ISSN - 2277 - 2755

Journal of Agricultural Science and Environment

INFLUENCE OF *BRADYRHIZOBIUM* AND TWO *GLOMUS* SPECIES ON THE GROWTH AND YIELD OF SOYBEAN

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ABSTRACT

A screenhouse experiment was conducted to investigate the effect of *Bradyrhizobium japonicum* on the response of soybean to inoculation with two species of mycorrhiza (*Glomus mosseae* and *Glomus deserticola*). The study was carried out in a screenhouse with soybean as test crop. The two species of arbuscular mycorrhiza fungi (AMF) were inoculated to the potted soil with or without *B. japonicum.* Single super phosphate (SSP) and zero amendment served as conventional and absolute control respectively. The experimental design was randomized complete block with 4 replicates. AMF/*Bradyrhizobium* interaction increased mycorrhizal fungi root colonization significantly (p = 0.05) by at least 35.9% at early growth stage (3 weeks after planting (WAP)) and 59.5% at later growth stage (9 WAP). *G mosseae*/*Bradyrhizobium* interaction significantly increased N and P uptakes by 68.9 and 80.0%, respectively, as well as plant height, number of leaves, number of branches, canopy spread and leaf area between 2 and 5 WAP. Soybean biomass increased significantly due to interaction of *G mosseae* and *Bradyrhizobium* by 42.2-53.4% between 3 and 9 WAP and nodule weight increased by 61.9-93.3% between 6 and 9 WAP. Grain yield per plant was similar in all AMF treatments and SSP but less in sole *Bradyrhizobium* inoculation by 37.5% and in control by 33.3%. AMF/*Bradyrhizobium* interactions produced higher N by up to 81% in the residual soil and the P content was similar to SSP but higher than in control by up to 32.3%. Interaction of *G mosseae* and *Bradyrhizobium* increased spores of mycorrhizal fungi in the soil by 41% at 3 WAP and 74.7% at 9 WAP. It was concluded that although *Bradyrhizobium* had a positive and synergistic influence on the activities of the two species of mycorrhizal fungi inoculated on soybean, the influence was, however, more pronounced on *G mosseae* than *G deserticola*.

Keywords: Soybean, *Bradyrhizobium japonicum*, *Glomus mosseae*, *G deserticola,* Residual soil

INTRODUCTION

The soils of Nigerian moist savanna are low in many of the essential nutrients including nitrogen and phosphorus (FFD, 2002). The cropping systems are currently dominated by low input practice, high input such as inorganic fertilizers, pesticides and other labour saving devices are mostly inaccessible to the resource-poor farmers. Grain legumes mainly soybean, cowpea and groundnut, are commonly grown in intercrop or rotation, where they play important role as sources of nitrogen to the succeeding or associated crops. Yusuf *et al.* (2009) attributed 124-279 kg ha^{-1} to fixed N effect and 193-513 kg ha^{-1} to rotation effect in maize fol-

lowing soybean or cowpea. These legumes require adequate level of P for nodulation and optimal nitrogen fixation, growth and yield (Sanginga *et al.*, 1996)). However, soils in these zones contain low levels of P, commonly below 10 mg kg^{-1} which is inadequate to meet the needs of most field crops (FFD, 2002). Therefore, there is need to develop practices that will help to improve P utilization by legumes and consequently improve their efficiencies in intercrops and rotations. Previously, varieties which have low P requirements were selected to compensate for inadequate level of P in the soils (Sanginga *et al.,* 1996; Abelgadir, 1998). This is possible due to high diversity between and within leguminous species in their response to P application in savanna soils (Babalola and Amapu, 2006).

The variations in P requirements by these legumes and their varieties could be traced to their responsiveness to arbuscular mycorrhizal fungi (Nwoko and Sanginga, 1999). Mycorrhizal dependency, which is the degree to which a host relies on the mycorrhizal fungi to produce maximum growth at a given level of soil fertility (Gerdeman, 1975) has been well demonstrated in soybean species (Khalil *et al*., 1994; Nwoko and Sanginga, 1999). These fungi extend the nutrient absorption area of crop species, thereby increasing the volume of soil that is exploited (Bolan *et al*., 1987; Voets *et al*., 2009). Arbuscular mycorrhizal colonization also helps the legumes to cope with drought, salinity stresses and premature nodule senescence (Auge, 2001; Ruiz-Lozano, 2003; Porcel *et al*., 2003). The interaction of these symbiotic associations possibly evolved from a set of pre-adaptations during co-evolution of the two associations (Provorov *et al*., 2002).

It has also been demonstrated that soybean growth and yield improved through rhizobial inoculation because the association between soybean, rhizobia and mycorrhizal fungi produces a synergistic effect on soybean productivity by improving N and P nutrition in the crop (Barea *et al.,* 2005a, 2002). The possible benefit of this association is even more emphasized by the fact that N fixation improvement through rhizobial inoculation is often promoted in soils when phosphorus level is adequate (Barea and Azcon-Aguilar 1983). This is because P is a component of the cellular structure including DNA and RNA therefore important in germination, root development, nodulation, nitrogen fixation, growth and grain production. It is expedient at this time to examine low input strategies that are capable of improving plant N and P nutrition, with attendant increase in residual soil fertility so that legumes used in intercrop or rotation can be more relevant in improving nutrition of associated or succeeding crop. In this sense, mycorrhizae associations could be the most poorly understood and untapped resource in the moist savanna soil of Nigeria. Since AM symbiosis is known to benefit plant growth and health, there is need to ascertain its effectiveness in particular plant production systems, with a view of manipulating and incorporating them into production practices (Barea *et al*., 2005b). Several studies have been reported in the humid savanna (Atayese *et al.,* 1992; Awotoye *et al.,* 1993, Osonubi *et al.,* 1991; Fagbola *et al.,* 1998) using species of Glomus. Fagbola *et al*. (1998) identified *G. mosseae* and *G* – from soil collected in Ibadan, however there is no such report on *G. desertiicola*. Given the fact that soil microbial interaction could be employed for efficient crop production, the aim of this study is to determine the influence of *G. mosseae* and *G. desertiicola* on the activity of *Bradyrhizobium*

japonicum in the production of soybean.

