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Rhizobia inoculation and liming increase cowpea productivity in Maranhão State

Thiago Palhares Farias[1], André Trochmann[1], Bruno Lima Soares[2] and Fatima Maria Souza Moreira[1*]

*[1]Departamento de Ciências do Solo, Universidade Federal de Lavras, Cx. Postal 3037, 37200-000, Lavras, Minas Gerais, Brazil. [2]Departamento de Agricultura, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. *Author for correspondence. E-mail: fmoreira@dcs.ufla.br*

ABSTRACT. The objetives of the study were to evaluate the agronomic efficiency of strains approved as inoculants for cowpea and of three new strains under selection, as well as to evaluate the influence of liming on the symbiosis and productivity. Two field experiments were conducted using cowpea (BRS Guariba) from June to September 2013. In the first experiment, a completely randomized block experimental design was used, with four replicates and seven treatments (INPA03-11B, UFLA03-84, UFLA03-153, UFLA03-154, UFLA03-164 and two controls). In the second experiment, a 7 x 2 factorial design was used, with the above described treatments being the first factor and liming as the second factor. Parameters evaluated were: nodule dry matter, shoot dry matter, shoot N content and accumulation, number of pods per plant, number of seeds per pod, weight of 100 seeds, grain yield and grain N content and accumulation. Strains UFLA03-153 and UFLA03-164 were more efficient for cowpea inoculation than the strains approved as inoculants, UFLA03-84 and INPA03-11B. Although the tested rhizobia strains and cultivar BRS Guariba are tolerant to soil acidity, productivity washigher when soil was limed. Yields obtained with fertilized and inoculated treatments were well above Maranhão state average.

Keywords: *Vigna unguiculata*, *Bradyrhizobium*, acidity tolerance, efficiency, production system, biology nitrogen fixation.

Inoculação com rizóbio e calagem aumentam a produtividade de feijão-caupi no Maranhão

RESUMO. Os objetivos do estudo foram avaliar a eficiência agronômica de estirpes aprovadas como inoculantes para feijão-caupi e de três novas estirpes sob seleção, bem como avaliar a influência da calagem sobre a simbiose e a produtividade. Dois experimentos de campo foram conduzidos utilizando feijão-caupi (BRS Guariba) de junho a setembro de 2013. No primeiro experimento, o delineamento experimental utilizado foi em blocos ao acaso, com quatro repetições e sete tratamentos (INPA03-11B, UFLA03-84, UFLA03-153, UFLA03-154, UFLA03-164 e dois controles). No segundo experimento, um fatorial 7 x 2 foi utilizado, sendo o primeiro fator composto pelos tratamentos acima e a calagem o segundo fator. Os parâmetros avaliados foram: matéria seca de nódulos, matéra seca de parte aérea, teor e acúmulo de N na parte aérea, número de vagens por planta, número de grãos por vagem, peso de 100 sementes, rendimento de grãos e teor e acúmulo de N no grão. As estirpes UFLA03-153 e UFLA03-164 foram mais eficientes para a inoculação do feijão-caupi do que as estirpes aprovadas como inoculantes, UFLA03-84 e INPA03-11B. Apesar de as estirpes e da cultivar testadas serem tolerantes à acidez do solo, a produtividade de feijão-caupi foi maior quando o solo recebeu calagem. As produções obtidas com os tratamentos de inoculação fertilizados foram bem acima da média do estado do Maranhão.

Palavras-chave: *Vigna unguiculata*, *Bradyrhizobium*, tolerância a acidez, eficiência, sistema de produção, fixação biológica de nitrogênio.

Introduction

The expansion of cowpea [*Vigna unguiculata* (L.) Walp.] throughout Brazil has consolidated the importance of this crop at national level. Cowpea, in addition to being a traditional crop in northern and northeastern Brazil, is being integrated into the strategic plan for grain production in the Brazilian Cerrado (Freire Filho et al., 2011). Cowpea is grown throughout the State of Maranhão, in different production systems. This state is the fourth biggest producer of cowpea in Northeast Brazil, but it has low productivity indexes, estimated as 510 kg ha^{-1} (Instituto Brasileiro de Geografia e Estatística [IBGE], 2015). The crop is traditional in family-based agricultural systems but has been introduced to the production systems of the Maranhão "Cerrado" over the past few years, and the southern part of this region is becoming the largest cowpea producer in the State.

This crop establishes symbioses with nitrogen-fixing bacteria, which allow plants to satisfy their nutritional nitrogen needs, resulting in low production costs and increased productivity. However, the efficiency of the symbiosis depends on both the environment and genetic factors of both symbionts (Moreira & Siqueira, 2006).

