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Effects of Arbuscular Mycorrhizal Fungus Inoculation and Phosphorus Fertilization on the Growth of *Gliricidia sepium* in Sterile and Non-Sterile Soils

[1]E.O. Iniobong, [2]M.G. Solomon and [3]O. Osonubi
[1]Department of Botany, [2]Department of Soil Science, University of Calabar,
P.M.B. 1115,Calabar, Nigeria
[3]Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria

Abstract: A pot experiment was conducted to investigate the effects of Arbuscular Mycorrhizal Fungus (AMF), *Glomus deserticola*, on the growth of *Gliricidia sepium* under 0, 50 and 100 mg Pkg^{-1} levels of phosphorus fertilization. Inoculation with AMF increased dry matter yield at all levels of applied Phosphorus (P) in both sterile and non-sterile soils while P fertilization at 100 mg P kg^{-1} soil level in conjunction with AMF inoculation increased the dry matter yield by 269.2 and 107.7% in sterile and non-sterile soils, respectively. P fertilization increased arbuscular mycorrhizal fungi colonization but decreased mycorrhizal dependency. Colonization by indigenous AMF was, however, significantly ($p<0.01$) reduced at 100 mg P kg^{-1} soil fertilization. Foliar nitrogen, phosphorus and potassium yields were significantly ($p<0.01$) increased by AMF inoculation.

Key words: *Gliricidia sepium*, mycorrhiza, phosphorus, sterile and non-sterile soils

INTRODUCTION

Most soils in the tropics are deficient in nitrogen and phosphorus. This constitutes a serious constraint to adequate food production. The use of inorganic fertilizers by resource poor small scale farmers and sometimes large scale farmers in some poor developing countries is made difficult by their scarcity, high cost or acidification of the low activity clay soils such as found in these sub humid tropics. The use of organic mulch has been found profitable (Whitbread *et al*., 2003; FAO, 2005). *Gliricidia sepium* leaves have been found to be profitable nutrient-rich mulch (Okon *et al*., 2007).The successful early establishment of *Gliricidia sepium* as a hedgerow species on the field for in-situ mulch provision depends to a very great extent on the fertility of the soil.

Arbuscular Mycorrhizal Fungi (AMF) are known to improve plant growth in marginal soils (Das *et al*., 2007; Oyetunji and Osonubi, 2007). They have been referred to as bio-fertilizers (Dixon *et al*., 1997) primarily due to their effects on plant P nutrition (Nagy *et al*., 2005). *Glomus* species for instance, have been known to specifically favour plant growth through improved mineral nutrition (Shockley *et al*., 2004; Okon *et al*., 2007).

For the establishment of hedgerow tree seedlings in a degraded nutrient poor alfisol such as found in southwestern Nigeria, it is necessary that an appropriate mycorrhizal fungus be used to promote early plant establishment and growth. Bagayoke *et al*. (2000) have shown that the beneficial effects of AMF can be improved by the addition of low levels of phosphate fertilizer. The exact level of P fertilization which will increase the beneficial effect of AMF will depend on the tree species. With successful establishment of hedgerow trees, efficient absorption of nutrients from the lower depths of the soil and subsequent recycling of these nutrients for the success of alley cropping system will be achieved.

Data are lacking on the mycorrhizal dependency of *Gliricidia sepium* under different levels of P fertilization in these sub-humid tropical soils. Besides, information on P tolerance limits of indigenous AMF of this environment with respect to their growth benefits to *G. sepium* are scarce. It is important to establish the extent to which *G. sepium* will respond to AMF inoculation at low levels of P fertilization. This will give adequate knowledge as to how best their quick establishment on the field can be achieved.

This study was undertaken to evaluate the effects of P fertilization and AMF inoculation on: AMF colonization of roots, dry matter yield, nodulation, nutrient yield and mycorrhizal dependency of *Gliricidia sepium* in sterile and non-sterile soils.

