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Response of cowpea (*Vigna unguiculata*) to AM and bradyrhizobium in a heavy and light textured soil

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ABSTRACT

A field experiment with cowpea *(Vigna unguiculata* (L.) Walp.) cv. Ife Brown was carried out in 1995 in a heavy textured loamy sand Iwo series soil and in 1996 in a light textured soil of the same series to determine its response to single or dual inoculation with arbuscular mycorrhiza (AM) fungus, *Glomus mosseae* and a mixture of Bradyrrhizobium strains IRj 2184A and IRc 256. Single or dual inoculation increased the plant height, nodule number and weight, dry matter accumulation of shoot, percent nitrogen(N) and phosphorus (P) in plant tissue, and the percent N_2 derived from atmosphere when compared with ninoculated control in the 2 years. Grain yield was enhanced by 20% due to dual inoculation in 1995 and only 1% in 1996. The rate and intensity of AM infection were significantly improved by inoculation.

Keywords: Arbuscular mycorrhizae, *bradyrhizobium*, cowpea, *Vigna unguiculata.*

INTRODUCTION

Cowpea (*Vigna unguiculata*) is an important grain legume crop in the sub-saharan Africa not only because of its high nutritional status, but also because of its ability to improve nitrogen fertility of the soil through N_2 fixation. However, yield potentials of some cowpea varieties are yet to be realized due to their low levels of atmospheric N_2 fixation and poor utilization of available soil nutrients. The use of Bradyrhizobial inoculants had, however, led in some cases to improvement of N_2 fixation and grain yield in some tropical soils. Adu and Nnadi (1990) in a 2 year study reported non-significant effect of inoculation on nodulation and grain yield of cowpea planted in the Savanna region of Nigeria. However, with appropriate choice of compatible inoculant strain or multistrain and responding cowpea varieties, increase in growth and nodulation have been reported (Danso 1975; Balasubramanian *et al.* 1980; Keyser *et al.* 1981; Minchin *et al.* 1981 and Barker and Sajise 1985). Non-optimal utilization of the available soil nutrients may also impair growth and yields. Therefore, arbuscular mycorrhiza (AM) inoculation prior to planting had been carried out in order to ensure adequate utilization of soil nutrients. Chhabra *et al.* (1990) reported increased plant height and total dry matter of root and shoot with AM inoculation. Aziz and Babte (1989) working in an eroded soil emphasized the importance of restoring lost nutrients before the benefits of AM inoculation can be effectively exploited. Results of the effect of dual inoculation of AM and Bradyrhizobium on cowpea (*Vigna unguiculata*) especially in soils of both heavy and light textures are poorly documented. Hence this study was designed to determine the effect of single and dual inoculation of AM (*Glomus mosseae*) and Bradyrhizobium on cowpea in two soils characterized by high and low organic matter.

MATERIALS AND METHODS

A field experiment with cowpea (*Vigna unguiculata*) cv. Ife Brown was carried out in 1995 in a high textured loamy sand Iwo series soil and in 1996 in a light textured soil of the same series. The chemical characteristics of the soils used are listed in Table 1.The experiment was conducted adopting a randomized complete block design replicated 4 times. The plot size was 6mx5m. The seeds were sown at 100 kg ha^{-1}, 4-5cm deep in furrows with a plant spacing of 30cm along the furrows and 60cm apart. Recommended cultural practices were followed to raise good and healthy crop.

At planting, single and dual inoculation of Bradyrhizobium strain and *Glomus mosseae* were done to form four treatments including uninoculated control. The mixture of two Bradyrhizobial strains IRj 2184A and IRc 256 were obtained from the Soil Microbiology Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and were grown in a Yeast Extract Mannitol (YEM) broth on a rotary shaker at 28°C for seven days. The culture was centrifuged at 5000 rpm three times in sterile distilled water by resuspension and recentrifugation. The cells were finally suspended in sterile distilled water at a density of about 10^7 to 10^8 cells per ml as determined by plate count agar on YEM agar. The suspension was inoculated into sterile peat at 40 mls 5g $peat^{-1}$. The peat culture containing about 10^6 to 10^7 cells gram $peat^{-1}$ (Vincent 1970) was inoculated on seeds at 5g inoculant kg $seeds^{-1}$. Soil culture of G. *Mosseae,* having 100 spores g $soil^{-1}$, obtained from the Department of Botany and Microbiology, University of Ibadan, Nigeria containing infective fungal propagules (external hyphae, fungal root fragments and spores was mixed with seeds at 20g soil planting $hill^{-1}$.

