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QUANTIFICATION OF THE BIOLOGICAL FIXATION OF NITROGEN IN *CANAVALIA ENSIFORMIS* BY THE METHODS OF NATURAL ABUNDANCE OF ^{15}N AND DIFFERENCE OF TOTAL N

Cuantificación de la fijación biológicade nitrógeno en *Canavalia ensiformis* crecida en un suelo pardo mullido carbonatado mediante los métodos de abundancia natural de ^{15}N y diferencia de N total

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ABSTRACT. In this work were traced as objectives to evaluate the growth and N accumulation in *Canavalia ensiformis*, grown in a Cambisol soil of El Salvador municipality, county of Guantánamo, coinoculated with four isolated of *Rhizobium* and two strains of HMA, as well as to quantify the process of nitrogen simbiotic fixation of this species, by the methods of the natural abundance of ^{15}N and the difference of total N. The dry mass growth and the N absorption of the jackbean sowed in monoculture were superior in presence of the isolated of *Rhizobium* Can 5, coinoculated with *Rhizoglomus intraradices*, in these plants, the BNF oscillated from 59 to 72 kg ha^{-1} in the dry and rainy period, respectively. The methods of BNF quantification, difference of total N and natural abundance of ^{15}N were similar to each other, although the difference of total N overestimates the quantification values regarding the natural abundance.

Key words: green manures, coinoculation, biological fixation of the nitrogen, mycorrhizae, *Rhizobium*

RESUMEN. En el presente trabajo, se trazaron como objetivos evaluar el crecimiento y acumulación de N en *Canavalia* ensiformis, crecida en un suelo Pardo mullido carbonatado del municipio El Salvador, provincia Guantánamo, coinoculada con cuatro aislados de *Rhizobium* y dos cepas de HMA, así como cuantificar el proceso de fijación simbiótica de nitrógeno de esta especie, mediante los métodos de la abundancia natural de ^{15}N y la diferencia de N total. El crecimiento en masa seca y la absorción de N de la canavalia sembrada en monocultivo fue superior en presencia del aislado de *Rhizobium* Can 5, coinoculado con *Rhizoglomus intraradices*, plantas que realizaron una FBN que oscila de 59 a 72 kg ha^{-1} en el período poco lluvioso y lluvioso, respectivamente. Los métodos de cuantificación de la FBN, por la diferencia de N total y abundancia natural de ^{15}N fueron semejantes entre sí, aunque la diferencia de N total sobreestima los valores de cuantificación respecto a la abundancia natural.

Palabras clave: abonos verdes, coinoculación, fijación biológica del nitrógeno, micorrizas, *Rhizobium*

INTRODUCTION

Nitrogen (N) is often the main limiting nutrient for most cultivated species. Successful manipulation of the incorporation of atmospheric N (N_2) through the biological N-fixing process (FBN, according its acronyms in Spanish) is an economically viable and environmentally beneficial agricultural practice.

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The use of N-fixing species in agricultural systems reduces the need for nitrogen fertilizers and improves soil conditions (1).

A sustainable way to incorporate N into agricultural systems is the insertion within plant crop rotations that establish symbiosis with nitrogen fixing bacteria present in the soil, capable of performing the biological fixation of nitrogen. Among these types of plants are legumes, which are used as green manures and cover crops, capable of maintaining or improving the physical, chemical and biological characteristics of the soil, which is important from the point of view of nitrogen fertilizer saving and the reduction of production costs (2).

In spite of the importance of the legumes in the production and in the nitrogen economy, in the quantification of the FBN different methodologies have been used. However, there is not yet a single method suitable for the determination of this process, because there is no precise information as to what the actual fixing capacity of said element is. Each of these methods has advantages and limitations, they may depend on the availability and proper determination of the FBN for any legume species grown under all possible variant soil and environments [A]. The evaluation and quantification of the FBN is based mainly on the increase of dry matter production, differences in total N yield, nodulation, solutes in the xylem, enzymatic activity (acetylene reduction) and the use of ^{15}N. The selection of one of them depends basically on the experimental objective that is pursued (3).

The generality of the methods developed up to the present tries to approach to have an approximate idea of the contribution of N coming from the atmosphere. In all cases and agricultural systems, non-fixing reference plants with similar soil N extraction capacity are used to differentiate the different sources of the element in the agricultural system under study.

