INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–305/2013/15–2–342–346
http://www.fspublishers.org

*Full Length Article*

# Effects of *Diversispora spurca* Inoculation on Growth, Root System Architecture and Chlorophyll Contents of Four Citrus Genotypes

**Yan Li[1], Ying-Ning Zou[1*] and Qiang-Sheng Wu[1]**
[1]*College of Horticulture and Gardening, Yangtze University, 88 Jingmi Road, Jingzhou, Hubei 434025, People's Republic of China*
*For correspondence: zouyingning@163.com

## Abstract

A pot experiment was conducted to study the effects of arbuscular mycorrhizal fungi, *Diversispora spurca*, on growth, root system architecture (RSA), and chlorophyll contents of four citrus genotype plants, namely, kumquat (*Fortunella margarita*), red tangerine (*Citrus tangerine*), sour orange (*C. aurantium*) and trifoliate orange (*Poncirus trifolaita*), in order to assess whether the effects are dependent on host genotype. After 180 days of mycorrhizal inoculation, the order of root mycorrhizal colonization was kumquat > sour orange > trifoliate orange > red tangerine, and mycorrhizal dependency kumquat > trifoliate orange > sour orange > red tangerine. Mycorrhizal colonization significantly increased plant height, stem diameter (except sour orange), leaf number of plant, shoot and root dry weights of all the plants, and mycorrhizas had host-specific differences in growth performance. Mycorrhizal inoculation markedly increased length, projected area, surface area, volume, branch and cross of the root systems, and the increases were kumquat > red tangerine ≈ trifoliate orange > sour orange. Significantly higher chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were found in the mycorrhizal plants than in the non-mycorrhizal plants. Results suggested that inoculation with *D. spurca* promoted growth and improved both RSA and chlorophyll contents, while the defferences were host-specific. 

**Keywords:** Arbuscular mycorrhizal fungi; Citrus; mycorrhizal dependency; Root

## Introduction

Arbuscular mycorrhizal fungi (AMF), an ancient group of fungi belonging to the phylum of Glomeromycota, can establish mutualistic association with ~80% of terrestrial plants, in order to obtain photosynthates of the host plant for their asexual life cycle (Harrison, 2005). In return, AMF are primarily responsible for water and nutrient transfer from soil to the host plant.

Citrus plants are broadly grown all over the world. Meanwhile, China is one of the important citrus producing countries, where the citrus industry is expanding. As a rule, citrus roots exhibit short and poorly distributed root hairs and thereby are highly dependent on arbuscular mycorrhizal symbiosis (Wu *et al*., 2011a). A large number of reports have shown that inoculation with AMF can have many beneficial effects on citrus plants. Inoculation with *Glomus mosseae* greatly increased growth of sour orange and rough lemon under the condition of a low soil phosphorus (P) content and also caused an increase of P and copper (Cu) in leaves (Krikun and Levy, 1980). In a low P soil, *G. intraradices* increased leaf $^{14}CO_2$ incorporation by 67%, total chlorophyll content by 28%, and ribulose bisphosphate carboxylase activity by 42% in sour orange seedlings (Nemec and Vu, 1990). Additionally, in trifoliate orange seedlings, AMF colonization improved tolerance to drought stress (Wu *et al*., 2011b) and high temperature stress but not low temperature stress (Wu and Zou, 2010; Wu, 2011), and salt stresses (Wu *et al*., 2010). Therefore, arbuscular mycorrhizal symbiosis is critical for growth of citrus trees.

Although AMF specifically colonize the roots of the host plant and also exhibit low host specificity, the compatibility between AMF and the host plant is existent. Spatial arrangement of the root system architecture (RSA) in soil can determine the capacity of a plant to uptake water and mineral nutrients (de Dorlodot *et al*., 2007). It is well documented that improvement of RSA caused by AMF in trifoliate orange seedlings was dependent on AMF species (Wu *et al*., 2011a). However, to data, the information about RSA variation of AMF on citrus genotype is unclear. The present work was carried out to assess whether the effects of AMF on growth, RSA, and chlorophyll contents were dependent on citrus genotype.

