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# DIVISÃO 2 - PROCESSOS E PROPRIEDADES DO SOLO

## Comissão 2.1 - Biologia do solo

### SYMBIOTIC CAPABILITY OF CALOPO RHIZOBIA FROM AN AGRISOIL WITH DIFFERENT CROPS IN PERNAMBUCO<sup>(1)</sup>

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#### SUMMARY

Biological nitrogen fixation by rhizobium-legume symbiosis represents one of the most important nitrogen sources for plants and depends strongly on the symbiotic efficiency of the rhizobium strain. This study evaluated the symbiotic capacity of rhizobial isolates from calopo (*Calopogonium mucunoides*) taken from an agrisoil under *Brachiaria decumbens* pasture, sabiá (*Mimosa caesalpiniiifolia*) plantations and Atlantic Forest areas of the Dry Forest Zone of Pernambuco. A total of 1,575 isolates were obtained from 398 groups. A single random isolate of each group was authenticated, in randomized blocks with two replications. Each plant was inoculated with 1 mL of a bacterial broth, containing an estimated population of  $10^8$  rhizobial cells mL<sup>-1</sup>. Forty-five days after inoculation, the plants were harvested, separated into shoots, roots and nodules, oven-dried to constant mass, and weighed. Next, the symbiotic capability was tested with 1.5 kg of an autoclaved sand:vermiculite (1:1) mixture in polyethylene bags. The treatments consisted of 122 authenticated isolates, selected based on the shoot dry matter, five uninoculated controls (treated with 0, 50, 100, 150, or 200 kg ha<sup>-1</sup> N) and a control inoculated with SEMIA 6152 (=BR1602), a strain of *Bradyrhizobium japonicum*. The test was performed as described above. The shoot dry matter of the plants inoculated with the most effective isolates did not differ from that of plants treated with 150 kg ha<sup>-1</sup> N. Shoot dry matter was positively correlated with all other variables. The proportion of effective isolates was highest among isolates from *sabiá* forests. There was great variation in nodule dry weight, as well as in N contents and total N.

**Index terms:** *Calopogonium mucunoides*, biological N fixation, forage legumes, tropical legumes.

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## RESUMO: CAPACIDADE SIMBIÓTICA DE RIZÓBIOS DE CALOPOGÔNIO DE DIFERENTES COBERTURAS VEGETAIS DE UM ARGISSOLO DE PERNAMBUCO

A fixação biológica do nitrogênio pela simbiose leguminosa-rizóbio é uma das mais importantes fontes de N para as plantas e depende fortemente da eficiência simbiótica da estirpe rizobiana. Este trabalho avaliou a capacidade simbiótica de isolados rizobianos de calopogônio (*Calopogonium mucunoides*) provenientes de um Argissolo sob pastagens de *Brachiaria decumbens*, bosques de sabiá (*Mimosa caesalpiniiifolia*), e áreas de Mata Atlântica da Zona da Mata seca de Pernambuco. Foram obtidos 1.575 isolados distribuídos em 398 grupos. Realizou-se a autenticação para apenas um isolado aleatório de cada grupo, em blocos casualizados com duas repetições. A inoculação foi feita com 1 mL por planta de caldo bacteriano com população estimada de  $10^8$  células rizobianas  $\text{mL}^{-1}$ . Aos 45 dias após a inoculação, as plantas foram colhidas, separadas em parte aérea, raízes e nódulos, secas em estufa até massa constante e pesadas. A fase seguinte, teste de capacidade simbiótica, foi montada em blocos casualizados, com duas repetições, em sacos de polietileno contendo 1,5 kg da mistura areia:vermiculita autoclavada (1:1). Foram isolados e selecionados 122 tratamentos, após a autenticação, com base na matéria seca da parte aérea, bem como cinco tratamentos-controle não inoculados, recebendo o equivalente a 0; 50; 100; 150; e 200  $\text{kg ha}^{-1}$  de N, e um tratamento inoculado com a estirpe SEMIA 6152 (=BR1602) de *Bradyrhizobium japonicum*. O experimento foi conduzido da mesma forma que a fase anterior. A matéria seca da parte aérea das plantas inoculadas com os isolados mais efetivos não diferenciou significativamente do tratamento adubado com 150  $\text{kg ha}^{-1}$  de N. A matéria seca da parte aérea correlacionou-se positivamente com todas as variáveis analisadas. A maior proporção de isolados eficientes foi obtida com os isolados de bosques de sabiá. Houve grande variação nos valores de matéria seca de nódulos, bem como na concentração e no acúmulo de N nas plantas.

