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Symbiotic efficiency of native rhizobia in legume tree *Leucaena leucocephala* derived from several soil classes of Brazilian Northeast region

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Abstract

The effective symbiotic activity of rhizobia can contribute increment of biological nitrogen fixation (BNF), which is of great importance in low technology regions. This study aimed to evaluate the symbiotic efficiency of native rhizobia from Brazilian Northeast soils for selection of the most efficient and competitive strains for *Leucaena leucocephala* plant. The greenhouse test was conducted in factorial scheme (7x3), with seven fertilization treatments and three soil classes (Luvisol, Ultisol and Oxisol). The fertilization treatments were: (a) four native rhizobia isolates, obtained from nodules of *L. leucocephala* grown in Brazilian semiarid soils; (b) recommended strain (SEMIA 6069); (c) control without rhizobia inoculation and without N fertilization; (d) control without rhizobia, but with N fertilization (100 mg kg^{-1}). The plants were harvested at 90 days and the following characteristics were determined: plant height, diameter, shoots dry matter, roots dry matter and nodules dry matter, number of nodules, nitrogen concentration and accumulation in shoots, and efficiency of nitrogen fixation. The isolates 36F and 45G are very promising on Biologic Nitrogen Fixation (BNF). They showed high symbiotic efficiency (higher than 90%). These selected strains provided the best characteristics such as plant height (57 and 52 cm), diameter (4.03 and 4.02 mm), dry matter of shoot (3.2 and 3.6 g $plant^{-1}$) and nitrogen accumulation in shoots (60 and 65 mg plant-1).

Keywords: Biological nitrogen fixation, inoculants, sustainable agriculture, strain selection, symbiosis.
Abbreviations: BNF_Biological nitrogen fixation**;** cfu_colony forming units**;** FACEPE_Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco.

Introduction

The adequate management of biological nitrogen fixation (BNF) is an important strategy to promote the sustainability and food security in many tropical regions. In general, in semiarid regions such as those found in the Brazilian Northeast, the soils have low available N. Usually, these agricultural systems do not use mineral fertilizers and predominated N-dependent crops are produced from the mineralization of organic matter and the BNF process (Freitas et al., 2010, Martins et al., 2015). Naturally, after successive agricultural cropping, the soil may become depleted, degraded, and finally abandoned for regenerations of the natural vegetation. In these conditions the soil restoration is necessary, especially for enrichment of nutrients, mainly nitrogen that needs a faster recovery of the vegetation (Silva et al., 2017).

In these sites, the process of BFN is the main form to supply N enrichment of the soil (Freitas et al., 2015). However, a viable and sustainable alternative for agricultural systems and rehabilitation of degraded areas of the semiarid region is the planting of legumes with the ability to associate symbiotically with nitrogen-fixing bacteria (Yuan et al., 2016). These legumes can be used in the agroforestry systems, mainly in the soils with low nitrogen content, to produce high quality forage for animal feed, wood and firewood, live fences, shade trees for planting crops and green manure (Freitas et al., 2011; Estringana et al., 2013; Martins et al., 2015).

The success for introduction of tree legumes in the semiarid region depends on the presence and extent of native rhizobia that have ability to establish more effective symbiosis due to the soil and local adaptation (Silva et al., 2016). The tree legume *L. leucocephala* can associate with a wide range of native rhizobia strains. In these areas effective nodulation is generally observed. In some cases the nodules formation are occurred but the symbiosis may be not effective (Faye et al., 2007, Silva et al., 2017). The contribution of natural fixed N due to symbiosis with native rhizobia has revealed varied rates of fixation in tree legumes, demonstrating the variability in the efficiency of indigenous rhizobia. This fact evidences the need of specific researches to study the process of effective inoculation and to observe the competitive behavior or the native strains to optimize the process (Souza et al., 2012; Silva et al., 2017).

In recent studies, selection of effectiveness and competitiveness of rhizobia have been carried out in several herbaceous legumes in Brazilian semiarid soils, especially peanut (Sizenando et al., 2016), lima bean (Antunes et al., 2011) and cowpea (Xavier et al., 2017). However, few studies were conducted to evaluate the effectiveness of native isolates in nodulation of tree legume (*Erythrina velutina* Willd.) (Menezes et al., 2017).

Thus, the objective of this study was to evaluate the symbiotic efficiency of native rhizobia from the Brazilian Semiarid region for inoculation of *Leucaena leucocephala* (Lam.) grown in different soil types.

