

Effect of glucose, root exudates and N forms in mycorrhizal symbiosis using *Rhizophagus intraradices*

Y. Wang¹, S. Tang¹, H. Jin^{1*}

¹College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua, Zhejiang 321004, People's Republic of China *Corresponding author: hrjin@zjnu.cn

Abstract

We investigated the effects of glucose and root exudates in combination with different nitrogen (N) forms on the spore germination rate and hyphal growth of the arbuscular mycorrhizal (AM) fungus *Rhizophagus intraradices*. Spores were cultured for 20 days at 26 °C in agar medium containing either glucose or root exudates at different concentrations. Nitrogen was supplied in the form of 4 mM NaNO₃, 4 mM NH₄Cl, 4 mM NH₄NO₃ or 2 mM urea. Three different controls without added N were used: deionized water, glucose, and root exudates. After 20 days, Glucose concentrations of 10, 25, 40 and 50 g L⁻¹ inhibited the spore germination; however, when the concentration of glucose was 5 g L⁻¹, the mean hyphal length was longer than that in the control. Glucose plus different forms of N sources also has no positive effect on the germination as well as hyphal growth. The addition of root exudates significantly improved both the spore germination rate and hyphal lengths in all four media (root exudates +4 mmol NaNO₃, root exudates +4 mmol NH₄Cl, root exudates +4 mmol NH₄NO₃ and root exudates +2 mmol urea). After incubation for 20 days, the highest spore germination rates (80.33%) were detected in the root exudate + urea medium, while that in the root exudate + NaNO₃ medium was lower than in the control. In all these four media, hyphal growth was initially rapid and slowed in the later stages of incubation. Finally, the greatest hyphal length (15.15 mm spores⁻¹) was observed in the root exudate + NH₄NO₃ medium, while the lowest was observed in the exudate + NaNO₃ medium.

Keywords: Arbuscular mycorrhiza, hyphal length, nitrogen sources, root exudate, spore germination

1. Introduction

Arbuscular mycorrhizal (AM) symbiosis is an association between obligate biotrophic fungi and more than 80% of land plants; these fungi represent the largest component of the soil fungal community (Gosling, 2006). Endophytes promote the growth of plants in various ways similar to rhizosphere bacteria

(Etesami *et al.*, 2014). The beneficial roles that AM fungi play in agricultural production are well known these fungi contribute to plant health by improving plant nutrition, and in most instances, plant resistance against pathogen attacks (St.-Arnaud *et al.*, 1995; Gianinazzi-Pearson *et al.*, 1996).

They also play an important role in the modulation of plant resistance to water and salt stress (Miransari *et al.*, 2008), acidity and phytotoxic levels of Al in the soil environment (Alex Seguel *et al.*, 2013); and in improving soil structure through the exudation of glomalin (Wu *et al.*, 2008). Some plants colonized with AMF can be more appropriate to decontaminate As-contaminated water than soils (Caporale A.G. *et al.*, 2014). Dormant AM fungal spores are not only adapted to adverse environments but are also the most effective means of colonization. The colonization ratio of AM fungi is largely correlated with spore germination.

Spore germination is the precondition of symbiosis with plants. During the pre-symbiotic phase, many factors, such as a rhizosphere environment, high flavonoid content, presence of soil microorganisms and plant cell suspension culture, can induce spore germination and promote hyphal growth without a host (Gianinazzi-Pearson *et al.*, 1989; Graham *et al.*, 1982). In addition, root exudates can increase the length and degree of branching of AM fungi hyphae (Tamasloukht *et al.*, 2003) and play an important role in plant-microbe interactions in the rhizosphere (Karin Hage-Ahmed *et al.*, 2013). Some studies also suggest that root exudates or host extracts can stimulate spore germination, but some literature has indicated negative or inconsequential effects (Beilby *et al.*, 1982; Hepper *et al.*, 1983; Bécard *et al.*, 1988). Research has revealed the influence of different forms of N on AM growth in whole plant system, (Hawkins *et al.*, 2001; Azcón *et al.*, 1996; Cuenca *et al.*, 1994). Further, AM fungal spores can absorb glucose as a source of C from the environment (Bago *et al.*, 1999; Bücking *et al.*, 2008).

