The Journal of Animal & Plant Sciences, 21(1): 2011, Page: 20-25
ISSN: 1018-7081

ROOT MORPHOLOGICAL MODIFICATION OF MYCORRHIZAL CITRUS (*CITRUS TANGERINE*) SEEDLINGS AFTER APPLICATION WITH EXOGENOUS POLYAMINES

Q. S. Wu, Y. N. Zou, Y. H. Peng and C. Y. Liu

College of Horticulture and Gardening, Yangtze University, No. 88 Jingmi Road, Jingzhou City, Hubei Province 434025,
People's Republic of China
Corresponding author. E-mail: wuqiangsh@163.com

ABSTRACT

The present work tried to determine the effects of the three polyamine (PA) species (100 mg L-1), putrescine (Put), spermidine (Spd) and spermine (Spm), on root colox`nization, root morphology and phosphorus (P) uptake of three-month-old citrus (Citrus tangerine) seedlings inoculated with arbuscular mycorrhizal fungus (AMF), Glomus mosseae. Exogenous PAs significantly increased root colonization three months after application. Sole AMF inoculation notably improved root morphology (total length, total projected area, total surface area and total volume) and growth performance (plant height, stem diameter and leaf number per plant) in comparison with non-mycorrhizal control, whilst PAs application to mycorrhizal seedlings increased more these root morphological and growth traits. AMF significantly reduced guaiacol peroxidase (G-POD) activity of root, whereas G-POD activities of mycorrhizal leaf and root were elevated by Pas, to a certain extent. Compared with sole AMF treatment, exogenous PAs significantly inhibited acid and neutral phosphatase activities of rhizosphere. Moreover, exogenous PAs notably reduced available P content of rhizosphere but significantly increased root P content. It concluded that exogenous PAs could stimulate both root colonization and morphology, and root morphological improvement of mycorrhizal seedlings by PAs was related to not G-POD activity but to root P level and would result in an increment of growth performance.

Key words: Arbuscular mycorrhizal fungi, citrus, putrescine, root morphology, spermidine, spermine

INTRODUCTİON

Citrus, belonging to the genus *Citrus* of the family Rutaceae, is the most widely produced fruit tree, and it is grown in more than 80 countries (Ladaniya, 2008). China is the second production country of citrus fruit and leads in tangerine/mandarin production, with more than 8.6 million-ton output. The presence of root hairs has been found in many citrus species, but root hairs of citrus are relatively shorter than those of other tree species (Poerwanto *et al*, 1989). Therefore, citrus must depend on arbuscular mycorrhiza (AM), a symbiosis between arbuscular mycorrhizal fungi (AMF) and roots of plants, which can improve the supply of water and nutrients to the host plant (Parniske, 2008; Wu *et al*, 2009). However, in the open field, mycorrhizal colonization of citrus is less than 10% (Wu *et al*, 2009).

Polyamines (PAs), namely putrescine (Put), spermidine (Spd) and spermine (Spm), are low molecular weight, aliphatic polycations found in the cells of all living organisms (Kusano *et al*, 2008). Plant PAs have involved in a variety of divergent processes, such as gene expression, protein and DNA synthesis, cellular homeostasis, cell division and differentiation, growth and developmental processes such as embryogenesis, organogenesis, senescence, and also responses to abiotic and biotic stresses (Moschou *et al*, 2008). Generally, PA concentration was equivalent in AM and non-AM plants when growth parameters were similar, irrespective of their different phosphorus (P) contents (Parádi *et al*, 2002). A recent experiment showed that exogenous PAs (100 mg L^{-1}), especially Put, obviously increased AM colonization of *Glomus versiforme*-colonized *Poncirus trifoliata* (Wu and Zou, 2009). Optimal concentrations (50-200 mg L^{-1}) of exogenous PAs had significantly positive effects on spore germination and hyphal growth of *G. mosseae* and *Gigaspora margarita in vitro* culture conditions (Zhang *et al*, 2003). However, it is unknown whether PAs regulate AM colonization of citrus. Moreover, PAs involve in root development, and PA accumulation has also been correlated to adventitious root formation (Couée *et al*, 2004). Greater root morphology will benefit plants absorbing more water and nutrients, thus increasing plant production. Root morphology modification depends on PA species. Spd and Spm contents showed positive correlations with primary root growth of *Pringlea antiscorbutica*, whereas Put level showed neutral or negative effects on the trait (Hummel *et al*, 2002). To date, information regarding the effect of PAs on root development of citrus is lacking.

