International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 4 (12), pp. 001-005, December, 2010. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Arbuscular mycorrhizal fungus inoculum production in rice plants

Tanzima Yeasmin*, Parmita Zaman, Ataur Rahman, Nurul Absar and Nurus Saba Khanum

Department of Biochemistry and Molecular Biology, University of Rajshahi.Rajshahi-6205. Bangladesh.

Accepted 21 June 2007

Mangifera indica **showed highest percentage (100%) of mycorrhizal colonization in Rajshahi University Campus, Bangladesh that was used as a stock plant in pot culture experiment. These root pieces have the ability to serve as a source of mycorrhizal inoculum for crop plants. After using mycorrhizal inoculum, the soil nutrients as well as root colonization for rice plants were greatly affected. Soil nutrients were increased (nitrogen-0.03 times and phosphorus 8 times compared to sterile soil), whereas the percentage of rice roots colonization of arbuscular mycorrhiza (AM) was also increased 9 times after mycorrhizal inoculation. Mycorrhizal enrichment greatly improved the soil nutrients such as nitrogen and phosphorus as well as growth of rice plants.**

Key words: Arbuscular mycorrhiza, rice, soil phosphorus, nitrogen.

INTRODUCTION

Beneficial effects of AM fungi as efficient scavengers of nutrients and as bio-control agents have been well established in horticultural crop production. However, the utilization of these fungi is limited by the lack of availability of inoculum in large quantity.

Mycorrhiza is a type of mutualistic association between plant and fungus. Arbuscular mycorrhizal fungi absorb nitrogen, phosphorus, potassium, calcium, sulfur, ferric, manganese, copper and zinc from the soil and then translocate these nutrients to the plants with whose roots they are associated (Gredemann, 1975). Their most consistent and important nutritional effects are to improve uptake of immobile nutrients such as phosphorus, copper and zinc (Manjunath and Habte, 1988). Arbuscular mycorrhizal fungi have their greatest effect when a host plant associated with them, which is of deficient in phosphorus. (Koide, 1992). It is a fact that mycorrihzal fungus is able to increase growth in a number of agricultural crops (Mosse, 1973; Gredemenn, 1975; Tinker, 1975). Sanni (1976) demonstrated the increase in the growth of rice plants after inoculation with *Giaspora gigentia.* Utilization of AM inoculum in crops like banana and papaya cultivation was demonstrated in four farmers of two Indian villages and the farmers in these villages were also convinced about its usefulness (Mohandas et al., 2004). Mycorrhizal colonization may have increased plant resistance to potential as Arsenic (As) toxicity at the highest level of As contamination as studied by Liu et al., (2005). Further it was reported by them that tomato plants might have potential for Phyto-extraction of As from moderately contaminated soils or Phyto-stabilization of more highly polluted sites. The mycorrhizal benefit of the indigenous fungi corresponded with a requirement for phosphate by the plants that were colonized by AM fungi already present in the soil equivalent to half that required by non-mycorrhizal plants (Gazey et al., 2006). Doud et al. *(*2002) found that addition of supplemented nutrients such as water, inorganic nutrient solution minus phosphorus and fish protein, was unnecessary but concentrated inoculum of AM fungi in a form readily used as an amendment to horticultural plotting media for the production of vegetable seedlings.

To get maximum agricultural benefit, inoculation of the soil with suitable type of AM fungi is necessary. Hence, the only way to culture and maintain arbuscular mycorrhizal fungus production, the use of AM inoculum that is produced by additional application of these fungi should become commercially feasible. For this reason, pot experiment inoculum production was conducted in the pres-

*Corresponding author. E-mail: yeasmin_bio@yahoo.com. Tel: 750041-4109(Office).-0721-750041-3758

Table1. Percentage and intensity of root colonization in crops plants.

