Journal of the American Pomological Society 71(4): 226-235 2017

Effects of Arbuscular Mycorrhizal Fungi and Phosphate-Solubilizing Fungus on the Rooting, Growth and Rhizosphere Niche of Beach Plum *(Prunus maritima)* Cuttings in a Phosphorus-deficient Soil

Xue M. Zai[1*#], Huan S. Zhang[2*#] and Zhen P. Hao[1]

Additional index words: *Funneliformis mosseae, Apophyzomyces spartima,* beach plum, rooting, rhizosphere niche, P-deficient soil

Abstract

The effects of an arbuscular mycorrhizal fungus (AMF), *Funneliformis mosseae*, and a phosphate-solubilizing fungus (PSF), *Apophysomyces spartima*, and their interactions, on rooting, cutting biomass, phosphorus (P) concentrations, rhizosphere microorganisms, phosphate concentrations, phosphatase activities and pH of beach plum (*Prunus maritima*) hardwood cuttings in a low phosphorus soil were evaluated. AMF colonization was also assessed. AMF, PSF, or both inoculations strongly promoted rooting, P concentrations, root and shoot dry weight (DW), numbers of bacteria and actinomycetes on the rhizoplane and in roots, numbers of nitrogen-fixing bacteria on the rhizoplane, available phosphate concentrations, and acid phosphatase enzyme activities in the rhizosphere soil of beach plum cuttings. Dual inoculation with AMF and PSF also resulted in significantly higher root colonization, rooting percentage, number of lateral fine roots, DW of roots and shoots, number of bacteria and actinomycetes on the rhizoplane and in roots, number of nitrogen-fixing bacteria on the rhizoplane, available phosphate concentrations, acid phosphatase activities, and the lowest pH values than following a single inoculation with AMF or PSF. Compared to FM + AS autoclaved treatment , AMF, PSF, or both inoculations had no influence on numbers of bacteria and actinomycetes in the rhizosphere, nitrogen-fixing bacteria in the rhizosphere and roots, fungi in the rhizosphere, roots and on the rhizoplane of beach plum cuttings. These results showed that AMF inoculation, plus the application of PSF, synergistically improved soil microenvironment and increased P availability in the rhizosphere of beach plum, which improved the rooting and growth of beach plum cuttings in a low phosphorus soil.

After nitrogen (N), phosphorus (P) is the major nutrient limiting plant growth despite being abundant in soils in both inorganic and organic forms. To increase the availability of P for plants, large amounts of fertilizer are used on a regular basis. But after application, a large proportion of fertilizer phosphorus is quickly transferred to the insoluble form. Therefore, very little of the applied P is used by plants, making continuous application necessary. Bioremediation using target plant species associated with a managed community of soil microorganisms has attracted increasing attention (Barea et al., 1996; Osorio and Habte, 2013; Zhang et al., 2011, 2014a). The arbuscular mycorrhizal (AM) fungi are relevant members of the rhizosphere mutualistic microsymbiont populations, which are known to serve many critical ecosystem functions, including the improvement of plant establishment by producing plant hormones (such as cytokinins, cell auxin, vitamin B and indole acetic acid), enhancement of soil enzyme

[1] Horticulture Dept., Jinling Institute of Technology, 130 Village Centers Qixia District, Naning 210038, PR China
[2] Nanjing Institute for Comprehensive Utilization of Wild Plants, Nanjing 210052, PR China
[*] Corresponding author: zaixueming680825@163.com
[#] These authors contributed equally to this study

activities, and plant nutrient uptake (Barea and Azcon-Aguilar, 1982; Liu et al., 2000; Zhang et al., 2011). AM fungi influenced the population of microorganisms in the rhizosphere by improving photosynthesis and mineral nutrition (Mechri et al., 2014), changed the physicochemical and biological properties of the soil, and enlarged the C pool of soil by increasing available P, hydrolyzable N, organic matter content, and several enzyme activities in soil (Zhang et al., 2014a, 2014c).

Inoculation of plants with P-solubilizing microorganisms (PSM) is also a promising technique because it can increase P availability in soils fertilized with rock phosphates (Vassilev et al., 2012). Higher concentrations of phosphate-solubilizing bacteria (PSB) are commonly present in the rhizosphere compared to non-rhizosphere soil (Youssef and Eissa, 2014). Besides providing phosphorus in soluble form to the plants, PSB also augments plant growth by stimulating the efficiency of biological nitrogen fixation (BNF) by nitrogen-fixing microorganisms (Mohammadi and Sohrabi, 2012). P-solubilizing fungi (PSF) have been reported to possess greater ability to solubilize insoluble phosphate than PSB (Whitelaw, 1999; Vassilev et al., 2012; Zhang et al., 2014a). The released P cannot be transferred to the roots by the PSM, but may be taken-up by the external mycelium of the AMF (Salvioli et al., 2012). AMF and PSM could interact positively in promoting P uptake of green gram plants (Zaidi and Khan, 2006), and leucaena seedlings (Osorio and Habte, 2013), leading to improved yield When the soil was P-deficient. Combined AMF and PSM inoculation could also alleviate the deleterious effects of abiotic stresses on plant growth by enabling greater nutrient (e.g., P, N and K) absorption, higher ionic accumulation in different root tissues, and maintenance of lower root Na^+/K^+ than inoculation with either microorganism alone (Zhang et al., 2014a).