The aim of the present study was to determine whether PAs modify mycorrhizal colonization, root morphology and P uptake of citrus seedlings inoculated with *G. mosseae.* Additionally, the study tried to clarify the mechanism of root morphological modification based on the analysis of guaiacol peroxidase (G-POD).

MATERİALS AND METHODS

Experimental design: In a completely random arrangement, five treatments with five replicates each for a total of 25 pots (three seedlings/pot) were as follows: 1) non-exogenous PA plus non-mycorrhizal control (non-AMF), 2) *G. mosseae* only (AMF), 3) exogenous Put plus *G. mosseae* (AMF+Put), 4) exogenous Spd plus *G. mosseae* (AMF+Spd) and 5) exogenous Spm plus *G. mosseae* (AMF+Spm). Put, Spd and Spm (Sigma, USA) were exogenously applied (300 mL of 100 mg L^{-1} PA) to the soil media after three months of AMF inoculation, respectively. The other treatments received 300 mL distilled water.

Plant growth and mycorrhizal infection: The experiment was performed in a plastic greenhouse, lacking the light and temperature equipments. The photo flux density ranged from 600 to 850 µmol m^{-2} s^{-2} during the entire experiment. The average day/night temperature was 26/18°C; the relative humidity was 65%-95%.

Seeds of citrus (*Citrus tangerine* Hort. ex Tanaka) were allowed to germinate on wet filter paper in petri dishes at 25°C, after surface-sterilization with 70% alcohol for 5 min. The seven-day-old seedlings were transferred to plastic pots (18 cm in depth and 20 cm in mouth diameter) with 3.2 kg of autoclaved (121°C, 0.11 MPa, 2 h) growth mixture of soil, vermiculite and sphagnum (5:2:1, v/v/v) on March 20, 2009. The pot substrate had been inoculated with *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe (BGC XZ02A) before transplant by placing 16 g of inoculum (the mixture of soil, spores, hyphae and infected roots) 5 cm below the surface of the substrate. Non-AMF pots supplied with 16 g sterilized substrate as control. These inocula were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. The experiment was performed from March 20 to September 20, 2009.

Parameter analysis: The seedlings were harvested after three months of PAs application, and plant height, stem diameter and leaf number per plant were recorded.

These intact roots were rinsed with distilled water and scanned using root automatism scan apparatus, EPSON Expression/STD 4800 scanner. The acquired root images were analysed using WinRHIZO Pro 2007b (Regent Instruments Inc., Quebec, Canada) to obtain root parameters, such as length, projected area, surface area, average diameter, total volume, etc.

After root scanned, a small quantity of 1-cm length fresh root pieces were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol, according to Phillips and Hayman (1970). The AM colonization was quantified according to the following formula:

$$\text{AM colonization (\%)} = \frac{\text{root length infected}}{\text{root length observed}} \times 100$$

Plant P content was analyzed by the vanadate-molybdate-yellow method (Chapman and Pratt, 1961). Rhizospheric soils, which were loosely adhered to roots, were air-dried and ground through a 1 mm sieve for the analyses. Determinations of available P and phosphatase activity were according to Olsen *et al* (1954) and Zhao and Jiang (1986), respectively. Hereinto, acid, neutral or alkaline phosphatase was extracted by sodium acetate buffer (pH 5.0), citric acid-disodium hydrogen phosphate buffer (pH 7.0) or borate buffer (pH 10.0), respectively.

Plant fresh material (0.3 g) was homogenized in 8 ml of 0.1 mol L^{-1} phosphate buffer (pH 7.8) in an ice bath. The homogenate was centrifuged at 4,200*g* for 10 min, and the supernatant was used for the assays. G-POD activity was determined using the method described by Amako *et al* (1994).

Soluble sugar content was determined using an anthrone method of Yemm and Willis (1954).

Statistical analysis: The experimental data were statistically analyzed by one-way variance (ANOVA) with SAS 8.1 software. The Least Significant Differences (LSD) were used to compare the means at 5% level.