Results

Height and shoot diameter of Leucaena leucocephala

The seedlings of *L. leucocephala* were developed normally and the survival rate was 100% under all treatments. At 30 days after sowing, the seedlings presented uniformity and no differences were observed among treatments. The mean height of plant recorded as 14.9 cm (Table 3). At this stage, the height varied from 14.1 to 15.7 cm. At 60 days after sowing, the *L. leucocephala* presented an average height of 28.2 cm. Also, no significant difference of rhizobia inoculation was observed. However, at 90 days, influence of rhizobia inoculation was observed. The higher height with the isolates 36F and 45G, presented growth of 57 and 52 cm that were higher than absolute control and mineral N control (Table 1).

Regarding the shoot diameter, all treatments were similar at 30 days of sowing, with a mean diameter of 1.32 mm. At 60 days of growth, the seedlings also did not showed response to rhizobia inoculation, and showed an average diameter of 2.44, mm. On the other hand, at 90 days, the treatment with rhizobia inoculation influenced the diameter. The best responses received from isolates 36F, 43K and 45G, that presented shoot diameter of 4.03; 4.11 and 4.02 mm, respectively, similar to the treatment with mineral nitrogen.

Nodulation and root biomass of Leucaena leucocephala

The plants of *Leucaena leucocephala* showed an average of 23 nodules, indicating no difference to the treatment without rhizobia inoculation (Table 2). The nodules biomass was influenced by the treatment with rhizobia inoculation, and the isolate 45G provided the highest biomass (47 mg plant^{-1}). No significant differences were observed in the dry matter of roots, suggesting that the isolates did not influence the root growth of *Leucaena leucocephala*. The dry matter of roots showed an average of 2.29 g plant^{-1} (Table 2).

N accumulation and symbiotic efficiency

The effect of inoculation was not observed on the nitrogen contents in the aerial biomass of the two legumes. However, due to the great difference in the shoot biomass, there was a significant effect on the total N accumulation of nitrogen in shoots (Table 3), especially when inoculated with the isolates 36F and 45G. These results showed the effectiveness of FBN that was higher than 90%, and did not differ to the treatments with mineral nitrogen. Positive and significant correlations were obtained with the highest correlation coefficients obtained from shoot dry matter and total nitrogen accumulated in the shoots (Table 4).

Discussion

The height and diameter of plants have been recently used for evaluation of symbiotic efficiency of rhizobia in tree legumes (Ceccon et al., 2012; Ramos et al., 2013; Primieri et al., 2016; Menezes et al., 2017). These parameters indicate the quality of the seedlings, the potential of survival and growth after transplanting. There are usually more vigorous and resistant plants in the field and due to these characteristics (Caione et al., 2012).

In the present study, we verified that the inoculation practice allowed the plants to obtain greater growth in height and diameter, compared to non-inoculated plants (control), or those contained rhizobia but did not receive N fertilizer. However, significant contrasts were only observed in the results obtained at 90 days of growth. The absence of significant differences in the first two evaluations may be correlated with the time needed to establish the symbiosis. Normally, some tree legumes require 20 to 30 days to show evidence of root nodules, and this fact delayed the BNF process (Mendes et al., 2013).

The existence of compatible native rhizobia populations was demonstrated by the natural nodulation in the not inoculated soils. The rhizobia population varied in size according to the soil which evidenced the differences in nodulation between the treatments. Many studies have shown that nodulation is affected by several factors such as type of specific rhizobia bacteria and the climatic conditions (Lira Junior et al., 2015). The symbiosis cannot be established in the absence of native rhizobia populations capable to nodulate certain legume species. Generally, populations of rhizobia capable of nodulation the specific native legumes are abundant in soils of regions, where the species are native (Bala et al., 2003). However, *L. leucocephala* is an exotic legume species, native to Central America, and yet has nodulation in all soils, possibly due to the high promiscuity of this species, which is able to establish symbiosis with rhizobia of the genus *Rhizobium* (Pereyra et al., 2015), *Mesorhizobium* (Rangel et al., 2016), *Sinorhizobium* (Xu et al., 2014), *Bradyrhizobium* (Wang et al., 2006), and *Cupriavidus* (Florentino et al., 2009),