Highly weathered soils predominate in Brazil, which can present chemical restrictions to agricultural production. Acidity is one of the most limiting features, and it can restrict the productivity of leguminous plants and their biological nitrogen fixation (BNF) (Kopittke, Blamey, Wang, & Menzies, 2011).

Many plants have developed morpho-physiological mechanisms to adapt to environmental adversities, making the effects dependent on the species and even on the genotype (Bian, Zhou, Sun, & LI, 2013). Due to its tropical origin, certain genotypes of cowpea developed tolerance to soil acidity (Jemo, Abaidoo, Nolte, & Horst, 2007). Because BNF evolved under the same conditions, various species and strains of N_2-fixing bacteria have also adapted to the same constraints (Soares et al., 2014).

However, depending on the level of tolerance, leguminous plants and N_2-fixing bacteria present varied responses to acidity (Zahran, 1999). Thus, if limiting factors such as soil pH, aluminum and manganese toxicity, and nutrient deficiency restrict the development of the macrosymbiont, the diazotrophs will not be able to efficiently fix N_2. Chemical soil restrictions connected to acidity therefore need to be corrected, and liming is often used because it acts directly on chemical attributes linked to acidity, thereby improving soil fertility. However, the recommendation of liming for production of cowpea inoculated with N_2-fixing bacteria must be based on the different symbiont responses because their magnitude will define the need for soil correction and its economic viability.

Selection of new bacterial strains more efficient in nitrogen fixation in cowpea is important for the optimization of crop nutrition techniques. N_2-fixing efficiency is the main criterion to recommend a new rhizobia strain, and the process of selecting new strains should be rigorous to differentiate the levels of efficiency between different strains (Moreira & Siqueira 2006).

Despite the importance of this crop, few studies have been performed for the selection of new rhizobia strains that are efficient at nitrogen fixation in symbiosis with cowpea at Maranhão, and this practice is little used in this State, especially in family-based agriculture systems. Besides, even large producers who adopt inoculation for cowpea production mostly use strains approved for soybean. Furthermore, no studies on the influence of liming on this symbiosis and on cowpea productivity have been reported for Maranhão. Therefore, the objective of the study was to evaluate the agronomic efficiency of two strains approved as inoculants for cowpea, and three new strains under selection, as well as the influence of liming on the symbiosis and cowpea productivity in Maranhão State, Brazil.

Material and methods

Two experiments were conducted in the State of Maranhão (MA), one in the Center Region, in an area of family-based agriculture at Dom Pedro (MA), elevation 171 m, latitude 5° 05'S, longitude 44° 21' W, with typic Dystrophic Red-Yellow Argisol, and one in the North Region, at the Federal Institute of Education, Science and Technology of Maranhão (Instituto Federal de Educação, Ciência and Tecnologia do Maranhão - IFMA), São Luís Campus, Maracanã, at São Luís, Maranhão State, elevation 41 m, latitude 2° 36'S, longitude 44° 16'W, with typic Dystrophic Yellow Latosol, between June and September 2013. The climate of the first region is sub-humid ($C_2W_2A'a'$), and that of the second region is humid ($B_1WA'a'$), according to the Thornthwaite classification. Minimal and maximal temperature, and rainfall data for the duration of the experiments are presented in Figure 1. The chemical characteristics of the soil at 0-20 cm are shown in Table 1.

A completely randomized experimental blocks design was used at Dom Pedro, with four replicates and seven treatments [five inoculant strains: INPA03-11B, UFLA03-84, UFLA03-153, UFLA03-154 and UFLA03-164 + two non-inoculated controls – one with application of 80 kg ha^{-1} urea-N (W/N) and another without mineral N (N/N)]. A randomized experimental design blocks was used at São Luís, with four replicates and a 7 x 2 factorial scheme [two liming treatments (with and without)].

Three new nitrogen-fixing bacterial strains were tested, based on previous selection in experiments under axenic conditions and using pots containing soil (Soares et al., 2014). The tested strains were UFLA03-153, UFLA03-154 and UFLA03-164, which were isolated from areas of bauxite mining in the Poços de Caldas mountain range (Serra de Poços de Caldas), Minas Gerais state, and identified as *Bradyrhizobium* sp., *Burkholderia fungorum* and *B. elkanii*, respectively (Oliveira-Longatti et al., 2014; Soares et al., 2014). In addition, two strains previously approved as inoculants for cowpea, UFLA03-84 (*Bradyrhizobium* sp.) and INPA03-11B (*B. elkanii*), were also tested (Soares et al., 2006). Seeds were treated with 250 g peat-based inoculants per 10 kg seeds. The inoculants consisted of cultures of the various tested strains at the log phase, grown on a growth medium: peat mixture (7:4, w:v) with 10^{-9} CFU g^{-1}. Experimental plots consisted of six rows, 4 m long, sown with cowpea cv. BRS Guariba, with 0.50 m spacing between lines and eight plants per linear meter.