RESULTS AND DİSCUSSİON

AM colonization: In the study, all the PAs significantly increased AM colonization of *G. mosseae*-colonized *C. tangerine* seedlings (Fig. 1). The ranking of the three PAs for AM colonization was as follows: Put > Spm > Spd. The result is in agreement with the finding of Wu and Zou (2009), who observed that exogenous PAs, especially Put and Spm, significantly increased mycorrhizal colonization of *G. versiforme*-colonized *Poncirus trifoliata*. In addition, a PA biosynthesis inhibitor showed a negative effect on AM colonization and hyphal length of host plants (El-Ghachtouli *et al*, 1996). The inhibition was reversed when exogenous Put applied to the PA biosynthesis inhibitor treatment. It was enough to indicate that PAs regulate mycorrhizal development of host plant.

Soluble sugar contents of leaf and root: Owing to the heterotrophic nature of the AM endophyte, the carbohydrate status of the hosts ought to change when roots sustain the AMF growth (Ocampo and Azcon, 1985). In the present work, sole AMF significantly increased soluble sugar contents of leaf and root in comparison with the non-AMF control (Fig. 2), implying that AMF colonization can induce the accumulation of soluble sugar to maintain fungal growth. The result is similar to both mycorrhizal *Poncirus trifoliata* (Wu *et al*, 2006) and *Trifolium subterraneum* (Same *et al*, 1983). However, compared with the sole AMF, exogenous PAs exhibited two different results for soluble sugar: in the leaf, the soluble sugar content was increased (Figure 2); in the root, the soluble sugar content was notably decreased (Fig. 2). It has been confirmed that PAs are present in chloroplasts, thylakoid membranes, photosystem II membranes, and the light-harvesting complex (Kotzabasis *et al*, 1993). Aziz (2003) observed that exogenous Spd induces an increase in soluble sugar content of both leaves and inflorescences of *Vitis vinifera*. It was concluded that the increase of soluble sugar in leaf due to PAs was related to the maintenance of photosynthetic activity by PAs. However, in the root, exogenous PAs markedly reduced soluble sugar content compared with sole AMF treatment. There is a fact that estimates of the amounts of carbon allocated to the fungus vary from 4% to 20% of the plant's total carbon budget (Jifon *et al*, 2002). The decrease in soluble sugar content of mycorrhiza root due to PAs may be due to the translocation of carbohydrate produced by the host to the fungal partner (Manoharan *et al*, 2008), exhibiting a better mycorrhizal development of citrus seedlings inoculated with dual AMF and PAs.

Root morphology: Data presented in Table 1 showed that alone AMF inoculation did not affect root average diameter but significantly increased total root length, total root projected area, total root surface area, and total root volume. This is consistent with the finding of Wu *et al* (2010) in *Poncirus trifoliata* seedlings exposed to non-salt or salt stress. The root morphological modification might be that PAs control cell division and differentiation in root apex and during lateral and adventitious root formation (Couée *et al*, 2004).

G-POD activities of leaf and root: Plant peroxidases (PODs) are enzymes that catalyze the oxidation of numerous artificial and physiological electron donors utilizing hydrogen peroxide (Hatzilazarou *et al*, 2006). In addition, POD activity plays a key role in rooting of microshoot of *Psoralea corylifolia in vitro* (Rout *et al*, 2000). Generally, the decline of root G-POD activity was accompanied by both a decrease in lignin content and an increase in NAA (Chen *et al*, 2002). Our results showed that although AMF inoculation increased G-POD activity in the leaf, the differences were not significant. In the root, sole AMF significantly inhibited G-POD activity (Fig. 3), implying that root morphological modification due to mycorrhization was the result of the decrease in the root G-POD activity. When the mycorrhizal seedlings were applied by the exogenous PAs, there were significant differences in the leaf G-POD activity only between sole AMF and AMF+Spm (Fig. 3). In the root, both Put and Spd treatments significantly increased the activity of G-POD compared with sole AMF. Changes in POD activity in relation to auxin have been demonstrated previously in *Psoralea corylifolia* (Rout *et al*, 2000). In plant tissues auxin especially NAA, is positively correlated with the process of root development (Chen *et al*, 2002). Therefore, it was concluded that root morphological modification was related to G-POD activity in sole AMF inoculation but not in PAs treatments. Since PODs exist in numerous isoenzymatic forms (Hatzilazarou *et al*, 2006), further experiments need to clarify the relationship between POD type and root morphological modification due to mycorrhization.

