

Regulation of Root Length and Lateral Root Number in Trifoliolate Orange Applied by Peroxide Hydrogen and Arbuscular Mycorrhizal Fungi

Chun-Yan LIU, Yong-Ming HUANG, Ying-Ning ZOU*, Qiang-Sheng WU

Yangtze University, College of Horticulture and Gardening, No 88 Jingmi Raod, Jingzhou, Hubei 434025, China;
zouyingning@163.com (*corresponding author)

Abstract

Root system morphology (RSM) in plants plays a key role in acquiring nutrients from the soil and is also altered by abiotic or biotic factors including soil microorganisms and signal molecules. The present study was made to evaluate the effects of an arbuscular mycorrhizal fungus (AMF, *Glomus versiforme*) and exogenous peroxide hydrogen (H_2O_2 , 0, 1 and 100 μM) on root length, lateral root number and activities of polyamine-metabolized enzymes in trifoliolate orange (*Poncirus trifoliata*) seedlings. After 5 months of inoculation with AMF, root mycorrhizal colonization was significantly increased by application of 1 μM H_2O_2 , but markedly restrained by 100 μM H_2O_2 . Inoculation with AMF significantly increased the taproot length and the number of second- and third-order lateral roots under 1 and 100 μM H_2O_2 application. The AMF infection significantly increased 0-1 cm classed root length and total root length, regardless of H_2O_2 concentration. In general, inoculation with AMF increased arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) activity of roots under 0, 1 and 100 μM H_2O_2 , increased diamine oxidase (DAO) activity of roots under 0 μM H_2O_2 and decreased DAO activity of roots under 1 and 100 μM H_2O_2 . Root polyamine oxidase (PAO) activity was similar between AMF and non-AMF seedlings, irrespectively of H_2O_2 concentration. Results suggest that lower concentration of H_2O_2 (1 μM) might be regarded as a signal to stimulate mycorrhizal and lateral root development through increase of ADC and ODC and decrease of DAO, while high concentration of H_2O_2 (100 μM) as a toxic compound of reactive oxygen species restricted AMF colonization.

Keywords: arginine decarboxylase, *Glomus versiforme*, ornithine decarboxylase, root morphology

Introduction

Root is a vital plant organ which absorbs water and nutrients from the soil. Meanwhile, root system morphology (RSM), the configuration and structure of the root system, can determine the transport capacity of root in soil nutrient and water (de Dorlodot *et al.*, 2007). In general, rhizospheric nutritional status and microorganisms would highly impact RSM (Osmont *et al.*, 2007; Chapman *et al.*, 2011; Sato and Miura, 2011).

Arbuscular mycorrhizal fungi (AMF), a kind of soil inhabitant microorganism, can form a mutual symbiont with the roots of ~80% of land plants, thus enhancing the uptake of mineral nutrition and water from the soil to the host plant, as well as withstanding various adversities (Koide and Mosse, 2004). Studies showed that inoculation with AMF could alter the RSM traits and promote the formation of lateral roots of higher order for the host plant (Yao *et al.*, 2009). The AMF-mediated RSM improvement may be related to increased plant hormone biosynthesis proposed by Kaldorf and Ludwig-Müllerb (2000) in maize colonized by *Glomus intraradices*. Wu *et al.* (2011) found that the RSM alteration by mycorrhization may be due to the AMF-induced alteration of carbohydrates and peroxidase. However, other studies also revealed that AMF

colonization did not alter RSM traits. Atkinson *et al.* (2003) found that root branching of *Trifolium repens* was not significantly affected by inoculation with *G. mosseae*. Herdlera *et al.* (2008) reported that the total root length and the root diameter of rice plants were notably decreased by the *G. intraradices* inoculation.