There have been several reports on the effects of glucose, nitrogen sources and root exudates on amino acids metabolism *in vitro* spore germination of AM

fungi (Gachomo *et al.* 2009; Jin *et al.*, 2011). The availability of exogenous inorganic N (ammonium and nitrate) and organic N (urea, arginine, and glutamine) to the AM fungal spores using only CO₂ for germination generated more than 5 times more internal free amino acids than those in the absence of exogenous N. A supply of exogenous nitrate to the AM fungal spores with only CO₂ gave rise to more than 10 times more asparagine than that without exogenous N. In contrast, the extra supply of exogenous glucose to the AM fungal spores generated a significant enhancement in the uptake of exogenous N sources, with more than 3 times more free amino acids being produced than those supplied with only exogenous CO₂. Meanwhile, arginine was the most abundant free amino acid produced and it was incorporated into the proteins of AM fungal spores to serve as an N storage compound. Root exudates stimulated N assimilation and metabolism of amino acids in a short-term germination (1 or 2 d), but in a long-term germination (1 or 2 weeks), N assimilation was not affected significantly by root exudates. However, the effect of glucose or root exudates together with different forms of N, with respect to the spore germination and hyphal growth of AM fungi, is virtually unknown. Spores have been widely used in *in vitro* cultures, owing to the simple methods involved (Fortin *et al.*, 2002). On the basis of previous research, we conducted *in vitro* culture of isolated AM spores to study these effects.

2. Materials and Methods

2.1. Spores and root exudates preparation

Glomus Rhizophagus intraradices spores were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences; Serial number: BGC AH01.

Disinfection was carried out based on Bécard *et al.* (1988). Spores were isolated by the wet-sieving technique (Gerdemann & Nicolson, 1963), washed by centrifugation for 1-2 min in sterilized distilled water containing a drop of Tween 20, surface-sterilized twice by soaking for 10 min in a tube containing 2 % (w/v) chloramine-T, rinsed five times for 1-2 min in a funnel with a 38 μm sieve, soaked in an antibiotic solution (100 mg L^{-1} gentamicin and 200 mg L^{-1} streptomycin), and finally rinsed with sterile water. Root exudates were prepared by growing Ri T-DNA-transformed carrot roots on M medium in Petri dishes at 28 °C for 4 weeks. The roots were then transferred to a sterilized liquid temperature degree medium containing 3% sucrose, and grown at 24 °C, for 4 weeks. Thereafter, they were cleaned with sterilized deionized water twice, and then incubated at 24 °C, in sterilized temperature degree deionized water for one week. Finally, root exudates were collected and stored at -20 °C.

2.2. Spore germination in the presence of glucose or root exudates, together with different forms of N

Three factors were considered. Glucose concentration, root exudates concentration and forms of nitrogen. Different concentrations of glucose, 5 g L^{-1} , 10 g L^{-1} , 25 g L^{-1} , 40 g L^{-1} , 50 g L^{-1} , or concentration of root exudates (50 ml L^{-1} , 100 ml L^{-1} , 200 ml L^{-1} , 300 ml L^{-1} , 400 ml L^{-1}) were added in a 0.8% aqueous agar medium respectively, in which 4 mM NaNO_3 , 4 mM NH_4Cl , 4 mM NH_4NO_3 , or 2 mM urea were included in separate treatments. We tested glucose concentration, root exudates concentration and the combination glucose + nitrogen, root exudates +

nitrogen. Control CK1 consist 0.8% water agar, 5 g L^{-1} Glucose without added nitrogen was used as the control CK2, while a medium containing 50 ml L^{-1} root exudates is denoted as the control CK3. All the medium were adjusted to pH 6.5, 120 , sterilized for 25 min.

The spores were then spread on petri dishes at the rate of 30 spores per dish, with three replicates. The dishes were incubated in the dark for 5, 10, 15, or 20 d at 26 °C.

Spore germination rates were measured and are expressed as a percentage of the total number of spores examined. Spores which produced germ tubes longer than their diameters were considered to have germinated. Hyphal growth was estimated by the line-hypha intersect method modified from Newman's technique (Newman, 1966).

