Rhizobium strains competitiveness on bean nodulation in Cerrado soils

Ruy Raposeiras[(1)], Ivanildo Evódio Marriel[(2)], Maria Rita Scotti Muzzi[(1)], Edilson Paiva[(2)], Israel Alexandre Pereira Filho[(2)], Lilia Costa Carvalhais[(1)], Raul Vinícius Magalhães Passos[(1)], Patrícia Pereira Pinto[(1)] and Nadja Maria Horta de Sá[(1)]

[(1)]Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Dep. de Botânica, Pampulha, Caixa Postal 486, CEP 31270-901 Belo Horizonte, MG, Brazil. E-mail: rraposeiras@yahoo.com.br, mrita@icb.ufmg.br, liliacarvalhais@hotmail.com, raulvinicius@ig.com.br, nadja@icb.ufmg.br [(2)]Embrapa Milho e Sorgo, Caixa Postal 151, CEP 35701-970 Sete Lagoas, MG, Brazil. E-mail: imarriel@cnpms.embrapa.br, edilson@cnpms.embrapa.br, Israel@cnpms.embrapa.br

Abstract – The objective of this work was to identify the most competitive and effective *Rhizobium* strains in order to increase common bean yield by nitrogen fixation as alternative or complementation to the nitrogen fertilization. Competitiveness tests were lead in axenic conditions, in Cerrado soil pots and in three field experiments, with native *Rhizobium* strains that were previously identified, according to their effectiveness and genetic variability. The identification of strains in nodules was performed using serological tests (axenic conditions) – agglutination and enzyme linked immunosorbent (Elisa) assays – and random amplified polymorfic DNA (RAPD) (Cerrado soil). Plant yield was determined using the dry weight (greenhouse conditions), total N and grain yield (field experiments). Among the analyzed *Rhizobium* strains, native strain SLA 2.2 and commercial strain CIAT 899 were the dominant nodules in plants of the most productive plots, presenting yield productivity similar or higher to those obtained in treatments where 20 kg ha^{-1} of N were applied.

Index terms: *Phaseolus vulgaris*, nodule occupancy, Elisa, RAPD.

Capacidade competitiva de estirpes de *Rhizobium* na nodulação do feijoeiro em solos de Cerrado

Resumo – O objetivo deste trabalho foi identificar as estirpes de *Rhizobium* mais efetivas e competitivas, a fim de maximizar a produtividade do feijoeiro por meio da fixação de nitrogênio, como alternativa à adubação nitrogenada. Foram conduzidos testes de competitividade em condições axênicas, em vasos com solo do Cerrado e em três experimentos de campo, com estirpes de *Rhizobium* nativas, previamente selecionadas quanto à efetividade e à variabilidade genética. A identificação das estirpes nos nódulos foi efetuada por meio das técnicas de aglutinação e ensaio imunoabsorvente de ligação de enzimas (Elisa), em condições de casa de vegetação, e pela técnica de DNA polimórfico amplificado ao acaso (RAPD), em solo de Cerrado. A produtividade das plantas foi determinada pela produção de matéria seca, teor de N e produção de grãos (condições de campo). A estirpe nativa SLA 2.2 e a estirpe comercial CIAT 899 foram dominantes nos nódulos das plantas das parcelas mais produtivas, com índices de produtividade iguais ou superiores aos obtidos nos tratamentos em que foram aplicados 20 kg ha^{-1} de N.

Termos para indexação: *Phaseolus vulgaris*, ocupação nodular, Elisa, RAPD.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most traditional crops in Brazil and very expressive in "Cerrados", an edaphic type of savanna in the central region of the country. However, yield is very low, due to poor cropping practices, such as an inefficient supply of fertilizer, especially nitrogen (N). Biological nitrogen fixation (BNF) by rhizobia can supply partly or all N required by plant legumes. Bean plants are considered very promiscuous, nodulating with various fast growing *Rhizobium* such as *R. leguminosarum* bv. *phaseoli* (Jordan, 1984), *R. tropici* (Martinez-Romero et al., l991), *R. etli* (Segovia et al., 1993), *R. gallicum* and *R. giardini* (Amarger et al., 1997).

Tropical soils present a high number of these rhizobial strains, but they are considered of low efficiency (Pereira et al., 1991). On the other side, this population

is very competitive, jeopardizing inoculation responses of bean with effective *Rhizobium*. Competitiveness in rhizobia refers to the relative ability of a given strain to infect a legume and cause nodule formation in the presence of other strains. There is competition at different levels: in rhizosphere, for survival and multiplication (Chatel et al., 1968); for accessible infection sites on roots and during penetration through the roots and nodule development, a stage presumably including several phases (Sessitsch et al., 2002).

In order to increase bean yield by N_2 fixation, as alternative for nitrogenous fertilizers, it is necessary to identify the most competitive and effective strains, which requires the determination of the nodule occupancy using a suitable marker. Different methods were employed in the identification of rhizobia isolated from nodules, especially in plants cultivated in field, including antibiotic resistance (Somasegaran & Hoben, 1985), agglutination immunology (Vincent, 1970), immunodiffusion (Somasegaran & Hoben, 1985), immunoblot assay (Khan et al., 1999), enzyme linked immunosorbent assay (Elisa) and others based on DNA analysis (Sá et al., 1997; Handley et al., 1998).

