

ROLES OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND NUTRIENT ACQUISITION OF PEACH (*PRUNUS PERSICA* L. BATSCH) SEEDLINGS

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) can form mutualistic symbioses with the roots of 80% of plants, which increase soil water and nutrient uptake for the host plants. However, little information is known on potential roles of different AMF for growth and nutrient acquisition of peach. In a potted experiment, we determined the growth performance, nutrient concentrations and mycorrhizal nutrient efficiency of the peach (*Prunus persica* L. Batsch) seedlings inoculated with *Glomus mosseae*, *G. versiforme*, and *Paraglomus occultum*, respectively. After 100 days of mycorrhizal inoculations, mycorrhizal colonization of one-year-old seedlings ranged from 23.4% to 54.9%. Generally, the formed mycorrhizal symbiosis significantly improved plant growth performance, such as plant height, stem diameter, shoot, root or total dry weight. Compared with the non-AMF seedlings, a range of 9.2–28.5% K of leaves, 86.0–120.9% K of roots, 118.3–417.3% Ca of leaves, 31.9–55.7% Mg of leaves, 76.6–140.4% Mg of roots, 101.3–169.6% Fe of leaves, 37.6–66.5% Fe of roots, 178.3–321.7% Cu of roots, 33.3–91.7% Mn of roots, 50.0–58.3% Zn of leaves, and 200.0–450.0% Zn of roots were respectively higher in the mycorrhizal seedlings. The mycorrhizal benefit role on nutrient uptake generally was the best in the *G. mosseae* treatment. It suggests that arbuscular mycorrhizas could improve growth performance and part nutrient acquisition of peach, which were absolutely dependent on AMF species.

Key words: arbuscular mycorrhiza; peach; mycorrhizal nutrient efficiency, nutrient uptake.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs, which can form mutualistic symbioses with the roots of around 80% of plant species (Giovannetti, 2008). AM symbiosis can establish extraradical mycelia, which disperse outside the roots to have access to a greater quantity of water and soil minerals for the host plants. In return, the symbiosis receives plant carbohydrates for the completion of its life cycle (Genre and Bonfante, 2010; Nasim, 2010). The mycorrhizal symbiont almost occurs in all fruit tree species grown in nursery or field (Calvet *et al.*, 2004).

It is well documented that AM symbiosis can increase plant growth and nutrient uptake, improve fruit quality and enhance several abiotic stresses such as low temperature stress, drought, salt stress, etc. (Azcon-Aguilar and Barea, 1997; Mena-Violante *et al.*, 2006; Miransari, 2010). Inducing better root colonization can enhance mycorrhizal beneficial roles on fruit trees. However, the beneficial roles depend on both fungal and fruit tree species. In a field, small differences in the ability to form arbuscular mycorrhizas (AMs) exist among ten grapevine rootstocks, but other factors, including crop load and soil moisture, have a large influence on root colonization (Schreiner, 2003). Bâ *et al.*

(2000) reported that five tropical fruit trees (*Adansonia digitata*, *Aphania senegalensis*, *Anacardium occidentale*, *Balanites aegyptiaca*, and *Sclerocarya birrea*) acquired benefits from AMF (*Glomus aggregatum* and *Glomus intraradices*) inoculation, while other six tropical fruit trees not. In apple (*Malus domestica*), *G. mosseae* singly and in combination with *G. macrocarpum* were more effective in increasing plant biomass than *G. macrocarpum* (Miller *et al.*, 1989). In eighteen *Prunus* rootstocks inoculated with three AMF, *G. etunicatum*, *G. intraradices*, and *G. mosseae*, only *G. intraradices* proved to be the most infective endophyte, achieving the highest root colonization in most of the rootstocks evaluated (Calvet *et al.*, 2004). A previous study by Wu *et al.* (2007) has revealed that five *Glomus* species differed in their ability to improve water relations of red tangerine (*Citrus tangerine*) seedlings. These studies taken together indicate that the beneficial roles of AMF lie on different AMF and plant species.