Beach plum (*Prunus maritima*) is an extremely salt tolerant, drought-resistant, multi-stemmed, deciduous shrub which grows wild on the eastern US coastline. Beach plum is popular for its white flowers in spring, lasting until late Autumn, as well as having edible fruit (Uva and Whitlow, 2003). Due to its strong adaptation to arid soils and its potential as an economic plant, beach plum was first introduced into China by Nanjing University in 2001. However, a shortage of propagules is now a serious problem that limits widespread cultivation in China. In addition to tissue culture methods, we are trying to obtain a large number of propagules through cuttings. Unfortunately, earlier studies revealed that conventional methods of cutting propagation often resulted in poor rooting, a prolonged nursery phase, poor growth, and low survival of rooted cuttings after transplanting. Roots of beach plum can form symbiotic associations with AMF and inoculation with AMF, such as *F. mosseae*, may improve the rooting and growth of beach plum cuttings (Zai et al., 2007). No information is available on the effects of AMF and PSF on the growth responses of beach plum cuttings in low phosphorus soil. This study was performed to determine the effects of an AMF (*F. mosseae*), and a PSF (*A. spartima*), alone and in combination on the rooting and the rhizosphere niche of beach plum hardwood cuttings in a P-deficient soil.

Materials and Methods

Fungal inocula. The mycorrhizal fungus used was *F. mosseae*, in the form of sandy soils containing AMF spores, hyphae, and colonized maize root fragments. The original inoculum (BGCJX01), which was separated from *Osmanthus fragrans* tree rhizosphere in Jiangxi Province of China, was provided by professor Y. S. Wang (Institute of Plant Nutrition and Fertilisers, Chinese Academy of Agriculture), and was propagated on maize plants growing in sandy soil for 10 weeks. The *A. spartima* was isolated from the topsoil (0~10 cm) samples of a *Spartina alterniflora* community in North Jiangsu province. It had

been previously identified as a phosphate-solubilizing fungus that could significantly enhance available P concentrations (Zhang et al., 2014b). The inoculum of *A. spartima* was prepared using the method of Zhang et al. (2011). To prepare liquid inoculum of *A. spartima*, the first step was to activate strains on slants. The fungus was inoculated on solid Martin culture medium (di-potassium hydrogenphosphate 1g, magnessium sulfate 0.5 g, sodium chloride 11.5 g, peptone 5 g, glucose10 g, gelose10 g, 1/30,000 bengal red water solution 100 ml, and demineralized water 900 ml), which had been autoclaved for 30 min at 121°C and then incubated in the dark at 28°C for 4 d. After activation, 3 ml sterile water was added to test tube, and the mixture was poured into 50 ml Martin broth (MB) which was added to 1.15% NaCl; *A. spartima* was grown on a rotating shaker at 180 rpm for 48 h, and this was the starter culture. It was added (5% of volume) to MB and then we added 1.15% NaCl, and the MB was cultured on a shaker for 96 h at 180 rpm. At the end it contained 2.3×10^5 colony forming units per ml and the solution was stored at 4°C until use.

Preparation of cuttings and low phosphorus substrate. Twelve hundred hardwood cuttings of beach plum of uniform size, (15 cm-long; 0.8 cm in diameter), each with two buds, were collected from the beach garden of Fu Jiabian (Nanjing, P.R. China) in January 2012. All cuttings were dipped in 1% (w/w) captan (Red Sun Group, Nanjing, P.R. China) for 10 min as a preventative measure against mildew. The P-deficient substrate used was a 1:1 (v/v) mixture of quartz sand and nutrient soil (a commercial soil purchased from the Red Sun Group, Nanjing, P. R. China), which had been sterilized by autoclaving twice for 1 h at 121ºC. The nutrient soil had the following characteristics: pH, 7.05; EC, 0.71 dS per m; organic matter, 13.5 $g\cdot kg^{-1}$; hydrolyzable N, 48.0 $mg\cdot kg^{-1}$; available P, 14.7 $mg\cdot kg^{-1}$; available K, 14.8 $g\cdot kg^{-1}$.