In the present work, the objectives of this study were to evaluate the growth and accumulation of N in *Canavalia ensiformis*, grown in a soft carbonated brown soil of El Salvador municipality, Guantánamo province, coinciding with four *Rhizobium* isolates and two AMF strains; as well as quantify the symbiotic nitrogen fixation process of this species, using natural abundance methods of ^{15}N and the total N difference.

[A] Zapata, H. I. *Acumulación de materia seca y fijación biológica de Nitrógeno en diferentes especies del género Lupinus cultivadas en suelos de Zapopan, Jalisco* [en línea]. Tesis de Maestría, Universidad de Guadalajara, 2015, Jalisco, México, 92 p., [Consultado: 31 de enero de 2015], Disponible en: <http://biblioteca.cucba.udg.mx:8080/xmlui/bitstream/handle/123456789/5926/Zapata_Hernandez_Isidro.pdf?sequence=1>.

MATERIALS AND METHODS

Two field experiments were carried out in different periods (rainy season and low rainy season) from 2010 to 2011, on a soft brown Sialitic carbonated soil (4), in two productive areas belonging to the El Salvador municipality, Guantanamo province.

The first experiment was carried out at the University of Guantanamo, in the rainy season (April to July 2010), at the Guantánamo - El Salvador road, located at the km 6 ½ km. The second experiment was carried out at the UBPC (Basic Unit of Cooperative Production) "Hermanos Sánchez", located in the eastern portion, Km 4 ½ Guantánamo highway - El Salvador, belonging to the Honduran Agricultural Farm, during the low rainy season (November 2010 to February 2011). The main physical - chemical and chemical characteristics of the soil are summarized in Table I.

Table I. Some physical-chemical and chemical characteristics of the arable layer of the soil at the beginning of the experiment (0-20 cm depth)

Experimental site	pH H_2O	OM (%)	P (mg kg^{-1})	Na^+	K^+	Ca^{2+}	Mg^{2+}
					(cmol kg^{-1})		
Polygon FAM	6,9	3,04	216	0,56	0,61	40,0	12,5
UBPC Hermanos Sánchez	6,8	2,83	177	0,50	0,51	35,0	19,0

Chemical determinations: pH H_2O potentiometer: soil/solution ratio of 1: 2,5; OM (organic matter) Walkley Black, P: 0,1 N solution of H_2SO_4 with soil to solution ratio 1: 25, Cations NH_4Ac at pH 7 (5)

The soil characteristics for the two experiments were similar, with slightly acidic pH and average organic matter (OM) content. Phosphorus (P) was high and probably due to the analytical method used for its determination; which contains an extractive solution of sulfuric acid which, upon reaction with the carbonate and calcium, releases these element towards the solution of the soil and, therefore, the amount thereof is overestimated. The contents of potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) had high values for this soil; However, Na only occupied 1% of the exchange complex, so it does not show adverse effects on crops.

Experimental design

It was used an area of 0,11 ha, distributed in 60 plots for both campaigns, each experimental plot counted with four furrows at a distance of 0,70 m of ridge by 4 m long for an area of 11,2 m^2. The canavalia was planted manually, two seeds per nest, at a distance of 0,30 m. Cultural attention was given according to the recommendations established for Cuba (6).

In the calculation area (two central grooves), three complete plants per linear meter were selected, including the rhizosphere soil and the rootlets of the plants, for each treatment and replication, leaving the two outer grooves as edge area, at 70 days after germination, during the flowering stage. In the case of reference plants, plants were grown in 1 m^2 on one side of the canavalia plots, taking four replicates.

In both periods a bifactorial arrangement of random blocks and four replicates were used. Five levels of the *Rhizobium* inoculation factor (four *Rhizobium* isolates plus one non-inoculation treatment) and three levels of mycorrhizal inoculation factor (two strains and one control without inoculation) were studied for a total of 15 treatments. *Megathyrsus maximus, basonym Panicum maximum* (guinea grass), was used as reference plant, which grows as weeds in the experimental areas.

Methods of inoculation of the biofertilizers used

Mycorrhizal inoculants were the *Glomus cubense* (Y. Rodr. and Dalpé), INCAM - 4 (7) and *Rhizoglomus intraradices* (Smith and Schenck), INCAM - 11 (8) strains from the arbuscular mycorrhizal fungi collection (AMF) of the Department of Biofertilizers and Plant Nutrition, of the National Institute of Agricultural Sciences (INCA). The strains were stored in a substrate developed for these purposes by the INCA mycorrhizal laboratory (Patent Register No. 2264) at 4 ºC. The HMA inoculums used in the experiments had an average titre of 50 g^{-1} spores of inoculant soil, certified in the INCA mycorrhizal laboratory.

