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Growth performance and rhizospheric traits of peach (*Prunus persica*) in response to mycorrhization on replant versus non-replant soil

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ABSTRACT

Replant disease is considered as a major bottleneck towards improved production of stone fruits such as peach (*Prunus persica* L. Batsch). Studies were carried out to investigate the effects of inoculation with an arbuscular mycorrhizal fungus (AMF), *Funneliformis mosseae* on the plant and rhizosphere related parameters on replant versus non-replant soil. In replant soil with AMF treatment, root mycorrhizal colonization was reduced by 48.2% over non-replant soil. This was well translated through significant decrease in growth attributing characters like plant height, stem diameter, leaf number, and shoot and root dry weight on replant soil, but upon AMF inoculation, a complete reversal of these responses was observed, coupled with an increase in total chlorophyll concentration. Mycorrhizosphere quality indicators, viz. easily extractable glomalin-related soil protein, total glomalin-related soil protein and soil organic carbon concentration were significantly higher in mycorrhizosphere than non-mycorrhizosphere under both replant as well as non-replant soil. These glomalin-related soil proteins in combination with soil organic carbon cemented the water-stable aggregates at 2.00–4.00 and 1.00–2.00 mm size fractions, eventually leading to higher mean weight diameter in mycorrhizosphere. Profiling of soil enzyme activities showed higher catalase and peroxidase activity but lower polyphenol oxidase activity in mycorrhizosphere compared to non-mycorrhizosphere of replant soil. Our results suggested that inoculation with AMF negated the major ill effects of replant disease in peach.

Key words: Glomalin, Mycorrhizas, Peach, Replant disease, Water-stable aggregate

Replant disease as a complex syndrome is commonly observed when the same plant species is repeatedly grown on a same soil site (Pacholak *et al.* 2004). In stone fruit trees (i e *Prunus* spp.), replant disease frequently leads to poor plant growth, depleted plant resistance to abiotic as well as biotic stress, coupled with restricted root development (Tewoldemedhin *et al.* 2011). The causal factors for such replant disease are highly varied which comprise of changes in soil properties, environmental degradation, reduction in beneficial microbial communities, nutrient exhaustion, and the accumulation of some toxic substances (Pacholak *et al.* 2004, Benzri *et al*. 2005, Rutto and Mizutani 2006, Jiménez *et al.* 2011), in addition to elevated population of plant-parasitic nematodes, fungi, and *Phytophthora* spp., singly or collectively leading to such problem eventually cutting the productive life of orchard (Browne *et al.* 2006). Peach (*Prunus persica* L. Batsch) tree is one of important deciduous fruit trees grown worldover and usually faces replant obstacles on replant soils (Benzri *et al*. 2005, Bent *et al.* 2009, Manici and Caputo 2010). Under such conditions, sustaining the production cycle of previous years remains always questionable.

Arbuscular mycorrhizal fungi (AMF), one of the most prevalent beneficial fungi in the soil, form mutualistically beneficial association with most plant roots, in which extraradical hyphae penetrate through the contact regions of roots in soils, thereby, host plants are heavily benefitted by improving water and nutrient uptake from the fungal partner to the host partner (Wu *et al.* 2013). In addition, AMF also release a glycoprotein into the soil known as glomalin-related soil protein (GRSP), which improves soil aggregate stability (Bedini *et al.* 2009) by cementing the soil water-stable aggregates (WSA) (Bedini *et al.* 2010). Qi *et al.* (2002) earlier reported that inoculation with three AMF species, viz. *Funneliformis mosseae*, *G intraradices*, and *G. veriforme* significantly increased both plant growth and root vigor of ginkgo seedlings on replanted soils. Mehta and Bharat (2013) in their recent investigation showed that AMF inoculation markedly decreased the population of pernicious bacteria and actinomycetes in the rhizosphere of replanted apple plants, thus, reducing the menace of replant disease. Nogales *et al.* (2009) in another study revealed that inoculation with *G. intraradices* significantly enhanced effect on the growth of 140 Ruggeri vines after the first year in replanted vineyard, but such beneficial effect was ceased

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from third year onwards. These studies, by and large, concentrated on plant growth response and changes in rhizosphere microbial community. The current state of knowledge on response of AMF on soil aggregate stability and soil enzyme activities is highly missing with respect to peach crop suffering from replant disease.

