Journal of Tropical Mycorrhiza
https://journal.ami-ri.org/index.php/JTM
E-ISSN 2829-467X
Vol. 2 No. 1 April 2023

Dual cultivation with endomycorrhizal host plants makes effective endomycorrhizal symbiosis in Intsia bijuga (Colebr.) Kuntze in greenhouse experiments

Nirina Rakotoarimanga[1], Abel Andriantsimahavandy[2], Robin Duponnois[3]
[1]Laboratoire de microbiologie de l'Environnement, Centre National de Recherches sur l'Environnement (LME/CNRE) BP 1739, Antananarivo Madagascar
[2]Faculté des sciences, Université d'Antananarivo, BP 906, Antananarivo Madagascar
[3]Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UM2, Campus International de Baillarguet, TA A-82/J, Montpellier, France

*Corresponding author : manganir@yahoo.fr

ABSTRACT. This work aimed to highlight the establishment of endomycorrhizal symbiosis in Intsia bijuga under certain conditions related to the presence of endomycorrhizal plants and show the effectiveness of symbiosis.The experiments were conducted at greenhouse. Single and dual cultivation of Intsia bijuga with each of the three different species of endomycorrhizal host plants (Zea mays, Crotalaria juncea and Dalbergia trichocarpa) on sterilized poor soil and forest soils (sterilized and unsterilized) were performed. On sterilized poor soil, the culture was inoculated by inoculums of endomycorrhizal fungi Glomus intraradices (syn. Rhizophagus irregularis). The effectiveness of the endomycorrhizal establishment was evaluated by the colonization of the roots by endomycorrhizae, the rate of Nitrogen and Phosphorus accumulated in leaves, and plant mycorhizal dependency. Dual cultivation of Intsia bijuga with each of the three endomycorhizal host plants allowed endomycorhizal establishment in its roots and Intsia bijuga development was significantly improved by endomycorrhizal establishment. The biomass and the rates of N and P were significantly high in endomycorrhizal plants. Mycorrhizal dependency of Intsia bijuga in dual cultivation either with Crotalaria juncea or with Dalbergia trichocarpa (legumes) was lower than those with Zea mays. Endomycorrhizal symbiosis was effective in Intsia bijuga cultivated with endomycorrhizal host plants.

Keywords: *Intsia bijuga; endomycorrhizae; dual cultivation; endomycorrhizal host plant; endomycorrhizal dependency.*

INTRODUCTION

Intsia bijuga, a Fabaceae family plant, which grows on the coasts of Madagascar, Seychelles, and other Southeast Asian countries, establishes ectomycorrhizal symbiosis (Tederooso, 2007; Nugroho et al., 2010, Rakotoarimanga, 2010). No study has reported yet that this plant was associated with endomycorrhizal fungi (Smith and Read, 2008; Nugroho et al., 2010). Mycorrhizal association is known for its positive role in the development of plants on poor soils for its ability to improve, especially plant nitrogen and phosphate nutrition (Rhodes and Gerdemann, 1975; Bolan, 1991; Smith and Read, 1997; Harrison 2005; Duponnois et al., 2005a; Lambers et al., 2008; Willis et al., 2013). Ninety seven percent of plants are associated with mycorrhizal fungi (Smith and Read, 2008) and the few plants that are not endomycorrhizal (Field et al., 2015) belong to the families of Cyperaceae, Juncaceae,

Amaranthaceae, Brassicaceae, Chenopodiaceae and Caryophyllaceae (Strullu, 1991, Norman et al., 1995). Endomycorrhizal fungi are symbionts mandatory and the continuity of their life cycle and survival depends primarily on their ability to infect plants (Nair, Safir, and Siqueira, 1991). Some authors showed that spore germination and development of mycorrhizal hyphae could be stimulated by the presence of the host plant via root exudates (Gianinazzi-Pearson and Gianinazzi Branzati 1989; Bécard and Piché, 1989; Nagata et al., 2015). Moreover, other authors reported that chemical signals of root exudate are insufficient to initiate and develop mycorrhizal fungi life cycle (Harrison, 1999, Smith and Read, 1997; Blee and Anderson, 1998; Rich, Schorderet and Reinhardt 2014). In fact, the establishment of endomycorrhizal association follows these steps: the recognition between symbiont (fungus) and host (plant), initiated via chemical signals emitted by the two partners, the development of hyphae, hyphae attachment of plant root walls followed by the formation of apressorium, penetration into the tissue and root cells and finally, the development of the fungus within the root leading the exchange (arbuscules) and reserve (vesicles) (Harrison, 1999, Smith and Read, 1997; Blee and Anderson, 1998; Gutjahr and Parniske, 2013). The formation of arbuscules and vesicles is a sign of the effectiveness of endomycorrhizal association establishment (Harrison, 1999, Smith and Read, 1997; Blee and Anderson, 1998; Bonfante and Genre, 2010 ; Strullu-Derrien et al., 2014). In this work, we try to demonstrate the possible formation of endomycorrhizal symbiosis in *Intsia bijuga* when cultivated next to the endomycorrhizal plants and the effectiveness of symbiosis.

