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Comparative effects of *Glomus mosseae* and P fertilizer on foliar mineral composition of *Acacia senegal* seedlings inoculated with *Rhizobium*

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Summary. A factorial experiment with two controlled factors was conducted in the greenhouse with *Acacia senegal* seedlings. The substrate was a degraded sandy soil (Dior soil) poor in available P (11 ppm - Olsen). The first controlled factor was soil sterilization, with two levels: (A) sterilized soil; (B) non-sterilized soil. The second factor was fertilization, with six levels: (1) uninoculated control; (2) inoculation with *Rhizobium* (ORS 1007); (3) inoculation with *Glomus mosseae*; (4) double inoculation with ORS 1007 and *G. mosseae*; (5) inoculation with ORS 1007 and 30 ppm phosphorus per plant; (6) inoculation with ORS 1007 and 60 ppm phosphours. The combination of the two factors and their levels led to 12 different plant treatments (A1-A6 and B1-B6). Compared to the control B1, the B5 and B6 treatments containing phosphorus increased: nodule dry weight about 7 times; leaf dry weight about 4 times; total N, P and Mg 4-5 times; total K and Ca 3-4 times. The mycorrhizal inoculation had the same positive effect on plant growth and mineral composition but with lower values. Plants inoculated with *Rhizobium* alone gave the lowest results. The A1 treatment gave lower values than B1. Foliar mineral contents varied within a narrow range (20-30%).

Key words: *Acacia* - *Glomus* - *Rhizobium* - Phosphorus

Introduction

Among tree legumes, *Acacia senegal* K. Willd. (New 1984) has a privileged position in the sahelian ecosystem. It provides valuable products such as gum arabic (ITC 1983), fodder, fire and timberwood for the economy of the sahelian countries. The combined effect of extended drought and excessive exploitation by man and livestock has severely damaged the ecosystem, resulting in the decline of natural populations of this species. It is necessary to work for the reconstitution of the plant cover of the area and, among other leguminous woody perennials, *A. senegal* could play a major role in reforestation programmes and in agroforestry ecosystems.

In this regard, deeper knowledge is required of the physiology, mineral supply and nitrogen-fixing symbiosis of this tree. This paper deals with the effects of different biological and mineral treatments on the growth and leaf mineral composition of *A. senegal*. Treatments included *Rhizobium* inoculation, *Glomus mosseae* inoculation and phosphorus fertilization.

Materials and methods

Seeds of *A. senegal* were soaked in concentrated sulphuric acid for 14 min, rinsed in sterilized water, pre-germinated in sterilized vermiculite and then transferred to polyethylene bags. Each bag contained 1.5 kg of a mixture of soil and polystyrene beads (2:1 by volume). The bags were placed in a greenhouse and watered to field capacity at 2-day intervals. A "Dior" soil of Kebemer's dunes (94.3% sand), widely represented in Senegal (Maignien 1965), was used. This soil type had a low available phosphorus level (11 ppm, Olsen method).

The factorial experiment, with randomization within the blocks, had three replicates of four plants and two controlled factors. The first factor was "soil sterilization" (F1) divided into two levels: (A) sterilized soil; (B) non-sterilized soil. The second controlled factor was "fertilization" (F2) with six levels: (1) control without inoculation or fertilizer; (2) *Rhizobium* inoculation (strain ORS 1007); (3) endomycorrhizal inoculation with *G. mosseae*; (4) double inoculation with ORS 1007 and *G. mosseae*; (5) *Rhizobium* inoculation (ORS 1007) + 30 ppm phosphorus; (6) rhizobial inoculation with ORS 1007 + 60 ppm phosphorus. The experiment was conducted in the greenhouse under non-sterile conditions. Combination of intervention levels of the two controlled factors gave 12 treatments (A1-A6 and B1-B6). A1 was the absolute control with no additives in sterilized soil, while B1 served as a control in natural non-sterile soil conditions.

The ORS 1007 fast-growing strain of *Rhizobium* used for inoculation was previously isolated from *Acacia laeta* Benth. nodules. Cross-inoculation tests demonstrated that ORS 1007 was also effective on *A. senegal* (Badji et al. 1988). For *Rhizobium* treatments, the seedlings were inoculated with a 2-ml inoculum

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(10^9 rhizobium ml^{-1}) on the same day that they were transferred from vermiculite to bags in the greenhouse.

For endomycorrhizal treatments, 500 mg chopped roots of *Vigna unguiculata* (L.) Walp., endomycorrhized by *G. mosseae*, were placed into the plantation hole just before transplanting. The final level of endomycorrhizal infection was verified from roots of harvested seedlings using the "slide method" (Giovanetti and Mosse 1980).