The objective of this work was to evaluate competitiveness of efficient bean nodulating strains of *Rhizobium* isolated from Cerrado soils employing agglutination, Elisa and RAPD (Random amplified polymorphic DNA) methods of identification.

Material and Methods

Rhizobium strains and bean plants

The strains of *Rhizobium tropici* (SLBR 3.12, SLBR 2.1, FJ 2.12, SLP 3.3, SLP 4.9, SLA 2.2, SLA 3.2, CPAC H_{2O} and CPAC H_{41}), *R. leguminosarum* bv. *phaseoli* (SLP 4.7), previously selected by effectiveness (Pinto et al., 1995), and commercial strains (CIAT 899 and PRF 81) were used. *Phaseolus vulgaris* cv. Carioca and cv. Diamante Negro were used as host plants. Rhizobial cells were grown in liquid manitol medium yeast extract (YM) at 29°C, in shaker, for 48 hours and maintained in the same medium containing agar (YMA) at 4ºC (Vincent, 1970). The number of cells was determined by measuring the optical density at 625 nm and verified by viable cell count after serial dilution on YMA.

Nodule occupancy and N_2 fixation under greenhouse conditions

Seeds of *Phaseolus vulgaris* (cv. Carioca) were surface-sterilized ($HgCl_2$, 0.1%) and aseptically germinated before planting in Leonard jars filled with sterilized sand and vermiculite (1:2, v/v) (Vincent, 1970). Pairs of strains in a 1:1 ratio were used as double inoculants or either single strain. The inoculum consisted of 1 mL from each strain (10^8 viable cells mL^{-1}), standarded at 625 nm. Plants were watered as needed with a N-free plant nutrient solution. Controls included non-inoculated plants, without or with mineral N supplied as KNO_3 (30 mg of N $plant^{-1}$ $week^{-1}$). After 30 days, plants were harvested and nodule number and plant shoot dry weight were evaluated. Three experiments were accomplished under controlled greenhouse conditions. The nodule occupancy was determined by agglutination and Elisa assays as described below.

The best *Rhizobium* strains selected in vitro were used to inoculate bean seeds as above (cv. Carioca and cv. Negro Argel) planted in pots filled with Cerrado soil where different plants, including bean, have been cultivated for the last ten years. Soil with clay texture presented pH 5.8; H+Al, 5.0 eqm 100 cm^{-3}; Ca, 3.4 eqm 100 cm^{-3}; Mg, 0.79 eqm 100 cm^{-3}; K, 259 ppm; P, 16 ppm; organic matter, 3.35%. The rhizobial population in this soil was around 10^3 to 10^4 cells g^{-1} of soil, estimated according to Vincent (1970). Controls included non-inoculated plants, with or without mineral N supplied by NH_4SO_4 (three applications in total of 100 ppm of N). After 40 days, plants were harvested and nodules number, plant shoot dry weight and plant total N were evaluated as described by Tedesco (1978). The nodule occupancy was determined by agglutination, Elisa and RAPD assays as described. These experiments were performed in a randomized block design, with five replicates, and the data were submitted to analysis of variance.

Field experiment

Three experiments were performed in two sites. Site A was cultivated only with maize and sorghum in the last ten years. The soil presented: pH 6.3; H+Al, 2.25 eqm 100 cm^{-3}; Ca, 5.08 eqm 100 cm^{-3}; Mg, 0.71 eqm 100 cm^{-3}; K, 5.5 ppm; P, 5 ppm; organic matter, 2.3%; and no rhizobial population. Site B, where different plants, including bean, were cultivated in the last ten years, presented the same characteristics described before in pot culture.

Rhizobium strains selected in pot culture, SLA 2.2, CIAT 899 and PRF 81 from Iapar and Embrapa Cerrados were used as inoculants. The peat-based inoculants were prepared by Embrapa Cerrados at a concentration of 10^8 viable cells g^{-1} of peat. The cv. Carioca was inoculated with pairs of strains at a 1:1 ratio as double inoculants or either single-strain. The experiments were performed in a randomized block design with five replicates, including plots that were not inoculated, with or without mineral N. In site A, in the first experiment, 20 kg ha^{-1} of N were applied and, in the second, 20, 40 and 60 kg ha^{-1} of N. In site B, 20, 40 and 60 kg ha^{-1} of N were used. Yield was evaluated 45 days after planting by shoot dry weight, plant total N (Tedesco, 1978) and yielded grains. One sample of nodule per treatment was collected and maintained in silica for strains identification by RAPD. The data were subjected to analysis of variance.