Hydrogen peroxide (H_2O_2) as one of reactive oxygen species (ROS) generally acts a key regulator in a broad range of physiological processes, such as senescence (Peng *et al.*, 2005), stomatal movement, cell cycle and growth and development (Deng *et al.*, 2012). The H_2O_2 excess accumulation in plants could be used as ROS to induce oxidative burst in cells, thereby triggering cell death. On the other hand, H_2O_2 is also regarded as an important signal compound concerning the interaction of plants with pathogenic microorganisms (Apel and Hirt, 2004; Laloi *et al.*, 2004), as well as many other processes referring to root biology, such as gravitropism, root elongation growth and root hair development (Zolla *et al.*, 2010; Jiang *et al.*, 2012). However, the interaction between AMF and H_2O_2 on RSM traits of the host plant is poorly known.

Diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm), three types of polyamine (PAs), are formed either by the direct decarboxylation of L-ornithine through ornithine decarboxylase (ODC) or by

decarboxylation from L-arginine to agmatine by arginine decarboxylase (ADC). The decomposition of PAs takes place under the two enzymes, diamine oxidase (DAO) and polyamine oxidase (PAO) (Kaur-Sawhney *et al.*, 2003). It is well documented that PAs play important roles in mycorrhizal formation, plant growth, organ development, fruit formation and leaf senescence (Kusano *et al.*, 2008; Wu *et al.*, 2012b). In root development, accumulation of PAs is involved in the formation of adventitious root (Couée *et al.*, 2004). Exogenous PAs could significantly promote the spore germination and hyphal growth of *G. mosseae* and *Gigaspora margarita*, *in vitro* culture conditions (Zhang *et al.*, 2003). Wu *et al.* (2012a) observed that AMF-mediated RSM improvement was related only to ADC and ODC, without any PAs concentrations. It seems that PA synthetase and degraded enzymes, except PA levels, were associated with AMF-mediated RSM. In addition, PA degradation may also act as a pathway to generate H₂O₂, which is considered to be an active secondary messenger (Vandenabeele *et al.*, 2003). However, so far, in plants, the potential relationship between RSM and PA-metabolized enzymes under H₂O₂ and mycorrhization conditions has not been examined in detail.

The aims of the present work were to (1) clarify the integrated effects of AMF and H₂O₂ on root length, lateral root number and PA-metabolized enzymes of citrus and to (2) evaluate the relationship between RSM and PA-metabolized enzymes.

Materials and methods

Plant culture

Seeds of trifoliolate orange [*Poncirus trifoliata* (L.) Raf.] were surface-sterilized with 70% ethanol solution for 5 min, washed with distilled water and then sown into a plastic pot (18 cm upper diameter × 13 cm below diameter × 17 cm height) including 2.5 kg autoclaved (121 °C, 0.11 Mpa, 2 h) soil. The inoculums of *Glomus versiforme* (40 g) including spore and extraradical hyphae were placed below 5 cm of the soil surface at the time of sowing. The non-AMF pots were also supplied with 40 g sterilized inoculums as the control.

Experimental design

The experiment consisted in a completely randomized design with two factors: inoculation with or without an AMF (*G. versiforme*) and application of exogenous H₂O₂ with 0, 1 and 100 μM. Each treatment had three replicates, reaching a total of 18 pots. Three months after mycorrhizal inoculation, different concentrations (0, 1 and 100 μM) of H₂O₂ were weekly applied into the designed pots, nine times during the experiment.

Measurement of plant growth and root colonization

The AM and non-AM plants were harvested after 63 days of exogenous H₂O₂ treatments. Before harvest, plant height, stem diameter and leaf number were directly determined.

Root mycorrhizal colonization was measured by the method of Phillips and Hayman (1970) after clearing with

10% KOH and staining with 0.05% trypan blue.

Determinations of root system morphology and lateral root number

The root systems were carefully taken out from the plastic pots and the intact root systems were scanned immediately by the Epson Perfection V700 Photo Dual Lens System (Seiko Epson Corp, Japan). The images of root systems were analyzed by a WinRHIZO version from 2007 (Regent Instruments Incorporated, Canada) and the 0-1, 1-2, 2-3, 3-4 and >4 cm classed root lengths were automatically obtained. Taproot lengths were measured by a flexible rule and the number of lateral roots was artificially quantified.

Determinations of PA-metabolized enzyme activities

Activities of PA synthetases (ADC and ODC) and PA-degrading enzymes (PAO and DAO) were determined according to the methods previously described by Wu *et al.* (2012a).

