INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–093/MFA/2010/12–4–576–580
http://www.fspublishers.org

Full Length Article

Exogenous Putrescine, not Spermine or Spermidine, Enhances Root Mycorrhizal Development and Plant Growth of Trifoliate Orange (*Poncirus trifoliata*) Seedlings

QIANG-SHENG WU[1], YING-NING ZOU AND XIN-HUA HE†
College of Horticulture and Gardening, Yangtze University, No. 88 Jingmi Road, Jingzhou, Hubei Province 434025, People's Republic of China
†*School of Natural Sciences, Edith Cowan University, Joodalup, WA 6027 Australia*
[1]Corresponding author's e-mail: wuqiangsh@163.com

ABSTRACT

In a pot experiment, with an arbuscular mycorrhizal fungus (AMF) *Glomus versiforme* inoculation, we investigated effects of three polyamines (PAs) putrescine (Put), spermidine (Spd) and spermine (Spm) on root development, plant growth and biomass production of 4-months-old trifoliate orange (*Poncirus trifoliata*). Compared to the sole AMF inoculation, addition of Put increased mycorrhizal colonization and numbers of entry points, arbuscules and vesicles significantly. Addition of Spm significantly increased mycorrhizal colonization and arbuscules, whilst addition of Spd had no effect on mycorrhizal development. Mycorrhizal plants had significantly higher root total length, projected area, surface area and volume than non-mycorrhizal plants. Compared to the sole AMF treatment, total root length, projected area, surface area and root volume were significantly increased by the Put treatment. Only total root surface area and root volume were significantly increased by the addition of Spm, while no obvious improvement on root development was observed by the Spd supply. Compared to the sole AMF treatment, all the growth parameters and biomass production of both shoots and roots were significantly enhanced by Put treatment, but not by Spd treatment. Our results demonstrated that citrus plant performance and root morphology could be enhanced by dual application of *G. versiforme* and exogenous Put.

Key Words: Arbuscular mycorrhiza; *Glomus versiforme*; Polyamine; *Poncirus trifoliata*; Putrescine; Root morphology

INTRODUCTION

Polyamines (PAs), including diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm), are organic compounds with two or more primary amino groups that exist ubiquitous in plant cells. They play important roles in regulation of DNA replication and cell division, controlling of morphogenesis and senescence, resistance to environmental stresses (Galston & Sawhney, 1990; Martin-Tanguy, 2001; Kaur-Sawhney *et al*., 2003; Couée *et al*., 2004). It is documented that PAs have profound effects on plant growth and development (Farooq *et al*., 2007). Arbuscular mycorrhizal (AM) symbiosis, a common association between soil AM fungi (AMF) and plant roots, improves water and nutrient uptake like phosphorus, nitrogen and micronutrients and thus enhance plant growth (Smith & Read, 2008; Goussous & Mohammad, 2009).

Studies have shown the effects of PAs on mycorrhization or AMF on PAs contents. On one hand, percentage of mycorrhizal infection and appressoria numbers in pea myc$^+$ (*Glomus intraradices*) lines were significantly increased by exogenous Put, Spm and Spd (El Ghachtouli *et al*., 1995). So did the spore germination and hyphal growth of *G. mosseae* and *Gigaspora margarita in vitro* (El Ghachtouli *et al*., 1996b; Zhang *et al*., 2003). Hyphal growth of *G. fistulosum* in maize (*Zea mays*) root segments was stimulated by 1.5 µmol L^{-1} Spm, but inhibited by 150 and 500 µmol L^{-1} Spm (Hrselová & Gryndler, 2000). On the other hand, Put, Spd or Spm contents were increased in *G. fasciculatum* inoculated alfalfa (*Medicago sativa*) (Goicoechea *et al*., 1998) and *G. intraradices* inoculated narrow-leaf bird's-foot trefoil (*Lotus glaber*) (Sannazzaro *et al*., 2007), but generally decreased in *G. fasciculatum* inoculated narrowleaf plantain (*Plantago lanceolata*) (Parádi *et al*., 2002; 2003a & b).