Experimental method. There were four treatments, each replicated ten -times. Each treatment consisted of 30 pots with one cutting per pot (30 cm × 25 cm × 20 cm), for a total of 1200 pots. The four treatments were: inoculated with *F. mosseae* (FM 10 g, 952 spores per g inoculum); inoculated with *A. spartima* (AS, 10 ml); inoculated with 10 g *F. mosseae* and 10 ml *A. spartima* (FM + AS); inoculated with 10 g sterilized *F. mosseae* plus 10 ml sterilized *A. spartima* (FM + AS autoclaved). The sterilized inocula in FM + AS autoclaved treatment had been autoclaved at 121 °C for 90 min three times. Inocula were placed in the above substrate below the beach plum cutting prior to planting. Each pot was placed on a 2-cm-deep plate in a greenhouse under controlled conditions (16 h photoperiod at a photosynthetic photon flux of 220 $\mu mol\cdot m^{-2}\cdot s^{-1}$ of photosynthetically active radiation at 28 °C, and 8 h of darkness at 18 °C, with relative humidity kept at 65 - 85 %). Cuttings were sprayed with 20 l per day of half strength Hoagland's solution (Hoagland and Arnon, 1950) for 30 d, then with 20 l per day of full-strength Hoagland's solution for 60 d.

Soil samples collection. After 90 d, three plants were removed randomly from pots according to Riley and Barber (1969, 1970), along with their roots. Any soil which could be shaken-off gently was collected, placed in sterilized Petri dishes and labelled "bulk soil". Soil that adhered loosely within 1 - 4 mm of the root surface was labeled "rhizosphere soil". The soil closest to the root surface (approx. 0 - 2 mm) was removed with a sterilized brush and termed "rhizoplane soil". "Rhizosphere soil", "rhizoplane soil", and "roots" were later referred to as the three rhizosphere niches. The cleaned plant roots were then washed three- or four-times with 3 l sterile distilled water and were cut into 3-cm-long pieces using heat-sterilized and cooled scissors. The root pieces from each plant were placed on sterile filter paper in a sterile 90-mm Petri dish. From these, 1.0 g root samples were used to analyze any associated microorganisms.

Measurements. Ninety d after planting the cuttings, rooting percentage values were recorded. Plant from each pot was selected at random and the number of lateral fine roots, the length of the longest primary root, the DW of roots and shoots were recorded. P concentration of the beach plum plant tissues were determined by an ammonium molybdate blue method (Jones et al., 1991). The percentage of root colonization was determined after clearing the roots with 10% (w/v) KOH and staining with trypan blue (Phillips and Hayman, 1970).

The number of microorganisms in the above three rhizosphere soil niches was determined by the dilution plate method (Li et al., 2008). A modified potato dextrose agar medium (Li et al., 2008) was used to culture the fungi, and beef extract peptone medium (Li et al., 2008) was used for the bacteria. Gause's synthetic agar medium (Li et al., 2008) was used for actinomyces, and rhizobium agar medium (Li et al., 2008) was used for nitrogen-fixing bacteria. All media were sterilized at 121 ºC for 30 min, and the culture dishes were inverted. After inoculation, the plates for actinomyces were cultured upside-down at 25 ºC in an incubator. Bacterial numbers were calculated after 5 d in culture. Plates for bacteria or nitrogen-fixing bacteria were cultured upside-down in a 30 ºC incubator and numbers were counted after 30 h.

The available P concentrations in soil were determined using sodium bicarbonate-extractable P colorimetric method (Olsen et al., 1954). Available P was extracted from the soil with 0.5 M $NaHCO_3$ at a nearly constant pH of 8.5 for 30 min, and the intensity of the color of the supernatant was determined at 660 nm (Beckman DU20, Fullerton, CA, USA). Acid phosphatase activity was determined according to the improved method of Hoffman (Zhou, 1987). Briefly, 5 g of soil was incubated in 10 ml of 0.2 M borate buffer (pH 5.0) and 5 ml of 20 mM buffered disodium phenylphosphate solution at 37 ºC for 12 h. The intensity of the color of the supernatant was determined at 570 nm (Beckman DU20, Fullerton, CA, USA), and the activity of acid phosphatase expressed as a phenol number of milligrams per gram of soil. The urease activity was determined using urea as substrate, and the soil mixture was incubated at 37 ºC for 5 h, and the amount of ammonium released over 24 h was assayed colorimetrically at 578 nm (Beckman DU20, Fullerton, CA, USA), and expressed as mg ammonium per gram of soil (Zhou, 1987). The protease activity was determined by indophenol colourimetry and expressed in mg NH_2-N released per g soil per 24 h (Zhou, 1987). Briefly, 1 ml of the reaction mixture, containing 20 µg protease extract, 3 mg azocasein, and 0.1 M Tris-HCl (pH 8), was incubated for 1 h at 30 °C. The reaction was stopped with 2 ml ice-cold 7 % perchloric acid. The protein was allowed to precipitate for 15 min at room temperature and was then centrifuged at 4000 g for 10 min at room temperature. The intensity of the color of the supernatant was determined at 440 nm (Beckman DU20, Fullerton, CA, USA) after the addition of 0.3 ml of 10 N NaOH. The concentration of hydrolysable-N in each soil sample was calculated by extraction of NO_3-N with 0.01 M $CuSO_4$, according to the manual for colorimetric determination (Kalra and Maynard, 1991). 100 ml extraction of NO_3-N were placed in a cylinder with a stopper and add 1 ml alkaline mixture and shaken. It was the supernatant 10 ml, was added 2 ml Seignette salt and 2 ml Nessler reagent was shaken and left to stand l0 min later which was centrifuged at 3500 rpm for 10 min then read color intensity at 425 nm (Beckman DU20, Fullerton, CA, USA)

