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Dependence of promiscuous soybean and herbaceous legumes on arbuscular mycorrhizal fungi and their response to bradyrhizobial inoculation in low P soils.

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Abstract

As the production of grain and herbaceous legumes is often limited by low levels of available P in moist savanna soils, the potential for managing arbuscular mycorrhizal fungi (AMF) by selecting lines or accessions dependent on AMF as a strategy to improve plant P nutrition and productivity is required. The interactions between AMF and *Bradyrhizobia* sp. and their effects on growth and mycorrhizal colonization of ten recent selections of promiscuous soybean breeding lines and two herbaceous legumes (*Lablab purpureus* and *Mucuna pruriens*) were investigated. The pots contained soil (available P = 5.33 μg P soil^{-1}, Bray 1) collected at Fashola from a derived savanna in Nigeria. Mycorrhizal colonization differed among promiscuous soybean lines (ranging from 16 to 33%) and was on average 20% for mucuna and lablab. Shoot weight of plants single or dually inoculated with AMF and *Bradyrhizobia* sp. were higher than those of uninoculated plants and the differences between lines and species were significant. Three groups of plants were obtained according to their mycorrhizal dependency (MD): (i) the highly dependent plants with (MD) >30%, e.g.,soybean line 1039 and mucuna; (ii) the intermediate group, with MD between 10 and 30%, e.g., soybean line 1576 and lablab, and (iii) the majority of soybean lines (five lines out of 10) that were not mycorrhizal dependent. This great variability in MD and response to P application among promiscuous soybean and herbaceous legumes offers a potential for the selection of plant germplasm able to grow in P deficient soil.

Keywords: *Bradyrhizobium* sp.; Derived savanna; *Lablab purpureus*; *Mucuna pruriens*; Mycorrhizal colonization; Soybean breeding lines

1. Introduction

Low-input cropping systems are currently practiced in marginal soils of the moist savanna zones where grain legumes (soybean and cowpea) and herbaceous legumes such as mucuna (*Mucuna pruriens*) and lablab (*Lablab pruriens*) play a role as a source of N to the succeeding or associated cereal crops. These legumes require P for N_2 fixation processes and growth. Phosphorus is not usually supplied to these legumes because farmers lack the means to purchase P fertilizers. The strategy adopted so far to compensate for the lack of P fertilizer has been to select legumes

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adapted to low P soils (Sanginga et al., 1996; Abdelgadir, 1998). Our studies have shown diversity between and within leguminous species in their response to P application in moist savanna soils. These legumes may also vary in responsiveness to arbuscular mycorrhizal fungi (AMF).

The value of AMF in extending the nutrient absorptive area of crop species has been thoroughly documented (Bagyaraj et al., 1984; Bolan et al., 1987). The mycorrhiza could, however, be the most important untapped and poorly understood resource for P acquisition in agriculture, especially in the moist savanna zones of West Africa. As the production of many crops, especially legumes, is often limited by low levels of available P in these zones, the potential for managing AMF by selecting species or lines dependent on AMF as a strategy to improve P nutrition and crop productivity is required.

Mycorrhizal dependency (MD) has been defined by Gerdeman (1975) as "the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility". The importance of AMF associations in agricultural crops and their significance in nodulating nitrogen-fixing plants are well documented (Barea and Azcón-Aguilar, 1983). Khalil et al. (1994), studying the mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars, showed that soybean had a higher MD than corn. Considerable variation occurred within soybean cultivars where the relative growth of improved cultivars was less enhanced with mycorrhizal colonization than the unimproved ones. Differences in the relative MD between crop species, or even cultivars are also related to other plant factors such as root structure, plant growth rates (Sieverding, 1986) and microorganisms in the rhizosphere which could affect the demand for P (Xie et al., 1995).

The selection of agriculturally important plant germplasms more tolerant of low P because of greater dependency on AMF may increase plant P nutrition and productivity on P-deficient soils in the moist savanna zone in West Africa. However, in breeding programs where the selection of cultivars usually occurs under conditions of high fertility, the improved cultivars or breeding lines chosen may have less than ideal synergic microbial associations. Consequently, breeders might inadvertently select against plants capable of obtaining nutrients in a low-input system.

