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Original article

# Spatial changes of arbuscular mycorrhizal fungi in peach and their correlation with soil properties

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## ABSTRACT

Arbuscular mycorrhizal (AM) fungi have beneficial effects on host plants, but their growth is influenced by various factors. This study was carried out to analyze the variation of AM fungi in soils and roots of peach (*Prunus persica* L. var. Golden Honey 3, a yellow-flesh variety) trees in different soil layers (0–40 cm) and their correlation with soil properties. The peach tree could be colonized by indigenous AM fungi (2.2–8.7 spores/g soil and 1.63–3.57 cm hyphal length/g soil), achieving 79.50–93.55% of root AM fungal colonization degree. The mycorrhizal growth, root sugars, soil three glomalins,  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , available P and K, and soil organic matter (SOM) had spatial heterogeneity. Soil spores, but not soil hyphae contributed to soil glomalin, and soil glomalin also contributed to SOM. There was a significant correlation of soil hyphae with spore density, soil  $\text{NO}_3\text{-N}$ , and SOM. Root mycorrhiza was positively correlated with spore density,  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and easily extractable glomalin-related soil protein. Notably, spore density positively correlated with  $\text{NO}_3\text{-N}$ , available K, SOM, and root fructose and glucose, while negatively correlated with available P and root sucrose. These findings concluded that mycorrhiza of peach showed spatial distribution, and soil properties mainly affected/changed based on the soil spore density.

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## 1. Introduction

A kind of beneficial microorganisms in the soil, viz., arbuscular mycorrhizal (AM) fungi, establish a mutual beneficial symbiosis in roots of higher plants for the absorption of water and nutrients (Bücking and Kafle, 2015). In general, the colonization and distribution of AM fungi depend upon environmental conditions rather than host plants, and thus root mycorrhizal colonization and soil spore density are primarily governed by soil factors (Gong et al.,

2012). In the rhizosphere of *Ulmus pumila* var. *sabulosa* in the hunsandak sandy land, the spatial distribution of AM fungi was mainly influenced by soil physicochemical properties including soil catalase, easily extractable glomalin-related soil protein (EEG), available K, soil organic matter (SOM), and alkaline phosphatase (Ma et al., 2018). In *Amorpha fruticosa* grown on the Loess Plateau (China), soil EEG, root AM colonization, soil spore density, and soil pH value played major roles in determining soil ecology (Xie and Tang, 2012).

Peach is a deciduous fruit tree that is widely planted around the world and has been shown to be an AM-dependent plant. Previous studies indicated the promoted effects in growth performance and P, Zn, and Cu uptake of peach seedlings grown in fumigated nursery soils and Zn-deficient soils after AM fungi inoculation (Gilmore, 1971; Lambert et al., 1979). AM fungi-inoculated peach seedlings recorded higher nutrient acquisition than non-inoculated seedlings (Wu et al., 2011). An AM fungus *Acaulospora scrobiculata* dramatically alleviated the obstacle of continuous cropping of peach in the soil through changing root exudate compositions and up-

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regulating the expression level of disease-resistant genes (Lü et al., 2019; Gao et al., 2020). In addition, AM fungi-inoculated peach plants showed stronger flooding tolerance than non-AM plants through accelerating the accumulation of proline and improving the root growth (Zheng et al., 2020). Field inoculation with AM fungal biofertilizer of a commercial product in a nectarine (*Prunus persica laevis*) orchard increased root survivorship and leaf P levels, indicating the positive impact on field peach (Baldi et al., 2016). Such results provide the evidence regarding the positive roles of AM symbiosis in peach trees. However, it is important to understand which soil factors affect the mycorrhizal development in the rhizosphere and endosphere of peach trees.

In peach, the yellow-fleshed peach is characterized by round and yellow fruits, along with a red blush. In yellow-fleshed peach cultivars, the 'Golden Honey 3' cultivar is almost round with most of the fruit surface being dark red and the flesh being orange-yellow with numerous red pigments near the core (Niu et al., 2018). As a result, the 'Golden Honey 3' variety is widely cultivated and increasingly popularized in China. However, there is no relevant information in the mycorrhizal status of this variety and the soil factors influencing it. The objectives of the present study were to assess the symbiosis of the 'Golden Honey 3' variety with AM fungi and also to analyze the relationship between various soil characteristics and AM status.