*Termos de indexação:* *Calopogonium mucunoides*, fixação biológica de N, leguminosas forrageiras, leguminosas tropicais.

## INTRODUCTION

Pastures cover more than 172 million hectares in Brazil and constitute the main feed source for animals destined for meat, milk and other animal products (IBGE, 2010). Due to the very low production cost in Brazil, the animal industry is highly competitive in the worldwide market, where Brazil is the main beef exporter and has now shifted from being a milk importer to an exporter (Caleman & Cunha, 2011).

Approximately 30 million ha of Brazilian pastureland are in some state of degradation, resulting in very low biomass yields, limiting animal consumption. Deficiencies in N and P are generally the main reason for pasture degradation (Boddey et al., 2004), particularly N deficiency caused by high N losses from soil-plant-animal systems (Lira et al., 2006). An interesting alternative that could retain N in pasture soils are legume-grass consortiums, in view of their ability to fix atmospheric  $\text{N}_2$  (Santos et al., 2005; Fontoura et al., 2011).

Calopo (*Calopogonium mucunoides* Desv.) is a promising legume for tropical grass-legume consortiums due to its low palatability and consequently lower acceptance by animals, when the grass is at full growth. These characteristics allow legumes to grow, reproduce and persist in pastures (Carvalho & Pires, 2008). Studying different forage

legumes for soil cover in the semi-arid region of Minas Gerais, (Teodoro et al., 2011) found that calopo fully covered the soil, retained soil moisture well and inhibited spontaneous vegetation growth. The authors also reported that calopo increased the availability of N and other nutrients, apart from raising soil organic matter contents.

Due to the economic and ecological importance of the rhizobium-legume symbiosis, rhizobium strain selection has been intensively studied throughout the world for several legume species (Lima et al., 2009; Freitas et al., 2011). The selection of strains with a high biological N fixation (BNF) potential could increase dry matter accumulation and thus N accumulation by legumes (Antunes et al., 2011).

One method of increasing the rhizobium isolate diversity and the chance of finding strains with a higher BNF efficiency is to collect soil from different regions, of different vegetation covers or cultivation systems, due to the established effects of these factors on the rhizobial population size, diversity and symbiotic efficiency (Jesus et al., 2005; Santos et al., 2005; Lima et al., 2009; Chang et al., 2011). Furthermore, Jesus et al. (2005) found that several land use systems affected the rhizobial diversity of siratro (*Macroptilium atropurpureum*), a N-fixing legume, in the western Amazon, where the bacterial richness and diversity were highest under cassava,

whereas that under native forest and peach palms did not differ significantly. On the other hand, Santos et al. (2005) found that vegetation covers affected the symbiotic efficiency of rhizobium strains of peanut. The total N accumulation and dry shoot biomass and efficacy were higher for strains from caatinga areas than those from leucaena, sabiá or peanut areas.

This study aimed to characterize calopo (*C. mucunoides* Desv) rhizobial isolates from *Brachiaria decumbens* pasture soils as well as from sabiá (*Mimosa caesalpiniiifolia*) and Atlantic forest areas in the Dry Forest Zone of Pernambuco, in a sterile substrate.

## MATERIAL AND METHODS

Soil was sampled in March 2010, at the Itambé Experimental Station of the Agronomic Institute of Pernambuco (IPA) in the Dry Forest Zone of Pernambuco (07° 25' S, 35° 06' W, 190 m asl), where the average annual rainfall is 1,200 mm, average annual temperature 24 °C and average relative humidity 80 %. The regional climate is AS according to the Köppen classification (IPA, 1994). Reference soils from Itambé are classified as Red-yellow dystrophic agrisoil with a loamy/clayey texture in the prominent A horizon, a semideciduous tropical forest and a slightly undulating relief (Jacomine et al., 1973) updated according to Embrapa (2006).

Soil samples were collected from the 0-0.2 m layer of *B. decumbens* pastures, sabiá plantations (*Mimosa caesalpiniiifolia*), and Atlantic Forest areas, from three sites per area. The pastures were planted at the end of the 1980s and were grazed from then on, whereas the sabiá forests were planted in 1998 and 1990, and the Atlantic Forest is approximately 60 years old (Lira, 2011, personal information<sup>6</sup>). These samples were analyzed for physical (Ruiz, 2005) and chemical (Embrapa, 1999) properties (Table 1), and a portion of each sample was refrigerated at 4 °C until inoculation in the nodulation experiment.