Inoculation with native isolates of *Rhizobia* has significantly increased shoot biomass and symbiotic effectiveness compared to the recommended and non-inoculated plants. The accumulation of nitrogen in shoot dry matter is normally used to estimate the symbiotic efficiency of nitrogen fixation of the native rhizobia according to Lima et al. (2012) and Calheiros et al. (2015). This method is easy to use and is relatively inexpensive. It is especially more suitable for use in soils with low nitrogen content (Rondon et al., 2007). The obtained results in our study showed that the native isolates presented symbiotic efficiency and better performance, ranging from 92 to 105%, in accordance with the reported by above mentioned authors. The best performance of native isolates compared with the recommended strains and the commercial inoculants has been reported in the literature (Calheiros et al., 2015) with calopogonium species, using isolates native to Northeast Brazil (84 to 131%). Menezes et al. (2017) reported the

Table 1. Height and diameter of *L. leucocephala* seedlings grown in pots with the different soils in response to rhizobia inoculation at 30, 60 and 90 days after planting date.

	Days after planting date		
Isolates	30	60	90
	Plant height (cm)		
25A	15.4^{a} ± 3.7	27.5^{a} ± 7.6	47.0^{b} ± 17.9
36F	14.1^{a} ± 3.0	29.6^{a} ± 6.3	57.2^{a} ± 9.1
43K	14.3^{a} ±3.6	26.2^{a} ± 6.5	47.8^{b} ± 11.1
45G	15.5^{a} ± 3.7	31.4^{a} ± 5.7	52.6^{a} ± 9.2
SEMIA 6069	15.3^{a} ± 2.2	28.8^{a} ± 4.9	45.6^{b} ± 10.9
N mineral fertilizer	15.7^{a} ± 3.3	29.3^{a} ± 4.4	47.8^{b} ± 15.3
Control without N	14.2^{a} ± 1.3	24.6^{a} ± 2.3	42.7^{b} ± 6.7
Average	14.9	28.2	47.2
CV (%)	12.8	18.16	14.6
	Plant diameter (mm)		
25A	1.21^{a} ± 0.11	2.25^{a} ± 0.18	3.44^{b} ± 0.17
36F	1.32^{a} ± 0.06	2.42^{a} ± 0.12	4.03^{a} ± 0.18
43K	1.36^{a} ± 0.10	2.45^{a} ± 0.17	4.11^{a} ± 0.55
45G	1.29^{a} ± 0.13	2.55^{a} ± 0.23	4.02^{a} ± 0.17
SEMIA 6069	1.34^{a} ± 0.04	2.44^{a} ± 0.09	3.36^{b} ± 0.12
N mineral fertilizer	1.34^{a} ±0.15	2.62^{a} ± 0.12	4.01^{a} ± 0.43
Control without N	1.37^{a} ± 0.15	2.39^{a} ± 0.07	3.64^{b} ± 0.14
Average	1.32	2.44	3.79
CV (%)	10.5	9.46	7.85

*Data with the same letter are not significantly different for Scott-Knott (p<0.05).

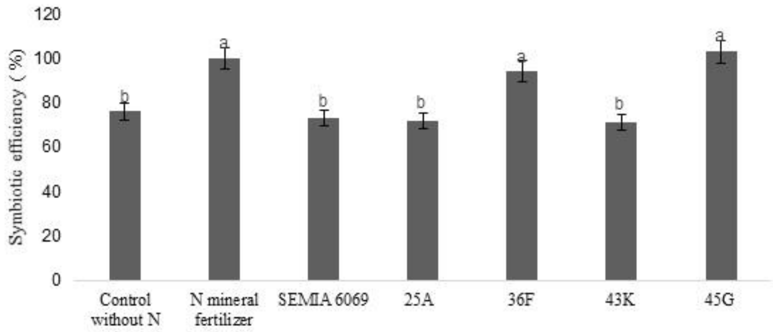

Fig 1. Relative effectiveness of the rhizobia native. The means were compared by the Scott-Knott test (p<0.05).