For mineral fertilizer treatments, phosphorus was supplied as KH_2PO_4 in one application 2 weeks after transplanting. The plants were allowed to grow for 25 weeks before harvest. Growth and mineral composition were estimated by the following parameters: dry weight of leaves; content of leaves (% dry wt.) of N, P, K, Ca and Mg; foliar mineral mass (mg) of N, P, K, Ca and Mg.

The Newman-Keuls test ($P=0.05$) was used for statistical analysis.

Results and discussion

The results provide evidence of global effects of the two controlled factors (Table 1) and the 12 treatments (Table 2). The level of endomycorrhization was controlled and found to be satisfactory for the plants inoculated with *G. mosseae*. The treatments have a large impact on nodulation (Colonna et al. 1990). In sterilized soil, the nodule dry weight per four plants increased uniformly from treatment A1 to treatment A6, varying from 11- to 559 mg (a 50-fold increase). In non-sterilized soil, it varied from 67- to 524 mg (an 8-fold increase) from treatments B1 to B6. The measurements of acetylene reducing activity showed that these nodules were all active.

Main factor effects

Sterilization (F1). There was no significant effect of soil sterilization on leaf dry weight, Ca content or Ca and Mg mass (Table 1). There was a significant negative effect on leaf N, P, K content and mass and a significant positive effect on leaf Mg content (Table 1). Destruction of native rhizobia and endomycorrhizal propagules due to soil sterilization might explain the depressive effect.

Fertilization (F2). Leaf dry weight and leaf mineral mass for N, P, K, Ca and Mg (Table 1) increased progressively and generally significantly with the different intervention levels (1–6) of this controlled factor (F2). Inoculation with *Rhizobium* (level 2), with *G. mosseae* (level 3) and with both microorganisms (level 4) significantly increased most leaf weights and mineral masses compared to control values (level 1). Fertilization with phosphorus (levels 5 and 6) induced even greater increases: the values obtained were significantly higher than those reached for levels 1, 2, 3 and 4 (Table 1). Results were less obvious for leaf mineral contents. Phosphorus fertilization had a significant positive effect on N, P and Mg contents. Generally the lowest values were noted for the control, except for leaf K% and Ca%.

Treatment effects (factor interaction)

Mass parameters (per four plants). Treatment A1 in sterilized soil corresponds to an "absolute" control in which the plants rely solely on their capacity for absorption. Treatment B1 in non-sterilized soil corresponds to a "natural condition" control in which the soil microorganisms were not initially destroyed. These two treatments serve as references for comparison with the other treatments.

In treatment A1, the plants grew only with the help of the low mineral resources available in the soil. Some nodule formation (11 mg) was observed, however, since the experiment was not conducted under fully sterile conditions. After 25 weeks, the dry leaf biomass was only 272 mg and the mineral masses in the leaves reached 9, 0.3, 3, 7 and 0.7 mg for N, P, K, Ca and Mg, respectively. The lowest values obtained for all measured parameters resulted from this treatment (Table 2). *A. senegal*, reduced to sole dependence upon its capacity for absorption, grew very poorly in this soil type.

Compared to the absolute control (A1), treatment A2, rhizobial inoculation (ORS 1007) in sterilized soil, increased nodulation (35 mg dry wt.) by a factor of 3 and the leaf dry weight and mineral masses by a factor of 2 (Table 2). The results from the natural conditions control (B1) were not attained. The increases remained weak, suggesting that an element, other than nitrogen played a limiting role, hindering nodule development, nitrogen fixation, plant growth and uptake of other minerals. This limiting element could be phosphorus.

Inoculation with *G. mosseae* (A3) was a more efficient treatment than the previous one. Although the soil had been sterilized initially, *G. mosseae* allowed *Rhizobium* contained in the irrigation water or in the air to form a nodule mass equal to 117 mg per four plants, or 10 times more than for A1. The nodulation, growth (measured by the leaf dry weight, Table 2) and mineral uptake (measured by mineral masses in leaves, Table 2) were improved compared to control A1 and to treatment A2. The plant-bacterial symbiosis appears to work more effectively in the presence of *G. mosseae*, which probably affects P supply (Sanginga et al. 1989). These results support previous reports on the effects of phosphorus on plant growth and function (Bieleski 1973; Walker 1980; Mengel 1984). The values for mineral masses and dry weight exceed all those reported for treatment B1.

The dual inoculation with ORS 1007 and *G. mosseae* in sterilized soil (A4) increased the nodule mass per four plants to 386 mg. All parameters of mass were increased. Plant and symbiosis function improved and nitrogen and phosphorus were supplied in larger quantities to the plant. A synergistic effect between *Rhizobium* and *G. mosseae* was apparent.