A total of 1,575 rhizobial isolates were obtained, 544 of which were taken from *B. decumbens* pastures, 666 from sabiá woodlots and 365 from Atlantic forest zones. Morphological clustering at a similarity level of 100 % resulted in 398 groups.

A randomly chosen isolate from each group was authenticated in polyethylene bags with 0.5 kg of an autoclaved 1:1 sand:vermiculate mixture and a polypropylene base, treated daily with Hoagland's solution without N (Hoagland & Arnon, 1950). The experiment was arranged in a randomized block design with two replications.

Calopo seeds were chemically scarified with concentrated H<sub>2</sub>SO<sub>4</sub> for 20 min and washed several times with distilled water (Costa et al., 2008), followed by immersion in ethanol for 10 s. The seeds were then disinfested with 5 % sodium hypochlorite for 5 min and washed with distilled water. Plantlets were subsequently germinated in Petri dishes with paper towels for three days and transplanted.

Two days after transplantation, each plantlet was inoculated with 1.0 mL of bacterial broth containing an estimated population of 10<sup>8</sup> cells mL<sup>-1</sup>. In addition to the inoculation treatments, uninoculated treatments were performed, both with and without Hoagland's solution with N, or plantlets were inoculated with SEMIA 6152 (=BR1602, *Bradyrhizobium japonicum*, GenBank accession AY904756, recommended by Embrapa Agrobiologia based on field experiments), which is currently recommended for inoculant production (Brasil, 2011).

Forty-five days after inoculation, the plants were harvested and separated into shoots, roots and nodules, placed in paper bags, dried in convection ovens at 65 °C to constant weight and then weighed to determine shoot, root and nodule dry matter (SDM, RDM and NDM).

To test the symbiotic efficiency, a randomized block design was applied with two replications. These assays were carried out in polyethylene bags with 1.5 kg of the sand:vermiculate mixture under the same conditions as in the first experiment. Seed germination and inoculation were also performed as per the first experiment.

The treatments consisted of 122 isolates selected in the first experiment based on shoot dry matter, five uninoculated treatments fertilized with 0, 50, 100, 150 or 200 kg ha<sup>-1</sup> N, on a substrate mass basis (equivalent to 0, 37.5, 75.0, 112.5 or 150.0 mg/bag of N) and a treatment inoculated with SEMIA 6152. Nitrogen was supplied as ammonium nitrate in four rates, applying the first at transplantation and the others in 8-day intervals.

Harvesting was conducted 45 days after inoculation, and the same variables were determined as in the first experiment. SDM was ground in a Wiley mill with 1.0 mm sieves, and N contents were determined according to Malavolta et al. (1989). A regression was performed for the treatments in which N was supplied to estimate the N dose required to reach the average SDM in each treatment (ND).

The relative efficiency (RE) was calculated based on the total N in SDM (STN), according to the following equation:

$$RE = \left( \frac{STN_{treatment}}{STN_{100 \text{ kg of N ha}^{-1}}} \right) \times 100$$

<sup>6</sup> Mario de Andrade Lira: Personal communication based on research activities conducted at the Itambé Experimental Station since the end of the 1960s and from contact with the authors in July 2011.

**Table 1. Means and 95 % confidence intervals of the physical and chemical characteristics of samples of the 0-20 cm soil layer from areas of *Brachiaria decumbens*, sabiá woodlots and Atlantic Forest at the Itambé Experimental Station**

Characteristic	<i>B. decumbens</i> pastures	Sabiá plantations	Atlantic forest
Physical			
Sand (g kg <sup>-1</sup> )	661 ± 42	657 ± 85	647 ± 73
Silt (g kg <sup>-1</sup> )	26 ± 18	26 ± 18	55 ± 12
Clay (g kg <sup>-1</sup> )	312 ± 60	288 ± 90	297 ± 84
Textural class	Sandy clayey loam	Sandy clayey loam	Sandy clayey loam
Chemical			
pH (H <sub>2</sub> O)	5.61 ± 0.37	5.00 ± 0.38	4.86 ± 0.39
C (g kg <sup>-1</sup> )	5.26 ± 1.49	5.38 ± 0.39	6.75 ± 0.80
P (mg dm <sup>-3</sup> )	23.33 ± 11.0	7.00 ± 0.00	5.67 ± 0.65
K <sup>+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.79 ± 0.44	0.34 ± 0.28	0.18 ± 0.05
Ca <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	2.30 ± 0.28	2.38 ± 0.92	3.37 ± 0.45
Mg <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	2.35 ± 0.26	2.15 ± 0.91	2.67 ± 0.56
Na <sup>+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.06 ± 0.04	0.11 ± 0.16	0.06 ± 0.03
Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.13 ± 0.14	0.53 ± 0.46	0.38 ± 0.41
H+Al (cmol <sub>c</sub> dm <sup>-3</sup> )	6.27 ± 1.60	7.55 ± 1.30	7.55 ± 1.30