Rhizobium strains identification in competition assays

Agglutination and Elisa assays were used for evaluating the specificity of antibodies. The agglutination reactions were accomplished in glass plates using 10 to 20 mL of antigen (pure culture of each strain of nodule exsudate) for 10 µL of antibody per reaction. The Elisa assays were performed in polystyrene plates according to Ahmad et al. (1981), using an Elisa plates' reader (Universal Microplate Reader, ELX 800), at 405 nm. For antibody preparation, pure cultures of the *Rhizobium* strains were grown in defined medium (Somasegaran & Hoben, 1985) for four days at 24ºC. The cells were recovered by centrifugation, washed in phosphate buffered saline (PBS) and disrupted by ultra-sonication (sonifier Branson 450, output 3, out cycle 75%) with two cycles of 30 seconds and one-minute interval. The rabbits immunization process was accomplished according to Somasegaran & Hoben (1985). Titers for the antisera were equal or higher than 1:800 and for the analysis, a dilution to 1:30 (v/v), with 0.85% of saline solution was performed. Controls included the strains isolated without antiserum.

Bacterial genomic DNA isolated from nodules from field and greenhouse experiments was extracted using the method described in Sá et al. (1997). Amplification reactions were performed with a Perkin-Elmer 9600 hermocycler, in which reactions consisted of 40 cycles, each cycle including the following steps: denaturation at 94ºC, for 15 seconds, annealing at 35ºC for 30 seconds and polymerization at 72ºC for one minute. An additional cycle for extension was conduced at 72ºC for 7 minutes. Each reaction mixture of 25 µL was composed of 2.5 mM of $MgCl_2$, 10 mM of Tris-HCl, 0.01 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 1U of Taq DNA polymerase, 0.4 µM of primer, 11 µL of H_2O; and 25 ng of DNA. The following five primers from Operon kit (Operon Technologies Inc.) were used: OPACO4 (ACGGGGACCTG), OPACO3 (CACTGGCCCA), OPAE10 (AGCAGCGAGG), OPAA10 (TGGTCGGGTG) and OPAE10 (CTAAGCGCA). After the reaction, the products were electrophoretically separated on 5% polyacrylamide gels. The gels were silver stained (Santos et al., 1993) and photographed. For strain identification, the fingerprint of each original strain was compared with the fingerprinting of the isolated ones for each primer tested. The strains that showed likelihood ≥70% of polymorphic products were considered similar to the original strain.

Results and Discussion

Total numbers of nodules, nodule occupancy data (by agglutination and Elisa assays) and dry matter of plants inoculated with *Rhizobium* strains are shown in Table 1. Three experiments were accomplished under controlled greenhouse conditions. In the first one, CIAT 899, SLP 4.7, SLP 3.3 and SLBR 3.12 strains were tested. Among these strains, CIAT 899 and SLP 4.7 were the most competitive, varying from 80 to 98% of nodule occupancy index in agglutination and Elisa tests. The SLBR 3.12 was the least competitive strain. A high level of cross reactions was observed.

In the second experiment, which included CIAT 899, FJ 2.12, SLA 2.2, SLAP 4.9, SLA 3.2 and SLBR 2.1, the most competitive strains were SLA 2.2, followed by SLA 3.2. The values of nodule occupancy ranged between 60 to 100% (SLA 2.2) and 30 to 95% (SLA 3.2). The most competitive strain, CIAT 899, in the first experiment, kept its higher competitiveness in relation to the other strains (FJ 2.12, SLP 4.9 and SLBR 2.1). The competition between FJ 2.12 and SLBR 2.1 showed high numbers of nodules without reaction and with cross reaction (80%).

In the last experiment, CPAC H_{20} was the most competitive, occupying 60% or more of the nodules, in both techniques. In score of higher percentage of nodule occupation, the CPAC H_{20} was followed by SLA 2.2 and CIAT 899. The CPAC H_{41} strain was the least competitive in this group. The most competitive strains

CPAC H_{20}, SLA 2.2 and CIAT 899 confirmed the high effectiveness when alone or when co-inoculated with other strains. The SLA 3.2 strain, despite being very competitive, showed little effectiveness in beans with low dry matter production. Although the SLP 3.3 presented low competitiveness, it was the most effective strain when inoculated alone. The nodule number parameter in experiments 1 and 3 did not show statistical differences (Duncan, 5%). However, a higher number of nodules does not necessarily correspond to a higher yield of plant dry matter, as demonstrated by SLBR 2.1 alone or in co-inoculation with CIAT 899 strain. This fact indicates that the presence of great number of nodules not always is guarantee of effectiveness of an inoculum (Provorov et al., 1994) and that the compatibility between strains in mix inoculants is an important factor to be considered (Brown & Ahmad, 1996).

In a pot culture, the nodule occupancy data, obtained by RAPD analysis (Figures 1 and 2), using specially OPAA10 and OPACO4 primers, showed that SLA 2.2

Table 1. Number of nodules, *Rhizobium* strains nodule occupancy by agglutination (A) and Elisa (E) assays and dry weight (g plant^{-1}) of bean plants under axenic greenhouse conditions, in three experiments, each consisting of strains: CIAT 899, SLP 4.7, SLP 3.3, SLBR 3.12, FJ 2.12, SLA 2.2, SLP 4.9, SLA 3.2, SLBR 2.1, CPAC H_{41} e CPAC H_{20}[(1)].

