

Biological nitrogen fixation by *Rhizobium* sp. native gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) under greenhouse conditions

Fijación biológica de nitrógeno por *Rhizobium* sp. nativo de matarratón (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) bajo condiciones de invernadero

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ABSTRACT

An experiment was conducted under greenhouse conditions in order to evaluate the association and biological nitrogen fixation (BNF), six native strains of *Rhizobium* sp isolated from nodules gliricidia and a commercial strain (Rhizobiol©). Suspensions of 10⁶ and 10⁸ cells/mL for each of the strains studied and applied in two separate trials: the first with seeds and the second with seedlings 35 days of age gliricidia. In the seed test is calculated the cumulative percentage of germination for 7 days. In both trials after 42 days in seeds and seedlings 52 days in length was measured and stem diameter, leaf number, dry weight of aerial parts, number of nodules/plant and percentage of nitrogen. All treatments stimulated germination, growth and BNF, being better in the native strains at a concentration of 10⁸ cells/mL, compared with the commercial strain and the control. These results indicate the positive effect exerted by *Rhizobium* sp., in promoting growth and BNF, which could be used for further studies to establish the production of a biofertilizer, allowing potentiate the production of crops by gliricidia for food arich cattle as source of protein in the Caribbean Biotechnology Center.

Key words: biofertilizer, leguminous tree, sustainable agriculture, Caribbean.

RESUMEN

Se realizó un experimento bajo condiciones de invernadero con el fin de evaluar la asociación y fijación biológica de nitrógeno (FBN) en seis cepas nativas de *Rhizobium* sp. aisladas de nódulos de matarratón y una cepa comercial (Rhizobiol©). Las suspensiones de 10⁶ y 10⁸ células/mL para cada una de las cepas estudiadas y aplicadas en dos ensayos separados: el primero con semillas y la segundo con plántulas de 35 días de edad matarratón. En el ensayo de semilla se calcula el porcentaje acumulado de germinación durante 7 días. En ambos ensayos, después de 42 días en las semillas y 52 días en plántulas se determinó la altura y diámetro del tallo, número de hojas, peso seco de la parte aérea, número de nódulos por planta y el porcentaje de nitrógeno. Todos los tratamientos estimularon la germinación, el crecimiento y FBN, siendo mejor en las cepas nativas a una concentración de 10⁸ células/mL, en comparación con la cepa comercial y el control. Estos resultados indican el efecto positivo ejercido por *Rhizobium* sp., en la promoción del crecimiento y FBN, que podrían ser utilizados para estudios adicionales para determinar la producción de un biofertilizante, permitiendo potenciar la producción de cultivos de matarratón como alimento de ganado bovino como fuente de proteína en el Centro de Biotecnología del Caribe.

Palabras clave: biofertilizante, árbol leguminoso, agricultura sostenible, Caribe.

Introduction

In the department of Cesar, located northwest of the country on the plains of Colombian Caribbean, its main economic lines are the farm from which it derives 47% of their income. The cattle industry ranks first order, with an estimated population of 1,513,149 heads (Rey *et al.*, 2005; Rojas *et al.*, 2009). For this reason it is estimated that Cesar contributes about 38% of domestic production of meat and 43% of milk production depends mainly on the grazing of native and introduced grasses occupies 1.4 and 1.9 million have respectively (Rey *et al.*, 2005). However, currently being carried out through integrated management system

spontaneous cultivation of legumes associated with these grasses, because soil conditions limit the availability and quality of forage during dry periods in the year.

The cattle population is one of the most demanding with respect to food, is currently promoting the use legumes because grasses (conventional plant) are scarce and insufficient in the dry months. As the implementation of this system (or agroforestry legume-grass) is relatively new, the constant waves of summer during the year have caused disruptions in the lives of animals, reflecting disease which has led to low production yield in the single purpose in cattle (dairy), in addition to that you consider that there are

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currently more than 100 animals formed homogeneously pure breeds such as Guzerat, Gyr-Gir and Brahman, this has led to implementing such a system as an alternative to counter the problem of shortage of fodder (Centro Biotecnológico del Caribe, 2009).

Gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) cultivation, the alternative has proven to be more objective and conducive to overcome the shortage of fodder in dry seasons. The relevance is that the leaves of these legumes, have high concentrations of crude protein, energy and digestibility by cattle (Pedraza *et al.*, 2001; Rey *et al.*, 2005; Mahecha, 2002; Diouf *et al.*, 2008). Furthermore, it is believed that the implementation of this system, legume-grass (agroforestry), provides key components to improve the physicochemical and biological properties of soil, having the ability to fix atmospheric nitrogen (N_2), leaving aside the use indiscriminate synthetic fertilizers have generated some extent a negative impact on soil ecology, which is currently expressed in erosion, desertification and depletion of vegetation cover (Mahecha *et al.*, 2002).

The ability of legumes to fix N_2 , is given through a partnership that is built with own soil symbiotic bacteria belonging to the genus *Rhizobium*, which lets you pick from nodular symbiosomas all nitrogen necessary for their physiological functions and formation of structures vegetative, besides this, let legume fixed nitrogen made available to other plants nearby (Döbereiner, 1997; Ferrera and Alarcón, 2007; Mahecha, 2002).

Isolation and selection of native strains of *Rhizobium* sp., intended for creation of bio-fertilizers, is seen as a safe, effective and economical way to secure and restore soil productivity, as well as fix nitrogen produce phytohormones, are involved in the solubilization of phosphorus and backlash (Akhtar and Siddiqui, 2009; Mourad *et al.*, 2009; Puertas *et al.*, 2006; Santillana *et al.*, 2005; Bardin *et al.*, 2004). This complex set of features makes the host plant set up in any type of soil; improve all biological processes manifest in maximum productivity.

The present investigation was to assess the potential for infectivity, promoting plant growth and effectiveness in N_2 fixation of native strains of *Rhizobium* sp. isolated from nodules gliricidia, by application of two bacterial concentrations (10^6 and 10^8 cells/mL) in seeds and seedlings under greenhouse conditions, this will continue with a period of consolidating field that supports a byproduct based symbiotic nitrogen-fixing microorganisms that contribute to a sustainable agro-ecological system.

Materials and methods

This research was conducted at the Microbiology Laboratory and greenhouse Caribbean Biotechnology Center (CBC), located in the municipality of Valledupar (Cesar) a $10^{\circ}27'20''$ N and $73^{\circ}15'30''$ W, with an average temperature of 28.4°C and a height of 169 m, annual rainfall of 961 mm and relative humidity of 67%. In the area, wind speeds reach 14.76 km h^{-1} , moderate rainfall spread between April and November with peak in May and October, this is provided by a slightly warmer climate (Ideam, 2005; Alcaldía de Valledupar, 2011)

Six strains were isolated native *Rhizobium* sp. from nodules of seedlings established crops gliricidia in the CBC for the evaluation and more commercial strain Rhizobiol© (CS). The strains were reactivated in Petri dishes containing yeast extract mannitol agar (YMA) and incubated at a temperature of 28°C for 3-5 d (Wang and Martínez, 2003). From these cultures were adapted inocula at concentrations of 10^6 and 10^8 cells/mL suspended in sterile distilled water.

Seed bioassay

Seed germination in greenhouse

Bags were made with 1 kg of previously sterilized soil, seeds were placed gliricidia three, 1% hypochlorite and 70% alcohol to a depth of 2 cm. Experimental design was applied in a randomized complete block of 7×2 , with three replicates per treatment and five experimental units each repetition, using as factor the native strains and the CS, two concentrations 10^6 and 10^8 cells/mL.

The inoculation was done aseptically using 2 mL of the respective bacterial suspension 10^6 and 10^8 cells/mL of each of the strains of *Rhizobium* sp. and the CS; prepared from pure cultures by counting in Newbauer chamber, applying directly on the seed inoculum. The control treatment (without inoculation) alone was added 2 mL of sterile distilled water.

Observations were made after 7 d, checking the number of seeds germinated seeds considering that emerged to the surface. For this assay were established 15 treatments including the control without inoculation. From the data obtained, we calculated the percentage of germination expressed as a percentage cumulative germination (PG) at 7 d after inoculation.