MATERIALS AND METHODS

The experiment was conducted in the screenhouse of University of Agriculture, Abeokuta and the pots were arranged in randomized complete block design (RCBD) with 4 replicates. RCBD was employed because of lack of uniformity in the conditions obtained in the screenhouse. It consisted of seven treatments which included two species of *Glomus*; *G. mosseae and G. deserticola*, *Bradyrhizobium japonicum, G. mosseae/ Bradyrhizobium* and *G. deserticola/ Bradyrhizobium* combinations as well as single super phosphate (SSP) and a no inoculation or fertilizer control. The SSP was applied at planting based on the national recommendation rate of 40 kg P_2O_5 for soybean (FFD, 2002).

Source of Materials

Two species of *Glomus* (*G mosseae* and *G deserticola*) as well as *Bradyrhizobium japonicum* (IRJ 2180A) and the seeds of soybean var TGX-1448-2F were all obtained from International Institute for Tropical Agriculture (IITA) Ibadan (Grain Legume Programme).

Culturing of AMF Inoculant

The spore inoculants of *G mosseae* and *G deserticola* containing about 70 and 60 spores per 100 g soil respectively were inoculated into potted sterile soil and planted to maize. Initially the maize seedlings were watered daily and allowed to grow for 3 months; the inflorescences were removed just before tasselling. Watering was stopped 10 days before the termination of culturing, thereafter maize roots were cut into tiny pieces (1cm) and mixed with the potted soil and this was used as the mycorrhizal spore inoculant (Khalil *et al.*, 1994). At the end of culturing, the spore densities were 60 and 40 spores per 100 g soil for *G. mosseae* and *G. deserticola,* respectively.

Pot Experiments

The soil used was collected from one of the research fields of UNAAB at 0-20 cm depth, it is a loamy sand with pH 5.9, organic carbon 0.35 %, available P 9.0 mg kg^{-1}, total N 0.2 %, CEC 1.9 and spore count 34 spores/100 g soil. Ten kilogram of soil was weighed into plastic pots, 50 g soil inoculant of *G mosseae* or *G deserticola* were applied into the potted soils and *B japonicum* at 13 x 10^4 cfu ml^{-1} was applied to soybean seedlings. *B japonicum* inoculation was achieved by applying 1 ml of the broth culture to each seedling at one week after planting as described by Vincent (1970).

Measurements

The data which were collected on soybean weekly from two weeks after planting (WAP) included plant height, number of leaves, number of branches, leaf area and canopy spread. The other crop data were per cent AMF colonization on root, N and P uptake, shoot and root biomass, number and count of nodules at 3, 6, 8 and 9 WAP. Grain yield and soil N, P, organic matter and spore count were determined at harvest.

Assessment of mycorrhizal root colonization

Fine root samples of soybean were collected and stored in vials at 4 ^{0}C for 72 hours. The root samples were washed thereafter with tap water and cut into 10-20 mm length. About 0.25 g of fresh and fine root sample was taken and cleaned in 10 % KOH in a water bath. The sample was rinsed in water and stained with 0.05 % tryphan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at room temperature (Phillips and Hayman, 1970). Mycorrhizal fungi coloniza-

tion was quantified using the modified intersect method described by Mcgonigle *et al*. (1990). The infection was expressed as percentage root colonized.

Spore extraction and count

Soil samples were taken across the depth, mixed, 100 g soil was sub-sampled from each pot and extracted for VAM spores by a wet-sieving method. This was followed by 20-60% weight/volume sucrose density centrifugation gradient at 3000 rpm for 4 min (Daniels and Skipper 1982). The spores were examined and counted under a dissecting microscope (15-45x).

Plant and soil analysis

The plants were sampled at flowering (6 WAP), oven-dried at 80^{0}C for 24 hrs, ground and digested in triple acid of perchloric acid, sulphuric acid and nitric acid (2:1:1) as described by Juo *et al*. (1974). The P in solution was determined in ascorbic acid (Murphy and Riley, 1962) modified by Anderson and Ingram (1998) while N was measured by Micro-Kjeldhal method (Bremner, 1996). At the end of the experiment, soil sample was taken from each pot, air-dried, passed through a 2 mm sieve and analysed for organic carbon, N and P. The organic carbon was determined using the dichromate titration method (Nelson and Summers, 1996), N was measured by the Micro-Kjeldahl method (Bremner, 1996) and P was extracted by the Bray 1 extractant (Bray and Kutz, 1945), the P in solution was determined by the molybdenum blue method.

Data analysis

Data were analysed using analysis of variance with the SAS/GLM procedure (SAS, 1989) and the means were separated using least significant difference (LSD) at 5 % probability.