Twenty grams of dry soil from each sample were diluted with deionized water (1:5 soil–water, w/v), and pH was measured by pH meter (PHS-P).

Statistical analysis. The design was completely randomized, and all data were statistically analyzed by analysis of variance (ANOVA) using SAS's General

Table 1. Effects of inoculation with arbuscular mycorrhizal fungi (FM) and P-solubilizing fungus (AS) on the rooting, growth, P concentration, and percentage root colonization of beach plum cuttings in a P-deficient soil.

Treatment [y]	Percentage root colonization (%)	rooting percentage (%)	Length of the longest primary root (cm)	Number of lateral roots	Root dry weight (g/plant)	Shoot dry weight (g/plant)	P concentration (mg/kg)
FM+AS autoclaved	0 c [x]	31.4 d	12.2 c	9.3 c	0.12 c	1.78 d	0.61 c
FM	59.3 b	41.3 b	17.6 b	16.8 b	0.21 b	3.10 c	0.72 b
AS	0 c	33.9 c	16.8 b	9.9 c	0.23 b	3.42 b	0.73 b
FM+ AS	70.7 a	48.2 a	19.9 a	21.7 a	0.32 a	4.11 a	0.84 a
Significant (P>F)	<0.001	0.002	<0.001	0.001	0.000	<0.001	0.042

[x] Mean values in each column followed by the same lower-case letter were not significantly different by Duncan's multiple tests at $P = 0.05$.

[y] FM + AS autoclaved, inoculated with 10 g sterilized *F. mosseae* and 10 ml sterilized *A. spartima;* FM, inoculated with 10 g *F. mosseae;* AS, inoculated with *A. spartima* 10 ml; FM + AS, inoculated with *F. mosseae* 10 g and *A. spartima* 10 ml.

Linear Model (GLM) Procedure (Version 9.0, SAS Institute, 2002) (Littell et al. 2007). For multiple comparisons of means, Duncan's multiple tests was employed and the significance levels were chosen to be P = 0.05.

Results and Discussion

Rooting and growth. Because the inocula in the FM + AS autoclaved treatment had been sterilized, the treatment was considered to be the control treatment. Inoculation with FM, AS or their combination improved rooting, P content and growth of beach plum cuttings compared to FM + AS autoclaved treatment (Table 1). Among the three different inocula tested, cuttings inoculated with FM + AS had the greatest rooting percentage, number of fine lateral roots, P concentration, root DW, and shoot DW. Cuttings inoculated with FM + AS had greater root colonization than those inoculated only with FM ($P < 0.05$).

Co-inoculation with AMF and PSF had a synergistic effect on the increase in plant DW. Similar effects have been found in *Leucaena leucocephala* (Osorio and Habte, 2001) and *Kostelelzkya virginica* (Zhang et al., 2011, 2014a, 2014b). It has been reported that AMF *G. versiforme* possesses inorganic phosphate transporters on its hyphae which help in the direct absorption of phosphate from soil (Salvioli et al., 2012). The contribution of the PSF to growth promotion was probably due to increasing soil P pool available for AM fungal extraradical hyphae to pass on to the plant (Khan et al., 2007). *A. spartima* (AS) was isolated from the top 0 - 10 cm of soil from a community of *Spartina alterniflora* in North Jiangsu Province, and which had been proved to be a kind of efficient phosphate-solubilizing fungus (Zhang et al., 2014b). The effectiveness of PSF in increasing soil solution P by dissolving rock phosphate and desorbing sorbed P may play an important role in P-deficient soils (Osorio and Habte, 2013). This positive effect of AS inoculation on soil P pool available ($P < 0.001$, Table 3) improved mycorrhizal responses of FM-inoculated beach plum plants, and increased P concentration in beach plum cuttings ($P < 0.05$, Table 1).