Plant No.	Family name	Scientific name	Total infection (%)	% of intensity of colonization		
				Poorly (+)	Moderately (++)	Highly (+++)
1	Gramineae	*Avena sativa*	25	15	10	×
2		*Oriza sativa*	10	10	0	0
3		*Saccharum officinarum*	28	20	5	3
4		*Triticum aestivum*	30	15	10	5
5		*Zea mays*	30	10	15	5

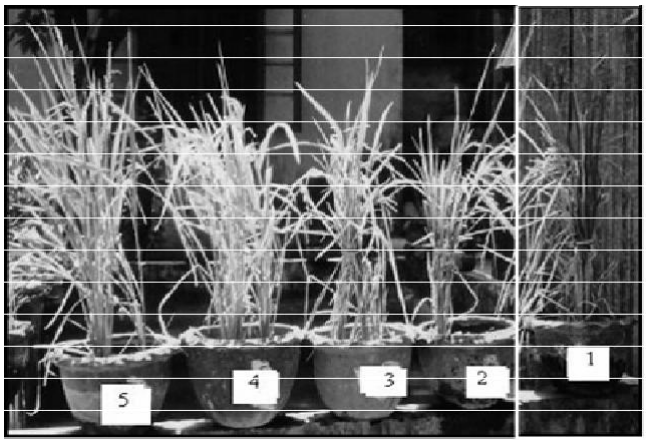

Figure 1. Experimental pots treatment with 1. Control soil, 2. Chemical fertilizer, 3. Urea, 4. AM inoculum, 5. Phosphorus with AM inoculum.

ent study for evaluation of the effect of the indigenous mycorrhiza on the growth and nutrition of rice plants.

The plants that naturally facilitate the higher colonization of AM can be generally considered to be used as stock plants (Parmita, 2005). Stock plants to culture the AM fungi for inoculum practice with utilization as biofertilizer were selected in order to increase agricultural crops as well as to minimize the use of chemical fertilizer and thereby reduce the environmental pollution.

METHODS AND MATERIALS

Endomycorrhizal inoculum was produced by the most common method. The first step was to identify a stock plant that had abundant colonization of AM fungus. The roots of the sampled plants were dug very carefully to get most of the finer roots. Root samples were cleaned, cut into 1 cm segments (Hayman, 1974) and stained according to the method described by Phillips and Hayman (1970). The root segments were then observed under a microscope. The infected and uninfected root segments were collected and percent of infection was calculated.

After confirming mycorrhizal association in the *Mangifera indica* stock plant roots, 100 g rhizospore containing soils were studied. Soil was collected from the natural land at a depth of 50 cm from the soil surface level locations during dry season. The experimental soils were prepared by mixing two parts of soil with one part of sand. The sand was pretreated with acid and washed with distilled water subsequently. About 4 kg capacity pots were used and 3 kg soil-sand samples were taken in each pot. Soil samples were taken and mixed with small amount of mycorrhizal soil to sterilized pots. The pots were seeded with plants, which would highly infect for the desired mycorrhiza. The pots were then left without water and thoroughly dry. At this point, the soil in the pots could be used for inoculum production. The soil contained a mixture of mycorrhizal spores, fungal hyphae and root pieces, which were colonized by mycorrhizae (Noyd, 1995).

Five pots of soil were used for the culture of rice plants. BRRI-29 variety of rice, collected from Bangladesh Rice Research Institute, Rajshahi was used as test crop. Five sets of experiments were conducted: 1st pot contained sterilized soil without any kind of fertilizer as control while 2nd pot contained only chemical fertilizer. The soil was fertilized with nitrogen, potassium, phosphate, zinc and calcium at the rate of 220, 85, 120, 10 and 70 kg/ha, and the source being urea, murate of potash, triple super phosphate, zinc sulfate and gypsum respectively. The 3rd pot was fertilized with 220 kg/ha urea and the 4th pot was mixed with stock plant (*M. indica*) mycorrhizal inoculum. In the last pot, phosphate was used as triple super phosphate, 120 kg/ha as well as mycorrhizal fungi inoculum. For mycorrhizal plants (grown in non–sterile soils), mycorrhizal dependency values were calculated according to the method devised by Plenchette et al. (1983). It has been designated as relative field mycorrhizal dependency (RFMD) index and was determined by expressing the difference between dry weights of mycorrhizal with that of non-mycoorrhizal plants. The results have been expressed as a percentage of dry weight of mycorrhizal plants.

RESULTS AND DISCUSSION

The occurrences and intensity of root colonization of AM in crop species of Gramineae family were presented in Table.1.All the five species have showed the colonization of AM. However, the amount of infection was varied from species to species and ranging from 10to 30%. Further the percentage of intensity of colonization was classified into abundant, moderate and poor types. The relatively the low intensity of mycorrhizal infection in the roots of crop species indicates the necessity and importance of artificial inoculation with mycorrhiza as biological input in crops cultivation.

