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Mycorrhizal Effects on Growth and Root Morphology are Associated with Changes in Chlorophyll and Carbohydrates in Trifoliate Orange Seedlings

Ge-Ge Chi[1,2] and Qiang-Sheng Wu[1,2,3]*

[1] College of Horticulture and Gardening, Yangtze University, Jingzhou, Hubei 434025, China
[2] Institute of Root Biology, Yangtze University, Jingzhou, Hubei 434025, China
[3] Department of Chemistry, Faculty of Science, University of Hradec Kralove 50003, Hradec Kralove, Czech Republic

Corresponding author.

Abstract

The effects of an arbuscular mycorrhizal fungus, *Diversispora spurca*, on plant growth, root morphology, chlorophyll concentration, and carbohydrate contents in trifoliate orange grown in a rootbox divided by 37- or 0.45-μm mesh were studied. After 18 weeks of mycorrhizal inoculation, root colonization was higher under 37-μm mesh than under 0.45-μm mesh. Mycorrhizal inoculation significantly increased growth performance (plant height, stem diameter, leaf number, shoot and root biomass) and root morphology (the number of 1st-, 2nd-, and 3rd-order lateral roots, root length, projected area, surface area, volume, tips, forks, and crossings), and the effects were superior under 37-μm than 0.45-μm. In addition, mycorrhizal colonization dramatically increased chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll concentrations and also promoted sucrose and glucose contents in leaf, root, and total plant. These results indicate that mycorrhiza-promoted effects on growth and root morphology are potentially associated with changes in chlorophyll and carbohydrates.

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Keywords

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Introduction

Roots are a support system in plants, considering as the nutrient uptake organ (Elsen et al., 2003). Root morphology refers to the spatial distribution of the root system in soils, which could be affected by various factors, including soil moisture, soil bulk density, soil microorganisms, etc. Arbuscular mycorrhizal fungi (AMF) are a kind of beneficial microorganisms in the soil, which establish symbiotic associations with plant roots. Mycorrhizal symbiosis develops extraradical hyphae outside the root to contribute roles in nutrient and water absorption, plant growth, and substance communications. As mycorrhizas are established in roots, it is not clear whether the formation of mycorrhizal symbioses stimulates root morphology in the host plant.

Carbohydrates are one of the most important plant photosynthates, participating in the process of plant growth and metabolism. AMF help plants to absorb water and nutrients, and in return it needs to obtain carbohydrates, especially glucose, from host plants to mycorrhizas (Schubert et al., 2004). Studies in the past showed that AMF inoculation could improve the carbon metabolism of plants, such as the increase of plant carbohydrate concentrations and leaf photosynthesis

(Jiang et al., 2008). As stated by Leake et al. (2004), AMs would consume 4–20% of carbohydrates produced by photosynthesis of the host plant (Bago et al., 2003). As a result, mycorrhizal association can enhance a sink demand for carbohydrates from the host plant to the fungal partner (Zhu and Miller, 2003).

The objective of this study was to determine the effect of AMF colonization on growth performance, root morphology, carbohydrates, and chlorophyll concentrations in trifoliate orange grown in a two-chambered rootbox.

Materials and methods

Experimental apparatus

The experimental apparatus was divided into upper and down parts. The upper part is characterized with 8 cm length of side and 11.3 cm height. In the location of the 4.3 cm tall, a 1-cm width organic glass was fixed in the periphery of cube. The 37-μm and 0.45-μm nylon mesh was covered at the bottom of the upper part. Hereinto, the mycorrhizal extraradical hyphae pass through 37-μm mesh, but do not allow root entering, and the 0.45-μm mesh can't allow the entering of roots and hyphae. The down part is characterized with 8.8 cm length of side and 6.5 cm height. The upper part was placed on the down part, resulting in the 4.3 cm overlapping region.

Plant growth

The four-leaf-old seedlings with uniform size (nucellar seedlings) and non-mycorrhization were transplanted into the upper part of the experimental apparatus, filled with autoclaved (121°C, 0.11Mpa, 40 min) 4-mm size air-dried soil. Approximate 1150 spores of *Diversispora spurca* were inoculated in the upper part. The non-AMF treatment also received the same quantity of the autoclaved inocula as the control. The soil (Xanthi-Udic Ferralsols, FAO system) was collected from the Citrus Orchard of the Yangtze University campus. The rootboxes were placed in a glass house from March 31 to August 4, 2014.