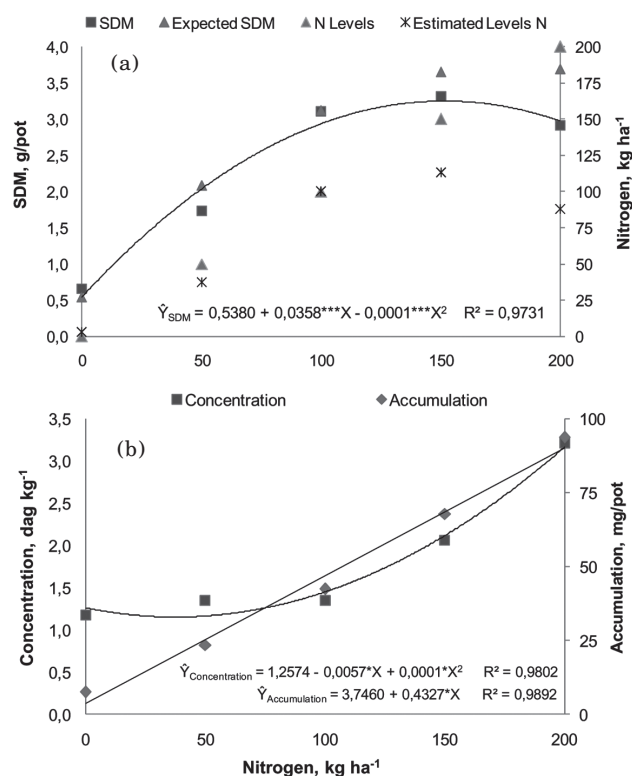
x±y, where x = average and y = confidence interval; pH (H<sub>2</sub>O) (1:2.5); P, K and Na: Mehlich-1; Ca, Mg, and Al: KCl 1 mol L<sup>-1</sup>; H+Al: calcium acetate 0.5 mol L<sup>-1</sup> at pH 7.0.

All data were subjected to ANOVA and grouped through the Scott Knott test for comparison of means, applying the test as a function of the symbiotic efficiency and vegetation cover using SISVAR 5.3 (Ferreira, 2008), followed by determination of the 5 % confidence interval for each Scott Knott class. Correlation coefficients between the variables were calculated using Statistica 8.0 (Statsoft, 2008). The effect of vegetation cover on the proportion of efficient isolates was estimated by a chi-square test of SDM using Minitab 16 (Minitab, 2009).

## RESULTS AND DISCUSSION

The shoot dry matter of Calopo increased significantly after N application (Figure 1a,b). The agronomic efficiency was highest in the treatment with 179 kg ha<sup>-1</sup> N, resulting in a maximum SDM of 3.74 g/pot. In contrast, SDM decreased after the highest N application (Figure 1a), which may have been due to a nutritional imbalance, as both the N concentration and N accumulation continued to increase with increasing N rates (Figure 1b). The response to N application was parabolic for N concentration and linear for N accumulation, associated with high determination coefficients.

The rhizobial isolates had a significant effect on all variables (p<0.05), except RDM (Table 2). The average SDM of plants inoculated with the best-performing isolates did not differ significantly (p>0.05)



**Figure 1. SDM accumulation and expected SDM (left axis) and N doses measured and estimated by SDM using the equation (right axis) (a) and N contents and SDM accumulation in calopo, as a function of applied N (b).**



**Table 2. Average shoot (SDM), root (RDM) and nodule (NDM) dry matter, shoot N content (SNC) and total N (STN), relative efficiency (RE) and N dose (ND) associated with a given SDM yield under inoculation with rhizobial isolates from *B. decumbens* pastures (BD), sabiá plantations (SP) and Atlantic Forest (AF) areas in the Dry Forest Zone of Pernambuco**

