

SYMBIOTIC EFFECTIVENESS AND COMPETITIVENESS OF CALOPO RHIZOBIAL ISOLATES IN AN *ARGISSOLO* *VERMELHO-AMARELO* UNDER THREE VEGETATION COVERS IN THE DRY FOREST ZONE OF PERNAMBUCO

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ABSTRACT

Biological N fixation in forage legumes is an important alternative to reduce pasture degradation, and is strongly influenced by the inoculant symbiotic capability. This paper evaluates the effectiveness of Calopo (*Calopogonium mucunoides*) rhizobial isolated from soil under three vegetation covers of an *Argissolo Vermelho-Amarelo* of the Dry Forest Zone of Pernambuco. An experiment was conducted evaluating 25 isolates, aside from 5 uninoculated controls with 0; 309; 60; 90 and 120 kg ha⁻¹ N, and a treatment inoculated with the SEMIA 6152 strain. The first cut was performed 45 days after inoculation and a second and third cut after 45-day-intervals. Shoot N content was quantified at all cuts. Shoot dry mass was affected by N rates at all cuts. Shoot dry mass increased from the first to the second cut in inoculated plants. There was no difference between rhizobial isolates from the different plant covers for any of the variables. Most variables were significantly and positively correlated.

Keywords: *Calopogonium mucunoides*, biological fixation, forage legumes.

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RESUMO: EFETIVIDADE SIMBIÓTICA E COMPETITIVIDADE DE RIZÓBIOS DE CALOPOGÔNIO ISOLADOS DE TRÊS COBERTURAS VEGETAIS, EM ARGISSOLO VERMELHO-AMARELO DA ZONA DA MATA PERNAMBUCANA

A fixação biológica de N por leguminosas forrageiras é uma alternativa importante para reduzir a degradação de pastagens e é muito influenciada pela capacidade simbiótica do inoculante. Este trabalho avaliou a efetividade de isolados rizobianos de calopogônio (*Calopogonium mucunoides*) de um Argissolo Vermelho-Amarelo da Zona da Mata Seca de Pernambuco com três diferentes coberturas vegetais. Foi conduzido um experimento testando 25 isolados, além de cinco controles não inoculados com 0; 30; 60; 90; e 120 kg ha⁻¹ de N, e um tratamento inoculado com a estirpe SEMIA 6152. O primeiro corte foi realizado 45 dias após a inoculação, sendo o segundo e o terceiro cortes realizados 45 dias após o precedente. Os teores de N na parte aérea foram quantificados em todos os cortes. A matéria seca da parte aérea foi influenciada pelas doses de N em todos os cortes. A matéria seca da parte aérea aumentou do primeiro para o segundo corte nas plantas inoculadas. Não houve diferença entre os isolados rizobianos das diferentes coberturas vegetais para nenhuma das variáveis. Houve correlação positiva e significativa para a maioria das variáveis.

Palavras-chave: Calopogonium mucunoides, fixação biológica, leguminosas forrageiras.

INTRODUCTION

Forage legumes intercropped with grasses allow partial or total substitution of N fertilizer, which is usually expensive and when insufficient, one of the main reasons for low pasture yield (Carvalho and Pires, 2008). On the other hand, in tropical regions there are no well-established grass-legume pastures, mostly due to difficulties with legume survival, which indicates that the use of native legumes might reduce the problem (Lira et al., 2006). Calopo (*Calopogonium mucunoides*) is a forage legume, native to South America, that can be used in humid tropical regions, and is found in a native or naturalized status in State Forest Zone of Pernambuco (Souza et al., 2007).

According to Herridge et al. (2008), forage legumes in pastures can fix from 110 to 227 kg ha⁻¹ yr⁻¹ of N, and an estimated 10 to 15 Gg yr⁻¹ N on a worldwide basis. Higher legume yields and consequently higher biological N fixation rates may be obtained through the selection of rhizobial strains with higher biological nitrogen fixation (BNF) efficiency (Antunes et al., 2011; Rahmani et al., 2011).

The selection of rhizobial strains effective for BNF usually require several steps, such as soil or nodule sampling from areas in which the target species naturally occurs, strain isolation, purification, and characterization, as well as the estimation of BNF efficiency on both sterilized and unsterilized substrates before the final field evaluation. Ideally a large number of strains should be included in these stages, to increase the chance of finding higher efficiency strains for inoculant production (Lima et al., 2009; Chagas Junior et al., 2010; Rufini et al., 2011; Araújo et al., 2012; Calheiros et al., 2013).

As an example of the possible effect of plant cover on rhizobial efficiency, Santos et al. (2005) studied groundnut with rhizobia isolated from different plant covers in a semi-arid region in Pernambuco. These authors found large variation in strain efficiency for BNF, due both to the groundnut cultivar and the plant cover from which the strain was initially isolated.

This paper aims to evaluate the symbiotic efficiency of Calopo rhizobial strains isolated from signal grass pastures, “sabiá” woodlots and Atlantic Forest in nearby areas of the same soil type in a Tropical Subhumid Area, the Dry Forest Zone of Pernambuco State.