In pot culture experiment, (Figure.1) the root pieces of *M. indica* (Figure 2a, b and c) which showed highest (100%) colonization on AM fungi in natural condition, were tested for their ability to act as source of mycorrhizal inoculum for rice.

The incidence of mycorrhizal infection in the rice roots was counted and percentage infection was calculated. It

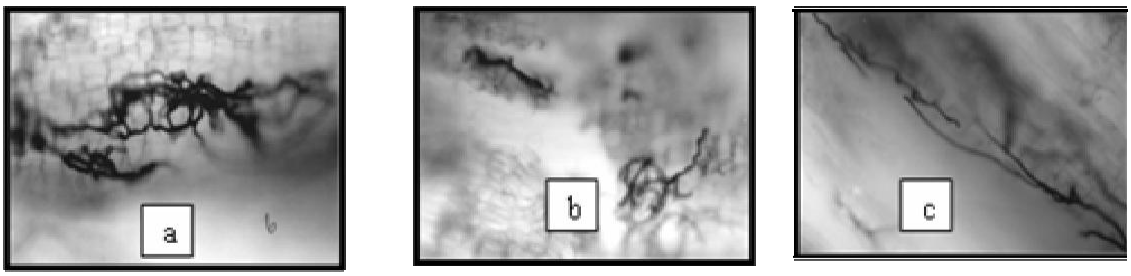

Figure 2. (a) Highly infected (+++) *Mangifera indica* roots (Intensity of infection 18 %); (b) Moderately infected (++) *Mangifera indica* roots (Intensity of infection38%) (++); (c) Poorly infected (+) *Mangifera indica* roots (Intensity of infection 44 %).

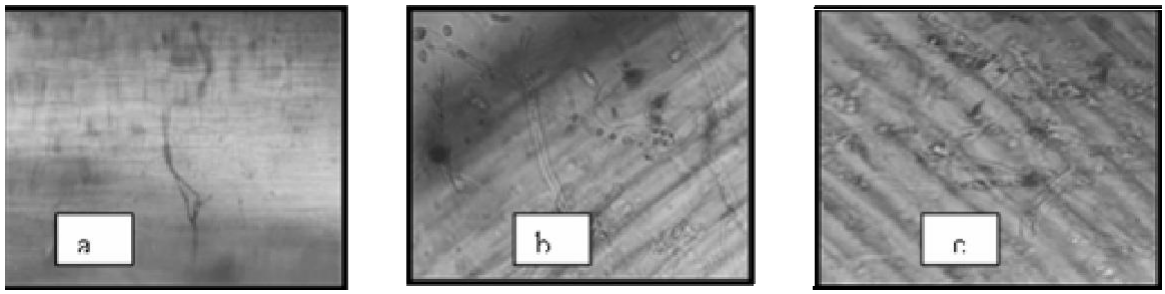

Figure 3. Root colonization of rice plant rice plant without AM inoculum×100; 2b.Root colonization of rice plant after AM inoculum×100; 2c. Root colonization of after AM

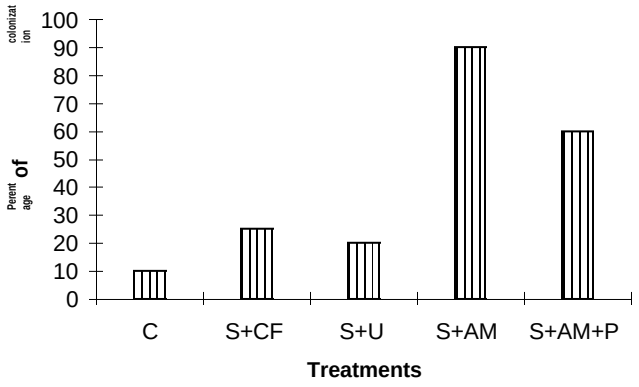

Figure 4. Percentage of root colonization under different treatments: C = Control, S+CF = Soil with chemical fertilizer, S+U = Soil with Urea, S+AM = Soil with Arbuscular mycorrhizal fungus and S+AM+P = Soil with Arbuscular mycorrhizal fungus and phosphorus.

was found that roots of rice plants grown in control soils (Figure 3) were infected in very small percentages. However, considerable mycorrhizal infection was observed in the roots of rice plants grown with arbuscular mycorrhizal inoculum (Figures 2b and 2c).