Peach, belonging to a species of *Prunus* of the subfamily Prunoideae of the family Rosaceae, is widely distributed and produced all around the world, including China. A small quantity of experiments have been conducted to evaluate the potential effects of AMF on peach. Inoculation with *G. fasciculatus* was equally or more effective in overcoming soil-fumigation nutrient-deficiency effects in peach nursery seedlings than the

standard nursery practice of side-dressing P and Zn at planting time (Larue *et al.*, 1975). Mycorrhizal symbiosis helped peach seedlings to overcome soil-fumigation nutrient-deficiency effects in nursery, uptake nutrients, and alleviate flooded stress, but not overcome peach replant problem (Rutto *et al.*, 2002; Rutto and Mizutani, 2006).

The objective of the present study was to evaluate the potential roles of three AMF species on peach seedlings, in terms of mycorrhizal colonization, plant growth and nutrient acquisition.

MATERIALS AND METHODS

The uniform peach (*Prunus persica* L. Batsch) seedlings with 9–10 leaves after sand stratification and germination in sand bed were selected and transferred into 16 plastic pots (18.5 cm upper mouth diameter × 9.5 cm bottom mouth diameter × 11.5 cm height) filled with 2.7 kg of autoclaved (121°C, 0.11 MPa, 1 h) soil/vermiculite/sphagnum (5:1:1, v/v/v) growth substrate, whose characteristics were pH 6.3, 9.8 g kg⁻¹ organic matter, and 17.71 mg kg⁻¹ available phosphorus.

At the time of transplanting, the designed mycorrhizal seedlings were inoculated with *Glomus mosseae*, *G. versiforme* and *Paraglomus occultum*, respectively. Inocula of the three AMF species consisted of 25 g of rhizosphere soils containing spores, external hyphae and mycorrhizal root fragments from *Sorghum vulgare*, which were commercially provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. The non-AMF control received an equal amount of autoclaved inoculum with a 2 ml aliquot of a filtrate of mycorrhizal inoculum to provide a general microbial population free of mycorrhizal propagules.

The experiment consisted of four treatments each replicated four times: *G. mosseae*, *G. versiforme*, *Paraglomus occultum* and non-AMF control. A total of 16 pots was used, and each pot cultivated one peach seedling. The mycorrhizal and non-mycorrhizal seedlings were arranged in a non-environmentally controlled plastic greenhouse from April 1 to July 10, 2010, where photo flux density is 576–869 μmol m⁻² s⁻¹, average day/night temperature 24.6/16.7°C, and relative humidity 70–95%, respectively.

The experiment ended after 100 days of mycorrhizal inoculations, and the growth performance including leaf number per plant, stem diameter, plant height, leaf, stem and root dry weights was recorded. For root mycorrhizal colonization, root samples were collected, stained with 0.05% trypan blue in lactic acid (Phillips and Hayman, 1970), and evaluated using the previous formula described by Wu *et al.* (2008).

About 0.1 g of dried materials was used to analyze their nutrient concentrations. The materials were

digested in 25 mL 1 mol L⁻¹ HCl for 2 h at 40°C. K, Mg, Ca, Fe, Cu, Mn, and Zn concentrations were determined directly using an Atomic Absorption Spectrometer (AI 1200, Aurora Instruments Limited, Canada). Mycorrhizal nutrient efficiency was introduced to describe the mycorrhizal effect on nutrient uptake. Quantification was performed using the following formula (Wu and Zou, 2009):

$$\text{Mycorrhizal nutrient efficiency(\%)} = \frac{E_i - E_{ni}}{E_i} \times 100$$

Where E_i and E_{ni} were the values of nutrient elements of citrus seedlings infected by AMF and non-AMF.

Statistical analysis: The data were statistically analyzed by analysis of variance (ANOVA) with SAS version 8.1 software. Fisher's protected least significant difference (LSD, $P < 0.05$) was used to compare the significant difference among these treatments.

RESULTS AND DISCUSSION

The present study observed the mycorrhizal fungal structures in the roots of the peach seedling colonized by exogenous AMF (Fig. 1), and the mycorrhizal colonization varied between 23.4 and 54.9% or among the AMF species (Table 1). *G. mosseae*-colonized seedlings exhibited the highest colonization, *G. versiforme* higher, and *Paraglomus occultum* lowest. However, no mycorrhizal colonization was observed in the roots of the non-AMF seedlings (Table 1).