With reference to the total seeds sown and germinated seeds total, as indicated by the following formula:

$$PG = \frac{\text{Total germinated seeds} \times 100}{\text{Total seeds sown}} \quad (1)$$

Infectivity and growth promotion effectiveness in biological nitrogen fixation (BNF)

After 2 weeks after it is determined the percentage of germination, seedlings were thinned each pot leaving the most vigorous (Mora, 1995; Matos *et al.*, 2007). The seedlings were maintained under greenhouse conditions with characteristics in 70-80% humidity, temperature of 31-34°C where the air volume, brightness and attack by pests are controlled, spray irrigation was applied twice daily for 2 h. After 42 d it took the data on stem length (SL) with the help of a tape measure and stem diameter (SD) with the help of a caliper, was recorded the number of true leaves (NTL) dry weight of aerial parts (DW) based on the methodology proposed by Reyes *et al.* (2008). We determined the number of nodules/plant (NNP) and percentage of leaf nitrogen by the Kjeldahl method (Jiménez, 2007).

Seedling bioassay

Were taken perforated polythene bags and filled with 1 kg of previously sterilized soil. Each pot was planted with three seeds *gliricidia* previously disinfected with iodine 70%, 1% sodium hypochlorite and 70% alcohol and subjected to pretreatment in sterile distilled water for 2 h before planting. After two weeks of emerged seedlings were thinned each pot leaving the most vigorous (Mora, 1995; Rincón, 2000). After a period of 35 d inoculation was performed by carefully removing the soil at the level of the stem base and adding 2 mL of the respective bacterial suspension (10^6 and 10^8 cells/mL), each of the strains of *Rhizobium* sp. and the CS, prepared from pure cultures (Matos and Zúñiga, 2002). The control treatment alone was added 2 mL of sterile distilled water. For this assay were established 15 treatments including the control without inoculation. Also in the seed test variables were measured for this test.

The results for both tests was performed comparing averages by multiple range test of Duncan ($P \leq 0.05$) and ANOVA, using the statistical package Statgraphics Plus version 5.1 and tested for the statistical package SPSS version 15.

Results and discussion

Seed bioassay

Seed germination

According to the results of all treatment levels were 100% except G21-106 and G34-106 who obtained values of 93.3% in both cases, with values over the control (80%), this is

possibly due to the production of phytohormones such as indole acetic acid (IAA) and gibberellins (Peña and Reyes, 2007). The concentration factor led to differences between treatments obtained a germination rate of 100% in all treatments the concentration treated with 10^8 cells/mL versus 80% obtained by CS-106 concentration 10^6 cells/mL, obtaining notice that the more cell the greater the effect on germination.

Some investigators have reported favorable results when assessing the effect of strains of *Rhizobium* sp. in the germination of seeds. Santillana *et al.* (2005) evaluated strains of *Rhizobium* sp., isolated from *Vicia faba* and *Pisium sativum* var. macrocarpum at concentrations of 10^8 cells/mL in seeds of *Solanum lycopersicum*, finding values of 100% in 9/19 strains evaluated with respect to the control of 80%.

Infectivity and growth promotion effectiveness in BNF

The Tab. 1 presents the means for measuring values obtained after 42 of observation. The stem length (SL), shows statistically significant differences ($P \leq 0.05$) better treatment G32-108 (34.77 cm), compared with the control (26.20 cm) and the commercial strain in both concentrations and CS-106 CS-108 with values of 26.4 cm and 26.9 cm respectively, with these results we can deduce that the strains of *Rhizobium* sp. native generated a positive impact in terms of their length. The statistically significant differences ($P \leq 0.05$) for SL, showing that treatments with higher bacterial concentrations (10^8 cells/mL), differ statistically from the other treatments except for G35-108 (29.70 cm) and CS-108 (26.93 cm), who are not significantly different ($P \leq 0.05$) compared with the control (26.20 cm). For the ST, was found better results in treatment G21-108 and G21-106, with respective values of 3.7 mm and 3.83 mm (Tab. 1), presenting significant differences only for the latter with compared to the control (3.0 mm) in both the commercial strain CS-106 concentrations (3.17 mm) and CS-108 (3.0 mm).

The NTL, no significant differences compared with the control, however the strains G32-108 (43.33), G35-108 (43.0), G36-108 (43.0), G21-108 (42.33), G38-108 (42.33) showed higher values compared with the control (36.67), CS-106 (37.0) and CS-108 (39.33) and can be noted that the inoculum of 10^8 cells/mL had a positive influence on this variable.

These results contrast with the DW, the best treatments for this analysis were G21-108 (1.28 g), G32-108 (1.287 g), G32-106 (1.30 g) and the highest value was for G38-108 (1.32 g) with respect to the control (0.81g), CS-106 and CS-108,

TABLE 1. Stem length (SL), stem thickness (ST), number of true leaves (NTL), dry weight (DW), number of nodules/plant (NNP) and percentage of nitrogen of plants gliricidia in the test with seed set in the greenhouse for a period of 42 d.