MATERIAL AND METHODS

Soil was collected in March 2010, at the Experimental Station of Itambé of the Agronomic Institute of Pernambuco (07° 25' S, 35° 06' W, 190 m asl) in the Zona da Mata Seca of the State of Pernambuco. The climate classification according to Köppen is AS', and the soil was classified as a *Argissolo Vermelho-Amarelo*, with prominent A horizon with clayey loam texture and slightly undulating relief, according to Jacomine et al. (1973), and updated according to Embrapa (2006).

Composite soil samples were collected from the 0.0-0.2 m layer in each of three distinct areas of signal grass (*Brachiaria decumbens*) pastures, “sabiá” (*Mimosa caesalpiniiifolia*) woodlots and Atlantic Forest. Signal grass pastures were established at the end of the 1980's and were pastured since then, and in different stages of degradation, with some invasion of native herb legumes. The “sabiá” woodlots were established in 1987 and 1990, and the Atlantic Forest at least 1960 before.

A soil subsample from each area was conserved at about 4 °C until inoculation of the trap plants. Another part of the samples was used for physical and chemical analyses (Table 1), according to Ruiz (2005) and (Embrapa, 1999), respectively.

Calopo plant traps were used for nodulation studies, from which 1,575 assumedly rhizobial isolates were obtained, and after authentication and initial symbiotic efficiency tests, 25 isolates were identified as the most efficient, with shoot dry mass higher than achieved with 50 kg ha⁻¹ N. These isolates were selected for the unsterilized soil experiment described in this paper.

This experiment was conducted in soil from the same experimental station (chemical and physical properties listed in table 1). Apart from the 25 selected strains, six control treatments were carried out. Five of these were uninoculated, treated with 0, 30, 60, 90, or 120 kg ha⁻¹ N, and the other was inoculated with *Bradyrhizobium japonicum*, strain SEMIA 6152=BR1602 (Genbank access AY904756), currently recommended for Calopo inoculant production (Brasil, 2011).

Nitrogen was supplied as ammonium nitrate, in one third 15 days after seedling establishment, and the remainder evenly divided after each cut. The amounts per experimental unit were established according to the average soil mass. Soil pH was corrected with calcium carbonate to 70 % base saturation, equivalent to 3.65 t ha⁻¹ of lime, 20 days before seedling transplantation. Phosphorus and potassium were supplied according to fertilizer recommendations for Pernambuco (IAP, 2008), at the beginning of the experiment and after each cut. The other nutrients were applied in Hoagland's

nutrient solution (Hoagland and Arnon, 1950), only at the beginning of the experiment.

Calopo seeds were chemically scarified with concentrated sulfuric acid for 20 min, repeatedly washed with distilled water, and afterwards surface-disinfected with sodium hypochlorite for 5 min, and again repeatedly washed with distilled water. Seeds were germinated in Petri dishes with paper towel for two days, and then transferred three-by-three to polyethylene bags. Two days after transplantation, each seedling was treated with 1 mL of bacterial broth with about 10⁸ rhizobial cells mL⁻¹. Plants were irrigated as necessary to maintain soil moisture near field capacity.

The first cut was performed 45 days after inoculation, and the second and third within 45-day-intervals. Shoot dry mass (SDM) was obtained by oven-drying at 65 °C to constant mass. The material was ground in a Willey mill, sieved (<1.0 mm), and used for N content determination according to Malavolta et al. (1989). After the third cut, root (RDM) and nodule (NDM) dry masses were obtained similarly to SDM.

For the N-fertilized treatments, regressions were estimated for SDM, shoot N content (SNC) and shoot N accumulation (SNA) using supplied N as the independent variable. The equivalent N rate (END) was then estimated for each pot based on observed SDM and SNA, on the basis of the respective regression.

Relative efficiency (RE) was calculated based on SNA, using the equation:

$$RE = \frac{\text{SNA per bag}}{\text{Average SNA of the treatment fertilized with 60 kg ha}^{-1} \text{ of N}} \times 100$$

The data were subjected to analysis of variance and the Scott-Knott test as appropriate, using

Table 1. Means and 95 % confidence interval of soil chemical and physical characteristics of signal grass pastures, "sabiá" woodlots and Atlantic Forest areas used for rhizobial isolation, and of the soil used in the inoculant efficiency experiment