MATERIALS AND METHODS

This pot experiment was conducted in the University of Ibadan, department of Botany and Microbiology nursery in southwestern Nigeria (Latitude 7°3′N,

Corresponding Author: E.O. Iniobong, Department of Botany, University of Calabar, P.M.B. 1115, Calabar, Nigeria

Longitude 3°54′E). The experimental conditions were 12 h day length with the noon photon flux density (PAR) of 1,200 mol m^{-2} S^{-1}, day and night temperatures of 25-37°C and 24-28±2°C, respectively and 45-65% relative humidity. The soil used was sandy loam containing 0.09% total N; 12.66 mg kg^{-1} extractable P ($Bray^{-1}$); 0.57 meq 100 g^{-1}Ca; 0.97 meq 100 g^{-1} Mg; 0.13 meq 100^{-1} K and 0.51 meq 100 g^{-1} Mn.

Fifty-four 10 L plastic pots were each filled with 9 kg soil previously sterilized by steaming in between electrodes at 121°C for 3 h, while another similar set of pots were filled with non-sterile soil. Both groups were sub-divided into equal halves of 27 pots each and designated AMF inoculated (M^+) and uninoculated (M^-). Treatments consisted of 0, 50 and 100 mg P kg^{-1} of soil as KH_2PO_4 in sterile and non-sterile soils.

Four *Gliricidia sepium* seeds surface sterilized in 1% mercuric chloride for 10 min were planted in each pot according to its designated treatment combination. AMF inoculation was done at the time of sowing by applying 20 g crude inoculum consisting of 440-610 spores in 100 g dry soil of *Glomus deserticola* (Trappe, Bloss and Menge, INVAM, CA 113) directly under the seeds while the M^- designated pots received a similar but sterile inoculum which has been autoclaved at 121°C under 1.1 kgcm^{-2} for 15 min. In addition to AMF inoculation, seeds in each pot were given 3 mL of 7days old suspension of *Rhizobium* species (isolated from nodules of mature *Gliricidia sepium* stands) in yeast mannitol broth containing approximately 10 bacterial cells per mililiter using a dispensing micropipette. On germination, the seedlings were thinned out to two per pot. One month after emergence, 500 mL of each P treatment level were applied to the plants according to their respective designations. The experimental layout consisted of a randomized complete block design with each treatment combination replicated thrice.

Growth measurements: Five months after planting, plants heights and girths were measured at soil level. The plants were carefully harvested after watering thoroughly to loosen the soil. Nodules were detached from the roots while any dislodged nodule was recovered from the soil using a 0.5 mm sieve. The nodules were cleaned and oven dried at 65°C for 48 h to determine the dry weight.

Representative feeder root samples from each replicate were collected for percentage AMF colonization assessment using the gridline intersect method of Giovanetti and Mosse (1980) after clearing and staining using the method of Koske and Gemma (1989). Each seedling was then separated into leaves, stem and roots and oven dried at 70°C for 24 h (leaves) and 72 h (stems and roots) to determine the dry weights.

Foliar nutrient contents were determined from oven dried leaves using Juo (1979) method of plant analysis. Total N was determined colorimetrically by micro-Kjedahl analysis. P content was determined by the molybdenum blue method while K was determined by atomic absorption spectrophotometry after wet ashing the leaves in nitric perchloric acid moisture. Foliar nutrient yields for these elements were then calculated as the products of nutrient content and dry matter yield per plant for 0 and 100 mg P kg^{-1} replicates only because of high cost of analyses. Mycorrhizal dependency was expressed as the difference between total dry matter yields of inoculated and uninoculated plants in percentage of the total dry matter yield of inoculated plants (Plenchette *et al*., 1983). All data were subjected to analysis of variance by SAS (Statistical Analysis System, 1989) procedure to determine the least significant differences. Duncan's multiple range test was used to separate the means of the measured parameters.

RESULTS

Arbuscular mycorrhizal fungi colonization: Inoculation increased mycorrhizal colonization with increased P fertilization in both sterile and non-sterile soils (Table 1). In uninoculated non-sterile soil, however, AM colonization at 100 mg Pkg^{-1} was greatly reduced compared to its colonization at 50 mg P kg^{-1}.

Plant dry matter yield: Increasing the level of P from 0-100 mg P kg^{-1} soil significantly increased the growth of plants in both sterile and non-sterile soils (Table 1). Inoculation with AMF significantly increased the total dry matter yield irrespective of P level or soil treatments (Table 1). Phosphorus fertilization also significantly increased dry matter yield especially when in conjunction with AMF inoculation. Except for uninoculated treatments, growth was generally significantly more rapid in sterile soil than in non-sterile soil.

