J Plant Growth Regul (2015) 34:499–508
DOI 10.1007/s00344-015-9485-x

Effects of Mycorrhizal Symbiosis on Growth Behavior and Carbohydrate Metabolism of Trifoliate Orange Under Different Substrate P Levels

Qiang-Sheng Wu · A. K. Srivastava · Yan Li

Received: 12 October 2014 / Accepted: 8 January 2015 / Published online: 19 February 2015

Abstract The carbohydrate regulatory function of arbuscular mycorrhizal fungi (AMF), associated changes in root morphology, and substrate P level are important in the efficiency of AMF. A pot experiment was carried out to study the effects of AMF (*Funneliformis mosseae*) on growth response, root morphology, and sucrose metabolism of trifoliate orange [*Poncirus trifoliata* (L.) Raf.] seedlings under varying P substrate levels (0, 3, and 30 mM). Mycorrhizal inoculation stimulated growth performance, biomass production (root and shoot fresh weight), and various root morphological traits, regardless of substrate P level. AMF-induced sucrose accumulation in leaves was more highly positively correlated with leaf sucrose synthase (synthesis direction) activity in AMF than in non-AMF seedlings. Root glucose and fructose concentrations were significantly increased by AMF inoculation, independent of P level. Root colonization was more highly correlated with root glucose than with root fructose. AMF inoculation represented varied effects on activity of acid invertase and neutral invertase in leaves and roots at all the three P levels. These results indicated that AMF-accelerated better growth response and root morphological traits were independent of substrate P level, and AMF-induced sucrose cleavage was dependent on substrate P levels, plant tissue types, and sucrose-cleaving enzyme types.

Q.-S. Wu (✉) · Y. Li
College of Horticulture and Gardening/Institute of Root Biology, Yangtze University, Jingzhou 434025, Hubei, China
e-mail: wuqiangsh@163.com

A. K. Srivastava
National Research Centre for Citrus, Amravati Road, Nagpur 440 033, Maharashtra, India

Keywords Arbuscular mycorrhiza · Citrus · Glucose · Invertase · Root morphology · Sucrose

Introduction

Arbuscular mycorrhizal fungi (AMF), an important group of soil inhabiting microorganisms, can establish symbiotic associations with roots of approximately 80 % of land plants, based upon the reciprocal exchange of nutrients (Grace and others 2009). As obligate biotrophs, arbuscular mycorrhizal (AM) symbiosis needs to acquire 4–20 % additional carbohydrates from the host plant for the process of symbiosis remain metabolic active. In this regard, hexoses originated from sucrose cleavage are predominantly taken up by the AM fungus within the root and converted into trehalose and glycogen, the typical fungal carbohydrates (Bago and others 2003). Studies in the past have shown that root AMF colonization could induce significant changes in sucrose-cleaving enzymes (Schubert and others 2003; Wu and others 2013), involved in releasing hexoses to either the colonized root cells to cope with its relatively higher metabolic activity or to the fungus itself (Schubert and others 2003; Ferrol and Pérez-Tienda 2009). These sucrose-cleaving enzyme transcripts such as acid invertase and sucrose synthase (breakdown direction) act on the arbuscule-enriched root cortical cells of the host (Blee and Anderson 2002). Therefore, increased carbon (C) allocation to AM roots is generally associated with a stimulation of activities of sucrose-cleaving enzymes in AM roots (Wright and others 1998).

On the other hand, AMF functioning is affected by substrate phosphorus (P) levels, with positive effects at low soil P levels and negative effects at high soil P levels (Srivastava and others 2002; Schmidt and others 2010; Beltrano and others 2013). For example, at high P supply,

inoculation with *Glomus intraradices* strongly depressed plant growth of Volkamer lemon (*Citrus volkameriana*), due to the greater C expenditure by AM roots than non-AM roots (Peng and others 1993). Until now, there was no evidence to evaluate whether AMF-mediated sucrose metabolism of the host is affected by substrate P levels.

It has been shown that AMF colonization can impact root development, with consequential effects on the anatomy, physiology, morphology of roots (Gutjahr and others 2009). Plant root morphology by and large determines root–soil contact areas, which is so important for increased P uptake (Gohoonia and Nielsen 2004; Wu and others 2012). In red tangerine, AMF inoculation improved root morphological traits by regulating endogenous polyamine metabolism (Wu and others 2012). However, it is still inconclusive as to whether AMF-induced root modification is dependent on substrate P level.

Trifoliate orange [*Poncirus trifoliata* (L.) Raf.], a close *Citrus* plant, possesses a shallow root system with few or no root hairs, thereby, being strongly dependent on AMs to absorb nutrients from the soil (Zobel and others 2006). In this background information, studies were undertaken to evaluate the effects of an AM fungus, *Funneliformis mosseae*, on growth, root morphology, and sucrose metabolism of trifoliate orange seedlings in response to different substrate P levels.

Materials and Methods

Experimental Setup

The experiment was conducted in 3^2 randomized factorial design with inoculation with or without *F. mosseae* (AMF and non-AMF) and three substrate P levels (0 (P_0), 3 (P_3), and 30 (P_{30}) mM KH_2PO_4). Each treatment was replicated four times. The experiment was set up under glass-house conditions (photosynthetic photon flux density is 768 μmol/m^2/s, day/night temperature 28 °C/21 °C, and relative air humidity 85 %) at the College of Horticulture and Gardening, Yangtze University, Jingzhou, China.

Seeds of trifoliate orange were sown in autoclaved sand after surface-sterilized with 70 % ethanol for 15 min, and grown in a controlled growth chamber at 28 °C/20 °C (day/night), 745 μmol/m^2/s photosynthetic photon flux density, and 80 % relative humidity. After 3 weeks, 5-leaf-old seedlings with uniform size (nucellar seedlings) were transplanted into plastic pots (20 cm length × 15 cm width × 18 cm height) filled with acid washed sand (<4 mm size), collected from the riverside of the Yangtze River near the Jingzhou city.

The strain of *F. mosseae* (Nicol. & Gerd.) Schüßler & Walker used for the experiment was isolated from the rhizosphere of *Incarvillea younghusbandii* in Dangxiong, Tibet, China. The inoculum including AMF-infected roots, spores (~23 spores/g), hyphae, and sand were derived from a pot culture with the identified fungal spores and *Trifolium repens* as the host for 16 weeks. A 60 g (corresponding to ~1380 spores) sample of AMF inoculum was placed in the rhizosphere of trifoliate orange seedlings in each pot at the time of transplanting. The non-AMF treated pots were also supplied with 60 g of autoclaved (121 °C, 0.11 Mpa, 2 h) AMF inoculum plus 2 mL filtrate (25 μm) of inoculum to keep other microbial communities except *F. mosseae*.