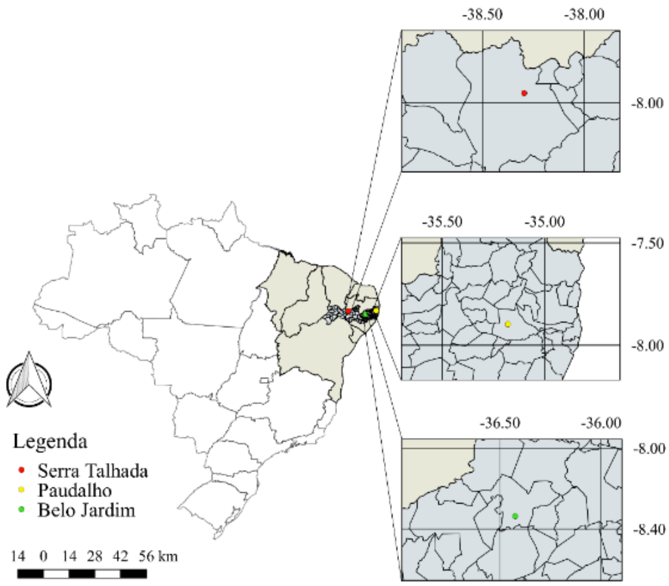

Fig 2. Localities where are collected the soil samples used in the greenhouse experiments

Table 2. Nodules number, dry biomass of nodules of *L. leucocephala* grown in different soils (Ultisol, Oxisol and Luvisol) from Brazilian Northeast, in function of rhizobia inoculation.

	Soil class				Soil class			
Isolate	Ultisol	Oxisol	Luvisol	average	Ultisol	Oxisol	Luvisol	average
	--------Number of nodules -------------				---- Dry biomass of nodules (mg plant^{-1})			
25A	21^{A}ª ± 15	36^{A}ª ± 18	11^{A}ª ± 5	23ª ±12	39^{A}ª ± 13	35^{A}ª ± 12	37^{A}ª ± 15	37^{b} ± 02
36F	18^{A}ª ± 9	33^{A}ª ± 14	12^{A}ª ± 6	21ª ± 11	27^{A}ª ± 05	36^{A}ª ± 08	33^{A}ª ±0 8	32^{b} ± 04
43K	26^{A}ª ± 13	33^{A}ª ± 31	13^{A}ª ± 7	24ª ± 10	33^{A}ª ± 12	42^{A}ª ± 26	19^{A}ª ± 18	31^{b} ± 12
45G	31^{A}ª ± 08	42^{A}ª ± 18	16^{A}ª ± 2	30ª ± 13	55^{A}ª ± 06	38^{A}ª ± 12	47^{A}ª ± 03	47^{a} ± 09
SEMIA 6069	26^{A}ª ± 10	27^{A}ª ± 18	14^{A}ª ± 3	22ª ± 8	38^{A}ª ± 04	39^{A}ª ± 08	21^{A}ª ± 3	33^{b} ± 10
N mineral fertilizer	21^{A}ª ± 14	20^{A}ª ± 15	10^{A}ª ± 14	17ª ± 6	29^{A}ª ± 16	26^{A}ª ± 13	11^{A}ª ± 10	22^{b} ± 10
Control without N	24^{A}ª ± 9	30^{A}ª ± 7	26^{A}ª ± 7	27ª ± 3	30^{A}ª ± 13	39^{A}ª ± 11	35^{A}ª ± 13	35^{a} ± 5
Average	24 A ± 4	32 A ± 7	14 B ± 5	23	32A ± 10	34A ± 05	36A ± 13	34
CV (%)	30.78				35.16			
	Biomass of roots (g plant^{-1})				Biomass of shoots (g plant^{-1})			
25A	2.8 ^{A}ª ±0.5	2.0 ^{A}ª ±0.3	2.6 ^{A}ª ± 0.4	2.4ª ± 0.2	2.8 ^{bA} ± 0.4	2,0 ^{bA} ± 0.3	2,6 ^{bA} ±0.3	2,5 ^{b} ± 0.4
36F	2.6 ^{A}ª ± 0.5	2.2 ^{A}ª ± 0.5	2,6 ^{A}ª ± 0.7	2.5ª ± 0.2	3.7 ^{aA} ± 0.3	2.7 ^{aB} ± 0.4	3.1 ^{aB} ± 0.1	3.2 ^{a} ± 0.5
43K	2.5 ^{A}ª ± 0.2	2.0 ^{A}ª ± 0.1	2.1 ^{A}ª ± 0.1	2.2ª ± 0.2	2.9 ^{bA} ± 0.2	1.9 ^{bB} ± 0.7	2.5 ^{bA} ± 0.2	2.4 ^{b} ± 0.5
45G	2.9 ^{A}ª ± 0.5	2.1 ^{A}ª ± 0.7	2.3 ^{A}ª ± 0.9	2.4ª ± 0.4	3.8 ^{aA} ± 1.0	3.3 ^{aA} ± 0.5	3.7 ^{aA} ±0.2	3.6 ^{a} ± 0.3
SEMIA 6069	3.3 ^{A}ª ± 0,5	1.8 ^{A}ª ± 0.2	1.9 ^{A}ª ± 0.8	2.3ª ± 0.8	2.7 ^{bA} ± 0.5	2.4 ^{bA} ± 0.3	2.5 ^{bA} ± 0.4	2.5 ^{b} ± 0.2
N mineral fertilizer	2.4 ^{A}ª ± 0.6	1.9 ^{A}ª ± 0.4	1.8 ^{A}ª ± 0.1	2.0^{a} ± 0.3	3.9 ^{aA} ±0.3	3.0 ^{aB} ± 0.3	3.6 ^{aA} ± 0.3	3.5 ^{a} ± 0.4
Control without N	2.9 ^{A}ª ± 0.1	2.0 ^{A}ª ± 0.4	1.4 ^{A}ª ± 0.7	2.1^{a} ± 0.8	2.9 ^{bA} ±0.4	2.1 ^{bA} ± 0.2	2.7 ^{bA} ±0.4	2.6 ^{b} ± 0.4
Average	2.7^{A} ± 0.3	2.0 ^{A} ± 0.14	2.1^{A} ± 0.4	2.3	3.3 ^{A} ± 0.5	2.5^{C} ± 0.5	2.9 ^{B} ± 0.5	2.9
CV (%)	22.08				14.56			