Monocots like grasses with rapidly developing roots are the ideal stock plants for AM, but any host plant which is readily colonized by AM and which can be easily grow in the greenhouse can also serve as the stock plant (Ferguson and Woodhead, 1982).

Figure 4 revealed the positive result as the plant of inoculated pot showed the highest intensity of AM infection than those of non-inoculated pots, with and without added phosphorus, had 60% and 90% infection, respecttively in their roots. Mosse (1975) also reported such an influence of phosphorus. It was found that the extent of root intensity is higher in mycorrhizal non-phosphorus rice plant than in mycorrhizal rice receiving added phosphorus. It may suggest that the reduction of mycorrhizal infection in presence of added phosphorus is owing to a self regulatory mechanism of plant discarding the mycorrhizal fungus when its phosphorus requirement is more than that satisfied (Hayman, 1982) . Grazey et al. (2006) also reported that the mycorrhizal benefit was independent of the plant-available phosphorus in the soil. Further, there was no additional benefit of inoculation on plant growth other than that due to the increased phosphorus uptake. Moreover again phosphorus is applied at high concentrations, as commonly done when growing plants in soil where AM fungi are absent, then it can cause nutritional disorder because of its antagonistic interacttions with other nutrients or it inhibits mycorrhizal formation (Lambert, 1979). Similar result was found in phosphorus added AM inoculum in the present study.

The soil analysis of rice plant under different treatments is shown in Table.2. Rice soil were treated with different

Table 2. Soil analysis under different treatments.

Sample no.	Soil treatments	pH	Organic matter (%)	Total nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Magnesium (ppm)	Calcium (ppm)
1	Control	7.95	2.81	0.16	29.10	0.28	3.10	18.10
2	Urea	7.61	3.36	0.19	259.51	0.18	2.80	17.50
3	Urea and muriate of potash	7.80	3.05	0.17	47.86	0.25	2.40	17.60
4	Inoculated with AM fungi roots	7.27	3.29	0.19	205.88	0.24	2.80	17.40
5	Triple super phosphate and AM inoculum	7.18	3.08	0.18	111.85	0.37	2.00	14.40

Table 3. Shoot dry weights and the percent RFMD for rice plants.

Sample no.	Soil treatments	Shoot dry weight (g)	∗∗Per cent RFMD	Percentage of Mycorrhizal root colonization
1	Control	35.25±.02		10
2	Urea	38.8±.03	9.14±.02	12
3	Urea and muriate of potash	40±.02	13.47±.025	15
4	Inoculated with AM fungi roots	64.7±.03	45.51±.03	90
5	Triple super phosphate and AM inoculum	37±.025	2±.02	10

∗ ∗ % RFMD = (Dry weight of mycorrhizal plant) – (Dry weight of non mycorrhizal plant)/ (Dry weight of mycorrhizal plant) X100

chemical fertilizers as well as with mycorrhizal inoculum. is shown in Table.2. Rice soil was treated with different chemical fertilizers as well as with mycorrhizal inoculum. Increased for applying of chemical fertilizer, mycorrhizal inoculum and phosphate added with inoculum. However, the better uptake of nitrogen by rice plant due to indigenous vesicular arbuscular mycorrhizae (VAM) infection might be justified as the exudates from mycorrhizal roots stimulate the fixation of atmosphere nitrogen by free living rhizosphore microorganism (Mosse, 1981).

In many tropical soils, phosphorus availability is limited due to phosphorus fixation. Plants inoculated with AM fungi are better able to obtain phosphorus when they are later planted into low phosphorus soil. Phosphorus markedly increased with chemical phosphate and root inoculum and along AM root inoculum of *M. indica* as control soil. So it suggested that infection with VA fungus is able to increase phosphorus as well as chemical phosphate. Phosphorus and nitrogen uptake by rice plants were also estimated to evaluate the effect of indigenous VA mycorrhiza. It was found that root infection by VAM fungi greatly improves phosphorus and nitrogen uptake by rice plants growth. The effect of mycorrhizal infection on phosphorus uptake was most pronounced. Improvement of phosphorus uptake by mycorrhizal plants was reported by Islam and Ayanaba (1981) . Draft and Nicolson (1966) applied different amounts of phosphate to soil and sand cultures, and found that the mycorrhizal infection and the beneficial effects of VA mycorrhiza on the host plant were inversely related to the amount of available phosphate. Murdoch (1967), Jenson and Jakobson (1980) reached a similar conclusion by adding phosphates of varying availability to soils. From soil analysis results we found that AM inoculation significantly enriched the soil nutrients than non-inoculated soil. From our study it was also observed that AM inoculated soil became more fertile without any type of other chemical fertilizers.