	Signal grass pastures	"Sabiá" woodlots	Atlantic Forest	Experimental soil
pH(H ₂ O)	5.61 ± 0.37	5.00 ± 0.38	4.86 ± 0.39	4.50
CO (g kg ⁻¹)	5.26 ± 1.49	5.38 ± 0.39	6.75 ± 0.80	16.49
P (mg dm ⁻³)	23.33 ± 11.0	7.00 ± 0.00	5.67 ± 0.65	3.07
K ⁺ (cmol _c dm ⁻³)	0.79 ± 0.44	0.34 ± 0.28	0.18 ± 0.05	0.25
Ca ²⁺ (cmol _c dm ⁻³)	2.30 ± 0.28	2.38 ± 0.92	3.37 ± 0.45	1.50
Mg ²⁺ (cmol _c dm ⁻³)	2.35 ± 0.26	2.15 ± 0.91	2.67 ± 0.56	1.00
Na ⁺ (cmol _c dm ⁻³)	0.06 ± 0.04	0.11 ± 0.16	0.06 ± 0.03	0.05
Al ³⁺ (cmol _c dm ⁻³)	0.13 ± 0.14	0.53 ± 0.46	0.38 ± 0.41	0.85
H+Al (cmol _c dm ⁻³)	6.27 ± 1.60	7.55 ± 1.30	7.55 ± 1.30	6.41
Sand (g kg ⁻¹)	661 ± 42	657 ± 85	647 ± 73	645
Silt (g kg ⁻¹)	26 ± 18	55 ± 18	55 ± 12	25
Clay (g kg ⁻¹)	312 ± 60	288 ± 90	297 ± 84	330
Textural class	Clayey-sandy loam			

Mean ± 95 % confidence interval; pH in water (1:2.5); P, K and Na extracted by Mehlich-1; Ca, Mg and Al extracted by 1 mol L⁻¹; H+Al extracted by 0.5 mol L⁻¹ calcium acetate at pH 7.0. All determinations according to Ruiz (2005) and Embrapa (1999).

SIsvar 5.3 (Ferreira, 2008) for each cut separately. Correlation coefficients among the variables were calculated using Statistica 8.0 (Statsoft, 2008).

RESULTS AND DISCUSSION

Shoot dry mass was affected by N rates in all three cuts; maximum values were achieved by 137.5; 82.0 and 98.0 kg ha⁻¹ N in the first, second and third cut, respectively, and with maximum SNA values of 1.68, 1.78 and 1.92 g/plant of N in the first, second and third cut, respectively (Figure 1).

In most treatments, SDM increased from the first to the second cut, but not from the second to the third (Tables 2 to 4). This may be due to organic matter decomposition (Buso & Kliemann, 2003), since it was independent from fertilization and inoculation. This conjecture is strengthened

by a lower increase at higher N rates, congruent with lower dependency from soil organic matter decomposition. The soil organic carbon content (16.49 g kg⁻¹ ≈ 28.5 g kg⁻¹ of organic matter) is close to the limit of 30 g kg⁻¹ of organic matter considered high for soils in Pernambuco (Luz et al., 2002), and substantially higher than in the initial sampling, but with lower pH and P content (Table 1).

Significant differences (p<0.05) were found for all variables in the first cut (Table 2), in which only isolate UFRPE Cm243 (p>0.05) allowed SDM as high as that for plants fertilized with up to 120 kg ha⁻¹ N and 1.22 and 1.38 times higher than the recommended strain and uninoculated plants, respectively.

In plants inoculated with the UFRPE isolates UFRPE Cm244, 253, 677, and 153, SNC did not differ (p>0.05) from N-fertilized plants, and was higher than for isolate UFRPE Cm 243, probably due a dilution effect for the latter. Values of SNA of plants inoculated with UFRPE isolates UFRPE Cm243; 677; 323; 244; 81; 362; 321; 90; 88; 163, and 308 were significantly higher (p<0.05) than for the other strains and did not differ from plants receiving 30 kg ha⁻¹ N, but lower than for other N-fertilized treatments (Table 2).

There was a 32 % average SDM increase in the second cut, compared to the first (Tables 2 and 3), which was higher for most of the inoculated plants. This stronger effect for inoculated plants may be due to higher RDM, as observed by Xavier et al. (2005) for alfalfa plants inoculated with *Sinorhizobium meliloti* in a greenhouse experiment in Minas Gerais, Brazil. On the other hand, those authors did not observe the SDM increase shown in our data.

Average SDM for the 15 highest-yielding isolates (Table 3) was 48 % higher than observed in the uninoculated, unfertilized treatment, and SDM for the highest yielding isolate (UFRPE Cm 244), although not different from the others of the group, was 88 % higher than in the uninoculated, unfertilized treatment. Since there was no significant difference (p>0.05) between SNC among all treatments, plants with those isolates also had the highest SNA, and RE ranged from 82 to 131.5 %, similar to RE of N-fertilized plants. The only isolate with RE below 50 %, e.g. whose SDM was below 50 % of plants receiving 100 kg ha⁻¹ N, was UFRPE Cm603.

In contrast to these large increases in SNA due to inoculation, Soares et al. (2006) found that a single strain achieved SNA similar to that of plants receiving 70 kg ha⁻¹ N in common bean in a field experiment in Minas Gerais, Brazil, while for the remaining isolates, SNA did not differ from the unfertilized treatment, even when inoculated with a strain recommended for inoculant production.