RESULTS

Roots of plants in pots inoculated with mycorrhizal fungi alone and in mixture with *B. japonicum* had significantly higher mycorrhizal fungi colonization at 3 and 9 WAP compared with those not inoculated or with SSP application (Table 1). The plants inoculated with *B. japonicum* however had higher colonization than the control at 9 WAP but lower colonization than those with mycorrhiza/*B. japonicum* mixture at both samplings and *G. mosseae* alone at 3 WAP. At 9 WAP, plants in pots inoculated with mycorrhiza alone had lower per cent colonization than those with mixture of mycorrhizal fungi and *B. japonicum*. Root colonization in pots with SSP application was comparable to those of sole *B. japonicum* inoculation and control.

Uptake of N by soybean was significantly higher with dual inoculations of *G. mosseae* and *Bradyrhizobium* compared with sole *B. japonicum* and AMF inoculations (Table 1).

The sole inoculations of *G. mosseae* and *B. japonicum* resulted in similar nitrogen uptake and higher than in SSP and control. Inoculation of *G. deserticola* with or without *B. japonicum* resulted in comparable N uptake to SSP application and control but *G. mosseae*/*Bradyrhizobium* combination gave higher N uptake than other treatments. Single inoculation of the two species of *Glomus* resulted in P uptake comparable to the maximum with combined inoculations of *G. mosseae* and *Bradyrhizobium* (Table 1). The two single inoculations of *Glomus* were also comparable to *G. deserticola*/*Bradyrhizobium* inoculation and SSP application, but gave significantly higher P uptake than in the plants inoculated with sole *B. japonicum*, and the control. Sole inoculation of *Bradyrhizobium* resulted in lower P

uptake than SSP, both of which gave higher uptake than the control.

The trend in weekly plant growth is presented in Figures 1 to 5. Plant height was only significantly affected by the treatment at 3 WAP, when *G. mosseae*/*Bradyrhizobium* combination resulted in taller plants than *G. desertícola*/*Bradyrhizobium* combination (Figure 1). The numbers of leaves were significantly higher in pots treated with *G. mosseae,* alone and in combination with *B. japonicum* compared with other treatments at 3 WAP and the control at 5 WAP. Combination of *G. desertícola* with *B. japonicum* had comparable number of leaves to plants inoculated with *B. japonicum* alone and the control, inoculation with *G. desertícola* and application of SSP resulted in the lowest number of leaves at 3 WAP. Furthermore, at 2, 4 and 6 WAP number of leaves was not significantly different in all the treatments (Figure 2).

The number of branches in soybean was comparable in plants inoculated with *G. mosseae* alone and in combination with *B. japonicum* at 3 WAP and plants inoculated with *G. mosseae* alone produced significantly higher number of branches compared to other treatments while plants inoculated with *G. mosseae*/*B. japonicum* combination had values comparable to the control but higher than other treatments. Similarly, at 5 WAP, plants inoculated with *G. mosseae* alone and in combination with *B. japonicum* produced comparable number of branches to the control but significantly higher than other treatments. However at 6 WAP number of branches was significantly higher in plants inoculated with *G. mosseae* compared with SSP but comparable to other treatments but number of branches was similar in all treatments at 2 and 5 WAP (Figure 3).

The canopy spread of soybean was significantly higher in plants inoculated with *G. mosseae* and its combination than with *G. desertícola* but comparable to the other treatments at 2 WAP and thereafter, no significant differences were shown among the treatments (Figure 4).

Furthermore, the leaf area was significantly higher in plants inoculated with *G. mosseae* alone and in combination with *B. japonicum* compared to plants inoculated with *B. japonicum* alone and *G. desertícola* alone but comparable to other treatments at 2 WAP (Figure 5). Also, leaf area was significantly higher in plants inoculated with *G. mosseae* alone compared with *B. japonicum* alone at 3 WAP and the control at 5 WAP.

Dual inoculation of *G. mosseae* and *B. japonicum* resulted in shoot biomass significantly higher than all the other treatments at 3 WAP and comparable to those of *G. mosseae* alone and control at 6 WAP (Table 2). At 9 WAP, single inoculation of *G. mosseae* and dual inoculation of *G. desertícola* and *B. japonicum* resulted in significantly higher shoot biomass compared with application of SSP and the control while dual inoculation of *G. mosseae* and *B. japonicum* resulted in significantly higher biomass than the control. Although root dry weight did not differ significantly among the treatments, the values were higher in mycorrhizal fungi treatments and SSP than in sole *B. japonicum* and control at 3 WAP while at 9 WAP, AMF inoculations had higher values than *B. japonicum* inoculation, SSP application and control at 6 and 9 WAP (Table 2).

The number and weight of nodules were significantly influenced by treatments at 6, 8 and 9 WAP (Table 3). Number of nodules on soybean roots was significantly higher

with dual inoculations of *G. mosseae* and *B. japonicum* compared with inoculations of the two mycorrhiza species alone as well as application of SSP and the control which also had similar number at 6, 8 and 9 WAP. The trend indicated for *G. mosseae* was also observed for dual inoculation of *G. desertícola* and *B. japonicum* at 6 WAP. Furthermore, at 6, 8 and 9 WAP number of nodules was comparable or similar in all plants inoculated with *B. japonicum,* and plants inoculated with *B. japonicum* alone was comparable to the control at 6, 8 and 9 WAP and SSP at 6 and 8 WAP. As observed with nodule number, dual inoculation of *G. mosseae* and *Bradyrhizobium* resulted in significantly higher weight of nodules per plant compared with all other treatments at 6, 8 and 9 WAP, with the exception of *Bradyrhizobium* at 9 WAP. Dual inoculation of *G. desertícola* and *Bradyrhizobium* as well as that of *Bradyrhizobium* alone resulted in significantly higher nodule weight than the two single mycorrhizal fungi inoculations, SSP application and the control at 6 WAP. Similarly dual inoculation of *G. desertícola* and *Bradyrhizobium* caused higher nodule weight than inoculation of *G. desertícola* alone as well as application of SSP and the control at 8 WAP while *Bradyrhizobium* inoculation resulted in higher values than *G. mosseae* as well as SSP application and control at 9 WAP.