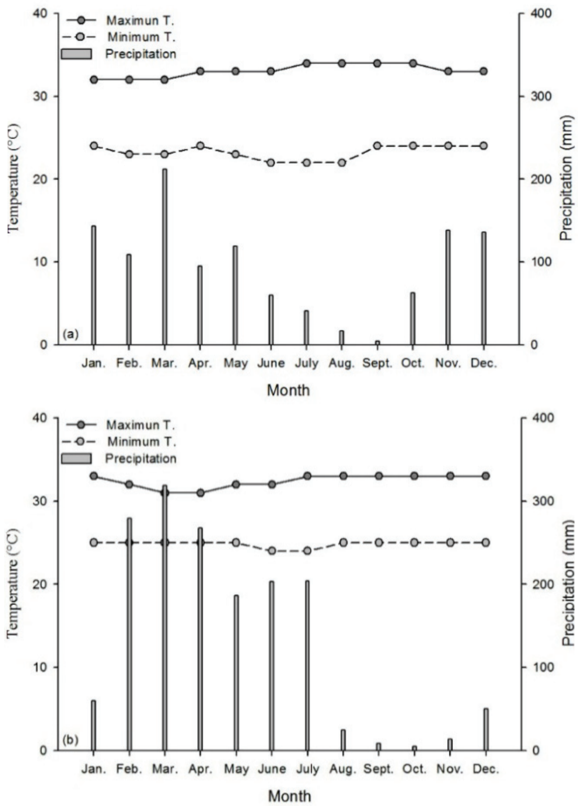

Figure 1. Minimum and maximun temperatures and mean precipitation by month in 2013 at Dom Pedro, Maranhão State (a) and São Luís, Maranhão State (b); Source: Instituto Nacional de Meteorologia – INMET.

Before planting, soils were sampled for assessment of the native rhizobia population density (approximate number g $soil^{-1}$, CFU) in a greenhouse experiment. Cowpea cv. BRS Guariba was grown as the trap plant, for 25 days, in wide-mouthed glass bottles containing Hoagland and Arnon (1950) nutrient solution inoculated with six soil dilutions (10^{-1} to 10^{-6}).

At Dom Pedro, there was no need for correction of soil acidity. Plantation fertilization was performed with application of 90 kg ha^{-1} P_2O_5 as triple superphosphate (45% P_2O_5). Potassium was not applied because the soil analysis revealed high potassium content. At São Luís, 2.53 t ha^{-1} dolomitic limestone, 40 kg ha^{-1} P_2O_5 as triple superphosphate (45% P_2O_5), and 40 kg ha^{-1} K_2O as potassium chloride (60% K_2O) were applied. The need for liming for neutralization of soil acidity was determined by increasing the base saturation to 70%.

Table 1. Chemical composition of soil samples (layer 0-20 cm)[1].

Soil Property	Unity	Municipality	
		Dom Pedro	São Luís
pH (H_2O)		6,2	5,2
P (Mehlich I)	mg dm^{-3}	3,84	24,62
K (Mehlich I)	mg dm^{-3}	288,0	22,0
Ca	$cmol_c$ dm^{-3}	2,60	0,50
Mg	$cmol_c$ dm^{-3}	1,30	0,20
Al	$cmol_c$ dm^{-3}	0,00	0,60
H+Al	$cmol_c$ dm^{-3}	2,08	3,62
SB	$cmol_c$ dm^{-3}	4,64	0,76
t	$cmol_c$ dm^{-3}	4, 64	1,36
T	$cmol_c$ dm^{-3}	6,72	4,38
m	%	0,00	44,12
V	%	69,02	17,27
OM	dag kg^{-}	3,84	1,99
Zn	mg dm^{-3}	4,05	1,04
Fe	mg dm^{-3}	182,02	212,56
Mn	mg dm^{-3}	116,40	1,60
Cu	mg dm^{-3}	0,89	0,24
B	mg dm^{-3}	0,35	0,26
S	mg dm^{-3}	4,38	3,32

(1)Analysis performed by the Soil Fertility Laboratory, Soil Science Department - UFLA. Ca and Mg were extracted with 1 mol L^{-1} KCl, S was extracted with monocalcium phosphate in acetic acid, H+Al was extracted with SMP, (OM) organic matter was extracted through Na2Cr2O7 4N + H2SO4 10 N oxidation, micronutrients were extracted with Mehlich 1, and B was extracted in hot water.