The plants were acclimatized for 2 weeks of AMF inoculation daily with 100 mL distilled water per pot, and then three P levels (0, 3, and 30 mM KH_2PO_4) were imposed daily through 100 mL standard Hoagland solution per pot until the harvest. The three P levels (0, 3, and 30 mM KH_2PO_4) in standard Hoagland solution used here were referred to as low, medium, and high P levels for trifoliate orange growth, according to a number of studies (Jifon and others 2002; Guo and others 2003).

Observations and Analysis

Growth attributing parameters such as plant height, stem diameter, and leaf number per plant were recorded before harvest. Whole seedlings were harvested by uprooting and measured the shoot and root fresh weights. Subsequently, the root systems were washed with tap water to remove soil particles, placed in Regent's water-proof trays without root overlap, and scanned by an Epson Perfection V700 Photo (Seiko Epson Corp, Nagano, Japan). The acquired images of roots were analyzed using the software WinRHIZO 2007b (Regent Instruments Incorporated, Quebec, Canada) to estimate total length, surface area and volume. The number of different order lateral roots was also manually counted.

Root segments 1.0 cm long were stained using the protocol as described by Phillips and Hayman (1970), and the root mycorrhiza was observed under a microscope for the presence of mycorrhizal mycelium. Root AM colonization was finally expressed as the percentage of colonized root lengths against observed total root lengths.

Fructose, glucose and sucrose in leaves and roots were extracted with 4 mL 80 % ethanol after 50 mg ground (0.5 mm sieve) dry samples were incubated for 40 min at 80 °C, and centrifugated at 2500×*g* for 5 min. The centrifuged residues were extracted again as per the above procedure, and the two supernatants were combined for analysis of carbohydrate concentration. The concentration of sucrose, glucose, and fructose was colorimetrically determined according to the procedure outlined by Zhang and Zai (2004). Sucrose was assayed with a mixture of 0.15 mL

supernatant and 0.15 mL 2 mol/L NaOH at 100 °C for 5 min, which were then mixed together with 2.1 mL 30 % HCl and 0.6 mL 0.1 % resorcinol at 80 °C for 10 min and followed by measurement of absorbance of assay at 480 nm using sucrose as one standard. Glucose concentration was determined by mixing 0.5 mL of supernatant with 1.0 mL of prepared solution (1 mg/mL *o*-dianisidine dihydrochloride, 1 mg/mL horseradish peroxidase, and 1 U/mL glucose oxidase) at 30 °C for 5 min, then added 2 mL 10 mol/L H_2SO_4 solution to terminate the reaction, and followed by measurement of absorbance at 460 nm using one glucose standard. Fructose was assayed using a mixture of 0.8 mL 0.1 % resorcinol, 0.4 mL H_2O, and 0.4 mL supernatant at 80 °C for 10 min. The absorbance of the mixed solution was determined at 480 nm using fructose as one standard solution.

A 0.2 g fresh tissue sample was homogenized in 4 mL 100 mM Hepes–NaOH buffer (pH 7.5), containing 20 mM EDTA, 1 mM NaF, 1 mM benzamidine, 20 mM cysteine and 1 % polyvinyl pyrrolidone, and centrifuged at 10,000×*g* for 30 min. The supernatants were dialyzed with 100 mM Hepes–NaOH buffer (pH 7.5) in a 21 mm dialysis bag for 12 h at 4 °C. The activity of acid invertase (AI), neutral invertase (NI), and sucrose synthase (SS, synthesis direction) in the supernatants, was assayed using the method described by Wu and others (2013). Meanwhile, an assay of SS (synthesis direction) activity is based on Tris–HCl buffer containing fructose and uridine diphosphate glucose, which can synthesize sucrose by SS (synthesis direction), and the enzyme reaction is ended by addition of NaOH.

Statistical Analysis

Data generated were statistically analyzed using the SAS software (v 8.1). Variance (ANOVA) was used to compare the significant difference with *t* tests at $P < 0.05$. The Pearson's correlation coefficients between different variables were performed using the Proc Corr's procedure of SAS.

Results

Root Mycorrhizal Colonization and Plant Growth

The varying magnitude of colonization of trifoliate orange seedlings was observed by *F. mosseae* in response to different P levels. Root AM colonization was rated as $P_0 > P_3 > P_{30}$ (Fig. 1). Compared with the P_3 treatment, P_0 treatment significantly decreased plant height, stem diameter, leaf number, and shoot and root fresh weight, irrespective of AMF or non-AMF seedlings (Fig. 2a–e). On the other hand, P_{30} treatment did not significantly alter plant height and stem diameter, but significantly increased root fresh weight and decreased leaf number and shoot fresh weight, regardless of AMF and non-AMF seedlings. In addition, AMF inoculation significantly increased the different growth traits such as plant height, stem diameter, leaf number, and shoot and root fresh weight, irrespective of substrate P levels, except for a non-significant difference in plant height between the AMF and non-AMF seedlings exposed at the P_{30} level (Fig. 2a–e).

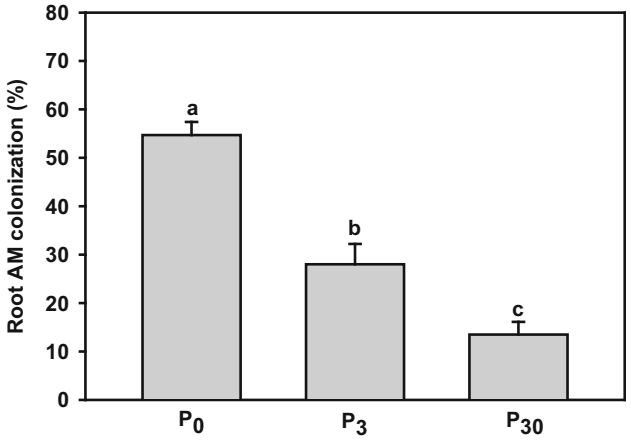

Fig. 1 Root colonization of trifoliate orange seedlings by *F. mosseae* under 0, 3, and 30 mM P level (defined as P_0, P_3, and P_{30}). Data (mean ± SD, $n = 4$) followed by *different letters above the bars* among treatments indicate significant differences at the 5 % level

Root Morphology

Root total length was significantly higher under P_3 and P_{30} than under P_0 treatment, irrespective of AM or non-AM status (Fig. 3a). Other root traits such as root surface area and volume significantly increased with increasing levels of substrate P (Fig. 3b, c). AMF colonization significantly increased the root total length, surface area, and volume, irrespective of substrate P level, except that root surface area and root volume were not significantly different between the AMF and non-AMF seedling grown under P_3 and P_{30}, respectively (Fig. 3a–c). Mycorrhizal seedlings showed significantly higher numbers of first-, second-, and third-order lateral roots than under non-mycorrhizal seedlings, regardless of substrate P level, although no significant difference in the number of first-order and second-order lateral roots was observed between AMF and non-AMF seedlings under P_{30} and P_3, respectively (Fig. 3d–f).

