Plant and Soil 68, 321–329 (1982). 0032-079X/82/0683-0323$01.35. Ms. 5088

Effect of inoculation with *Glomus mosseae* on nitrogen fixation by field grown soybeans

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Key words Alginate A value Endomycorrhiza *Glomus mosseae* Inoculant Nitrogen fixation *Rhizobium japonicum* Soybean

Summary This field study was undertaken to determine the effect of inoculation with *Glomus mosseae* on N_2 fixation and P uptake by soybean. The inoculation with *Glomus mosseae* was achieved using a new type of inoculant, alginate-entrapped (AE) endomycorrhizal fungus. N_2 fixation was assessed using the A value method. In P-fertilized plots, inoculation with AE *Glomus mosseae* increased the harvest index based on dry weight (+ 20%) and N content of seeds (+ 17%), the A value (+ 31%) and % N derived from fixation (+ 75%). Inoculation with AE *Glomus mosseae* decreased the coefficient of variation for the A value and for the dry weights of the different plant parts.

Introduction

Most experiments on the effect of inoculation of soybean with vesicular-arbuscular (VA) fungi reported up to now have been carried out under greenhouse conditions. These experiments have clearly shown growth increases of soybean in response to infection by effective strains of VA fungi[4]. These growth increases have been attributed to a better exploitation of the labile pool of soil P[10]. Pot experiments[1,4,23] have also clearly indicated that VA mycorrhizae can greatly assist nodulation and N_2 fixation of soybeans inoculated with rhizobia, a result similar to that already reported for other legumes, such as Stylosanthes[16]. This stimulation of the legume N_2-fixing activity is probably at least partly the result of the improvement of the P uptake by the plant. The few field experiments carried out up to now on soybean in Florida[21], in North Carolina[21] and in Bangalor, India[2] have confirmed the beneficial effects of inoculation by VA fungi. However, the reported positive effect on the soybean yields were not always statistically significant[2].

The first aim of the experiment reported here was to gain further understanding of the effect of VA infection on P uptake and N_2 fixation in the field using the A value method[8] which is recognized as giving accurate and reliable estimations of N_2 fixation[12]. The second was to use for the first time in the field a polymer-entrapped VA inoculant. This type of inoculant, already proposed for Rhizobium[13], has been recently adapted to VA fungi[7].

Materials and methods

Field experiments were carried out at the ISRA (Institut Sénégalais de Recherches Agricoles) research station of Sefa, South Senegal, in 1980. The soil was a leached ferruginous tropical soil[6] (alfic eutrustox) in which soybean had never been grown (Table 1).

Table 1. Properties of soil from experimental plots

	0–20 cm	20–40 cm
Texture		
Sand (50–2000 μm) (%)	73.3	69.7
Clay (< 2 μm) (%)	12.2	15.3
pH, H_2O (1/2.5)	5.9	5.8
Organic C (%)	0.481	0.408
Organic N (%)	0.046	0.041
Exchangeable cations		
S (meq/100 g)	2.01	1.94
T (meq/100 g)	2.74	2.93
V = S/T × 100	73	66
Total P, ppm	217	216
Available P (Truog), ppm	5.4	2.9

S: Total exchangeable cations.
T: Total exchange capacity.

Experimental design

Split-plot experimental design was used with six replicates. All the plots were inoculated with *Rhizobium japonicum*. There were two main treatments: one in which no P was added, and another in which P was added at the rate of 22 kg P/ha. The surface of main-plot was 28 m^2. There were two subtreatments one involving inoculation with alginate-gel without *Glomus mosseae*, the other involving inoculation with AE *Glomus mosseae*. The surface of subplot was 2,5 m^2. All main plots received starter nitrogen fertilizer (17 kg N/ha), and K as KCl (90 kg K/ha) was applied at the time of sowing and at the time of flowering. This experimental design was part of a larger experiment, presented elsewhere[9], designed to study the effect of different fertilizers and Rhizobium inoculation on soybean yield.

Two statistical analyses were performed. The first, involving the whole split-plot design (which comprised both P-fertilized and non P-fertilized plots) was performed according to the methods described by Lecompt[14] and Snedecor and Cochran[24]. The second interpretation, based on the P-fertilized plots alone, was performed using the paired data method[19].