Table 1. Some chemical characteristics of the soils used in the two field experiments.

	1995	1996
Organic carbon (%)	2.2	0.81
Nitrogen (%)	0.25	0.07
Available P (mg kg^{-1})	15.10	2.99
Exchangeable ions (meq 100g $soil^{-1}$)		
Potassium (K)	0.57	0.42
Calcium (Ca)	5.21	3.61
Magnesium (Mg)	0.81	0.49
Sodium (Na)	1.52	0.42
Hydrogen ion concentration (H^+)	0.09	0.06
CEC	8.17	4.99

Two weeks after planting (WAP) $10kgNhta^{-1}$ as ammonium sulphate with 10.09% ^{15}N atom excess (a.e.) was applied in solution into a 1x1m microplots. The seedlings were thinned to 2 plants $hill^{-1}$ before fertilizer application. Non-nodulating clay soybean used as the reference crop was equally treated with labelled N. The plant height was taken at 4WAP and expressed in centimeter. At 6WAP, the shoots and nodules were dried at 70°C for 24hrs and dry matter expressed in grammes and the nodule number was counted. Pods of harvested plants were shelled 75DAP and the weight expressed in kilogrmmes (kg). The dry shoots were ground and analysed for %N on a Kjeldahl digest (Eastin1978) and the N isotope ratio analysis was performed on emission spectrometer (Fiedler and Proksch 1975). Atmospheric N_2 fixed was estimated using isotope dilution method (McAuliffe *et al.* 1958; Fried and Middleboe 1977). Plant tissue phosphorus was determined after dry ashing and digestion with HCl by the Chloromolybdate blue method (Bray and Kurtz 1954). The rate and intensity of AM root infection were determined by the method of Kormanic *et al.* (1980). Intensity was scored as follows: Intensity1=small infection sites widely scattered along roots; intensity 2= larger infection sites more uniformly distributed through the infested roots but rarely coalescing, and intensity 3= feeder roots almost solidly infected with no easily identified isolated patches of infection.

Data were subjected to statistical analysis using analysis of variance (ANOVA) and the treatment means were compared by the Duncans Multiple Range Test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Single inoculation of cowpea with Bradyrhizobium significantly increased the plant height and nodule number and weight in both experimentation years. These parameters were significantly (p=0.05) increased in 1996 when the test crop was dually inoculated with AM and Bradyrhizobium (Table 2). Eventhough the levels of soil nutrients were low in 1996, growth enhancement due to inoculation was clearly shown. This may be due to the abilities of the test microorganisms to effectively mobilize the available soil nutrients (Harris *et al.* 1985 and Mahmoud and Ikram 1987). The dry matter of shoot responded posively to inoculation. While the increases resulting from inoculation were marginal in dry matter of shoot in 1995 and 1996, about 2 fold significant increases were obtained when Bradyrhizobiun alone was used (Table 2). Dual or single inoculation showed significant increases in seed yield in 1995. The highest seed yield was observed due to dual inoculation. In 1996, seed yields were also increased by inoculation but dual inoculation performed poorly when compared to Bradyrhizobium alone, which registered the highest grain yield. Generally, the benefits derivable from AM fungi in health of their vegetative partners have been amply demonstrated by Perrin (1992) and their use in agriculture, horticulture and reforestation was emphasized by Plenchette (1992).

Table 2. Effect of AM and Bradyrhizobium inoculation on plant height, nodule number and weight, dry matter of shoot and grain yield of cowpea (*Vigna unguiculata*).

Treatments	Plant height (4wap), cm		Nodule no. (6 wap)		Nodule wt. (6 wap), g		Shoot dry matter, g		Grain yield, kg ha^{-1}	
	1996	1995	1996	1995	1996	1995	1996	1995	1995	1996
Uninoculated control	35.73b	20.51b	23.34b	20.42b	0.213b	0.111b	2.41b	1.74b	835.01b	700.42b
Bradyrhizobium	42.61a	34.22a	39.52a	40.71a	0.422	0.312a	4.52a	3.92a	1000.41a	858.21a
AM	37.34b	34.22a	33.01ab	36.51a	0.332b	0.113b	2.91ab	2.51ab	1009.04a	770.63ab
AM+Bradyrhizobium	36.43b	33.54a	32.34ab	34.22a	0.342ab	0.313a	3.43ab	3.12a	1020.0a	710.32b

Mean values with same letter in the vertical columns are not significantly different (P>0.05) according to DMRT.