In our study, we evaluated the response of AMF on plant biomass production, chlorophyll concentration, GRSP fractions, SOC, aggregate stability, and soil enzyme activities in the rhizosphere of peach seedlings grown on replant (RP) soil and non-replant (NRP) soil.

MATERIALS AND METHODS

A pot experiment was conducted through the 2×2 completely randomized factorial design, with a total of four treatments, each replicated four times. The first factor was inoculation with and without an AMF as *Funneliformis mosseae*. Another factor was replant treatments as plants grown on RP soil and NRP soil.

Six-leaf-old peach seedlings of uniform growth germinated in a sterilized sand for 4-months (November 2011 to March 2012), were transplanted in the plastic pots (20 cm upper diameter × 18 cm height × 15 cm bottom diameter), each pot carrying 2.7 kg soil. The RP soil used in the experiment was collected from the 0–20 cm depth of 16-year-old Yuhualu variety (*P. persica* cv. Yuhualu) of peach grafted on *P. persica* at Boksugol, Jingzhou, China. While NRP soil was collected from the another 16-year-old peach orchard of the same region, but without any apparent replant disease problem. Both RP and NRP soils were then sterilized through autoclaving (0.11 Mpa, 121°C, 2 hr) for the elimination of native AMF spores.

Spores of *Funneliformis mosseae* (Nicol. & Gerd.) Schüßler & Walker were isolated from the rhizosphere of *Incarvillea younghusbandii* in Dangxiong and propagated with white clover (*Trifolium repens*) as a host plant for 16-weeks at 22/18°C (day/night). A 25 g of inoculum carrying 23 spores/g was inoculated into the designed soil before transplanting. The AMF and non-AMF seedlings were grown in a polyacrylene greenhouse of Yangtze University campus from 28 March to 21 July, 2012 under growing conditions consisting of photosynthetic photon flux density of 768 mol/m^2/s, day/night temperature of 28/21°C, and relative air humidity of 85%.

After 116 days of the AMF inoculation, all the seedlings were harvested. Growth parameters such as plant height, stem diameter, and leaf number per plant were recorded before harvesting. Seedlings were separated into shoots and roots, and the dry weight of shoots and roots was measured after drying of 72 hr at 75°C. The soils from the pots after air-drying and sieving through 4 mm size mesh sieve were divided into two parts, one part was used for the analysis of soil enzyme activities, and the other part was utilized for analysis of WSAs and fraction concentrations.

The concentration of chlorophyll *a* and *b*, total chlorophyll, and carotenoids was measured using the method as described by Wu *et al.* (2012). Root mycorrhizas were stained with the protocol of Phillips and Hayman (1970) and were estimated by the formula as proposed by Wu *et al.* (2008). The easily extractable glomalin related soil protein (EE-GRSP) and total GRSP (T-GRSP) concentrations were determined as per the procedure described by Zou *et al.* (2014). While determination of SOC was undertaken following the protocol as suggested by Rowell (1994).

The 30 g of 4 mm sieved dry soil was used to determine the different WSA% in 0.25, 0.50, 1.00, and 2.00 mm size fractions which were analyzed following the method of Kemper and Rosenau (1986). The mean weight diameter (MWD, an indicator of aggregate stability) of 0.25-4.00 mm size aggregates was calculated using the formula:

$$\text{MWD}= \sum_{i=1}^{n} XiWi$$

where *Xi* is the diameter of the *i* sieve (mm), *Wi* is the proportion of the *i* size fraction of WSA in the total sample of WSA, and $n = 4$ (in this study) represents the number of WSA size fractions.

Determination of soil catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO) activities was carried out following the protocol as outlined by Yan (1988).

