Artículo Científico

Response of *Arachis pintoi* to inoculation with selected rhizobia strains in Brazilian Cerrado soils under field conditions[1]

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Introduction

The Cerrado soils in Brazil are often low in available nitrogen. A multiple purpose leguminous plant species adapted to these soils may have an important impact on pasture and crop productions by increasing nitrogen availability in this ecosystem through biological nitrogen fixation.

Among the leguminous plants evaluated for its utilization in the Cerrado region is *Arachis pintoi,* known as tropical white clover or forage peanut and considered a multiple purpose legume (Thomas, 1993; Hard, 1995) that can be utilized as forage associated with grasses, as soil cover in perennial crops and as green manure.

The genus *Arachis* is native to South America (Gregory et al., 1973). It ranges, geographically, from the Equator to 34° S and from the Atlantic coast to the eastern foothills of the Andes including semi-arid regions to areas that receive 2000 mm or more of rain annually (Elkan et al., 1981).

Arachis pintoi is a promiscuous species nodulating with native rhizobia strains with abundant and apparently active nodules, although, these symbioses may be ineffective. (Pinto et al., 1999; Oliveira et al., 1996; Silvester-Bradley et al., 1988). It has been observed that *A. pintoi* inoculated with selected strains has increased shoot weight and shoot nitrogen content when compared to uninoculated control (Purcino et al., 2000; Pinto et al., 1999; Silvester-Bradley et al., 1988). Strain BR 1405 recommended for *A. hypogea* was not effective for *A. pintoi* and strain CIAT 3101 recommended for *A. pintoi* inoculation by Centro Internacional de Agricultura Tropical (CIAT), Colombia, was not effective in Cerrado soils (Purcino et al., 2000; Pinto et al., 1999). Since an increase in the *A. pintoi* productivity and shoot quality will contribute to enhance its utilization in the Cerrado region, the objective of this work was to evaluate and select under field conditions, strains of rhizobia with high nitrogen fixing efficiency and adapted to these soils.

Materials and methods

Two field experiments were carried out in 1998. Experiment one was located at 19º 28' S and 45º 15' W, 732 m above sea level at EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais), Sete Lagoas, Minas Gerais State, Brazil; whereas experiment two was conducted at Embrapa Cerrados, Brasilia, DF, Brazil, placed at 15º 35º S and 47º 42' W, 1000 m above sea level. Average temperature is 21.9° C on both locations and average annual rainfall are 1350 and 1500 mm in the first and second location, respectively.

The soil physical analyzes of experimental areas showed that both soils were classified as clay soils and chemical analyzes results are presented on Table 1. The soil at EPAMIG was degraded and had been cultivated with corn for 10 years. At planting, this area was limed to elevate base saturation up to 40%. The Embrapa Cerrados experimental area had been previously cultivated with several leguminous plants. Before planting, both areas were fertilized with 50 kg/ha of a micronutrient mix FTE BR-12.

The experiments were carried out as a complete randomized block design with seven treatments (Table 2) replicated four times. Plots were 3 m long and consisted of four rows planted 0.50 m apart, with 10 seeds/m of *A. pintoi* ecotype BRA 031143. The

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Table 1. **Soil chemical analyzes of experimental areas.**

Location	pH	H + Al	Al	Ca	Mg	K	P	O.M.	Sat. Al.
		(meq/100 cc)				(ppm)		(ppm)	
EPAMIG	4.8	6.79	0.90	0.88	0.11	117	5	2.28	41
Embrapa-CPAC	5.5	5.30	0.14	2.10	0.40	80	11	3.00	5

Bradyrhizobium strains used in this study had been previously selected as effective for *A. pintoi* in greenhouse experiments (Purcino et al., 2000; Pinto et al., 1999) and their origin is described in Table 2.