Experimental design

Four treatments with five replicates were arranged with a complete randomized design, for a total of 20 rootboxes. These treatments are as follows: (i) inoculation with *D. spurca* in the upper part of 37-μm mesh (AMF/37μm), (ii) inoculation with *D. spurca* in the upper part of 0.45-μm mesh (AMF/0.45μm), (iii) inoculation without *D. spurca* in the upper part of 37-μm mesh (non-AMF/37μm), and (iv) inoculation without *D. spurca* in the upper part of 0.45-μm mesh (non-AMF/0.45μm).

Variable measurements

Shoots and roots were respectively harvested on August 4, 2014, and their fresh biomass was determined. Plant height, stem diameter, and leaf numbers were measured before plant harvest. The root systems were washed with tap water to remove soil particles, placed in Regent's water-proof trays without root overlaps, and scanned by an Epson Perfection V700 Photo (Seiko Epson Corp, Nagano, Japan). The obtained images of roots were analyzed using the WinRHIZO 2007 (Regent Instruments Incorporated, Quebec, Canada) to determine projected area, surface area, average diameter, volume, tips, forks and crossings. The number of different order lateral roots was also manually counted.

Root AMF colonization was measured by the protocol of Phillips and Hayman (1970) in trypan blue staining. Determination of leaf chlorophyll was followed by Lichtenthaler (1987).

The concentration of sucrose, glucose, and fructose in leaves and roots was measured as per the protocol described by Zhang and Zai (2004).

Statistical analysis

Data were statistically analyzed using the SAS software (Version 8.1). Variance (ANOVA) was used to compare the significant difference with Duncan's Multiple Range tests at $p<0.05$.

Results and discussion

Root mycorrhizal colonization

Root mycorrhizal colonization was observed only in AMF-inoculated seedlings, but not in non-AMF-inoculated seedlings, irrespective of 37-μm and 0.45-μm mesh. The seedlings had 39.3% of root mycorrhizal colonization under AMF/37μm conditions and 32.2% under AMF/0.45μm conditions, respectively. Meanwhile, root colonization was significantly higher under AMF/37μm conditions than under AMF/0.45μm conditions, suggesting that 37-μm mesh benefits better mycorrhizal colonization in roots than 0.45-μm mesh.

Table 1: Effects of *Diversispora spurca* on plant growth performance of trifoliate orange grown in 37-μm or 0.45-μm mesh root-box.

Treatments	Plant height (cm)	Stem diameter (mm)	Leaf number (per/plant)	Biomass in root+hyphae zone (g FW/plant)	
				Shoot	Root
AMF/0.45μm	28.2±3.9b	2.85±0.19b	27.2±1.6b	2.13±0.22b	1.19±0.18b
AMF/37μm	42.5±2.8a	3.40±0.16a	32.0±2.7a	3.92±0.62a	1.72±0.24a
Non-AMF/0.45μm	23.2±3.6c	2.60±0.18c	21.0±3.2c	1.53±0.40c	0.95±0.11c
Non-AMF/37μm	22.3±2.5c	2.54±0.19c	23.0±3.7c	1.40±0.10c	0.88±0.06c

Note: Data (means ± SD, n = 5) followed by different letters indicate significant differences (Duncan test, p<0.05) among treatments.

Table 2: Effects of *Diversispora spurca* on root morphology and number of lateral roots of trifoliate orange grown in 37-μm or 0.45-μm mesh root-box.

Treatments	Length (cm)	Projected area (cm^2)	Surface area(cm^2)	Average diameter (mm)	Volume (cm^3)	Tips	Forks	Crossings	Number of lateral roots (#/plant)		
									1st	2nd	3rd
AMF/0.45μm	341±29b	20.3±1.1b	63.6±3.4b	0.59±0.02b	0.95±0.04b	211±52b	1370±227b	250±50b	39±5b	166±18b	29±4b
AMF/37μm	530±134a	30.5±6.5a	95.7±20.4a	0.58±0.03b	1.38±0.25a	389±43a	2497±310a	465±133a	53±3a	249±16a	37±5a
Non-AMF/0.45μm	199±77c	13.0±5.1c	41.0±16.1c	0.65±0.04a	0.67±0.27c	147±29c	574±358c	94±58c	30±3c	116±15c	23±4c
Non-AMF/37μm	204±31c	12.1±2.0c	37.9±6.4c	0.59±0.03b	0.56±0.11c	115±17c	651±129c	131±25c	32±7c	102±16c	22±2c

Note: Data (means ± SD, n = 5) followed by different letters indicate significant differences (Duncan test, p < 0.05) among treatments.