## Materials and Methods

### Plant Culture

The experiment was performed in a plastic greenhouse of Yangtze University, Jingzhou, China. The citrus genotypes

**To cite this paper:** Li, Y., Y.N. Zou and Q.S. Wu, 2013. Effects of *Diversispora spurca* inoculation on growth, root system architecture and chlorophyll contents of four citrus genotype. *Int. J. Agric. Biol*., 15: 342–346

used here included kumquat (*Fortunella margarita* L. Swingle), red tangerine (*Citrus tangerine* ex. Tanaka), sour orange (*C. aurantium* L.) and trifoliate orange (*Poncirus trifolaita* L. Raf.). Seeds of citrus plants were sown in a plastic pot (20 cm upper mouth diameter × 15 cm bottom mouth diameter × 18 cm height) filled with 3.0 kg of autoclaved (121°C, 0.11MPa, 2 h) growth substrates of soil, vermiculite, and pearlite (5:1:1, v/v/v). The potted growth mixture had been inoculated with *Diversispora spurca* before transplant by placing 15 g of mycorrhizal inoculum in the rhizosphere. Non-AMF treatment was provided by the 15 g of sterilized mycorrhizal inoculum. The mycorrhizal inoculum including spores, extraradical hyphae, and infected fragmentized roots was provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China. The seedlings were grown from March 27 to September 7, 2010 in a greenhouse.

### Experimental Design

The experiment was a 2×4 factorial randomized block design including four citrus genotypes (kumquat, red tangerine, sour orange and trifoliate orange) and two mycorrhizal inoculations (with or without *D. spurca*), each with 3 replicates for a total of 24 pots (two seedlings per pot).

### Determinations of Variables

After 180 days of AMF inoculation, the mycorrhizal and non-mycorrhizal citrus seedlings were harvested. Plant height, stem diameter, and leaf number per plant were recorded before the harvest. The seedlings from each pot were divided into shoot and root. The roots were rinsed with distilled water and scanned with the EPSON Flatbed Scanner V700. The root pictures were analyzed with the WinRHIZO Pro 2007b (Regent Instruments Inc., Quebec, Canada), and the RSA traits including branch, cross, surface area, projected area, volume, and total length were obtained. Leaf chlorophyll was extracted by 80% of acetone, and its content was measured by the method of Lichtenthaler and Wellburn (1983).

A small quantity of 1~2 cm root segments were cleared with 10% of KOH and stained with 0.05% of trypan blue solution (Phillips and Hayman, 1970). Root mycorrhizal colonization was quantified based on the formula of Wu *et al*. (2007). Entry points, vesicles and arbuscules were observed with the LEICADME biomicroscope and expressed as each number per cm root.

### Statistical Analysis

The data were analyzed by ANOVA with software of SAS 8.1 version. Significant differences of means were compared with the Least Significant Difference (LSD) at 5% level.

## Results

### Mycorrhizal Colonization

Roots were not infected by the non-AMF treatment in the non-AMF citrus plants. All the citrus plants were well colonized by the *D. spurca*, and root mycorrhizal colonization in the inoculated plants ranged from 23 to 47% (Fig. 1).

### Plant Growth

Inoculation with *D. spurca* significantly enhanced plant height, stem diameter (except sour orange), leaf number of plant, shoot and root dry weights of all the citrus seedlings (Table 1). Additionally, the order of mycorrhizal dependency of the four citrus plants was kumquat (204%) > trifoliate orange (136%) > sour orange (131%) > red tangerine (127%).

Root/shoot ratio of mycorrhizal kumquat and red tangerine plants was significantly higher than those of non-mycorrhizal plants (Table 1). However, in this study, we also found no significant differences of root/shoot ratio between mycorrhizal and non-mycorrhizal sour orange and trifoliate orange plants.

