Artículo Científico

Indigenous rhizobia associated with *Arachis pintoi* in Cerrado soils of Brazil

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Introduction

The beneficial role of leguminous forage species in pasture-base animal production systems is due to its atmospheric nitrogen (N_2) fixing capacity when associated with *Rhizobium* and *Bradyrhizobium* bacteria, resulting in highly nutritional forage. Besides improving animal production, leguminous forage plants contribute to soil fertility, transferring nitrogen to the soil via: (1) nodule, root and shoot decay and its incorporation into organic matter residues, and (2) as animal manure. Additionally, forage species may contribute to increase ground cover, therefore, helping to control weed infestation and soil erosion (Aguirre et al., 1988). In the Cerrado area, Brazil, *Arachis pintoi* is considered an outstanding leguminous plant due to its ability to adapt to acid and low-fertility soils (Rincón et al., 1992), thus representing and interesting alternative for pasture systems in this ecosystem.

Although *A. pintoi* is a promiscuous legume species which nodulates with native rhizobia in tropical soils, inoculation with selected strains may promote increased forage production, as observed by Thomas (1993) in studies carried out at the International Center for Tropical Agriculture (CIAT) in Colombia. Usually *A. pintoi* nodulates with the native strains found in Cerrado soils, but lack of information about the indigenous rhizobial populations specific to this plant and their role in the nitrogen fixation process requires further investigation.

The presence, number and biological N_2 fixing capacity are among the factors which determine the need for inoculation of leguminous plants (Singleton and Tavares, 1986; Thies et al., 1991), a practice that is more valuable in low-fertility soils such as those of those Cerrado region.

Therefore, the objective of this work was to select and identify effective indigenous Cerrado soils rhizobia strains capable of improving *A. pintoi* forage production.

Materials and methods

The plant-infection technique (Somasegaran and Hoben, 1985; Vincent, 1970) was used to estimate the native rhizobial population capable of symbiosis with *A. pintoi*. Soil sample were collected during the summer rainy season from the 0-20 cm layer of: (1) virgin Cerrado soil, (2) Cerrado soil cultivated with different forage leguminous species, and (3) Cerrado soil cultivated with *A. pintoi* (Table 1). Samples were diluted in series (10^{-1} to 10^{-9}) and 1 ml aliquots were applied to Leonard jars containing pre-germinated *A. pintoi* ecotype BRA031143 seeds. Results were expressed as the most probable number (MPN) of rhizobia per gram of soil (Somasegaran and Hoben, 1985).

Rhizobia strains were isolated from *A. pintoi* nodules collected from noninoculated plants cultivated in Cerrado and lowland soils of Sete Lagoas and Planaltina, Brazil. One hundred and seventy five sampled colonies were tested for their inoculation capacity in *Macroptilium atropurpureum* cv. Siratro, in nitrogen-free Jensem medium under axenic conditions (Vincent, 1970).

Nitrogen-fixing efficiency was initially determined in Leonard jars under axenic conditions (Hungria, 1995) in experiments conducted in a greenhouse using a completely randomized block design in triplicate. In addition to 38 native strains selected from the previous experiment, commercial strains furnished by CIAT-Colombia (CIAT 2139, CIAT 3806, CIA T3101) and a strain indicated for the genus *Arachis*, BR 1405 supplied by CNPAB-EMBRAPA were used for comparison.

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Table 1. **Soil characteristics at sites where samples were collected to count MPN[a] of rhizobia associated with *Arachis pintoi*.**

Site	pH	Al	Ca	Mg	K	P	O.M.	Sat. Al	Characteristic
		(meq /100 cc)			(ppm)		(%)		
Virgin Cerrado	4.7	0.6	0.87	0.12	36	13	3.80	60	Characteristic Cerrado vegetation with typical shrubs
Cultivated Cerrado (1)	5.8	0	3.11	0.40	59	10	3.02	0	Cerrado cultivated for more than 15 years with different leguminous forage
Cultivated Cerrado (2)	5.4	0.1	2.63	0.36	69	9	2.68	3	Cerrado under *A. pintoi* cultivation for 3 years, previously under corn cultivation

a. MPN = most probable number of rhizobia per gram of soil.

Control treatments consisted of Cerrado soil suspension inoculation and nitrogen fertilization (250 mg/jar, N). Plants were grown en Leonard jars with a modified Norris nutrient solution (Vargas, personal communication), containing higher calcium and lower phosphorous levels. Sixty days after emergence, the plants were collected and evaluated for nodulation and dry matter (DM) production.