Before planting, seeds of *A. pintoi* ecotype BRA 031143 were surface sterilized by soaking in a solution of 50% distilled water and 50% NaOCl at 2% of concentration for 8 min. Then, the seeds were rinsed eight times, dried at room temperature and inoculated with selected *Rhizobia* strains (Purcino et al., 2000; Pinto et al., 1999). The inoculants were prepared from pure culture of each strain, grown in yeast mannitol broth. Broth cultures were applied to sterilized peat (whose pH had been previously raised to 6.5 with $CaCO_3$) to reach about 50% moisture. The mixtures were allowed to mature at room temperature for 30 days. Plate counts showed 1.5 x 10^9 cells/g of peat and MPN counts 5 x10^8 cells/g. Immediately before planting, the seeds were inoculated by preparing a peat slurry with a 25% sucrose sticker solution, at a rate of 1 kg of inoculant per 40 kg of seeds.

The plants in these experiments were clipped twice. The first cut occurred 115 days after germination (DAG) and the second, 9 months after the first one. Shoot dry matter productions were determined after drying in a forced air oven at 65° C until stable weight and shoot N content by Kjeldahl´s method (Tedesco, 1978). Total N in the shoot was determined by the equation shoot dry matter x shoot N content. Data were submitted to analyses of variance and means were ranked according to the Duncan's test at 0.05% level.

Results and discussion

At EPAMIG, the maximum and minimum average temperature were 30° C and 18° C, respectively, and rainfall was 923 mm, about 29% lower than the average of the last 20 years and it negatively influenced *A. pintoi* productions. In Brasilia, the maximum and minimum average temperature were 27.6° C and 16.5° C, respectively, and the average annual rainfall was 1269 mm, about 10% lower than the average of the last 28 years.

Although *A. pintoi* nodulates with native *Rhizobia* strains, seed inoculation with selected strains can enhance plant dry matter and nitrogen content in shoot in greenhouse experiments (Pinto et al., 1999; Purcino et al., 2000; Sylvester-Bradley et al., 1988). Similar results were found for this legume plant under Cerrado field conditions (Tables 3 and 4).

The effect of inoculation on shoot dry matter production, shoot N content and total shoot nitrogen for *A. pintoi* at the EPAMIG experimental farm in the first cut, 115 DAG, is presented on Table 3. Seed inoculation with strains MGAP13, NC230 and NC70 increased dry matter production by 62%, 47% and 26%, and total nitrogen by 62%, 61% and 38%, respectively, in relation to control treatments. In the second cut no significant differences among

Table 2. **Treatments utilized and the origin of the strains.**

Treatments	Origins
T 1 Control	Seeds without inoculation and no N fertilizer added to soil.
T 2 Nitrogen	N fertilizer added to soil (urea) equivalent to 60kg/ha divided in three applications.
T 3 Strain MGAP 13	Native to the Cerrado area of EPAMIG Experimental Farm and isolated from *A. pintoi* plant-nodule by Universidade Federal de Minas Gerais, Brazil.
T 4 Strain CAT 3101	Isolated from Centrosema plant-nodule by CIAT, Colombia, and recommended as inoculant for *A. pintoi*.
T 5 Strain NC 70	All NC strains were collected in the centre of diversity of the genera *Arachis* in South America and belong to the Soil Microbiology Laboratory Collection of North Carolina State University, EUA.
T 6 Strain NC 229	—
T 7 Strain NC 230	—

Table 3. **Effect of inoculation on shoot dry matter production, shoot N percent and total shoot N production of *Arachis pintoi* in a Cerrado area. 1st cutting. EPAMIG, Brazil. 1999.**

Treatments	Dry matter		N on shoot	N total on shoot	
	Kg/ha	% in relation to control	(%)	Kg/ha	% in relation to control
Control	6492 c*	—	2.58	167 c	—
Nitrogen	6497 c	0.7	2.76	179 bc	7
Strain MGAP13	10560 a	63	2.56	270 a	62
Strain CIAT3101	6368 c	-2	2.74	174 bc	4
NC 70	8172 ab	26	2.82	230 ab	38
NC 229	5413 c	-17	2.63	142 c	-15
NC 230	9523 ab	47	2.84	269 a	61
CV (%)	20.5	—	8.50	15.3	—

* Values with similar letters in the same column do not differ significantly by Duncan's test ($P < 0.05$).