In the non-sterilized soil of treatment B1, *A. senegal* in the presence of the existing soil microflora was placed in simulated field conditions. This treatment served as the "natural conditions" control, where the plant benefits from the actions of indigenous rhizobial and endomycorrhizal partners. The results (Table 2)

Table 1. Global effects of soil sterilization (F1) and soil fertilization (F2) on leaf dry weight, leaf mineral mass and mineral contents of *Acacia senegal* seedlings cultivated on "Dior" soil. Means within columns with the same letter are not significantly different ($P=0.05$) by the Newman-Keuls test

	Leaf dry weight[a] mg	Leaf mineral mass[a] and mineral contents (% dry wt.)									
		N		P		K		Ca		Mg	
		mg	%	mg	%	mg	%	mg	%	mg	%
F1: "Soil sterilization" factor											
A - Sterilized soil	1419	52.19 b	3.59 b	2.45 b	0.16 b	17.54 b	1.28 b	39.90	2.83	4.67	0.31 a
B - Non-sterilized soil	1559	61.15 a	3.88 a	2.78 a	0.18 a	22.66 a	1.53 a	35.79	2.43	4.00	0.26 b
F2: "Fertilization" factor											
1 - Control	423 d	15.15 d	3.37 b	0.64 e	0.14 b	6.31 e	1.36	11.58 d	2.86	1.10 e	0.26 b
2 - *Rhizobium* (ORS.1007)	983 c	37.29 c	3.77 a	1.49 d	0.16 b	11.95 d, e	1.54	21.11 c, d	2.52	2.20 d, e	0.28 a, b
3 - *Glomus mosseae*	1181 b, c	45.59 b, c	3.78 a	1.82 c, d	0.16 b	17.55 c, d	1.49	31.83 b, c	2.82	3.47 c, d	0.31 a, b
4 - ORS.1007 + *G. mosseae*	1551 b	58.66 b	3.80 a	2.34 c	0.15 b	20.54 c	1.37	38.51 b	2.46	4.19 c	0.27 a, b
5 - ORS.1007 + 30 ppm P	2232 a	85.78 a	3.88 a	4.30 b	0.20 a	28.35 b	1.29	57.40 a	2.55	6.74 b	0.30 a, b
6 - ORS.1007 + 60 ppm P	2566 a	97.56 a	3.82 a	4.94 a	0.20 a	35.93 a	1.42	66.65 a	2.55	8.31 a	0.32 a
Least significant range	628.23	22.69	0.35	0.93	0.05	9.29	0.38	19.95	0.70	2.07	0.05
Variation coefficient (%)	24.1	22.9	5.4	20.5	15.5	26.4	15.3	30.1	15.3	27.2	11.8

[a] For four plants

Table 2. Effects of 12 treatments on leaf dry weight, leaf mineral mass and mineral contents of *Acacia senegal* seedlings cultivated on "Dior" soil. Means within columns with the same letter are not significantly different ($P=0.05$) by the Newman-Keuls test