Inoculation with *Bradyrhizobium* significantly delayed days to first flowering compared with control, SSP and dual inoculation of *G. desertícola* and *Bradyrhizobium* as well as days to 50 % flowering compared with all the other treatments (Table 4). Grain yield per plant was significantly higher in all the plants inoculated with mycorrhizal fungi than in sole *Bradyrhizobium* inoculation and absolute control but comparable to plants with SSP application, although numerically higher in mycorrhizal plants by 32-42 % than SSP application.

The contents of N, P organic matter and mycorrhizal fungi spores in the residual soil were significantly affected by treatments (Table 5). Soil nitrogen was comparable among the dual inoculation treatments but significantly higher in dual inoculation of *G. desertícola* and *Bradyrhizobium* than in the remaining treatments.

The levels of soil N contents were similar in the *G. mosseae* treatments, but significantly higher than those of sole inoculations of *G. desertícola*, *Bradyrhizobium*, SSP and control. Among the treatments, soil in pots with dual inoculation of *G. desertícola* and *Bradyrhizobium* had P content comparable to the maximum in pots treated with SSP. Soils treated with dual inoculations of mycorrhizal fungi and *Bradyrhizobium* had similar P content but significantly higher than those of their corresponding pots with sole inoculations of mycorrhizal fungi and that of the un-amended control (Table 5). Soil organic matter was highest in pots with sole inoculation of AM fungi and SSP but similar in pots with dual inoculations and lowest in sole *Bradyrhizobium* inoculation and control. The soil with dual inoculations of *G. mosseae* and *Bradyrhizobium* had significantly higher spore counts than the other treatments at 3 and 9 WAP (Table 5). Furthermore, spores in soil with dual inoculation of *G. desertícola* and *Bradyrhizobium* were significantly more than those of other treatments at 3 WAP with the exception of sole *G. desertícola* at 9 WAP. Both of the sole mycorrhizal fungi treatments had more soil AMF spores than non mycorrhizal fungi treatments at 3 and 9 WAP except that treatment with sole *Bradyrhizobium* had comparable spore to sole *G. mosseae* treatment at 9 WAP.

Table 1: Effects of AMF and Bradyrhizobial inoculations on mycorrhizal root colonization and nutrient uptakes in soybean

Treatment	Rate of root colonization AMF (%)		Nutrient uptake (mg/kg)	
	3 WAP	9 WAP	N	P
G. mosseae	35.3	58.3	0.42	3.0
G. deserticola	34.3	64.0	0.27	3.4
G. mosseae/Brad	39.3	77.0	0.75	4.0
Gdeserticola/Brad	35.7	78.3	0.37	2.5
B. japonicum	26.7	48.0	0.41	1.9
SSP	21.3	40.1	0.22	2.9
Control	21.7	31.7	0.22	0.8
LSD (0.05))	7.86	10.56	0.17	1.02