Plant growth performance

Earlier studies had confirmed that inoculation of AMF induced the increase in plant growth (Wu et al., 2010). The present study also indicated that significantly higher plant height, stem diameter, leaf number, and shoot and root biomass ranked as the trend of AMF/37μm > AMF/0.45μm > non-AMF/0.45μm ≈ non-AMF/37μm in the decreasing order (Table 1), suggesting that mycorrhizal inoculation heavily stimulated plant growth performance in trifoliate orange, regardless of 37-μm and 0.45-μm mesh. The mycorrhizal effect was superior under 37-μm mesh than under 0.45-μm mesh. We concluded that extraradical hyphae in the upper part could pass through the 37-μm mesh, other than the 0.45-μm mesh, to the down part, resulting in extensive root absorptive areas.

Root morphology

Root projected area, surface area, volume, tips, forks and crossings were significantly increased by the inoculation with *D. spurca*, irrespective of 37-μm and 0.45-μm mesh (Table 2), which is in agreement with the findings by Wu et al. (2011) in trifoliate orange inoculated with *Glomus mosseae* and *Glomus versiforme*. Compared with AMF/0.45μm, AMF/37μm treatment more highly increased all root morphology parameters, except for root average diameter (Table 2), suggesting that AMF has a positive effect. The improvement of root morphology under mycorrhization might lead to the promotion of plant growth performance. Our study also found that AMF inoculation significantly increased the number of first-, second-, and third-order lateral roots (Table 2), implying that AMF induced lateral root formation and development.

Chlorophyll levels

The present study showed that chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll concentrations were dramatically increased by mycorrhizal inoculation under both 37-μm and 0.45-μm mesh relative to non-mycorrhizal treatment (Fig. 1), which is consistent with the results of Li et al. (2013) on four citrus genotypes inoculated with *D. spurca*. Compared with the AMF/0.45μm treatment, AMF inoculation under 37-μm mesh conditions represented greater chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll concentrations, which is consistent with the AM responses to plant growth performance and root morphology. Higher chlorophyll concentrations in AMF plant would help the host to enhance the photosynthesis, and thus more photosynthetic carbohydrates are produced for the utilization of AM sinks (Hajbagheri and Enteshari, 2011).

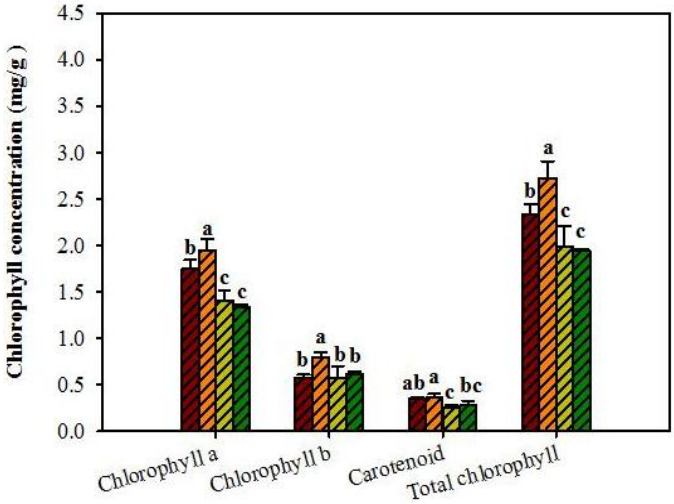

Fig. 1: Effects of *Diversispora spurca* on chlorophyll a, chlorophyll b, carotenoid and total chlorophyll concentrations in trifoliate orange grown in 37-μm or 0.45-μm mesh root-box. Date (mean ± SD, n = 5) followed by different letters above the bars indicate significant differences (Duncan test, p<0.05) between the treatment.