As a bacterial inoculant, four *Rhizobium* isolates (Can 2, Can 3, Can 4 and Can 5) were obtained from canavalia nodules belonging to the collection of rhizobia strains belonging to the Department of Physiology of the National Institute of Agricultural Sciences (INCA), at a concentration of 107 to 108 CFU g^{-1} (9).

The co-inoculation of these biofertilizers was carried out at the time of planting, by the method of coating the seeds (10), using a dose of 5,95 kg ha^{-1} of the mycorrhizal inoculant, equivalent to 10 % of the weight of the seeds and 100 g of each bacterial inoculant. First, a homogeneous paste was made in a proportion of 1 kg of mycorrhizal inoculant per 10 kg of seed with the separate AMF strains and subsequently each *Rhizobium* isolate was added. Simple inoculation of the bacterial inoculum was carried out by means of a sugar solution, and then the seed was covered until completely covered, dried in the shade for 5 to 10 minutes and then seeding.

Evaluations and methodologies used

Aerial dry mass

To determine aerial dry mass (t ha^{-1}), the organs of the aerial part of the plants (leaves and stems) were taken. Each organ (g per plant) was weighed together and then separately on a Sartorius digital METTLER scale, from which a fraction of 100 grams was taken and dried in the oven at 70 °C until reaching constant mass, dry mass of each organ and expressed in t ha^{-1}.

N content

The concentration of N was determined as a percentage of the dry mass of the aerial part by wet digestion with H_2SO_4+Se and through the methodology of Nessler (5). N extraction (kg ha^{-1}) was calculated from the dry mass data of the aerial part and its corresponding element concentration (% N), by the following formula.

$$\text{Extraction of N} = \frac{\text{MS PA x Conc N}}{100}$$

Where: MS PA = aerial dry mass (t ha^{-1})

Conc N = concentration (% N) of the element in the dry mass of the aerial part

From these data, the percentage of FBN was calculated by the method of the total N difference using formula (3):

$$\text{\% FBN} = \frac{\text{Content N Fix - Content N Ref x 100}}{\text{Content N Fix}}$$

Where: Fix = fixing plant,

Ref = non-fixing plant

Quantification of the natural abundance of ^{15}N

The estimated FBN percentage was calculated from the natural abundance of ^{15}N of the legume and of the reference plant (guinea grass), using the following equation (11):

$$\text{\% FBN} = \frac{(\delta\, ^{15}\text{N ref} - \delta\, ^{15}\text{N fix}) \times 100}{(\Delta\, ^{15}\text{N fix-B})}$$

Where: δ ^{15}N ref = natural abundance of ^{15}N of the reference plant δ ^{15}N fix = natural abundance of ^{15}N of the leguminous plant

B = abundance of ^{15}N of the same legume grown exclusively with N from the FBN. In the present study, the value used was -1,35 (12)

Statistical analysis

The results were processed through an analysis of variance (ANOVA) of double classification, taking into consideration the effect of the factors and their interaction. The averages were compared by Duncan's score (13), for a 5 % significance, after verifying that they met the normal distribution adjustment using the Kolmogorov-Smirnov test (14), and of homogeneity of variance by the test of Levene (15).