2.3. Data analysis

Data were processed with an analysis of a repeated-measures ANOVA, and means were compared using Duncan's multi range test using Sigma Stat software. A significance level of 0.05 was used, unless stated otherwise.

3. Results

When glucose was present in the medium (at any concentration), all the spore germination rates after 20 days were lower than or not significantly different from those in the control. Glucose concentrations of 10, 25, 40 and 50 g L^{-1} inhibited hyphal growth, while a concentration of 5g L^{-1} promoted it. In general, both spore germination rates and hyphal growth decreased gradually in response to elevation in the concentrations of glucose.

Table 1. Effect of different concentrations of glucose on the spore germination and hyphal length of *R. Intraradices*

Glu	5d		10d		15d		20d	
	A	B	A	B	A	B	A	B
5	15.33±0.67a	1.28±0.07b	32.67±1.56ab	2.17±0.12b	40.00±2.12b	5.47±0.10a	55.67±2.19a	6.67±0.31a
10	13.67±0.56ab	1.17±0.05b	20.00±1.05bc	1.95±0.08b	29.67±1.63b	4.22±0.28ab	34.67±1.56b	5.00±0.23ab
25	14.00±0.38ab	0.83±0.04b	16.67±1.03c	1.00±0.04c	16.67±1.12c	1.83±0.07bc	28.00±1.32b	3.33±0.13bc
40	6.33±0.36bc	0.83±0.04b	9.33±0.78c	0.67±0.03c	12.67±1.09c	1.17±0.05c	16.00±0.98c	1.67±0.08c
50	4.33±0.45c	0.67±0.03b	7.33±0.56c	1.25±0.04c	11.00±1.32c	1.33±0.05c	14.67±1.21c	1.67±0.07c
CK1	13.00±0.53ab	2.61±0.11a	47.67±2.10a	3.33±0.12a	58.00±2.45c	5.00±0.23a	63.00±2.45a	5.67±0.18a

Glu means glucose (g L^{-1}), A means Germination rate (%), B means Hyphal length (mm/spore), CK1 means no glucose, nor nitrate, nor exudates, only 0.8% water agar contained. Means followed by the same letter in a column are not significantly different ($P < 0.05$).

Addition of 50 ml L^{-1} root exudates, the spore germination rate after 20d was higher than in the control. However, this gradually decreased in response to increasing root exudate concentrations. For example, at a root exudate concentration of 400 ml L^{-1} , the spore germination rate was only 28% comparing with 63% in the control. On the

other hand, root exudate concentrations of 50-100 ml L^{-1} promoted hyphal growth; the maximum hyphal length was 12.33 mm spores⁻¹ observed in the 50 ml L^{-1} root exudates treatment. However, inhibition of hyphal growth was observed when 300 and 400 ml L^{-1} root exudates concentrations were used.

Table 2. Effect of different concentrations of root exudates on spore germination and hyphal length in *R. intraradices*

RE	5d		10d		15d		20d	
	A	B	A	B	A	B	A	B
50	14.67±0.71a	3.17±0.14a	37±1.22ab	7.33±0.32a	60.67±3.12ab	9.00±0.43a	77.67±3.45a	12.33±0.57a
100	12.67±0.58a	2.33±0.11abc	21.33±1.02b	3.17±0.14b	27.33±1.45bc	7.00±0.35ab	40.00±2.34b	9.33±0.42ab
200	8.67±0.39bc	2.33±0.09abc	22.67±0.87b	2.83±0.13b	28.00±1.34bc	4.00±0.20b	36.67±1.67b	6.67±0.34c
300	8.00±0.35c	1.17±0.04bc	24.00±0.96b	1.00±0.04c	27.33±1.27c	3.33±0.12b	37.33±1.41b	4.00±0.21c
400	7.33±0.31c	0.67±0.03c	20.00±0.84b	1.17±0.05c	25.33±1.23c	2.33±0.09b	28.00±1.23b	3.33±0.15c
CK1	13.00±0.61ab	2.61±0.12a	47.67±2.28a	3.33±0.14b	58.00±2.56c	5.00±0.21b	63.00±3.21b	5.67±0.27bc

RE means root exudate (ml L^{-1}), A means Germination rate (%), B means Hyphal length (mm/spore), CK1 means no glucose, nor nitrate, nor exudates, only 0.8% water agar contained. Means followed by the same letter in a column are not significantly different ($P < 0.05$).