Veg. cover	Isol.	SK class <sup>(1)</sup>	SDM	RDM	NDM	SNC	STN	ER <sup>(2)</sup>	ND <sup>(3)</sup>
			g/bag	g/bag	mg/bag	dag kg <sup>-1</sup>	mg/bag	%	kg ha <sup>-1</sup>
SP	5	1	3.37±0.20 a	0.44±0.09 c	359.70±44.16 a	2.69±0.22 a	90.88±10.82 a	214.28±25.52 a	125.63±19.74 a
BD	1		3.32±0.20 a	0.35±0.05 c	289.00±60.76 a	2.71±0.06 a	90.01±7.39 a	212.24±17.44 a	114.49±15.19 a
SP	3	2	2.86±0.32 b	0.36±0.09 c	240.83±51.31 b	2.61±0.32 a	75.21±14.83 b	177.33±28.56 b	87.72±19.70 c
SP	7		2.56±0.12 c	0.35±0.06 c	273.71±42.42 b	2.67±0.17 a	68.70±6.15 b	161.99±14.49 b	70.71±5.69 d
BD	1	3	2.62±0.42 c	0.38±0.16 c	346.00±3.92 a	2.22±0.02 b	58.05±9.80 b	136.88±23.10 b	73.32±19.94 d
AF	1		2.36±0.28 c	0.34±0.31 c	226.50±238.14 b	2.90±0.78 a	68.82±26.51 b	162.28±16.30 b	61.39±12.08 d
SP	5		2.03±0.12 d	0.37±0.10 c	192.60±59.0 b	2.33±0.31 b	47.39±7.60 c	111.75±17.92 c	48.20 ±4.75 e
BD	1	4	1.92±0.28 d	0.28±0.07 c	208.50±59.78 b	2.36±0.15 b	45.32±9.52 c	106.87±22.45 c	43.94±10.52 e
AF	1		2.28±0.38 d	0.29±0.16 c	230.0±186.20 b	2.54±0.81 a	58.62±28.10 b	138.23±20.04 b	58.15±15.81 d
SP	10		1.46±0.09 e	0.45±0.08 c	94.35±17.92 c	1.74±0.26 c	26.01±4.67 d	61.33±11.00 d	28.09±2.91 f
BD	6	5	1.49±0.12 e	0.31±0.09 c	142.17±41.58 c	2.28±0.47 b	34.63±8.27 c	81.66±19.49 c	29.11±4.02 f
AF	3		1.55±0.17 e	0.37±0.11 c	123.17±57.05 c	2.00±0.62 b	32.03±12.19 c	75.53±28.75 c	30.92±5.70 f
SP	8		1.23±0.11 f	0.49±0.11 c	46.06±25.64 d	1.50±0.28 c	18.74±4.49 d	44.18±10.60 d	20.74±3.35 g
BD	6	6	1.07±0.12 f	0.37±0.08 c	36.67±16.58 d	1.43±0.25 c	15.38±3.40 d	36.25±8.01 e	15.53±3.78 g
AF	1		1.07±0.29 f	0.52±0.42 c	10.50±4.90 d	1.17±0.08 c	12.50±2.56 d	29.49±6.03 e	15.60±9.00 g
SP	30		0.74±0.05 g	0.34±0.02 c	30.90±6.95 d	1.41±0.09 c	10.61±1.13 d	25.01±2.68 e	6.03±1.27 h
BD	23	7	0.72±0.06 g	0.32±0.03 c	31.96±12.85 d	1.40±0.12 c	10.18±1.44 d	24.01±3.40 e	5.77±1.57 h
AF	10		0.71±0.09 g	0.35±0.05 c	18.65±6.70 d	1.42±0.17 c	9.86±1.68 d	23.26±3.95 e	5.53±2.25 h
Semia 6152			0.62±0.14 g	0.34±0.23 c	0.60±0.78 d	1.44±0.01 c	8.90±1.88 e	21.00±4.44 e	2.32±3.88 h
N 0			0.66±0.09 g	0.35±0.08 c	-	1.18±0.59 c	7.62±2.81 e	17.97±6.62 e	-
N 50			1.74±0.16 e	0.63±0.09 b	-	1.35±0.10 c	23.54±3.81 d	55.50±8.97 d	-
N100			3.11±0.33 b	0.96±0.24 a	-	1.35±0.47 c	42.41±19.19 c	100.00±36.02 c	-
N 150			3.31±0.12 a	0.79±0.10 b	-	2.06±0.72 b	67.89±21.40 b	150.07±31.96 b	-
N 200			2.92±0.05 b	0.49±0.42 c	-	3.21±0.30 a	93.62±7.17 a	220.72±16.91 a	-