Statistical analyses

Data were subjected to the analysis of variance (ANOVA) with SAS v8.1, and the significant differences among treatments were used for comparison to the Duncan test at P<0.05.

Results and discussion

Mycorrhizal colonization

After inoculation with *G. versiforme*, root colonization of the trifoliolate orange seedlings ranged from 55.28 to 75.91% (Fig. 1). Compared to 0 μM H₂O₂ control, 1 μM H₂O₂ significantly increased AMF colonization by 16.6%, whereas 100 μM H₂O₂ level notably decreased mycorrhizal infection by 15.1%. There is increasing evidence indicating that during the early establishment of a symbiotic relationship, many plant signals and fungal perception are involved in the process (Requena *et al.*, 2007). It seems that the low concentration of H₂O₂ (1 μM) might be regarded as a signal molecule to stimulate mycorrhizal development, while high concentration of H₂O₂ (100 μM) as a toxic compound of ROS restricted AMF colonization.

Growth performance

The present study showed that exogenous H₂O₂ and AMF colonization did not significantly alter the stem diameter of the seedlings (Tab. 1). Leaf number per plant was significantly higher in AMF than in non-AMF seedlings under 0 and 100 μM H₂O₂, except treatments under 1 μM H₂O₂ (Tab. 1). Compared to non-AMF seedlings, AMF seedlings showed notably higher plant height under 0 μM H₂O₂ conditions, but lower under 100 μM H₂O₂ conditions. In addition, compared to 0 μM H₂O₂, 1 and 100 μM H₂O₂ generally increased plant height and leaf number, suggesting that the low or high concentration of H₂O₂ did not absolutely interfere with the

plant growth performance. Previous studies had shown that AMF could facilitate the growth of the host plants, attributed to extraradical hyphal absorption of water and mineral nutrients (Sharif and Claassen, 2011). Although low concentrations of H₂O₂ promoted root AMF colonization, the H₂O₂-induced increase of root colonization, they did not show significant effects on plant height, stem diameter and leaf number (Tab. 1). There was a significantly interacted effect of AMF and H₂O₂ on leaf

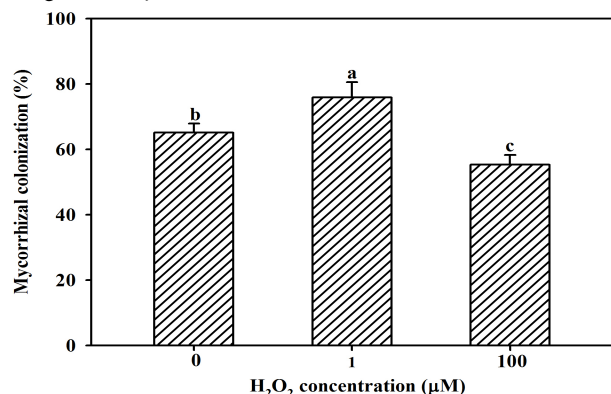


Fig. 1. Effect of exogenous H₂O₂ on AMF colonization of *Glomus versiforme*-inoculated trifoliolate orange (*Poncirus trifoliata*) seedlings. Data (means±SE, n=3) followed by the different letter above the bars are significantly different at P<0.05.

Tab. 1. Effect of *Glomus versiforme* alone or in combination with exogenous H₂O₂ on growth performance of trifoliolate orange (*Poncirus trifoliata*) seedlings

H ₂ O ₂ concentration (µM)	Inoculation	Plant height (cm)	Stem diameter (cm)	Leaf number per plant
0	Non-AMF	17.0±1.7d	0.227±0.005a	15±0c
	AMF	20.9±0.3bc	0.237±0.018a	18±1b
1	Non-AMF	20.3±0.6c	0.237±0.011a	19±0ab
	AMF	21.5±1.1bc	0.236±0.016a	18±0b
100	Non-AMF	24.2±0.8a	0.249±0.027a	18±0b
	AMF	22.3±1.1b	0.250±0.013a	20±2a

Note: The data (means±SE, n=3) in a column followed by different letters are significantly different at 5% level.

number.