Table 1. Effect of *Glomus mosseae* alone or in combination with PAs on root morphology of citrus (*Citrus tangerine*) seedlings

Treatments	Average diameter (mm)	Total length (cm)	Total projected area (cm^2)	Total surface area (cm^2)	Total volume (cm^3)
Non-AMF	1.63^{a}	126.6^{c}	10.34^{c}	64.99^{c}	1.04^{c}
AMF	1.63^{a}	151.3^{b}	12.37^{b}	75.12^{b}	1.77^{b}
AMF+Put	1.67^{a}	176.9^{a}	14.81^{a}	92.46^{a}	2.04^{a}
AMF+Spd	1.69^{a}	169.3^{a}	13.56^{ab}	90.85^{a}	1.95^{a}
AMF+Spm	1.57^{a}	188.7^{a}	14.64^{a}	91.97^{a}	1.93^{a}

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

Table 2. Effect of *Glomus mosseae* alone or in combination with PAs on plant P, rhizospheric available P and rhizospheric phosphatase of citrus (*Citrus tangerine*) seedlings

Treatments	Available P of rhizosphere (mg kg^{-1})	Phosphatase activity of rhizosphere (mg hydroxybenzene g^{-1})			Plant P content (mg g^{-1})	
		Acid	Neutral	Alkaline	Leaf	Root
Non-AMF	6.09^{a}	1.01^{b}	0.20^{a}	0.019^{b}	4.73^{b}	1.41^{c}
AMF	4.97^{b}	1.45^{a}	0.13^{b}	0.056a	6.25^{a}	2.56^{b}
AMF+Put	4.00^{cd}	0.96^{b}	0.11^{b}	0.044a	6.65^{a}	3.76^{a}
AMF+Spd	4.41^{c}	0.88^{b}	0.08^{b}	0.049a	6.46^{a}	3.69^{a}
AMF+Spm	3.63^{d}	0.96^{b}	0.10^{b}	0.038^{ab}	6.77^{a}	3.64^{a}

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

Table 3. Effect of *Glomus mosseae* alone or in combination with PAs on growth performance of citrus (*Citrus tangerine*) seedlings

Treatments	Plant height (cm)	Stem diameter (cm)	Leaf number per plant
Non-AMF	14.9^{d}	0.308^{c}	18.6^{c}
AMF	16.8^{c}	0.326^{b}	21.9^{b}
AMF+Put	18.0^{bc}	0.348^{a}	23.0^{ab}
AMF+Spd	19.8^{ab}	0.344^{a}	25.2^{a}
AMF+Spm	19.9^{a}	0.334^{ab}	22.1^{b}

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

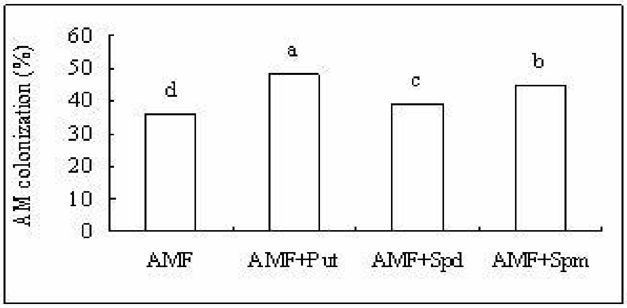

Figure 1. Effect of exogenous Pas on AM colonization of *Glomus mosseae*-INOCULATED CITRUS (*Cıtrus tangerine*) seedlings. Data followed by the same letter above the bars are not significantly different among these treatments at *P*<0.05.