Treatment	Nodules per plant	No. of nodules analyzed (A/E)	Nodules (%) reacting as inoculant strain (s)												Plant dry matter
			CIAT 899 (A/E)	SLP 4.7 (A/E)	SLP 3.3 (A/E)	SLBR 3.12 (A/E)	FJ 2.12 (A/E)	SLA 2.2 (A/E)	SLP 4.9 (A/E)	SLA 3.2 (A/E)	SLBR 2.1 (A/E)	CPAC H_{41} (A/E)	CPAC H_{20} (A/E)	No. or cross reaction (A/E)	
Experiment 1															
CIAT 899	119^{ns}	30/20	87/100											13/0	0.434ab
SLP 4.7	78^{ns}	60/20		95/100										5/0	0.377 bc
SLP 3.3	143^{ns}	40/20			93/100									7/0	0.543a
SLBR 3.12	131^{ns}	40/20				70/100								30/0	0.453ab
CIAT 899 x SLP 4.7	72^{ns}	30/20	83/45	80/25										17/30	0.264c
CIAT 899 x SLP 3.3	87^{ns}	40/20	93/60		0/30									7/10	0.315bc
CIAT 899 x SLBR 3.12	112^{ns}	20/20	20/67			10/25								70/8	0.285c
SLP 4.7 x SLBR 3.12	90^{ns}	40/20		98/80		0/15								2/5	0.328bc
SLP 4.7 x SLP 3.3	82^{ns}	40/20		98/70	30/20									2/10	0.263c
SLP 3.3 x SLBR 3.12	91^{ns}	40/20			73/70	23/20								4/10	0.326bc
Control with N	-	-												-	0.450ab
Experiment 2															
CIAT 899	51abc	20/20	90/100											10/0	0.340a
FJ 2.12	36abd	20/20					85/100							15/0	0.231abd
SLA 2.2	76a	20/20						100/100						0/0	0.447a
SLP 4.9	53abc	20/20							70/100					30/0	0.311ab
SLA 3.2	38abd	20/20								100/100				0/0	0.209bd
SLBR 2.1	72a	20/20									100/100			0/0	0.222abd
CIAT 899 x FJ 2.12	50abc	20/22	100/86				0/0							0/14	0.336a
CIAT 899 x SLA 2.2	45abd	20/22	10/08					90/62						0/30	0.249abcd
CIAT 899 x SLP 4.9	43abd	20/22	100/80						0/0					0/20	0.247abcd
CIAT 899 x SLBR 2.1	61ab	20/22	50/60								0/30			50/10	0.271abc
CIAT 899 x SLA 3.2	29bd	20/22	-/10							-/60				-/30	0.282abc
FJ 2.12 x SLA 2.2	43abd	2022					0/09	100/55						0/36	0.247abcd
FJ 2.12 x SLP 4.9	42abd	20/22					80/80		10/10					10/10	0.304ab
FJ 2.12 x SLA 3.2	35abd	20/22					0/0			60/100				40/0	0.169d
FJ 2.12 x SLBR 2.1	37abd	20/22					20/20				0/0			80/80	0.275abc
SLA 2.2 x SLP 4.9	64ab	20/22						85/92	5/4					10/4	0.303ab
SLA 2.2 x SLA 3.2	57abc	20/22						60/70		30/30				10/0	0.250abcd
SLA 2.2 x SLBR 2.1	54abc	20/22						80/80			0/0			20/20	0.249abcd
SLP 4.9 x SLA 3.2	39abd	20/22							0/18	90/64				10/18	0.278abc
SLP 4.9 x SLBR 2.1	40abd	20/22							50/40		50/60			0/0	0.228abd
SLA 3.2 x SLBR 2.1	23d	20/22								95/55	0/18			5/27	0.224abd
Control with N	-	-												-	0.414a
Experiment 3															
CIAT 899	80^{ns}	15/20	60/100											40/0	0.320bcd
SLA 2.2	70^{ns}	15/20						100/100						0/0	0.339bcd
CPAC H_{41}	82^{ns}	15/20										60/100		40/0	0.241d
CPAC H_{20}	59^{ns}	15/20											100/100	0/0	0.531a
CIAT 899 x CPAC H_{41}	57^{ns}	15/20	50/60									50/40		0/0	0.232d
CIAT 899 x CPAC H_{20}	66^{ns}	15/20	0/30										100/70	0/0	0.354bcd
SLA 2.2 x CPAC H_{41}	57^{ns}	15/20						100/80				0/20		0/0	0.404bc
SLA 2.2xCPAC H_{20}	78^{ns}	15/20						40/40					60/60	0/0	0.251cd
CPAC H_{41} x CPAC H_{20}	52^{ns}	15/20										0/25	100/75	0/0	0.414ab
Control with N	-	-												-	0.353bcd

[(1)]Means followed by the same letter in the column did not differ significantly at 5% of probability by the Duncan test; CV: 46,6 and 30,4% (Experiment 1), 42,3 and 26,6% (Experiment 2), 44,4 and 29,5% (Experiment 3), for nodules per plant and plant dry matter, respectively. [ns]Nonsignificant.