Number of lateral roots

Our results showed that the root systems of the five months old trifoliolate orange seedlings were primarily dominated by first-order and second-order lateral roots (Tab. 2). Mycorrhizal inoculation significantly increased taproot length compared to non-mycorrhizal seedlings, irrespectively of exogenous H₂O₂ levels.

A study showed that inoculation with *Paraglomus occultum* significantly increased number of the lateral roots in *Citrus tangerina* plants (Wu et al., 2012b). The present study indicated that the AMF and exogenous H₂O₂ significantly influenced the number of the second-order and third-order lateral roots, but did not alter the number of the first-order lateral roots. This result is in compliance with the previous reports by Zai et al. (2007), who found that there

was no significant influence on the number of first-order lateral roots between AM and non-AM trifoliolate orange seedlings, but the number of the second-order lateral roots were markedly increased by mycorrhization.

Different classed root lengths

In the present study, the trifoliolate orange seedlings in total root lengths depended on 0-1 cm classed root lengths (Tab. 3). Compared to non-AMF control, *G. versiforme* significantly increased 0-1 cm classed root lengths by 12.6, 10.6 and 7.3% under 0, 1 and 100 µM H₂O₂, respectively. In addition, the AMF seedlings showed higher total root lengths than the non-AMF controls, regardless of exogenous H₂O₂ concentration. The results imply that AMF highly induced elongation of lateral roots, which is not dependent on exogenous H₂O₂ level. Yao et al. (2009) reported that the fine roots lengths in trifoliolate orange seedlings colonized by *Gigaspora margarita*, *G. mosseae*, *G. versiforme* and *G. caledonium* were with 35.0, 35.1, 30.5 and 45.5% higher than in non-AMF seedlings. In addition, AMF and exogenous H₂O₂ generally did not alter 1-2, 2-3, and >4 cm classed root lengths, suggesting that the AMF and exogenous H₂O₂ mainly regulated the fine roots lengths

Tab. 2. Effects of *Glomus versiforme* and exogenous H₂O₂ on taproot length and number of lateral roots in trifoliolate orange (*Poncirus trifoliata*) seedlings

H ₂ O ₂ concentration (µM)	Inoculation	Taproot length (cm)	Number of lateral roots		
			First-order	Second-order	Third-order
0	Non-AMF	18.2±0.6c	40±2a	74±3c	3±0cd
	AMF	26.5±1.5a	39±6a	60±3bc	7±0ab
1	Non-AMF	21.8±1.2b	38±11a	81±2b	4±2c
	AMF	26.2±0.9a	43±9a	89±5a	5±1bc
100	Non-AMF	22.7±0.9b	44±1a	66±3d	2±2d
	AMF	26.3±1.5a	44±1a	78±3bc	8±1a

Note: The data (means±SE, n=3) in a column followed by different letters are significantly different at 5% level.

(e.g., 0-1 cm classed root lengths).

Activities of PA synthetases ADC and ODC

Studies demonstrated that PAs are an important regulatory factor involved in the process of mycorrhizal formation (Wu et al., 2012b) and that the formation of PAs derives either from L-ornithine by ODC or from L-arginine by ADC, being disintegrated by PAO or DAO (El Meskaoui and Trembaly, 2009). In the present study, ADC activity of both leaves and roots was generally significantly higher under AMF than under non-AMF treatment under 0 and 100 µM H₂O₂ levels, while under 1 µM H₂O₂ concentration AMF seedlings recorded higher ADC activity of roots and lower ADC activity of leaves than non-AMF seedlings (Fig. 2a, 2b). ODC activity of leaves was similar between AM and non-AM seedlings under 1 and 100 µM and was significantly higher in AM than in non-AM seedlings grown in 0 µM H₂O₂ level (Fig. 2c). The *G. versiforme* increased the ODC activity of roots by 135.9, 3.8 and 7.8% under 0, 1 and 100 µM H₂O₂, respectively (Fig.