<sup>(1)</sup> Classes formed using the Scott Knott test based on the SDM yields associated with the different vegetation covers <sup>(2)</sup> Relative efficiency: STN of each treatment/STN of plants fertilized with 100 kg ha<sup>-1</sup> N x 100. <sup>(3)</sup> The estimated N dose necessary for the observed SDM. Means of two replications followed by the same letter did differ significantly (p>0.05) based on the Scott Knott test.

from that of plants fertilized with 150 kg ha<sup>-1</sup> N. The SDM yield of these plants was 4.6 times higher than that of plants inoculated with the worst-performing isolates and 5.1 and 5.4 times higher than that of uninoculated, unfertilized plants and plants inoculated with the recommended strain, respectively (Table 2).

Previous research on calopo also identified isolates leading to higher SDM yields than plants treated with 145 mg/plant of N, which is approximately equivalent to 360 kg ha<sup>-1</sup> N, under greenhouse conditions and in Leonard jars (Xavier & Franco, 1995). These findings confirm the potential of this species to improve N availability in pasture.

Seven symbiotic efficiency classes were grouped, based on the calopo SDM yields. The first four were efficient because they obtained higher SDM yields than the 50 kg ha<sup>-1</sup> N treatment. These classes consisted of 20 representative isolates from sabiá woodlots, three from *B. decumbens* pasture and two

from Atlantic Forest areas. The best-performing class consisted of six isolates, including five from sabiá woodlots (UFRPE Cm153, 326, 362, 363, and 556) and one from a *B. decumbens* pasture (UFRPE Cm799).

The recommended strain, SEMIA 6152, was not efficient with respect to BNF. This treatment was included in the lowest SDM yield group and did not differ from the unfertilized treatment. This pattern was observed for most isolates, confirming the need to evaluate native rhizobia, which are better adapted to the local environmental conditions where the strain will be used as inoculant (Teixeira et al., 2010; Sarr et al., 2011).

Nodule dry matter varied largely (Table 2); the strains with highest efficiency achieved NDM yields 32 times higher than those with the lowest efficiencies (p<0.05) and approximately 550 times higher than those of the recommended strain. No nodulation was observed in any of the uninoculated treatments,

confirming that no external contamination occurred during the experiment. Similar to the differences between these isolates and the recommended strains observed for NDM, Antunes et al. (2011) also identified native isolates with a higher efficiency than that of strains recommended for faba bean.

The highest proportion of efficient strains was found in the sabiá plantations (Table 3), most likely due to the high N demand at these sites (Carvalho & Pires, 2008), confirming the effect of vegetation cover on the rhizobial symbiotic efficiency. This effect was further supported by the low proportion of efficient strains obtained from the Atlantic Forest, where the N demand was lowest (Martins, 2009), although the soil organic matter contents did not differ significantly between the vegetation covers (Table 1).

Significant effects of vegetation cover on the BNF potential of native rhizobia were previously observed for cowpea, where 86 % of the higher efficiency strains were isolated from caatinga and pasture soils, while the lowest proportion (36 %) was isolated from cassava fields (Nascimento et al., 2010). Effects of vegetation cover were also found for peanut, since in this crop isolates from caatinga induced higher N accumulation, shoot dry mass and efficacy than isolates from leucaena, sabiá and peanut fields (Santos et al., 2005).

Isolates from *B. decumbens* pastures had a lower symbiotic efficiency than those from sabiá plantations (Table 3), although there were no major differences in the soil characteristics of these areas, except for a higher P content in the pasture soils.

Plants inoculated with 18 of the isolates showed ca. 84 % of the shoot N content (SNC) of plants fertilized with 200 kg ha<sup>-1</sup> N (Table 2). In contrast, plants inoculated with strains resulting in a higher SDM differed from the others, achieving ca. 97 % of the STN of plants fertilized with 200 kg ha<sup>-1</sup> N. Similar results were found for peanut, where native strains from Serra Talhada, Pernambuco, resulted in higher STN than that of plants treated with 100 mg kg N, equivalent to the highest dose tested in this experiment (200 kg ha<sup>-1</sup> N) (Santos et al., 2005).