When glucose with different forms of N were included in the 0.8% agar medium, spore germination rate increased in proportion to time but was higher than the control only in medium D. After incubation for 20 days, spore germination rates reached their maxima (%) of 39, 46.67, 50.67, 57.33 and 55.67 in media A (glucose + NaNO₃), B (glucose + NH₄Cl), C (glucose + NH₄NO₃),

D (glucose + urea) and CK2, respectively. In the C and D mediums, spore germination rate were observed to be rapid initially and higher than the control CK2 within 15d, However, a constantly increasing germination rate was observed in medium D but have no difference from the control after incubation for 20 days.

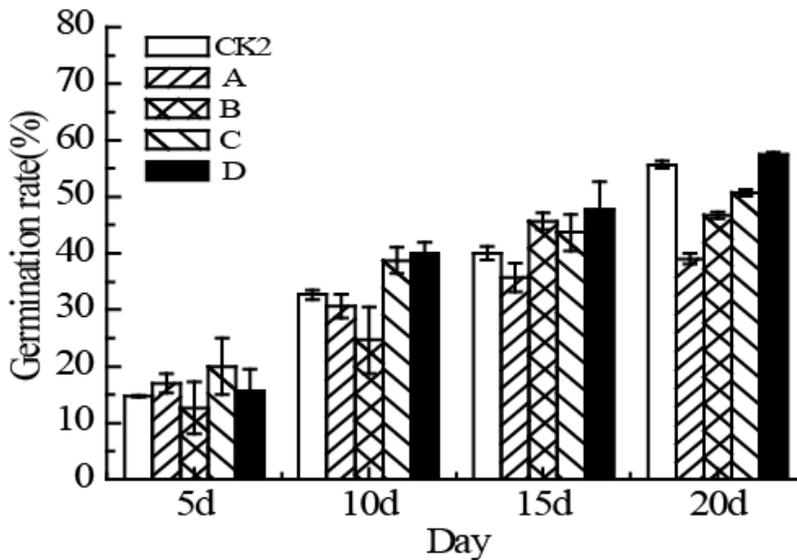


Figure 1. Effect of different N forms together with glucose on spore germination rates in *R. intraradices*. Error bars indicate standard deviations, based on three replicates. CK2: glucose; A: glucose + NaNO₃; B: glucose + NH₄Cl; C: glucose + NH₄NO₃; D: glucose + urea.

Hyphal growth was observed to increase in response to increasing germination time in A, B, C, and D media. However, the greatest hyphal length (9.33 mm spores⁻¹) was observed only in medium

C; no significant differences were detected among the others. Although hyphal growth in medium B was rapid during the first ten days, very little subsequent growth was observed.

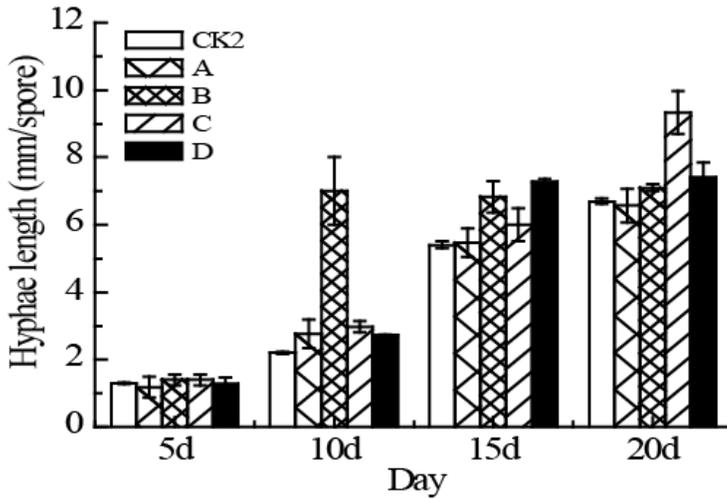


Figure 2. Effects of different N forms together with glucose on the hyphal length of *R. intraradices*. Error bars indicate standard deviations, based on three replicates. CK2: glucose; A: glucose + NaNO₃; B: glucose + NH₄Cl; C: glucose + NH₄NO₃; D: glucose + urea