MATERIAL AND METHODS

Fungal strains: A strain of *Glomus intraradices* Schenk & Smith (DAOM 181602, Ottawa Agricultural Herbarium) (syn. *Rhizophagus irregularis*, Krüger et al., 2011) maintained in culture on the roots of corn (*Zea mays*. L.) grown in a pot was used for mycorrhization.

Plant material and soil culture: Each of the three endomycorrhizal host plants was grown in a pot with Intsia bijuga seedling: *Zea mays*. L. (maize; grass) (Amerian et al., 2001), *Crotalaria juncea*. L. (crotalaire; shrub) (Trindade et al., 2006) and *Dalbergia trichocarpa* Baker (redwood; tree) (Rakotoarimanga, 2010).

Two soil types were used: poor sandy and forest which were collected under *Intsia bijuga* plantation. The physico-chemical characteristics of the sandy soil were as follow: pH (H_2O):6.0, carbon: 0.3%, total nitrogen: 0.3%, Olsen phosphorus: 2.1mg.kg^{-1}, total phosphorus: 41.4mg.kg^{-1} and those of the soil forest were: pH (H_2O): 4.7, carbon: 1.8%, total nitrogen: 0.1%, Olsen phosphorus: 5.2mg.kg^{-1}, total phosphorus: 17.1mg.kg^{-1}.

Experimental setup:

Each seedling of *Intsia bijuga* seedling was grown in a pot with either *Zea mays*, *crotalaria juncea* or *Dalbergia trichocarpa* seedlings at the rate of one seedling in each pot.

Experiment 1: Culture on sterilized poor sandy soil

Culture was performed on the poor sandy soil autoclaved at 140 ° C for 45 minutes. Plastic pots 10cm in diameter and 20cm in height were filled with 750g of soil. Soil culture

was mixed with 15g of *Glomus intraradices* mycorrhized maize soil and *Glomus intraradices* mycorhizal root fragments (13g of soil and 2g of root fragments). *Intsia bijuga* seedling or both *Intsia bijuga* and *crotalaria juncea* seedlings, *Intsia bijuga* and *Zea mays* seedlings, or *Intsia bijuga* and *Dalbergia trichocarpa* seedlings were planted in each pot. Ten replicates per treatment were performed. The same experiment was repeated but the soil was mixed with a mixture of 13g of non mycorrhizal corn soil and non-mychorrizal maize root fragments (2g).

Experiment 2: Culture on forest soil

Another culture experiment as before was performed on forest soil unsterilized and sterilized in an autoclave at 140 °C for 45 minutes, but seedlings did not receive *Glomus intraradices* inoculum.

Ten replicates of each treatment were arranged in a completely randomized design in a greenhouse under natural light (daylight approximately 12 h, mean temperature 25°C day) and watered two times a week with tap water without fertilizers. After four months culturing, *Intsia bijuga* plants were harvested. Roots were stained by the method of Philips and Hayman (1970) to highlight the possible formation of mycorrhizal symbiosis by the presence of hyphae and/or vesicles. Then the percentages of root colonization by endomycorrhizal fungi were determined (Brundrett et al., 1985). The roots and shoots of each *Intsia bijuga* plant were dried separately at 65°C for a week and then weighed to measure shoot and root biomass. Mycorrhizal dependency of *Intsia bijuga* was obtained by calculating the ratio of the difference between the total biomass of mycorrhizal plant and the total biomass of non-mycorrhizal plant, on the total biomass of mycorrhizal plant, expressed as a percentage (Plenchette et al. 1983). One gram of dried leaves in each *Intsia bijuga* plant was taken to be crushed and reduced to ashes at 500 ° C to assess the level of phosphorus content in leaves,. The ash was dissolved in 2 ml of 6N HCl and 10 ml of HNO_3. N and phosphorus was determined by colorimetric method (John, 1970). Another gram of dried leaves of each *Intsia bijuga* plant was crushed and dissolved in 15ml of H_2SO_4 containing $50g.L^{-1}$ salicylic acid to be determined by the Kjeldahl method the nitrogen accumulated.