Carbohydrate Concentrations

Different P treatments in the order of their increasing efficiency of leaf sucrose were ranked as $P_3 > P_0 \approx P_{30}$ and

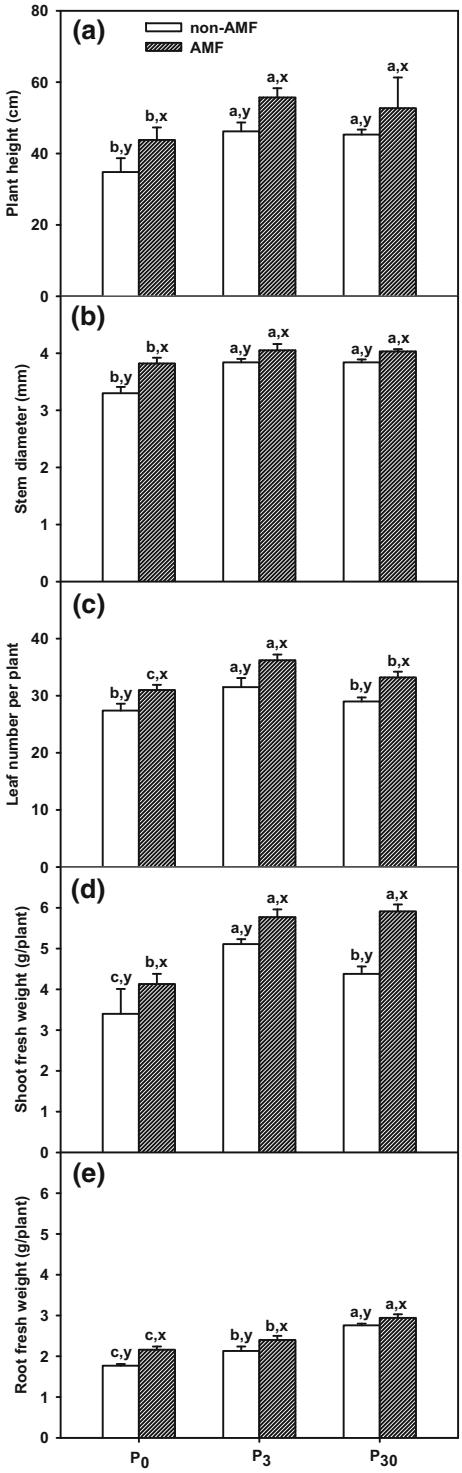

Fig. 2 Effect of *F. mosseae* on plant growth performance of trifoliate orange (*P. trifoliata*) seedlings grown under 0, 3, and 30 mM P level (defined as P_0, P_3, and P_{30}). Data (mean ± SD, $n = 4$) are significantly different ($P < 0.05$) if followed by *different letters above the bars* between P levels for the same *F. mosseae* treatment (**a–c**) or between *F. mosseae* treatments for the same P level (*x*, *y*). *Black columns* and *white columns* indicate the seedlings inoculated with AMF and non-AMF, respectively

$P_{30} > P_0 > P_3$ under non-mycorrhization and mycorrhization, respectively (Fig. 4a). The effect of different P treatments in relation to root sucrose concentration was classified as $P_{30} \geq P_3 \geq P_0$ and $P_0 > P_3 \approx P_{30}$ under non-mycorrhization and mycorrhization, respectively (Fig. 4b). The concentration of glucose in both leaves and roots followed a different pattern of response accruing through mycorrhization versus non-mycorrhization. Significantly higher glucose concentration in leaves was ranked as $P_0 \approx P_3 > P_{30}$ under non-mycorrhization and as $P_0 \geq P_3 \geq P_{30}$ under mycorrhization (Fig. 4c), whereas significantly higher glucose concentrations in root was ranked as $P_0 > P_3 > P_{30}$ under both mycorrhization and non-mycorrhization (Fig. 4d). The concentration of fructose in response to different P levels followed almost the same pattern as sucrose, irrespective of AMF treatments, with significantly highest fructose concentration in leaves observed at P_{30} (Fig. 4e). However, in roots, the pattern of fructose accumulation in response to substrate P levels was ranked as $P_0 > P_3 > P_{30}$ under mycorrhization and $P_0 \approx P_{30} > P_3$ under non-mycorrhization, respectively (Fig. 4f).

Compared with non-AMF seedlings, AMF seedlings recorded 33.8 and 58.5 % higher leaf sucrose concentrations under P_0 and P_{30}, respectively (Fig. 4a). However, AMF inoculation produced no variation in sucrose concentration in roots with P_3 treatment, but root sucrose significantly increased by 18.0 % and decreased by 23.1 % under P_0 and P_{30} treatment, respectively (Fig. 4b).

AMF inoculation induced no change in leaf glucose concentration under P_0 and P_3 but significantly increased leaf glucose by 24.8 % under P_{30} (Fig. 4c). In roots, AMF seedlings observed 27.9, 12.7, and 21.3 % higher glucose concentrations with P_0, P_3, and P_{30}, respectively, as compared with non-AMF seedlings (Fig. 4d).

Leaf fructose concentration was 10.2, 14.3, and 17.3 % significantly higher in AMF seedlings than in non-AMF seedlings under P_0, P_3, and P_{30} treatments, respectively (Fig. 4e). On the other hand, root fructose concentration was 73.1 and 73.4 % significantly higher in AMF seedling than in non-AMF seedlings under P_0 and P_3, respectively. However, root sucrose concentration was 18.1 % significantly lower under mycorrhization than under non-mycorrhization with P_{30} (Fig. 4f).