Generally, mycorrhizal fungal efficiency is measured in terms of growth status of the host plant (Wu *et al.*, 2007). Compared with the non-AMF treatment, plant height, stem diameter, shoot, root or total dry weight was significantly increased by 30.3%, 17.2%, 34.4%, 64.5% or 45.4% respectively with the inoculation of *G. mosseae*, 11.8%, 10.9%, 19.4%, 31.2% or 23.5% with *G. versiforme*, and 6.4%, 1.9%, 12.1%, 24.8% or 16.8% with *Paraglomus occultum* (Table 1). These results suggested different efficacy of mycorrhizal fungal species on enhancing peach growth. In the three AMF species used here, greater positive efficacy on plant growth performance generally ranked as *G. mosseae* > *G. versiforme* > *Paraglomus occultum*. Moreover, the enhanced efficacy of mycorrhizas was more obvious for root than for shoot or total dry weight. Our results agree with the findings of Yao *et al.* (2005) in litchi (*Litchi chinensis*) and Wu *et al.* (2007) in red tangerine (*Citrus tangerine*). Although AMF are non-specific associated with host plants (Selosse *et al.*, 2006), the present study clearly showed the best symbiosis between peach and *G. mosseae*, suggesting that different AMF play different roles on plant growth, due to functional diversity of AMF.

Although AM symbiosis exhibits a wide range of benefits to the host plant, the most widely cited benefit

is the enhancement of nutrient acquisition (Hodge *et al.*, 2010). Experimental evidence from this study showed that for macroelement, mycorrhizal seedlings recorded higher K concentrations of leaves and roots, Ca concentration of leaves, and Mg concentrations of leaves and roots than non-mycorrhizal ones, and also exhibited lower root Ca concentration (Table 2). The results might ascribe to the reason that fungal hyphae extend from the root surface into the soil, increase the surface areas of root and thus acquire more macroelement beyond the depletion zone (Schnepf *et al.*, 2010). Moreover, the mycorrhizal benefit for K, Ca, and Mg enhancements was dependent on AMF species (Table 2). Among the three AMF, greater mycorrhizal efficiency on leaf and root K ranked as *G. mosseae* > *G. versiforme* > *Paraglomus occultum*, leaf Ca as *G. versiforme* > *G. mosseae* > *Paraglomus occultum*, and leaf and root Mg as *G. mosseae* > *Paraglomus occultum* > *G. versiforme*. The result is in agreement with the finding of Diop *et al.* (2003) in the *G. aggregatum*-, *G. versiforme*-, and *G. mosseae*-colonized *Solanum aethiopicum* plants. Moreover, in our study, mycorrhizal efficiencies of K and Mg were higher in roots than in leaves, suggesting better K and Mg acquisition of roots by AMF.

A significant more Fe concentration was observed in mycorrhizal than in non-mycorrhizal leaves and roots (Table 2). Herein, inoculation with *G. mosseae* exhibited higher Fe concentrations of leaf and roots than

inoculations with *G. versiforme* and *Paraglomus occultum*, respectively, which showed similar Fe levels. The similar result was also reported by Wang and Xia (2009) in trifoliolate orange (*Poncirus trifoliata*) seedlings inoculated with *G. versiforme* at different pH levels of nutrient solution. However, Caris *et al.* (1998) reported that inoculation with *G. mosseae* had a significant influence on the labelled ⁵⁹Fe concentrations in shoots of sorghum (*Sorghum bicolor*) plants but not peanut (*Arachis hypogea*) plants. These data indicated that the Fe increment due to mycorrhiza is dependent of both fungal and plant species, and might be the results of both the external hyphae and the increases of root Fe(III) chelate reductase activities (Caris *et al.*, 1998; Wang and Xia, 2009).