At full bloom, 35 days after emergence (DAE), ten plants were collected from the third and fourth rows of each plot. The following variables were quantified: nodule number (NN) and dry matter (NDM), shoot dry matter (SDM), relative efficiency (Efr) and shoot N content (SNC) and accumulation (SNA). At full maturity, 75 DAE, all plants were collected from the fourth and fifth rows of each plot. The variables quantified were number of pods per plant (NPP), number of seeds per pod (NSP), weight of 100 grains (p100), grain yield (GY), nitrogen grain content (GNC) and accumulation (GNA).

Shoot and nodule dry matters were determined following drying at 60°C in a forced-air circulation oven until constant weight was reached. N levels were determined according to the micro Kjeldahl method. N accumulation was calculated using the shoot and grain N contents. The former was calculated by multiplying shoot nitrogen content by the shoot dry matter and the latter by multiplying the grain nitrogen content by the grain yield with humidity corrected to 13%. Relative efficiency was calculated by dividing the shoot dry matter of inoculated plants by the shoot dry matter of plants with 80 kg ha^{-1} N and multiplying by 100.

The data were subjected to a variance analysis, at $p < 0.05$. When significant differences were observed, averages were compared using the Scott-Knott test, at $p < 0.05$, using the R software (R Development Core Team, 2013).

Results and discussion

Dom Pedro experimental area

Table 2 lists the mean values of the variables analyzed at the flowering stage (35 DAE) and maturation stage (75 DAE) of the experiment performed at Dom Pedro. The treatments had a significant effect on all the variables.

At the first assessment (35 DAE), there were no differences *(p >0.05)* in the number of nodules between the treatments inoculated with the strains UFLA03-84, UFLA03-153, UFLA03-154 and UFLA03-164 and the uninoculated control without mineral nitrogen (N) supplementation (Table 2). Nodulation of the control without N revealed the nodulating ability of native rhizobia populations, estimated to have a density of 140 CFU g^{-1} soil, that are adapted to the local environmental and soil conditions.

The nodule dry matter was not significantly different ($p > 0.05$) among the treatments inoculated with the three new strains (UFLA03-153, UFLA03-154 and UFLA03-164) and the strain approved as an inoculant (UFLA03-84) (Table 2). Studies performed by Soares et al. (2014) using potted soil in Minas Gerais and by Ferreira et al. (2013) and Costa, Nóbrega, Martins, Amaral, and Moreira (2011) using field experiments in Piauí showed similar results. The Scott-Knott test indicated that the uninoculated treatments without mineral N supplementation and the treatments inoculated with INPA03-11B (another strain approved as an inoculant strain) had intermediate nodule dry matter values (Table 2).

The greatest shoot dry matter was observed in the treatments inoculated with UFLA03-164, which did not significantly differ ($p < 0.05$) from the treatments with application of 80 kg ha^{-1} mineral N. The remaining treatments exhibited shoot dry matter values that did not differ from each other (Table 2).

There were no significant differences in the relative efficiencies between the treatment inoculated with UFLA03-164 and the control with N fertilization (Table 2). According to Moreira and Siqueira (2006), BNF is dependent on the interaction between rhizobia, the legume host and the environment, further adding to the effects of genetic variation among symbionts. This interaction can explain the low efficiency of the strains UFLA03-84 and INPA03-11B, which did not provide different results *(*$p > 0.05$*)* from the uninoculated treatment without mineral N supplementation.

Table 2. Average number of nodules per plant (NN), nodule dry matter (NDM, mg $plant^{-1}$), shoot dry matter (SDM, g $plant^{-1}$), relative efficiency (Efr, %), shoot nitrogen content (SNC, %), and shoot nitrogen accumulation (SNA, mg $plant^{-1}$) at 35 days after emergence (DAE), number of pods per plant (NPP) (Unit), number of seeds per pod (NSP) (Unit), weight of 100 seeds (w100seeds, g), grain yield (GY, kg ha^{-1}), grain nitrogen content (GNC, %), and grain nitrogen accumulation (GNA, mg $plant^{-1}$) 75 DAE, of BRS Guariba cultivar at Dom Pedro municipality, Maranhão[(1)].