In this study, we hypothesized that soybean breeding lines are more responsive than the herbaceous legumes such as mucuna and lablab to AMF associations, and therefore, will satisfy their P requirement in a low P soil, whereas, the herbaceous legumes would not support the same aggressive and effective AMF association, nor the same level of colonization, because of low nodulation due to the lack of effective bradyrhizobia. Promiscuous soybean and herbaceous legumes have been used as test plants because of their importance as N sources in maize-based cropping systems and also because little or no work has been reported on their mycorrhizal dependency and interactions with P and bradyrhizobial inoculation in the moist savanna zone of West Africa.

The objectives of this study were to (i) determine the response of soybean and herbaceous legumes to bradyrhizobial and AMF inoculation, and (ii) assess their mycorrhizal dependency and relate it to their P response in a low P soil.

2. Materials and methods

2.1. Soil collection and preparation

Soil samples were collected from a field located in the derived savanna at Fashola (7°50′N; 3°55′E). This site has a bimodal rainfall pattern averaging about 1230 mm/annum. Selected physico-chemical characteristics (0–15 cm depth) of the site measured according to IITA analytical procedures (IITA, 1982) are given in Table 1.

Soil was sieved through a 2 mm screen and flame-sterilized at 120°C for 40 min by passing it through a gas propelled furnace (Machine Terra Force, Kent Horticultural Engineers, Tom Bridge Road, Kent ME 185 NY).

2.2. Response of soybean to AMF and bradyrhizobial inoculation

The experiment had two treatment factors and was arranged in a randomized complete block design with four replications. Factor 1 consisted of two soil treatments (sterilized and non sterilized), while the second

Table 1
Physico-chemical characteristics of soils collected (0–15 cm depth) at Fashola derived savanna in Nigeria

Soil characteristics	
Sand (%)	83.00
Silt (%)	16.00
Clay (%)	1.00
pH (H_2O)	6.00
Organic C (%)	0.48
Total N (%)	0.04
Available P (ppm) Bay1	3.80
NH_4 - Acetate extractable cations (meq/100 g)	
Ca	4.15
Mg	0.42
Mn	0.03
K	0.11
Na	0.03
Total acidity (meq/100 g)	0.01

factor comprised nine treatments consisting of (i) two of bradyrhizobia (*Bradyrhizobium*1 a mixture of R25 + IRj 2180 used as reference strains for soybean at IITA or a slow growing *Bradyrhizobium*2 strain, 6.1 SG, isolated from promiscuous soybean grown at Zonkwa in the southern Guinea savanna of Nigeria), (ii) two AMF types (*Glomus mossae* or a local AMF, which is a mixture of cultures isolated from Zonkwa) and their combinations. Uninoculated soils constituted the control. 5 kg of soil from Fashola was used for this experiment and received only 30 kg K as KCl and a combination of micronutrient (Vincent, 1970).

Soybean seeds of TGX 1660 – 19F were surface-sterilized by rinsing in 95% ethanol for 10 s to remove debris, followed by agitating in 3% hydrogen peroxide (H_2O_2) for 3–5 min. after which they were rinsed with six changes of sterile distilled water. Four seeds were planted and thinned to two seedlings per pot 1 week after emergence. Harvesting was done 8 weeks after planting. Shoots were cut at the soil surface and the fresh weight recorded before they were oven-dried at 80°C for 2 days and their dry weights determined.

Roots were placed in a sieve and carefully washed under a gentle stream of water from a tap. Nodules were detached from the roots and preserved in transparent polyethylene bags in a refrigerator at 4°C. Nodules were counted and their fresh weight taken. About 1 g root was taken from each root sample and placed in glass vials for clearing and staining for mycorrhizal colonization rating (Phillips and Hayman, 1970; Giovanetti and Mosse, 1980). The roots of each sample were also oven-dried at 80°C for 2 days and their dry weight was determined.

2.3. Mycorrhizal dependency of soybean and herbaceous legumes

This was also an experiment with two treatment factors arranged in a randomized complete block design with four replications. The first factor consisted of host plants: (i) 10 promiscuous soybean breeding lines (Table 4), (ii) maize, (iii) lablab and mucuna, and the second factor had the following four treatments: (i) uninoculated control with or without P application and (ii) AMF inoculation with or without P application. It was conducted in pots containing 5 kg soil collected at Fashola.