Treatments	SL (cm)	ST (mm)	NTL	DW (g)	NNP	N (%)
G21-106	30.70 abcde	3.83 c	41.67 a	1.19 b	26.95 bcde	0.359 bc
G21-108	32.63 cde	3.70 bc	42.33 a	1.28 b	43.67 fgh	0.355 bc
G32-106	33.77 de	3.30 abc	41.67 a	1.30 b	27.67 bcde	0.327 a
G32-108	34.77 e	3.53 abc	43.33 a	1.29 b	48.87 gh	0.817 d
G34-106	33.70 de	3.43 abc	39.67 a	1.07 b	30.40 cde	0.340 a
G34-108	32.90 cde	3.50 abc	39.67 a	1.11 b	38.60 efg	0.554 abc
G35-106	28.77 abcd	3.20 ab	39.67 a	1.06 b	16.62 b	0.691 cd
G35-108	29.70 abcde	3.30 abc	43.00 a	1.19 b	25.83 bcd	0.625 cd
G36-106	31.27 abcde	3.10 a	39.67 a	1.04 b	19.60 bc	0.648 cd
G36-108	32.77 cde	3.17 ab	43.00 a	0.84 b	24.57 bcd	0.591 bcd
G38-106	28.23 abc	3.37 abc	34.33 a	0.97 b	36.33 dfg	0.721 cd
G38-108	31.70 bcde	3.10 a	42.33 a	1.32 b	53.93 h	0.832 d
CS-106	26.37 a	3.17 ab	37.00 a	0.92 b	22.20 bc	0.314 a
CS-108	26.93 ab	3.00 a	39.33 a	0.82 b	32.10 cdef	0.630 cd
Control	26.20 a	3.00 a	36.67 a	0.81 b	0 a	0.332 a

Means in the same line followed by different letters indicate a significant difference according to Duncan test ($P \leq 0.05$). G = *Rhizobium* sp. native strain for gliricidia, CS = commercial strain. 106 and 108 = 10^6 or 10^8 = concentration of cells/mL.