Shoot dry weight was considered as the index of growth parameter. For mycorrhizal plants, mycorrhizal dependency values were calculated and have been designated as relative field mycorrhizal dependency (RFMD) index. The shoot dry weights of rice plants growth both in different treatment soils and the percent RFMD for mycorrhizal rice plants are found to varied bet-ween 35.25 ± 02 to 64.7 ±. 03 and 2±.02 to 45.51 ± .03, respectively as presented in Table 3.

Marked differences were observed in the shoot dry weights of mycorrhizal and that of the non-mycorrhizal plants. Percent of RFMD for rice plants were 9.14, 13.47, 45.51 and 2 in the soil treated with urea, urea with muriate of potash, inoculated with AM fungi roots and triple super phosphate with AM inoculum. The differences in growth were also quite marked (Figure1). Similar findings were also reported by Khan et al (1988).

In fact, the nutritionally significant function of mycorrhiza depends on soil exploration by fungal hyphae. Because of a very large surface to volume ratio, hyphae produce an extra and well-distributed absorbing surface.

This extra surface is particularly important for phosphate uptake by the plant because this ion is readily sorbed on clay complexes and diffused very slowly in soil that helps in developing a depletion zone around the actively absorbing rootlet. Hyphae of the mycorrhizal infection can also induce a better uptake of nutrients other than phosphate. However, direct mycorrhizal effects on mine-ral nutrition may well be limited to those elements, which have a poor mobility (Khan et al., 1988). Improvement of phosphorus uptake by mycorrhizal plants was reported by Islam and Ayanaba (1981), Bagyaraj and Sreeramulu (1982), Jensen (1983), Nielsen and Jensen (1983) and Jensen (1984).

It may be concluded from the present data that the indigenous AM mycorrhizal spores and mycelia present in Rajshahi University campus soils are capable of infect-ing rice roots. AM infection improves the soil nutrient, which is necessary for rice plant growth. *M. indica* is widely distributed in Bangladesh and the present results also suggested that the rice plant may be considered as an initial stock plant which may be used for inoculum production in Bangladesh climatic condition. Further many areas of Bangladesh are highly Arsenic contaminated. Use of AM inoculum in crops production might be useful for the peoples as mycorrhizal colonization have increased the plant resistances against Arsenic toxicity (Liu et al., 2005). In future, the most modern and advanced technology should be considered for large-scale inoculum production of AM fungus under field condition.

REFERENCES

Bagyaraj DJ, Sreeramulu KR (1982). Preinoculation with VAM improved growth and yield of chili transplanted in the field and saves phosphate fertilizer. Plant and soil. 69: 376-381.

Draft MJ, Nicolson TH (1966). Effect of endogone mycorrhiza on plant growth. New Phytol. 65: 343-350.

Doud DD, Nagahashi G, Pfeffer PE, Reider C, Kavser WM (2002). On-farm production of AM fungus inoculum in mixtures of compost and vermiculite. *Mycorrhiza.* 12(6): 285-90.

Ferguson JJ, Woodhead SH (1982). Production of endomycorrhizal inoculum. In methods and principle of mycorrhizal resarch. N. C. Schenek (Ed.). The American Phytopathology Society. Minnesota. pp.47-54.

Gazey C, Abbott LK, Robson AD (2006).Indigenous and introduced arbuscular mycorrhizal fungi contribute to plant growth in two agricultural soils from south-western Australia. Bioresour Technol. 97(6): 809-18.

Gredemann JW, Nicolson TH (1975). Spores of Mycorrhizal endogone species extracted from soil wet sieving and decanting. Trans. Brit. Mycol. Soc.46.235-244

Hayman DS (1974). Plant responses to vesicular arbuscular mycorrhizae VI. Effect of light and tem. New Phytologist. 73*:* 71-80

Hayman DS (1982). *Endomycorrhizae*, In interactions between non-pathogen soil microorganisms and plants. Eds. Elseveir. Amsterdam. pp. 401- 442.