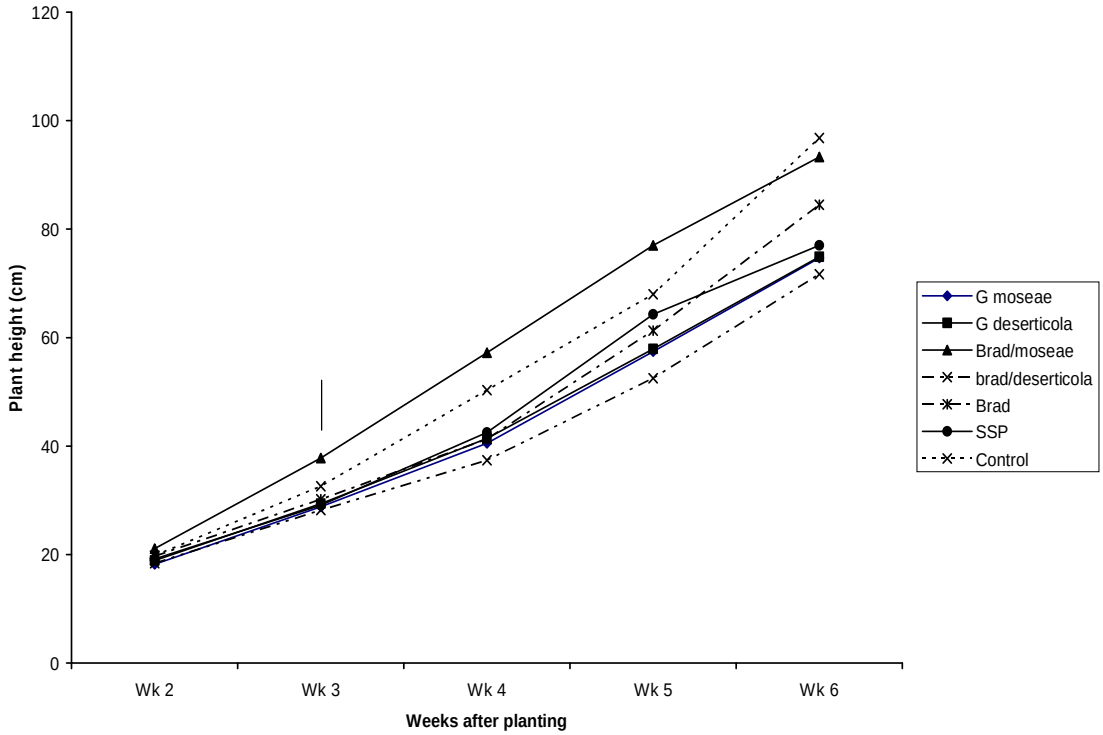

Figure 1: Effect of AMF and bradyrhizobial inoculations on soybean plant height

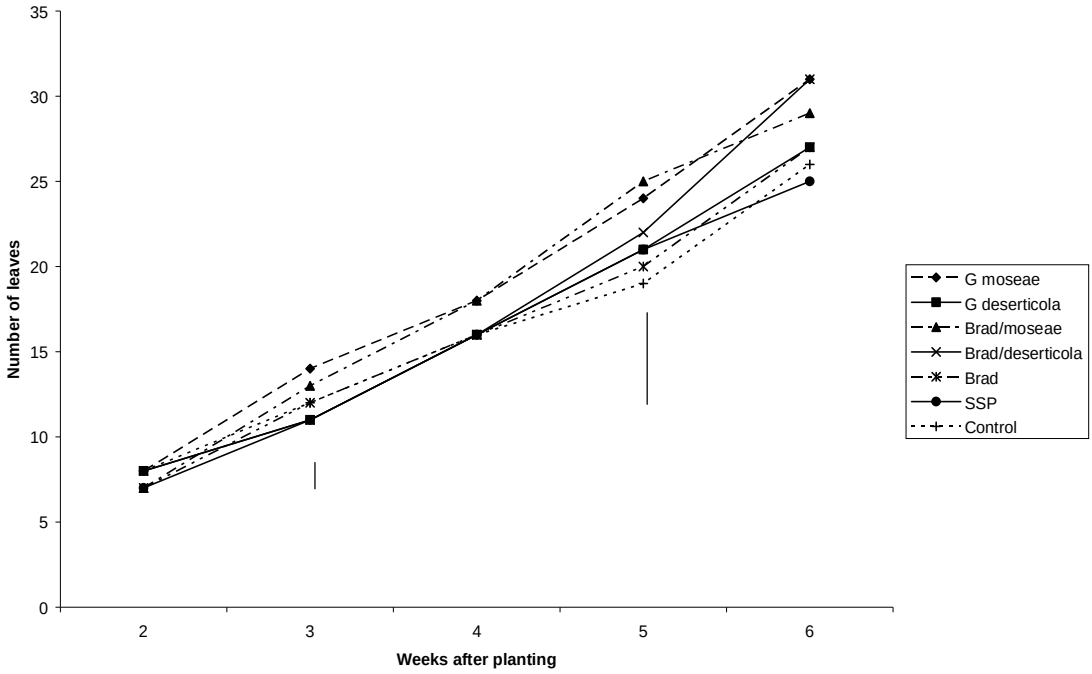

Figure 2: Effect of AMF and bradyrhizobial inoculations on number of leaves of soybean

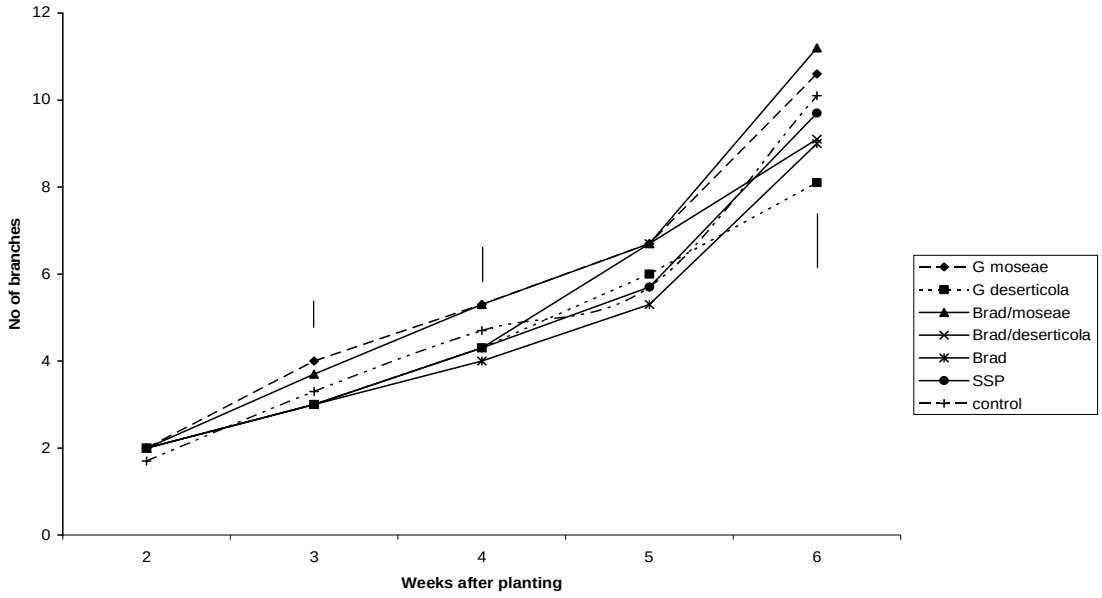

Figure 3: Effect of AMF and bradyrhizobial inoculations on number of branches of soybean