Treatments and materials

N fertilizer was applied at the rate of 17 kg/ha as a solution of $(^{15}NH_4)_2SO_4$ with an atom excess of 4.85%.

The soybean cultivar used was cv. 44A/3 obtained by ISRA. The Rhizobium peat-base inoculant (strain USDA 138) which contained $3 \,.\, 10^8$ living cells per g, was applied to the seedling bed at the rate of 220 g per 28 m^2 plot.

Glomus mosseae was multiplied on roots of *Vigna unguiculata* which had been inoculated with roots fragments heavily infected with the VA fungus. After two month's growth the roots of *Vigna unguiculata* were harvested, washed on a sieve to remove extra soil and the mixture of infected roots, spores, extramatrical hyphae was then thoroughly homogenised in a Waring blender for 3 seconds three times (100 g in 1000 ml of water). The suspension thus obtained was entrapped in calcium alginate according the method already described for Rhizobium[13]. The resulting product consisted of wet beads of alginate-entrapped (AE) *Glomus mosseae* which were stored in a cold room. Each bead contained *ca* 12 mg (fresh weight) of infected roots, spores and hyphae, inoculation was performed by introducing 10–15 beads 3–4 cm deep into the soil around each seedling when they were 15 days old.

Rainfall

The total rainfall before sowing (June to July 19) was 153 mm and 504 mm during the growth cycle (July 19 to October 15). The rainfall distribution was fairly even; however, a dry period did occur from September 10 to October 1 at the time of pod filling.

Amount of nitrogen fixed by soybean

This was evaluated according to the A value method[8] which involves simultaneous determination of the A values by uninoculated soybean (Au) and by inoculated soybean (Ai) using ^{15}N-labelled nitrogen fertilizer.

For the sake of clarity, we give hereafter the definition in short of the 'A' value method used for the determination of fixed-N, clearly by Fried and Broeshart[8]:

1 – The available amount of N in a source is designated by 'A', which is a *concept.*

2 – The 'A' value is expressed in equivalent units of kg N/ha as nitrogen fertilizer applied (ammonium sulfate in our experiment).

3 – Symbiotic N_2 fixation by legume crop is confronted with 3 sources of N: a, Soil N; b, Fertilizer N (ammonium sulfate in our experiment); c, N supplied by N_2 fixing mechanism in nodules.

4 – We need a non nodulating (non nod.) crop with the same growth-period for the determination of A 'Soil' value.

5 – From nodulating crop, we can determine (using labelled fertilizer) the A 'Soil + fixation' value.

6 – In our experiment, we are in the situation where nodulating crop and non nodulating crop 'see' the same available amount of soil N but received different amount of fertilizer N.

7 – A 'Fix.' = A 'Soil + fix.' – A 'Soil'

If:

A 'fertilizer' = rate of fertilizer applied (17 N)

% Ndff = % of N derived from fertilizer

% Ndf fixation = % of N derived from fixation

% Ndf soil = % of N derived from soil

We can write:

$$\frac{\%\text{Ndff}}{\text{A fertilizer}} = \frac{\%\text{ Ndf fixation}}{\text{A fixation}} = \frac{\%\text{ Ndf Soil}}{\text{A Soil}}$$

8 – Fixed-N (kg/ha) = % Ndf fixation × total N (kg N/ha).

In our experiment the non nod. crop was non inoculated crop belonging to the same variety as the nodulating crop.

A value of uninoculated soybean (Au) were obtained from the larger experiment mentioned above, were:

Au = 331 for plots with P fertilization.

Au = 263 for plots without P.

Table 2. Influence of fertilization and inoculation with *Glomus mosseae* on plant dry weight, total N and P contents of field-grown soybeans

Treatments		Plant dry wt (kg/ha)			Total N (kg/ha)			Total P (kg/ha)		
P fertilization (kg P/ha)	*Glomus mosseae*	Seeds	Straw	Seeds + straw	Seeds	Straw	Seeds + straw	Seeds	Straw	Seeds + straw
0	0	1486 (33)	3098 (27)	4584 (28)	95	23	119	5.8	1.0	6.9
0	+	1381 (30)	3391 (14)	4772 (18)	87	25	112	5.4	1.1	6.5
22	0	1546 (12)	4128 (15)	5674 (11)	100	43	143	7.7	2.9	10.6
22	+	1848 (4)	4164 (8)	6012 (6)	116	38	154	9.2	2.5	11.7
Main effect of P fertilization		(21)	(22)	(20)	(22)	(30)	(22)	(24)	(37)	(22)
Main effect of inoculation with *G. mosseae*		(15)	(8)	(9)	(15)	(20)	(15)	(19)	(35)	(21)

In brackets, coefficient of variation (%). Data related to straw, including husk. The only significant effect was the main effect of P fertilization (F test, blocks with split-plot).