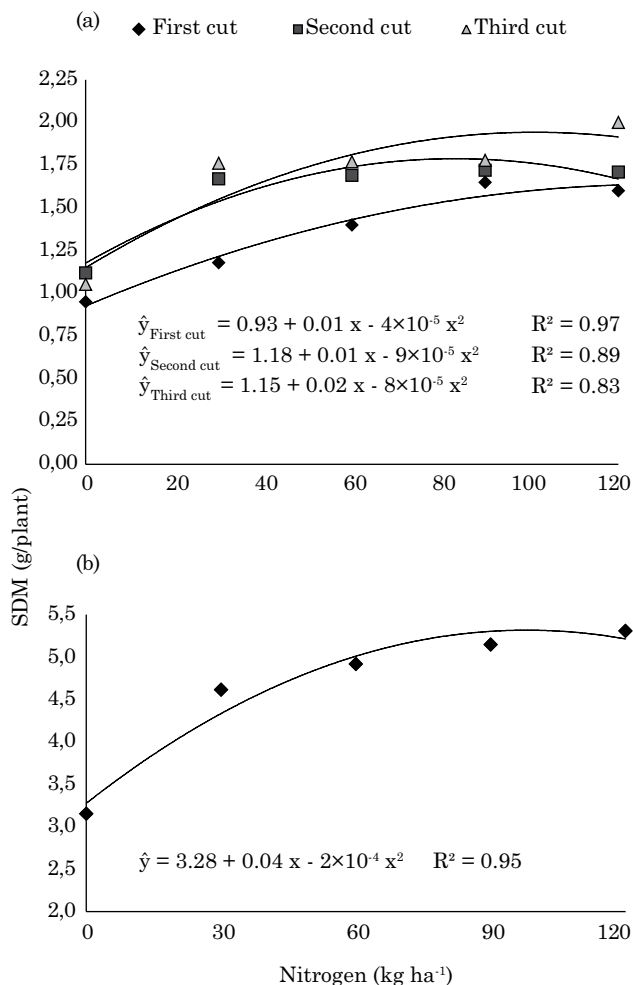


Figure 1. Shoot dry mass (SDM) of calopo plants by cuts (a) and accumulated in the three cuts (b) after three cuts in response to applied nitrogen.

Table 2. Shoot dry mass (SDM), shoot N concentration (SNC) and shoot N accumulation (SNA), relative efficiency (RE), and estimated equivalent N rate (END) in the first cut of calopo inoculated with isolated rhizobial previously obtained by calopo plant trap

Treatment	SDM	SNC	SNA	RE ⁽¹⁾	END ⁽²⁾
	g/plant	dag kg ⁻¹	mg/plant	%	kg ha ⁻¹
UFRPE Cm72	0.99 b	2.62 b	25.78 c	58.47 c	5.53 c
UFRPE Cm81	1.19 b	2.53 b	29.49 b	66.86 b	27.48 b
UFRPE Cm88	1.09 b	2.64 b	28.22 b	63.99 b	16.38 c
UFRPE Cm90	1.26 b	2.30 b	28.79 b	65.30 b	35.42 b
UFRPE Cm153	0.94 b	2.73 a	26.02 c	58.99 c	1.82 c
UFRPE Cm155	1.10 b	2.36 b	26.27 c	59.56 c	18.14 c
UFRPE Cm163	1.09 b	2.54 b	28.11 b	63.75 b	16.59 c
UFRPE Cm243	1.31 a	2.58 b	33.89 b	76.85 b	41.28 b
UFRPE Cm244	1.01 b	3.00 a	30.18 b	68.43 b	7.43 c
UFRPE Cm253	0.95 b	2.76 a	25.58 c	58.01 c	2.42 c
UFRPE Cm308	1.12 b	2.52 b	28.07 b	63.64 b	19.16 c
UFRPE Cm309	0.98 b	2.54 b	25.03 c	56.76 c	4.89 c
UFRPE Cm321	1.08 b	2.60 b	28.81 b	65.32 b	16.03 c
UFRPE Cm323	1.21 b	2.58 b	30.53 b	69.22 b	29.95 b
UFRPE Cm326	1.06 b	2.27 b	24.83 c	56.29 c	13.46 c
UFRPE Cm362	1.22 b	2.39 b	29.43 b	66.75 b	31.63 b
UFRPE Cm363	1.05 b	2.35 b	24.84 c	56.32 c	12.93 c
UFRPE Cm504	1.08 b	2.40 b	26.37 c	59.80 c	16.48 c
UFRPE Cm556	0.89 b	2.55 b	23.14 c	52.47 c	-
UFRPE Cm603	1.00 b	2.49 b	24.58 c	55.69 c	7.04 c
UFRPE Cm677	1.11 b	2.75 a	30.71 b	69.63 b	18.60 c
UFRPE Cm683	0.99 b	2.48 b	24.88 c	56.42 c	6.75 c
UFRPE Cm799	1.02 b	2.34 b	23.23 c	52.67 c	9.66 c
UFRPE Cm1302	1.06 b	2.16 b	22.89 c	51.91 c	12.32 c
UFRPE Cm1535	0.95 b	2.56 b	24.36 c	55.25 c	2.02 c
SEMIA 6152 ⁽³⁾	1.07 b	2.61 b	27.74 b	62.90 b	14.60 c
CV (%)	17.14	12.62	16.21	16.21	

Letters in the same column followed by the same letter are not significantly different ($p < 0.05$) by Scott-Knott. ⁽¹⁾ RE: SNA for each pot/average SNA for the treatment with 60 kg ha⁻¹ N. ⁽²⁾ Estimated based on the SDM regression for the applied N treatments. ⁽³⁾ *Bradyrhizobium japonicum* strain recommended for Calopo production in Brazil.