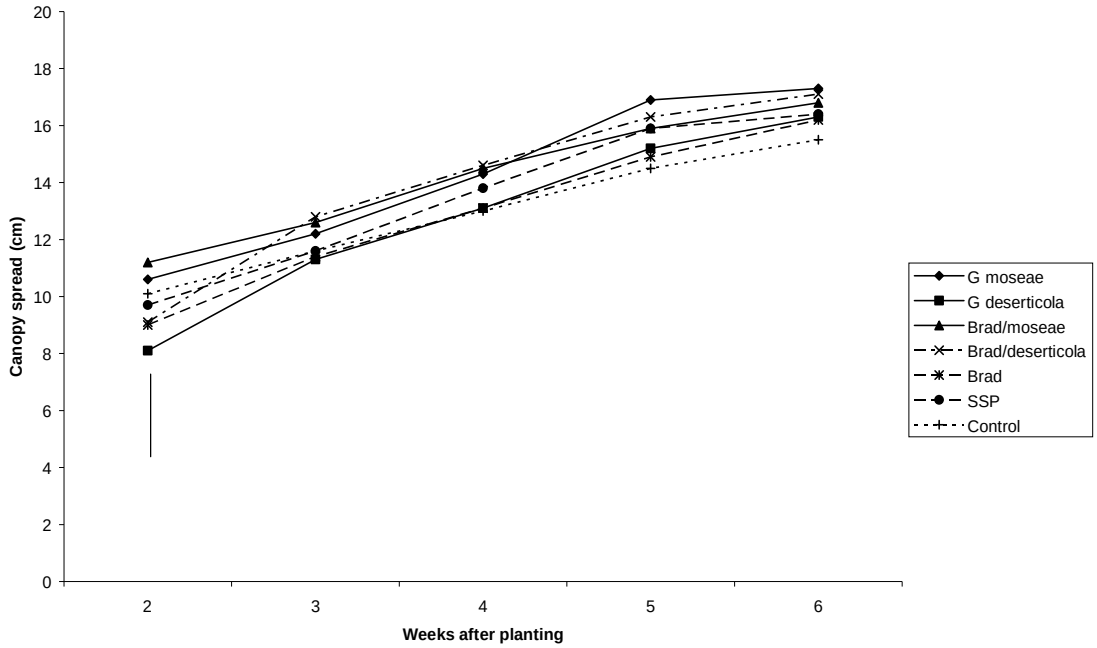

Figure 4: Effects of inoculations of AMF and bradyrhizozium on canopy spread of soybean

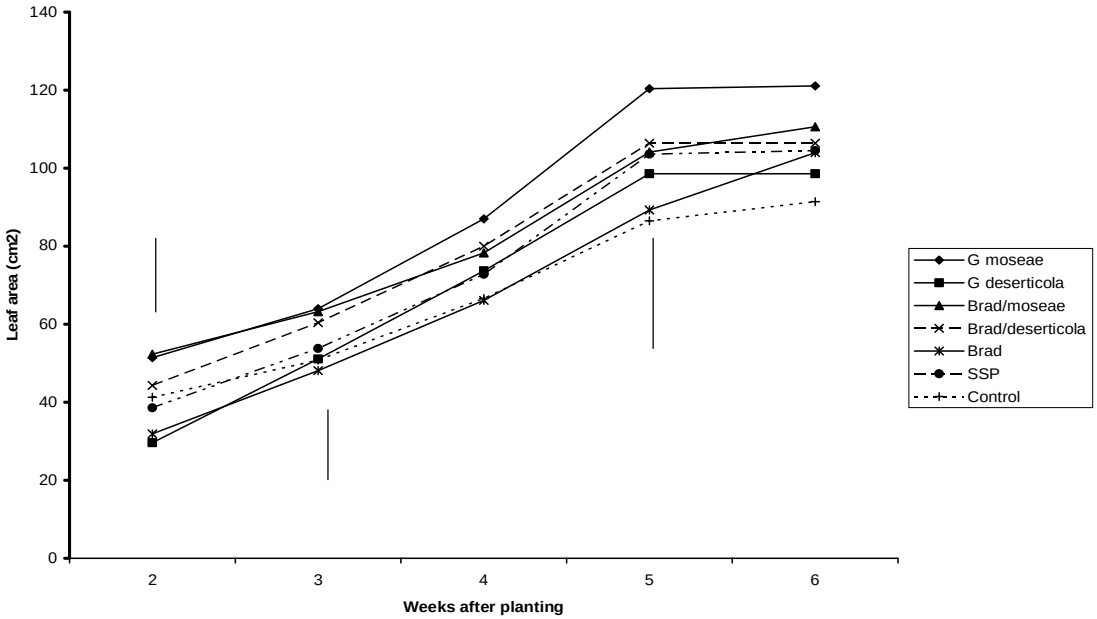

Figure 5: Effects of AMF and Bradyrhizobium inoculations on leaf area of soybean

Table 2: Effects of AMF and bradyrhizobial inoculations on soybean biomass at 3, 6 and 9 WAP