Many studies support the idea that AMF

can modify root morphology through their effects on phytohormone levels (Niemi et al., 2002; Scagel, 2004). Kaldorf and Ludwig-Müller (2000) inoculated maize plants with *G. intraradices,* and showed that the levels of both free and bound indole-3-butyric acid (IBA) were increased at different stages of colonization, accompanied by increased percentages of fine lateral roots. Zhang et al. (2014a) reported that after *Kostelelzkya virginica* had been inoculated with a phosphate-solubilizing fungus, *Mortierella* sp., or combined inoculation both with *Mortierella* sp. and *F. mosseae*, root dry weight and root/shoot dry weight ratio increased significantly. Inoculations with FM, AS, or both may cause higher levels of endogenous hormones in the cuttings, and that the levels of endogenous hormones in the cuttings co-inoculated with FM and AS were higher than in cuttings inoculated with FM and AS alone. The hormones modified root morphology, formed more lateral fine roots, and enhanced plant growth through increased nutrient and water uptake (Zai et al., 2007; Wu et al., 2011). Certainly, the hypothesis by which inoculations with FM, AS, or both could increase endogenous hormone levels in plants also needs further study.

Counts of microorganisms. Among the niches of rhizosphere soil, rhizoplane soil and roots, the rhizoplane had the highest number of bacteria and actinomycetes (Table 2). Compared to FM + AS autoclaved treatment, cuttings in other treatments had greater numbers of bacteria and actiomycetes on the rhizoplane and in roots and greater numbers of nitrogen-fixing bacteria on the rhizoplane ($P < 0.001$). The number of bacteria and actinomycetes on the rhizoplane and in the roots of cuttings in the FM + AS treatment was greater than in cuttings only inoculated with FM or AS alone ($P < 0.001$). The number of nitrogen-fixing bacteria on the rhizoplane of cuttings in the FM + AS treatment was greater than in cuttings only inoculated with FM or AS alone ($P < 0.001$).

The establishment of a plant-AMF symbiosis may alter the composition of root exudates which play key roles in qualitative

Table 2. Effects of inoculation with arbuscular mycorrhizal fungi (FM) and P-solubilizing fungus (AS) on numbers of microorganisms in rhizosphere of beach plum cuttings in a P-deficient soil.

Treatment [y]	Numbers of bacteria (10^6 CFU/g)			Numbers of fungi (10^4 CFU/g)			Numbers of actionmyces (10^5 CFU/g)			Numbers of nitrogen-fixing bacteria (10^6 CFU/g)		
	Rhizosphere	Rhizoplane	Roots	Rhizosphere	Rhizoplane	Roots	Rhizosphere	Rhizoplane	Roots	Rhizosphere	Rhizoplane	Roots
FM+AS autoclaved	44.9 [x]	97.3 d	67.1 c	6.87	7.47	1.79	1.69	1.93 d	1.62 d	7.05	10.22 d	2.85
FM	46.2	166.8 b	90.5 b	7.15	7.83	1.83	1.78	2.72 b	2.43 b	7.24	12.47 b	3.02
AS	45.3	145.2 c	86.3 b	6.94	7.66	1.78	1.75	2.26 c	1.97 c	7.16	11.32 c	2.88
FM+ AS	47.1	225.6 a	132.7 a	7.25	7.95	1.91	1.82	3.74 a	3.14 a	7.33	14.78 a	3.05
Significant (P>F)	0.671	<0.001	<0.001	0.098	0.068	0.052	0.145	<0.001	<0.001	0.065	<0.001	0.061

[x] Mean values in each column followed by the same lower-case letter were not significantly different by Duncan's multiple tests at $P = 0.05$.

[y] FM + AS autoclaved, inoculated with 10 g sterilized *F. mosseae* and 10 ml sterilized *A. spartima;* FM, inoculated with 10 g *F. mosseae;* AS, inoculated with *A. spartima* 10 ml; FM + AS, inoculated with *F. mosseae* 10 g and *A. spartima* 10 ml.

Table 2. Effects of inoculation with arbuscular mycorrhizal fungi (FM) and P-solubilizing fungus (AS) on available phosphate concentrations, phosphatase activities, pH values, hydrolysable-N concentrations, protease and urease activities in rhizosphere of beach plum cuttings in a P-deficient soil.

Treatment [y]	Available phosphate (mg/kg)	pH	Acid phosphatase (g phenol/g soil/ 24 h)	Hydrolysable-N (mg/kg)	Protease (mg NH_2-N/g/24 h)	Urease (mg NH_3-N/g/24 h)
FM+AS autoclaved	7.64 c [x]	7.05 a	0.201 d	55 c	0.713 d	0.603 d
FM	10.25 b	6.60 b	0.374 c	68 b	1.014 b	1.332 b
AS	9.30 b	6.42 b	0.417 b	64 b	0.881 c	0.927 c
FM+ AS	13.72 a	6.01 c	0.813 a	77 a	1.902 a	1.774 a
Significant (P>F)	<0.001	0.002	<0.001	0.012	<0.001	<0.001

[x] Mean values in each column followed by the same lower-case letter were not significantly different by Duncan's multiple tests at $P = 0.05$.