Plants inoculated with UFRPE isolates (UFRPE Cm155; 244; 362; 504, and 1302) had the highest END values, with an average of 71.92 kg ha⁻¹ N, significantly higher ($p < 0.05$) than observed for plants receiving up to 120 kg ha⁻¹ N.

Constrasting to the SDM increase from the first to the second cut, there was a small (6 %) decrease from the second to the third, probably due to the flowering that occurred a few days after the initial

regrowth from the second cut. In the third cut, only isolates UFRPE Cm72; 81; 163; 321; 323; 556; 677 and 1302 did not differ from the SDM yields of N-fertilized plants (Table 4). There was no significant effect of inoculation on either RDM or NDM, most likely due to the high random variability observed in the experiment. On the other hand, this lack of significant difference in NDM, coupled with the increase in both SDM and SNA, indicates

Table 3. Shoot dry mass (SDM), shoot N concentration (SNC) and shoot N accumulation (SNA), relative efficiency (RE), and estimated equivalent N rate (END) in the second cut of calopo inoculated with isolated rhizobial previously obtained by calopo plant trap

Treatment	SDM	SNC	SNA	RE ⁽¹⁾	END ⁽²⁾
	g/plant	dag/kg	mg/plant	%	kg/ha
UFRPE Cm72	1.49 a	2.80 a	41.80 a	84.42 a	25.50 c
UFRPE Cm81	1.11 b	2.86 a	32.19 b	65.01 b	-
UFRPE Cm88	1.10 b	3.04 a	32.22 b	65.07 b	-
UFRPE Cm90	1.07 b	3.21 a	34.02 b	68.71 b	-
UFRPE Cm153	0.86 b	3.14 a	26.72 b	53.97 b	-
UFRPE Cm155	1.88 a	2.95 a	54.90 a	110.88 a	72.53 a
UFRPE Cm163	1.45 a	2.85 a	41.37 a	83.55 a	21.33 c
UFRPE Cm243	1.65 a	2.46 a	40.61 a	82.00 a	46.02 b
UFRPE Cm244	2.11 a	3.06 a	65.11 a	131.49 a	81.67 a
UFRPE Cm253	0.97 b	2.89 a	26.75 b	54.03 b	-
UFRPE Cm308	1.60 a	3.19 a	50.57 a	102.12 a	44.04 b
UFRPE Cm309	1.64 a	2.91 a	47.45 a	95.83 a	42.96 b
UFRPE Cm321	1.10 b	3.09 a	34.11 b	68.88 b	-
UFRPE Cm323	1.28 b	2.99 a	38.18 b	77.09 b	11.16 c
UFRPE Cm326	1.61 a	2.99 a	48.37 a	97.68 a	39.10 b
UFRPE Cm362	1.78 a	3.02 a	53.83 a	108.71 a	71.44 a
UFRPE Cm363	1.52 a	2.78 a	41.85 a	84.51 a	31.41 b
UFRPE Cm504	1.79 a	2.89 a	51.39 a	103.78 a	73.74 a
UFRPE Cm556	1.24 b	2.98 a	37.41 b	75.55 b	6.44 c
UFRPE Cm603	0.71 b	3.17 a	22.84 b	46.12 b	-
UFRPE Cm677	1.37 a	3.16 a	43.58 a	88.01 a	16.34 c
UFRPE Cm683	0.85 b	3.32 a	28.07 b	56.70 b	-
UFRPE Cm799	1.68 a	2.80 a	46.49 a	93.88 a	49.97 b
UFRPE Cm1302	1.80 a	2.60 a	46.37 a	93.65 a	60.20 a
UFRPE Cm1535	1.51 a	2.85 a	42.41 a	85.65 a	30.20 b
SEMIA 6152 ⁽³⁾	1.41 a	2.49 a	34.48 b	69.63 b	17.66 c
CV (%)	14.92	9.99	16.79	16.79	

Letters in the same column followed by the same letter are not significantly different ($p < 0.05$) by Scott-Knott. ⁽¹⁾ RE: SNA for each pot/average SNA for the treatment with 60 kg ha⁻¹ N. ⁽²⁾ Estimated based on the SDM regression for the applied N treatments. ⁽³⁾ *Bradyrhizobium japonicum* strain recommended for Calopo production in Brazil.

a higher BNF efficiency for the isolates used in the experiment, compared to the natively occurring, and may also imply that they are at least as competitive as those naturally found in the soil, otherwise this effect would not be observed.

Plants inoculated with isolates UFRPE Cm 72; 163; 323 and 677 had the highest SNA, and RE over 90 %, not differing ($p > 0.05$) from N-fertilized plants. Six other isolates (UFRPE Cm81; 253; 321; 556; 603 and 683) also increased SNA with the cuts,

and only isolates UFRPE Cm88 and 1535 had RE values below 50 % (Table 4).