Treatment	Shoot biomass (g/plant)			Root biomass (g/plant)		
	3 WAP	6 WAP	9 WAP	3 WAP	6 WAP	9 WAP
G. mosseae	0.40	2.61	6.30	0.06	0.32	0.64
G. deserticola	0.37	2.45	4.88	0.06	0.23	0.44
G. mosseae/Brad	0.66	3.48	6.04	0.07	0.31	0.60
G. deserticola/ Brad	0.31	2.38	6.49	0.06	0.2	0.55
B. japonicum	0.31	2.04	3.90	0.04	0.26	0.34
SSP	0.42	2.01	3.30	0.07	0.24	0.33
Control	0.33	2.76	2.81	0.04	0.27	0.37
LSD (0.05)	0.14	0.97	2.89	0.06	0.17	0.32

Table 3: Effects of AMF and bradyrhizobial inoculations on soybean nodulation at 6, 8 and 9 WAP

Treatment	Nodule number			Nodule weight (g/plant)		
	6 WAP	8 WAP	9 WAP	6 WAP	8 WAP	9 WAP
G .mosseae	0.3	13.0	7.0	0.03	0.11	0.05
G. deserticola	2.0	10.0	9.0	0.02	0.06	0.09
G. mosseae/Brad	25.0	63.0	42.0	0.15	0.33	0.35
G. deserticola/ Brad	26.0	33.0	22.0	0.09	0.21	0.15
B. japonicum	17.0	36.0	33.0	0.10	0.13	0.26
SSP	4.0	6.0	7.0	0.02	0.02	0.06
Control	2.0	10.0	9.0	0.01	0.08	0.04
LSD (0.05)	17.67	31.59	26.02	0.05	0.11	0.20

Table 4: Effects of AMF and bradyrhizobial inoculations on days to flowering and grain yield of soybean

	Days to flowering		Grain yield
Treatment	1st	50 %	g plant-1
G. mosseae	39.0	40.0	14.4
G. deserticola	39.0	41.0	14.2
G. mosseae/Brad	39.0	40.0	15.0
G. deserticola/Brad	38.0	41.0	14.4
B. japonicum	40.0	42.0	9.0
SSP	38.0	41.0	10.8
Control	36.0	41.0	9.6
LSD (0.05)	1.97	1.14	4.35

Table 5: Effects of AMF and bradyrhizobial inoculations on Nitrogen, Phosphorus, organic matter contents and spore counts of the residual soil

	Nutrients		SOM	Spore counts (100 g-1 of soil)	
Treatment	N (%)	P(mg/kg)	(%)	3 WAP	9 WAP
G. mosseae	0.82	8.7	0.49	38.33	60.67
G. deserticola	0.20	8.8	0.57	39.67	71.0
G. mosseae/Brad	1.15	9.9	0.34	53.67	134.33
G. deserticola/Brad	1.24	11.1	0.40	47.0	89.33
B. japonicum	0.41	9.4	0.25	32.33	45.33
SSP	0.36	11.7	0.50	31.0	33.33
Control	0.21	6.7	0.30	31.67	34.0
LSD (0.05)	0.358	1.162	0.096	5.641	24.908

DISSCUSSION

Soil inoculation with both *G mosseae* and *G desertícola* fungi increased root colonization by mycorrhizal fungi at both the seedling and at later stages of soybean development. Although the effect of interaction of mycorrhizal fungi and *Bradyrhizobium* was not apparent on root colonization at the seedling stage of soybean, root colonization was enhanced by inoculations of *Bradyrhizobium* following mycorrhizal fungi at later stages of soybean development. Recently, under an *in vitro* condition. Voets *et al*. (2009) noted enhanced and fast colonization by AMF inoculation but attributed it to extraradical mycelium network of the inoculated fungi. Also, application of *Glomus mosseae* alone and with *Bradyrhizobium* caused higher N uptake than sole *Bradyrhizobium* inoculation and all mycorrhizal fungi treatments caused higher P uptake than sole *B. japonicum* treatment although the difference observed with *G. desertícola*/*B. japonicum* was not significant. In addition to the parameters inter-alia, all treatments caused higher P uptake than control and inoculation with *G. mosseae*/*Bradyrhizobium* combination caused higher P uptake than with SSP application. The result suggests that sole inoculation of *G. mosseae* caused the same P uptake as SSP but combination of *G. mosseae* and *B. japonicum* caused higher N and P uptake than in SSP and sole *Bradyrhizobium*. Nitrogen uptake was lowest in pots with SSP, *G. desertícola* and control. Moreover, consistent increase in nodulation was observed in all *B. japonicum* and combination of *Bradyrhizobium* with *G. mosseae* increased the persistence of the nodules. Inoculation with AM fungi was found to protect soybean against drought and prevent premature nodule senescence (Porcel *et al*., 2003). The synergistic nature of mycorrhizal fungi/*Bradyrhizobium* interaction which has been widely reported (Barea *et al.,* 2002, 2004) and was probably developed because the two organisms have similar evolution pattern (Parniske, 2000, Provovo 2002, Gianinazzi-Pearson, 1997). The association caused persistent mycorrhizal colonization of root as shown in the present study leading to enhanced P and N uptake in *G. mosseae*/*Bradyrhizobium* interaction. Barea *et al.* (2004) noted that nodulation and rhizobial activity improve within the nodule and earlier studies revealed that nitrogen fixation improvement through rhizobial inoculation is better achieved with adequate P (Barea and Azcon-Aguilar, 1983, Barea *et al*. 1992, 2002). The enhanced N fixation might have been a result of improved nodulation and inoculation of *Bradyrhizobium* either in combination or sole it allows the nodules to persist longer than in non-bradyrhizobial treatments, but it appears that only the association with *G. mosseae* caused increased nodule weights. However, adequate P uptake is important for optimum nodulation (Babalola and Amapu, 1999, Sanginga *et al*. 1996, Barea *et al*. 1992, 2002), therefore enhanced P uptake resulting from *G. mosseae*/*Bradyrhizobium* interaction might be responsible for higher nodulation observed with this treatment. Since inoculation of AMF improved P uptake (Turnau *et al.*, 2005) through hyphal exploration, phosphate solubilization and mineralization of organic P (Barea *et al*., 2004, 2005; Gininazzi *et al*., 2002; Turnau *et al.,* 2005), thereby, providing favourable condition for nitrogen fixation by *B japonicum*.