Data were analyzed by two-factor variance (ANOVA) in SAS software (8.1v). The significant differences between treatments were compared with the help of Duncan's Multiple Range Test at 0.05 level of significance.

RESULTS AND DISCUSSION

Root mycorrhizal colonization

Earlier studies (Gur and Cohen 1988) indicated that the RP peach rhizosphere accumulated lots of toxic substances and growth inhibiting substances, such as cyanogenic glucoside, tannic acid, and hydrogen cyanide, which could potentially inhibit spore germination and mycelial growth of *Gigaspora margarita* (Rutto and Mizutani 2005). As a result, the present study showed a significant decrease in root AM colonization on RP soil by 33%, as compared with NRP soil (Table 1).

Plant performance

The RP treatment significantly reduced the AMF colonization, plant height, stem diameter, leaf number and shoot and root dry weights than the NRP treatment (Table 1). However, compared with the non-AMF inoculated seedlings, the AMF seedlings exhibited marked improvements in various growth parameters, viz. plant height, stem diameter, leaf number, and shoot and root dry weights higher by 10, 17, 14, 23, and 21%, respectively, on the NRP soil and 12, 16, 20, 23, and 22% higher on the RP soil. No significant difference in response of various growth parameters was observed when non-AMF seedlings on NRP soil was compared with AMF seedlings on RP soil (Table 1). These results suggested some reversal mechanism by AMF against replant disease. Pro-inoculation with *Gigaspora margarita* was observed significantly effective in improving the growth of replanted

Table 1 Effect of arbuscular mycorrhizal fungus (AMF) *Funneliformis mosseae* on growth performance of peach seedlings grown on replant (RP) and non-replant (NRP) soils

Replant treatments	AMF treatments	Root AM colonization (%)	Plant height (cm)	Stem diameter (mm)	Leaf number	Shoot dry weight (g)	Root dry weight (g)
NRP	+AMF	80.3±6.8a	94.0±2.5a	5.50±0.10a	139±8a	15.78±0.62a	5.32±0.30a
	-AMF	0.0±0.0c	85.3±4.9b	4.70±0.54b	122±6b	12.86±1.29b	4.40±0.21c
RP	+AMF	54.2±6.9b	85.7±5.9b	4.69±0.37b	125±5b	13.61±0.41b	4.82±0.17b
	-AMF	0.0±0.0c	76.8±2.4c	4.05±0.05c	104±2c	11.06±0.60c	3.96±0.13d
Significance							
RP		**	**	**	**	**	**
AMF		**	**	**	**	**	**
RP×AMF		**	NS	NS	NS	NS	NS

Note: Data (means±SE, n=4) followed by different letters indicate significant differences between treatments at P<0.05 *; ** P<0.01; NS, non significant.

peach tree (Rutto and Mizutani 2006). In another study, inoculation with *G. fasciculatum* and *Glomus* spp. significantly increased plant height, stem diameter, leaf area, shoot/root fresh and dry weight in RP apple (Metha and Bharat 2013). These results in addition to our study suggested that AMF possessed the potential to enhance plant growth on even RP soil.

Chlorophyll concentrations

Concentration of all fractions of total chlorophyll, viz. chlorophyll *a*, chlorophyll *b*, and carotenoid was significantly reduced in non-AMF seedlings on RP soil compared to NRP soil, irrespective of AMF or non-AMF seedlings (Table 2). Compared to the non-AMF seedlings, the AMF seedlings recorded 26, 100 and 34% higher chlorophyll *a*, chlorophyll *b*, and total chlorophyll concentrations, respectively, on NRP soil. Under the RP conditions, however, the AMF produced no significant difference in concentration of various chlorophyll fractions. Qi *et al*. (2002) reported that inoculation with three AMF species, *F. mosseae*, *G. versiforme* and *G. intraradices*, notably increased total chlorophyll concentration of Ginkgo seedlings, irrespective of sterilized or non-sterilized RP soils. It seems that the AMF effect on chlorophyll concentration might be dependent on the synergy between AMF species and host plant species. It seems that AM function on chlorophyll concentration was inhibited by RP in peach seedlings (Rumberger *et al*. 2004).