AM infection was noticed even in uninoculated control. About 3-fold increase in infection rate was obtained due to dual or single inoculation of AM when compared to uninoculated control in 1995 while in 1996, a 9 fold increase was recorded due to dual inoculation and AM alone (Table 3). Similarly,

Table 3. Effect of AM and Bradyrhizobium inoculation on the rate and intensity of AM infection, tissue N and P of cowpea (*Vigna unguiculata*).

Treatments	AM infection rate (%) 1995	AM infection rate (%) 1996	*AM infection intensity 1995	*AM infection intensity 1996	N (%) 1995	N (%) 1996	Ndfa (%) 1995	Ndfa (%) 1996	P (%) 1995	P (%) 1996
Uninoculated control	12.51^{a}	2.42^{a}	1	1	1.87^{b}	1.40^{ab}	30.21^{b}	35.12^{b}	0.74^{b}	0.64^{c}
Bradyrhizobium	14.21^{a}	4.51^{a}	1^{a}	1^{a}	2.97^{a}	2.72^{a}	40.11^{a}	59.71^{a}	0.83^{ab}	0.79^{bc}
AM	39.42^{b}	22.52^{b}	2	2	2.71^{a}	2.14^{a}	49.7^{a}	63.14^{a}	0.85^{ab}	0.93^{a}
AM+Bradyrhizobium	31.21^{b}	19.41^{a}	2	1	2.85^{a}	2.20^{a}	42.14^{a}	61.22^{a}	0.99^{a}	0.94^{a}

Mean values with same letter in the vertical columns are not significantly different (P>0.05) according to DMRT.
* Not analysed statistically

the intensity of infection of mycorrhizal roots vried in different prorportions. While small infection sites were widely scattered along roots in uninoculated cowpea roots, larger infection sites were uniformly distributed through the infected roots in inoculated plants. However the sites did not coalese in any form. This result indicated the presence of mycorrhiza fungi in soil prior to inoculation. It was however, practically impossible to distinguish among different fungi within roots using clearing and staining procedure adopted, but it was not difficult to infer from this data the success of *G. mosseae* inoculation. Significant increases were obtained in N content in inoculated plants in the 2 years (Table 3). However, it was higher in 1995 than in 1996. Conversely, the percent nitrogen derived from atmosphere (%Ndfa) was generally lower in 1995 than in 1996. The inadequate soil N may have led to increased N_2 fixation. The average N_2 fixed was about 60%. This was about 3-fold increase over the value obtained by Awonaike *et al.* (1991). Dual or single inoculation also had positive effect on P, with plants dually inoculated having the highest amount of P. Results of this study confirm many reports about the effectiveness of symbiotic association between AM and cowpea with respect to nutrient uptake and yield. Chhabra *et al.* (1990) reported increases in cowpea plant height, total dry matter of shoot, NPK content and extensive mycorrhizal development in roots. Ikombo *et al.* (1991) found strong colonization (85%) of roots with AM fungus at 36 days after sowing. They concluded that recovery of cowpea from early P deficiency stress resulted from increased P absorption following the development of a mycorrhizal association with the roots. Islam *et al.* (1980) had earlier reported high mycorrhizal infection and P uptake of cowpea roots, when grown in a field earlier put to AM inoculated cowpea. Similarly, Islam and Ayanaba (1981) reported increased percentage of infected roots, shoot dry weight and nodule yield of cowpea cv Tvx 1836-44G and VITA-4 after inoculation with *G. mosseae.*

Results obtained in this study indicated that dual inoculation of cowpea with AM and Bradyrhizobium or single inoculation with AM has the potential of enhancing cowpea for optimal utilization of soil nutrients in fertile soil environment and soil low in fertility. The data of this study had shown that even in soil of low nutrient status, it is practically possible for cowpea to improve the benefits of using the available soil nutrients through mycorrhiza inoculation. The claim by Aziz and Habte (1989) that it was important to restore lost nutrients before the benefits of AM inoculation could be effectively exploited, needs a review.

It should however, be stated that lack of expression might be obtained if the nutrient status falls below their critical levels (yet to be determined). The finding of Aziz and Habte (1989) might have been based on response obtained in soils that had its nutrient values below these critical levels.

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