Statistical analysis

All data were subjected to a one-way analysis of variance using the STATISTICA Computer program, and means were compared with the Newman–Keuls multiple range test (p= 0, 05).

RESULT AND DISCUSSION

The results of the two experiments showed that dual cultivation of *Intsia bijuga* with each of the three endomycorhizal host plants (*Zea mays*, *Crotalaria juncea* and *Dalbergia trichocarpa*) allowed endomycorhizal establishment in its roots. Indeed, after staining with trypan blue (Phillips and Hayman, 1970), endomycorrhizal structures (vesicles and hyphae) were recorded in *Intsia bijuga* root fragments cultivated with each of these three plants inoculated with *Glomus intraradices* and/or cultivated on non-disinfected forest soil. No vesicles or hyphae were observed in *Intsia bijuga* roots grown alone. *Intsia bijuga* development was significantly improved by endomycorrhizal establishment. Mycorrhizal

plants' biomass, nitrogen and phosphorus leaf contents were significantly high (Tables 1 and 2.).

Table 1. Growth response, endomycorrhizal rate, mycorrhizal dependency and on –leaf mineral content of *Intsia bijuga* after 4 months in dual cultivation with *Crotalaria juncea*, *Zea mays* and *Dalbergia trichocarpa* in poor sand soil

Soil	Culture (Treatment)	Ib Total Biomass (g dry weight)	Ib Endomycorrhizal colonization (%)	Ib Mycorrhizal dependency	Ib Nitrogen (%)	Ib Phosphorus (%)
Poor sand soil sterilized	Ib	3,67a*	0a	0a	1,19ab	1,67abc
	Ib+C	3,55a	0a	0a	1,13a	1,58a
	Ib+Z	3,78a	0a	0a	1,18b	1,72bc
	Ib+D	3,75a	0a	0a	1,18b	1,62ab
	Ib+Gi	3,88a	0a	0a	1,18b	1,65ab
	Ib+Gi+C	5,75c	24,20bc	34,09c	2,10d	4,87d
	Ib+Gi+M	5,43c	22,39b	30,13c	2,06d	4,89d
	Ib+Gi+D	5,98c	28,00d	37,24c	2,09d	4,94d

(*): Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance (P<0.05)

Ib: *Intsia bijuga*; Gi: *Glomus intraradices*; C: *Crotalaria juncea*; Z: *Zea mays*; D: *Dalbergia trichocarpa.*

On non-disinfected forest soil, the mycorrhizal dependency of *Intsia bijuga* in dual cultivation either with *Crotalaria juncea* or *Dalbergia trichocarpa* was lower than those with *Zea mays* (Table 2.)

Table 2. Growth response, endomycorrhizal rate, mycorrhizal dependency and on –leaf mineral content of *Intsia bijuga* after 4 months in dual cultivation with *Crotalaria juncea*, *Zea mays* and *Dalbergia trichocarpa* in forest soils

Soil	Culture (Treatment)	Ib Total Biomass (g dry weight)	Ib Endomycorrhizal colonization (%)	Ib Mycorrhizal dependency	Ib Nitrogen (%)	Ib Phosphorus (%)
Forest soil sterilized	Ib	4,57b	0a	0a	1,22b	1,69bc
	Ib+C	4,63b	0a	0a	1,20b	1,66ab
	Ib+Z	3,88a	0a	0a	1,20b	1,71bc
	Ib+D	4,55b	0a	0a	1,21b	1,64ab
Forest soil unsterilized	Ib	4,91b	0a	0a	1,34c	1,77c
	Ib+C	5,72c	24,60bc	13,95b	2,10d	4,88d
	Ib+Z	5,82c	24,79bc	33,10c	2,06d	4,92d
	Ib+D	5,78c	26,10cd	31,04b	2,09d	4,93d

(*): Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance (P<0.05)