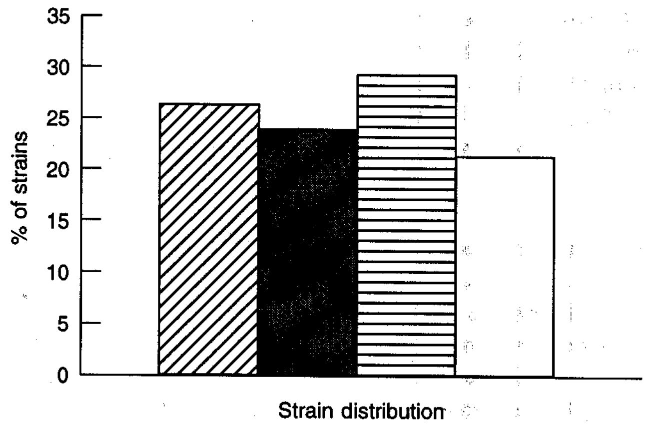
In another greenhouse experiment, strains were evaluated in pots containing virgin Cerrado soil limed to pH 5.4, incubated with rice husk to immobilize soil nitrogen and fertilized with 2 g of simple super phosphate, 166 mg KCl and 20 mg of FTE BR12 per kg of soil. Pre-germinated seeds of *A. pintoi* BRA 031143, considered the most efficient nitrogen fixing ecotype (Purcino, 1997), were planted. Treatments consisted of inoculation with selected native and commercial strains, and controls in which the plants were grown in the absence and presence of NH_4NO_3 (300 mg/kg soil). The entire experiment was carried out as a completly randomized block design, with treatments in triplicate. Ninety days after planting, the plants were harvested and analyzed for nodule weight, shoot dry mass production and nitrogen level using the Kjedahhl's method (Tedesco, 1978).

Results and discussion

The presence and quality of native rhizobial populations are basic parameters for the development of ecological and inoculation viability studies. Rhizobial numbers vary enormously depending on soil type, environment and type of soil cultivation (Hisch, 1996). Variations in the average native rhizobial populations associated with *A. pintoi* were in the three sample sites used in this study. For the virgin Cerrado soil, the four samples collected during the summer months contained numbers that varied from 1 x 10 to 1.7 x 10^2/g soil. For the soil that had been previously cultivated with other leguminous forage or with *A. pintoi*, a variation between 1.7 x 10^2 to 3.4 x 10^3/g soil was observed. These levels are compatible with other results reported for tropical soils (Esiobu, 1994; Thies et al., 1991) but were lower than results obtained for Cerrado soils which may reach 10^6 cells/g soil (Vargas et al., 1994). Our data confirm previous results indicating that the presence and history of legume cover are the two most influential factors affecting rhizobial number in soil (Lowendorf, 1980; Wooner et al., 1988).

In Colombia, working with *A. pintoi*, Sylvester-Bradley et al. (1988) found that although this legume is promiscuous with native *Bradyrhizobium* strains, this symbiosis can be ineffective. A similar situation seems to occur in the Cerrado soils used in this study.

Date et al. (1993) have proposed en equation (100 x inoculated plant dry mass/N fertilized plant dry mass) and the following criteria to quantify nitrogen fixing effectiveness: values < 35% for ineffective strains, 35%-50% for low effectiveness, 50%-80% for effective strains and > 80% for high effectiveness. According to these criteria, the effectiveness of the rhizobia strains tested in the Leonard jar experiment varied significantly (Figure 1). Twenty six percent of the native strains were ineffective, 24% had low effectiveness, 29% were effective and 21% were highly effective (Figure 1). The high incidence of strains with low nitrogen fixing capacity was also apparent in plants inoculated with an extract of a Cerrado soil. Dry mass production of these plants was very low, with values below 50% of the nitrogen fertilized controls. On the other hand, however, native strains with high nitrogen fixing capacity were also found, and some of them produced more plant growth than nitrogen fertilized plants. In this category,



Strains:

- < 35%: MGAC13, MGAC15, MGAC20, MGAC22, MGAC24, MGAC27, MGAV02, MGAV19, MGIIV2, MGIIV5
- 35% - 50%: MGAC04, MGAC05, MGAC30, MGAV10, ALS35, MGAC06, MGAV08, MGAV03, MGAV14
- 50% - 80%: MGAC01, MGAC04, MGAC18, MGAP06, MGAP14, MGAP16, MGACCV22, CPAC01, CPAC08, ALS20, ALS24
- > 80%: MGAC02, MGAC03, MGAP11, MGAP12, MGAP13, ALS23, ALS25, MGAP08

Figure 1. *N fixation efficiency evaluated under axenic conditions of native Cerrado soil rhizobia strains isolated from* Arachis pintoi *according to Date's et al. (1993) equation (100 x inoculated plant mass/N fertilized plant dry mass).*

the most promising strains were the native MGAP 13, MGAP 12, MGAP 14, CPAC 08 and MGAC 03. Under axenic conditions, commercial strains CIAT 2139 and CIAT 3101 confirmed their high effectiveness, CIAT 3806 had low effectiveness and BR 1405 was ineffective.