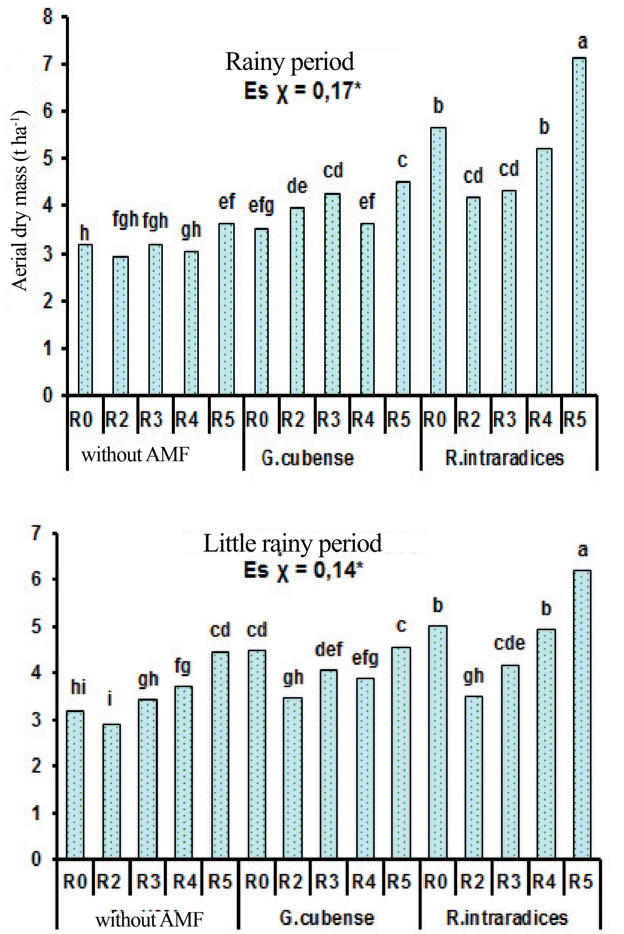
For the case of the quantification results of the FBN (expressed in kg ha^{-1}), a comparison analysis of paired samples was carried out using the t test (α = 0,05), using as null hypothesis that the samples Aare the same and as an alternative hypothesis that the samples differ from each other. All statistical analyzes were performed using the statistical package STATGRAPHICS Centurion XV (16).

RESULTS AND DISCUSSION

When analyzing the effect of *Rhizobium*-AMF coinoculation on the aerial dry mass of canavalia, interaction between the study factors at 70 days after germination was observed. The inoculation of *R. intraradices* combined with Can 5 isolate promoted an increase of the above mentioned indicator, which manifested itself superiorly to the rest of the other strains; This allowed yields of 7,12 and 6,2 t ha^{-1} of aerial dry mass to be obtained for both periods (Figure).

On the other hand, it was found that the strain *R. intraradices* in the treatments without bacterial inoculation and in combination with the isolate Can 4, showed the second better positive effect in relation to the other treatments in the two moments studied; while with the simple and combined application of *Rhizobium* and *G. cubense*, aerial dry mass production decreased to 4,57 and 4,7 t ha^{-1}, respectively, indicating that this strain of AMF had a lower symbiotic effect than *R Intraradices*.

In general, it was observed that the highest aerial dry mass production was obtained in the rainy season, results that correspond to long days and high precipitation, in this case oscillated between 164 and 190 mm in the region, which favored the vegetative increase of this species in a less prolonged period. However, during the low rainy season, where rainfall ranged from 65,9 to 96 mm, with short days and low temperatures,



R0: without *Rhizobium*, R2, R3, R4 and R5: *Rhizobium* isolates (Can 2, Can 3, Can 4 and Can 5). * Medias with different letters differ from each other, according to ($p < 0.05$)

Figure. Dry mass production area of canavalia grown on a Brown Sialitic soil during two different climatic periods

there was a decrease in production of the indicator mentioned above. This result was to be expected, since in experimental results obtained in studies with other leguminous plants have been shown to grow faster in the summer, as they accumulate more nutrients, due to the increase of sunlight and humidity in this time of the year. It is also known that increasing rainfall favors the growth of plants used as green manure (17); therefore, to higher phytomass production, the nutrient content is increased.

Likewise, it has been reported that adequate inoculation of a legume with effective strains causes an increase in the aerial mass of plants (18), although numerous reports indicate that canavalia is a promiscuous genus that can establish effective symbiosis with several strains of *Rhizobium* (9).

In this sense, positive results of the inoculation with *G. cubense*, -strain INCAM-4-, on the mycorrhizal function of canavalia and its effect of permanence in the maize crop for two different seasons - spring and cold - have been reported. The growth of the plants of this species was superior in spring with respect to the winter, reaching between 2,24–4,58 t ha^{-1} and 2,82–5,38 t ha^{-1} of dry mass without and with mycorrhizal inoculation respectively, in the presence of a low initial number of native spores in the soil and without previous applications of the mycorrhizal inoculant. In relation to the cold season, dry mass production decreased between 1,34–1,35 t ha^{-1} and 1,60-1,70 t ha^{-1} respectively (19). However, it is worth noting that the results reported by these authors are inferior to those found in this research, because the edaphoclimatic conditions were different and could influence both the development of the plants and the symbiotic activities of the microorganisms.

