

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

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Summary This field study was undertaken to determine the effect of inoculation with *Glomus mosseae* on N₂ fixation and P uptake by soybean. The inoculation with *Glomus mosseae* was achieved using a new type of inoculant, alginate-entrapped (AE) endomycorrhizal fungus. N₂ 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 fungi⁴. These growth increases have been attributed to a better exploitation of the labile pool of soil P¹⁰. Pot experiments^{1,4,23} have also clearly indicated that VA mycorrhizae can greatly assist nodulation and N₂ 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 N₂-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 Florida²¹, in North Carolina²¹ and in Bangalor, India² have confirmed the beneficial effects of inoculation by VA fungi. However, the reported positive effect on the soybean yields were not always statistically significant².

The first aim of the experiment reported here was to gain further understanding of the effect of VA infection on P uptake and N₂ fixation in the field using the A value method⁸ which is recognized as giving accurate and reliable estimations of N₂ fixation¹². 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 fungi⁷.

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 soil⁶ (alfic eutruxox) in which soybean had never been grown (Table 1).

Table 1. Properties of soil from experimental plots

| | 0-20 cm | 20-40 cm |
|-----------------------------------|---------|----------|
| <i>Texture</i> | | |
| Sand (50-2000 μm) (%) | 73.3 | 69.7 |
| Clay (< 2 μm) (%) | 12.2 | 15.3 |
| pH, H ₂ O (1/2.5) | 5.9 | 5.8 |
| Organic C (%) | 0.481 | 0.408 |
| Organic N (%) | 0.046 | 0.041 |
| <i>Exchangeable cations</i> | | |
| S (meq/100 g) | 2.01 | 1.94 |
| T (meq/100 g) | 2.74 | 2.93 |
| V = S/T \times 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 m². 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 m². 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 elsewhere⁹, 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 Lecompt¹⁴ and Snedecor and Cochran²⁴. The second interpretation, based on the P-fertilized plots alone, was performed using the paired data method¹⁹.

Treatments and materials

N fertilizer was applied at the rate of 17 kg/ha as a solution of (¹⁵NH₄)₂SO₄ 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 $3 \cdot 10^8$ living cells per g, was applied to the seedling bed at the rate of 220 g per 28 m² 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 Rhizobium¹³. 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 method⁸ which involves simultaneous determination of the A values by uninoculated soybean (Au) and by inoculated soybean (Ai) using ¹⁵N-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 Broeshart⁸:

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 ferti- zation (kg P/ha) | <i>Glomus mosseae</i> | 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 inocu- lation with <i>G. mosseae</i> | | (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 $A_i - A_u$ 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

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 plants 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 <i>Glomus mosseae</i> | |
| 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 inoculation¹⁷. 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* decreased 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.

| P. fertilizer | G.m. | D.W. | N | P |
|---------------|------|------|------|------|
| 0 | 0 | 0.32 | 0.80 | 0.84 |
| + | | 0.29 | 0.78 | 0.83 |
| % | | 0.1 | 0.5 | 1.0 |

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 N₂ fixation, VA mycorrhizae conserve the stock of soil N and thus contribute to improving the N balance which is often negative in

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