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Nutrient acquisition and fruit quality of Ponkan mandarin in response to AMF inoculation

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) inhabited in citrus rhizosphere playing multiple roles in plant growth, nutrient acquisition, and stress tolerance are well known under potted conditions. However, information regarding response of AMF application in field citrus trees is scarce. In this study, single inoculation of *Funneliformis mosseae* and mixture of *Diversispora versiformis*, *F. mosseae*, and *Rhizoglomus intraradices* into the rhizosphere were compared with non-inoculated (control) three-yr-old *Citrus reticulata* Blanco var. Ponkan cv. Jinshuigan grafted on trifoliate orange grown under open field condition. After eight months of inoculation, root mycorrhiza colonization and soil hyphal length varied respectively from 43% to 56% and from 3.66 to 5.57 cm/g soil and also 1.7-2.2 times and 13.1-19.9 times higher in AM-treated trees than non-inoculated trees. Two AMF treatments collectively increased root P, K, Cu, Zn, and Mn concentrations, sarcocarp P, Mn, and Fe concentrations, peer coloration value, and fruit diameter longitudinal axis, as well as root vitality by 36%-76%. In addition, single *F. mosseae* inoculation dramatically increased juice soluble solid content and sarcocarp Zn concentrations than non-AMF treatment; mixed-AMF inoculation considerably increased single fruit weight and sarcocarp K and B contents. On the whole, these beneficial effects were superior under mixed AMF treatment than under single AMF treatment. Our study, hence, suggested that mixed culture of AMF inoculation, played an important role in quality production of Pokan mandarin as a viable biofertilizer use option.

Key words: Arbuscular mycorrhiza, Fruit quality, Nutrient composition, Pokan mandarin

Arbuscular mycorrhizal fungi (AMF) representing *Glomeromycotina* have the ubiquitous ability to form a symbiosis with roots of most of terrestrial plants. The mycorrhizal symbiosis helps host plants absorb nutrients and conversely, the fungal growth is maintained by organic carbon and lipid supplied by host plants (Srivastava *et al*. 2002, Keymer and Gutjahr 2018, Wu *et al*. 2019b, Zhang *et al*. 2020). In this symbiotic relation, the fungi and root cortical cells have no direct contact and separated by symbiotic interface, through which water and nutrients are exchanged (Plassard *et al*. 2019). AM-symbiosis has important roles in plant physiological and ecological functionings (Wu *et al*. 2012, 2019a, Liu *et al*. 2018, Jansa and Kohout 2019, Lü *et al*. 2019, Zhang *et al*. 2018, 2019, Zou *et al*. 2019).

Citrus is an evergreen fruit tree, widely cultivated all over the world (Srivastava and Singh 2008). The countries such as Spain, Italy and Australia are known for exporting sweet oranges long with developing countries such as Egypt and South Africa (Zhang and Xiao 2019). Good tree growth depends on rhizosphere ecology dominated superior microbial species within rhizosphere (Nugullie *et al.* 2015). Wu *et al*. (2017a) utilized SSU rRNA in identifying the diversity of AMF in rhizosphere of 29-yr-old Satsuma mandarin, dominated by AMF like *Glomus* and *Claroideoglomus*. The response of AMF-inoculation in promoting plant growth, improving fruit quality, optimizing root architecture, enhancing nutrient absorption, enhancing adverse tolerance, improving soil structure, and increasing soil fertility is highlighted through number of previous studies (Srivastava *et al*. 2007, Ortas *et al*. 2017, Tuo *et al*. 2017, Wu *et al.* 2011). But, such responses of mycorrhizal symbiosis were observed mainly based on potted experiments. Yao *et al*. (1997, 1999) reported that application of *Funneliformis mosseae* into field *Citrus iyo* trees increased peel carotenoid content, juice soluble solid content and solid acid ratio. Other fruit quality parameters like fruit weight and total soluble solid content of sweet orange trees were reported producing favorable response by inoculation with *Funneliformis mosseae* (Sui *et al*. 2007). Similarly, AMF application stimulated the increase of fruit weight in papaya plants and fruit K and Cu concentration in strawberry plants (Castellanos-Morales

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et al. 2010; Vazquez-Hernandez *et al*. 2011). The use of AMF is gradually gaining popularity in fruit culture due to expensive chemical and inorganic fertilizer inputs aiding in higher cost of production (Ortas *et al*. 2017). As a result, large scale use of mycorrhizal fungi into sustainable field becomes imperative in fruit culture.