Oliveira et al. (1996), working with calopo, cunhã, stylo and tropical kudzu, all grown in monoculture or intercropped with one of two grasses in a greenhouse, similarly found increased SNA up to the third cut, with a decline after the third, in 8 of 13 treatments.

Plants inoculated with UFRPE Cm 677 did not differ ($p > 0.05$) from plants receiving 120 kg ha⁻¹ N,

Table 4. Shoot (SDM), root (RDM) and nodule (NDM) dry mass, shoot N concentration (SNC) and shoot N accumulation (SNA), relative efficiency (RE) and estimated equivalent N rate (END) in the third cut of calopo inoculated with isolated rhizobial previously obtained by calopo plant trap

Treatment	SDM	RDM	NDM	SNC	SNA	RE ⁽¹⁾	END ⁽²⁾
	g/plant		mg/plant	dag/kg	mg/plant	%	kg/ha
UFRPE Cm72	1.74 a	0.98 b	97.00 a	2.92 a	50.92 a	104.03 a	51.82 b
UFRPE Cm81	1.43 a	0.89 b	80.50 a	2.64 a	38.04 b	77.88 b	24.01 c
UFRPE Cm88	0.78 b	0.51 b	34.33 a	2.54 a	19.80 b	40.53 b	-
UFRPE Cm90	0.90 b	0.44 b	36.00 a	2.84 a	25.39 b	51.98 b	-
UFRPE Cm153	0.95 b	0.41 b	56.67 a	2.27 a	21.55 b	44.11 b	-
UFRPE Cm155	0.99 b	0.79 b	35.56 a	2.96 a	29.36 b	60.09 b	-
UFRPE Cm163	1.68 a	0.73 b	105.50 a	2.74 a	45.90 a	93.97 a	42.69 b
UFRPE Cm243	1.27 b	0.83 b	79.50 a	2.74 a	35.27 b	72.20 b	8.38 c
UFRPE Cm244	1.18 b	0.70 b	59.50 a	2.86 a	33.52 b	68.61 b	1.90 c
UFRPE Cm253	1.17 b	0.57 b	72.34 a	2.67 a	31.56 b	64.60 b	2.89 c
UFRPE Cm308	1.29 b	0.69 b	69.50 a	2.73 a	35.24 b	72.14 b	9.86 c
UFRPE Cm309	1.15 b	0.65 b	62.25 a	2.99 a	34.26 b	70.12 b	0.58 c
UFRPE Cm321	1.48 a	0.62 b	92.00 a	2.74 a	40.03 b	81.95 b	23.84 c
UFRPE Cm323	1.76 a	0.77 b	85.00 a	2.77 a	48.65 a	99.59 a	52.59 b
UFRPE Cm326	1.04 b	0.64 b	54.50 a	2.83 a	29.23 b	59.84 b	-
UFRPE Cm362	1.28 b	0.83 b	63.50 a	2.83 a	36.27 b	74.25 b	9.09 c
UFRPE Cm363	1.34 b	0.65 b	101.00 a	2.75 a	36.79 b	75.32 b	13.91 c
UFRPE Cm504	1.16 b	0.70 b	47.67 a	2.60 a	29.22 b	59.82 b	2.46 c
UFRPE Cm556	1.55 a	0.67 b	92.00 a	2.45 a	38.27 b	78.34 b	31.24 c
UFRPE Cm603	1.37 b	0.44 b	89.00 a	2.79 a	38.14 b	78.08 b	16.06 c
UFRPE Cm677	1.96 a	0.91 b	133.25 a	2.98 a	58.68 a	120.12 a	91.09 a
UFRPE Cm683	1.09 b	0.52 b	80.00 a	2.71 a	29.57 b	60.52 b	-
UFRPE Cm799	1.32 b	0.77 b	95.17 a	2.36 a	30.92 b	63.28 b	12.42 c
UFRPE Cm1302	1.47 a	0.91 b	82.78 a	2.60 a	37.47 b	76.71 b	24.19 c
UFRPE Cm1535	0.89 b	0.53 b	45.83 a	2.70 a	24.37 b	49.88 b	-
SEMIA 6152 ⁽³⁾	1.26 b	0.97 b	45.00 a	2.54 a	32.26 b	66.03 b	8.12 c
CV (%)	13.33	30.05	54.31	11.71	14.84	14.84	

Letters in the same column followed by the same letter are not significantly different ($p < 0.05$) by Scott-Knott. ⁽¹⁾ RE: SNA for each pot/average SNA for the treatment with 60 kg ha⁻¹ N. ⁽²⁾ Estimated based on the SDM regression for the applied N treatments. ⁽³⁾ *Bradyrhizobium japonicum* strain recommended for Calopo production in Brazil.

while isolates UFRPE Cm72; 163 and 323 had lower results, but were not different either from those receiving up to 90 kg ha⁻¹ N, while plants inoculated with the currently recommended SEMIA 6152 did not differ from unfertilized, uninoculated plants (Table 4).