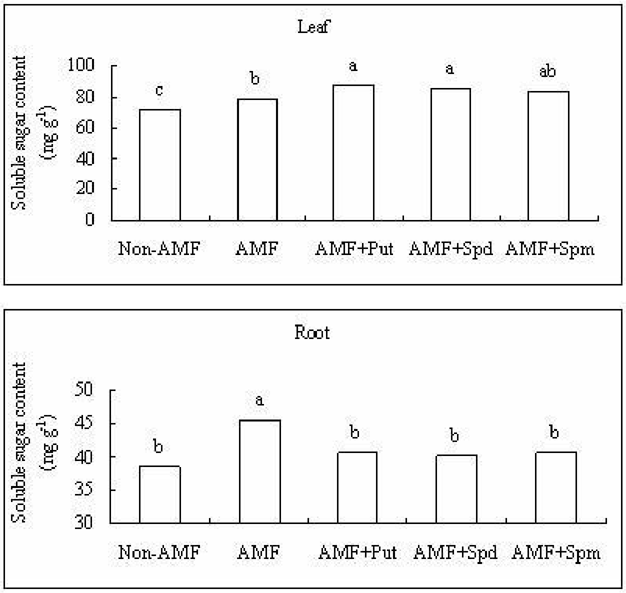

Figure 2. Effect of Glomus mosseae alone or in combination with PAs on leaf and root soluble sugar content of citrus (*Citrus tangerine*) seedlings. Data followed by the same letter above the bars are not significantly different among these treatments at *P*<0.05.

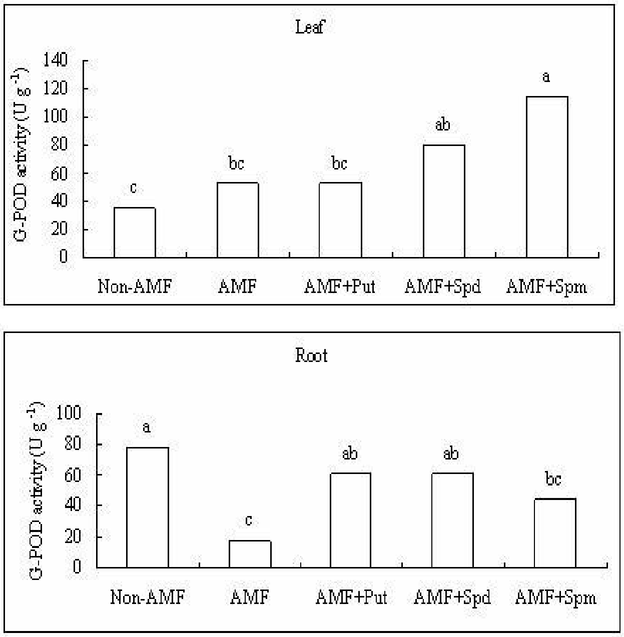

Figure 3. Effect of *Glomus mosseae* alone or in combination with Pas on leaf and root guaiacol peroxidase (G-POD) activities of citrus (*Citrus tangerine*) seedlings. Data followed by the same letter above the bars are not significantly different among these treatments at *P*<0.05.

Plant P, rhizospheric available P and rhizospheric phosphatase: Variations in root morphology and mycorrhizal colonization have been shown to be correlated with the plant utilization ability of soil available P (Machado *et al*, 2004; Hajiboland *et al*, 2009). The present study showed that sole AMF increased the activities of acid phosphatase and alkaline phosphatase of rhizosphere but inhibited neutral phosphatase activity in comparison with non-AMF control, thus inducing the decrease of available P of rhizosphere and the increase of Plant P content (Table 2), because acid phosphatase can catalyze the hydrolysis of organic phosphate esters to ortho-phosphate (Speir and Ross, 1978). Compared with sole AMF treatment, exogenous PAs did not affect alkaline phosphatase activity of rhizosphere but significantly inhibited acid and neutral phosphatase activities of rhizosphere (Table 2). Moreover, exogenous PAs notably reduced available P content of rhizosphere but significantly increased root P content. The results might ascribe that root morphological improvement could be expected to improve P acquisition and facilitate an exploration of a greater soil volume (Zobel *et al*, 2006). Padilla and Encina (2005) also reported that mycorrhizal colonization increased the total number of roots and the number of first-order and second-order laterals in adult cherimoya (*Annona cherimola*) plants, thus alleviating P deficiency. Therefore, the P increment in the citrus seedlings may result from two reasons: 1) due to mycorrhization attributed to the stimulation of rhizospheric acid phosphatase activity, root morphological modification and directly mycorrhizal uptake; 2) due to PAs because of both root morphological improvement and mycorrhizal uptake. Additionally, root P level can positively affect root morphology, and in turn better root morphology can help roots to uptake more P (Schroeder and Janos, 2005; Hu *et al*, 2010). It seems that root morphological modification by exogenous PAs could be due to the P increments in the roots. There is a need to evaluate the interactive relation between root morphology and root P level.