In this study, we used single and mixed AMF inoculations into citrus trees raised in open fields for evaluating mycorrhizal responses on nutrient concentrations of roots and fruits in addition to fruit quality. Such experiment will provide better in field evidence in favor of AMF as biofertilizers to be used in citriculture.

MATERIALS AND METHODS

Plant culture and mycorrhizal inoculation: The experiment was carried out in a three-year-old *Citrus reticulata* Blanco var. Ponkan mandarin cv. Jinshuigan grafted on trifoliate orange (*Poncirus trifoliata* L. Raf.) raised in 3×4 m spacing at the Agricultural Science and Technology Industrial Park, Yangtze University (Jingzhou, China).

Single inoculum of *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler was used as an AMF treatment. In addition, mixed inoculums of *Diversispora versiformis* (P. Karst.) Oehl, G.A. Silva & Sieverd., *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, and *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl, were considered as another AMF treatment. These AMF strains were provided by the Bank of Glomeromycota in China (Beijing, China), and propagated with identified spores in pot culture with white clover as host plants for three months. These AMF inocula were made up of spores, mycorrhizal hyphae, and colonized root segments. A total of 600 g mycorrhizal inocula were applied into a citrus rhizosphere.

In the 40 cm horizontal distance from the tree trunk, two dressing furrows in the southern and northern direction of selected citrus trees were characterized with 40 cm long, 15 cm width, and 30 cm depth. Each fertilization slot was supplied with 300 g AMF inoculum plus 3.4 kg organic fertilizer with rotten chicken droppings. For non-AMF treatment, 600 g autoclaved inoculum containing 300 g *Funneliformis mosseae* inoculum and 300 g mixed AMF inoculums were applied. After AMF inoculation, the surface of dressing furrow sown white clover with the density of 300 seeds/m^2. All the treated trees were subjected to standard field management in terms of pruning, fertilization, and water supplement. Each of three treatments had five replicates, with a total of 15 trees. Fruits of treated trees were harvested on November 8, 2018, and root systems and rhizospheric soils were collected for analysis.

Observation and analysis: Root mycorrhizas were stained according to the procedure outlined by Phillips and Hayman (1970). Root mycorrhizal colonization was calculated with the following formula: root colonization (%) = AMF colonized root segment lengths / total observed root segment lengths × 100. Soil hyphal length of mycorrhizas was determined by Bethlenfalvay and Ames (1987). Root vitality was analyzed as per the triphenyl tetrazolium chloride method described by Lindstrom and Nystrom (1987). Fruit weight was determined using electronic scales. Fruit horizontal and vertical diameter was measured with vernier caliper. Peel coloration value was assayed using colorimeters (CR10, Japan). Fruit rigidity and juice soluble solid content were analyzed through fruit sclerometer (GY-B, China) and hand saccharometer (WYT-4, China), respectively. The concentration of different nutrients such as P, K, Ca, Mg, Fe, Cu, Mn, B, and Zn in roots and sarcocarps were analysed using ICP Specmometers (IRIS Advantage, Thermo, USA) after digesting them in diacid mixture as per the standard procedure. The data were analyzed with one-factor variance (ANOVA) in SAS 8.1, and the Duncan's multiple range test was used to compare the significance between treatments at P = 0.05 level.