Treatment	NN Unid.	NDM mg $planta^{-1}$	SDM g $planta^{-1}$	Efr %	SNC %	SNA mg $planta^{-1}$
UFLA03-153	24,50a	137,45a	4,40b	53,02b	4,18b	184,91b
UFLA03-154	30,50a	158,30a	5,75b	68,93b	3,96c	232,48b
UFLA03-164	38,50a	185,84a	8,55a	104,01a	4,37b	375,38a
UFLA03-84	28,75a	160,42a	6,75b	80,87b	4,07c	274,56b
INPA03-11B	18,25b	97,85b	6,05b	74,40b	3,85c	232,98b
Control N/N[2]	34,25a	110,95b	4,90b	60,40b	4,26b	209,28b
Control W/N[2]	8,00c	13,57c	8,28a	100,00a	5,47a	454,47a
CV (%)	23,27	24,78	23,40	23,57	4,34	22,31

Treatment	NPP Unid.	NSP Unid.	p.100 g	GY kg ha^{-1}	GNC %	GNA mg $planta^{-1}$
UFLA03-153	4,09b	8,91a	21,38b	1.245,07b	4,22b	328,44b
UFLA03-154	3,43c	8,80a	20,90b	1.021,54c	4,04b	259,21c
UFLA03-164	3,93b	9,07a	21,28b	1.227,92b	4,27b	328,81b
UFLA03-84	3,35c	6,92b	21,66b	796,56c	4,57a	226,61c
INPA03-11B	3,04c	9,39a	21,11b	959,85c	4,54a	270,83c
Control N/N	2,93c	9,57a	21,74b	959,76c	4,35b	259,81c
Control W/N	4,83a	8,95a	23,22a	1.621,38a	4,87a	497,13a
CV (%)	12,46	9,93	4,38	15,82	2,40	16,75

[(1)]Means followed by the same lowercase letter within the same column or by the same uppercase letter within the same row belong to the same group according to the Scott-Knott test, at $p < 0.05$.
[2]N/N - uninoculated and with no mineral Nitrogen, W/N- uninoculated and with 80 kg ha^{-1} mineral N.

The N content in the shoot dry matter was higher for the treatment with N fertilization (80 kg ha^{-1}) compared to the other treatments (Table 2). There was no difference ($p > 0.05$) between the treatments inoculated with UFLA03-164 and UFLA03-153 and the uninoculated control without N supplementation, which exhibited intermediate levels. The treatments with UFLA03-154 had N levels in the shoot dry matter that were similar to the treatments inoculated with UFLA03-84 and INPA03-11B, forming a group that had the lowest mean values for this variable (Table 2). N levels in all treatments can be considered adequate for cowpea growth as all were above 3% (Anele et al., 2010; Costa et al., 2011).N accumulation in the shoot dry matter for the treatment inoculated with strain UFLA03-164 was not significantly different from the uninoculated treatment without mineral N supplementation (Table 2). Soares et al. (2014) in experiments with potted soil in Minas Gerais and Ferreira et al. (2013) in field experiments in Piauí have both reported similar results.

At the second evaluation time-point (75 DAE), the treatments showed significant effects for the variables analyzed during the maturation stage (Table 2). The treatment with the greatest number of pods per plant treatment was the uninoculated control supplemented with 80 kg ha^{-1} mineral N, followed by the treatments inoculated with strains UFLA03-153 and UFLA03-164. The Scott-Knott test ($p > 0.05$) indicated that there were no differences in the number of seeds per pod except for in the treatment inoculated with strain UFLA03-84, a result that may be related to the genotype of the cultivar BRS Guariba. The control treatment with N supplementation yielded the highest weight of 100 seeds (Table 2). The other treatments were not significantly different from each other. In the overall comparison for the three variables described above, the strain UFLA03-84 had the lowest values.

The highest grain yields for the inoculated treatments were observed for UFLA03-153 and UFLA03-164, which were not significantly different from each other (Table 2). The treatment with N supplementation (80 kg ha^{-1}) had the highest grain yield of all the treatments. Greater grain production as a result of N fertilization and increased yield of cowpeas inoculated with rhizobia were also reported by Santos, Aguiar, and Moura, (2011). These authors studied the effects of soil management on the efficiency of rhizobia-inoculated cowpeas in Maranhão and reported yields of approximately 1,700 kg ha^{-1}. This high yield was also obtained with 98 kg ha^{-1} of single superphosphate. Gualter, Boddey, Rumjanek, Freitas, and Xavier (2011) also examined the efficiency of rhizobial strains inoculated onto cowpeas in Maranhão and reported a maximum yield of 893 kg ha^{-1} on family farms. The treatments that these authors performed also included liming and phosphorus and potassium fertilization. The same authors observed a maximum yield of 679 kg ha^{-1} for the treatments inoculated with strains approved as inoculants, such as INPA03-11B, which was well below the yield observed for the same treatment at Dom Pedro (Table 2).