In our study, mycorrhizal peach seedlings recorded significantly lower Cu and Mn concentrations of leaves but higher Cu and Mn concentrations of roots, compared with non-mycorrhizal seedlings (Table 2). In leaves, mycorrhizal efficiency of Cu was -80.0, -20.0 or -66.7% with the *G. mosseae*, *G. versiforme*, or *Paraglomus occultum*, respectively; Mn -77.3, -61.3, or 26.7%. In roots, mycorrhizal efficiency of *G. mosseae* presented 321.7% for Cu and 91.7% for Mn; *G. versiforme* 178.3% for Cu and 33.3% for Mn; *Paraglomus occultum* 221.7% for Cu and 33.3% for Mn. These data imply that mycorrhizal efficiencies of Cu and

Table 1. Influences of three different arbuscular mycorrhizal fungi (AMF) on root colonization and growth performance of peach (*Prunus persica* L. Batsch) seedlings

Mycorrhizal inoculation	Mycorrhizal colonization (%)	Plant height (cm)	Stem diameter (cm)	Leaf number per plant	Dry weight (g plant ⁻¹)		
					Shoot	Root	Total
<i>G. mosseae</i>	54.9a	55.22a	0.430a	47.5a	3.32a	2.32a	5.64a
<i>G. versiforme</i>	43.9b	47.37b	0.407a	44.5a	2.95ab	1.85b	4.79b
<i>Paraglomus occultum</i>	23.4c	45.10bc	0.374b	42.0a	2.77bc	1.76b	4.53b
Non-AMF	0.0d	42.37c	0.367b	43.3a	2.47c	1.41c	3.88c

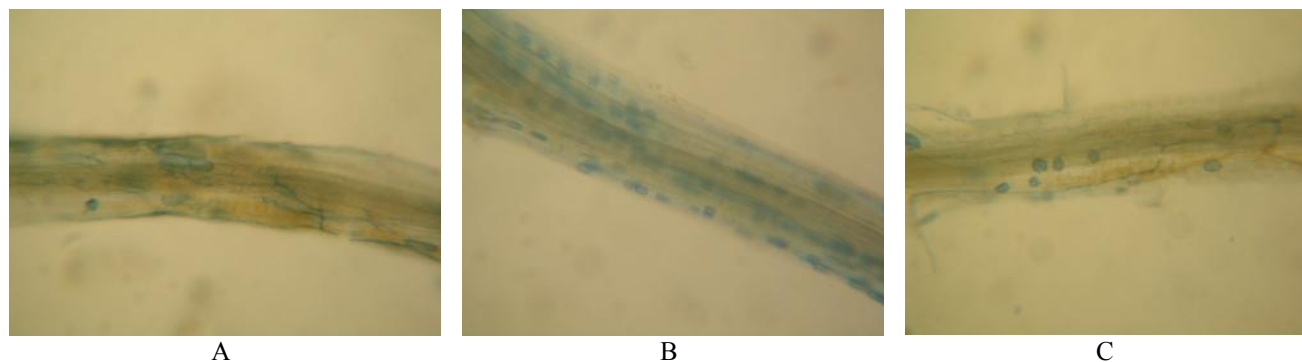
Within each column values followed by same letter are not significantly different ($P < 0.05$).

Table 2 Influences of three different arbuscular mycorrhizal fungi (AMF) on nutrient concentrations and mycorrhizal nutrient efficiency of peach (*Prunus persica* L. Batsch) seedlings

Mycorrhizal inoculation	K		Ca		Mg		Fe		Cu		Mn		Zn	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Nutrient concentration														
<i>G. mosseae</i>	32.16a	10.76a	3.51b	1.37b	2.88a	4.11a	2.13a	3.63a	0.03d	0.97a	0.17c	0.23a	0.18a	0.22a
<i>G. versiforme</i>	29.50b	10.08ab	5.38a	1.46b	2.44b	3.02b	1.72b	3.00b	0.12b	0.64c	0.29c	0.16b	0.19a	0.13b
<i>Paraglomus occultum</i>	27.31c	9.06b	2.27c	1.72a	2.68ab	3.84a	1.59b	3.06b	0.05c	0.74b	0.55b	0.16b	0.19a	0.12b
Non-AMF	25.02d	4.87c	1.04d	1.72a	1.85c	1.71c	0.79c	2.18c	0.15a	0.23d	0.75a	0.12c	0.12b	0.04c
Mycorrhizal nutrient efficiency														
<i>G. mosseae</i>	28.5	120.9	237.5	-20.3	55.7	140.4	169.6	66.5	-80.0	321.7	-77.3	91.7	50.0	450.0
<i>G. versiforme</i>	17.9	107.0	417.3	-15.1	31.9	76.6	117.7	37.6	-20.0	178.3	-61.3	33.3	58.3	225.0
<i>Paraglomus occultum</i>	9.2	86.0	118.3	0.0	44.9	124.6	101.3	40.4	-66.7	221.7	-26.7	33.3	58.3	200.0

Within each column values followed by same letter are not significantly different ($P < 0.05$).