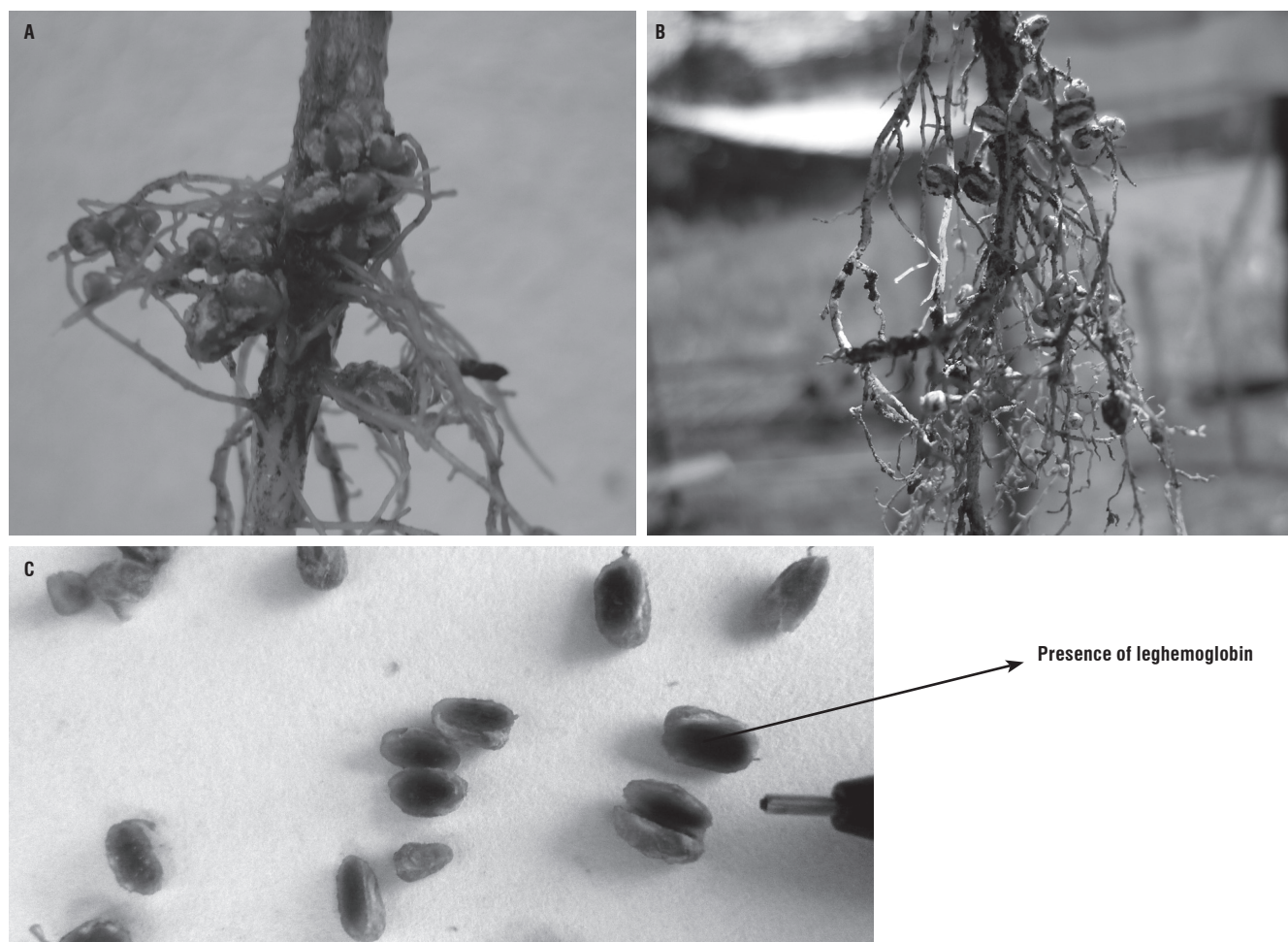


FIGURE 1. Nodulation in seedlings of gliricidia. (A) Nodulation reached for the test with seedlings, (B) Nodulation distributed throughout the root system to the test seed (C) effective nodules sectioned. Photos: J.G. Cubillos.

with values respectively 0.92 and 0.82 g (Tab. 1), also showed good results for G34-106 (1.07 g), G34-108 (1.107 g), G35-108 (1.190 g), G21-106 (1.190 g), however there were no significant differences according to Duncan test ($P \leq 0.05$). These results are similar to those found by Mora (1995), who managed to obtain significant differences in dry matter of common bean plants huasteco black variety (*Phaseolus vulgaris* L.), inoculated with strains of *Rhizobium leguminosarum* biovar phaseoli native, with respect to control without inoculation.

The NNP and %N, makes clear in the first instance, that the strains of *Rhizobium* sp., generated positive effect on the nodulation process (Fig. 1), relative to uninoculated control (Tab. 1), which showed no nodulation. The highest values were driven by G34-108 (38.60 NNP), G21-108 (43.67 NNP), G32-108 (48.87 NNP) and the highest value was obtained for G38-108 (53.93 NNP), showing significant differences according to Duncan test ($P \leq 0.05$), with respect to CS-106 and CS-108 with values of 22.2 and 32.10 NNP, respectively. This shows the points made by some researchers (Rincón *et al.*, 2000), which indicated that in most soils can be found indigenous populations of rhizobia, which will differ in their ability to infect legumes according to the chemical and soil physical and due to the slow process of natural selection are subject to stress conditions and excretion of substances carried out by the roots as flavonoids and glycoproteins that attract or select the different strains of *Rhizobium* sp., living in the rhizosphere of these plants.

For the %N, was found better results for CS-108 (0.63%), G36-106 (0.648%), G35-106 (0.691%), G38-106 (0.721%), G32-108 (0.817%) and the highest percentage was for G38-108