RESULTS AND DISCUSSION

Mycorrhizal response of citrus trees: Citrus trees without AMF inoculation showed 24.88% of root colonization and 0.28 cm hyphal length/g soil, while inoculated-AMF trees observed 42.57% to 55.70% of mycorrhizal colonization and 3.66 to 5.57 cm hyphal length/g soil (Table 1). Significantly higher mycorrhizal growth in root colonization and soil hyphal length was observed in the following decreasing order of mixed-AMF > *F. mosseae* > non-AMF. These observations indicated that AMF inoculation magnified the mycorrhizal growth in roots and soils of citrus trees grown in open field. Superior AMF growth in field citrus has bigger capacity to confer greater impacts on soil quality parameters, which aided in an increased plant growth, nutrient acquisition, and stress tolerance in inoculated plants (Jansa *et al*. 2016, Wu *et al*. 2017b).

Changes in root vitality of citrus trees after AMF inoculation: Roots participate in various physiological roles in nutrient and water acquisition, resource storage, and hormonal synthesis, whilst the degree of root vitality is used to determine the magnitude of response of such roles of roots (Scattolin *et al*. 2008). The present work indicated that single and mixed AMF treatments significantly increased root vitality by 36% and 76%, respectively, as compared to non-AMF-inoculated treatment (Table 1).

Table 1 Mycorrhizal growth and root vitality of Ponkan mandarin grown under open field in response to inoculation with single and mixed AMF compared to non-AMF treatment

Inoculation treatments	Root mycorrhizal colonization (%)	Soil hyphal length (cm/g soil)	Root vitality (mg/g/h)
F. mosseae	42.57±7.78b	3.66±0.99b	2.07±0.21b
Mixed AMF	55.70±9.77a	5.57±1.45a	2.78±0.23a
Non-AMF	24.88±4.13c	0.28±0.15c	1.58±0.34c

Data (means ± SD, n = 5) are significantly different (p < 0.05) if followed by different letters in the column.

Druege *et al*. (2006) earlier reported that, AMF inoculation with the *Glomus intraradices* isolate H510 had the positive contribution towards improved root activity of poinsettia (*Euphorbia pulcherrima*) plants by altering carbohydrate metabolism and plant hormonal dynamics in roots.

Changes in root nutrient composition after AMF inoculation: An important function of AMs is to help host plants absorb nutrients, especially P, from the soil and delivered to AMF-colonized roots (Plassard *et al*. 2019), because extraradical hyphae of AMs spread in the soil around the roots to absorb nutrients that roots having restricted access (Heidari and Karami 2014). Our study showed that inoculated citrus plants represented superior root nutritional levels than uninoculated plants (Table 2). Hereinto, single and mixed AMF treatments significantly increased root P, K, Cu, Zn, and Mn concentration, as compared to non-AMF treatment. Findings of Shao *et al*. (2018) in *Camellia sinensis* and Wu *et al*. (2011) in *Prunus persica* plants earlier reported similar observations. While, mixed-AMF-inoculation treatment also increased root Mg concentrations, compared to non-AMF treatment. Root P, Cu, and Mn concentration was significantly higher in *F. mosseae*-inoculated citrus plants than in mixed-AMF-inoculated plants. It seems that mixed AMF inoculation represented relatively greater nutrient acquisition of roots than single AMF treatment. The improved nutrition acquisition under mycorrhization is attributed to direct absorption of extraradical hyphae to nutrients, AMF-stimulated root morphological optimization, endogenous polyamine balance, and indole-3-acetic acid accumulation (Wu *et al*. 2012, Tuo *et al*. 2017, Liu *et al*. 2018, Zhang *et al*. 2019). Acquisition of nutrients by roots was enhanced by mycorrhizas, thereby, altering the nutrient pool of inoculated soil. Lü *et al*. (2019) reported that in peach, mycorrhizosphere had higher soil organic carbon content, soil bacterial population, soil urease and acid phosphatase activity, and root exudate compositions, coupled with a lower abundance of allelochemical substances as compared with non-mycorrhizosphere. Mycorrhizas also released a specific glycoprotein, glomalin, into soils to contribute towards enhancement in soil organic carbon pools (Zou *et al*. 2016). As a result of these positive effects of arbuscular mycorrhizas, host plants were nutritionally healthier.