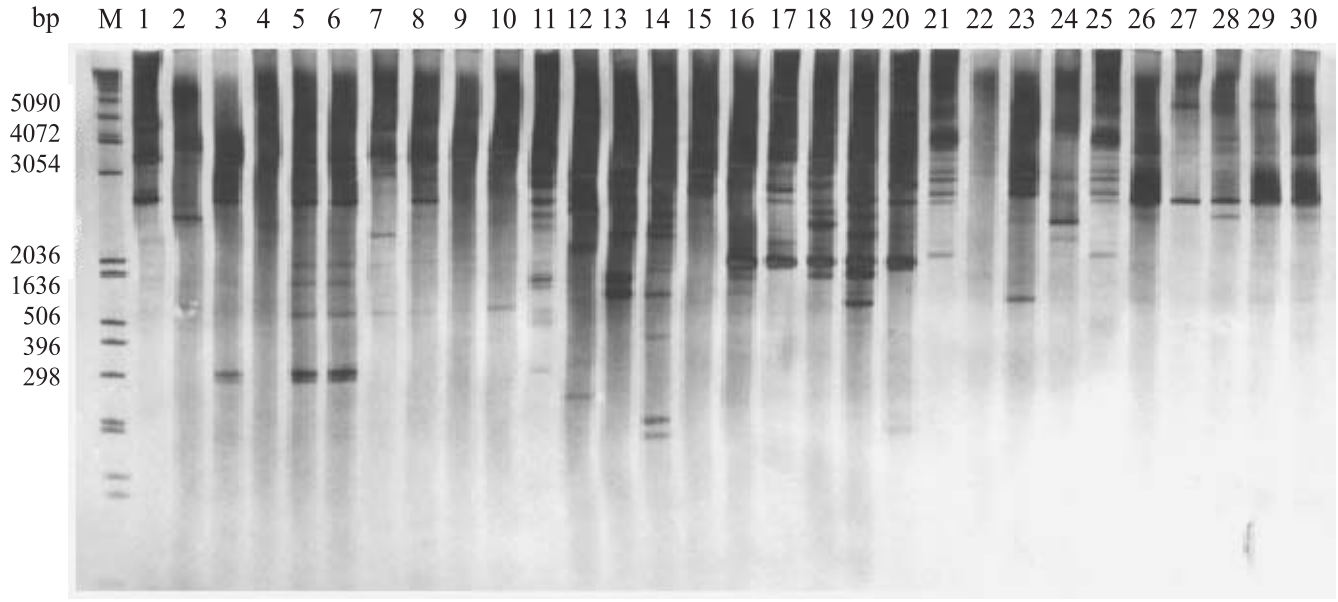

Figure 1. Amplification example of genomic DNA of *Rhizobium* strains from bean nodules (cultivar Diamante Negro) inoculated with SLA 2.2 and CPAC H_{20}. M-DNA marker, SLA 2.2 (1-11-21), CPAC H_{20} (2-12-22), strains from nodules formed by co-inoculation of SLA 2.2 and CPAC H_{20} using primer OPAA10 (3-4-5-6-7-8-9-10), using primer OPAC10 (13-14-15-16-17-18-19-20), and co-inoculation of SLA 2.2 and CPAC H_{20} with primer OPAC04 (23-24-25-26-27-28-29-30).