The germination rate of AM fungal spores in E (root exudate + NaNO₃), F (root exudate + NH₄Cl), G (root exudate + NH₄NO₃), H (root exudate + urea) medium increased with elongation of culture time. Finally the

germination rate in E, F, G medium with different sources of nitrogen and root exudates had no difference from the control except H medium with root exudate plus urea in which the germination rate still increased up to 80.33%.

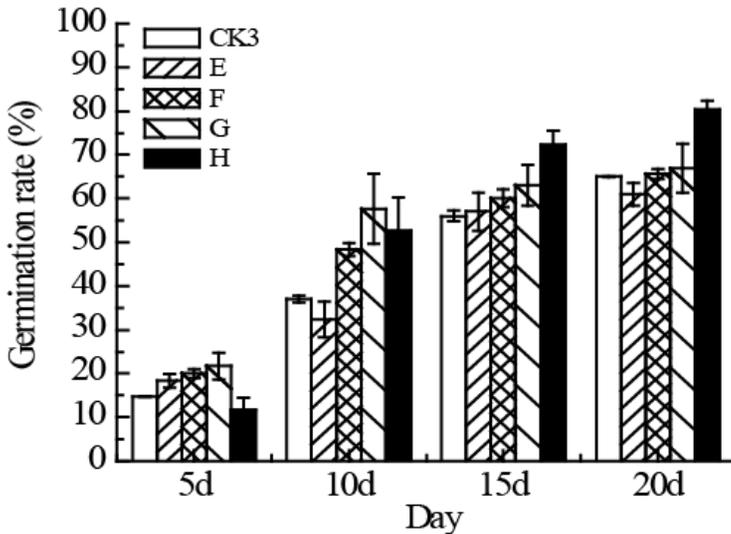


Figure 3. Effects of different N forms together with root exudates on the spore germination rate of *Glomus intraradices*. Error bars indicate standard errors based on three replicates. CK3: root exudate; E: root exudate + NaNO₃; F: root exudate + NH₄Cl; G: root exudate + NH₄NO₃; H: root exudate + urea

The presence of both root exudates and N stimulated hyphal growth. In the early phase of growth, small differences in hyphal length were detected among media, but the only significant difference was observed between the medium G with root exudate plus ammonium nitrate and the others, with the former exhibiting the greatest hyphal growth between

10 and 15 days of incubation. Overall, the greatest, although not statistically different, hyphal length (15.15 mm spores⁻¹) was observed in the medium G at 20 days. Meanwhile, media E, F, H and the control displayed hyphal lengths of 10.33, 13.48, 14.57 and 12.33 mm spores⁻¹, respectively, indicating that F and H exhibited intermediate values.