Root nodulation: Nodulation significantly increased with increased level of P fertilization (Table 1). The highest nodulation was observed when P was applied in conjunction with AMF inoculation in non-sterile.

Mycorrhizal Dependency (MD): Mycorrhizal dependency decreased with increased level of P fertilization (Table 1). There was a positive relationship between AMF colonization and mycorrhizal dependency with the coefficient of correlation being r = 0.57 (Fig.1).

Nutrient yield: Inoculation with Glomus deserticola significantly increased the foliar nutrient yields of N, P and K (Table 2).

Table1: Effect of arbuscular mycorrhizal fungi and phosphorus fertilization on root colonization, dry matter yield, nodulation and mycorrhizal dependency of *Gliricidia sepium*

AM	Soil	mg P kg $^{-1}$	AMF Colonization (%)	Shoot dwt (g plant $^{-1}$)	Root dwt (g plant $^{-1}$)	Nodulation Dwt (mg plant^{-1})	MD
M^+	ST	0	83.0c	14.9d	15.2e	1030c	54.8a
		50	92.6b	18.4b	19.7c	1230b	44.3b
		100	97.3a	24.1a	28.6a	1290b	39.6c
	NST	0	59.1e	11.3e	14.5e	640d	44.5b
		50	72.4d	14.6d	19.1c	950c	37.1d
		100	85.7c	19.5b	23.7b	1580a	24.0g
M^-	ST	0	_	6.6h	7.8g	260g	_
		50	_	8.5g	12.7g	360f	_
		100	_	12.1e	19.6c	1180bc	_
	NST	0	38.0f	8.9f	11.8f	350f	31.2e
		50	60.3e	11.5e	17.6d	460ef	26.9f
		100	13.5g	17.2c	23.1b	1040c	17.3h
Interactions							
M ×P			***	***	**	***	**
M×ST			**	NS	*	NS	NS
M×P×ST			***	**	**	**	**

M^+: Inoculated; M^-: Uninoculated; ST: Sterile soil; NST: Non-Sterile soil. Means within each column followed by different letters are significantly different at p<0.05 according to Duncan's multiple range test. M: Mycorrhiza; P: P fertilization; * p<0.05; ** p<0.01; *** p<0.001; NS: Not Significant

Table 2: Effect of arbuscular mycorrhizal fungi inoculation on foliar nutrient yield of *Gliricidia sepium*

AM	Soil	mg P kg $^{-1}$	N	P	K
			----------(mg plant^{-1})----------		
M^+	ST	0	205.3c	0.5b	5.3b
		100	361.7a	0.9a	7.7a
	NST	0	110.6f	0.2e	2.5g
		100	234.5b	0.5b	4.2c
	ST	0	79.4gf	0.2e	2.4g
		100	136.9e	0.4c	3.5e
	NST	0	83.8g	0.2e	2.8f
		100	169.3d	0.3d	4.0d
Interactions M×P			***	***	***
M×ST			**	***	**
M×P×ST			**	**	*

M^+: inoculated; M^-: uninoculated; ST: Sterile soil; NST: Non-Sterile soil. Means witn each column followed by different letters are significantly different at p<0.05 according Duncan's multiple range test.M: Mycorrhiza; P: P fertilization; *p<0.05; **p<0.01; ***p<0.001; NS: Not Significant

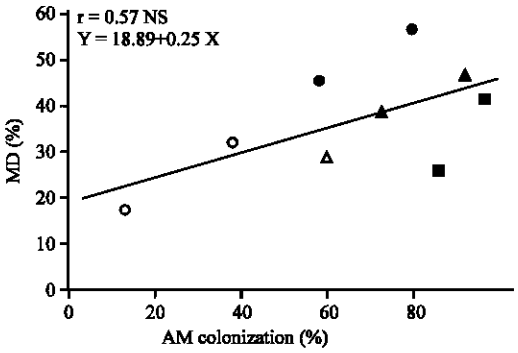

Fig. 1: The relationship between AM colonization and mycorrhizal dependency of *Gliricidia sepium*. 0, 50 and 100 mg Pkg^{-1} represented by circle, triangle and square, respectively. Closed symbols are AM inoculated while open symbols represent AM uninoculated