(0.832%), showing significant differences compared with the control without inoculation (0.331%) and CS-106 (0.314%). It was found that G38-108 (0.832%), with significant differences in the concentration commercial strain 10^6 cells/mL CS-106 (0.314%), but not statistically different CS-108 (0.63%). On the other hand the values obtained by CS-106 and control without inoculation (0.331%), not significantly different from each other (Tab. 1). It can be inferred that the strains of *Rhizobium* sp. native generated a very positive effect in terms of biological nitrogen fixation in plants gliricidia, applied at concentrations of 10^6 and 10^8 cells/mL directly on the seeds, being even favorable results in the treatment with commercial strain concentration of 10^8 cells/mL. Nitrogen levels for the best treatments (G32-108 = 0.817% and G38-108 = 0.832%) could be found up to 150% more nitrogen accumulated, compared to the control without nitrogen. Similar results were reported by Matos and Zúñiga (2002), who found that native strains of *Rhizobium* sp. were highly efficient nitrogen content increased up to 76.8% in Pallar Criollo Iqueño (*Phaseolus lunatus*) and 76.2% in Pallar ICA 450; with respect to a control added with nitrogen. Moreover Urzúa *et al.* (2001), noted a correlation between the aerial dry matter production, produced by forage vetch (*Vicia benghalensis*) and accumulation of nitrogen in greenhouse conditions.

Seedling bioassay

Infectivity and growth promotion effectiveness in BNF

In Tab. 2 presents the means for measuring values obtained after 17 d after application at 35 d, measurements were made (Mora, 1995).

TABLE 2. Means for stem length (SL), stem thickness (ST), number of true leaves (NTL), dry weight (DW), number of nodules/plant (NNP) and percentage of nitrogen of plants gliricidia in the test with seedlings, established in the greenhouse for a period of 52 d.

Treatments	SL (cm)	ST (mm)	NTL	DW (g)	NNP	N (%)
G21-106	28.53 a	4.79 ab	45.0 a	2.213 c	19.87 b	0.627 c
G21-108	30.22 abc	4.86 b	50.33 abc	2.193 c	25.2 bcd	1.02 d
G32-106	32.14 abcd	4.77 ab	51.67 abc	2.05 bc	27.43 bcd	0.413 b
G32-108	38.97 e	4.55 ab	53.0 abc	1.94 bc	31.23 bcd	0.36 b
G34-106	32.53 abcd	4.76 ab	52.33 abc	2.083 bc	29.2 bcd	0.417 b
G34-108	38.89 e	4.68 ab	58.33 c	1.97 bc	32.83 bcd	0.427 b
G35-106	33.66 bcd	4.83 ab	49.33 abc	2.063 bc	37.23 d	0.423 b
G35-108	36.10 de	4.14 a	48.0 ab	1.86 bc	30.5 bcd	0.463 b
G36-106	29.36 abc	4.34 ab	50.0 abc	1.633 ab	27.0 bcd	0.363 b
G36-108	35.80 de	4.27 ab	52.0 abc	2.233 c	23.33 bcd	0.363 b
G38-106	31.20 abcd	4.44 ab	50.33 abc	1.603 ab	30.33 bcd	0.467 b
G38-108	34.24 cde	4.47 ab	55.33 bc	1.603 ab	36.37 cd	0.383 b
CC-106	28.33 a	4.61 ab	46.67 ab	1.86 bc	20.33 bc	0.08 a
CC-108	29.2 abc	4.61 ab	50.0 abc	1.79 bc	32.77 bcd	0.09 a
Control	28.89 ab	4.22 ab	44.0 a	1.22 a	0 a	0.073 a

Means in the same line followed by different letters indicate a significant difference according to Duncan test ($P \leq 0.05$). G = *Rhizobium* sp. native strain for gliricidia, CS = commercial strain. 106 and 108 = 10^6 or 10^8 = concentration of cells/mL.

The result obtained for SL in the seedlings at 52 d, shows that all treatments had a positive effect on this variable, except G21-106 (28.53 cm), G21-108 (30.22 cm), G36-106 (29.36 cm), CS-106 (28.33 cm) and CS-108 (29.2 cm) (Tab. 2), who did not differ significantly according to Duncan test ($P \leq 0.05$), compared with the control (28.89 cm), but there were differences with the G36-108 treatments (35.8 cm), G35-108 (36.1 cm), G34-108 (38.89 cm), G32-108 (38.97 cm), showing these last two treatments higher values (Fig. 2). Also is found that the concentration produced a significant effect on the SL showing better results in 10^8 cells/mL except the treatment with the commercial strain CS-108 and G21-108 (Tab. 2). Similar results were reported by Matos and Zúñiga (2002), who found that the length of the stem in two varieties of Pallar Criollo iqueño (*P. lunatus*) and ICA 450, was significantly higher compared with the control, after being inoculated with native strains of *Rhizobium* sp., and *Bradyrhizobium* sp.