Sucrose Relevant Enzyme Activities

Compared with non-AM seedlings, AMF seedlings accounted for 21.7 and 5.3 % higher leaf AI activity under P_0 and P_3 but 25.3 % significantly lower leaf AI activity under P_{30} (Fig. 5a). In roots, AMF colonization showed no change in AI activity under P_3, but significantly increased

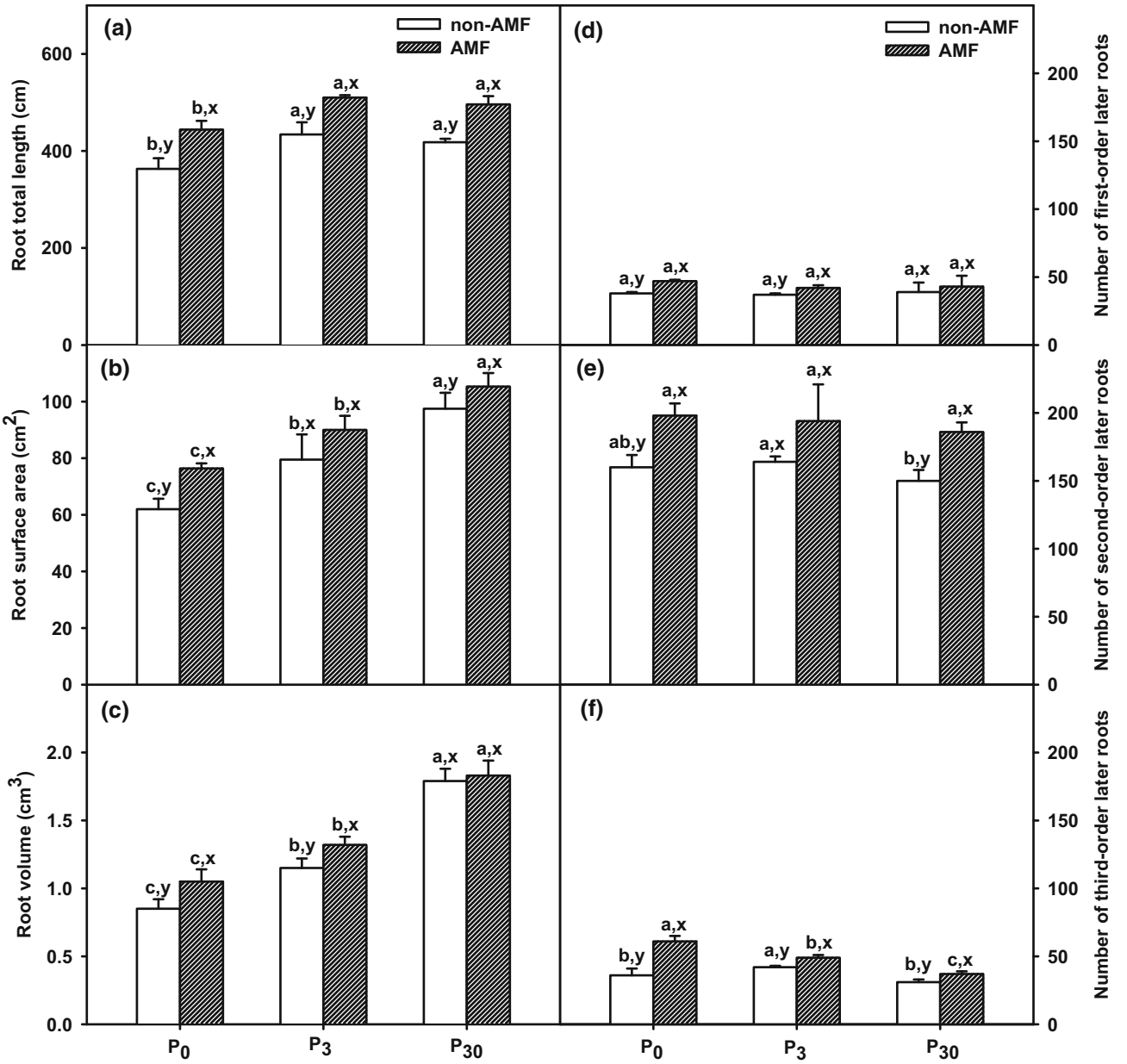

Fig. 3 Effects of *F. mosseae* on root morphological traits of trifoliate orange (*P. trifoliata*) seedlings grown under 0, 3, and 30 mM P level (defined as P_0, P_3, and P_{30}). Data (mean ± SD, $n = 4$) are significantly different ($P < 0.05$) if followed by *different letters above the bars* between P levels for the same *F. mosseae* treatment (**a–c**) or between *F. mosseae* treatments for the same P level (*x*, *y*). *Black columns* and *white columns* indicate the seedlings inoculated with AMF and non-AMF, respectively

AI activity by 27.0 % under P_{30} and decreased by 25.7 % under P_0 (Fig. 5b). In addition, the significantly higher rank of leaf AI activity was $P_3 > P_0 \approx P_{30}$ under mycorrhization and $P_{30} > P_3 > P_0$ under non-mycorrhization. The effect of different P treatments in relation to root AI activity was classified as $P_3 > P_{30} > P_0$ and $P_3 > P_0 > P_{30}$ under mycorrhization and non-mycorrhization, respectively.

Mycorrhizal seedlings had 45.2 and 26.9 % significantly higher leaf NI activity under P_0 and P_{30} (Fig. 5c). Although root NI activity was 56.2 and 48.0 % significantly higher in AMF than non-AMF seedlings with P_0 and P_3 treatments, respectively (Fig. 5d). No significant difference in root NI activity was observed between AMF and non-AMF seedlings under P_{30}. Under P_3, AMF colonization significantly decreased leaf NI activity by 35.7 %. Under mycorrhization, the significantly higher rank of leaf and root AI activity was $P_{30} > P_0 > P_3$ and $P_3 > P_0 > P_{30}$, respectively. Under non-mycorrhization, leaf and root NI activity significantly increased with the increase of substrate P level.