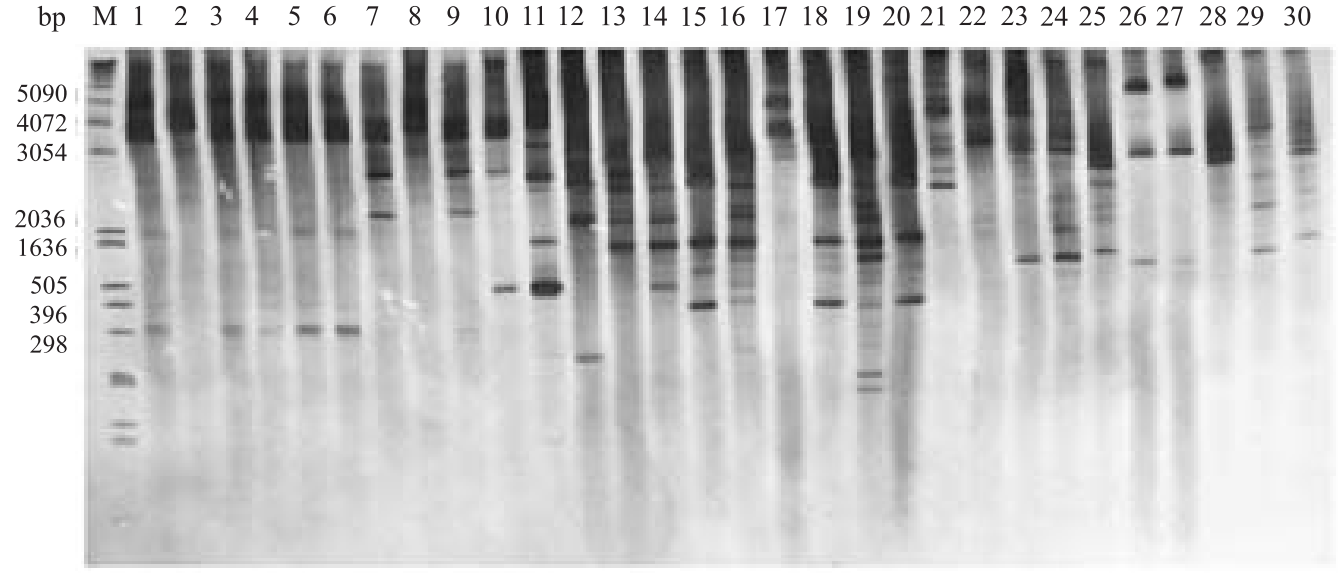

Figure 2. Amplification example of genomic DNA of *Rhizobium* strains from bean nodules (cultivar Carioca) inoculated with CIAT 899 and CPAC H_{20}. M-DNA marker, CIAT 899 (1-11-21), CPAC H_{20} (2-12-22), strains from nodules formed by co-inoculation of CIAT 899 and CPAC H_{20} using primer OPAA10 (3-4-5-6-7-8-9-10), using primer OPAC10 (13-14-15-16-17-18-19-20), and co-inoculation of CIAT 899 and CPAC H_{20} with OPAC04 (23-24-25-26-27-28-29-30).

was the most competitive among the strains in both cultivars, not only when inoculated alone (60 to 75%) but also when co-inoculated with any of the other strains (48 to 87%) (Table 2). Although the CAPC H_{20} was very competitive in axenic conditions, in pot culture it showed less competitiveness. When inoculated alone, only 20–30% of nodule occupancy in cv. Carioca and 30% in cv. Diamante Negro. When co-inoculated with SLA 2.2 or CIAT 899, this strain showed levels of nodule occupancy between 10 and 20% in cv. Carioca and it was not detected in cv. Diamante Negro. These results evidence that the competitiveness of each *Rhizobium* strain depends not only on its genetic intrinsic characteristics for development of this ability but it is also influenced by environmental factors and the genotype of the host legume (Brutti et al., 1999). The CIAT 899 strain was detected in nodules of both cultivars between 25 to 35%, when inoculated alone. When co-inoculated with other strains, the result was variable. It was more competitive in relation to CPAC H_{20} (40–50%) and less competitive than SLA 2.2. As shown in Table 3, there were positive effects in inoculation of both cultivars, estimated by plant dry weight. However, this effect was apparently more accentuated in cv. Carioca, where dry weight production was higher in treatments in which plant growth was symbiosis dependent.

Several studies observed nodule occupancy range with host cultivars (Handley et al., 1998). In beans, Buttery et al. (1997) showed significantly different effects between cultivars and *Rhizobium* strains in plant growth, nitrogen content and seed production. Other studies also reported the ability of a cultivar to select a particular strain and emphasized that this preference could be of major significance in resolving strain competition problem in *Phaseolus vulgaris.*

Although the plants that received mineral N fertilizer produced significantly greater shoot dry weights and N content, some treatments, especially with inoculation with SLA 2.2, showed significant production improvement. This effect in CIAT 899 inoculation was smaller, especially with "Diamante Negro". No effect was observed with CPAC H_{20} strain inoculation, whose treatment was equivalent statistically to control without inoculation in both cultivars. The nodules number did not show statistical differences at 5% of probability.

Under field conditions, in site A (Table 4), the SLA 2.2 strain confirmed its higher competitiveness observed in previous experiments through the highest nodule occupancy, both when inoculated alone (68 to 84%) or when co-inoculated with other strains (50 to 87%), identified by RAPD. The CIAT 899 strain also presented high levels of nodule occupancy when inoculated alone (60 to 70%) or when co-inoculated with PRF 81 strain (70 to 83%). However, when co-inoculated with SLA 2.2, it showed low levels of nodule occupancy (25 to 32%). PRF 81 was the least competitive strain. In this site, most yield plots were inoculated with SLA 2.2 (Table 5). In these treatments, plant dry weight, seed yield and plant nitrogen were equivalent or superior to that of treatments with 20 kg ha^{-1} of N mineral. The CIAT 899 strain inoculation showed lower yield than that of SLA 2.2, close to the treatments with 20 kg ha^{-1} of N mineral application, however, higher than the control without inoculation. Effect of PRF 81 inoculation was not observed. This treatment was statistically equivalent to control without inoculation.

In the second experiment, same site, including two more levels of mineral N, SLA 2.2 confirmed its highest competitiveness either alone or inoculated with CIAT 899 (Table 6). The CIAT 899 strain increased its nodule occupancy level in relation to the first experiment. The PRF 81 showed again the least nodule occupancy with all primers used in its identification. In treatments without inoculation with 20 and 40 kg ha^{-1} of N, nodule occupancy of 0 to 25% were detected, according to the primer, especially of SLA 2.2 and CIAT 899 (Table 6).