## 2. Materials and methods

### 2.1. Experimental materials

The tested plant material was the five-year-old 'Golden Honey 3' (*Prunus persica* L. var. Golden Honey 3) grafted on *P. persica* L. Batsch at an inter-planting spacing of 3 × 4 m. The orchard was located in the Special Fruit Garden of Yangtze University (30°21'27"N, 112°3'5"E) along with a total annual solar radiation of 104–110 kcal/cm<sup>2</sup>, an annual sunshine hours of 1800–2000 h, and an annual rainfall between 1100 and 1300 mm. The orchard soil was the Xanthi-Udic-Ferralsols according to FAO system.

### 2.2. Experimental sampling

The root and soil sampling was carried out in December 2020. Fifteen peach trees with consistent cultivation and management were selected. Fine roots were collected within the canopy, and the soil attached to the roots was gently shaken off as the rhizospheric soil. Samples of the three trees were mixed into one replicate, with a total of five replicates. Meanwhile, root samples and rhizospheric soils were collected at four soil layers, including 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm. Soils from the same layer of the three trees were uniformly mixed, naturally dried and sieved by a mesh with a diameter of 2 mm.

### 2.3. Variable determinations

Mycorrhizal hyphal length in the soil was analyzed immediately after soil samples were collected from the field using the method described by Bethlenfalvy and Ames (1987). Root AM fungi colonization was carried out by Phillips and Hayman (1970) after staining 0.05% trypan solution. Root AM structures were examined microscopically, and the colonization degree of AM fungi in roots was estimated using the formula described by Cheng et al. (2021). Root glucose, fructose, and sucrose contents were measured by Wu et al. (2015b).

Spore density in the soil was measured using the sucrose centrifugation-wet sieve decantation method outlined by Ianson and Allen (1986). SOM content was assayed using the potassium

dichromate oxidation method (Lao, 1988). Soil's NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, available K, and available P contents were analyzed with a HM-TYD Soil Fertilizer Nutrient Tester (Shandong Hengmei Electronic Technology Co., Ltd, Weifang, China) according to the users' guidelines.

GRSP fractions including EEG and DEG (difficultly extractable GRSP) were extracted from soils with 20 mM citrate buffer (1 : 8, g/v) for half an hour and 50 mM citrate buffer for an hour at 121 °C, respectively (Wu et al., 2015a), and the concentration of these GRSPs was analysed according to Bradford's assay (1976).

### 2.4. Data analyses

The data obtained from the experiment were statistically analyzed using SAS. Significant ( $P = 0.05$ ) differences between four soil layers were performed by the Duncan's new multiple range test. Correlation coefficients between selected variables were achieved using Pearson's Product Moment ( $r$ ).

## 3. Results

### 3.1. Spatial distribution of AM fungi in the rhizosphere and endosphere

In the rhizosphere of yellow-fleshed peach variety 'Golden Honey 3', soil mycorrhizal hyphae (Fig. 1a) and mycorrhizal fungal spores (Fig. 1b) were observed, ranging in 1.63–3.57 cm/g soil and 2.2–8.7 spores/g soil, respectively (Table 1). Roots could be colonized by indigenous AM fungi (Fig. 1c), varying in 79.50%–93.55% (Table 1). A good symbiotic relationship could be formed between peach roots and indigenous AM fungi. The root mycorrhizal colonization degree and soil spore density decreased gradually with increasing soil depths (0–40 cm). The length of soil hyphae gradually decreased with the increase of 0–30 cm soil layer and then increased in the 30–40 cm depth soil.