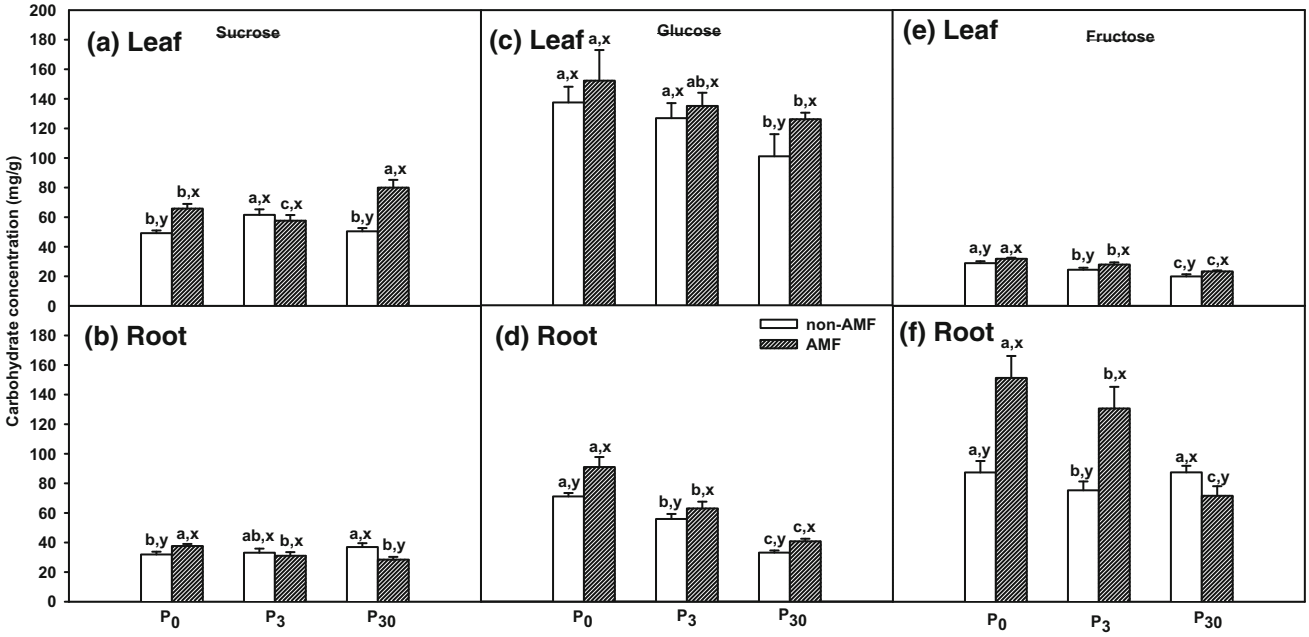

Fig. 4 Effects of *F. mosseae* on sucrose, glucose, and fructose concentrations in leaves and roots of trifoliate orange (*P. trifoliata*) seedlings grown under 0, 3, and 30 mM P level (defined as P_0, P_3, and P_{30}). Data (mean ± SD, $n = 4$) are significantly different ($P < 0.05$) if followed by *different letters above the bars* between P levels for the same *F. mosseae* treatment (**a–c**) or between *F. mosseae* treatments for the same P level (*x*, *y*). *Black columns* and *white columns* indicate the seedlings inoculated with AMF and non-AMF, respectively

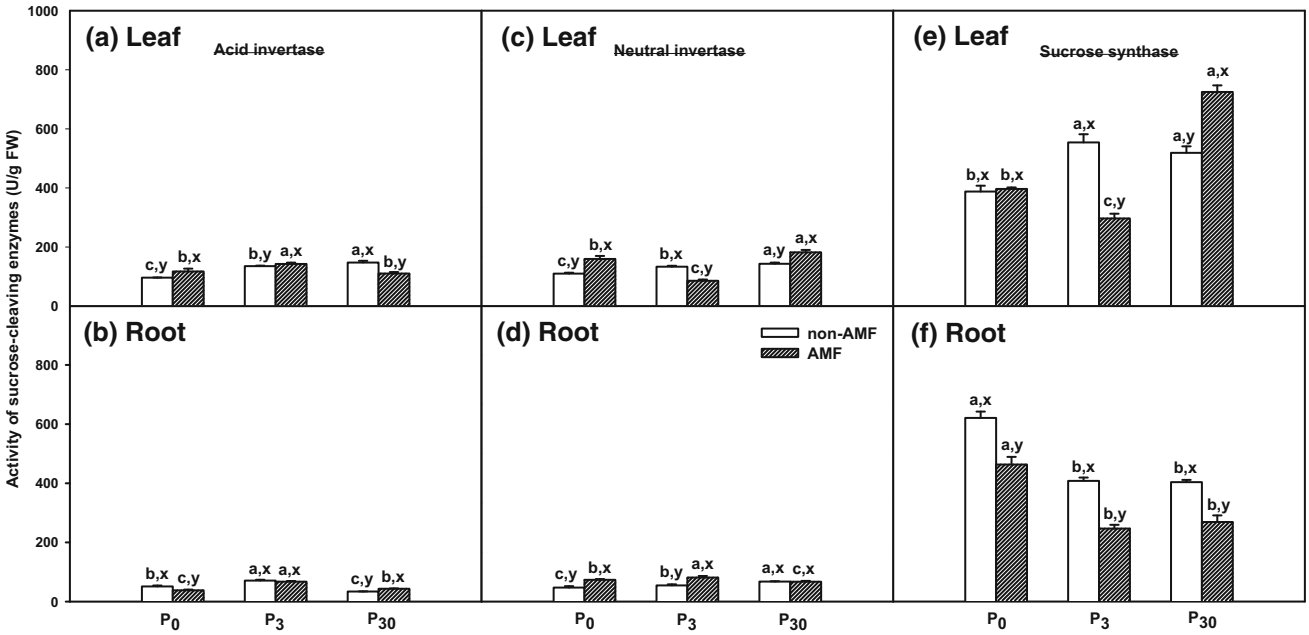

Fig. 5 Effects of *F. mosseae* on acid invertase, neutral invertase, and sucrose synthase (synthesis direction) activities in leaves and roots of trifoliate orange (*P. trifoliata*) seedlings grown under 0, 3, and 30 mM P level (defined as P_0, P_3, and P_{30}). Data (mean ± SD, $n = 4$) are significantly different ($P < 0.05$) if followed by *different letters above the bars* between P levels for the same *F. mosseae* treatment (**a–c**) or between *F. mosseae* treatments for the same P level (*x*, *y*). *Black columns* and *white columns* indicate the seedlings inoculated with AMF and non-AMF, respectively

Compared with non-AMF treatment, AMF treatment showed three types of responses on leaf SS activity: no change under P_0 treatment, a significant increase under P_{30}, and a significant decrease under P_3 (Fig. 5e). Significantly higher leaf SS activity ranked as $P_{30} > P_0 > P_3$ under mycorrhization and as $P_3 \approx P_{30} > P_0$ under non-

mycorrhization. In roots, AMF seedlings recorded 25.4, 39.5, and 33.4 % lower SS activity than non-AMF seedlings under P_0, P_3, and P_{30}, respectively (Fig. 5f). Root SS activity was significantly higher under P_0 than under either P_3 or P_{30}, irrespective of AM or non-AM seedlings.