Based on the aforementioned, it was reported that in the soybean plants (*Glycine max*) the dry mass values of the aerial part were superior when the coinoculation *Bradyrhizobium elkanii* - AMF, they oscillated between 8,68 - 15, 48 g per plant, an aspect that demonstrates the agronomic importance of the practice of *Rhizobium*-AMF coinoculation in legumes (20).

Also, in investigations carried out on different types of soil and various combinations of co-inoculation of Rhizobium isolates and strains of AMF in canavalia, significant results are achieved; For example, in brown soils without carbonates, it was possible to verify that its growth and development benefit, regardless of the indicator evaluated. In this sense, the effect of the coinoculation of Can 5 and *G. hoi-like* isolates led to an increase in the height of the plant, but not for the combination of Can 2003a with *G. hoi-like*, with which the lowest value was reached. For the case of the fresh mass, the greatest increase was reached with the combination Can 2003b - *R. intraradices*; however, this indicator was significantly depressed when using *G. hoi - like* and the isolated Can 5 (21). Similarly, it occurred in the Red Ferralitic soil, but with the combination of the isolated Can 2 and *G. cubense*; While for the case of the Gley Nodular Ferruginoso soil, the best behavior was obtained with the coinoculation of the isolates Can 3, Can 4 and Can 5 and *Funneliformis mosseae* (22).

These same authors report results of dry mass of canavalia planted in summer, -optimal season-, in conditions of high temperatures and humidity, in which they have an exuberant growth. These values ranged from 2,5 to 10 t ha^{-1}.

Table II shows the effect of coinoculation on the N content of canavalia in the two seasons. The combination *R. intraradices* with the isolate Can 5 showed repeatedly the best results on the nitrogen extraction of canavalia, with an interaction between the factors under study, with values of 136 kg ha^{-1} for the rainy season and 93,44 Kg ha^{-1} for the slightly rainy season, in correspondence with the results obtained in the Figure.

It should be noted that an increase of this indicator was observed in the presence of *R. intraradices* without inoculation of rhizobia, with respect to the isolates Can 2, Can 3 and Can 4 for both periods, due to the apparent existence of resident strains of *Rhizobium*, which were probably more effective in establishing the symbiosis in the presence of *R. intraradices* than the inoculated isolates, except Can 5. Likewise, this increase in treatments without bacterial inoculation and without AMF, possibly due to the compatibility of resident strains, Both *Rhizobium* and AMF, capable of establishing symbiosis with macrosymbiont, as effective as the coinoculated.

Table II. Effect of simple and combined inoculation of *Rhizobium* and AMF on canavalia N extraction (kg ha^{-1}) at 70 days DAG

Treatments		Rainy season	Little rainy season
Without AMF	R0	71,76 fghi	49,49 ij
	R2	63,43 i	45,95 j
	R3	71,47 ghi	52,58 ghij
	R4	66,03 hi	59,11 efgh
	R5	78,24 defg	71,04 cd
Glomus cubense	R0	77,06 efgh	66,97 cde
	R2	79,14 defg	58,66 efgh
	R3	87,69 de	71,60 c
	R4	81,26 defg	62,54 def
	R5	90,08 d	70,32 cd
Rhizophagus intraradices	R0	121,13 b	84,99 b
	R2	84,66 de	51,11 hij
	R3	83,98 def	54,99 fghi
	R4	104,09 c	61,46 efg
	R5	136,26 a	93,44 a
Es χ		3,77*	2,81*

*Medias with different letters in the same column differ from each other, according to ($p < 0.05$). R0: without *Rhizobium*, R2, R3, R4 and R5: *Rhizobium* isolates (Can 2, Can 3, Can 4 and Can 5 respectively). DAG: days after germination

Another cause that could influence these results was the effect of the AMF-leguminous symbiosis, especially that of the *R. intraradices* strain, which has been the most recommended for this type of soil (23), since it could have influenced so that the absorption processes of nutrients benefit, causing the greater growth of the canavalia. In this sense, it is suggested that the associations of *Rhizobium*-arbuscular mycorrhizae act synergistically in the levels of mineral nutrition and plant growth. The main effect of AMFs on enhancing *Rhizobium* activity is through a generalized stimulation of host nutrition, although some more localized effects at the root or nodule level may occur (24), because the double inoculation elicits a synergistic effect with respect to the colonization of both microorganisms (25).

