orange). Data (means ± SE, $n = 3$) are significantly different ($P < 0.05$) if followed by different letters between plant species for the same *Fortunella margarita* treatment (a, b, c) or between *F. mosseae* treatments for the same plant species (x, y).

2. Materials and methods

2.1. Plants, mycorrhizal inoculums and experimental design

Seeds of three citrus genotypes (kumquat, red tangerine and trifoliate orange) were collected from a Citrus Orchard on the Yangtze University campus. The AM fungus, *F. mosseae* (Nicol. & Gerd.) Schüßler and Walker (2010) was kindly provided by Professor Ren-Xue Xia from the College of Horticulture and Forestry, Huazhong Agricultural University, Wuhan, China. The inoculums, propagated through the identified spores with white clover (*Trifolium repens*) for 16 weeks, were a mixture of infected root segments, spores (~28 spores/g), extraradical hyphae and river sand.

The experiment was a completely randomized design with each of these three citrus genotypes and two mycorrhizal treatments (with or without the *F. mosseae*). Each treatment had three replicates or pots (20 cm diameter in upper mouth × 15 cm diameter in bottom mouth × 18 cm in height) for a total of 18 pots.

2.2. Plant growth

Seeds were surface-sterilized for 10 min in 70% of ethanol and sown in pots containing 2.8 kg sterilized soil from the above-mentioned Citrus Orchard. The soil is classified as Xanthi-Udic Ferralsols (FAO system) with pH 6.2, 9.4 g kg^{-1} organic matter, 123.5 mg kg^{-1} available nitrogen, 16.2 mg kg^{-1} Oslen-P, and 23.6 mg kg^{-1} available potassium.

For AMF treatments 20 g inoculums of *F. mosseae* were added to each pot at below 5 cm depth before sowing. The non-AMF treatments received 20 g autoclaved inoculums and 2 mL inoculum filtrate (25 μm filter) for other microbial communities. The pots were then placed in a plastic greenhouse on the Yangtze University campus from March 26 to August 16, 2011. The greenhouse had a photo flux density of 721–967 μmol m^{-2} s^{-1} with 14–35/8–30 °C (day/night) and 70–95% relative air humidity. Plants were thinned to three seedlings per pot one month after sowing. The AM and non-AM seedlings received 100 mL Hoagland solution twice per month.

2.3. Plant harvest and determination of root mycorrhizal colonization

Shoots and roots were separately harvested on August 16, 2011 and oven-dried at 75 °C for 48 h. Mycorrhizal colonization was quantified after the clearance of 1.0 cm fresh root segments with 10% KOH and staining with 0.05% trypan blue in lactophenol (w/v) (Phillips and Hayman, 1970). The root mycorrhizal colonization was counted as the percentage of infected root length against total observed root length.