Data with same letter are not significantly different (capital letters compare the soils), and (low letters compare the fertilization treatments) for Scott-Knott ($p<0.05$).

Table 3. Concentration and total N accumulation in shoot dry biomass of *L. leucocephala* grown in different soils (Ultisol, Oxisol and Luvisol) from Brazilian Northeast, in function of rhizobia inoculation.

Isolate	Ultisol	Oxisol	Luvisol	Average
		Soil class		
	Concentration N in shoot dry matter (dag kg^{-1})			
25A	1.9^{Aa} ± 0.1	1.9^{Aa} ± 0.1	1.9^{Aa} ± 0.2	1.9^{a} ± 0.1
36F	1.9^{Aa} ±0.2	2.0^{Aa} ± 0.2	1.7^{Aa} ± 0.1	1.9^{a} ± 0.1
43K	2.0^{Aa} ± 0.1	1.7^{Aa} ± 0.1	1.9^{Aa} ±0.1	1.8^{a} ± 0.1
45G	1.8^{Aa} ± 0.2	1.8^{Aa} ± 0.2	1.8^{Aa} ±0.1	1.8^{a} ± 0.1
SEMIA 6069	1.9^{Aa} ± 0.1	1.8^{Aa} ± 1	1.9^{Aa} ± 0.1	1.8^{a} ± 0.1
N mineral fertilizer	1.9^{Aa} ± 0.1	1.8^{Aa} ± 0.2	1.8^{Aa} ± 0.2	1.8^{a} ± 0.1
Control without N	1.9^{Aa} ± 0.2	1.8^{Aa} ± 0.1	2.0^{Aa} ± 0.1	1.9^{a} ± 0.1
Average	1.9^{A} ± 0.1	1.8^{A} ± 0.1	1.8^{A} ± 0.1	1.9
CV (%)		8.6		
	Total N accumulated in shoot dry matter (mg $plant^{-1}$)			
25A	54^{Aa} ± 7	38^{Aa} ± 4	47^{Aa} ± 6	46^{b} ± 8
36F	71^{Aa} ±8	54^{Aa} ±7	55^{Aa} ± 3	60^{a} ± 10
43K	59^{Aa} ± 3	33^{Aa} ± 11	46^{Aa} ±5	46^{b} ± 13
45G	70^{Aa} ± 28	60^{Aa} ± 9	64^{Aa} ± 3	65^{a} ± 5
SEMIA 6069	49^{Aa} ± 14	42^{Aa} ± 4	47^{Aa} ± 9	46^{b} ± 4
N mineral fertilizer	73^{Aa} ± 8	55^{Aa} ± 7	64^{Aa} ± 2	64^{a} ± 9
Control without N	57^{Aa} ± 3	37^{Aa} ± 5	53^{Aa} ± 10	49^{b} ± 10
Average	62.2^{A} ±9	46^{C} ±11	54.3^{B} ± 8	54
CV (%)		17.02		

Data with same letter are not significantly different (capital letters compare the soils), and (low letters compare the fertilization treatments) for Scott-Knott ($p<0.05$).