### 3.2. Spatial distribution of root sugars

The sucrose content in the root of 0–10 cm depth soil was significantly lower than that in the root of other three soil layers, while no significant differences in root sucrose content were found among the root isolated from 10 to 40 cm soil layers (Table 1). The root fructose content was the highest in the root grown in the surface soil (0–10 cm) and the lowest in the 30–40 cm depth soil. The root glucose content showed significant differences among all treatments and decreased after the soil deep was increased.

### 3.3. Spatial distribution of soil nutrients

Soil nutrients in rhizosphere of yellow-fleshed peach showed certain spatial distribution (Table 2). As the depth of the soil increased, SOM and NO<sub>3</sub><sup>-</sup>-N content gradually decreased, while soil available P content increased. NH<sub>4</sub><sup>+</sup>-N and available K in the soil showed diversified variations in soil layers, along with the highest value in the 0–10 depth soil.

### 3.4. Spatial distribution of soil glomalin

Soil EEG, DEG and TG contents decreased with the increase of soil depth, whilst the significantly increasing trend of EEG and TG concentrations was shown as the soil of 1–10 cm > 10–20 cm ≈ 20–30 cm > 30–40 cm layer (Fig. 2).

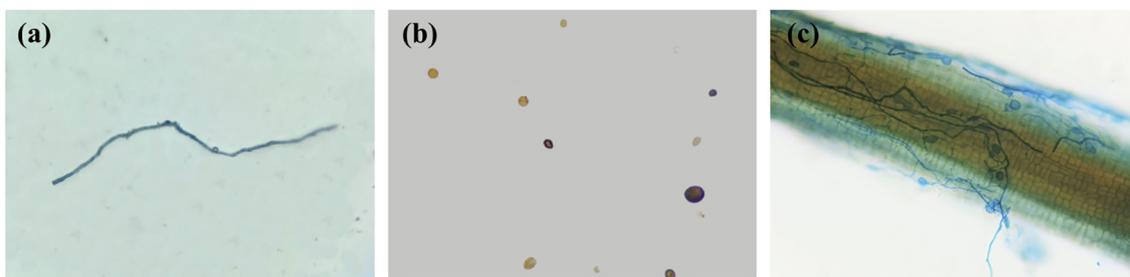


Fig. 1. Soil mycorrhizal hyphae (a), indigenous spores (b), and root mycorrhizal fungal colonization (c) of the yellow-fleshed peach variety 'Golden Honey 3'.

Table 1  
Changes in mycorrhizal status and root sugar contents in the yellow-fleshed peach variety 'Golden Honey 3'.

Soil depth (cm)	Mycorrhizal status			Root sugar (mg/g DW)		
	Root mycorrhizal colonization (%)	Soil hyphal length (cm/g soil)	Soil spore density (spores/g soil)	Sucrose	Fructose	Glucose
0–10	93.55 ± 8.75a	3.57 ± 0.31a	8.7 ± 2.0a	19.97 ± 3.97b	60.43 ± 4.35a	18.45 ± 0.48a
10–20	88.04 ± 13.36ab	2.93 ± 0.43ab	6.0 ± 0.8b	44.71 ± 13.79a	51.60 ± 3.61b	17.47 ± 0.57b
20–30	83.56 ± 16.41ab	1.63 ± 0.41c	2.5 ± 1.3c	43.70 ± 7.48a	53.09 ± 3.13b	16.17 ± 0.57c
30–40	79.50 ± 17.42b	2.77 ± 0.54b	2.2 ± 0.8c	46.32 ± 19.69a	41.46 ± 5.61c	14.82 ± 0.75d

The data followed by different letters mean significant differences at the 0.05 level.

Table 2  
Spatial distribution of soil physical–chemical properties in the yellow-fleshed peach variety 'Golden Honey 3'.

Soil depth (cm)	NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	SOM (g/kg)
0–10	72.18 ± 3.13a	73.83 ± 7.49a	39.99 ± 8.11c	311.70 ± 12.46a	11.67 ± 2.38a
10–20	68.02 ± 4.24a	61.84 ± 1.35b	40.94 ± 8.31bc	230.53 ± 6.78c	8.08 ± 0.50b
20–30	71.98 ± 2.32a	55.74 ± 2.81bc	50.77 ± 4.23ab	274.95 ± 5.58b	5.49 ± 0.68c
30–40	53.26 ± 6.65b	50.67 ± 3.08c	52.81 ± 3.59a	225.03 ± 7.32c	3.89 ± 0.88c

The data followed by different letters mean significant differences at the 0.05 level.