The rate of P fertilizer application was 12 mg of single superphosphate per kg of soil. All the plants received the equivalent of 30 kg K ha^{-1} as KCl and a combination of micronutrients at the level recommended by Vincent (1970) for legumes.

Seed preparation, planting, harvesting, and sampling were carried out in the same method as in Experiment 1 and the same parameters were assessed.

2.4. Bradyrhizobial inoculation

1 ml of inoculum containing approximately 10^8 cells of *Bradyrhizobia* was applied as appropriate to each seedling at one week after planting following the procedure described by Vincent (1970).

2.5. Arbuscular mycorrhizal fungi colonization

The AMF was grown in pots containing sterilized soils regularly watered by Jensen's solution (Vincent, 1970) with *Zea mays* as the host plant. After 60 days of growth with normal watering followed by 20 days of plant drying without watering (this was done in order to enhance mycorrhizal infection) , the infected soil, containing about 100 spores g^{-1} of soil together with infected root fragments chopped into approximately 0.5 cm pieces, was used as AMF inoculant.

The percentage mycorrhizal colonization was determined after clearing and staining the roots (Phillips and Hayman, 1970) by the gridline-intersect method

(Giovannetti and Mosse, 1980). Root pieces were spread out evenly in square plastic (10.2 by 10.2 cm) Petri dishes and scanned at 40× magnification. Three sets of observations were recorded along 100 root gridline intersections. Each observation was made on a rearrangement of the same root subsample. The percentage of roots colonized was expressed as the number of intersections with colonized roots from the 100 intersections counted.

The mycorrhizal dependency (MD) or response to mycorrhizal colonization was calculated by using the following formula (Plenchette et al., 1983):

$$\mathrm{MD} = \frac{(\text{Dry weight of mycorrhizal plants} - \text{Dry weight of non mycorrhizal plants})}{\text{Dry weight of mycorrhizal plants}} \times 100$$

2.6. Statistical analysis

All the data collected were subjected to analysis of variance (ANOVA) using the SAS-GLM procedure (SAS, 1989). The significance of the main factors and interaction effects was determined on the basis of the F ratios. The Duncan's multiple range test was performed to evaluate differences between treatment means.

3. Results

3.1. Response of soybean to AMF and bradyrhizobial inoculation

3.1.1. Nodulation and mycorrhizal colonization.

Nodulation and mycorrhizal colonization were both significantly affected by the soil and microsymbiont treatments ($p < 0.01$). As no interactions occurred between the inoculation treatments (with AMF or bradyrhizobia) and the soil treatments (sterilized and non sterilized) for nodulation and mycorrhizal colonization, only the main factor effects are considered in Tables 2 and 3. The numbers and weights of nodules and degree of mycorrhizal colonization of plants (averaged across microsymbiont treatments) in sterilized soils were about double those in non sterilized soils (Table 3).

The number and weight of nodules were significantly influenced by the strains of bradyrhizobia and their combinations with AMF types (Table 4). *Bradyrhizobium* 1 doubled the number of nodules while *Bradyrhizobia* sp. 2 did not show any significant effect. However, the numbers and weights of nodules on plants inoculated with *Bradyrhizobium*2 and AMF 1 were significantly greater than those on plants inoculated with *Bradyrhizobium*2 alone. These interactions were not significant between *Bradyrhizobium*1 and the two AMF types. Inoculation with AMF alone did not affect nodulation.

Plants inoculated with AMF1 and AMF2 had a greater mycorrhizal root infection rate than uninoculated plants (Table 3). Although bradyrhizobia did not significantly interact with any type of AMF, the percentage mycorrhizal root infection of plants not inoculated with AMF increased by 57% due to bradyrhizobial inoculation.