Consequent to increased N and P nutrition because of mycorrhizal fungi inoculation and AMF/*Bradyrhizobium* interactions, growth and development of soybean also improved in those treatments. Although not significant, soybean plant inoculated with *G. mosseae* consistently had highest root bio-

mass. This is an indication that growth of soybean roots also increased with *G. mosseae* and its combination with *Bradyrhizobium* compared with other treatments. Schreiner *et al.* (2007) showed that growth and efficiency of grapevine roots increased due to mycorrhizal fungi colonization.

The number of days to first and 50 % flowering was least in control and highest in sole *Bradyrhizobium* mycorrhizal inoculation. The luxuriant vegetative growth resulting from inoculation with *Bradyrhizobium* and subsequent improved N nutrition might have caused delayed flowering. Inoculation with mycorrhizal fungi caused increase in grain yield; this was, however, comparable with SSP application. This should be considered as an advantage because SSP is a soluble, conventional and expensive phosphorus fertilizer with 18 % P_2O_5 applied at recommended rate of 40 kg ha^{-1} and obtaining an alternative P source with comparable but numerically higher yield and at less cost will be more assessable to the resource-poor farmers.

The content of N in the residual soil improved with dual inoculations of mycorrhizal fungi and *Bradyrhizobium* as well as sole inoculation of *G mosseae*, but inoculation of sole *Bradyrhizobium* did not improve soil N content. This is because biological nitrogen fixation takes place in the legume root tissue from where the fixed N is immediately transported to other parts of the plant, while the activities of AMF extended to the soil and might have impacted positively on the soil. As expected, more P was observed in soil treated with SSP than other treatments, while mycorrhizal fungi/ *Bradyrhizobium* combination had more P than in the sole inoculation of each species, showing that interactions of mycorrhizal fungi and *Bradyrhizobium* impacted positively on soil P. Phosphorus content was higher in all treatments than in the control, indicating that where no input is applied inoculation of mycorrhiza fungi and *Bradyrhizobium* either alone or in combination will improve soil P and alleviate low P condition in the soil. It has been earlier reported that in infertile soils, AM fungi efficiently absorb P from low mobile minerals through the modification of host root development (Kang *et al.,*1980).

Due to higher biomass production, soil organic matter increased in pots with sole AMF and SSP treatments but in pots with *G. deserticola*/*Bradyrhizobium* combination it was higher than in soils with sole *Bradyrhizobium* and control. The residual soil in pots with *G. mosseae*/*Bradyrhizobium* combination consistently contained more mycorrhizal fungi spore than the other treatments, followed by soil with *G. deserticola*/*Bradyrhizobium* and lowest in soils without AMf inoculation. Pots with sole inoculations of AMF however consistently gave less spores than pots with AMF/*Bradyrhizobium* combination. This tends to reveal that AMF/*Bradyrhizobium* interaction led to the production of more spores, however without the interaction, inoculation of mycorrhizal fungi alone could still increase soil spore although to a lower extent. Earlier work by Guevara and Lopez (2007) showed that root colonization could be limited by richness and abundance of spore, therefore this increase in spores of mycorrhizal fungi can benefit succeeding crops by influencing nutrient cycling in soil-plant systems, improve plant health through increased protection against biotic and abiotic stresses and also improve soil structure through aggregate formation (Linderman, 1992, Gininazzi *et al.,* 2002; Barea *et al.,* 2005b; Turnau *et al.,* 2005).

CONCLUSION

The interaction between AM fungi and *Bradyrhizobium* caused improved root colonization by mycorrhizal fungi at early and later stages of soybean development. Consequently uptakes of N and P also improved, particularly with *G. mosseae*/*Bradyrhizobium* combination. This led to improved growth and development of soybean with *G. mosseae*/*Bradyrhizobium* interaction. Number of nodules was similar in all *Bradyrhizobium* inoculations however, *G. mosseae*/*Bradyrhizobium* combination increased the weights of nodules. Initially, all the treatments delayed flowering probably due to luxuriant growth but by 50 % flowering only sole inoculation of *Bradyrhizobium* persisted in this delay. Grain yield per plant was higher in all treatments with mycorrhizal fungi than in sole *Bradyrhizobium* and control. The interaction between AM fungi and *Bradyrhizobium* also impacted positively on the soil N, P and organic matter, increasing soil N more than in sole *Bradyrhizobium* and P than was obtained with their sole but similar to SSP. The residual soil spore was also increased by this interaction, giving more spore than with sole inoculations of mycorrhizal fungi and treatments without mycorrhizal fungi. However, the impact of *Bradyrhizobium* interaction was more pronounced with *G. mosseae* than with *G. desertícola* in promoting soybean growth and yield. In addition, the interaction of the two species of *Glomus with Bradyrhizobium* produced similar increase on the residual soil nutrients but *G. mosseae*/*Bradyrhizobium* interaction produced more spores in the residual soil.

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(Manuscript received: 23rd January, 2009; accepted: 12th May, 2009).