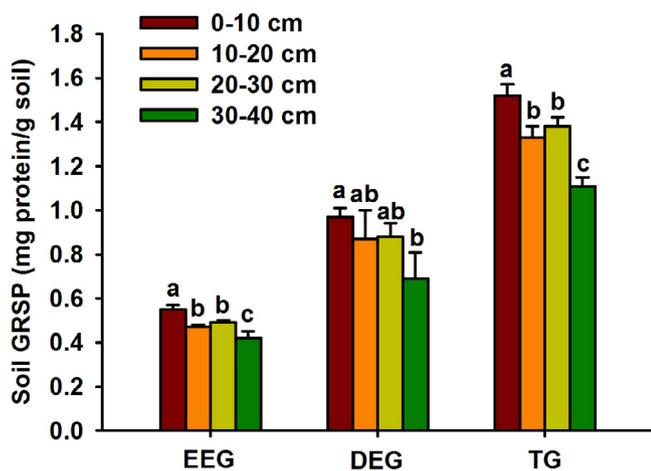


Fig. 2. Spatial distribution of soil glomalin-related soil protein concentrations in a yellow-fleshed peach variety 'Golden Honey 3'. Data with different letter at the bar indicated significant differences at the 0.05 level.

### 3.5. Correlation studies

There was not any significant correlation between root glucose, fructose and sucrose contents and root colonization and soil hyphae (Table 3). Root sucrose was negatively correlated with EEG, DEG, TG, and spore density, while root glucose and fructose showed positive correlations with EEG, DEG, TG, and spore density significantly. In addition, root colonization was positively correlated with NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, TG, and spore density.

was also positively correlated with NO<sub>3</sub><sup>-</sup>-N, available K, SOM, EEG, DEG, and TG, whereas it negatively correlated with available P. There was a positive correlation of soil hyphal length with NO<sub>3</sub><sup>-</sup>-N and SOM.

### 4. Discussion

The present study showed that the yellow-fleshed peach variety 'Golden Honey 3' could be colonized by indigenous AM fungi (2.2–8.7 spores/g soil and 1.63–3.57 cm hyphal length/g soil), reaching 79.50%–93.55% of root AM fungi colonization. Correlation studies also showed the positive correlation between soil hyphal length, soil spore number, and mycorrhizal colonization, implying that the three indicators of mycorrhizal development were interrelated with each other. Similar results were observed in *Citrus unshiu* grafted on *Poncirus trifoliata* conducted by Wu et al. (2006). Moreover, root colonization and spore number decreased as soil depth increased, possibly because surface soils accumulated more dead leaves and humus, better soil nutrients and gas phase conditions than sub-surface soils, which is suitable for the growth of aerophilic AM fungi (He and Hou, 2008; Xu et al., 2013). However, other studies showed spatial changes in mycorrhizal colonization and spore density (Wang et al., 2021). As a result, soil conditions, plant species, and indigenous AM fungal species all affect the mycorrhizal formation of host plants. Meanwhile, the SOM content required for AM fungal growth is relatively high in the 0–20 cm deep soil, hence AM fungi grow best in that soil layer. The peach rhizosphere had 2.2–8.7 spores/g soil, which was consistent with that observed by Guo and He (2013) in the rhizosphere (wind-sand soil) of *Caragana korshinskii* in a farming-pastoral zone, Inner