The different treatments could be divided into two different groups ($p < 0.05$) based on the grain N content (Table 2). The first group had higher values and consisted of the uninoculated treatment with mineral N supplementation, UFLA03-84 and INPA03-11B. The second group consisted of the remaining treatments. The highest grain N accumulation was observed for the uninoculated treatment with 80 kg ha^{-1} mineral N supplementation. The second group, in which the treatments had intermediate values of grain N accumulation, consisted of strains UFLA03-164 and UFLA03-153. The last group consisted of the treatments inoculated with strains UFLA03-154, UFLA03-84 and INPA03-11B and the uninoculated control without mineral N supplementation (Table 2). Considering all parameters the BNF efficiency for strains UFLA03-164 and UFLA03-153 can be considered satisfactory in the municipality of Dom Pedro.

São Luís experimental area

Table 3 lists the mean values of the variables analyzed at the flowering stage (35 DAE) and maturation stage (75 DAE) of the experiment performed at São Luís.

The interaction between liming and inoculants did not have a significant effect ($p > 0.05$) on the variables analyzed at flowering (Table 3). The N source had a significant effect on all the variables analyzed, and the Scott-Knott test ($p < 0.05$) revealed the agronomic efficiency of the strain UFLA03-153.

The average acidity of the soil, the high level of Al^{3+} saturation and Ca^{2+} and Mg^{2+} deficiency did not limit the nodulation of the strains evaluated (Table 3). According to Correa and Barneix (1997), rhizobia decrease their growth rate under acidic conditions as an adaptive response to their environment. However, high concentrations of H^+ and Al^{3+} can lead to a significant reduction in population density and survival of rhizobia in the soil. An analysis of the number and dry matter of nodules showed that liming did not have a

Table 3. Average number of nodules per plant (NN), nodule dry matter (NDM, mg plant^{-1}), shoot dry matter (SDM, g plant^{-1}), relative efficiency (Efr, %), shoot nitrogen content (SNC, %), and shoot nitrogen accumulation (SNA, mg plant^{-1}) at 35 days after emergence (DAE), number of pods per plant (NPP), number of seeds per pod (NSP), weight of 100 seeds (w100seeds, g), grain yield (GY, kg ha^{-1}), grain nitrogen content (GNC, %), and grain nitrogen accumulation (GNA, mg plant^{-1}) at 75 DAE, of BRS Guariba cultivar at São Luís municipality, Maranhão State[(1)].

Treatment	NN Unid.	NDM mg planta^{-1}	SDM g planta^{-1}	Efr %	SNC %	SNA mg planta^{-1}
UFLA03-153	23,38a	165,32a	4,75b	63,08b	4,25b	201,19b
UFLA03-154	28,25a	206,03a	4,83b	61,54b	4,41b	213,70b
UFLA03-164	22,50a	177,57a	5,00b	64,50b	4,22b	213,73b
UFLA03-84	22,50a	164,24a	4,83b	61,05b	4,25b	205,84b
INPA03-11B	27,25a	174,18a	5,50b	69,01b	3,97b	219,37b
Control N/N[2]	24,88a	149,84a	4,15b	52,03b	3,88b	160,71b
Control W/N	7,25b	33,77b	8,03a	100,00a	4,80a	386,27[a]
Liming						
Without	21,32a	151,37a	5,07a	66,75a	4,16b	214,57[a]
With	23,25a	154,61a	5,52a	67,60a	4,35a	242,80[a]
CV (%)	23,25	16,34	22,52	24,13	8,24	25,95

Treatment	NPP Unid.	NSP Unid.	p.100 g	GY kg ha^{-1}	GNC %	GNA mg planta^{-1}	
						Limed	Without Liming
UFLA03-153	3,64b	9,34a	21,35b	1.153,41b	4,62a	367,41bA	300,91aB
UFLA03-154	3,56b	8,28b	20,92b	978,27c	4,22b	276,88cA	240,15bA
UFLA03-164	3,38b	8,61b	21,00b	970,20c	4,07c	274,11cA	220,28bA
UFLA03-84	3,68b	7,64b	20,83b	923,04c	3,94c	234,55cA	217,28bA
INPA03-11B	3,14b	9,59a	20,66b	980,36c	4,27b	274,94cA	248,18bA
Control N/N	3,59b	8,04b	21,12b	982,84c	4,14b	298,35cA	210,86bB
Control W/N	4,79a	8,00b	23,26a	1.430,08a	4,78a	504,38aA	354,90aB
Without liming	3,59a	8,15b	20,90b	959,31b	4,23b		
Limed	3,78a	8,85a	21,72a	1.160,18a	4,35a		
CV (%)	13,01	11,73	4,03	12,67	2,20	14,06	