For the ST was found that the strains did not generate a significant effect on this variable compared with the control, however the highest values in terms of stem diameter (Tab. 2) were found in G21-108 (4.86 mm), G21-106 (4.79 mm), G35-106 (4.83 mm), G32-106 (4.77 mm), G34-108 (4.68 mm) and G34-106 (4.76 mm) compared with the control (4.22 mm), CS-106 and CS-108 who gave values of

4.61 mm in both cases but there were no significant differences. With respect to NTL gave better results in the treatment G34-108, with 58 leaves/plant, which presents significant differences with respect to G21-106 (45.0), G35-108 (48.0), CS -106 (46.67) and the control (44.0), who had the lowest number of leaves. At this point there is the relationship between the variables SL and NTL for the treatment G34-108 who showed the highest values in both analyzes 38.89 cm and 58.33 leaves/plant, respectively. These results are due to greater leaf area, provide greater availability of photosynthate to the plant for nitrogen fixation, which is reflected in a better development (Matos and Zúñiga, 2002).

As relates to the DW found significant differences only in G36-108 (2.23 g), G21-106 (2.123 g), G21-108 (2.193 g), compared with the control (1.22 g). The treatments CC-106 (1.86 g) and CC-108 (1.79 g), showed no statistical differences with the native strains according to Duncan ($P \leq 0.05$). With the above we can say that there is strong relationship between the variables ST and DW, as the treatments G21-106 and G21-108, were filed within the highest values in both cases. These results are similar to those obtained by Urzúa *et al.* (2001) who found in the dry matter yield better results for native strains of *Rhizobium leguminosarum* bv. viceae inoculated in *Vicia benghalensis*, whose values