[y] FM + AS autoclaved, inoculated with 10 g sterilized *F. mosseae* and 10 ml sterilized *A. spartima;* FM, inoculated with 10 g *F. mosseae;* AS, inoculated with *A. spartima* 10 ml; FM + AS, inoculated with *F. mosseae* 10 g and *A. spartima* 10 ml.

and quantitative modifications in the microbial population in the mycorrhizosphere (Meyer and Linderman, 1986; (Sundareshwar et al., 2003; Manjula et al., 2005; Zhang et al., 2014c). The experiments conducted by Meyer and Linderman (1986) demonstrated that the rhizosphere and the rhizoplane of mycorrhizal and non-mycorrhizal maize seedlings had different bacterial communities. Pan et al. (2000) showed that after corn had been inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi, counts of bacteria, actinomyces, and nitrogen-fixing bacteria in the rhizosphere soil increased significantly, while the number of fungi was reduced slightly. Sundareshwar et al. (2003) reported that a bacterial community containing N-transforming or N-fixing bacteria in coastal wetlands was limited by P deficiency. Inoculations with AMF, PSF or both can enhance P uptake of plant, reactivate soil microbial community, and consequently improve soil quality (Zayed and Abdel-Motaal, 2005; Mohammadi and Sohrabi, 2012; Mechri et al., 2014; Aghababaei et al., 2014; Zhang et al., 2014c). A larger population of PSM was maintained in the presence of *G. fasciculatum*, and these effects may be attributed to higher metabolic activities of PSM for longer periods in the rhizosphere AM inoculation (Singh and Singh, 1993). AMF inoculation, plus the application of PSF, synergistically resulted in significantly higher number of bacteria and actinomycetes on the rhizoplane and in roots, and number of nitrogen-fixing bacteria on the rhizoplane than following a single inoculation with AMF or PSF ($P < 0.001$, Table 2).

Available phosphate concentrations, acid phosphatase activities, pH values, Hydrolysable-N concentrations, protease and urease activities in the rhizosphere soil.

Inoculations with FM, AS, or both increased available phosphate concentrations, acid phosphatase activities, hydrolysable-N concentrations, protease activities, and urease activities in the rhizosphere soil ($P < 0.05$, Table 3). Among the three different inocula tested, soils from the FM + AS treatment had the greatest available phosphate concentrations, acid phosphatase activities, hydrolysable-N concentrations, protease activities, and urease activities. Inoculations

with FM, AS, or both decreased pH values in rhizosphere soil ($P < 0.05$). Among the three different inocula tested, rhizosphere soil from cuttings inoculated with Fm + As had the lowest pH values.

When plants are under P stress, some plants have mechanisms to adapt actively by reducing the rhizosphere pH value to increase P absorption (Welsh, 2000). The release of organic acids, chelating substances (e.g., 2-ketogluconic acid), humic substances, mineral acids (sulphuric acid), siderophores, and proton extrusion by PSM have all been reported as important mechanisms for phosphate solubilization (AzcÓn and Barea, 1996). The released P cannot be transferred to the roots by the PSF, but may be taken-up by the external mycelium of the AMF. AMF and PSF's interaction might also promote absorption and utilization of P for plants to a certain extent (Gahooniat and Melsen, 1992).

Johansen et al. (1994) observed increased translocation of soil N to cucumber plants inoculated with G. intraradices when ^{15}N-labelled NH_4NO_3 was added to soil, indicating hyphal transport of inorganic N. P limitation of microbial growth also affects the transformation and availability of N (Sundareshwar et al., 2003). In the current study, the increase in hydrolysable-N concentrations in the rhizosphere soil of inoculated plants was probably due to an increase in the number of indigenous N-transforming or N-fixing bacteria promoted by the release of P by the FM, AS, or both inocula. Urease and protease activities promoted by microbe inoculation can also account for the increase in hydrolysable-N concentrations. Previous studies have shown that acid phosphatase is dominant in acid soils and that alkaline phosphatase is dominant in alkaline soils (Eizavi and Tabatabai, 1977). Acid phosphatase activity increased following soil inoculation with AMF and PSM, as reported by Kim et al. (1997). In the current study, FM, AS, or both inoculations acidified the rhizosphere soil. Only acid phosphatase activities were measured because all pH values in the rhizosphere were < 7.05 (Table 3). Osorio and Habte (2001) reported that acid production was the major mechanism in the solubilization of rock phosphate by PSF. The pH of the growth medium decreased as a result of acid production by PSF. Soil enzymes are believed to be primarily of microbial origin, but can also originate from plant root exudates and organic wastes (Nannipieri et al., 2002). Accordingly, the increases in enzyme activities in the current study were probably the result of enhancing the microbial populations in rhizosphere soil and root growth of beach plum plants inoculated with FM, AS, or both (Table 1, Table 2).