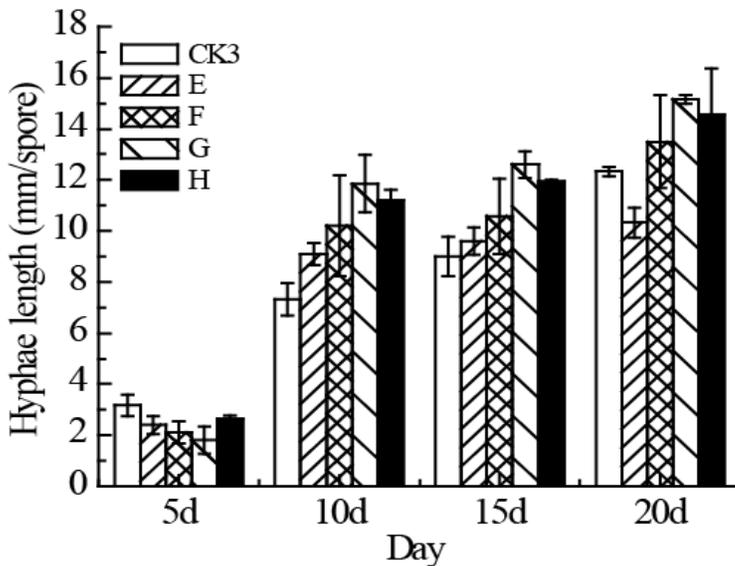


Figure 4. Effects of different N forms together with root exudates on hyphal length in *Glomus intraradices*. Error bars indicate standard errors, based on three replicates. CK3: root exudate; E: root exudate + NaNO₃, F: root exudate + NH₄Cl, G: root exudates + NH₄NO₃, H: root exudate + urea

4. Discussion

Spores of AM fungi are generally capable of spontaneous germination provided certain physical and physiological conditions are fulfilled (Smith *et al.*, 2008). *Rhizophagus intraradices* is frequently used in spore germination experiments because of these characteristics as well as its strong adaptability, small volume, thin cell walls, and sensitivity to the environment. When glucose levels were increased

from 5 to 50 g L⁻¹, the spore germination rates correspondingly decreased. One possible explanation is that spores of *R. intraradices* may be quite sensitive to high concentrations of glucose, which may interfere with the ability of the hyphae to absorb external nutrients, but the mechanism is unclear. However, when the concentration of glucose was 5 g L⁻¹, the mean hyphal length was longer than that in the control. Some studies have reported that AM fungal spores can absorb acetic acid, glucose, fructose

and CO₂ from the environment as carbon sources (Bago *et al.*, 1999; Becard *et al.*, 1988; Bücking *et al.*, 2008). Our results allow us to hypothesize that low concentrations of glucose could promote hyphal growth. In the media containing glucose combined with different forms of N, the mean germination rates were not higher than in the control at 20 days of incubation. Some studies have indicated that when AM fungal spores absorb glucose, the supply of this carbon source facilitates the biosynthesis of amino acids, including Arg as the main amino acid for new germ tube and hyphal growth. This in turn improves N absorption (Jin *et al.*, 2011). Therefore, this may explain why the simultaneous presence of glucose with N sources improved hyphal growth during the early stages of incubation. Some studies have also showed that ammonium and urea are more rapidly assimilated by AM fungi than nitrate and amino acids (Gachomo *et al.*, 2009). This helps explain why the ratios of the hyphal lengths in the media A (glucose + NaNO₃), B (glucose + NH₄Cl), C (glucose + NH₄NO₃), and D (glucose + urea) were 11:12:24:19. Root exudates at 50 ml L⁻¹ are beneficial to both spore germination and hyphal growth. There is evidence that host root exudates contain more than one type of compound that stimulates branching of AM fungal hyphae (Tawaraya *et al.*, 1996). Akiyama *et al.* (2005) isolated a branching factor strigolactone, 5-deoxy-strigol from the root exudates of *Lotus japonicus*. Strigolactones are a group of sesquiterpene lactones, previously isolated as seed-germination stimulants for the parasitic weeds *Striga* and *Orobanche*. The natural strigolactones 5-deoxy-strigol, sorgolactone and strigol, induced extensive hyphal branching in germinating spores of the AM fungus *Gigaspora margarita* at very low concentrations. Thus, the quality of root exudates is correlated with their stimulatory effects on the growth of AM fungi (Elias *et al.*, 1987).

However, as the concentrations of root exudates increased, the spore germination rate and hyphal lengths gradually correspondingly decreased in our studies. Previous studies have shown that high concentrations of some compounds may restrain spore germination (Hawkins *et al.*, 2001; Nagahashi *et al.*, 1996); this may be one explanation for our observations. In the media containing root exudates with different N forms, only root exudates + urea stimulated germination at 20 days. Some studies have indicated that root exudates improve the absorption of nitrogen of AM fungi in the short term (Jin *et al.*, 2011). In other words, a synergistic effect between root exudates and N compounds may have existed in our study. Compared with NO₃³⁻, NH₄⁺ as an N source is more likely to be used by AM fungi (Hawkins *et al.*, 2000; Toussaint *et al.*, 2004). This may explain why differences in hyphal length were detected among media F (root exudate + NH₄Cl), G (root exudate + NH₄NO₃), and H (root exudate + urea) at the final stage of incubation.