Table 2. Competitive occupancy of bean nodules by *Rhizobium* strains, determined by RAPD in two cultivars, Carioca and Diamante Negro, in pot culture under greenhouse conditions [(1)].

N	N_{ef}	N_{efmax}	Fraction of the N_{efmax}
1	1.000	–	0.500
5	1.667	–	0.833
7	1.750	–	0.875
10	1.818	–	0.910
12	1.846	–	0.923
15	1.875	–	0.938
18	1.895	–	0.947
20	1.905	–	0.952
25	1.923	–	0.962
30	1.935	–	0.968
40	1.951	–	0.976
50	1.961	–	0.980
60	1.967	–	0.984
100	1.980	–	0.990
∞	2.000	2.000	1.000

[(1)]N: number of nodules analyzed; X: other *Rhizobium* strains.

This fact is common in field experiments due to the complexity of avoiding contamination among the plots, especially in reinoculated areas (Table 6). In this study (Table 5), reinoculations with SLA 2.2 and CIAT 899 contributed for yields comparable to those obtained with 20 to 40 kg ha^{-1} of N. The dry weight and total N in treatments inoculated with these strains were comparable with treatments with 40 to 60 kg ha^{-1} of N. For PRF 81 strain, no response was observed after reinoculation. These results, especially those with CIAT 899, apparently indicate that the reinoculation may be important to help the establishment of some strains in the soil, resulting in increases in nodulation and yield (Vlassak et al., 1996). In site B, yield was higher in all treatments including the control, not inoculated (Table 5), probably due to the high fertility in this area, especially in phosphorus and organic matter contents. However, the inoculation response, in this site, is not so evident, as opposed to results with this soil in greenhouse experiments.

Many authors have pointed the limitations of the extrapolation on greenhouse results for the field, explaining that controlled conditions, inoculum proximity and multiplication can favor the inoculation response (Somasegaran & Bohlool, 1990). The range of soil organic matter can affect the inoculation. Brutti et al. (1999) confirm this effect in competitiveness experiments, in different soil with *Bradyrhizobium japonicum*, for soybean nodulation. On the other hand, results observed also indicated the influence of the indigenous rhizobial populations.

Site B, previously cultivated with different legumes, including beans, showed high rhizobial number (10^3 to 10^5 viable cells per g of soil). Many authors (Sessitsch et al., 2002) cite that the presence of well established rhizobial population is one of the principal limitations for inoculation success. This fact is more relevant in Brazilian soils, where naturalized bean rhizobial population is considered very high (Hungria et al., 1997). In sites with lower rhizobial population, especially due to the absence of host legume (Sá, 2001) and lower levels of fertility, as probably occurred in site A, the inoculation response is more evident. In this site, the SLA 2.2 strain showed more competitiveness and was the only one to contribute significantly for plant yield. In subsequent experiments with reinoculation, CIAT 899 strain reached similar results. This supremacy of SLA 2.2 strain may be associated with

Table 3. Nitrogen fixation, dry matter and nodulation of bean plants of cultivars Carioca and Diamante Negro inoculated with *Rhizobium* strains in pot culture under greenhouse conditions(1).

Treatment	Plant dry matter (g $plant^{-1}$)	Number of nodules	Plant total N (mg $plant^{-1}$)
		Carioca	
Mineral N	2.39a	-	94.6a
Control without N	1.26d	55^{ns}	36.3d
CIAT 899	1.48bc	92^{ns}	52.5bc
SLA 2.2	1.73ab	76^{ns}	63.1b
CPAC H_{20}	1.30bcd	65^{ns}	45.1cd
CIAT 899 x SLA 2.2	1.58abc	71^{ns}	57.0bc
CIAT 899 x CPAC H_{20}	1.63ab	98^{ns}	59.0bc
SLA 2.2 x CPAC H_{20}	1.61ab	77^{ns}	63.6b
CV (%)	13.9	30.9	2.6
		Diamante Negro	
Mineral N	2.34a	-	98.2a
Control without N	0.98bc	67^{ns}	30.6d
CIAT 899	1.25abc	65^{ns}	48.1bc
SLA 2.2	1.33abc	103^{ns}	52.3b
CPAC H_{20}	0.81cd	80^{ns}	28.3d
CIAT 899 x SLA 2.2	1.23abc	56^{ns}	40.6c
CIAT 899 x CPAC H_{20}	1.40ab	48^{ns}	46.8bc
SLA 2.2 x CPAC H_{20}	1.56ab	79^{ns}	51.3b
CV (%)	17.2	34.3	7.0

(1)Means followed by the same letter in each column did not differ significantly at 5% of probability by the Duncan test. ^{ns}Nonsignificant.

Table 4. Competitive occupancy of bean nodules by *Rhizobium* strains by RAPD in field (first experiment, site A)(1).