Mycorrhizosphere quality

A significantly higher reduction in rhizospheric SOC, EE-GRSP and TGRSP was observed on RP soil than on NRP soil (Fig. 1a-c). Nevertheless, compared with the non-AMF seedlings, AMF inoculation significantly increased the rhizospheric SOC, EE-GRSP and T-GRSP concentrations by 180, 18, and 7%, respectively, on RP soil and 40, 9, and 6% on NRP soil.

Distribution of WSAs in 2.00–4.00, 1.00–2.00, 0.50–1.00, and 0.25–0.50 mm size fractions and the MWD were significantly reduced under RP soil, irrespective of AMF or non-AMF seedlings (Table 3). Compared with the non-AMF seedlings, the AMF seedlings recorded 61 and 117% higher WSA in the 2.00–4.00 and 1.00–2.00 mm size fractions and 77% higher MWD on NRP soil, corresponding to 29, 29, and 22% higher on RP soil. On the other hand, AMF inoculation significantly increased WSAs in the 0.50–1.00 and 0.25–0.50 mm size fractions by 110 and 33% on NRP soil. Nevertheless, under the RP soil conditions, WSAs in the 0.50–1.00 and 0.25–0.50 mm size fractions displayed no significant difference between AMF and non-AMF

Table 2 Effect of arbuscular mycorrhizal fungus (AMF) *Funneliformis mosseae* on chlorophyll concentration of peach seedlings grown on replant (RP) and non-replant (NRP) soils

Replant treatments	AMF treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Carotenoid (mg/g)	Total chlorophyll (mg/g)
NRP	+AMF	1.10±0.05a	0.26±0.08a	0.25±0.08a	1.35±0.09a
	-AMF	0.87±0.12b	0.13±0.03b	0.27±0.02a	1.01±0.06b
RP	+AMF	0.70±0.05c	0.15±0.01b	0.07±0.01b	0.85±0.06c
	-AMF	0.64±0.05c	0.14±0.03b	0.02±0.01b	0.78±0.08c
Significance					
RP		**	NS	**	**
AMF		**	*	NS	**
RP×AMF		NS	NS	NS	*

Note: Data (means±SE, n=4) followed by different letters indicate significant differences between treatments at P<0.05 *; ** P<0.01; NS, non significant.