DISCUSSION

The reduction in AMF colonization of uninoculated plant roots at 100 mg P kg^{-1} levels observed in non-sterilized soil supports some earlier findings by Olsen *et al.* (1999) as well as Ryan and Angus (2003). The fact that this was not the case with inoculated plants shows that the sensitivity of AMF to P varies from species to species and that the inoculum potential was high. The reduction of colonization at 100 mg Pkg^{-1} soil level must have been due to an increased P concentration in the plant tissues beyond the tolerance level of the indigenous AMF in the non-sterile soil treatments. It is likely that *Glomus deserticola* requires a higher P level for effective functioning while the indigenous AMF may be more adapted to low and medium levels of P for proper functioning. Besides, the controlling effect of P on root colonization through its effect on host carbon metabolism could have effected a check or selection pressure to reduce colonization at high carbon cost to the host (Fitter, 1991). There is therefore, a need to establish a balance in terms of individual AM fungus' critical P tolerance limits and the capacity of the would-be host plant to tolerate its colonization if the benefits of the former is to be maximized.

The corresponding increase in growth and dry matter yield of AMF inoculated plants with increased P

fertilization up to 100 mg P kg^{-1} can be attributed to increased availability of phosphorus to the plants which when taken in could have played a regulatory role both for photosynthesis through chlorophyll production (Ekanayake *et al*., 2004) and nodule activities all of which would make available sufficient metabolites for rapid growth.

The reduced growth and lower dry matter yield in inoculated plants grown in non-sterile soil could have been caused by an initial suppression or inhibition of the *Glomus deserticola* propagules germination by indigenous soil microorganisms (Linderman, 1992), competition for available P by them (Fitter and Garbaye, 1994), grazing by soil fauna or possibly the masking over effects of the indigenous mycorrhizal flora on the introduced AM fungus (Daft, 1992).

The increased nodulation with increased level of P can be explained on the enhancing effect of sufficient phosphorus on nodulation and nodule activity (Bethlenfalvay, 1992). The increased nodulation in inoculated plants must have been caused by a direct preferential enhancement of nodule functions by AM fungi mediated through P uptake (Fitter and Garbaye, 1994). Also, the partitioning of photosynthates into the roots usually caused by AMF colonization must have favoured colonization by *Rhizobium* species.

Mycorrhizal dependency decreasing with increasing levels of fertilization goes on to confirm that the beneficial effects of AMF are P- mediated. Addition of P to the soil might have made it available to the plant roots for easy absorption and utilization for growth, thus explaining the lesser mycorrhizal dependency in P fertilized plants. This explanation lends support to the premise that the importance of AMF in agriculture is diminished when high rates of fertilizer (especially P) are supplied.

The positive relationship between AMF colonization and mycorrhizal dependency (Fig. 1), indicates that *Gliricidia sepium* requires AMF for successful establishment. The various levels of interactions between AMF, inoculation, P fertilization and soil sterilization on the different growth parameters (Table 2) indicate that an appropriate combination of these factors can enhance maximum growth of *Gliricidia sepium* for rapid hedgerow establishment on the field.

Foliar N, P and K yield increased significantly with AMF inoculation showing a possibly increased efficiency in extraction of nutrients beyond the depletion zone of the roots by the external hyphae (Jakobson, 1992). Similarly, the increased N yield in AMF inoculated plants could have been due to the AMF possibly absorbing both NO_3^- and NH_4^+ from the soil (Hamel, 2004) by assimilating ammonium via glutamine synthetase activity (Smith *et al*., 1985) or through enhanced nodulation activities caused by increased P uptake.

Our results have shown that addition of small amounts of phosphorus in conjunction with AMF inoculation can enhance early establishment of *Gliricidia sepium* seedlings on the field. This can go a long way to ease the difficulties of raising in-situ mulching materials and leguminous shrubs in agro-forestry systems.

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REFERENCES

Bagayoke, M., E. George, V. Romheld and A.B. Buerkert, 2000. Effects of mycorrhizae and phosphorus on growth and nutrient uptake of millet, cowpea and sorghum on a West African soil. J. Agric. Sci., 135: 399-407.

Bethlenfalvay, G.J., 1992. Mycorrhizae and crop productivity. In Mycorrhizae in Sustainable Agriculture. G.J. Bethlenfalvay and R.G. Linderman (Eds.). ASA Special Publication, 54: 1-27.