Changes in fruit quality after AMF inoculation: Many past studies had showed that AMF inoculation into rhizosphere of *Citrus sinensis* improved the fruit quality to varying proportions (Yao *et al*. 1997, 1999, Sui *et al*. 2007). In our study, single *F. mosseae* inoculation increased the fruit vertical diameter, soluble solid content, and peel coloration value, as compared with non-AMF treatment (Table 3, Fig 1). In addition, mixed-AMF inoculation considerably increased fruit weight, fruit horizontal and vertical diameter, and fruit coloration value than non-AMF inoculation (Table 3, Fig 1). As reported by Yao e*t al*. (1997, 1999), 100 spores of *Funneliformis mosseae* per m^2 were applied into the rhizosphere of *Citrus iyo* trees, which improved the carotenoid content in flavedo, as well as soluble solid content and solid acid ratio in fruit juice. Similarly, *Citrus sinensis* grafted on trifoliate orange inoculated with *F. mosseae* exhibited higher single fruit weight and soluble solid content, as well as lower titratable acid content in fruit juice (Sui *et al*. 2007). In strawberry fruits, *Glomus intraradices* imparted the colour changes through the changes in the levels of anthocyanins (Castellanos-Morales *et al*. 2010). However, other quality parameters like sugar content, rigidity, and color of fruits of mycorrhized papaya were similar to those of non-mycorrhized control (Vazquez-Hernandez *et al*. 2011). Under the current condition of organic citriculture, AMF as a biofertilizer has a promising utility in citrus orchard to decrease the amount of inorganic fertilizer usage, especially

Table 2 Root and fruit nutrient composition of Ponkan mandarin grown under open field in response inoculation with single and mixed AMF compared to non-AMF treatment

Inoculation treatment	Macronutrients (mg/g DW)				Micronutrients (mg/kg DW)				
	P	K	Ca	Mg	Cu	Zn	Mn	Fe	B
Roots									
F. mosseae	2.51 ± 0.10a	18.57 ± 1.18a	19.41 ± 2.03a	3.00 ± 0.25ab	83.35 ± 3.64a	103.9 ± 3.7a	278.4 ± 18.2a	4.71 ± 0.51a	34.62 ± 2.58a
Mixed AMF	2.36 ± 0.09b	17.94 ± 0.70a	21.72 ± 1.84a	3.39 ± 0.25a	67.48 ± 3.93b	91.7 ± 6.2a	207.3 ± 12.3b	4.05 ± 0.42a	32.76 ± 2.75a
Non-AMF	1.78 ± 0.09c	13.99 ± 1.06b	22.37 ± 1.47a	2.66 ± 0.38b	30.79 ± 8.57c	46.4 ± 11.9b	170.1 ± 29.2c	4.14 ± 0.62a	33.18 ± 3.03a
Fruits									
F. mosseae	1.16 ± 0.09a	10.22 ± 0.52ab	1.32 ± 0.55a	0.82 ± 0.04a	3.82 ± 0.35ab	9.3 ± 0.4a	4.72 ± 0.30b	203.9 ± 25.1a	11.34 ± 1.29ab
Mixed AMF	1.23 ± 0.04a	10.66 ± 0.75a	1.47 ± 0.28a	0.85 ± 0.04a	2.95 ± 1.09b	8.3 ± 0.9ab	7.26 ± 0.39a	220.6 ± 5.7a	12.63 ± 0.93a
Non-AMF	1.00 ± 0.05b	9.59 ± 0.45b	1.06 ± 0.04a	0.78 ± 0.04a	4.88 ± 0.67a	7.6 ± 0.4b	3.99 ± 0.42c	173.8 ± 4.5b	10.42 ± 0.48b

Data (means ± SD, n = 5) are significantly different (p < 0.05) if followed by different letters in the column.