	Leaf dry weight[a] mg	Leaf mineral mass[a] and mineral contents (% dry wt.)									
		N		P		K		Ca		Mg	
		mg	%	mg	%	mg	%	mg	%	mg	%
Sterilized soil											
A1 - Control	272.23 e	9.08 f	3.18 d	0.31 d	0.11 c	3.23	1.08	7.44 e	2.85	0.68 e	0.25 a, b
A2 - *Rhizobium* (ORS.1007)	454.97 e	17.01 f	3.72 a, b, c	0.65 d	0.14 b, c	6.87	1.61	11.93 e	2.67	1.40 e	0.31 a, b
A3 - *Glomus mosseae*	994.10 d, e	33.92 e, f	3.39 c, d	1.44 b, c, d	0.15 a, b, c	12.56	1.36	30.23 c, d, e	3.13	3.29 d, e	0.34 a
A4 - ORS.1007 + *G. mosseae*	1706.17 b, c, d	64.92 b, c, d, e	3.84 a, b, c	2.64 b	0.16 a, b, c	20.36	1.23	44.36 b, c, d	2.58	5.02 c, d	0.29 a, b
A5 - ORS.1007 + 30 ppm P	2286.83 a, b	83.54 a, b, c, d	3.68 a, b, c	4.12 a	0.18 a, b	26.13	1.15	66.86 a, b	2.92	7.76 b	0.34 a
A6 - ORS.1007 + 60 ppm P	2802.47 a	104.65 a	3.74 a, b, c	5.22 a	0.19 a, b	36.13	1.28	78.61 a	2.81	9.86 a	0.35 a
Non-sterilized soil											
B1 - Control	573.70 e	21.21 f	3.57 b, c, d	0.96 c, d	0.17 a, b, c	9.40	1.63	15.72 d, e	2.87	1.51 e	0.26 a, b
B2 - *Rhizobium* (ORS.1007)	1510.20 b, c, d	57.57 c, d, e	3.81 a, b, c	2.32 b	0.15 a, b, c	17.03	1.46	30.29 c, d, e	2.36	3.00 d, e	0.24 b
B3 - *G. mosseae*	1368.67 c, d	57.26 c, d, e	4.17 a	2.21 b	0.16 a, b, c	22.55	1.62	33.42 c, d, e	2.51	3.64 e	0.27 a, b
B4 - ORS.1007 + *G. mosseae*	1395.67 c, d	52.40 d, e	3.76 a, b, c	2.03 b, c	0.15 a, b, c	20.72	1.48	32.67 c, d, e	2.35	3.36 d, e	0.24 b
B5 - ORS.1007 + 30 ppm P	2177.20 a, b, c	88.02 a, b, c	4.07 a, b	4.47 a	0.21 a	30.57	1.42	47.94 b, c	2.17	5.72 b, c, d	0.26 a, b
B6 - ORS.1007 + 60 ppm P	2329.83 a, b	90.46 a, b	3.90 a, b, c	4.66 a	0.21 a	35.72	1.56	54.70 b, c	2.29	6.75 b, c	0.29 a, b
Least significant range	628.23	22.69	0.35	0.93	0.05	9.29	0.39	19.95	0.70	2.07	0.05
Variation coefficient (%)	24.1	22.9	5.4	20.5	15.5	26.4	15.5	30.1	15.3	27.2	11.8

[a] For four plants

should be comparable to those of treatment A4, which also contains rhizobia and endomycorrhizers. However, the results were in fact consistently inferior for B1 than A4. This indicates that the indigenous symbionts were not well adapted to the plant or that the populations of indigenous microbes suffer from the unfavourable climatic conditions of heat and drought. Even if the plant growth was not yet maximized (leaf dry weight = 573 mg), the leaf nitrogen content (3.5%) here was the lowest observed for the non-sterilized soil treatments. This may indicate an insufficient nitrogen supply due to poor absorption or fixation.

Compared to the "natural conditions" control (B1), the treatment B2 with *Rhizobium* caused the doubling of all the parameters measured (Table 2). Treatment B3 introduced *G. mosseae* inoculum into non-sterilized soil containing existing microbial symbionts. In this case, the results were similar to those of treatment B2 (Table 2). The synergy observed between *Rhizobium* and *G. mosseae* when introduced into sterilized soil (treatment A4) was not noted in non-sterilized soil (treatment B4). The values obtained for B4 were most similar to those of treatments B2, B3 and A3, but were less than those of treatment A4 (Table 2). It seems that some antagonism exists between introduced microorganisms and those already in the soil.

All parameters were greatly increased by the treatments containing phosphorus (A5, A6, B5, and B6). These increases were especially significant compared to the controls A1 and B1. Nodulation approached or exceeded 500 mg per four plants. Foliar dry weight ranged from 2200 to 2800 mg. Mineral masses in the leaves were 4–5 times higher than the B1 treatment. Nodulation, nitrogen fixation, growth and mineral supply were all enhanced in both sterilized and non-sterilized soils with phosphorus fertilization in addition to rhizobial inoculation. This type of treatment replaced the action of *G. mosseae* and permitted the better functioning of the plant symbiosis. Growth, as measured by foliar dry weight, was higher in sterilized soil (A5, A6; Table 2), and the quantities of Ca and Mg in the leaves were generally greater in sterilized soil (A5, A6; Table 2).

Mineral content parameters

Leaf mineral content varied much less with different treatments than did dry weight or mineral element weight. In comparison to the absolute control (A1), all treatments induced increases in N, P and K content. This agrees with the natural deficit of these elements in the used soil. Another consideration is that Mg and Ca contents tended to be higher in sterilized soil than in non-sterilized soil; the opposite was true for the K content. This fact needs further investigation. The leaf mineral content ranged from 20% to 30%.

Our results show that it would be useless to create performant symbionts by selecting different partners (plant, bacteria, endomycorrhiza) if their new potentialities can not be expressed in the natural environment. In this regard, it is advisable to determine the physiological conditions for optimum functioning and to identify the limiting factors of the environment. Here, the principal limiting factor was the P concentration of the soil. Dual inoculation and the action of endomycorrhizae may partially reduce this deficiency but P fertilization together with rhizobial inoculation was the most efficient treatment.

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