Trifoliate orange [*Poncirus trifoliata* (L.) Raf.], a close relative of *Citrus,* is greatly demanded as the main rootstock for citrus plantation in China. *P. trifoliata* has relatively short root hairs in the field (Poerwanto *et al*., 1989) and is dependent on AM *Glomus* species (Graham & Syvertsen, 1985; Davies & Albrigo, 1994). At present, no information is available as if PAs could affect the growth of trifoliate orange. Furthermore no studies have addressed the

To cite this paper: Wu, Q.S., Y.N. Zou and X.H. He, 2010. Exogenous putrescine, not spermine or spermidine, enhances root mycorrhizal development and plant growth of trifoliate orange (*Poncirus trifoliata*) seedlings. *Int. J. Agric. Biol*., 12: 576–580

interactions between AMF and PAs on plant growth, particularly on root development. Combined with or without an AM fungus *G. versiforme* inoculation, the objectives of the present work were to evaluate effects of three exogenously applied PAs (Put, Spd or Spm) on (1) root mycorrhization development, (2) root morphological traits and (3) plant growth of 4-months-old trifoliate orange seedlings.

MATERIALS AND METHODS

Plant and fungal materials: Seeds of trifoliate orange were sterilized with 70% alcohol for 5 min and rinsed with distilled water. Germinated seeds were sowed in plastic pots (20 cm diameter with 16 cm depth) containing an autoclaved mix of xanthi-udic ferralsols, vermiculite and perlite (2:1:1 volume), whose characteristics are pH 6.4 and available phosphorus 20.07 mg kg^{-1}. Before sowing, 15 g mycorrhizal inocula of *Glomus versiforme* (Karsten) Berch (BGC NM04B, a mixture of soil, spores, hyphae & root material from Beijing Academy of Agriculture and Forestry Sciences) were applied on the surface of the growth media at 5 cm depth. The non-mycorrhizal controls received the same amount of autoclaved *G. versiforme* inoculum (about 15 g) plus fungi-free inoculum filtrate. Plants were grown in a greenhouse in the Yangtze University's campus, with day/night temperature at 25/18±5°C and relative humidity at 75±15% between April and July 2008, irrigated with distilled water depending on the vigor. Three uniform five-leaf size seedlings were selected at 60 days of sowing in each pot for later PAs treatments.

Experiment design: An incomplete design was used considering the effect of dual mycorrhizal fungi and PAs on mycorrhization and plant growth, but not the effect of single PAs on plant growth. The incomplete five treatments were as follows: (1) non-exogenous PA plus non-mycorrhizal control (non-PA+non-AMF), (2) AM *G. versiforme* only (non-PA+AMF), (3) exogenous Put plus *G. versiforme* (Put+AMF), (4) exogenous Spd plus *G. versiforme* (Spd+AMF) and (5) exogenous Spm plus *G. versiforme* (Spm+AMF). Each treatment had three replicates each for a total of 15 pots (three seedlings/pot). A total of 320 mL of 0.01% Put, Spd or Spm (Sigma, USA) was exogenously applied to the designed pot when plants were 2-months-old seedlings. The control treatment received 320 mL distilled water.

Determination of mycorrhization and plant growth: Seedlings were harvested two months after the exogenous PAs treatments, and plant height, stem diameter, leaf area and leaf number per plant were recorded. All fresh roots of the three seedlings in each pot were carefully washed by tap water and directly placed on the Regent's water-proof trays. Image of the root system was acquired using an extra optimized Epson Expression/STD 4800 scanner and analyzed with the WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Root morphological traits including average diameter, total root length, projected area, surface area and volume were automatically imaged and recorded. Shoot and root were separately dried at 75°C for 48 h in a force hot-air oven.

After root scanning, one-cm fresh fine root pieces was cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol (Smith & Dickson, 1997). Percentage of AM colonization and mycorrhizal structures including entry points, vesicles and arbuscules were microscopically examined (Wu *et al*., 2008). These fine roots were also oven-dried (48 h at 75°C) and dry weight was recorded.

Statistical analysis: Data were arc-sine transformed if zero values existed and then analyzed by ANOVA (SAS, Version 8.1). Differences in means (n=3) were compared and considered by Fisher's Protected Least Significance Difference at $P < 0.05$.

RESULTS

No AM colonization was found in the non-PA+non-AMF controls, while inoculation with *G. versiforme* alone achieved 36 % root AM colonization (Table I; Fig. 1). Compared to the non-PA+AMF treatment, the dual Spd+AMF treatment did not increase AM colonization and numbers of arbuscules and vesicles and even reduced entry point numbers; while the dual AMF+Spm treatment significantly increased AMF colonization and arbuscule numbers, but not entry point and vesicle numbers. Among the three PA+AMF treatments, the Put+AMF treatment had significantly greatest AM colonization, numbers of entry point, arbuscule and vesicle, except arbuscules in the Spm+AMF treatment. The Spm+AMF treatment had similar AM mycorrhization to the Spd+AMF treatment, except arbuscules numbers.