Isolates UFRPE Cm72, 163, 243, 244, 323, 362, 677, and 1302 allowed higher total SDM after three cuts, not differing ($p > 0.05$) from the plants receiving 30 kg ha⁻¹ N, although only UFRPE Cm 244 and

677 did not differ from the 30 kg ha⁻¹ N treatment in SNA and RE after the third cut (Table 5).

Shoot dry mass, after each cut (Figure 1a) and accumulated after three cuts (Figure 1b), was affected by applied N, with the highest SDM after three cuts achieved by 104 kg ha⁻¹ N at 5.44 g/plant.

The isolates UFRPE Cm244 and 677 were generally effective, and their average SNA was around 83 and 85 % of that accumulated by plants receiving 120 kg ha⁻¹ N. These effects are similar

Table 5. Average shoot dry mass (SDM), shoot N accumulation (SNA) and relative efficiency (RE) of calopo rhizobial isolates after three cuts

Treatment	SDM	SNA	RE ⁽¹⁾
	g/plant	mg/plant	%
UFRPE Cm72	4.22 b	118.50 c	83.18 c
UFRPE Cm81	3.74 d	99.72 d	69.99 d
UFRPE Cm88	2.96 e	80.23 e	56.32 e
UFRPE Cm90	3.22 e	88.21 e	61.91 e
UFRPE Cm153	2.75 e	74.28 e	52.14 e
UFRPE Cm155	3.97 c	110.53 d	77.58 d
UFRPE Cm163	4.22 b	115.39 c	80.99 c
UFRPE Cm243	4.22 b	109.76 d	77.04 d
UFRPE Cm244	4.30 b	128.81 b	90.41 b
UFRPE Cm253	3.09 e	83.89 e	58.89 e
UFRPE Cm308	4.01 c	113.87 c	79.93 c
UFRPE Cm309	3.76 d	106.73 d	74.92 d
UFRPE Cm321	3.66 d	102.95 d	72.26 d
UFRPE Cm323	4.24 b	117.35 c	82.37 c
UFRPE Cm326	3.70 d	102.43 d	71.89 d
UFRPE Cm362	4.28 b	119.53 c	83.90 c
UFRPE Cm363	3.90 c	103.48 d	72.63 d
UFRPE Cm504	4.02 c	106.98 d	75.09 d
UFRPE Cm556	3.68 d	98.81 d	69.36 d
UFRPE Cm603	3.08 e	85.54 e	60.04 e
UFRPE Cm677	4.45 b	132.96 b	93.33 b
UFRPE Cm683	2.93 e	82.52 e	57.92 e
UFRPE Cm799	4.01 c	100.62 d	70.63 d
UFRPE Cm1302	4.33 b	106.74 d	74.92 d
UFRPE Cm1535	3.34 e	91.14 e	63.97 e
SEMIA 6152	3.73 d	94.48 d	66.31 d
CV (%)	7.44	7.88	7.88

Letters in the same column followed by the same letter are not significantly different ($p < 0.05$) by Scott-Knott. ⁽¹⁾ RE: SNA per pot/average SNA for the treatment with 60 kg ha⁻¹ N.

to those found by Fontoura et al. (2011) who found that inoculated *Lotus glaber* plants, cultivated in Leonard jars, accumulated similar levels of N as plants fertilized with 100 kg ha⁻¹ N.

Inoculation with isolates UFRPE Cm362 and 677 was equivalent to supplying 112 and 126 kg ha⁻¹ N,