Growth performance: Since AMF alone or in combination with exogenous PAs improved root morphology of citrus seedlings and stimulated the increase of plant P, it was reasonable to observe that sole AMF significantly increased plant height, stem diameter and leaf number per plant compared with non-AMF treatment, and exogenous PAs increased these growth traits to a certain extent compared with sole AMF (Table 3).

Conclusions: As stated above, exogenous PAs stimulated both the root colonization and the root morphology of the *G. mosseae*-inoculated citrus seedlings. The root morphological improvement by PAs was related to not G-POD activity but to root P level, and also helped the seedlings with the uptake of P from the rhizosphere, resulting in an increment of the growth performance.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No.: 30800747) and the Creative Experimental Project of National Undergraduate Students from Yangtze University (No.: 091048911).

REFERENCES

Amako, K., G. X. Chen, and K. Asade (1994). Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. Plant Cell Physiol. 35:497-504.

Aziz, A. (2003). Spermidine and related-metabolic inhibitors modulate sugar and amino acid levels in *Vitis vinifera* L.: possible relationships with initial fruitlet abscission. J. Exp. Bot. 54:355-363.

Berta, G., A. Fusconi, and J. E. Hooke. (2002). Arbuscular mycorrhizal modifications to plant root systems: scale, mechanisms and consequences. In: Gianinazzi, S., H. Schüepp, J.M. Barea,and K. Haselwandter (Eds.). Mycorrhizal Technology in Agriculture. Birkhäuser Verlag, Switzerland, pp 71-85.

Chapman, H. D., and P. F. Pratt (1961). Methods of Analysis for Soils, Plants and Waters. University of California, Riverside, USA.

Chen, L. M., J. T. Cheng, E. L. Chen, T. J. Yiu, and Z. H. Liu (2002). Naphthaleneacetic acid suppresses peroxidase activity during the induction of adventitious roots in soybean hypocotyls. J. Plant Physiol. 159:1349-1354.

Couée, I., I. Hummel, C. Sulmon, G. Gouesbet, and A. El Amrani (2004). Involvement of polyamines in root development. Plant Cell Tiss. Org. Cult. 76:1-10.

El Ghachtouli, N., J. Martin-Tanguy, M. Paynot, and S. Gianinazzi (1996). First report of the inhibition of arbuscular mycorrhizal infection of *Pisum sativum* by specific and irreversible inhibition of polyamine biosynthesis or by gibberellic acid treatment. FEBS Lett. 385:189-192.

Hajiboland, R., N. Aliasgharzad, and R. Barzeghar (2009). Phosphorus mobilization and uptake in mycorrhizal rice (*Oryza sativa* L.) plants under flooded and non-flooded conditions. Acta Agric. Slov. 93:153-161.

Hatzilazarou, S. P., T. D. Syros, T. A. Yupsanis, A. M. Bosabalidis, and A. S. Economou (2006). Peroxidase, ligin and anatomy during *in vitro* and *ex vitro* rooting of gardenia (*Gardenia jasminoides* Ellis) microshoots. J. Plant Physiol. 163:827-836.

Hu, Y. F., X. S. Ye, L. Shi, H. Y. Duan, and F. S. Xu (2010). Genotypic differences in root morphology and phosphorus uptake kinetics in *Brassica napus* under low phosphorus supply. J. Plant Nutri. 33:889-901.

Hummel, I., I. Couée, A. El Amrani, J. Martin-Tanguy, and F. Hennion (2002). Involvement of polyamines in root development at low temperature in the subantarctic cruciferous species *Pringlea antioscorbutica*. J. Exp. Bot. 53:1463-1473.

Jifon, J. L., J. H. Graham, D. L. Drouillard, and J. P. Syvertsen (2002). Growth depression of mycorrhizal *Citrus* seedlings grown at high phosphorus supply is mitigated by elevated CO_2. New Phytol. 153:133-142.

Kotzabasis, K., C. Fotinou, K. A. Roubelakis-Angelakis, and D. Ghanotakis (1993). Polyamines in the photosynthetic apparatus. Photosystem II highly resolved subcomplexes are enriched in spermine. Photosyn. Res. 38:83-88.

Kusano, T., T. Berberich, C. Tateda, and Y. Takahashi (2008). Polyamines: essential factors for growth and survival. Planta 228:367-381.

Ladaniya, M. S. (2008). Citrus Fruit: Biology, Technology and Evaluation. Academic Press, San Diego.