For plant growth and biomass production, compared to the non-PA+non-AMF control, plant height, stem diameter, leaf number, leaf area, shoot and root biomass production were significantly increased by the sole AM treatment, except the root/shoot ratio (Table II). Compared to the sole non-PA+AMF treatment, both the Put and the Spm treatments had significant greater plant height, stem diameter, leaf number, leaf area, root/shoot ratio, shoot and root biomass production, respectively except for the plant height, stem diameter and leaf numnber in the Spm+AMF treatment. There were no significant differences in plant growth and biomass production between the Spm+AMF and the Spd+AMF treatment.

For root morphological traits, no significant difference was found in the average root diameter (Table III), no matter with or without the application of sole AMF or dual PA+AMF. Compared to the non-PA+non-AMF control, the sole non-PA+AMF treatment increased total length, total projected area and total surface area, but not total root volume (Table III). All five investigated root morphological traits (root diameter, total length, projected area, surface area & volume) were significantly greatest in the dual Put+AMF

Table I: Effects of *G. versiforme* and/or exogenous PAs on the AM mycorrhization of trifoliate orange seedlings

Parameters	Treatments				
	Put +AMF	Spm + AMF	Spd + AMF	Non-PA + AMF	Non-PA + non-AMF
AM colonization (%)	61.3±1.0a	40.6±1.4b	36.6±3.6bc	36.1±2.4c	0
Arbuscules (no. cm^{-1} root)	3.1±0.3a	3.0±0.2a	1.9±0.2b	1.8±0.3b	0
Entry points (no. cm^{-1} root)	2.0±0.4a	1.2±0.2b	0.7±0.3c	1.2±0.2b	0
Vesicles (no. cm^{-1} root)	2.5±0.6a	1.4±0.2b	1.4±0.2b	1.2±0.3b	0

Table II: Effects of *G. versiforme* and/or exogenous PAs on plant growth and biomass production of trifoliate orange seedlings

Parameters	Treatments				
	Put +AMF	Spm + AMF	Spd + AMF	Non-PA + AMF	Non-PA + non-AMF
Plant height (cm)	17.27±1.27a	15.27±1.37b	15.07±0.67b	15.27±0.31b	12.70±1.31c
Stem diameter (cm)	0.214±0.009a	0.202±0.006b	0.200±0.005b	0.198±0.005b	0.180±0.006c
Leaf number	17.4±0.8a	15.5±0.9ab	16.4±1.4ab	14.8±0.9b	12.7±1.3c
Leaf area (cm^2 $plant^{-1}$)	17.3±0.9a	13.5±0.9b	12.5±0.6b	10.6±1.1c	8.9±1.0d
Shoot dry weight (g $plant^{-1}$)	0.34±0.03a	0.28±0.03b	0.26±0.02bc	0.24±0.02c	0.19±0.01d
Root dry weight (g $plant^{-1}$)	0.16±0.02a	0.12±0.01b	0.11±0.01bc	0.10±0.01c	0.07±0.01d
Root/shoot ratio	0.46±0.02a	0.45±0.02ab	0.42±0.02bc	0.41±0.01cd	0.39±0.01d

Table III: Effects of *G. versiforme* and/or exogenous PAs on root morphology of trifoliate orange seedlings

Parameters	Treatments				
	Put + AMF	Spm + AMF	Spd + AMF	Non-PA + AMF	Non-PA + non-AMF
Average diameter (mm)	0.47±0.03a	0.48±0.02a	0.46±0.01a	0.45±0.01a	0.45±0.04a
Total length (cm)	232.5±10.6a	212.7±14.5ab	198.2±14.3b	189.2±25.1b	132.0±17.5c
Total projected area (cm^2)	10.9±0.9a	10.0±1.1ab	8.2±1.5bc	8.3±0.7b	6.3±0.8c
Total surface area (cm^2)	34.3±2.9a	31.5±3.6ab	28.1±1.4bc	25.9±2.7c	18.6±2.7d
Total volume (cm^3)	0.41±0.06a	0.37±0.06ab	0.32±0.01bc	0.29±0.02cd	0.21±0.04d

Values are means ± SE (n = 3). Identical letters in the same row indicate no significant differences between treatments (P < 0.05)

treatment. In contrast, no significant differences in five investigated root morphological traits were observed between the sole non-PA+AMF and the dual Spd+AMF treatment, or between the Spd+AMF and the Spm+AMF treatments.