Correlationship Studies

Correlationship revealed that root AMF colonization was significantly positively correlated with all the three fractions of carbohydrates (glucose, sucrose, and fructose) concentrations of roots (Fig. 6). Correlation of root AMF colonization with root glucose was strongly higher than with root fructose. Leaf SS activity was significantly positively correlated with leaf sucrose concentration under both AMF or non-AMF conditions (Fig. 7). Interestingly, the correlation between leaf SS activity and leaf sucrose was higher under mycorrhization than under non-mycorrhization, suggesting that AMF inoculation aided in transforming the host plant, to metabolically more active.

Discussion

Our study showed that root mycorrhizal colonization of trifoliate orange by *F. mosseae* decreased with increasing substrate P levels. Earlier results by Beltrano and others (2013) showed a significant reduction in root AMF colonization with increasing P levels from 10 to 40 mg/kg soil in pepper plants inoculated with *Glomus intraradices*. A significant positive correlation of root AMF colonization with fructose, glucose, and sucrose concentrations of roots suggested the direct involvement of AMF in changes in root carbohydrate levels, irrespective of substrate P level. In addition, the response of root colonization to substrate P level is attributable to spore behavior, because external hyphae of spores were often mediated by substrate P level (Trindade and others 2006).

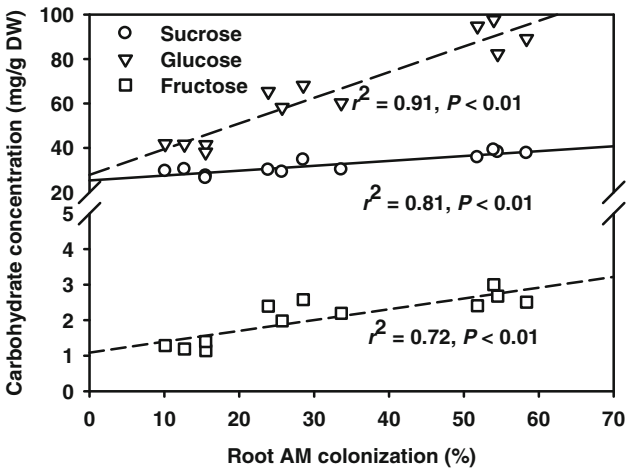

Fig. 6 Linear regression between root AM colonization and root sucrose, glucose, and fructose concentrations in *F. mosseae*-colonized trifoliate orange (*P. trifoliata*) seedlings grown under 0, 3, and 30 mM P level ($n = 12$)

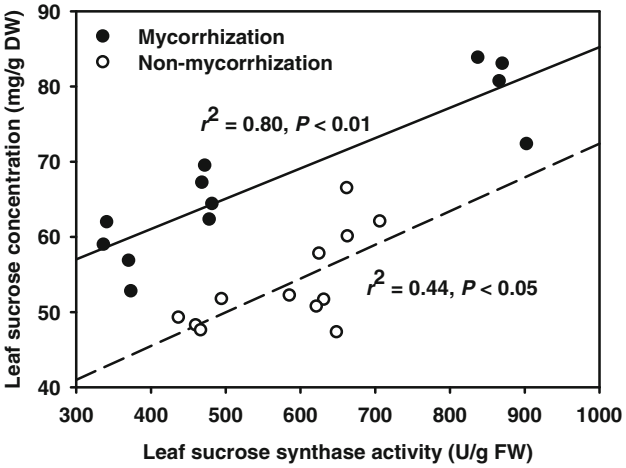

Fig. 7 Linear regression between leaf sucrose synthase (synthesis direction) activity and leaf sucrose concentration in *F. mosseae*-colonized trifoliate orange (*P. trifoliata*) seedlings grown under 0, 3, and 30 mM P level ($n = 12$)

In the present study, the trifoliate orange seedlings between P_3 and P_{30} conditions seemed to be little different in growth performance, indicating that high P is not required to accelerate the plant growth process, because the roots of trifoliate orange are relatively shallow and possess fewer and short root hairs, sometimes even no root hairs (Zhang and others 2013). We also observed that mycorrhizal seedlings maintained a significantly higher magnitude of growth, irrespective of substrate P level, suggesting that AMF-induced enhancement in plant growth is independent of the substrate P level, or this strain of *F. mosseae* is an efficient AM fungus for growth of trifoliate orange (Wu and others 2006), though the substrate P level strongly affected root mycorrhizal colonization in the present study. The present study further revealed that AMF inoculation improved plant growth performance, more distinctly under P_0 than under P_{30}. The AMF effect on plant growth responses resulted from the enhancement in uptake of native soil P by extraradical mycorrhizal mycelium (Grimoldi and others 2005). Possibly, AMF plants possessed a higher P-use-efficiency at a low P concentration than at a high P concentration (Ning and Cumming 2001).

Phosphorus application has been shown to increase root morphology in many plants (Li and others 2012). In our work, P_0 treatment generally inhibited root morphological characters, but P_{30} treatment failed to produce the same magnitude of response (except a higher surface area and volume and lower third-order lateral root number), in comparison to P_3 treatment, suggesting that trifoliate

orange was more sensitive at low P than at high P. On the other hand, such changes of root morphology in response to P availability are an important factor in P-use-efficiency (Zobel and others 2006). However, AMF trifoliate orange showed higher root morphological traits than non-AMF seedlings, irrespective of substrate P level. Our previous studies in trifoliate orange inoculated with AMF species such as *F. mosseae*, *G. versiforme,* and *Paraglomus occultum* showed similar kinds of responses on root morphological characters (Wu and others 2011). However, the substrate P level produced no significant effect of AMF on root morphology of trifoliate orange, because increase in root total length did not account for the effect of substrate P level on arbuscules, an exchanged interface of nutrients between AMFs and the host plant (Braunberger and others 1991). It is well documented that AMF plants contained a modified endogenous balance of growth regulators (Berta and others 1993; Wu and others 2012). These dynamic changes in hormone balance especially auxin, which has not been determined in this study, could trigger the desired increase in cell size and cell elongation of roots, thereby, inducing a favorable change in root morphology (López-Bucio and others 2002; Padilla and Encina 2005).