The amount of fixed N_2, expressed in kg N/ha, was obtained by multiplying the difference Ai–Au by the percent utilization of the fertilizer by the soybeans.

Plants were carefully harvested avoiding contamination with soil N. The samples were dried at 65–70°C for 24 h, weighed, ground into a 40 mesh powder, analysed for P content according to the usual vanadomolybdophosphate method and for total nitrogen content according to the Kjeldahl method.

^{15}N analyses were carried out at the Seibersdorf Laboratory (IAEA) using Dumas' method (the combustion performed in this technique converts total N directly to N_2) and emission spectrometry.

Assessment of mycorrhizal infection

The roots were stained according to the method of Phillips and Hayman[18], cut into 5 mm segments and observed under a dissecting microscope to determine the percentage of infected roots (frequency).

Results and discussion

Interpretation of data related to the split-plot design

This interpretation indicated that the only significant treatments were P fertilization, which increased the weight, total P and N content of soybean (Table 2), and inoculation with AE *Glomus mosseae*, which significantly decreased the amount of N fertilizer taken up by the plant (Table 3). The beneficial effect of P fertilization on soybean yield was to be expected but the result with inoculation with AE *Glomus mosseae* is worth underlining since this is the first time that the effect of inoculation with AE *Glomus mosseae* on N fertilizer uptake is demonstrated *in situ* using the A value method. The decrease in N fertilizer uptake observed occurred concurrently with an increase in N_2 fixation, which confirms that by improving P and water uptake during the dry period inoculation with AE *Glomus mosseae* can enhance N_2 fixation (Table 3). One striking effect of P fertilization was that it decreased the plant heterogeneity

Table 3. Sources of N and A value

Treatments		Fixed N_2		Soil N		Fertilizer N		A value
P fertilization (kg P/ha)	*Glomus mosseae*	%	kg/ha	%	kg/ha	%	kg/ha	kg/ha
0	0	37	44	58	69	4.0	4.8	422 (32)
0	+	38	42	58	65	3.9	4.3	431 (32)
22	0	24	34	73	104	3.8	5.4	439 (16)
22	+	41	63	56	86	2.9	4.4	576 (6)
Main effect of P fertilization							(15)	(24)
Main effect of inoculation with *G. mosseae*							(14)	(24)

In brackets, coefficient of variation (%).

The only significant effect was the main effect of *Glomus mosseae* on amount of N fertilizer taken up by soybeans (F test, blocks with split-plot).

expressed by the coefficient of variation (CV). Thus the CV for the dry weight of seeds which was 30–35% in plots without P was only 4–12% in plots with P (Table 2). The application rate of P was low (22 kg P/ha) but sufficient to decrease the heterogeneity of the distribution of available P in the soil.

The effect of either treatments (P application or inoculation with AE *Glomus mosseae* on mycorrhizal infection of the roots was not significant (Table 4), probably because the roots were sampled when the plant were too old (pod filling stage), which allowed plenty of time for the native VA fungi to invade the roots.

Table 4. Root infection of soybean by VA fungi

Treatments		Infection frequency (%)
P fertilization (kg P/ha)	Inoculation with *Glomus mosseae*	
0	0	38 (27)
0	+	46 (22)
22	0	36 (28)
22	+	42 (24)

No significant differences.
In brackets, coefficient of variation (%).

It was not possible to carry out a statistical analysis of the harvest index. However, table 5 shows that in the plots with no P fertilization the effect of inoculation with AE *Glomus mosseae* on the harvest index* expressed as dry weight, total N or total P was negative whereas it was positive in the plots with P fertilization. In other words the beneficial effect of inoculation with AE *Glomus mosseae* on the harvest index occurred only when a small amount of P (22 kg/ha) was applied to the soil, which confirmed the suggestion that P application may be necessary for the expression of the beneficial effect of VA inoculation[17]. The differences observed between plants with or without AE *Glomus mosseae* was probably accentuated by the drought period which occurred during pod-filling.