DISCUSSION

Supplement of either Put, Spm or Spd to soil growth media had stimulating effects on mycorrhizal colonization and numbers of appressoria in AM peas (El Ghachtouli *et al*., 1995) and hyphal growth of AM maize root segments at low 1.5 µmol L^{-1} Spm (Hrselová & Gryndler, 2000). Effects of PAs on mycorrhization of trifoliate orange were variable in our study (Table I). Four investigated mycorrhizal parameters (percentage of AM colonization, numbers of entry point, vesicule & arbuscule) were significantly increased by the Put+AMF treatment in 4-months-old trifoliate orange. Both AM colonization and arbuscule numbers were significantly increased by the Spm+AMF treatment. In contrast, AM mycorrhization was similar between the Spd+AMF and the non-PA+AMF treatment. Different results between ours and other studies might result from differences in the plant species, AM species and concentration or amount of PAs used. Put is the precursor of Spd and Spm biosynthesis (Couée *et al*., 2004) and generally occurs in AMF spores, as an important regulatory factor in plant-AM fungus interactions (El Ghachtouli *et al*., 1996a; Sannazzaro *et al*., 2004). In addition, *in vitro* hyphal growth of *G. mosseae* and *Gigaspora margarita* was inhibited at Put, Spm or Spd 500 mg L^{-1} (Zhang *et al*., 2003) or in maize root segments at 150 and 500 µmol L^{-1} Spm (Hrselová & Gryndler, 2000). Considering that their different chemical structure and different concentrations used in these studies, further studies are needed if the same PA concentrations could give a similar effect on AM mycorrhization among Put, Spm and Spd.

PAs play an important role in cell proliferation and growth and are involved in inducing primary, lateral and adventitious root development (Galston & Sawhney, 1990; Martin-Tanguy & Carré, 1993; Couée *et al*., 2004). Effects of PAs on root traits of trifoliate orange were variable in our study (Table III). In this study, in combination with the AM fungus *G. versiforme*, Spd application (Spd+AMF) did not affect the investigated root morphological traits and Spm application (Spm+AMF) only increased the root total surface area and total volume. In contrast, additional application of Put (Put+AMF), significantly increased in some of the investigated root traits e.g., total root length, total projected root area, total root surface area & total root volume (Table III). Our results agreed with that effects of the intracellular PA content on root growth of *Pringlea antiscorbutica* were dependent on the exogenously supplied PAs, Put, Spd or Spm (Hummel *et al*., 2002). Such improvements of root morphological traits (Table III) might relate to a better mycorrhizal development in trifoliate

Fig. 1: Root colonization of trifoliate orange (*Poncirus trifoliata*) seedlings by *G. versiforme*. Hereinto, blue stained areas of roots mean mycorrhizal infection

orange seedlings (Table I) by the Put+AMF treatment.

Our experiments showed that the sole AMF treatment enhanced plant growth of trifoliate orange seedlings, which was consistent with numerous studies (Smith & Read, 2008). Other studies demonstrated that the sole application of PAs could also improve plant growth (Galston & Sawhney, 1990; Martin-Tanguy, 2001; Kaur-Sawhney *et al*., 2003; Couée *et al*., 2004). Effects of PAs on the growth of trifoliate orange were variable in our study (Table II). Compared to the non-PA+AMF inoculation, the dual Put+AMF treatment significantly increased all the growth parameters, biomass production of both shoots and roots and root/shoot ratio and the dual Spm+AMF treatment only increased leaf area, root/shoot ratio, shoot and root biomass production (Table II). In contrast, plant growth, biomass and root/shoot ratio were similar to each other between the sole AMF and the dual Spd+AMF treatment. Increase of plant growth under the Put+AMF and Spm+AMF in trifoliate orange may result from greater improvements on both mycorrhization (Table I) and root morphological traits (Table III). However, it would be interesting to have treatments on how PAs could affect plant growth without AMF inoculation so that interactions between AMF and PAs could be investigated.

In conclusion, compared to the sole non-PA+AMF treatment, root AM development, root morphological traits and plant (shoot & root) growth of 4-months-old trifoliate orange seedlings were significantly enhanced by the dual diamine Put+AMF treatment, while some of these parameters were increased by the tetramine Spm+AMF treatment, but not by the triamine Spd+AMF treatment. Our results indicated that the dual Put+AMF application could be a feasible procedure for providing trifoliate orange rootstocks and further studies are needed to study the interactions between AM fungi and PAs on plant growth.

Acknowledgement: This work was supported by the National Natural Science Foundation of China (No.: 30800747). We thank the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences to provide *G. versiforme* inoculum.