Tab. 3. Effect of *Glomus versiforme* and exogenous H₂O₂ on the different classed root lengths of trifoliolate orange (*Poncirus trifoliata*) seedlings

H ₂ O ₂ concentration (μM)	Inoculation	Lengths of different classed roots (cm)					Total
		0-1 cm	1-2 cm	2-3 cm	3-4 cm	>4 cm	
0	Non-AMF	183.5±4.4b	6.1±2.8a	3.9±1.4a	0.4±0.6b	0.0±0.0a	193.4±3.6c
	AMF	206.8±5.8a	6.7±1.6a	3.9±0.9a	0.8±0.5ab	0.1±0.1a	218.6±6.3a
1	Non-AMF	188.6±0.9b	7.2±2.9a	4.4±1.3a	0.5±0.3ab	0.1±0.2a	200.8±1.7bc
	AMF	208.6±8.7a	5.9±1.3a	4.4±0.7a	1.5±0.7a	0.2±0.2a	220.8±10.4a
100	Non-AMF	193.6±4.2b	7.0±0.2a	3.9±1.5a	0.6±0.1ab	0.1±0.1a	205.5±5.3b
	AMF	207.7±7.4a	6.4±2.4a	4.1±1.4a	1.1±0.7ab	0.1±0.1a	219.5±5.4a

Note: The data (means±SE, n=3) in a column followed by different letters are significantly different at 5% level.

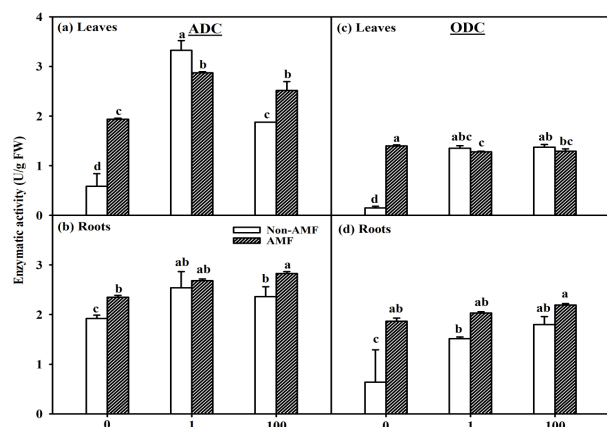


Fig. 2. Influence of *Glomus versiforme* and exogenous H₂O₂ on activity of PA synthetic enzymes (ADC and ODC) in leaves and roots of trifoliolate orange (*Poncirus trifoliata*) seedlings. Data (means±SE, n=3) followed by the same letter above the bars are not significantly different at $P<0.05$

2d). It seems that AMF plants presented higher ADC and ODC activity of roots, irrespectively of exogenous H₂O₂ concentrations. It is well known that ADC is generally associated with cell expansion and ODC with cell division (Paschalidis and Roubelakis-Angelakis, 2005). Wu *et al.* (2012a) revealed that AMF-mediated RSM was related to the change of ADC and ODC activity. Therefore, in AMF seedlings, greater activity of ADC and ODC would benefit the development of lateral roots.

Activities of PA-degrading enzyme DAO and PAO

As compared to that of the non-mycorrhizal seedlings, DAO and PAO activities in leaves of the mycorrhizal seedlings decreased by 31.0 and 19.4% (Fig. 3a, 3c) and DAO and PAO in roots by 19.6 and 7.0% under 100 μM H₂O₂ treatment, respectively (Fig. 3b, 3d). Under 1 μM H₂O₂ treatment, DAO and PAO activities in leaves and roots were similar between AMF and non-AMF seedlings. Under 0 μM H₂O₂ concentration conditions, PAO activity in leaves and roots of AMF and non-AMF seedlings was not significantly different, but lower leaf DAO and higher root DAO were found in AMF seedlings. DAO and PAO are not only involved in the degradation of PAs, but also in the production of H₂O₂, during root cell growth and