[(1)]Means followed by the same lowercase letter within the same column or the same uppercase letter within the same row belong to the same group according to the Scott-Knott test, at $p < 0.05$. [2]N/N - uninoculated and with no mineral Nitrogen, W/N- uninoculated and with 80 kg ha^{-1} mineral N.

significant effect ($p > 0.05$) on the treatments (Table 3), indicating that the evaluated strains as well as the native population of rhizobia are tolerant to the soil pH and aluminum levels. These results are consistent with those reported by Soares et al. (2014), who showed "*in vitro*" tolerance of strains UFLA03-153, UFLA03-154, UFLA03-164, UFLA03-84 and INPA03-11B to a pH of 5.0 and to aluminum.

The development of adaptation mechanisms to adverse soil conditions in slow-growing strains has been reported by several authors (Keyser & Munns 1979; Correa & Barneix 1997; Lima et al., 2009; Ferreira, Bomfeti, Soares, & Moreira, 2012).

The number and dry matter of nodules were not significantly different ($p > 0.05$) between treatments except for the control with N supplementation, which had the fewest and lowest dry matter of nodules (Table 3). The values for these two variables were similar to those observed by other authors in field experiments conducted in Maranhão (Gualter et al., 2011; Santos et al., 2011).

The evaluation of the shoot dry matter, relative efficiency and shoot N content and accumulation indicated that the interaction between liming and the inoculants did not significantly affect the treatments ($p > 0.05$) (Table 3). The cultivar BRS Guariba showed acid tolerance given that the low pH of 5.2 and low natural fertility of the soil did not limit nodulation. In addition, root growth inhibition or symptoms of nutrient deficiency, especially for N, were not visually observed during the experiment, confirming the adaptation of cowpea cultivars to the acidity and low fertility of the soil observed by other authors (Jemo et al., 2007). However, the liming factor had a significant effect on the N content of the shoots (Table 3). The shoot N content was relatively high compared to levels reported by other authors (Costa et al., 2011; Anele et al., 2010), indicating that N was not a limiting factor for plant growth (Table 3). The N source had a significant effect ($p < 0.05$), and the uninoculated treatment with 80 kg ha^{-1} mineral N supplementation exhibited the highest shoot dry matter (Table 3). The other treatments showed intermediate shoot dry matter values and did not significantly differ from each other according to the Scott-Knott test.

There was no significant difference in the relative efficiency

between the inoculated treatments and the uninoculated control treatment without mineral N supplementation (Table 3). This similarity demonstrates the ability of the native population to establish effective symbioses with cowpeas, a legume that is considered promiscuous (Guimarães et al., 2012; Jaramillo et al., 2013). Similar results were reported by Rufini et al. (2014), who studied the symbiotic efficiency of rhizobia inoculants on cowpeas grown in eutrophic Red Latosol soils in Minas Gerais.

The control with N supplementation (80 kg ha^{-1}) had significantly higher N content and accumulation in its shoot dry matter ($p < 0.05$) compared to the remaining treatments. There were no differences between the inoculated treatments and the control treatments without N supplementation, demonstrating the ability of the native populations to establish effective symbioses with cowpea in São Luís (Table 3). All the treatments had satisfactory N content, with levels greater than 3%.

In the second evaluation (75 DAE), except for grain N accumulation, the interaction between liming and the inoculants did not have a statistically significant effect ($p > 0.05$) on the variables analyzed in the maturation stage. This finding is consistent with the tolerance of the strains evaluated and the native rhizobia population to the pH and aluminum levels in the soil (Table 3). However, liming did have a significant effect on several variables, including the number of seeds per pod, seed weight, grain yield and grain N content, indicating that although tolerant, BRS Guariba is still responsive to increased nutrient availability in the soil, especially regarding yield gain. Some authors have found that liming has no effect on N_2 fixation or cowpea production in the northeast (Costa & Stamford 1991), but Cravo, Smyth, and Brasil, (2012) reported that cowpea yields increased significantly in response to liming in the northeast and Amazon regions, respectively.