Carbohydrate concentrations

In general, sucrose can be transferred into plant sinks and non-plant sinks, such as mycorrhizal sinks (Wu et al., 2013). Our results also showed that AMF significantly leaf glucose, fructose, and sucrose contents in trifoliate orange, irrespective of 37-μm and 0.45-μm mesh (Fig. 2a), which is a similar effect of AMF on chlorophyll levels. This indicated that AMF strongly stimulate the synthesis of chlorophyll and subsequently accelerate the production of photosynthates. On the other hand, AMF inoculation heavily increased glucose and sucrose contents while markedly decreased fructose contents in roots (Fig. 2b) and total plant (leaf + root) (Fig. 2c). Lerat et al. (2003) observed that root AMF colonization was generally linearly correlated with the C-sink strength of roots. The higher plant C assimilation rate would hence compensate for their greater below-ground C expenditure in AM symbioses (Eissenstat et al., 1993). In addition, root growth needs carbon sources as the metabolic substrate, and the increase in mycorrhiza-induced glucose and sucrose contents would provide greater metabolic substrates for root development, which is a key factor in regulating root morphology (Koch et al., 2000).

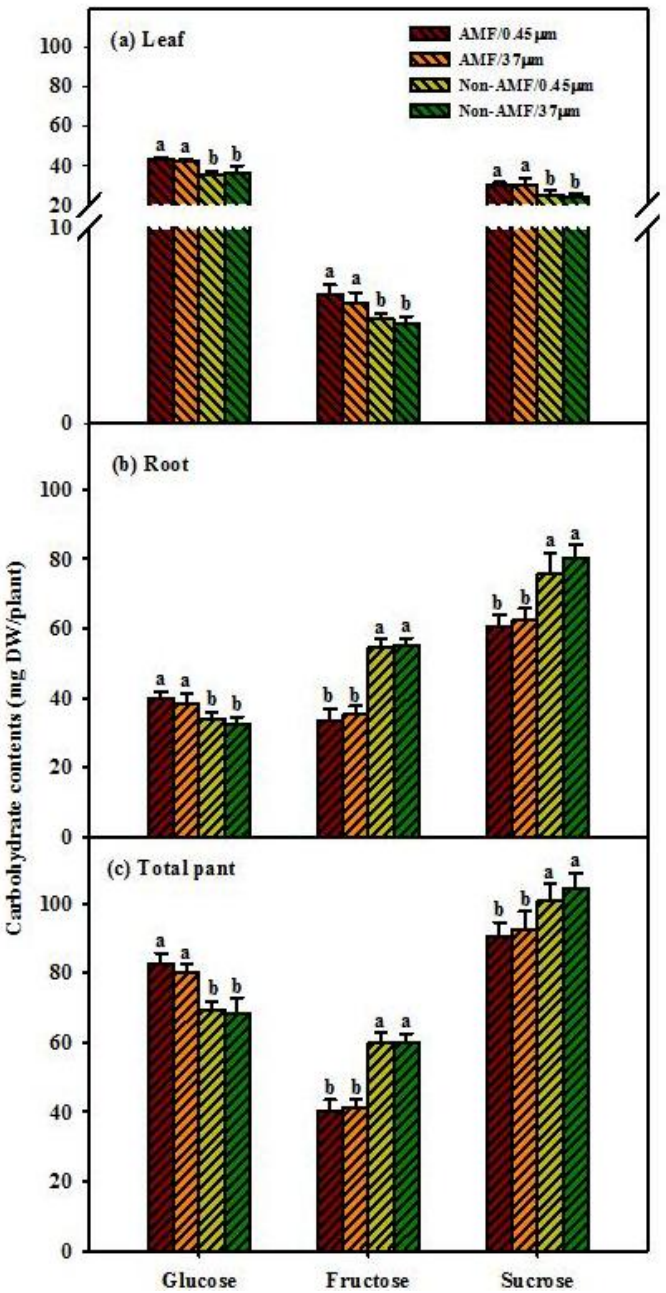

Fig. 2: Effects of *Diversispora spurca* on glucose, fructose, and sucrose concentrations in (a) leaf, (b) root, and (c) total plant (leaf + root) of trifoliate orange grown in 37-μm or 0.45-μm mesh root-box. Data (mean ± SD, n = 5) followed by different letters above the bars indicate significant differences (Duncan test, $P < 0.05$) between the treatments.

Conclusion

In our study, inoculation with *D. spurca* strongly improved plant growth performance and root morphology, which is potentially, associated with the increase in mycorrhiza-modulated chlorophyll and sucrose and glucose contents.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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