Table 4. Correlation between growth parameters in the two tree legumes

Variables	Diameter	Number of nodules	Biomass of nodules	Biomass of shoots	Biomass of roots	N in shoot	N accumulated	Symbiotic efficiency
	Leucena							
Height	0.583^{*}	0.058^{ns}	0.300^{*}	0.669^{*}	0.240^{ns}	-0.048^{ns}	0.623^{*}	0.359*
Diameter		0.016^{ns}	0.085^{ns}	0.637^{*}	0.164^{ns}	-0.060^{ns}	0.584^{*}	0.427*
Number of nodules			0.701^{*}	0.149^{ns}	-0.233^{ns}	-0.105^{ns}	0.111^{ns}	0.292*
Biomass of nodules				0.263^{*}	-0.031^{ns}	-0.225^{ns}	0.187^{ns}	0.261*
Biomass of shoots					0.012^{ns}	-0.064^{ns}	0.935^{*}	0.789*
Biomass of roots						-0.050	-0.028^{ns}	-0.069^{ns}
N in shoot							0.285^{*}	0.189^{ns}
N accumulated								0.820*

Significant by the t test at 5% of probability.

the highest competitive potential among several rhizobia isolates by *Erythrina velutina*, which is native to Brazilian semiarid regions compared to the recommended strain. They observed a very positive effectiveness of the native rhizobia.

Based on the interval used by Lalande et al. (1990), it was evident from our study that the native isolates were highly efficient (> 80%) in effectiveness of biological nitrogen fixation. These results demonstrate the possibility of selecting strains of native rhizobia within the natural diversity, highly adapted to the Brazilian Semiarid region. These native rhizobia are able to establish effective symbiosis. The selection of such isolates is essential for the inoculation of tree legumes because they contribute to increase the amount of fixable N_2. It is very important practice to N enrichment in places where the soils have low total N content and organic matter. Normally, in these areas the use of mineral fertilizer is inaccessible to most farmers.

Materials and methods

Experimental conditions

The greenhouse experiment was conducted for evaluation of symbiotic efficiency of native rhizobia on growth of tree legume *Leucaena leucocephala* in Brazilian Semiarid soils. The native rhizobia were isolated from nodules of *L. leucocephala* grown in various Brazilian Semiarid soils (Silva et al. 2016). In the present experiment, the seedlings of *L. leucocephala* were grown in pots (2 dm^{-3}) using superficial layer (0-20 cm) of the Brazilian Northeast, collecting soils of different classes (Luvisol, Ultisol and Oxisol) from agricultural areas (Fig 2). The soil samples were air dried, sieved (to 5 mm) and applied to the relative pots. In order to preserve the original conditions of the soils, no limestone (for acidity correction) and fertilizer (as nutrient) was added.

Table 5. Attributes of the soil and characteristics of the sites of samples collection.

	Soil Classes		
Characteristics	Luvisol	Ultisol	Oxisol
pH (H_2O)	6.76 ± 0.12	6.28 ± 0.29	5.56 ± 0.32
Total C (g kg^{-1})	7.7 ± 2.4	8.4 ± 0.9	3.0 ± 0.5
Available P (mg dm^3)	106 ± 13.8	69 ± 11.7	8 ± 2.2
Available K^+	292 ± 58	284 ± 42	135 ± 7.5
Exchangeable Ca^{2+} ($cmol_c$ dm^3)	2.85 ± 0.19	2.04 ± 0.92	1.34 ± 7.5
Exchangeable Mg^{2+} ($cmol_c$ dm^3)	0.86 ± 0.23	0.89 ± 0.21	0.58 ± 0.12
Exchangeable Al^{3+} ($cmol_c$ dm^3)	0.0 ± 0.0	0.0 ± 0.0	0.11±0.03
H+ Al ($cmol_c$ dm^3)	2.15 ± 0.47	2.70 ± 0.63	2.91 ± 0.15
Soil Density SD (g cm^3)	1.61 ± 0.04	1.55 ± 0.01	1.31 ± 0.07
Particles density Dp (g cm^3)	2.60 ± 0.03	2.64 ± 0.31	2.62 ± 0.22
Sand (g kg^{-1})	772 ± 25	713 ± 18	525 ± 25
Silt (g kg^{-1})	71 ± 23	98 ± 11	103 ± 10
Clay (g kg^{-1})	137 ± 5	189 ± 7	372 ± 19
Site of sample collection	Serra Talhada	Belo Jardim	Paudalho
Altitude (m)	429	608	86
Annual Rainfall (mm)	716	660	1239
Temperature	24	24	25

Table 6. Phenotypic characteristics of *L. leucocephala* rhizobia isolated from Brazilian Semiarid soils.