Regarding the effect of the N sources, a significant effect ($p < 0.05$) of the treatments was observed for all the other variables analyzed at this stage.

The Scott-Knott tests indicated that the treatment with 80 kg ha^{-1} mineral N supplementation yielded the greatest number of pods per plant (Table 3). The inoculated treatments were not significantly different from the control treatment without mineral N supplementation.

The number of seeds per pod significantly differed ($p < 0.05$) among treatments, with the strains UFLA03-153 and INPA03-11B showing the most seeds per pod. The remaining inoculated treatments did not differ from the uninoculated controls with and without mineral N supplementation regarding this variable (80 kg ha^{-1}).

The uninoculated treatment with 80 kg ha^{-1} N supplementation had the highest seed weights (Table 3). The inoculated treatments were grouped into a second group and did not significantly differ ($p > 0.05$) from the uninoculated treatment without mineral N supplementation.

Grain yield varied significantly ($p < 0.05$) among the treatments, and increased cowpea yields were observed for the uninoculated control with 80 kg ha^{-1} mineral N supplementation (Table 3). The strain UFLA03-153 was superior to the strains approved as inoculants (UFLA03-84 and INPA03-11B) and resulted in the highest grain yield among the inoculated treatments. Other authors observed similar grain yields between an uninoculated control without mineral N supplementation and the strains UFLA03-84 and INPA03-11B, with values close to the ones reported in São Luís (Rufini et al., 2014). The lowest mean yield was observed for the treatment inoculated with strain UFLA03-84.

The treatments could be divided into three significantly different groups ($p < 0.05$) based on the grain N content (Table 3). The first group with the highest N content consisted of the uninoculated treatment with mineral N supplementation and UFLA03-153. The second group consisted of the treatments UFLA03-154, INPA03-11B and the control without mineral N supplementation. The last group consisted of the treatments inoculated with strains UFLA03-164 and UFLA03-84 (Table 3).

Grain N accumulation was the one variable that was significantly influenced ($p < 0.05$) by the interaction of the liming and inoculants (Table 3). When soil correction was performed, the Scott-Knott test indicated three treatment groups, with the uninoculated control with mineral N supplementation showing the highest levels of grain N accumulation, followed by the treatment inoculated with UFLA03-153, with intermediate levels, and with the remaining treatments forming the last group. In the absence of soil correction, the treatment inoculated with strain UFLA03-153 was similar to the control with N supplementation, showing the highest levels of grain N accumulation, followed by the remaining treatments (Table 3). This pattern shows that the efficiency of chemical N fertilizer is more dependent on improvements in chemical properties of the soil than on the efficiency of the inoculants, confirming the tolerance of the evaluated strains to acidic soils. Similar results have been reported by other authors, who found no difference in the grain N content compared to treatments inoculated with the strains UFLA03-84 and INPA03-11B (Soares et al., 2006). Both with and without the liming, the non-inoculated treatment without mineral N supplementation had similar levels of grain N accumulation as the treatments inoculated with strains UFLA03-154, UFLA03-164, UFLA03-84 and INPA03-11B (Table 3). Other authors have observed similar results (Rufini et al., 2014).

It is noteworthy that the yields obtained in these experiments (Dom Pedro: 797-1,621 kg ha^{-1}; São Luís: 923-1,430 kg ha^{-1}) were well above the state average (510 kg ha^{-1}). This increase is not only due to the rhizobial inoculants but is also due to the phosphate fertilization, which supplies one of the primary limiting factors in tropical soils, especially in Dom Pedro. Although the yields obtained are higher with N fertilization than with rhizobial inoculants, the economic and ecological benefits of the latter outweigh the benefits of the former (Sousa & Moreira, 2011).

Notably, interaction between liming and inoculants only had a significant effect on one variable, and liming is therefore not recommended for use in combination with rhizobial inoculants. However, increased cowpea production was observed when the effects of liming were isolated.

Conclusion

Inoculation of cowpea with strains UFLA03-153 and UFLA03-164 is recommended for family-based agriculture systems and for intensive agriculture systems.

The strains UFLA03-153 and UFLA03-164 are more efficient for atmospheric nitrogen fixation than the strains approved as inoculants, UFLA03-84 and INPA03-11B, resulting in increased productivity.

Strain UFLA03-164 is recommended for the production of forage with high protein content in Dom Pedro.

Liming increases the grain yield of cultivar BRS Guariba in production systems.

The use of liming together with inoculation is not recommended for the production of cowpea in Dom Pedro and São Luís.

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