Ib: *Intsia bijuga*; Gi: *Glomus intraradices*; C: *Crotalaria juncea*; Z: *Zea mays*; D: *Dalbergia trichocarpa.*

Some authors mentioned that *Intsia bijuga* never establishes endomycorrhizal symbiosis nor nitrogen-fixing symbiosis (Smith and Read, 2008; Nugroho et al., 2010). Indeed, histological observation of *Intsia bijuga* roots collected on a plantation of 50 years and a nursery for 4 to 12 months made by Nugroho et al., (2010) showed no evidence of the

establishment of endomycorrhizal symbiosis. In our previous work, we found neither vesicles nor hyphae in the roots of *Intsia bijuga* plants collected in the lowland forest of Tampolo, located on the east coast of Madagascar (Rakotoarimanga, 2010). Some authors have shown that at the beginning of their growth, certain trees can be infected by mycorrhizal strains which rarely infect mature trees. These are the cases observed on *Eucalyptus*. In effect, Lapeyrie and Chilvers (1985) have highlighted the success of endomycorrhizae and ectomycorrhizae on roots of *Eucalyptus dumosa* seedlings grown in pots. In addition, Malajczuk et al., (1981), in their inoculation study of two species of Eucalyptus (*Eucalyptus diversicolor* and *Eucalyptus marginata*) by mycorrhizal fungus strain *Glomus fasciculatum*, found that mature trees are infested rarely by this strain.

According to Giovannetti et al. (1993), root exudates could influence the germination of mycorrhizal fungi and their ability to infect roots through appressoria formation, but these authors found that non endotrophic plant *Lupinus albus* was not infected by *Glomus mossae* even though the roots contact with endomycorhizal host plant root *Medicago sativa* in the same pot. However, some authors showed that the plant of the genus *Lupinus* was infected by endomycorrhizal fungi in situ (Trinick, 1977) or in laboratory conditions (Bedmar and Ocampo, 1986) when its roots were in contact with other endomycorrhizal host plants roots. Our results are similar to those obtained by Hirrel et al., (1978); Ocampo et al., (1980); Veiga et al., (2013) and Fernandez et al., (2019). These authors found that some plant species of Chenopodiaceae, Fabaceae and Cruciferaceae families, which are not endomycorrhizal host plants, become hosts when they are inoculated in the case of co-culture with other endomycorrhizal plants. However, arbuscules were not observed on colonized plant roots (Hirrel et al., 1978; Ocampo et al., 1980; Rinaudo et al., 2010 ; Veiga et al., 2013; Fernandez et al., 2019), Hirrel et al. (1978) found that vesicles and mycorrhizal fungal hyphae colonized dead tissues or old root. In this case (dead tissue colonization and absence of arbuscules), some authors considered that the fungus infected the plant as a saprophyte but not as symbiosis (Giovanetti and Lioi, 1990, Hall and Finch, 1974; Stasz and Sakai, 1984; Park and Linderman, 1980). Certainly, in the case of *Intsia bijuga*, we have not determined the absence or presence of arbuscules in the experiment, but the improvement of plants development whose roots were colonized by hyphae and vesicles, suggest efficient symbiosis. These results demonstrate *Intsia bijuga* endomycorrhizal dependency. Endomycorrhizal symbiosis enhances nutrients acquisition for a plant, especially nitrogen and phosphorus (Dommergues and Mangenot, 1970 Gianinazzi-Pearson, 1982; Plenchette, 1982; Strullu, 1991; Sabia *et al*., 2015; Puschel et al., 2016; Zhu *et al*., 2016). Nitrogen and phosphorus necessary for the development and survival of plants are carried by fungal strain due to the elongation of its hyphae exploring the soil (Tinker, 1984; Mousain, 1989; Morillou and Martin, 1997; Sato et al. , 2015) or by its ability to produce enzymes such as phosphatases (Jayachandran et., 1992; Mousain, 1989) or protease (Näsholm et al., 1998). Plant colonization by mycorrhizal fungi and the effectiveness of these depend on several factors. Indeed, the colonization of plants depends mainly on the amount of nitrogen and phosphorus in the soil (Chabot et al., 1998; Ruan et al., 2000; Ruotsalainen et al., 2002). According to Amijee et al. (1993), mycorrhizal infection is not effective when the rate of phosphorus by plants is high in the soil. Plants have not the same mycorrhizal dependency (Gerdemann, 1975). This latter depends on biotic and