Daft, M.J., 1992. Use of VA mycorrhizas in agriculture: Problems and prospects. In mycorrhizas in Ecosystems. D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander (Eds.). CAB International, UK., pp: 198-201.

Das, K., R. Dang, T.N. Shivananda and N. Sekeroglu, 2007. Influence of bio-fertilizers on the yield and nutrient content in Stevia rebaudiana Bert. Grown in Indian subtropics. J. Med. Plants Res., 1: 5-8.

Dixon, R.K., K.G. Mukerji, B.P. Chamola, K. Alpana and A. Kaushik, 1997. Vesicular arbuscular mycorrhizal symbiosis in relation to forestation in arid land. Ann. Forestry, 5: 1-9.

Ekanayake, I.J., O.J. Oyetunji, O. Osonubi and O. Lyasse, 2004. The effects of arbuscu-lar mycorrhizal fungi and water stress on leaf chlorophyll production of cassava (Manihot esculenta Crantz). Food Agric. Environ., 2: 190-196.

FAO., 2005. The importance of soil organic matter: Key to drought-resistant soil and sustained food production. FAO Soils Bulletin No.80, Rome, pp: 78.

Fitter, A.H., 1991. Cost and benefits of mycorrhizas: Implications for functioning under natural conditions. Experientia, 47: 350-355.

Fitter, A.H. and J. Garbaye, 1994. Interaction Between Mycorrhizal Fungi and Other Soil Organisms. In Management of Mycorrhizas in Agriculture, Horticulture and Forestry. Developments in Plant and Soil Sciences. A.D. Robson, L.K. Abbott and N. Malajczuk (Eds.). Kluwer Academic Publishers, Netherlands, 56: 123-13.

Giovanetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol., 84: 489- 500.

Hamel, C., 2004. Impact of arbuscular mycorrhiza fungi on N and P cycling in the zone. Can. J. Soil Sci., 84: 383-395.

Jakobsen, I., 1992. Phosphorus transport by external hyphae of vesicular arbuscular mycorrhizas. Mycorrhizas in Ecosystems. Read, D. J., D. H. Lewis, A.H. Fitter and I.J. Alexander (Eds.). CAB International, UK., pp: 48-54.

Juo, A.S.R., 1979. Selected methods for soil and plant analysis. IITA manual series No 1. IITA, Ibadan, Nigeria, pp: 70.

Koske, R.E. and J.N. Gemma, 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res., 92: 486-488.

Nagy, F., V. Karandoshov, W. Chague, K. Kalinkevich, M. Tamasloukht, G. H. Xu, I. Jakobsen, A.A. Levy, N. Amrhein and M. Bucher, 2005. The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. Plant J., 42: 236-250.

Okon, I.E., M.G. Solomon and O. Osonubi, 2007. The effects of arbuscular mycorrhizal inoculation and mulch on the yield of cassava under humid tropical condition (In the Press).

Oyetunji, O.J. and O. Osonubi, 2007. Assessment of influence of alley cropping system and Arbuscular Mycorrhizal (AM) fungi on cassava productivity in derived savanna zone of Nigeria. World J. Agric. Sci., 3: 489-495.

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

Ryan, M.H. and J.F. Angus, 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil; increased Zn-uptake but no increase in P-uptake or yield. Plant and Soil, 250: 225-239.

Statistical Analysis System (SAS) Institute, 1989. SAS User's guide. Cary, North Carolina, USA.

Shockley, F.W., R.L. McGraw and H.E. Garrett, 2004.Growth and nutrient concentration of two native forage legumes inoculated with *Rhizobium* and Mycorrhiza in Missouri, USA. Agrofor. Sys., 60: 137-142.

Smith, S.E., B.J. St John, F.A. Smith and D.J.D. Nicholas, 1985. Activity of glutamine synthetase and glutamate dehydrogenase in *Trifolium subterraneum* L. and *Allium cepa* L. Effects of mycorrhizal infection and phosphate nutrition. New Phytol., 99: 211-227.

Whitbread, A., G. Blair, Y.Konboon, R. Lefroy and K. Naklang, 2003. Managing crop residues, fertilizers and leaf litters to improve soil C, nutrient balances and the grain yield of rice and wheat cropping systems in Thailand and Australia. Agric. Ecosys. Environ., 100: 251-263.