AMF must obtain hexose from sucrose cleavage, primarily glucose, and transform it into trehalose and glycogen, typical fungal carbohydrates (Bago and others 2003). Our studies indicated that leaf sucrose concentration was significantly higher under mycorrhization than under non-mycorrhization with P_0 and P_{30} treatments, without any significant response at P_3. There observations suggested that AMF plants provided a greater sucrose source in leaves to load into the phloem for long-distance transport to the target sink organs such as AMs, which is dependent on the supply substrate P level. Doidy and others (2012) cloned three sucrose transporters from *Glomus intraradices*-colonized *Medicago truncatula*, which modulated C fluxes from the photosynthetic leaf source towards the AM symbiont. As a result, plants have been shown to direct 4–20 % of photoassimilates towards AM roots for sustaining growth and respiration requirements of intraradical as well as extraradical mycelia (Smith and Read 2008).

Sucrose synthase (SS) has the synthesis direction and breakdown direction, and can collectively catalyze the reversible reaction of sucrose into hexoses (Verma and others 2013). The present study indicated that the leaf sucrose concentration was more strongly positively correlated with leaf SS (synthesis direction) activity in AMF seedlings than in non-AMF seedlings, confirming that AMs would induce higher SS (synthesis direction) activity to synthesize sucrose in leaves as a source. There is certainly evidence that the net photosynthesis rate of the host plant is enhanced to meet the increased carbohydrate requirement for AM (Ferrol and Pérez-Tienda 2009). It is well known that under physiological conditions, SS is commonly considered to be involved in sucrose breakdown and glucose channeling to cellulose synthase, rather than sucrose synthesis alone (Amor and others 1995; Baier and others 2010). Schubert and others (2003) observed no significant change in root SS activity of soybean plants inoculated with *F. mosseae* at an earlier stage (<35 days), but after a later growth stage (>35 days) root SS activity was significantly higher under the mycorrhization with *F. mosseae* than with non-AMF. Baier and others (2010) further cloned a symbiosis-induced *M. truncatula* SS gene MtSucS1, which directly or indirectly helped in sustaining the normal maturation of arbuscules and shelflife of these arbuscules in efficient functioning of AMs. In this context, further studies are needed to consider SS (breakdown direction) activity and expression of related SS genes in different mycorrhizal structures, beside SS (synthesis direction) activity.

Plants deliver sucrose to AM roots, where sucrose is cleaved into glucose and fructose for the utilization of AMF (Smith and Read 2008). In the present study, mycorrhization induced higher glucose and fructose concentrations in roots, irrespective of substrate P level, except a lower root fructose concentration in AMF than in non-AMF seedlings with P_{30} treatment. It seems that more sucrose from source leaves in AMF plants was cleaved into hexoses for AMF development. A higher correlation of root AMF colonization with root glucose than with root fructose further suggested that AMs would preferably utilize glucose to transform it into typical fungal carbohydrates. This accounted for significantly lower root SS activity in AMF seedlings as compared with non-AMF seedlings. In fact, AMF inoculation down-regulated SS gene expression in roots of a AMF–plant system (Baier and others 2010).

When the plant delivers sucrose to AMF roots, sucrose cleavage by plant enzymes maintains a pool of hexoses available for transfer (Smith and Read 2008). Meanwhile, plant invertases primarily catalyze the irreversible hydrolysis of sucrose into glucose and fructose (Moscatello and others 2011). In our study, root AI activity remained unchanged between AMF and non-AMF seedlings under P_3, but higher in AMF than non-AMF seedlings under P_{30} and lower in AMF than in non-AMF seedlings under P_0. AMF inoculation showed no change in root NI activity under P_{30} but indicated significantly higher root NI activity under both P_0 and P_3 conditions. These results suggest that AMF inoculation had a varied change on root AI and NI activity in relation to different substrate P levels. Higher root AI activity as observed in our study in AMF seedlings than non-AMF control under P_{30} condition, was attributable to the strong expression of the acid invertase gene in arbuscule-infected roots under high P conditions

(Blee and Anderson 2002). However, the AMF inoculation showed no change in root AI activity under P_3 condition. Schubert and others (2003) earlier reported no significant difference in root AI activity between *F. mosseae*-colonized and non-AMF soybean. On the other hand, root AI activity was significantly lower in inoculated seedlings than uninoculated seedlings under P_0 condition, suggesting that low P altered AMF functioning on root AI activity. Based on this effect on root AI activity, AMF-induced root AI changes were observed dependent on substrate P level. Wu and others (2013) earlier reported divergent effects of *F. mosseae* on AI and NI activity between leaves and roots in three citrus genotypes. These studies further concluded that AMF-changed sucrose-cleaving enzyme activities are dependent on enzyme types, tissue types and substrate P levels. Such variation in sucrose-cleaving enzymes caused by mycorrhization was accountable to apoplast pH value, which partly controlled these sucrose-cleaving enzymatic activities (Schaeffer and others 1995). It seems that plant invertase has an important contribution in expanding the C sink capacity for AMs and subsequently the mycorrhizal plants.

Conclusion

In this work, AMF inoculation improved the growth performance and root morphological traits of trifoliate orange. These structural modifications were independent of substrate P level. However, AMF-induced sucrose cleavage was dependent on P levels, plant tissue type, and sucrose-cleaving enzyme types. In a better mycorrhizal root, more sucrose would be diverted into hexose primarily as glucose, thus, resulting in an increased allocation of glucose and decreased allocation of sucrose to roots. The functional characterization of such regulatory mechanisms would pave the way in providing a better understanding about the physiology as well as biochemistry involved in AMF functioning.

Acknowledgments This study was supported by the Key Project of Chinese Ministry of Education (211107) and the Open Fund of Institute of Root Biology, Yangtze University (R201401).