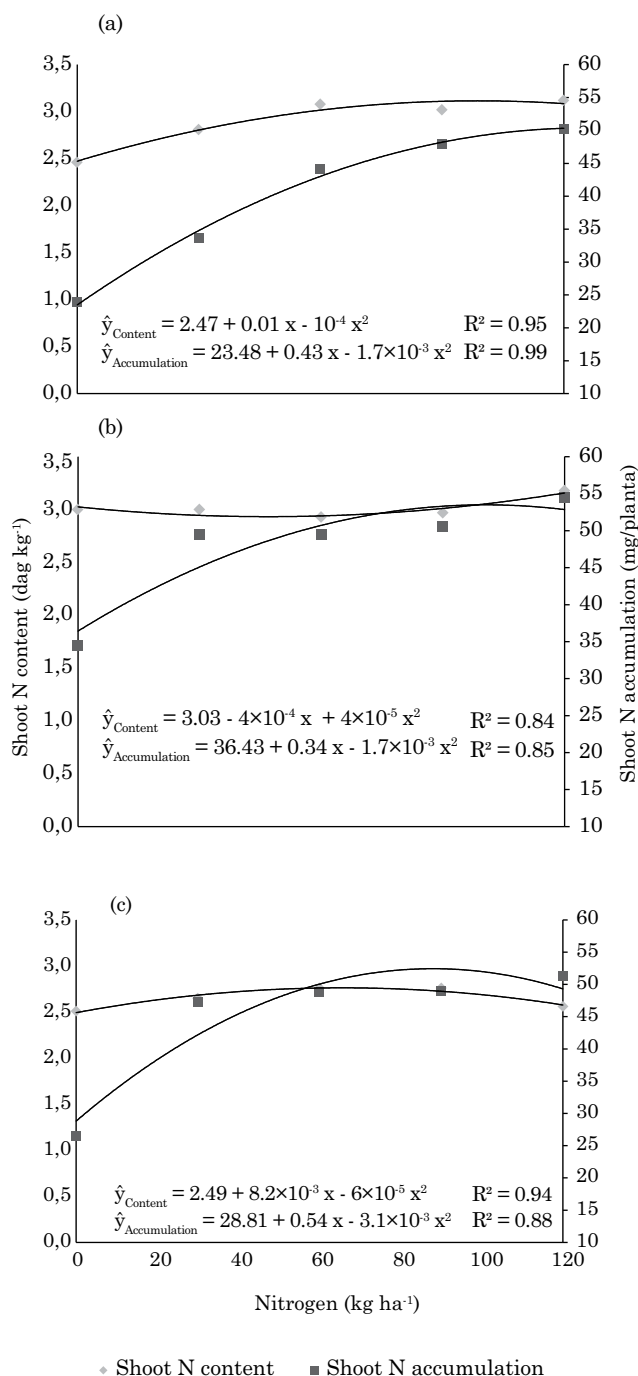


Figure 2. Shoot N content (SNC) and shoot N accumulation (SNA) of calopo plants in the first (a), second (b) and third (c) cuts in response to N fertilization rates.

respectively (Tables 2 to 4), over the three cuts, which would be the maximum N fertilizer rate recommended for Pernambuco, where average annual rainfall is at least 1,200 mm yr⁻¹ (IAP, 2008). Assuming an average N fertilizer cost of R\$ 2.79 kg⁻¹ of N (CONAB, 2012), inoculation with these strains would cost R\$ 313.00 and R\$ 352.00, per hectare,

Table 6. Shoot dry mass (SDM), shoot N content (SNC) and shoot N accumulation (SNA); relative efficiency (RE) and estimated N rate (END) for the first, second and third cut; and root (RDM) and nodule dry masses (NDM) for the third cut for Calopo plants grown on an unsterilized Argissolo Vermelho-Amarelo in the Zona da Mata Seca, Pernambuco

	First cut				Second cut				Third cut					
	SNC	SNA	RE	END	SNC	SNA	RE	END	SNC	SNA	RE	END	RDM	NDM
SDM	0.18*	0.80*	0.80*	0.93*	-0.32*	0.93*	0.93*	0.95*	0.08 ^{ns}	0.92*	0.92*	0.97*	0.60*	0.65*
SNC	-	0.72*	0.72*	0.24*	-	0.04 ^{ns}	0.04 ^{ns}	-0.31*	-	0.45*	0.45*	0.10 ^{ns}	0.05 ^{ns}	0.03 ^{ns}
SNA	-	-	1.00*	0.80*	-	-	1.00*	0.87*	-	-	1.00*	0.91*	0.54*	0.59*
RE	-	-	-	0.80*	-	-	-	0.87*	-	-	-	0.91*	0.54*	0.59*
END	-	-	-	-	-	-	-	-	-	-	-	-	0.67*	0.61*
RDM	-	-	-	-	-	-	-	-	-	-	-	-	-	0.26*

^{ns} and *: not significant ($p>0.05$) and significant ($p<0.05$) correlation coefficient, according to Student's t test.

respectively, aside from the non-financial advantages of substitution of N fertilizer.

Figure 2 presents shoot N concentration and accumulation for the three cuts, in the N-fertilized treatments. A quadratic regression was found for all situations, with high, significant determination coefficients. In the first cut, maximum SNC would have been achieved with 65 kg ha⁻¹ N, while maximum SNA would be extrapolated to 126 kg ha⁻¹ N. For the following cuts, SNC was not importantly affected by the N rate, while the effects of N application on SNA continued strong, but maximum SNA was estimated to occur with 100 and 87 kg ha⁻¹ N for the second and third cut, perhaps due to increased BNF, or increased organic matter cycling.

There were positive and significant correlations for most variables in all cuts (Table 6), with the highest correlation coefficients for SDM and SNA, RE and END; SNA and RE, as was expected due to their calculation formulas. On the other hand, SDM was negatively correlated with SNC and SNC with END, most likely indicating a dilution effect.

Positive and significant correlations are frequently found between SDM and SNA in inoculation studies, which is one of the bases for strain selection for inoculant production (Nascimento et al., 2010; Antunes et al., 2011), particularly in greenhouse experiments.

CONCLUSION

The currently recommended rhizobial strain was not as effective as strains UFRPE Cm 244 and 677 in the accumulation of shoot dry mass and shoot N content over three cuts. Inoculation with these two UFRPE isolates did not differ from the application of 30 kg ha⁻¹ N, but exceeded 90 % of the values achieved by plants fertilized with 60 kg ha⁻¹ N, indicating potential use for inoculant production.

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