Conclusions

Inoculation with *Funneliformis mosseae*, *Apophysomyces spartima*, or both improved soil microenvironment and increased P availability in the rhizosphere of beach plum, and also promoted rooting and nutrient uptake of cuttings. Inoculation of AMF in combination with PSF synergistically improved the rooting and growth of beach plum hardwood cuttings in a P-deficient soil.

Acknowledgments

This research was supported by the Natural Science Foundation of Jiangsu Province (BK20151098, BK20141064), the School Fund Project in Jinling Institute of Technology (Project No. 40410491).

Literature Cited

Aghababaei, F., F. Raiesi, and A. Hosseinpur. 2014. The combined effects of earthworms and arbuscular mycorrhizal fungi on microbial biomass and enzyme activities in a calcareous soil spiked with cadmium. Appl. Soil Ecol. 75: 33-42.

AzcÓn, C. and J. M. Barea. 1996. Interactions of arbuscular mycorrhiza with rhizosphere microorganisms. In: Mycorrhiza: Biological Soil Resource. (Guerrero, E., Ed.). FEN, Bogota, Colombia, USA. pp 47-68.

Barea, J.M. and C. Azcon-Aguilar. 1982. Production of plant growth regulating substances by the vesicular

arbuscular mycorrhizal fungus – *Glomus moseae*. Appl. Environ. Microbiol. 43: 810-813

Barea, J.M., R.M. Tobar, and C. AzcÓn-Aguilar. 1996. Effect of a genetically modified *Rhizobium meliloti* inoculant on the development of arbuscular mycorrhizas, root morphology, nutrient uptake and biomass accumulation in *Medicago sativa*. New Phytol. 134(2): 361-369.

Eivazi, F. and M.A. Tabatabai. 1977. Phosphatases in soils. Soil Biol. Biochem. 9: 167-172.

Gahooniat, T.S. and N.E. Melsen. 1992. The root induced pH change on the deletion of inorganic and organic phosphorous in the rhizosphere. Plant Soil 143: 183-189.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347: 1-32

Johansen, A.I., I. Jakobsen, and E.S. Jensen. 1994. Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. Plant Soil 160: 1-9.

Jones, J.R., J.B. Wolf, and H.A. Mills. 1991. Plant analysis handbook. Micro Macro Publishing, Athens, CA, USA. pp 195-203.

Kaldorf, M. and J. Ludwig-Müller. 2000. AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. Physiol. Plantarum 109: 58-64.

Kalra,Y.P. and D.G. Maynard. 1991. Methods Manual for Forest Soil and Plant Analysis. Northern Forestry Centre, Edmonton, Northwest Region, Canada. pp. 116

Khan, M.S., A. Zaidi, and P.A. Wani. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture – a review. Agron. Sustain Dev. 27, 29-43.

Kim, K.Y., D. Jordan, and G.A. Mcdonald. 1997. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol. Fert. Soils 26: 79-87.

Littell, R.C., W.W. Stroup, and R.J. Freund. 2007. SAS® for Linear Models, 4th ed. Cary, N.C.

Liu, R.J., M. Li, and X.X. Meng. 2000. Effects of AM fungi on endogenous hormones in corn and cotton plants. Mycosystema 19(1): 91-96.

Li, Z.G., Y.M. Luo, and Y. Teng. 2008. Research methods of soil and environmental microorganisms. Science Press, Beijing, P. R. China. pp 64-83.

Manjula, G., E.P. Philip, and H.R. Jin. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435: 819-823.

Mechri, B., A. G. Manga, M. Tekaya, F. Attia, H. Cheheb, F. B. Meriem, and M. Hammami. 2014. Changes in microbial communities and carbohydrate profiles induced by the mycorrhizal fungus (*Glomus intraradices*) in rhizosphere of olive trees (*Olea europaea* L.). Appl. Soil Ecol. 75: 124-133.

Meyer, J.R. and R.G. Linderman. 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol. Biochem. 18(2): 191-196.

Mohammadi, K. and Y. Sohrabi. 2012. Bacterial biofertilizers for sustainable crop production: a review. ARPN J. Agric. Biol. Sci. 7(5): 307-16.

Niemi, K., H. Hdggman, and T. Sarjala. 2002. Effects of exogenous diamines on the interaction between ectomycorrhizal fungi and adventitious root formation in Scots pine in Vitro. Tree Physiol. 22 (6): 373-381.

Nannipieri, P., E. Kandeler, and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Enzymes in the Environment. (Burns, R.G. and Dick, R., Eds.). Marcel Dekker, New York, NY, USA. pp. 1-33.

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available-phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No. 939. US Government Printing Office, Washington DC, USA. pp 19-27.

Osorio, N.W. and M. Habte. 2001. Synergistic influence of an arbuscular mycorrhizal fungus and a P-solubilizing fungus on growth and P uptake of *Leucaena leucocephala* in an Oxisol. Arid Land Res. Manag. 15(3): 263-274.