Table 4. **Effect of inoculation on shoot dry matter production, shoot N content and total shoot N production of *Arachis pintoi* in a Cerrado area. 2 nd cutting. Embrapa/CPAC. Brazil, 2000.**

Treatments	Dry matter production	N content on shoot	Total N on shoot	
	(kg/ha)	(%)	(kg/ha)	% in relation to control
Control	7202	1.74	125 b*	-
Nitrogen	7574	1.86	141 b	13
Strain MGAP13	9576	1.91	183 a	46
Strain CIAT3101	8202	1.70	139 b	11
NC 70	9030	1.82	164 ab	31
NC 229	7392	1.82	132 b	6
NC 230	8529	1.75	150 ab	20
CV (%)	15.7	75	16.5	-

* Values with similar letters in the same column do not differ significantly by Duncan's test ($P < 0.05$).

treatments were observed. These results indicate that inoculation is important for the establishment of *A. pintoi* in degraded soils such as at the EPAMIG experimental site.

At Embrapa Cerrados experimental farm, results from the statistical analyzes of shoot dry matter production showed that, neither inoculation with distinct rizobia strains nor applied N fertilizer promoted increases in this parameter. This may have been due to the high soil N content provided by previous cultivations with legumes and also from organic matter mineralization. On the other hand, however, in the second cut, inoculation with strain MGAP 13 increased total shoot N by 46%, in relation to the control treatment. Also, the strains NC 70 and NC 230 tended to produce plants with increased total shoot N content. On both areas, based on the results of the control treatment, the native strains nitrogen fixing capacity was equivalent to 60 kg/ha N fertilizer.

According to Date (1993) the effectiveness of rhizobial strains in terms of nitrogen fixing capacity can be determined by the equation:

$$\frac{100 \times \text{inoculated plant dry mass}}{\text{N fertilizer plant dry mass}}$$

where, values < 35% indicate ineffective strains; 35-50% low effectiveness, 50-80 effective strains and, > 80% high effectiveness.

Results from this equation showed that strains MGAP13 and NC230, utilized for *A. pintoi* seed inoculation, can be considered effective and lowly effective strains, respectively, under poor soil conditions in the Cerrado region. The inoculation with strains MGAP13 and NC230 had increased *A pintoi* dry matter productions by 62% and 47%, respectively, in relation to nitrogen fertilized plants.

Conclusion

In a degraded Cerrado soil, inoculation of *Arachis pintoi* seeds with strains MGAP 13 and NC 230 increased dry matter productions by 62% and 47%, and shoot N content by 62% and 61%, respectively, in the first cut 115 DAG, showing that inoculation has a positive effect on the establishment of this legume under poor soil conditions.

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Resumen

En condiciones de campo en la región Cerrados, Brasil, se evaluó la respuesta de *Arachis pintoi*, una leguminosa multipropósito nativa de América del Sur, a la inoculación con cepas de *Bradyrhizobium*. En el corte 115 días después de la siembra, la inoculación con *Bradyrhizobium* MGAP13, NC230 y NC70 aumentó la producción de MS de la leguminosa en 62%, 47% y 26%, y el nitrógeno total de la parte aérea en 62%, 61% y 38 %, respectivamente, en comparación con el tratamiento control. *Bradyrhizobium* BR1405, recomendada para *A. Hipogea*, y *Bradyrhizobium* CIAT3101, recomendada para *A. pintoi,* no fueron efectivas para esta leguminosa.

Summary

The response of *Arachis pintoi*, a multipurpose legume native of South America, to inoculation with *Bradyrhizobium* strains was evaluated under field conditions in the Cerrados region of Brazil. In the cutting performed 115 days after planting, inoculation with *Bradyrhizobium* MGAP13, NC230, and NC70 had increased DM production of the legume by 62%, 47% and 26%, and total shoot nitrogen by 62%, 61% and 38%, respectively, compared with the check treatment. *Bradyrhizobium* BR1405, recommended for *A. hipogea*, and *Bradyrhizobium* CIAT3101, recommended for *A. pintoi*, proved ineffective for this legume.

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