Table 3 Fruit quality of Ponkan mandarin trees grown under open field in response to inoculation with single and mixed AMF compared to non-AMF treatment

Inoculation treatment	Fruit weight (g/ fruit)	Horizontal diameter (cm)	Vertical diameter (cm)	Rigidity (kg×10^5/ cm^3)	Soluble solid content (%)	Coloration value
F. mosseae	44.79±2.07b	4.69±0.11ab	3.64±0.05ab	15.04±1.92a	11.04±0.40a	79.81±1.06a
Mixed AMF	49.69±1.94a	4.81±0.11a	3.70±0.05a	15.69±1.67a	10.35±0.37b	79.68±1.63a
Non-AMF	43.93±3.75b	4.62±0.07b	3.59±0.04b	14.92±0.93a	9.96±0.26b	74.58±0.92b

Data (means ± SD, n = 5) are significantly different ($p < 0.05$) if followed by different letters in the column.

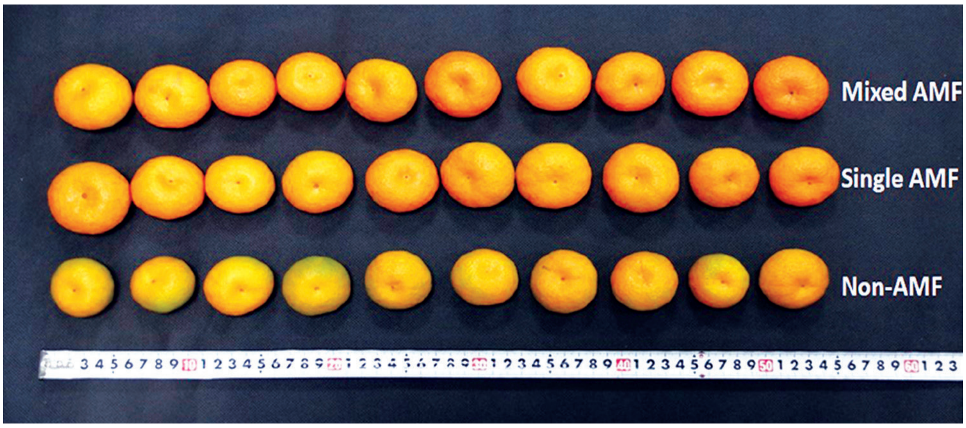

Fig. 1 Changes in fruit appearance of Pongan mandarin trees in response to single and mixed inoculation of AMF.

P, besides better fruit quality (Wu *et al*. 2017b).

In addition, nutritional levels of fruit juices were contrastingly different among three treatments. Compared with non-AMF inoculation, single *F. mosseae* inoculation considerably elevated the concentration of P, Zn, Mn, and Fe in fruit juices (Table 2). Mixed-AMF-inoculation treatment also dramatically increased P, K, Mn, Fe, and B contents in fruit juice. An inoculation of AMF in citrus trees for eight-months conferred the positive effect on fruit quality, which provided a new line of thought to improve fruit quality in citrus. The improved nutrient status in AMF-inoculated citrus is the result of collective effect arising from altered root morphology, carbohydrate supplement, and hormonal balance. As previously mentioned, mycorrhizal Ponkan mandarin trees were nutritionally richer through expanding sink capacity of mycorrhized roots, as compared with non-AMF-inoculated trees (Table 2), creating better diversion of nutrients towards fruits as much stronger nutrient sink. However, mycorrhizal effects on fruit quality of citrus will need to be observed on a long term basis following AMF inoculation in the field.

The fruit ripening process of mycorrhized Pongan mandarin, especially with mixed AMF inoculants, was comparatively faster than non-mycorrhized trees. Vazquez-Hernandez *et al*. (2011) reported no distinct response of *Funneliformis mosseae* on fruit ripening process of papaya plants. Postharvest evaluation further showed the lower weight loss in fruits of mycorrhized papaya plants than non-mycorrhizal plants. It implied that fruits of mycorrhizal trees possibly have a superior postharvest quality.

Our studies firstly showed that AMF-inoculated citrus trees developed relatively superior intrinsic and external fruit quality parameters than non-AMF-treated trees. These results offer an opportunity to utilize AMF as a biofertilizer for sustainable citriculture development by cutting down an extensive usage of inorganic fertilizers.

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