5. Conclusion

Spore germination of AM fungi is the prerequisite for successful symbiosis, thus, the external factors (glucose, root exudates, N sources) that affect the germination of AM fungi were explored. Root exudates were better than glucose at promoting spore germination, and exhibited interactions with certain forms of N to increase the spore germination rate and hyphal length.

Acknowledgements

The authors express their sincere thanks to the National Natural Science Foundation of China (Grant No. 41371291) and the Natural Science Foundation of Zhejiang Province of China (Grant No. LY12C01002)

and the Science and Technology Project of Jinhua (Grant No.2014-2-011).

References

- Akiyama, K., Matsuzaki, K., Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*. 435, 824-827
- Azcon, R., Gomez, M., Tobar, R. 1996. Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. *Biol. Fert. Soils*. 22,156-161.
- Bago, B., Pfeffer, P. E., Douds, D.D .1999. Carbon metabolism in spores of the arbuscular mycorrhizal fungus *Glomus intraradices* as revealed by nuclear magnetic resonance spectroscopy. *Plant Physiol*. 121, 263-271.
- Bécard, G., Fortin, J.A. 1988. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytol*. 108, 211-218.
- Beilby, J.P., Kidby, D.K. 1982. The early synthesis of RNA, protein, and some associated metabolic events in germinating vesicular-arbuscular mycorrhizal fungal spores of *Glomus caledonius*. *Can. J. Microbiol*. 28, 623-628.
- Bücking, H., Abubaker, J., Govindarajulu, M., Tala, M., Pfeffer, P.E., Nagahashi, G., Lammers, P.J., Shachar-Hill, Y. 2008. Root exudates stimulate the uptake and metabolism of organic carbon in germinating spores of *Glomus intraradices*. *New Phytol*. 180, 684-695.
- Caporale, A.G., Sarkar, D., Datta, R., Punamiya, P., Violante, A. 2014. Effect of arbuscular mycorrhizal fungi (*Glomus* spp.) on growth and arsenic uptake of vetiver grass (*Chrysopogon zizanioides* L.) from contaminated soil and water systems. *J. Soil Sci. Plant Nutr*. 14 (4), 955-972.
- Cuenca, G., Azcon, R. 1994. Effects of ammonium and nitrate on the growth of vesicular-arbuscular mycorrhizal *Eiyythrina poeppigiana* O.I. cook seedlings. *Biol. Fert. Soils*. 18, 249-254.
- Elias, K.S., Safir, G.R. 1987. Hyphal elongation of *Glomus fasciculatus* in response to root exudates. *Applied Environ. Microbiol*. 53, 1928-1933.
- Etesami, H., Mirseyed, H., Alikhani, H. 2014. In planta selection of plant growth promoting endophytic bacteria for rice (*Oryza sativa* L.). *J. Soil Sci. Plant Nutr*. 14 (2), 491-503.
- Fortin, J.A., Bécard, G., Declerck, S., Dalpé, Y., St-Arnaud, M., Coughlan, A.P., Piché, Y. 2002. Arbuscular mycorrhiza on root-organ cultures. *Can. J. Bot*. 80, 1-20.
- Gachomo, E., Allen, J.W., Pfeffer, P.E., Govindarajulu, M., Douds, D.D., Jin, H.R., Nagahashi, G., Lammers, P.J., Shachar-Hill, Y. and Bücking, H. 2009. Germinating spores of *Glomus intraradices* can use internal and exogenous nitrogen sources for *de novo* biosynthesis of amino acids. *New Phytol*. 184, 399-411.
- Gerdemann, J.W., Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. *Trans Br Mycol Soc*. 46, 235-244.
- Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Tahiri-Alaoui, A., Gianinazzi, S. 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytol*. 133, 45-57.
- Gosling, P., Hodge, A., Goodlass, G., Bending, G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems and Environment*. 113, 17-35.