Machado, C. T. T., and A. M. C. Furlani (2004). Kinetics of phosphorus uptake and root morphology of local and improved varieties of maize. Sci. Agric. (Piracicaba, Braz.) 61:69-76.

Manoharan, P. T., M. Pandi, V. Shanmugaiah, S. Gomathinayagam, and N. Balasubramanian (2008). Effect of vesicular arbuscular mycorrhizal fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions. Afr. J. Biotech. 7:3431-3436.

Moschou, P. N., K. A. Paschalidis, and K. A. Roubelakis-Angelakis (2008). Plant polyamine catabolism. Plant Signal. Behav. 3:1061-1066.

Ocampo, J. A., and R. Azcon (1985). Relationship between the concentration of sugars in the roots and VA mycorrhizal infection. Plant Soil 86:95-100.

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. United States Department of Agriculture, Washington, pp 1-9.

Padilla, I. M. G., and C. L. Encina (2005). Changes in root morphology accompanying mycorrhizal alleviation of phosphorus deficiency in micropropagated *Annona cherimola* Mill. plants. Sci. Hortic.106:360-369.

Parádi, I, Z. Bratek, B. Berecz, and K. Halász (2002). Influence of arbuscular mycorrhiza, P limitation and Cd-stress on polyamine contents of plants. Acta Biol. Sceged. 46:59-60.

Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat. Rev. Microbiol. 6:763-775.

Phillips, J. M., and D. S. Hayman (1970). Improved produres for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.

Poerwanto, R., H. Inoue, and I. Kataoka (1989). Effects of temperature on the morphology and physiology of the roots of trifoliate orange budded with satsuma mandarin. J. Jap. Soc. Hort. Sci. 58:267-274.

Rout, G. R., S. Samantaray, and P. Das (2000). *In vitro* rooting of *Psoralea corylifolia* Linn: Peroxidase activity as a marker. Plant Growth Regul. 30:215-219.

Same, B. I., A. D. Robson, and L. K. Abbott (1983). Phosphorus, soluble carbohydrates and endomycorrhizal infection. Soil Biol. Biochem. 15:593-597.

Schroeder, M. S., and D. P. Janos (2005). Plant growth, phosphorus nutritioni, and root morphological responses to arbuscular mycorrhizas, phosphorus fertilization, and intraspecific density. Mycorrhiza 15:203-216.

Speir, T. W., and D. J. Ross (1975). Effect of storage on the acitivities of protease, urease, phosphatase, and sulphatase in three soils under pasture. New Zealand J. Soil Sci. 18:231-237.

Wu, Q. S., Y. Levy, and Y. N. Zou (2009). Arbuscular mycorrhizae and water relationss in citrus. In: Tennant, P., and N. Benkeblia (Eds.). Citrus II. Tree For. Sci. Biotech. 3(Special Issue 1): 105-112.

Wu, Q. S., R. X. Xia, Y. N. Zou, and M. Y. Wang (2006). Effects of arbuscular mycorrhizal fungi on the growth and antioxidant enzymes of micropropagated citrus. Chin. J. Appl. Environ. Biol. 12:635-639.

Wu, Q. S., and Y. N. Zou (2009). The effect of dual application of arbusuclar mycorrhizal fungi and polyamines upon growth and nutrient uptake on trifoliate orange (*Poncirus trifoliata*) seedlings. Not. Bot. Horti Agrobot. Cluj 37:95-98.

Wu, Q. S., Y. N. Zou, and X. H. He (2010). Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. Acta Physiol. Plant. 32:297-304.

Yemm, E. W., and A. J. Willis (1954). The estimation of carbonhydrates in plant extracts by anthrone. Biochem. J. 57:508-514.

Zhang, Y., L. Y. Xie, B. Q. Xiong, and M. Zeng (2003). Effect of polyamine on growth and development of arbuscular mycorrhizal fungi *in vitro* culture condition. Mycosyst. 22:417-423.

Zhao, L. P., and Y. Jiang (1986). Research of determination of soil phosphatase activity. Chin. J. Soil Sci. 17:138-141.

Zobel, R. W., G. A. Alloush, and D. P. Belesky (2006). Differential root morphology response to no versus high phosphorus in three hydroponically grown forage chicory cultivars. Environ. Exp. Bot. 57:201-208.