Fig. 1 Peach (*Prunus persica* L. Batsch) roots colonized by exogenous arbuscular mycorrhizal fungi. **A:** *Glomus mosseae*; **B:** *Glomus versiforme*; **C:** *Paraglomus occultum*



Mn were absolutely dependent on mycorrhizal fungal species and plant tissues. Previous studies observed that mycorrhizal hyphae could take part in Cu and Mn uptake (Li *et al.*, 1991; Giovannetti, 2008). Therefore, mycorrhizal symbiosis significantly increased Cu and Mn concentrations of roots. However, compared with those of non-mycorrhizal leaves, lower Cu and Mn concentrations of mycorrhizal leaves might ascribe to the reason that mycorrhizal hyphae are only concerned with Cu and Mn uptake but not with Cu and Mn translocation.

It has been proven that mycorrhizal symbiosis can improve Zn nutrition as a secondary consequence of P nutrition (Subramanian *et al.*, 2009). In the present study, all the AM fungal species significantly increased the concentrations of Zn in leaf and root, and the mycorrhizal efficiency of Zn was higher for root (200.0–450%) than for leaf (50.0–58.3%) (Table 2). The result is consistent with the finding of Marques *et al.* (2006), who observed that inoculation with *G. claroideum* or *G. intraradices* enhanced the Zn accumulation in the tissues of *Solanum nigrum* plants.

Conclusions: Our results showed that mycorrhizal inoculation generally increased growth performance of the peach seedlings under the potted conditions, and also significantly elevated K, Mg, Fe and Zn concentrations of leaves and roots, Ca concentration of leaves, Cu and Mn concentrations of roots, which were obviously dependent on AMF species. In the three AMF, *G. mosseae* exhibited the best mycorrhizal efficiency on growth and nutrient acquisition of peach seedlings.

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REFERENCES

- Azcon-Aguilar, C., and J. M. Barea (1997). Applying mycorrhiza biotechnology to horticulture: significance and potentials. *Sci. Hort.* 68:1-24.
- Bâ, A. M., C. Plenchette, P. Danthu, R. Duponnois and T. Guissou (2000). Functional compatibility of two arbuscular mycorrhizae with thirteen fruit trees in Senegal. *Agrofor. Syst.* 50:95-105.
- Calvet, C., V. Estaun, A. Camprubi, A. Hernandez-Dorrego, J. Pinochet, and M.A. Moreno (2004). Aptitude for mycorrhizal root colonization in *Prunus* rootstocks. *Sci. Hort.* 100:39-49.
- Caris, C., W. Hordt, H. J. Hawkins, V. Romheld, and E. George (1998). Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. *Mycorrhiza* 8:35-39.
- Diop, T. A., T. Krasova-Wade, A. Diallo, M. Diouf, and M. Gueye (2003). *Solanum* cultivar responses to arbuscular mycorrhizal fungi: growth and mineral status. *Afr. J. Biotechnol.* 2:429-433.
- Genre, A., and P. Bonfante (2010). The making of symbiotic cells in arbuscular mycorrhizal roots. In: Koltai, H., and Y. Kapulnik (Eds.) *Arbuscular Mycorrhizas: Physiology and Function*. Springer-Verlag, Berlin Heidelberg, pp 57-71.
- Giovannetti, M. (2008). Structure, extent and functional significance of belowground arbuscular mycorrhizal networks. In: Varma, A. (Ed.) *Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics*. Third edition. Springer-Verlag, Berlin Heidelberg, pp 59-72.
- Hodge, A., T. Helgason, and A. H. Fitter (2010). Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecol.* 3:267-273.