References

Amor Y, Haigler CH, Jonhson S, Wainscott M, Delmer DP (1995) A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proc Natl Acad Sci USA 92:9353–9357

Bago B, Preffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y (2003) Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiol 131:1496–1507

Baier MC, Keck M, Godde V, Niehaus K, Kuster H, Hohnjec N (2010) Knockdown of the symbiotic sucrose synthase *MtSucS1* affects arbuscule maturation and maintenance in mycorrhizal roots of *Medicago truncatula*. Plant Physiol 152:1000–1014

Beltrano J, Rusitti M, Agrango MC, Ronco M (2013) Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. J Soil Sci Plant Nutr 13:123–141

Berta G, Fusconi A, Trotta A (1993) VA mycorrhizal infection and the morphology and function of root systems. Environ Exp Bot 33:159–173

Blee KA, Anderson AJ (2002) Transcripts for genes encoding soluble acid invertase and sucrose synthase accumulate in root tip and cortical cells containing mycorrhizal arbuscules. Plant Mol Biol 50:197–211

Braunberger PG, Miller MH, Peterson RL (1991) Effect of phosphorus nutrition on morphological characteristics of vesicular-arbuscular mycorrhizal colonization of maize. New Phytol 119:107–113

Doidy J, van Tuinen D, Lamotte O, Corneillat M, Alcaraz G, Wipf D (2012) The *Medicago truncatula* sucrose transporter family: characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi. Mol Plant 5:1346–1358

Ferrol N, Pérez-Tienda J (2009) Coordinated nutrient exchange in arbuscular mycorrhiza. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (eds) Mycorrhizas-functional processes and ecological impact. Springer, Berlin, pp 73–87

Gohoonia TS, Nielsen NE (2004) Root traits as tools for creating phosphorus efficient crop varieties. Plant Soil 260:47–57

Grace EJ, Smith FA, Smith SE (2009) Deciphering the arbuscular mycorrhizal pathway of P uptake in non-responsive plant species. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (eds) Mycorrhizas-functional processes and ecological impact. Springer, Berlin, pp 89–106

Grimoldi AA, Kavanova M, Lattanzi FA, Schnyder H (2005) Phosphorus nutrition-mediated effects of arbuscular mycorrhiza on leaf morphology and carbon allocation in perennial ryegrass. New Phytol 168:435–444

Guo YP, Chen PZ, Zhang LC, Zhang SL (2003) Phosphorus deficiency stress aggravate photoinhibition of photosynthesis and function of xanthophyll cycle in citrus leaves. Plant Nutr Fertil Sci 9:359–363 (in Chinese with English abstract)

Gutjahr C, Casieri L, Paszkowski U (2009) *Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling. New Phytol 182:829–837

Jifon JL, Graham JH, Drouillard DL, Syvertsen JP (2002) Growth depression of mycorrhizal *Citrus* seedlings grown at high phosphorus supply is mitigated by elevated CO_2. New Phytol 153:133–142

Li ZX, Xu CZ, Li KP, Yan S, Qu X, Zhang JR (2012) Phosphate starvation of maize inhibits lateral root formation and alters gene expression in the lateral root primordium zone. BMC Plant Biol 12:89

López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol 129:244–256

Moscatello S, Famiani F, Projetti S, Farinelli D, Battistelli A (2011) Sucrose synthase dominates carbohydrate metabolism and relative growth rate in growing kiwifruit (*Actinidia deliciosa*, cv Hayward). Sci Hortic 128:197–205

Ning JC, Cumming JR (2001) Arbuscular mycorrhizal fungi alter phosphorus relations of broomsedge (*Andropogon virginicus* L.) plants. J Exp Bot 52:1883–1891

Padilla IMG, Encina CL (2005) Changes in root morphology accompanying mycorrhizal alleviation of phosphorus deficiency in micropropagated *Annona cherimola* Mill. plants. Sci Hortic 106:360–369

Peng SB, Eissenstat DM, Graham JH, Williams K, Hodge NC (1993) Growth depression in mycorrhizal citrus at high-phosphorus supply. Plant Physiol 101:1063–1071

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Myc Soc 55:158–161

Schaeffer C, Wallenda T, Güttenberger M, Hampp R (1995) Acid invertase mycorrhizal and non-mycorrhizal roots of Norway spruce (*Picea abies* [L.] Kar) seedlings. New Phytol 129:417–424

Schmidt B, Mononkos M, Sumalan R, Biro B (2010) Suppression of arbuscular mycorrhiza's development by high concentrations of phosphorus at *Tagetes patula* L. Res J Agric Sci 42:156–162

Schubert A, Allara P, Morte A (2003) Cleavage of sucrose in roots of soybean (*Glycine max*) colonized by an arbuscular mycorrhizal fungus. New Phytol 161:495–501

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, New York

Srivastava AK, Singh S, Marathe RA (2002) Organic citrus: soil fertility and plant nutrition. J Sustain Agric 19:5–29

Trindade AV, Siqueira JO, Sturmer SL (2006) Arbuscular mycorrhizal fungi in papaya plantations of Espírito Santo and Bahia, Brazil. Braz J Microbiol 37:283–289

Verma A, Agarwal AK, Dubey RS, Solomon S, Singh SB (2013) Sugar partitioning in sprouting lateral bud and shoot development of sugarcane. Plant Physiol Biochem 62:111–115

Wright DP, Read DJ, Scholes JD (1998) Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. Plant, Cell Environ 21:881–891

Wu QS, Wang YS, Xia RX (2006) Comparison of arbuscular mycorrhizal fungi for drought resistance of trifoliate orange (*Poncirus trifoliata* L. Raf.) seedlings. Acta Hortic Sin 33:613–616 (in Chinese with English abstract)

Wu QS, Zou YN, He XH, Luo P (2011) Arbuscular mycorrhizal fungi can alter some root characters and physiological status in trifoliate orange (*Poncirus trifoliata* L. Raf.) seedlings. Plant Growth Regul 65:273–278

Wu QS, He XH, Zou YN, Liu CY, Xiao J, Li Y (2012) Arbuscular mycorrhizas alter root system architecture of *Citrus tangerine* through regulating metabolism of endogenous polyamines. Plant Growth Regul 68:27–35

Wu QS, Zou YN, Huang YM, Li Y, He XH (2013) Arbuscular mycorrhizal fungi induce sucrose cleavage for carbon supply of arbuscular mycorrhizas in citrus genotypes. Sci Hortic 160: 320–325

Zhang ZL, Zai L (2004) Experimental manual of plant physiology, 3rd edn. Higer Education Press, Beijing (in Chinese)

Zhang DJ, Xia RX, Cao X, Shu B, Chen CL (2013) Root hair development of *Poncirus trifoliata* grown in different growth cultures and treated with 3-indolebutyric acid and ethephon. Sci Hortic 160:389–397

Zobel RW, Alloush GA, Belesky DP (2006) Differential root morphology response to no versus high phosphorus, in three hydroponically grown forage chicory cultivars. Environ Exp Bot 57:201–208