Islam R, Ayanaba A (1981). Growth and yield responses of cowpea and maize to inoculation with *Glomus mosseae* in sterilize soil under field conditions. Plant and Soil. 63: 503-509.

Jakobson I (1980). The occurrences of VA-mycorrhiza in barley and wheat grown in some Danish soils with different fertilizer treatments. Plant and Soil. 35: 403-414.

Jensen A (1983).The effect of indigenous VA-mycorrhizal fungi on nutrient uptake and growth of barley in two Danish soils. Plant and soil. 70: 155-163.

Jensen A (1984).Responces of barley, pea and maize to inoculation with different VA-mycorrhizal fungi in irradiated soil. Plant and soil. 78: 315-523.

Khan AH, Islam A, Begum S, Imamul Huq SM (1988).Mycorrhizal status of some Bangladesh soils and the effects of indigenous VA-mycorrhizal fungi on the growth of rice plants. Bangladesh. J. Bot .17(1): 49-56.

Koide RT, Scheiner RP (1992). Regulation of the vesicular–arbuscular mycorrhizal symbiosis. Annul Rev of Plant Physiol. and Plant Molecular Biol. .43:557-5891.

Lambert DH, Baker DE (1979). The role of Mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. Soil Sc. Society Am. J. pp. 976-980.

Liu Y, Zhu YG, Chen BD, Christie P, li XL (2005).Yield and arsenate uptake of arbuscular mycorrhizal tomato colonized by Glomus mosseae BEG167 in As spiked soil under glasshouse conditions. Environ. Int .31(6): 867-73.

Manjunath A, Habte M (1988). The development of vesicular arbuscular mycorrhizal infection and the uptake of immobile nutrients in *Leucaena leucocephala*. Plant and soil.106: 97-103.

Mohandas S, Chandre Gowda MJ, Manamohan M (2004). Popularization of Arbuscular Mycorrhizal (AM) inoculum production and application on –farm. Ishs Acta Horticulturae 638: XXVI International Horticultural Congress: Sustainability of Horticultural Systems in the 21st Century. Ed Bertschinger L Anderson J.D. Toronto.Canada.

Mosse B (1973).Advances in the study of VA-mycorrhiza .Ann. Rev. Phytopathol. 11:1711-194.

Mosse B, Herper C (1975). Vesicular-Arbuscular Mycorrhizal infection in root organ culture. Physiol. Plant Pathol. 5: 215-223.

Mosse B (1981). Vesicular arbuscular Mycorrhiza research for tropical agriculture. Research bulletin 194. College of Agriculture and Human, Resources. Honolulu. University of Hawaii, p 82.

Murdoch CL, Jakobson JA, Gerdemann JW (1967). Utilization of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. Plant and Soil, 27(3): 329-334.

Sanni SO (1976). VA-mycorrhiza in some Nigerian soils .the effects of *Gispora gigantea* on the growth of rice .New Phytol.77: 673-674.

Nielsen JD, Jensen A (1983). Influence of VA-mycorrhizal fungi on growth and uptake of various nutrients as well as uptake ratio of fertilizer P for lucerne (*Medica sativa*). Plant and soil 70: 165-172.

Noyd RK (1995). Ecological interactions between native prairie grasses and AMF in the reclamation of ore tailings. Doctorial thesis. University of Minnesota. St. Paul. MN. USA.

Parmita Z (2005). Study of occurrence and association and of Arbscular Mycorrhizal fungus in medicinal plants *of* Rajshahi Univesity Campus. M.Sc Thesis. Rajshahi University .Bangladesh

Phillips JM, Hayman DS. (1970). Improved procedure for cleaning and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. British Mycol. Soc. 55:158-161.

Plenchette C, Foretin A, Furlan V (1983). Growth responses of several plant species to mycorrhizae in a soil of moderator P-fertility. Plant and Soil. 70: 199-209.

Tinker PB (1975) .In: Symbiosis .29th Symp: Soc. Exp. Biol.(Ed.) D.G. Jennings and D. L .Les. Cambridge University Press .p.325.