- Govindarajulu, M., Pfeffer, P.E., Jin, H.R., Abubaker, J., Douds, D.D., Allen, J.W., Bücking, H., Lammers, P.J., Shachar-Hill, Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature*. 435, 819-823.
- Graham, J.H. 1982. Effect of citrus root exudates on germination of chlamydospores of the vesicular-arbuscular mycorrhizal fungus *Glomus epigaeum*. *Mycologia*. 74, 831-835.
- Hawkins, H.J., George, E. 2001. Reduced ¹⁵N-nitrogen transport through arbuscular mycorrhizal hyphae to *Triticum aestivum* L. supplied with ammonium vs. nitrate nutrition. *Ann. Bot.* 87, 303-311.
- Hawkins, H.J., Johansen, A., George, E. 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant Soil*. 226, 275-285.
- Hepper, C. M., Jakobsen, I. 1983. Hyphal growth from spores of the mycorrhizal fungus *Glomus caledonius*: effect of amino acids. *Soil Biol Biochem*. 15, 55-58.
- Jin, H. R., Liu, J., Liu, J., Huang, X.W. 2012. Forms of nitrogen uptake, translocation and transfer via arbuscular mycorrhizal fungi: A Review. *Sci China Life Sciences*. 55(6), 474-482.
- Jin, H.R., Pfeffer, P.E., Shachar-Hill, Y., Douds, D.D., Piotrowski, E. & Lammers, P. J. 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. *New Phytol*. 168, 687-696.
- Jin, H.R., Jiang, D.H., Zhang, P.H. 2011. Effect of carbon and nitrogen availability on the metabolism of amino acids in the germinating spores of arbuscular mycorrhizal fungi. *Pedosphere*. 21 4, 432-442.
- Karin, H., Anna, M., Andreas, V., Franz, H., Siegrid, S. 2013. Alterations in Root Exudation of Intercropped Tomato Mediated by the Arbuscular Mycorrhizal Fungus *Glomus mosseae* and the Soilborne Pathogen *Fusarium oxysporum* f.sp. *Lycopersici*. *J Phytopathol*. 161, 763-773.
- Miransari, M., Bahrami, H. A., Rejali, F., Malakouti, M.J. 2008. Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on wheat (*Triticum aestivum* L.) growth. *Soil Biol Biochem*. 40, 1197-1206.
- Nagahashi, G., Douds, D.D., Abney, G.D. 1996. Phosphorus amendment inhibits hyphal branching of the VAM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation. *Mycorrhiza*. 6(5), 403-408.
- Newman, E.I. 1996. A method of estimating the total length of root in a sample. *Appl. Ecol*. 3, 139-145.
- Ryan, M.H., McNerney, J.K., Record, I.R., Angus, J.F. 2008. Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *J. Sci. Food Agric*. 88, 1208-1216.
- Smith, S.E., Read, D.J. 2008. *Mycorrhizal Symbiosis*, 3rd ed. London, Academic Press.
- Seguel, A., Cumming, J., Klugh-Stewart, K., Cornejo, P., Borie F. 2013. The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. *Mycorrhiza*. 23, 167-183.
- St.-Arnaud, M., Hamel, C., Caron, M., Fortin, J.A. 1995. Endo mycorrhizes VA et sensibilité des plantes aux maladies: Synthèse de la littérature et mécanismes d'interaction potentiels. In: *La symbiose mycorrhizienne. Etat des connaissances*, Orbis Publishing, Frelighsburg, Canada, pp: 51-87.

- Tamasloukht, M.B., Séjalon-Delmas, N., Kluever, A. 2003. Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea*. *Plant Physiol.* 131, 1-11.
- Tawaraya, K., Watanabe, S., Yoshida, E., Wagatsuma, T. 1996. Effect of onion (*Allium cepa*) root exudates on the hyphal growth of *Gigaspora margarita*. *Mycorrhiza.* 6, 57-59.
- Toussaint, J.P., St-Arnaud, M., Charest, C. 2004. Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an *in vitro* compartmented system. *Can J Microbiol.* 50, 251-260.
- Wu, Q.S., Xia, R.X., Zou, Y.N. 2008. Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *Eur. J. Soil Biol.* 44, 122-128.