Photoperiodicity may also have influenced this behavior, characteristic of this crop, which allows it to grow more in conditions of long days, high temperatures and precipitation, a peculiar aspect of the spring season or rainy season of the Cuban climate.

In Cuba, an increase in nutrient content in canavalia has been reported as a direct response to the inoculation with *G. cubense* in Red Ferralitic soil, compared to the control without inoculation, given the effects that AMF have on the improvement of absorption and nutrient content by the host plant (19).

With regard to nutrient extraction, it is known that legumes present more N than any other cover crop, which results in an important contribution of this element to the soil-plant system after cutting green manures (18).

Quantification of the biological fixation of N by Canavalia ensiformis

Table III shows the quantification result of the biological nitrogen fixation process, determined by the methods of the total N difference and the natural abundance of ^{15}N.

The result of the quantification of BNF in the rainy season remained above 40 % in the group of treatments without mycorrhizal inoculation, being the Can 5 strain. In the group of treatments with mycorrhizal inoculation, it was possible to show the best behavior and the highest percentages of fixation with the species *R. intraradices*; expected result, being considered to be the most efficient for these edaphic conditions (26). Similar results were found when evaluating the process using the natural abundance method of ^{15}N. In most cases except for some exceptions, the method quantified percentages of fixed N, higher than those evaluated by the total N difference method.

In both methods, the lowest values of quantification were detected in treatments without inoculation of rhizobia strains. This result evidences the low efficiency of the native rhizobia of the soil in performing this process in symbiosis with canavalia and reiterates the advantages of proceeding to inoculation with efficient strains of this legume, in order to guarantee a greater contribution of the element to the soil. In this regard, it is proposed that inoculation of legumes with efficient strains of *Rhizobium* can increase the FBN process by up to 30 % (18).

Similarly, when the process was evaluated in the low rainy season, although the quantities quantified were lower than those obtained in the rainy season, which is a logical behavior, it is taking into account the seasonality of canavalia. In the low rainy season, with short days, the growth decreases compared to the rainy season (17) and the biological fixation process is reduced by the effect of decreasing water availability[B].

In agreement with these results, it is known that some species of tropical legumes used as green manures in Brazil, can make a high contribution of N to the system via FBN, mainly due to the high planting densities. In the case of canavalia up to 80 % of this, N from the FBN quantified by the natural abundance method of ^{15}N (18). For the conditions of Cuba, it has been reported that this species when grown on Red Ferralitic soil may be able to have 62 % of the leaf N derived from the FBN process, which is the equivalent of more than 80 kg ha^{-1} of N derived from the air, confirming the importance of this legume as a substitute for nitrogen fertilization in agricultural systems (27).

When analyzing the paired samples of the quantification of FBN, expressed in kg ha^{-1}, a p value greater than 0,05 was observed, which shows that the samples are similar. This means that both methods are valid to determine the quantification of the FBN process, which validates the use of the total N difference method under the experimental conditions under which the determinations were made.

[B] Freixas, C. J. A. *Influencia de los inoculantes a base de Bradyrhizobium elkanii ICA 8001, sobre la fijación biológica del nitrógeno en plantas de soya (Glycine max (L.) Merr.), con y sin déficit hídrico*. Tesis de Maestría, Instituto Nacional de Ciencias Agrícolas, 2011, Mayabeque, Cuba, 55 p.

Table III. Quantification of the biological fixation of N by canavalia, at 70 DAS, through two methods of determination in the two climatic periods studied