3.1.2. Shoot and root dry weight

Shoot and root dry weight were both significantly affected by the soil and microsymbiont treatments ($p < 0.01$). As no interactions occurred between the inoculation treatments (with AMF or bradyrhizobia) and the soil treatments (sterilized and non sterilized) for shoot and root dry weight, only the main factor effects are considered in Tables 2 and 3. Shoot dry weight of plants (averaged across the microsymbiont inoculated treatments) in sterilized soil was signifi-

Table 2
Shoot growth and symbiotic characteristics of plants grown in sterilized and nonsterilized Fashola soil. (Average means across the microsymbiont inoculated treatments)*

	Sterilized soil	Non sterilized soil
Shoot dry weight (g plant^{-1})	17.84[a]	14.01[b]
Root dry weight (g plant^{-1})	5.32[a]	4.70[a]
Nodule number (No. plant^{-1})	73.33[a]	38.40[b]
Nodule dry weight (g plant^{-1})	2.30[a]	1.29[b]
Mycorrhizal colonization (%)	21.24[a]	12.82[b]

* Means followed by the same letter are not significantly different at p <00.5.

Table 3
Effect of bradyrhizobia and arbuscular mycorrhizal fungi on nodulation, mycorrhizal infection and shoot dry weight of soybean grown in Fashola soil (Average means across the sterilized and non sterilized soil treatments)*

	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Nodule (g plant^{-1})	Nodule weight (g plant^{-1})	AMF root (%)
Control	13.41^{d}	4.64^{a}	35^{c}	1.27^{bc}	4^{c}
Brady 1	15.75^{abc}	5.45^{a}	75^{ab}	1.94^{bc}	7^{c}
Brady 2	14.50^{cd}	4.24^{a}	35^{c}	1.02^{c}	7^{c}
AMF 1	15.80^{abc}	4.81^{a}	46^{bc}	1.30^{bc}	24^{a}
AMF 2	15.02^{bcd}	4.75^{a}	43^{c}	1.83b^{c}	20^{a}
Brady1 + AMF1	17.75^{a}	6.15^{a}	78^{a}	2.14^{b}	23^{ab}
Brady1 + AMF2	17.51^{a}	5.68^{a}	75^{ab}	3.08^{a}	24^{a}
Brady2 + AMF1	17.11^{ab}	5.00^{a}	73^{ab}	2.25^{ab}	22^{ab}
Brady2 + AMF 2	16.46^{abc}	4.37^{a}	45^{bc}	1.33^{bc}	24^{a}

* Means followed by the same letter are not significantly different at $p \leq 00.5$.

Table 4
Effects of soybean breeding lines and herbaceous legumes and dual inoculation with bradyrhizobia and arbuscular mycorrhizal fungi (AMF) on shoot dry weight of plants grown in Fashola soil

	Plant hosts	Control	*p*	P + AMF	AMF	Means
Soybean line	1039	2.13	4.26	3.74	4.36	3.72^{def}
Soybean line	1196	3.11	4.07	2.57	3.46	3.30^{f}
Soybean line	1251	3.49	3.28	3.80	3.44	3.50^{ef}
Soybean line	1293	3.85	3.86	4.52	3.75	3.99^{bcd}
Soybean line	1419	2.98	3.60	2.85	3.62	3.26^{f}
Soybean line	1420	2.33	1.71	2.59	3.36	2.50^{g}
Soybean line	1456	2.75	2.26	2.63	2.63	2.57^{g}
Soybean line	1511	4.02	3.61	4.22	3.62	3.87^{cde}
Soybean line	1516	2.59	2.77	1.69	2.54	2.40^{g}
Soybean line	1576	3.53	4.33	4.38	4.56	4.20^{cb}
	Mucuna	3.56	8.31	8.64	5.33	6.46^{a}
	Lablab	2.11	3.38	3.37	2.32	2.79^{g}
	Maize	3.09	3.75	5.88	4.88	4.40^{b}
	Means	3.04^{b*}	3.78^{a}	3.91^{a}	3.68^{a}	

* Means followed by the same letters are not statistically different at $p \leq 0.05$. SED(cultivar) = 0.15; SED (treatment) = 0.09; SED (Cultivar × treatment) = 0.32.

cantly higher than that of plants growing in nonsterilized soils (Table 2).