- Larue, J. H., W. D. Clellan, and W. I. Peacock (1975). Mycorrhizal fungi and peach nursery nutrition. *California Agric.* 5:6-7.
- Li, X. L., H. Marschner, and E. George (1991). Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover. *Plant Soil* 136:49-57.
- Marques, A. P.G. C., R. S. Oliveira, A. O. S. S. Rangel, and P. M. L. Castro (2006). Zinc accumulation in *Solanum nigrum* is enhanced by different arbuscular mycorrhizal fungi. *Chemosphere* 65:1256-1263.
- Mena-Violante, H. G., O. Ocampo-Jimenez, L. Dendooven, G. Martinez-Soto, J. Gonzalez-Castaneda, F. T. Davies, and V. Olalde-Portugal (2006). Arbuscular mycorrhizal fungi enhanced fruit growth and quality of chile ancho (*Capsicum annuum* L. cv San Luis) plants exposed to drought. *Mycorrhiza* 16:261-267.
- Miller, D. D., M. Bodmer, and H. Schuepp (1989). Spread of endomycorrhizal colonization and effects on growth of apple seedlings. *New Phytol.* 111:51-59.
- Miransari, M. (2010). Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. *Plant Biol.* 12:563-569.
- Nasim, G. (2010). The role of arbuscular mycorrhizae in inducing resistance to drought and salinity stress in crops. In: Ashraf, M., M. Ozturk, and M.S.A. Ahmad (Eds.). *Plant Adaptation and Phytoremediation*. Springer-Verlag, Berlin Heidelberg, pp 119-141.
- 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. Br. Mycol. Soc.* 55:158-161.
- Rutto, K. L., and F. Mizutani (2006). Peach seedlings growth in replant and non-replant soils after inoculation with arbuscular mycorrhizal fungi. *Soil Biol. Biochem.* 38:2536-2542.
- Rutto, K. L., F. Mizutani, and K. Kadoya (2002). Effect of root-zone flooding on mycorrhizal and non-mycorrhizal peach (*Prunus persica* Batsch) seedlings. *Sci. Hort.* 94:285-295.
- Schnepf A, Jones D, and T. Roose (2011). Modelling nutrient uptake by individual hyphae of arbuscular mycorrhizal fungi: temporal and spatial scales for an experimental design. *Bull. Math. Biol.* 73:2175-2200.
- Schreiner, R. P. (2003). Mycorrhizal colonization of grapevine rootstocks under field conditions. *Am. J. Enol. Vitic* 54:143-149.
- Selosse, M. A., F. Richard, X. H. He, and S. W. Simard (2006). Mycorrhizal networks: des liaisons dangereuses? *Trend. Ecol. Evol.* 21:621-628.
- Subramanian, K. S., V. Tenshia, K. Jayalakshmi, and V. Ramachandran (2009). Role of arbuscular mycorrhizal fungus (*Glomus intraradices*) – (fungus aided) in zinc nutrition of maize. *J. Agric. Biotech. Sustain. Dev.* 1:29-38.
- Wang, M. Y., and R. X. Xia (2009). Effects of arbuscular mycorrhizal fungi on growth and iron uptake of *Poncirus trifoliata* under different pH. *Acta Microbiol. Sin.* 49:1374-1379.
- Wu, Q. S., and Y. N. Zou (2009). Mycorrhizal influence on nutrient uptake of citrus exposed to drought stress. *Philipp. Agric. Scientist* 92:33-38.
- Wu, Q. S., R. X. Xia, and Y. N. Zou (2008). Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *Eur. J. Soil Biol.* 44:122-128.
- Wu, Q. S., Y. N. Zou, R. X. Xia, and M. Y. Wang (2007). Five *Glomus* species affect water relations of *Citrus tangerine* during drought stress. *Bot. Stud.* 48:147-154.
- Yao, Q., H. H. Zhu, and J. Z. Chen (2005). Growth responses and endogenous IAA and iPAs changes of litchi (*Litchi chinensis* Sonn.) seedlings induced by arbuscular mycorrhizal fungal inoculation. *Sci. Hort.* 105:145-151.