### Root System Architecture

In the present work, inoculation with *D. spurca* markedly increased root length, projected area, surface area, root volume, root branch and root cross of the four citrus genotype plants, compared with non-AMF treatment (Table 2; Fig. 2). The increases of RSA traits caused by AMF in the present work were the highest response to kumquat (91.3, 94.5, 94.6, 100.0, 163.8 and 166.7%, respectively), higher red tangerine (62.0, 71.6, 71.5, 82.3, 82.7 and 85.4%, respectively) and trifoliate orange (57.8, 80.1, 80.1, 103. 4, 120.4 and 70.0%, respectively), and the lowest sour orange (28.7, 40.2, 40.3, 50.1, 59.3 and 33.6%, respectively).

### Chlorophyll

Our study showed that the mycorrhizal colonization had a conspicuous effect on chlorophyll content (Table 3). Significantly higher chlorophyll *a* content (38.6–66.3%), chlorophyll *b* content (29.1–110.5%), carotenoid content (29.1–56.1%), and total chlorophyll content (37.5–70.6%) were observed in the four AMF citrus plants than in the non-AMF ones. The increased trends of chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents caused by AMF were the highest in trifoliate orange, higher in kumquat and red tangerine, and the lowest in sour orange.

## Discussion

In this study, we confirmed that different citrus genotypes varied widely in their dependency on *D. spurca*. The root colonization ranked as kumquat > sour orange >

**Table 1:** Effect of AMF (*Diversispora spurca*) on growth traits of four citrus genotype plants

| Citrus genotype | AMF inoculation | Plant height (cm) | Stem diameter (cm) | Leaf number per plant | Dry weight (g) | | | Root/shoot ratio | Mycorrhizal dependency (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Shoot | Root | Total | | |
| Kumquat | | | | | | | | | |
| | AMF | 18.0c,x | 0.293c,x | 18.9b,x | 0.87b,x | 0.47c,x | 1.35c,x | 0.54b,y | 204 |
| | Non-AMF | 11.6c,y | 0.233c,y | 10.5c,y | 0.32c,y | 0.23c,y | 0.55c,y | 0.71ab,x | |
| Red tangerine | | | | | | | | | |
| | AMF | 15.7c,x | 0.308b,x | 18.2b,x | 0.95b,x | 0.66b,x | 1.61b,x | 0.69a,y | 127 |
| | Non-AMF | 13.2c,y | 0.278b,y | 12.7b,y | 0.62b,y | 0.52b,y | 1.14b,y | 0.84a,x | |
| Sour orange | | | | | | | | | |
| | AMF | 23.3b,x | 0.343a,x | 16.0c,x | 2.20a,x | 1.38a,x | 3.57a,x | 0.63a,x | 131 |
| | Non-AMF | 19.5b,y | 0.356a,x | 13.5b,y | 1.77a,y | 1.05a,y | 2.83a,y | 0.60b,x | |
| Trifoliate orange | | | | | | | | | |
| | AMF | 32.3a,x | 0.316b,x | 25.0a,x | 0.94b,x | 0.61b,x | 1.56bc,x | 0.65a,x | 136 |
| | Non-AMF | 27.8a,y | 0.288b,y | 21.0a,y | 0.70b,y | 0.45b,x | 1.15b,y | 0.64b,x | |
| *Significance* | | | | | | | | | |
| Citrus genotype | | ** | ** | ** | ** | ** | ** | ** | |
| AMF | | ** | ** | ** | ** | ** | ** | ** | |
| Citrus genotype×AMF | | NS | * | ** | ** | NS | * | * | |