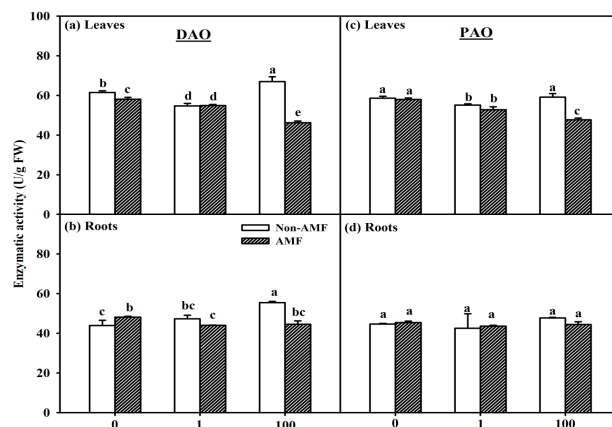


Fig. 3. Effects of *Glomus versiforme* and exogenous H₂O₂ on activity of PA-metabolic enzymes (DAO and PAO) in leaves and roots of trifoliolate orange (*Poncirus trifoliata*) seedlings. Data (means±SE, n=3) followed by the same letter above the bars are not significantly different at $P<0.05$

differentiation (Wisniewski *et al.*, 2000). Since no significant differences in PAO of roots were observed between AMF and non-AMF trifoliolate orange seedlings under 1 and 100 μM H₂O₂ application, higher total root length and number of second-order and third-order lateral roots in AMF seedlings were not related to PAO.

Conclusions

Low concentration (1 μM) of exogenous H₂O₂ may act as an important regulating factor of mycorrhizal development in trifoliolate orange seedlings inoculated with *Glomus versiforme*, but high concentration (100 μM) of exogenous H₂O₂ may confer oxidative damage and thus restrict root mycorrhizal colonization. In addition, under application of a low H₂O₂ concentration (1 μM) conditions, AMF colonization significantly increased the number of second-order later root and total roots length (mainly 0-1 cm classed roots length), which were related to the increase of ADC and ODC and the decrease of DAO in roots.

Acknowledgements

This research was supported by the National Natural

Science Foundation of China (31101513).