Isolates	Soil	Land use system	Phenotypic characteristic*		
			TC	pH	Color
Isolates of nodules of *Leucaena leucocephala* (Leucena)					
25B (*Ensifer*)	Luvisol	Caatinga	Fast	Acid	Cream
36F (*Rhizobium*)	Ultisol	Agriculture	Fast	Acid	Cream
43K (*Ensifer*)	Luvisol	Caatinga	Fast	Acid	Cream
45G (*Mesorhizobium*)	Luvisol	Agriculture	Fast	Acid	Cream

* Growth period; pH modification in culture (YMA medium) and color of the colony.

The samples were submitted to analyses (Donagema et al., 2011) and the results are shown in Table 5. Seeds of the *L. leucocephala* were mechanically scarified and subsequently disinfested in 70% alcohol (1 min) and 2% sodium hypochlorite (5 minutes), and then washed with sterilized water. The seeds were sown directly in to the soil pot (4 seeds pot^{-1}). Ten days after sowing only one seedling were left in each pot. The inoculation was performed according to Menezes et al. (2017), pipetting 1 ml (10^9cfu/ml) of the respective rhizobia isolates for seed. The growth of *L. leucocephala* was evaluated by measuring the height and the diameter of the shoots, using a ruler and a digital caliper, respectively. The plants were evaluated at 30, 60 and 90 days after sowing, and the harvest was performed at 90 days. The number of nodules and the dry biomass of shoot, roots and nodules were determined after oven drying at 65ºC for 72 hours. Shoots nitrogen concentration was measured using Kjeldahl method (Silva, 2009), and the total accumulated N calculated by multiplying the nitrogen content and the respective biomass. Symbiotic efficiency was determined as previously described by (Calheiros et al., 2015), based on the relation between the total N accumulation in biomass of inoculated plants/non-inoculated control plants supplemented with nitrogen, and then converted into a percentage.

Experimental design

The experiment was conducted in factorial scheme (7x3) using a complete randomized block design, with four replicates for treatment. The fertilization treatments were: (a) four native isolates, obtained from nodules of *Leucaena leucocephala* grown in Brazilian Semiarid soils; (b) recommended strain for *L. leucocephala*; (c) control without rhizobia inoculation and without N fertilization; (d) control without rhizobia but with N fertilization (100 mg kg^{-1}) as ammonium nitrate. The treatments were applied in the three different soils (Luvisol, Ultisol and Oxisol).

The native rhizobia *L. leucocephala* were screened in previous assays based on their ability to fragment amplification of the symbiotic genes *nif*H and *nod*C, and because they presented greater dry biomass of shoots, in the previous works conducted in sterilized substrate (Silva, 2017). The native isolates were 25A, 36F, 43K and 45G. The recommended strain for *L. leucocephala* was SEMIA 6069 (*Bradyrhizobium elkanii*). The phenotypic characteristics of native rhizobia are shown in Table 6.

Statistical analysis

Results of plant height, shoot diameter, dry biomass of shoot, root and nodules, number of nodules, and shoot nitrogen accumulation were submitted to statistical analysis using the Sisvar computational program (Ferreira, 2011). The F-test data of nodules number were transformed to $(x + 1)^{1/2}$. The means were compared by the Scott-Knott test (p<0.05). The correlation coefficients between the studied variables were calculated by Statistica 8.0.

Conclusion

The rhizobia isolates 36F and 45G showed to be the most effective on the FBN process, and may represent an excellent strategy to increase the amounts of fixed N, especially in sites where the technological systems need to be increased. The results indicate the potential of the rhizobia native strains that are most effective and competitive and may be used to promote good growth of the used tropical tree legumes. The realization of field experiments may be necessary in terms to prove the potential of the rhizobia inoculants produced by native strains.

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