**Table 2:** Effects of AMF (*Diversispora spurca*) on RSA traits of four citrus genotype plants

| Citrus genotype | AMF inoculation | Length (cm) | Projected area ($cm^2$) | Surface area ($cm^2$) | Volume ($cm^3$) | Branch number | Cross number |
|---|---|---|---|---|---|---|---|
| Kumquat | | | | | | | |
| | AMF | 219.31c,x | 14.90c,x | 46.81c,x | 0.80c,x | 1076.0c,x | 60.8c,x |
| | Non-AMF | 114.64c,y | 7.66c,y | 24.05c,y | 0.40c,y | 407.8c,y | 22.8b,y |
| Red tangerine | | | | | | | |
| | AMF | 508.45b,x | 30.39b,x | 95.46b,x | 1.44b,x | 3986.0b,x | 298.8ab,x |
| | Non-AMF | 313.82b,y | 17.71b,y | 55.65b,y | 0.79b,y | 2181.7b,y | 161.2a,y |
| Sour orange | | | | | | | |
| | AMF | 651.57a,x | 47.78a,x | 150.11a,x | 2.78a,x | 5269.0a,x | 246.7b,x |
| | Non-AMF | 506.33a,y | 34.07a,y | 107.02a,y | 1.84a,y | 3307.3a,y | 184.7a,y |
| Trifoliate orange | | | | | | | |
| | AMF | 510.87b,x | 27.92b,x | 87.72b,x | 1.20bc,x | 3713.3b,x | 344.3a,x |
| | Non-AMF | 323.74b,y | 15.50b,y | 48.70b,y | 0.59bc,y | 1684.7b,y | 199.0a,y |
| *Significance* | | | | | | | |
| Citrus genotype | | ** | NS | ** | ** | ** | ** |
| AMF | | ** | NS | ** | ** | ** | ** |
| Citrus genotype × AMF | | NS | NS | NS | NS | NS | NS |

**Note:** Means followed by different letters (a, b, c, etc.) in a column in AMF or non-AMF citrus plants or different letters (x, y) in a column between AMF and non-AMF citrus plants are significantly different (LSD, $P<0.05$); NS – not significant; * $P < 0.05$. ** $P < 0.01$

trifoliate orange > red tangerine, suggesting that host genotype is an important factor controlling the mycorrhizal colonization (Graham and Eissenstat, 1994).

It is well known that AMF application to horticultural plants has been studied and achieved the enhancing effects (Ortas, 2010), just like the data listed in Table 1. The AM fungal enhancement of citrus growth may be attributed to enhanced mineral nutrition of AM plants under the low P supply but not high soil P conditions (Eissenstat *et al.*, 1993; Peng *et al.*, 1993). The order of growth increases in the four citrus plants treated by exogenous AMF was kumquat > trifoliate orange > red tangerine > sour orange, suggesting that the enhancing effect of AMF on growth was dependent on citrus genotype. This result is in agreement with the report of Youpensuk *et al*. (2009), who found that growth performance was increased by mixed AMF in lime, pomelo, and tangerine varieties, but little or none in cleopatra, troyer and sweet orange. So obviously, AMF have host-specific differences in growth performance, though they can associate with all host plant generally.

Roots are a major organ for plants to absorb moisture

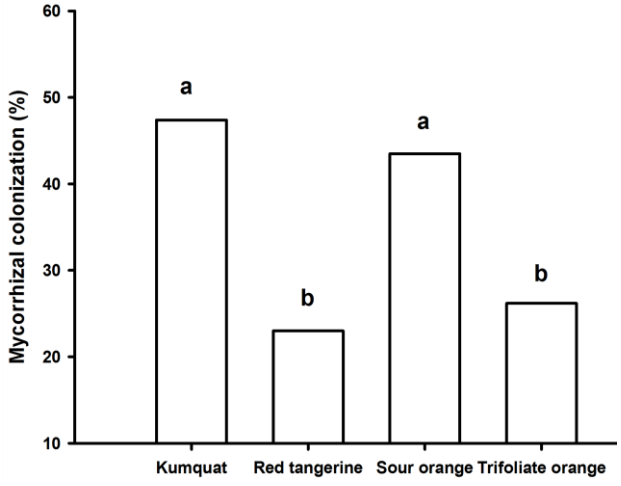


**Fig. 1:** Mycorrhizal colonization of four citrus genotype plants inoculated with *Diversispora spurca*. Means followed by the same letter within a column show no significant difference at 5% level