REFERENCES

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

Davies, F.S. and L.G. Albrigo, 1994. *Citrus*. CAB international, Wallingford, UK

El Ghachtouli, N., M. Paynot, D. Morandi, J. Martin-Tanguy and S. Gianinazzi, 1995. The effect of polyamines on endomycorrhizal infection of wild-type *Pisum sativum*, cv Frisson (nod^+myc^+) and two mutants (nod^-myc^+ and nod^-myc^-). *Mycorrhiza*, 5: 189–192

El Ghachtouli, N., J. Martin-Tanguy, M. Paynot and S. Gianinazzi, 1996a. 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

El Ghachtouli, N., M. Paynot, J. Martin-Tanguy, D. Morandi and S. Gianinazzi, 1996b. Effect of polyamines and polyamine biosynthesis inhibitors on spore germination and hyphal growth of *Glomus mosseae*. *Mycol. Res*., 100: 597–600

Farooq, M.F., S.M.A. Basra, M. Hussain, H. Rehman and B.A. Saleem, 2007. Incorporation of polyamines in the priming media enhances the germination and early seedlings growth in hybrid sunflower (*Helianthus annuus* L.). *Int. J. Agric. Biol*., 9: 868–872

Galston, A.W. and R.K. Sawhney, 1990. Polyamines in plant physiology. *Plant Physiol*., 94: 406–410

Goicoechea, N., G. Szalai, M.C. Antolín, M. Sánchez-Díaz and E. Páldi, 1998. Influence of arbuscular mycorrhizae and Rhizobium on free polyamines and proline levels in water-stressed alfalfa. *J. Plant Physiol*., 153: 706–711

Goussous, S.J. and M.J. Mohammad, 2009. Effect of two arbuscular mycorrhizae and N and P fertilizers on growth and nutrient uptake of onions. *Int. J. Agric. Biol*., 11: 463–467

Graham, J.H. and J.P. Syvertsen, 1985. Host determinants of mycorrhizal dependency of citrus rootstock seedlings. *New Phytol*., 101: 667–676

Hrselová, H. and M. Gryndler, 2000. Effect of spermine on proliferation of hyphae of *Glomus fistulosum*, an arbuscular mycorrhizal fungus, in maize roots. *Folia Microbiol*., 45: 167–171

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 antiscorbutica*. *J. Exp. Bot*., 53: 1463–1473

Kaur-Sawhney, R., A.F. Tiburcio, T. Altabella and A.W.Galston, 2003. Polyamines in plants: An overview. *J. Cell Mol. Biol*., 2: 1–12

Martin-Tanguy, J. and M. Carré, 1993. Polyamines in grapevine microcuttings cultivated *in vitro*-effects of amines and inhibitors of polyamine biosynthesis on polyamine levels and microcutting growthi and development. *Plant Growth Regul*., 13: 269–280

Martin-Tanguy, J., 2001. Metabolism and function of polyamines in plants: recent development (new approaches). *Plant Growth Regul*., 34: 135–148

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. Szegediensis*, 46: 59–60

Parádi, I., B. Berecz, K. Halász and Z. Bratek, 2003a. Influence of arbuscular mycorrhiza and cadmium on the polyamine contents of Ri T-DNA transformed *Daucus carota* L. root cultures. *Acta Biol. Szegediensis*, 47: 31–36

Parádi, I., Z. Bratek and F. Láng, 2003b. Influence of arbuscular mycorrhiza and phosphorus supply on polyamine content, growth and photosynthesis of *Plantago lanceolata*. *Biol. Plant*, 46: 563–569

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. Japanese Soc. Hort. Sci*., 58: 267–274

Sannazzaro, A., C. Álvarez, A. Menéndez, F. Pieckenstain, E. Albertó and O. Ruiz, 2004. Ornithine and arginine decarboxilase activities and effect of some polyamine biosynthesis inhibitors on *Gigaspora rosea* germinating spores. *FEMS Microbiol. Lett.*, 230: 115–121

Sannazzaro, A.I., M. Echeverría, E.O. Albertó, O.A. Ruiz and A.B. Menéndez, 2007. Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. *Plant Physiol. Biochem.*, 45: 39–46

Smith, S.E. and S. Dickson, 1997. *VA Mycorrhizas: Basic Research Techniques*. CRC for Soil and Land Management, Glen Osmond, Adelaide, Australia

Smith, S.E. and D.J. Read, 2008. *Mycorrhizal Symbiosis*, 3rd edition. Academic Press, San Diego

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. *European J. Soil Biol.*, 44: 122–128

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

(Received 01 March 2010; Accepted 11 May 2010)