**Table 3**  
Correlation coefficient between mycorrhizal growth and soil properties.

	Root colonization	Soil hyphal length	Spore density	EEG	DEG	TG
Spore density	0.57*	0.68**	1.00	0.73**	0.57*	0.65**
NH <sub>4</sub> <sup>+</sup> -N	0.57*	0.07	0.46	0.75**	0.65**	0.71**
NO <sub>3</sub> <sup>-</sup> -N	0.51*	0.61*	0.83**	0.74**	0.58*	0.65**
Available P	-0.21	-0.39	-0.63**	-0.48	-0.61*	-0.59*
Available K	0.44	0.19	0.53*	0.88**	0.63**	0.73**
SOM	0.50	0.57*	0.87**	0.75**	0.61*	0.68**
Root sucrose	-0.18	-0.22	-0.53*	-0.68**	-0.60*	-0.65**
Root fructose	0.48	0.24	0.65**	0.81**	0.65**	0.72**
Root glucose	0.48	0.44	0.88**	0.76**	0.76**	0.79**
EEG	0.57*	0.24	0.73**	1.00	0.81**	0.90**
DEG	0.33	0.12	0.58*	0.81**	1.00	0.98**
TG	0.41	0.16	0.65**	0.90**	0.98**	1.00

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

Mongolia, but, significantly lower than agricultural soils. Such low spore density in the peach rhizosphere may be explained by the time of sampling, where December is the period of dormancy of AM fungal spores and their own low spore production (He et al., 2002; He and Hou, 2008).

Glomalin is a metal ion-containing glycoprotein isolated from spores and hyphae of AM fungi, which considerably improved the soil aggregate stability and increased the SOM pool (He et al., 2020; Agnihotri et al., 2021). The present study revealed the positive correlation of fungal spore density, but not soil hyphae, with soil EEG, DEG, and TG significantly, indicating that spores could be the essential source of soil GRSPs in mycorrhizosphere. This is in agreement with the results of Li et al. (2020) in extremely dry desert shrubs. In addition, soil EEG, but not DEG and TG, positively correlated with root mycorrhizal colonization, because the EEG is the active and functional component in soil GRSP fractions (Meng et al., 2020). On the other, all soil GRSPs were significantly correlated with SOM, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N. He et al. (2020) reported that purified EEG and TG contributed 8.67% and 23.26% of SOM. Meng et al. (2020) reported the contribution of purified EEG and TG to soil total N, accounted for 15.6% and 33.8%, respectively. Relatively higher correlation coefficient was observed between soil NH<sub>4</sub><sup>+</sup>-N and GRSP than between soil NO<sub>3</sub><sup>-</sup>-N and GRSP, because mycorrhizal fungi preferred to absorb soil NH<sub>4</sub><sup>+</sup>, rather than NO<sub>3</sub><sup>-</sup> (Bücking and Kafle, 2015).

Our studies also observed that the spatial distribution of AM fungi in peach was associated with soil properties, which was proved by previous studies (Li et al., 2020). Our study showed a positive correlation between mycorrhizal colonization and soil N content. Soil NO<sub>3</sub><sup>-</sup>-N was also significantly correlated with hyphae length and spore number in the soil. It appeared that mycorrhizal hyphae and spores in the soil were more affected by soil NO<sub>3</sub><sup>-</sup>-N than by NH<sub>4</sub><sup>+</sup>-N. Despite the mycorrhizal fungal preference for NH<sub>4</sub><sup>+</sup>, hyphal length, hyphal N transport, and arbuscule number were lower under NH<sub>4</sub><sup>+</sup> supplement than under NO<sub>3</sub><sup>-</sup> supplement (Hawkins and George, 2001). Further study is needed to decipher the relationship between mycorrhizal growth and soil various N levels.

SOM showed significant correlation with soil spores and hyphae length, indicating that SOM is an important regulator of soil mycorrhizal fungi growth. This study also displayed a negative correlation between soil spore number and soil available P, because high P levels in the soil reduced the permeability of root cell membrane and decreased the amount of root exudates, thus, inhibiting mycorrhizal growth (Dhillion and Zak, 1993). Significantly, our study also observed the positive correlation of root colonization with root fructose and glucose, but the negative correlation with root sucrose. The host's sucrose can be assigned to the mycorrhizal symbiotic interface for hydrolysis glucose and fructose, which would be absorbed by the fungi (Sun and Xu, 2009; Wu et al., 2015b, 2017).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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