**Table 3:** Effects of AMF (*Diversispora spurca*) on chlorophyll *a*, *b*, carotenoid and total chlorophyll contents of four citrus genotype plants

| Citrus genotype | AMF inoculation | Chlorophyll *a* (mg/g) | Chlorophyll *b* (mg/g) | Carotenoid (mg/g) | Total chlorophyll (mg/g) |
|---|---|---|---|---|---|
| Kumquat | | | | | |
| | AMF | 2.79a,x | 0.73a,x | 0.78a,x | 3.52a,x |
| | Non-AMF | 1.87a,y | 0.55a,y | 0.58a,y | 2.42a,y |
| Red tangerine | | | | | |
| | AMF | 1.50c,x | 0.28c,x | 0.50c,x | 1.78c,x |
| | Non-AMF | 1.02b,y | 0.15cb,y | 0.38b,y | 1.17c,y |
| Sour orange | | | | | |
| | AMF | 2.19b,x | 0.44b,x | 0.89a,x | 2.64b,x |
| | Non-AMF | 1.59a,y | 0.34b,x | 0.57a,y | 1.92b,y |
| Trifoliate orange | | | | | |
| | AMF | 2.91a,x | 0.40b,x | 0.66b,x | 3.31a,x |
| | Non-AMF | 1.75a,y | 0.19c,y | 0.51a,y | 1.94b,y |
| *Significance* | | | | | |
| Citrus genotype | | ** | ** | ** | ** |
| AMF | | ** | ** | ** | ** |
| Citrus genotype×AMF | | NS | NS | * | NS |

**Note:** Means followed by different letters (a, b, c, etc.) in a column in AMF or non-AMF citrus plants or different letters (x, y) in a column between AMF and non-AMF citrus plants are significantly different (LSD, $P<0.05$); NS – not significant; * $P < 0.05$. ** $P < 0.01$

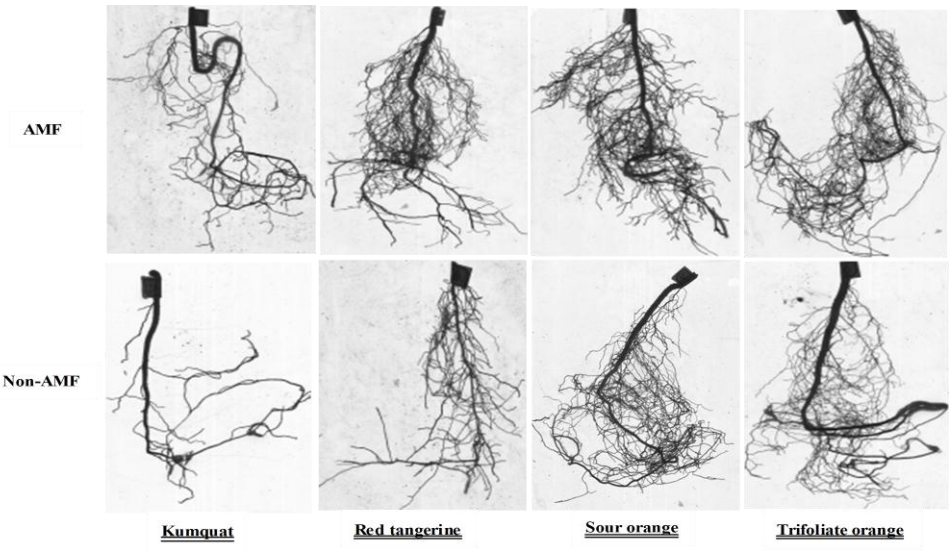


**Fig. 2:** Status of root system architecture in four citrus genotype plants inoculated with or without *Diversispora spurca*

and nutrients. RSA is an important parameter to judge the uptake capacity of the plant (Wu *et al*., 2011c). Our result showed that inoculation with *D. spurca* significantly enhanced various traits of RSA including length, surface and projected areas, volume, cross and branch, irrespectively of citrus genotype. The result is in agreement with the report conducted by Wu *et al*. (2011a). As stated in Table 2, among the four citrus genotypes, the highest root characteristics was sour orange, whereas the increase rate of RSA induced by *D. spurca* was the highest to kumquat compared with non-AMF plants. The alteration may be due to either the host plant's RSA status or the responses of host plant to specific mycorrhizal fungi.