Treatment	Primer OPAC03				Primer OPAE10				Primer OPAC10			
	CIAT 899	SLA 2.2	PRF 81	X	CIAT 899	SLA 2.2	PRF 81	X	CIAT 899	SLA 2.2	PRF 81	X
	Nodules (%) as original fingerprint strain											
CIAT 899	60			40	70			30	70			30
SLA 2.2		74		26		84		16		68		32
PRF 81			15	85			23	77			35	65
CIAT 899 x SLA 2.2	25	50		25	25	50		25	32	68		0
CIAT 899 x PRF 81	83		17	0	70		30	0	76		13	11
SLA 2.2 x PRF 81		87	13	0		80	20	0		87	6	7

(1)Number of nodules analyzed for each treatment: 30; X: other *Rhizobium* strains.

Table 5. Dry matter (g plant^{-1}), grain yield (kg ha^{-1}) and total N (g plant^{-1}) of bean plants inoculated with *Rhizobium* strains in two sites of Cerrado[(1)].

Treatment	1st experiment, site A			2nd experiment, site A			Site B		
	Plant dry matter	Grain yield	Total N	Plant dry matter	Grain yield	Total N	Plant dry matter	Grain yield	Total N
SLA 2.2	30.1a	2,129.6a	1.02a	54.6a	1,625.0bc	2.02b	30.0ab	3,142.0abc	1.01bc
CIAT 899	23.1bc	1,234.0cd	0.71de	51.0a	1,787.3bc	1.75c	36.5ab	3,399.0ab	1.14b
PRF 81	18.6c	1,182.0cd	0.54f	11.7c	1,087.3f	0.39g	29.2ab	2,384.0c	0.87cd
SLA 2.2 x CIAT 899	26.7ab	1,731.0ab	0.88bc	39.8ab	1,537.3bcd	1.26d	25.0b	3,117.0abc	0.70e
SLA 2.2 x PRF 81	26.6ab	1,582.6bc	0.79cd	34.0ab	1,266.6def	1.02e	27.8ab	2,992.0bc	0.82d
CIAT 899 x PRF 81	26.5ab	1,530.0bcd	0.80bcd	40.5ab	1,166.6ef	1.31d	29.7ab	2,612.0bc	0.88cd
Control without N	20.5c	1,134.0d	0.68e	14.0c	1,400.0cdef	0.41g	27.1ab	2,606.0bc	0.81d
20 kg ha^{-1} N	25.9ab	2,103.0a	0.89b	28.7ab	1,816.6bc	0.93ef	33.6ab	3,044.6abc	1.04bc
40 kg ha^{-1} N	-	-	-	38.2b	2,133.3 b	1.31d	44.3a	4,028.0a	1.19b
60 kg ha^{-1} N	-	-	-	58.6a	2,562.3a	2.13a	45.7a	4,128.3a	1.55a
CV (%)	7.1	9.1	6.2	48.2	8.8	3.8	19.3	11.3	5.6

[(1)]Means followed by the same letter in each column did not differ significantly at 5% of probability by the Duncan test.

Table 6. Competitive occupancy of bean nodules by *Rhizobium* strains by RAPD in field (second experiment, site A).

Treatment	Nodules analyzed	Primer OPAC03				Primer OPAE10				Primer OPAC10			
		CIAT 899	SLA 2.2	PRF 81	X[(1)]	CIAT 899	SLA 2.2	PRF 81	X	CIAT 899	SLA 2.2	PRF 81	X
		Nodules (%) as original fingerprint strain											
CIAT 899	27	72			28	81			19	65			35
SLA 2.2	28		60		40		83		17		67		33
PRF 81	22			0	100			33	67			33	67
CIAT 899 x SLA 2.2	25	20	55		25	38	62		0	23	70		7
Control without N	25	25	5	0	70	0	0	5	95	7	25	5	63
Mineral N (20 and 40 kg ha^{-1})	38	0	0	0	100	3	5	0	92	15	10	0	75

[(1)]X: other *Rhizobium* strains.

the fact that this strain comes from this soil region and the good performance of the commercial strain CIAT 899, after reinoculation, confirms its competitiveness power in some conditions. In opposition, PRF 81 strain, which comes from other region and is regarded as competitive and efficient in some soils, especially from the south region (Hungria et al., 1997), was not successful in this "cerrado" soil conditions.

The influence of one strain on the other, in a mixed inoculum, was observed together with the ability of individual competitiveness of each strain influenced by soil conditions and its interaction with rhizosphere and naturalized rhizobial population. In these studies, the inoculation response was more evident when the strain was inoculated alone. Since the Brazilian law requires the presence of at least two strains, it is important to co-select effective and competitive as well as compatible rhizobial for multistrain inoculant formulations.

Conclusions

1. *Rhizobium* strain identification in soil is only suitable by RAPD method; agglutination and Elisa assays are only effective under axenic conditions.

2. SLA 2.2 and CIAT 899 are competitive strains for bean inoculation in some Cerrado soil conditions, especially represented by low levels of fertility and reduced rhizobial population.

3. *Rhizobium* strain competitiveness, besides its interaction with soil conditions, host, rhizosphere and native population, may be also influenced by the presence of other strain in the same inoculant.

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