*Bradyrhizobium*1 significantly increased the shoot dry weight of soybean while *Bradyrhizobium*2 did not (Table 3). The interactions between *Bradyrhizobium*2 and AMF1 were, however, significant. Dual inoculation with *Bradyrhizobium*2 and AMF1 or AMF2 produced significantly larger and heavier plants compared to uninoculated plants and inoculated plants with *Bradyrhizobium*2 alone . Single inoculation with AMF1 also significantly increased shoot dry weight as compared to the control.

3.2. *Mycorrhizal dependency of soybean and herbaceous legumes*

Shoot dry weight was significantly affected by AMF colonization and P application treatments ($p < 0.01$). AMF colonization and P application significantly improved the average shoot weight of soybean lines and lablab and mucuna (Table 4). Line differences were significant within soybean. For example, the shoot dry weights of soybean lines 1039 and 1576 when inoculated with AMF were comparable to P application and were enhanced

Table 5
Effect of soybean breeding lines and herbaceous legumes and dual inoculation with bradyrhizobia and arbuscular mycorrhizal fungi (AMF) on % AMF root infection of plant grown in Fashola soil

	Plant hosts	Control	P	P + AMF	AMF	Means
Soybean line	1039	5	19	14	41	20^{cde}
Soybean line	1196	13	14	33	16	19^{cde}
Soybean line	1251	3	7	36	43	22^{cde}
Soybean line	1293	6	23	18	26	18^{de}
Soybean line	1419	35	31	30	37	33^{a}
Soybean line	1420	5	24	34	33	24^{bcd}
Soybean line	1456	19	31	37	38	31^{ab}
Soybean line	1511	25	33	42	23	31^{ab}
Soybean line	1516	2	20	21	20	16^{e}
Soybean line	1576	19	12	33	43	27^{abc}
	Mucuna	19	3	38	40	25^{bcd}
	Lablab	3	9	34	31	19^{de}
	Maize	10	13	18	44	21^{cde}
	Means	13^{c*}	18^{b}	30^{a}	34^{a}	

*Means followed by the same letters are not statistically different at $p \leq 0.05$. SED(cultivar) = 2.40; SED (treatment) = 1.33 ; SED (Cultivar × treatment) = 4.80.

2.04-fold and 1.39-fold, above the uninoculated control without P application, respectively. Growth of mucuna and lablab inoculated with AMF was increased by 1.49- and 1.09-fold, above the uninoculated control, respectively and there was an additional growth in response to P as indicated by the results of P + AMF treatment. Most of the plants that responded to AMF inoculation also responded significantly to P application.

Mycorrhizal colonization differed among soybean lines (ranging from 16 to 33%) and was on average 25% for mucuna and 19% for lablab (Table 5). As expected P application reduced the level of mycorrhizal colonization but inter and intra-specific differ-

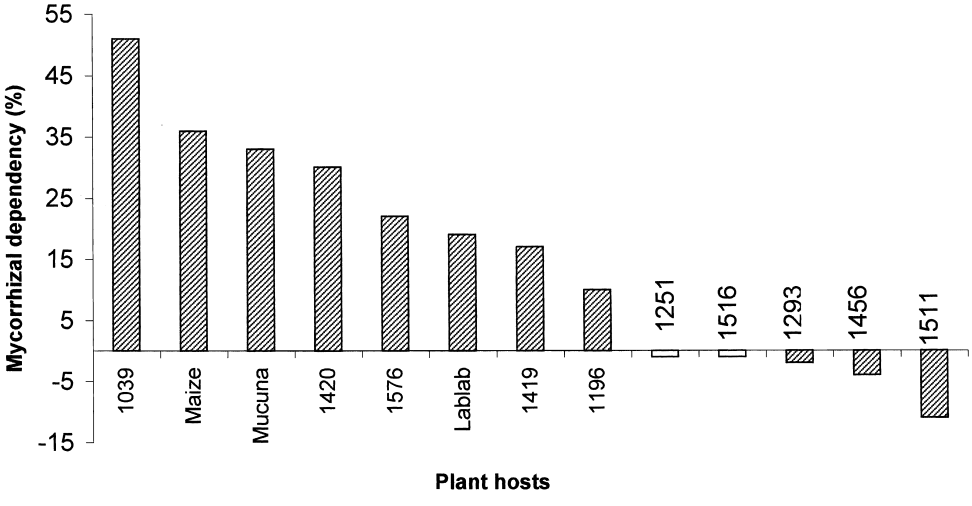

Fig. 1. Effect of soybean breeding lines and herbaceous legumes and dual inoculation with bradyrhizobial and arbuscular mycorrhizal fungi (AMF) on AMF dependency.

ences occurred. AMF inoculation increased the mycorrhizal colonization of seven soybean lines, lablab, mucuna, and maize. It did not affect soybean lines 1196, 1419, and 1511.