abiotic factors such as mycorrhizal fungi species, plant genotype, soil type, nitrogen and phosphorus (Plenchette, 1991; Johnson et al. 2015). In this study, mycorrhizal dependency of Intsia bijuga seems to be dependent on the plant species cultivated. Dependence is low when Intsia bijuga was cultivating with legumes on unsterilized forest soil. These results suggest that the ability of legumes to establish a nitrogen-fixing symbiosis with nitrogen-fixing bacteria in the soil improved its nitrogen richness (Mc Innes and Haq, 2007) and influenced the mycorrhizal dependency of *Intsia bijuga* (Plenchette, 1991).

CONCLUSION

By these results, this was the first time that the establishment of endomycorrhizal symbiosis in Intsia bijuga was demonstrated in greenhouse conditions. In this study, it was shown that Intsia bijuga development depends on the endomycorrhizal symbiosis establishment. Thus, dual cultivation of Intsia bijuga with endomycorhizal host plants makes effective endomycorrhizal symbiosis in Intsia bijuga.

REFERENCES

Amerian, M.R., Stewart, W.S. and Griffiths H. 2001. Effect of two species of arbuscular mycorrhizal fungi on growth, assimilation and leaf water relations in maize (*Zea mays*). Aspects of Applied Biology. 63.

Amijee, F., Stribley D.P. and Lane P.W. 1993. The susceptibility of roots to infection by an arbuscular mycorrhizal fungus in relation to age and phosphorus supply. New Phytologist 125: 581–586.

Bedmar, E.J. and Ocampo, J.A. 1986. Susceptibilidad de distintas variedades de guisante, veza y lupino a la infeccion por *Glomus mosseae*. Anales de Edafologia y Agrobiologia 45: 231-238.

Bonfante P, Genre A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nature Communications 1: 48.

Brundrett, M., Bougher, N., Dell, Grove, B. T. and Malajczuk, N. 1996. Working with Mycorrhizas in Forestry and Agriculture. ACIAR Monograph 32.

Chabot, R., Beauchamp, C.J., Kloepper J.W., and Antoun H. 1998. Effect of phosphorus on root colonization and growth promotion of maize by bioluminescent mutants of phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Soil Biology and Biochemistry. 12: 1615-1618.

Dommergues, Y. and Mangenot F. 1970. Ecologie microbienne du sol. Masson, Paris.

Field, K.J., Rimington, W.R., Bidartondo, M.I., Allinson, K.E., Beerling, D.J., et al. 2015. The first evidence of mutualism between ancient plant lineages (*Haplomitriopsida liverworts*) and *Mucoromycotina* fungi and its response to simulated Palaeozoic changes in atmospheric CO2. New Phytologist. 205: 743–756.

Fernández, I., Cosme, M., Stringlis, I.A., Yu, K., de Jonge, R., van Wees, S., Pozo, M.J., Pieterse, C.M.J., van der Heijden, M.G.A. 2019. Molecular dialogue between arbuscular mycorrhizal fungi and the nonhost plant Arabidopsis thaliana switches from initial detection to antagonism. New Phytologist. 223, pp. 867-881.

Gerdemann, J.W. 1975. Vesicular-arbuscular mycorrhizae. PP. 575-591 in the development and function of roots. (Torrey JG, Clarkson DT, Eds). Academic Press. New York and London.

Gianinazzi-Pearson, V. 1982. Importance des mycorhizes dans la nutrition et la physiologie des plantes. Dans : *Les mycorhizes, biologie et utilisations*, Gianinazzi-Pearson, V. et Gianinazzi, S. (éds). INRA Pub., pp. 51-59.

Giovannetti, M. and Lioi, L. 1990. The mycorrhizal status of *Arbutusunedo* in relation to compatible and incompatible fungi. Canadian Journal of Botany 68: 1239-1244.

Giovannetti, M., Avio L., Sbrana, C. and Citernesi, A.S. 1993. Factors Affecting Appressorium Development in the Vesicular-Arbuscular Mycorrhizal Fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe. New Phytologist 123: 115-122.