Some strains tested in axenic conditions were also evaluated in pots containing Cerrado soil. Results obtained are presented in Table 2. In general they presented a tendency similar to that encountered in axenic conditions, but except for strains MGAP 13, CIAT 3101, MGAP 12 and CPAC 08, produced less dry mass than nitrogen fertilized plants. The nodules weight and N/plant also had significant differences among the strains and in general, had a positive relationship with DM plant mass. The very low dry mass production and N/plant of the noninoculated plants which received no supplemental mineral N confirmed the low effectiveness of most native strains, as previously observed under axenic conditions. Nonetheless, three native (MGAP 13, MGAP 12, CPAC 08) and one commercial strain (CIAT 3101) produced dry mass and N level comparable to N fertilized plants.

When tested in soils, some strains effective under axenic conditions showed a reduction in biological N_2 fixation capacity, probably because of competition with

Table 2. **Dry mass, % of dry mass in relation to N-fertilized, nodule weight and N/plant of rhizobia strains isolated from *Arachis pintoi* after 90 days of plant growth in Cerrado soil.**

Rhizobia strains	Plant weight mass (g/plant)	% (plant weight mass) in relation to N fertilizer	Nodules weight (g/plant)	N/plant (g/kg)
MGAP 13	2.578 ab*	85	0.1393 a	2.68 ab
CIAT 3101	2.573 ab	85	0.1163 ab	2.65 ab
CPAC 08	2.350 abc	77	0.0839 abc	2.75 ab
MGAP 12	2.348 abc	77	0.0697 de	2.85 ab
MGAP 06	1.930 bcd	63	0.0800 cd	2.62 bc
MGAC 03	1.920 bcd	63	0.0766 cd	2.75 ab
CIAT mixed strains	1.680 bcd	55	0.0575 fgh	2.66 b
CIAT 2138	1.625 bcd	53	0.0833 c	1.98 e
CIAT 3806	1.577 bcd	52	0.0693 de	2.33 cd
MGAP 14	1.490 cd	48	0.0523 fghij	2.30 d
Mixed Cerrado native strains	1.467 cd	48	0.0475 hij	2.65 b
MGAC 02	1.382 cd	45	0.0458 ij	2.32 b
CPAC 01	1.380 cd	45	0.0476 hij	1.79 efg
MGAP 11	1.372 cd	43	0.0550 fghi	1.98 e
ALS 25	1.320 cd	43	0.0592 fgi	1.50 gh
MGAP 08	1.280 d	42	0.0416 ghij	1.58 fgh
MGAP 16	1.188 d	39	0.0516 fghij	1.62 fgh
ALS 23	1.138 d	37	0.0442 ij	1.85 ef
N-fertilized	3.043 a	100	0.0619 ef	2.99 a
Noninoculated	1.077 d	35	0.0432 j	1.47 h

* Means followed by the same letter in each column did not differ significantly (Tukey 5%).

the soil's native population. A similar situation was also observed in the treatment where a mix of effective strains were used as inoculum, producing plants with less dry mass when compared with plants inoculated with a single effective strain.

The presence of low effectiveness rhizobia strains associated with *A. pintoi* in Cerrado soils illustrates the necessity to select strains with higher efficiency and competitiveness for inoculation to increase to productivity of this legume.

Resumen

En el ecosistema Cerrado de Brasil se evaluó la población nativa de *Bradyrhizobium* con potencial para hacer simbiosis con *Arachis pintoi.* Las muestras de suelo se tomaron en la época de transición seca-lluvias a 20 cm de profundidad en suelos vírgenes, cultivados con diferentes especies de leguminosas y con *A. pintoi.* De cada una de estas muestras se preparon diluciones de 10^{-1} a 10^{-9} que se aplicaron a suelos en potes que contenían ecotipos pregerminados de *A. pintoi* BRA031143. En el suelo virgen, en la época seca el número de rizobios varió desde 1 x 10 hasta 1.7 x 10^2/g de suelo. En suelos cultivados con leguminosas, incluyendo *A. pintoi,* este valor fue 1.7 x 10^2 hasta 3.4 x 10^3/g de suelo. Se encontraron cepas nativas de rizobio con alta capacidad para fijar N y con algunas de ellas *A. pintoi* produjo más MS que con la aplicación de 250 g/pote de N. En este estudio, las razas nativas más promisorias fueron MGAP 13, MGAP 12, MGAP 14, CPAC 08 y MGAC 03. Igualmente fueron efectivas las razas de rizobio CIAT 2139 y CIAT 3101.

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