References

- Apel K, Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Ann Rev Plant Biol* 55:373-399.
- Atkinson D, Black KE, Forbes PJ, Hooker JE, Baddeley JA, Watson CA (2003). The influence of arbuscular mycorrhizal colonisation and environment on root development in soil. *Eur J Soil Sci* 54:751-757.
- Chapman N, Whalley WR, Lindsey K, Miller AJ (2011). Water supply and not nitrate concentration determines primary root growth in *Arabidopsis*. *Plant Cell Environ* 34:1630-1638.
- Couée I, Hummel I, Sulmon C, Gouesbet G, Amrani AE (2004). Involvement of polyamines in root development. *Plant Cell Tiss Org Cult* 76:1-10.
- de Dorlodot S, Forster B, Pages L, Price A, Tuberosa R, Draye X (2007). Root system architecture: opportunities and constraints for genetic improvement of crops. *Trend Plant Sci* 12:474-481.
- Deng XP, Cheng YJ, Wu XB, Kwak SS, Chen W, Eneji AE (2012). Exogenous hydrogen peroxide positively influences root growth and metabolism in leaves of sweet potato seedlings. *Aust J Crop Sci* 6:1572-1578.
- El Meskaoui A, Trembaly FM (2009). Effects of exogenous polyamines and inhibitors of polyamine biosynthesis on endogenous free polyamine contents and the maturation of white spruce somatic embryos. *Afr J Biotech* 8:6807-6816.
- Herdler S, Kreuzer K, Scheu S, Bonkowski M (2008). Interactions between arbuscular mycorrhizal fungi (*Glomus intraradices*, Glomeromycota) and amoebae (*Acanthamoeba castellanii*, Protozoa) in the rhizosphere of rice (*Oryza sativa*). *Soil Biol Biochem* 40:660-668.
- Jiang J, Su M, Wang L, Jiao C, Sun Z, Cheng W, Li F, Wang C (2012). Exogenous hydrogen peroxide reversibly inhibits root gravitropism and induces horizontal curvature of primary root during grass pea germination. *Plant Physiol Biochem* 53:84-93.
- Kaldorf M, Ludwig-Müller J (2000). AM fungi might affect the root morphology of maize by increasing indole-3-butyrac acid biosynthesis. *Physiol Plant* 109:58-67.
- Kaur-Sawhney R, Tiburcio AF, Altabella T, Galston AW (2003). Polyamines in plants: An overview. *J Cell Mol Biol* 2:1-12.
- Koide RT, Mosse B (2004). A history of research on arbuscular mycorrhiza. *Mycorrhiza* 14:145-163.
- Kusano T, Berberich T, Tateda C, Takahashi Y (2008). Polyamines: essential factors for growth and survival. *Planta* 228:367-381.
- Laloi C, Apel K, Danon A (2004). Reactive oxygen signalling: the latest news. *Curr Opin Plant Biol* 7:323-328.
- Osmont KS, Sibout R, Hardtke CS (2007). Hidden branches: developments in root system architecture. *Ann Rev Plant Biol* 58:93-113.
- Peng LT, Jiang YM, Yang SZ, Pan SY (2005). Accelerated senescence of fresh-cut Chinese water chestnut tissues in relation to hydrogen peroxide accumulation. *J Plant Physiol Mol Biol* 31:527-532.
- Phillips JM, Hayman DS (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.
- Requena N, Serrano E, Ocon A, Breuninger M (2007). Plant signals and fungal perception during arbuscular mycorrhiza establishment. *Phytochem* 68:33-40.
- Sato A, Miura K (2011). Root architecture remodeling induced by phosphate starvation. *Plant Signal Behav* 6:1122-1126.
- Sharif M, Claassen N (2011). Action mechanisms of arbuscular mycorrhizal fungi in phosphorus uptake by *Capsicum annuum* L. *Pedosphere* 21:502-511.
- Vandenabeele S, Van Der Kelen K, Dat J, Gadjev I, Boonefaes T, Morsa S, Rottiers P, Slooten L, Van Montagu M, Zabeau M (2003). A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. *Proc Natl Acad Sci* 100:16113-16118.
- Wisniewski JP, Rathbun EA, Knox JP, Brewin NJ (2000). Involvement of diamine oxidase and peroxidase in insolubilization of the extracellular matrix: implications for pea nodule initiation by *Rhizobium leguminosarum*. *Mol Plant Microbe Interact* 13:413-420.
- Wu QS, He XH, Zou YN, Liu CY, Xiao J, Li Y (2012a). Arbuscular mycorrhizas alter root system architecture of *Citrus tangerine* through regulating metabolism of endogenous polyamines. *Plant Growth Regul* 68:27-35.
- Wu QS, Zou YN, He XH, Luo P (2011). Arbuscular mycorrhizal fungi can alter some root characters and physiological status in trifoliolate orange (*Poncirus trifoliata* L. Raf.) seedlings. *Plant Growth Regul* 65:273-278.
- Wu QS, Zou YN, Liu CY, Lu T (2012b). Interacted effect of arbuscular mycorrhizal fungi and polyamines on root system architecture of citrus seedlings. *J Integr Agric* 11:1675-1681.
- Yao Q, Wang LR, Zhu HH, Chen JZ (2009). Effect of arbuscular mycorrhizal fungal inoculation on root system architecture of trifoliolate orange (*Poncirus trifoliata* L. Raf.) seedlings. *Sci Hortic* 121:458-461.
- Zhang Y, Xie LY, Xiong BQ, Zeng M (2003). Effect of polyamine on growth and development of arbuscular mycorrhizal fungi *in vitro* culture condition. *Mycosyst* 22:417-423.
- Zai XM, Qin P, Wan SW, Zhao FG, Wang G, Yan DL, Zhou J (2007). Effects of arbuscular mycorrhizal fungi on the rooting and growth of beach plum (*Prunus maritima*) cuttings. *J Hortic Sci Biotechnol* 82:863-866.
- Zolla G, Heimer YM, Barak S (2010). Mild salinity stimulates a stress-induced morphogenic response in *Arabidopsis thaliana* roots. *J Exp Bot* 61:211-224.