Gutjahr C., Parniske M. 2013. Cell and developmental biology of arbuscular mycorrhiza symbiosis. Annual Review Cell Developmental Biology. 29: 593–617

Hall, J.B. and Finch, H.C. 1974. Mycorrhiza in roots of avocado: effect upon chemotaxis of *Phytophthora cinnamoni* zoospores. *Proceedings of the American* Phytopathological Society 1: 86.

Harrison, M.J. 2005. Signaling in the arbuscular mycorrhizal symbiosis. Annual Review Microbiology. 59:19-42.

Hirrel, M.C, Mehravaran, H. and Gerdemann, J.W. 1978. Vesicular arbuscular mycorrhizae in the Chenopodiaceae and Cruciferae: do they occur? Canadian Journal of Botany 56: 2813-2817.

Jayachandran, K., Schwab, A.P. and Hetrick, B.A.D. 1992. Mineralization of organic phosphorus by vesicular-arbuscular mycorrhizal fungi. Soil and Biochemistry, 24 : 897-903.

John, M.K. 1970. Colorimetric determination in soil and plant material with ascorbic acid. Soil Sciences. 68: 171-177.

Johnson, N.C., Wilson, G.W.T., Wilson, J.A., Miller, R.M. and Bowker, M.A. 2015 Mycorrhizal phenotypes and the Law of the Minimum. New Phytologist, 205: 1473–1484.

Krüger, M., Krüger, C., Walker, C., Stockinger, H. and Schüßler, A. 2011. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytologist.193:970–984.

Lapeyrie, F.F. and Chilvers, G.A. 1985. An endomycorrhiza-ectomycorrhiza succession associated with enhanced growth of *Eucalyptus dumosa* seedlings planted in a calcareous soil. New Phytologist. 100: 93-104.

Malajkzuk, N., Linderman, R.G. Kough, R.G. and Trappe, J.M. 1981. Presence of vesicular arbuscular mycorrhizae in *Eucalyptus* spp. and *Acacia* spp. and their absence in *Banksia* spp. after inoculation with *Glomus fasciculatus*. New Phytologist 87: 567-572.

Martin, F. and Lorillou. 1997. Nitrogen acquisition and assimilation in ectomycorrhizal systems. In: *Trees-contributions to modern tree physiology* (ed. H. RENNENBERG, ESCHRICH, W. & ZIEGLER, H.) SPB Academic Publ., La Haye, Pays-Bas, p. 423-439.

Mc Innes, A. and Haq, K. 2007. Contributions of *Rhizobia* to soil Nitrogen fertility. L.K. Abott and D.V. Murphy (eds.). Soil biological fertility-A key to sustainable Land Use in Agriculture. 99-128.

Mousain, D. 1989. Etude de la nutrition phosphatée de symbiotes ectomycorhiziens. Ph'D Thesis, USTL Montpellier II. France.

Nagata M, Yamamoto N, Shigeyama T, Terasawa Y, Anai T, Sakai T, Inada S, Arima S, Hashiguchi M, Akashi R, Nakayama, H., Ueno, D., Hirsch, A.M., Suzuki, A. 2015. Red/far red light controls arbuscular mycorrhizal colonization via jasmonic acid and strigolactone signaling. Plant and Cell Physiology.

Nasholm, T., Ekblad A., Nordin, A., Giehler R., Hogberg, M. and Hogberg, P.1998. Boreal forest plants take up organic nitrogen. Nature 392: 914-916.

Nugroho, J.D., Irdika M., Agus, P. and Endang, S. 2010. Morphological Characteristics of Ectomycorrhizas on Merbau [*Intsia bijuga* (Colebr.) O. Kuntze]. Journal of Biosciences 17: 68-72.

Ocampo, J.A., Martin, J. and Hayman, D.S. 1980. Influence of plant interactions on vesicular-arbuscular mycorrhizal infection. I. Host and non-host plants grown together. New Phytologist. 84: 27-35.

Park, J.L. and Linderman, R.G. 1980. Association of vesicular arbuscular fungi with the moss *Funaria hygrometrica*. Canadian Journal of Botany. 58: 1898-1 904.

Phillips, J.M. and Hayman, D.S. 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.

Plenchette, C. 1982. Recherches sur les endomycorhizes à vésicules et arbuscules. Influence de la plante hôte, du champignon et du phosphore sur l'expression de la symbiose endomycorhizienne. Ph'D Thesis, Univ. Laval, Québec, Canada.