Climatic season	Mycorrhizal inoculation	Inoculation with rizobia	Quantification FBN			
			Difference of total N	Natural abundance ^{15}N	Difference of total N	Natural abundance ^{15}N
			%		kg ha^{-1}	
Rainy	without AMF	R0	49,89 (± 0,06)	63,00 (± 0,01)	35,80 (± 0,03)	31,43 (± 0,04)
		R2	43,31 (± 0,08)	41,90 (± 0,79)	27,47 (± 0,04)	18,15 (± 0,33)
		R3	49,69 (± 0,11)	56,00 (± 0,64)	35,51 (± 0,32)	27,82 (± 0,35)
		R4	45,54 (± 0,21)	92,11 (± 0,18)	30,07 (± 0,76)	41,95 (± 0,52)
		R5	54,04 (± 0,27)	93,43 (± 0,71)	42,28 (± 0,07)	50,49 (± 0,60)
	Glomus cubense	R0	53,34 (± 0,28)	63,30 (± 0,24)	41,10 (± 0,70)	33,76 (± 0,03)
		R2	54,56 (± 0,77)	95,06 (± 0,27)	43,18 (± 0,47)	51,86 (± 0,39)
		R3	58,99 (± 0,40)	97,42 (± 0,78)	51,73 (± 0,32)	57,47 (± 0,19)
		R4	55,75 (± 0,54)	94,97 (± 0,28)	45,30 (± 0,20)	52,94 (± 0,03)
		R5	60,08 (± 0,19)	97,32 (± 0,42)	54,12 (± 0,11)	58,47 (± 0,80)
	Rhizophagus intraradices	R0	70,31 (± 0,15)	85,40 (± 0,23)	85,17 (± 0,04)	60,05 (± 0,71)
		R2	57,52 (± 0,05)	98,48 (± 0,34)	48,70 (± 0,98)	56,65 (± 0,64)
		R3	57,18 (± 0,93)	98,42 (± 0,71)	48,02 (± 0,93)	56,28 (± 0,57)
		R4	65,45 (± 0,25)	98,77 (± 0,18)	68,13 (± 0,88)	64,65 (± 0,52)
		R5	73,61 (± 0,50)	98,99 (± 0,64)	100,30 (± 0,85)	72,87 (± 0,47)
Little rainy	Without AMF	R0	27,34 (± 0,24)	27,40 (± 0,10)	13,53 (± 0,81)	7,49 (± 0,43)
		R2	21,74 (± 0,03)	31,10 (± 0,46)	9,99 (± 0,78)	6,76 (± 0,39)
		R3	31,61 (± 0,90)	41,87 (± 0,39)	16,62 (± 0,76)	13,23 (± 0,36)
		R4	39,16 (± 0,08)	40,20 (± 0,41)	23,15 (± 0,74)	15,74 (± 0,25)
		R5	49,38 (± 0,53)	58,20 (± 0,55)	35,08 (± 0,65)	28,74 (± 0,15)
	Glomus cubense	R0	46,30 (± 0,15)	60,06 (± 0,81)	31,01 (± 0,59)	27,81 (± 0,04)
		R2	38,70 (± 0,77)	69,50 (± 0,22)	22,70 (± 0,52)	26,89 (± 0,94)
		R3	49,78 (± 0,40)	69,92 (± 0,81)	35,64 (± 0,46)	34,80 (± 0,86)
		R4	42,50 (± 0,05)	75,80 (± 0,62)	26,58 (± 0,39)	32,22 (± 0,74)
		R5	48,86 (± 0,71)	93,67 (± 0,67)	34,36 (± 0,33)	45,77 (± 0,08)
	Rhizophagus intraradices	R0	57,69 (± 0,38)	89,43 (± 0,06)	49,03 (± 0,70)	51,59 (± 0,03)
		R2	29,64 (± 0,07)	48,50 (± 0,33)	15,15 (± 0,47)	14,38 (± 0,99)
		R3	34,61 (± 0,78)	72,30 (± 0,95)	19,03 (± 0,32)	25,02 (± 0,096)
		R4	41,49 (± 0,50)	81,60 (± 0,15)	25,50 (± 0,21)	33,86 (± 0,92)
		R5	61,52 (± 0,25)	96,14 (± 0,03)	57,48 (± 0,12)	59,14 (± 0,90)
Test of t comparison of means for the variable FBN (kg ha^{-1})		χ				
		t		0,26		0,45
		p		0,80 N.S.		

R0: without *Rhizobium*, R2, R3, R4 and R5: *Rhizobium* isolates (Can 2, Can 3, Can 4 and Can 5, respectively). DAG: days after germination. Number in brackets = standard deviation of means. X = sample mean, t = statistical test t, p = p-value of test t

CONCLUSIONS

- Growth in dry mass and N uptake of canavalia planted in monoculture in a carbonated soft Sialitic Brown soil was superior in the presence of the *Rhizobium* Can 5 isolate, coinoculated with *Rhizoglomus intraradices*.
- Canavalia plants coinoculated with the isolate of *Rhizobium* Can 5 and *Rhizoglomus intraradices* produce a FBN ranging from 59 to 72 kg ha^{-1} in the rainy and rainy season, respectively.
- The methods of quantification of the FBN by the difference of total N and natural abundance of ^{15}N are similar to each other, although the difference of total N overestimates the quantification values with respect to natural abundance.

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