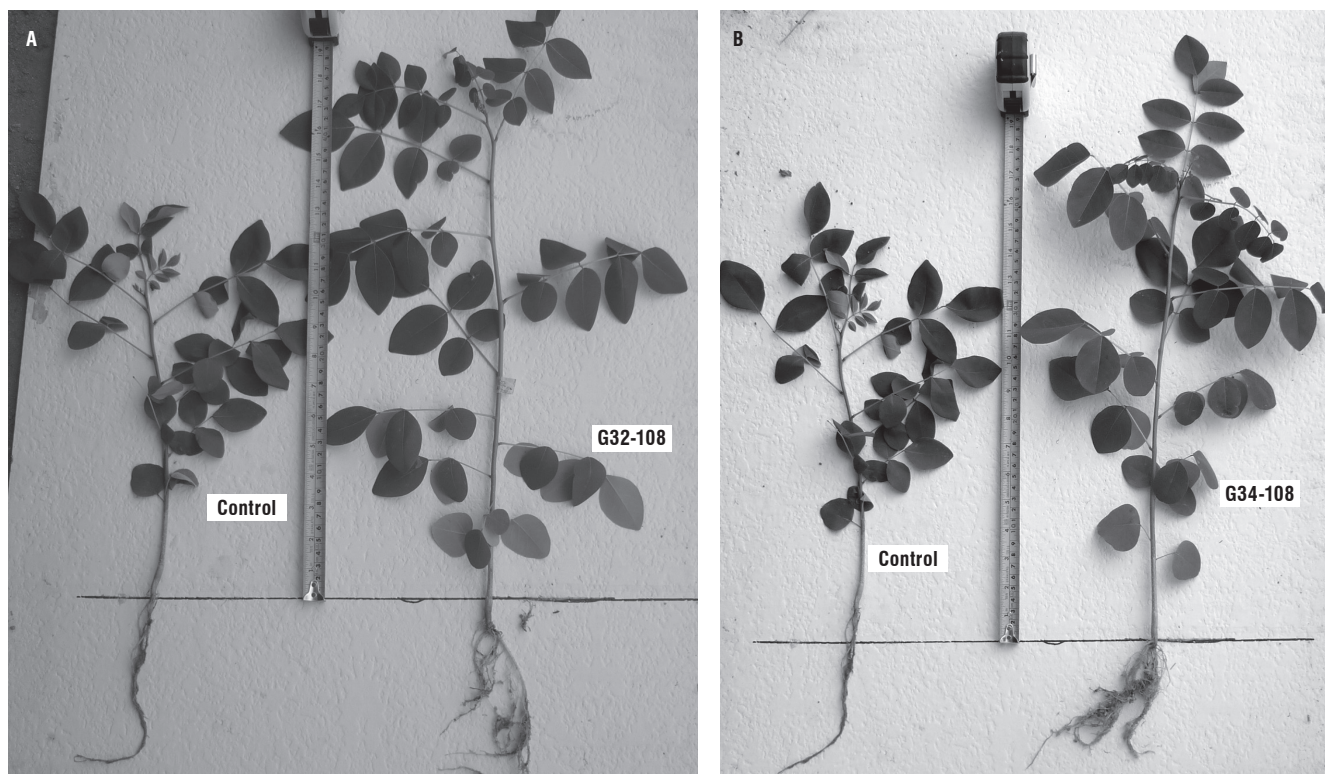


FIGURE 2. Gliricidia seedlings after being inoculated with strain native *Rhizobium* sp. G32-108 (A) and G34-108 (B) compared with the control. Photos: J.G. Cubillos.

were above the control and the reference strain *R. leguminosarum* bv. viceae, obtaining significant differences with native strains analyzed.

In analyzing the NNP, we found that the native strains of *Rhizobium* sp., generated a positive effect in nodulation compared to the control treatment who did not submit nodulation. This is attributed to good soil sterilization process, which eliminated any possible presence of indigenous strains in soil (Mora, 1995). To measure this variable did not take into account data such as the size of nodules and redness to cut them, only the total count of nodes present in the root of each seedling.

The highest levels of nodulation were found in G34-108 (32.83 NNP), CS-108 (32.77 NNP) G38-108 (36,37 NNP) and G35-106 (37 NNP), appearing only in last treatment significant difference compared with the commercial strain CS-106 (20.33 NNP), G21-106 (19.87 NNP) and with the control. But some researchers say the ability of native strains to form nodules, does not necessarily indicate the efficiency of biological nitrogen fixation (Mora, 1995).

Legumes is common “promiscuous” presented a nodulation “semi-effective” where there is a good nodulation, but set a small amount of N, which prevents them from reaching their yield potential. Thus, not all strains of *Rhizobium* sp. are effective in fixing nitrogen, although the infectivity is high (Mora, 1995).

For the %N values were lower in CS-106, CS-108 and the control with respective values of 0.08, 0.09 and 0.073%, with significant differences ($P \leq 0, 05$) with the other treatments, being highest levels in G21-108 and G21-106, with values of 1.02 and 0.63% respectively. Also is found good results for G34-108 (0.427%), G35-108 (0.463%) and G38-106 (0.467%), who despite being below G21-106 and G21-108, showed significant differences compared to CS-106 (0.08%) CS-108 (0.09%) and the control (0.073%). Is observed that G38-106 (0.467%), showed better results with respect to G38-108 (0.383%) but no significant differences ($P \leq 0.05$). This may be due to the settling time of the nodules, because the higher the concentration of rhizobia greater the competition for space and nutrients in the root, indicating more time to set when the inoculum was found in higher concentrations. According to Matos and Zúñiga (2002), who argue that early nodulation could induce an earlier onset of nitrogen fixation, therefore a greater amount of nitrogen accumulated in the plant. Infectivity early could increase the effect of the native strains inefficient. (Matos and Zúñiga, 2002; Rincón *et al.*, 2000; Mora, 1995).

Conclusions

The native strains of *Rhizobium* sp., generate a positive effect on seed germination gliricidia, stimulating germination to levels of 100% in most treatments compared with the control with 80%. Is found that the best results in treatments who receiving inoculation of 10^8 cells/mL, also had a significant effect on infectivity, effectiveness and growth promotion highlighting the strains G34, G38, G21, G32 which showed better results with respect to accumulated dry matter, percentage of nitrogen in concentrations 10^8 cells/mL, found results well above the commercial strain and the control.

The nodulation was found in relation to leaf nitrogen accumulation was much better in the test with seed, due to increased settling time of the rhizobia strains as were distributed throughout the radicle. Is found that commercial strain Rhizobiol© in the highest concentration also stimulate the growth and development of seedlings, but these values were below the native strains. The inoculum of 10^8 cells/mL significantly influenced the growth and development of seedlings, with respect to the concentration 10^6 cells/mL for both strains native to the commercial strain. This indicates the possibility of advancing the study and evaluation of field due to its better adaptation to agro-ecological and climatic conditions of the region and subsequently the development of a bioproduct to mechanize farming gliricidia, roll feed, emphasizing harmony with environmental policies, and improving the development and sustainability of agriculture in the Colombian Caribbean area.

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