The average SDM associated with the most efficient strains obtained from sabiá woodlots and *B.*

*decumbens* pastures indicated NDs (N dose required to reach the average SDM in each treatment) equivalent to 126 and 114 kg ha<sup>-1</sup> N, respectively (Table 2). These doses were equivalent to the highest N fertilization recommended for pastures in the State Pernambuco (IPA, 2008).

Interestingly, the ND for plants fertilized with 150 kg ha<sup>-1</sup> N was only 113 kg ha<sup>-1</sup> N (Figure 1a), which is equivalent to only 76 % of the supplied N, while the NDs of plants fertilized with 100 and 200 kg ha<sup>-1</sup> N were equivalent to 100 and 44 % of the supplied N, respectively. This loss of efficiency was attributed to the lower SDM observed at higher N levels, most likely due to a nutritional imbalance, because both SNC and STN increased at higher N doses (Figure 1b). In a field experiment with a dystrophic Red Latossol in Santo Antônio de Goiás found a similar reduction in N use efficiency was observed, with highest agronomic and physiological efficiencies at 30 kg ha<sup>-1</sup> N. Higher N doses lead to significant reductions in physiological efficiency, with approximately 60 % efficiency at flowering and less than 20 % at harvest, at rates of 240 kg ha<sup>-1</sup> N (Sant'ana et al., 2011).

Considering the average NDs of the most efficient strains (126 and 114 kg ha<sup>-1</sup> N) and an average cost of R\$ 2.79 kg<sup>-1</sup> N (CONAB, 2012), inoculation with these isolates would be approximately equivalent to a reduction in N fertilization costs of R\$ 352.00 and R\$ 318.00, in addition to other advantage associated with the BNF.

All variables were positively correlated with SDM (Table 4), but there was no significant correlation between RDM and the other variables. Nevertheless, SDM, STN, ER and ND all showed high significant correlations with each other, as expected, since STN, ER and ND were all derived from SDM. The low correlation with RDM was most likely due to the low range of values for this variable (Table 2), similar to what was found regarding the correlations between RDM and SDM, NDM, STN and ER for fava beans (Antunes et al., 2011).

This study identified calopo isolates with a high nodulation and BNF potential, which could be an excellent alternative to N fertilization in pastures with

**Table 3. Chi-square test for the observed and expected frequencies of efficient strains isolated from *B. decumbens* pastures, sabiá plantations and Atlantic Forest areas in the Dry Forest Zone of Pernambuco**

Vegetation cover	Efficient	Inefficient	Total	Proportion
	Observed (Expected)			
				%
<i>B. decumbens</i> pastures	3 (7.79)	35 (30.21)	38	7.89 b
Sabiá plantations	20 (13.93)	48 (54.07)	68	29.41 a
Atlantic forest	2 (3.28)	14 (12.72)	16	12.50 ab
Total	25	97	122	20.49

Different letters indicate significant differences (p<0.05) in the proportion of efficient strains.

**Table 4. Correlation coefficients between shoot (SDM), root (RDM) and nodule (NDM) dry matter, shoot N concentration (SNC) and total N (STN), relative efficiency (RE) and the N dose (ND)**

	RDM	NDM	SNC	STN	ER	ND
SDM	0.551 <sup>ns</sup>	0.891*	0.818*	0.979*	0.967*	0.995*
RDM	-	0.169 <sup>ns</sup>	0.044 <sup>ns</sup>	0.402 <sup>ns</sup>	0.432 <sup>ns</sup>	0.598 <sup>ns</sup>
NDM	-	-	0.911*	0.934*	0.879*	0.857*
SNC	-	-	-	0.915*	0.858*	0.782*
STN	-	-	-	-	0.969*	0.961*
ER	-	-	-	-	-	0.951*

<sup>ns</sup> and \*: not significant and significant at 5 % probability, respectively, according to t tests.

this legume species. These isolates should be further evaluated under field conditions to allow an assessment of their competitive ability compared with native strains, before these strains can be recommended for inoculant production.

## CONCLUSIONS

1. There was a strong effect of vegetation cover on the symbiotic potential of isolates from the three study areas.

2. The most efficient isolates found in this study lead to SDM yields equivalent to those obtained with up to 120 kg ha<sup>-1</sup> N. These isolates may have considerable effects on N availability within a pasture, and their use could result in significant reductions in fertilization costs.

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