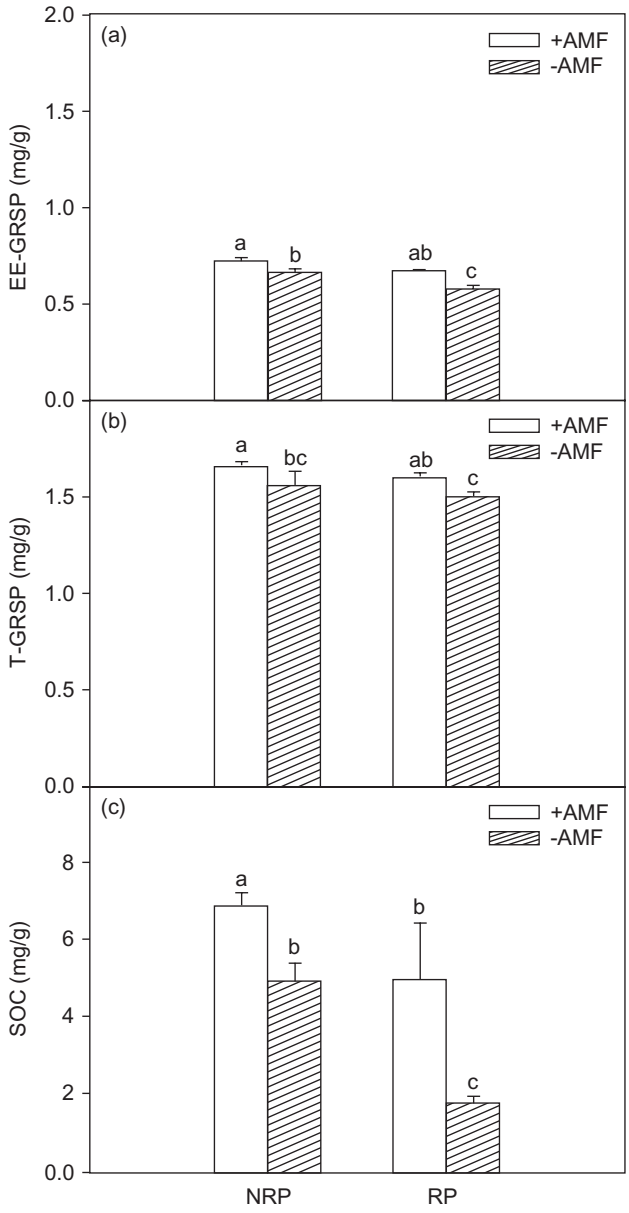

Fig 1 Effect of arbuscular mycorrhizal fungus (AMF) *Funneliformis mosseae* on mycorrhizosphere-based quality parameters of peach seedlings grown on replant (RP) and non-replant (NRP) soils. Data (means±SE, *n*=4) followed by different letters above the bars indicate significant differences between treatments at $P<0.05$.

seedlings. Contribution of higher GRSP and SOC concentrations in mycorrhizosphere of peach seedlings (Fig 1) helped in gluing and stabilizing WSAs (Rillig and Mummey 2006; Wang *et al.* 2014).The decrease of mycorrhizal colonization under the RP conditions (Table 1) reduced the production of GRSPs and soil hyphal length, resulting in consequent reduction in AM functioning on stabilizing WSAs on RP soil.

Soil enzyme activities

Soil CAT and POD activity was significantly lower on RP than NRP soil in AMF and non-AMF seedlings (Fig 2a-b). Whereas the soil PPO activity was significantly higher on RP than NRP soil in non-AMF seedlings (Fig 2c). The AMF inoculation significantly increased the rhizosphere soil CAT and POD activity by 7 and 49%, respectively, on RP soil with 8 and 41% on NRP soil. Nevertheless, the AMF inoculation significantly decreased soil PPO activity by 70% only on RP soil.

Soil enzyme activities generally reflect the change of soil fertility, and also express soil biological activities (Allison *et al.* 2007). CAT activity implies soil detoxification, and has a close relation to soil microbial abundance and plant roots (Liu *et al.* 2008). POD eliminates the soil allelochemicals and is involved the synthesis of soil humus (Kong 2007, Wang *et al.* 2010). PPO activity has a negative relationship with the transformation of aromatic organic compounds in humus composition, and can oxidize phenol, amine, and some heterocyclic compounds in soil (Wang *et al.* 2010). Compared to the NRP soil, the soil CAT and POD activities were significantly reduced on RP soil, but the PPO activity was increased only on non-AMF treated conditions (Fig 2). Earlier studies (Zhang *et al.* 2008) revealed that RP in fruit trees could decrease both soil organic matter and beneficial microbial activities, thus resulting in reduced soil enzyme activities. Our results also showed that AMF inoculation significantly increased rhizospheric CAT and POD activities on RP or NRP soil and decreased activity of PPO under the RP conditions (Fig 2), suggesting that AMF would aid in promoting the proliferation of beneficial microbial communities within the RP rhizosphere to alleviate RP disease.

Table 3 Effect of arbuscular mycorrhizal fungus (AMF) *Funneliformis mosseae* on the distribution of WSAs and MWD values in rhizosphere of peach seedlings grown on replant (RP) and non-replant (NRP) soils

Replant treatments	AMF treatments	Percentage of WSAs (%)				MWD (mm)
		2.00–4.00 mm	1.00–2.00 mm	0.50–1.00 mm	0.25–0.50 mm	
NRP	+AMF	10.8±0.4a	11.7±0.4a	14.3±0.7a	10.8±0.7a	0.645±0.007a
	-AMF	6.7±0.4b	5.4±0.6c	6.8±0.6b	8.1±0.5b	0.364±0.003b
RP	+AMF	6.2±0.4b	8.0±0.4b	5.1±0.7c	3.7±0.2c	0.358±0.006b
	-AMF	4.8±0.5c	6.2±0.8c	5.1±0.8c	4.4±0.6c	0.293±0.023c
Significance						
RP		**	**	**	**	**
AMF		**	**	**	*	**
RP×AMF		**	**	**	**	**

Note: Data (means±SE, *n*=4) followed by different letters indicate significant differences between treatments at $P<0.05$ *; ** $P<0.01$; NS, non significant.