Inoculation with AE *Glomus mosseae* descreased the coefficient of variation of the different parameters studied including the A value. For this latter parameter the coefficient decreased from 16 to 6% in the P treated plots.

The high variability of control plots (without AE *Glomus mosseae*) was attributed to the patchy distribution of VA fungi reflected by a high coefficient of variation of infection. Inoculation with AE *Glomus mosseae* improved the uniformity of the root infection (Table 4), and hence resulted in a more regular growth and ion uptake by the plants. A similar decrease in the variability of data

* Weight, total P or N content of seeds as a proportion of weight, total P or N content of crop.

Table 5. Estimation of the harvest index resulting from inoculation with *Glomus mosseae* (G.m.)

P. fertilizer	G.m.	D.W.	N	P
0	0	0.32	0.80	0.84
	+	0.29	0.78	0.83
	%	− 9.1	−2.5	−1.2
+	0	0.27	0.70	0.73
	+	0.31	0.75	0.79
	%	+14.8	+7.1	+8.2

% = relative increase or decrease in harvest index.
Harvest index = weight, total N or P content of seeds as a proportion of weight, total N or P content of crop.

following VA inoculation has recently been found by Morandi *et al.*[15] using raspberry. It is interesting to note that during their growth VA inoculated soybeans were visually better developed and greener than uninoculated plants.

Interpretation of data concerning P-fertilized plots alone

Table 6 shows that inoculation with AE *Glomus mosseae* significantly increased dry weight (+ 20%), total N content of seeds (+ 17%), the A value (+ 31%) and % N derived from fixation (+ 75%). The increase in total N was not surprising since the beneficial effect of VA fungi on the N content of seeds has already been reported[21,22]. Increase in the A value is probably related to the stimulation of N_2 fixation, which is itself a consequence of endomycorrhizal increase of ion uptake[3]. This has been clearly shown in glass-house conditions and, in a few instances, in the field using indirect methods of assessments, such as nodule number or nodule weight and acetylene reduction activity measurements[1,4,22].

Table 6. Significant effects of inoculation with *Glomus mosseae* (study restricted to the P-fertilized plots)

Inoculation with *Glomus mosseae*	Dry weight of seeds (kg/ha)	Total N of seeds (kg/ha)	A value (kg/ha)	Fixed N_2 (%)
0	1546 (100)	99.6 (100)	439 (100)	23.7 (100)
+	1848 (120)	116.2 (117)	576 (131)	41.4 (175)
LSD ($P = 0.05$)	234 (15)	15.8 (16)	109 (25)	17.2 (73)

% of control in brackets.
Fixed N_2 % = % N plant derived from fixation.

Conclusion

The present field study on soybeans confirms most of the experimental data obtained under glass-house conditions on the effect of inoculation with VA fungi, *i.e.* increased dry weight, total P and N. There is a strong indication that inoculation with *Glomus mosseae* increases the harvest index, which is a most valuable result. Moreover, by reducing uptake of fertilizer N and soil N and simultaneously promoting N_2 fixation, VA mycorrhizae conserve the stock of soil N and thus contribute to improving the N balance which is often negative in grain legume crops[25]. However, even under the best conditions (*i.e.* in P-fertilized plots inoculated with AE *Glomus mosseae*) the total amount of N_2 fixed is low (63 kg/ha) and only 41% of crop N is derived from N_2 fixation whereas higher figures have reported[12].

Although the present experiment was not specially designed to check the performance of the inoculum consisting of a polymer-entrapped VA fungus, the results reported here suggest that this new type of inoculum could be successfully used in the field. It would be interesting to compare it with other methods used to introduce VA fungi into soil, such as the soil pellet method described by Hall[11].

Acknowledgements We thank Dr. V. Gianinazzi-Pearson for reviewing the manuscript and Youssouph N'Diaye and Moussa Niang for technical assistance. The investigation was funded in part by IAEA (joint FAO/IAEA coordinated research programme).

Received 4 May 1982. Revised August 1982

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