Similarly, in the present work, colonization by *D. spurca* significantly increased leaf chlorophyll *a*, chlorophyll *b*, carotenoid, and total chlorophyll contents, irrespectively of citrus genotype, whereas the increases of chlorophyll contents induced by *D. spurca* was dependent on host genotype. The chlorophyll alteration may be attributed to the increased mineral nutrients (Misra *et al*., 2005). In general, leaf chlorophyll content is closely related to photosynthetic ability in plants (Dong *et al*., 2007). Since higher chlorophyll content was in the mycorrhizal citrus plants, it seems that the AMF citrus plants might maintain better photosynthetic characteristics than the non-AMF plants, thereby providing more photosynthetic production for sustaining mycorrhizal development and root growth.

In conclusion, inoculation with *D. spurca* significantly enhanced growth performance, RSA, and chlorophyll contents of citrus plants in different genotypes, and the effects were obviously dependent on the host genotype.

## Acknowledgements

This study was supported by the National Natural Science Foundation of China (31101513).

## References

de Dorlodot, S., B. Forster, L. Pages, A. Price, R. Tuberosa and X. Draye, 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends Plant Sci*., 12: 474–481

Dong, Y., Z. Yang, J. Xu, D. Lin, Y. Sugimoto, L. Luo and H. Mei, 2007. Quantitative trait loci for leaf chlorophyll content at two developmental stages of rice (*Oryza sativa* L.). *Commun*. *Biometry Crop Sci*., 2: 1–7

Eissenstat, D.M., J.H. Graham, J.P. Syvertsen and D.L. Drouillard, 1993. Carbon economy of sour orange in relation to mycorrhizal colonization and phosphorus status. *Ann*. *Bot*., 71: 1–10

Graham, J.H and Eissenstat DM. 1994. Host genotype and the formation and function of VA mycorrhizae. *Plant Soil*, 159: 179–185

Harrison, M.J., 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Annu*. *Rev*. *Microbiol*., 59: 19–42

Krikun, J. and Y. Levy, 1980. Effect of vesicular arbuscular mycorrhiza on citrus growth and mineral composition. *Phytoparasitica*, 8: 195–200

Lichtenthaler, H.K. and A.R. Wellburn, 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem*. *Soc*. *Trans*., 11: 591–592

Misra, A., A.K. Srivastava, N.K. Srivastava and A. Khan, 2005. Zn acquisition and its role in growth, photosynthesis, photosynthetic pigments and biochemical changes in essential monoterpene oils of *Pelargonium graveolens*. *Photosynthetica*, 43: 153–155

Nemec, S. and J.C.V. Vu, 1990. Effects of soil phosphorus and *Glomus intraradices* on growth, nonstructural carbohydrates, and photosynthetic activity of *Citrus aurantium*. *Plant Soil*, 128: 257–263

Ortas, I., 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. *Span*. *J*. *Agric*. *Res*., 8: 116–122

Peng, S., D.M. Eissenstat, J.H. Graham, K. Williams and N.C. Hodge, 1993. Growth depression in mycorrhizal citrus at high-phosphorus supply. *Plant Physiol*., 101: 1063–1071

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*. *Brit*. *Mycol*. *Soc*., 55: 158–161

Wu, Q.S., 2011. Mycorrhizal efficacy of trifoliate orange seedlings on alleviating temperature stress. *Plant Soil Environ*., 57: 459–464

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

Wu, Q.S. and Y.N. Zou, 2010. Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. *Sci*. *Hortic*., 125: 289–293

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

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

Wu, Q.S., Y.N. Zou and X.H. He, 2011b. Differences of hyphal and soil phosphatase activities in drought-stressed mycorrhizal trifoliate orange (*Poncirus trifoliata*) seedlings. *Sci*. *Hortic*., 129: 294–298

Wu, Q.S., G.H. Li and Y.N. Zou, 2011c. Improvement of root system architecture in peach (*Prunus persica*) seedlings by arbuscular mycorrhizal fungi, related to allocation of glucose/sucrose to root. *Not*. *Bot*. *Horti.c Agrobiol*., 39: 232–236

Youpensuk, S., L. Sittichai and R. Benjavan, 2009. Genotypic variation in responses of *Citrus* spp. to arbuscular mycorrhizal fungi. *J. Agric. Sci.*, 1: 59–65

**(Received 18 June 2012; Accepted 10 December 2012)**