Mycorrhizal dependency ranged from −11 to +51% (Fig. 1). Three MD groups were obtained: (i) the highly dependent group with MD higher than 30% (e.g. soybean line 1039 and mucuna); (ii) the intermediate group with MD ranging between 10 and 30% (e.g. soybean line 1576, 1420, and 1419, and lablab, and (iii) the non-MD group which comprised the majority of soybean lines e.g., five lines. Shoot growth response to P of uninoculated MD plants was higher than that of non MD plants (Table 4).

4. Discussion

In general, we have assumed that legumes will respond to P application and they will benefit most from AMF association under nutrient-poor conditions, especially when P is limiting in soils of the moist savanna (Ganry et al., 1982; Bationo et al., 1986) This could not, however, be generalized in the present study as two types of responses to either AMF inoculation or P application were obtained. For example, AMF inoculation did not affect the growth of soybean breeding lines such as 1456, 1511, and 1516 whereas soybean line 1039 and mucuna required AMF to grow well in the absence of P application. The variability in response to AMF and P between and within species should be exploited to establish grain or forage legumes on marginal soils being currently exploited by farmers in the moist savanna.

The effect of AMF inoculation on growth of soybean line 1039 and mucuna plants was similar to that of plants receiving the equivalent of 60 kg P ha^{-1}. This rate is higher than that used by growers on grain legumes such as cowpea and soybean in the moist savanna. The above results have implications for the breeding and resource management programs being conducted in the moist savanna by scientists of the International Institute of Tropical Agriculture (IITA) (IITA, 1982). As described in the introduction, the selection of cultivars in breeding programs has mostly occurred under conditions of high fertility (e.g. N and P fertilized conditions), so the improved cultivars chosen may have less than ideal synergic microbial associations. It is proven that high P depresses AMF root infection and this has also been shown in this study, in addition high N soil content inhibits nodulation and nitrogen fixation (Barea and Azcón-Aguilar, 1983; Bethlenfalvay et al., 1985). In selecting cultivars or lines in conditions of high fertility, breeders may have inadvertently selected against plants such as soybean line 1039 capable of obtaining nutrients (N, P) in low-input systems. Because of these results, the soybean breeding program at IITA has started selecting and breeding new soybean lines tolerant to low P under low-input cropping systems. These lines are useful in some marginal conditions of the moist savanna where these legumes are being introduced.

The MD of plant hosts (breeding line, species) could also have implications for cropping systems currently practiced in the moist savanna. It is well known that AMF establishment and/or function could be affected by crop rotation involving non-host plants (Johnson et al., 1992). Our results showing differences in the dependency within soybean and between herbaceous legumes indicate that attention should be paid to the choice of the appropriate germplasm to be incorporated in an association or rotation cropping systems established in P-deficient soils.

Our results suggest that the introduction of efficient AMF inoculum could significantly improve P nutrition of the legumes in moist savanna, but except for the few attempts made in West Africa (Ganry et al., 1982) the production of adequate quantities for field trials and the unresolved problems of competition constitute major handicap for the use of AMF inoculum.

In maize-based cropping systems, where soybean and herbaceous legumes are currently grown in order to improve soil fertility, the first concern will be to preserve the natural mycorrhizal potential as it exists in the field; the second is to try to improve this potential. Non-effective naturally occurring strains of AMF need, in some cases, to be replaced with more effective strains. Since AMF inoculation technology is still problematic, the main emphasis should be on choice of MD germplasm or species entering in rotation. If indigenous endophytes are efficient but sparse, they can also be stimulated by the introduction of MD germplasm, which will stimulate and eventually multiply the appropriate AMF to obtain a suitable inoculum to maintain an adequate mycorrhizal level.

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