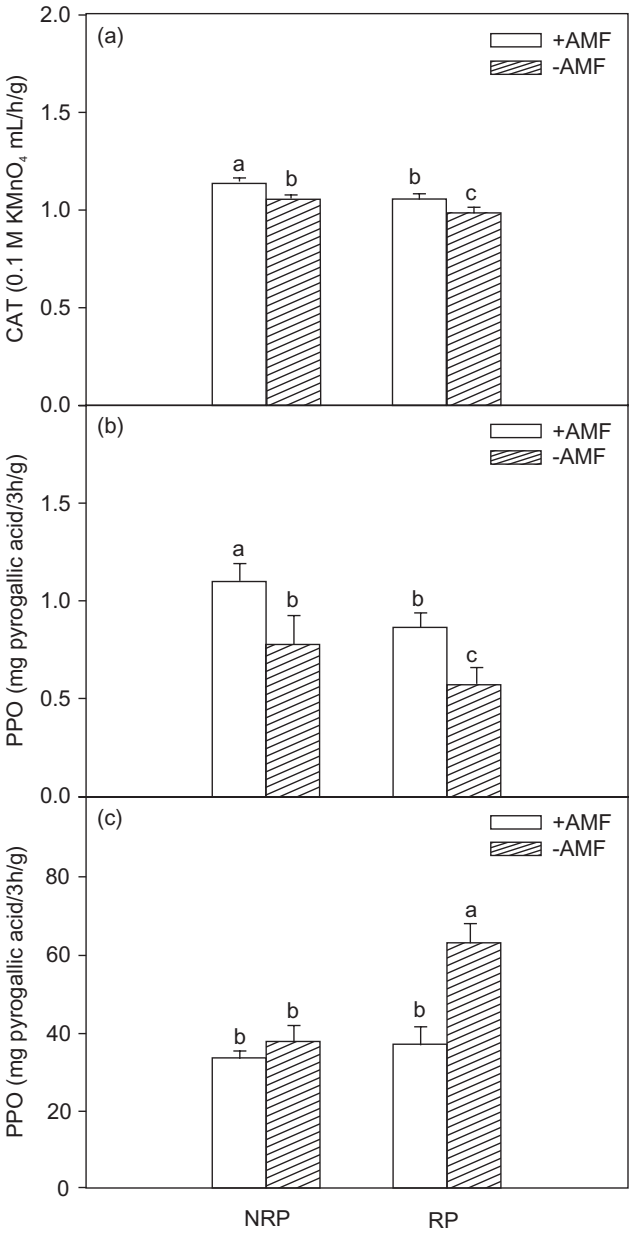

Fig 2 Effect of arbuscular mycorrhizal fungus (AMF) *Funneliformis mosseae* on soil enzyme activities in rhizosphere of peach seedlings grown on replant (RP) and non-replant (NRP) soils. Data (means±SE, n=4) followed by different letters above the bars indicate significant differences between treatments at P<0.05.

CONCLUSION

The RP treatment considerably inhibited plant growth and rhizospheric trait performance of peach seedlings, as compared with the NRP treatment. After inoculated with an AMF, *F. mosseae*, the growth performance (including morphological traits but not chlorophyll concentration) and rhizospheric WSA% at the size of 2.00–4.00 and 1.00–2.00 mm, GRSP and SOC concentrations, and CAT and POD activity were markedly enhanced in the peach seedlings, though a notable decrease of root AMF colonization was found after the RP treatment. Our results suggested that inoculation with AMF negated the major ill effects of replant disease in peach.

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