Plenchette, C. 1991. Utilisation des mycorhizes en agriculture et horticulture. In *les mycorhizes des arbres et plantes cultivées*. Tech. et doc.-Lavoisier, p. 131-179

Plenchette, C., Fortin, J. and Furlan, V. 1983. Growth responses of plant species to mycorrhizae in soil of moderate P-fertility. Mycorrhizal dependency under field conditions. Plant and soil 70: 199-209.

Puschel, D., Janouskova, M., Hujslova, M., Slavikova, R., Gryndlerova, H. and Jansa, J. 2016. Plant-fungus competition for nitrogen erases mycorrhizal growth benefits of *Andropogon gerardii* under limited nitrogen supply. Ecology and Evolution. 6: 4332–4346

Rakotoarimanga, N.C. 2010. Structure et fonctionnement de la microflore rhizosphérique associée à la symbiose mycorhizienne en faveur de *Intsia bijuga*, plante autochtone malgache de la forêt de Tampolo Ph'D Thesis, Univ. d'Antananarivo, Madagascar.

Rich, M.K., Schorderet, M., Reinhardt, D. 2014. The role of the cell wall compartment in mutualistic symbioses of plants. Front Plant Science.

Rinaudo, V., Bàrberi, P., Giovannetti, M., van der Heijden, M.G.A. 2010. Mycorrhizal fungi suppress aggressive agricultural weeds. Plant Soil. 333 pp. 7-20

Ruan, J., Zhang, F. and Wong, M. H. 2000. Effect of nitrogen form and phosphorus source on the growth, nutrient uptake and soil rhizosphere property of *Camellia sinensis* L. Plant and Soil. 223: 63-71.

Ruotsalainen, A. L., Väre, H. and Vestberg, M. 2002. Seasonality of root fungal colonization in low-alpine herbs. Mycorrhiza. 12: 29-36.

Sabia, E., Claps, S., Morone, G., Bruno, A., Sepe, L. and Aleandri, R. 2015. Field inoculation of arbuscular mycorrhiza on maize (*Zea mays* L.) under low inputs: preliminary study on quantitative and qualitative aspects. Italian Journal of Agronomy. 10: 30–33.

Sato, T., Ezawa, T., Cheng, W.G. and Tawaraya, K. 2015. Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus *Rhizophagus clarus*. Soil Science and Plant Nutrition. 61: 269–274.

Smith, S.E. and Read, D.J. 2008. Mycorrhizal symbiosis. 3rd edition. New York: Acad Pr.

Stasz, T.E. and Sakai, W.S. 1984. Vesicular-arbuscular mycorrhizal fungi in scale-leaves of Zingiberaceae. *Mycologia* 76: 754-757

Strullu, D.G. 1991. Les mycorhizes des arbres et plantes cultivées. Collection Tec. & Doc., Lavoisier, Paris.

Strullu-Derrien, C., Kenrick, P., Pressel, S., Duckett, J.G., Rioult, J.P., Strullu, D.G. 2014. Fungal associations in *Horneophyton ligneri* from the Rhynie Chert (c. 407 Ma) closely resemble those in extant lower land plants: novel insights into ancestral plant–fungus symbioses. New Phytologist 203: 964– 979.

Tinker, P.B. 1984. The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. Plant Soil 76: 77-91.

Trindade, A.V., Siqueira, J.O. and Stümer, S.L. 2006. Arbuscular mycorrhizal fungi in papaya plantations of Espírito Santo and Bahia, Brazil. Brazilian Journal of Microbiology. 37:283-289

Trinick, M. J. and Mosse, B. 1975. Rothamsted Report for 1974, part 1, p. 253.

Veiga, R.S.L., Faccio, A., Genre, A., Pieterse, C.M.J., Bonfante, P., Van der Heijden, M.G.A. 2013. Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant *Arabidopsis thaliana*. Plant, Cell and Environment. 36 pp. 1926-1937.

Willis, A., Rodrigues, B.F. and Harris, P.J.C. 2013. The Ecology of Arbuscular Mycorrhizal Fungi. Critical Reviews in Plant Sciences, 32(1)

Zhu, X.C., Song, F.B., Liu, S.Q. and Liu, F.L. 2016. Arbuscular mycorrhiza improve growth, nitrogen uptake, and nitrogen use efficiency in wheat grown under elevated CO2. Mycorrhiza. 26: 133–140.