Osorio, N.W. and M. Habte. 2013. Synergistic effect of a phosphate-solubilizing fungus and an arbuscular mycorrhizal fungus on leucaena seedlings in an Oxisol fertilized with rock phosphate. Botany 91(4): 274-281.

Pan, C.M., Q.R. Guo, and Q.J. Qiu. 2000. Effect of VAM fungus on the growth of core and micro-ecological environment of core rhizosphere. Soil Environ. Sci. 9(4): 304-306.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. British Mycol. Soc. 55(1): 158-IN18.

Riley, D. and S.A. Barber. 1969. Bicarbonate accumulation and pH changes at the soybean (*Glycine max* (L.) Merr.) root-soil interface. Soil Sci. Soc. Am. J. 33(6): 905-908.

Riley, D. and S.A. Barber. 1970. Salt accumulation at the soybean (*Glycine Max*.(L.) Merr.) root-soil interface. Soil Sci. Soc. Am. J. 34(1): 154-155.

Salvioli, A., I. Zouari, M. Chalot, And P. Bonfante. 2012. The arbuscular mycorrhizal status has an impact on

the transcriptome profile and amino acid composition of tomato fruit. BMC Plant Biol. 12(1): 1.

Scagel, C.F. 2004. Changes in cutting composition during early stages of adventitious rooting of miniature rose altered by inoculation with arbuscular mycorrhizal fungi. J. Am. Soc. Hortic. Sci. 129 (5): 624-634.

Singh, H.P. and T.A. Singh. 1993. The interaction of rock phosphate, Bradyrhizobium, vesicular-arbuscular mycorrhizae and phosphate-solubilizing microbes on soybean grown in a sub-Himalayan mollisol. Mycorrhiza 4: 37-43.

Sundareshwar, P.V., J.T. Morris, and E.K. Koepfler. 2003. Phosphorus limitation of coastal ecosystem processes. Science 299(5606): 563-565.

Uva, R.H. and T.H. Whitlow. 2003. Beach plum (*Prunus maritima* Marsh.): small farm sustainability through crop diversification and value added products. HortScience 38(5): 793.

Vassilev, N., B. Eichler-Löbermann, and M. Vassileva. 2012. Stress-tolerant P-solubilizing microorganisms. Appl. Microbiol. Biot. 95(4): 851-859.

Welsh, D.T. 2000. Nitrogen fixation in seagrass meadows: Regulation, plant-bacteria interactions and significance to primary productivity. Ecol. Lett. 3: 58-72.

Whitelaw, M.A. 1999. Growth promotion of plants inoculated with phosphate-solubilizing fungi. Adv. Agron. 69: 99-151.

Wu, Q.S., Y.N. Zou, and X.H. He. 2011. Arbuscular mycorrhizal fungi can alter some root characters and physiological status in trifoliate orange (*Poncirus trifoliata* L. Raf.) seedlings. Plant Growth Regul. 65(2): 273-278.

Youssef, M.M.A. and M.F.M. Eissa. 2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. E3 J. Biotechnol. Pharm. Res. 5(1): 1–6.

Zaidi, A. and M.S. Khan. 2006. Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on green gram-Bradyrhizobium symbiosis. Turk. J. Agric.For. 30(3): 223-230.

Zayed, G. and H. Abdel-Motaal. 2005. Bio-active composts from rice straw enriched with rock phosphate and their effect on the phosphorous nutrition and microbial community in rhizosphere of cowpea. Bioresource Technol. 96(8): 929-935.

Zai, X., P. Qin, S.W. Wan, F.G. Zhao, G. Wang, D.L. Yan, and J. Zhou. 2007. Effects of arbuscular mycorrhizal fungi on the rooting and growth of beach plum (*Prunus maritima*) cuttings. J. Hortic. Sci. Biotech. 82(6): 863-866.

Zhang, H.S., F.F. Qin, P. Qin, and S.M. Pan. 2014a. Evidence that arbuscular mycorrhizal and phosphate-solubilizing fungi alleviate NaCl stress in the halophyte *Kosteletzkya virginica*: nutrient uptake and ion distribution within root tissues. Mycorrhiza 24(5): 383-395.

Zhang, H.S., P. Qin, and W.M. Zhang. 2014b. Fungus and *Apophysomyces spartina* on P-uptake of Castor Oil Plant (*Ricinus communis* L.) and Rhizosphere Soil Enzyme Activities under Salt Stress. Agr. Sci. Tech. 15(4): 659.

Zhang, H.S., X.M. Zai, and X.H. Wu. 2014c. An ecological technology of coastal saline soil amelioration. Ecol. Eng. 67: 80-88.

Zhang, H.S., X.H. Wu, and G. Li. 2011. Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kostelelzkya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. Biol. Fert. Soils 47(5): 543-554.

Zhou, L.K. 1987. Soil enzymes. Beijing Science Press, Beijing, P. R. China. pp. 74-81.