Colegio de Postgraduados

Native rhizobia and their role in the nodulation, growth, and development of *Leucaena leucocephala* (Lam.) de Wit seedlings

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ABSTRACT

Objective: To evaluate the effect of native rhizobial strains on nodulation, growth, dry biomass production, total nitrogen (N) and carbon (C) content in plant tissue, as well as nitrogenase activity in seedlings of *Leucaena leucocephala*.

Design/Methodology/Approach: The study followed a completely randomized design. Seeds of *L. leucocephala* were germinated in an inert substrate, and six treatments were evaluated, corresponding to native rhizobial strains and an uninoculated control. Plants were harvested at 45, 75, and 105 days, and the following parameters were measured: (i) total number of nodules, (ii) plant height (root and shoot), (iii) dry plant weight, (iv) carbon and nitrogen content in dry biomass, and (v) nitrogenase enzyme activity.

Results: Treatments with strains 40, 41-2, and 46 showed the highest number of nodules at 75 days. At 105 days, plants inoculated with strain 74 had a significantly higher average dry biomass ($p<0.001$). The average nitrogen content was significantly higher ($p<0.0001$) in treatments with strains 26, 34b, 46, and in control. No significant differences were observed in carbon content among treatments. Nitrogenase activity was confirmed in all inoculated treatments but was absent in control.

Limitations/Implications: The results obtained with native *Rhizobium* sp. strains highlight their potential to enhance biological nitrogen fixation in *Leucaena leucocephala*, which could contribute to the development of more sustainable agroforestry systems, especially in nitrogen-deficient soils.

Findings/Conclusions: This study highlights the potential of certain native rhizobial strains for inoculating *L. leucocephala*, enhancing its growth and development. However, further research under field conditions is needed to confirm these findings.

Keywords: Biological nitrogen fixation, Fodder biomass, Native rhizobia strains, Nitrogenase, Sustainable livestock.

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INTRODUCTION

Food demand is expected to increase anywhere (between 35-56%) by 2050, while crop yields are growing too slowly (or even decreasing), making it increasingly difficult to meet this projected demand (Valin *et al.*, 2013; van Dijk *et al.*, 2021). In addition, tropical soils in many regions are deficient in nitrogen (N), and this deficiency reduces the productivity of

crops, pastures, and livestock, further exacerbating food production challenges (Soumare *et al.*, 2020; Udvardi *et al.*, 2021). One solution used so far has been N fertilization, which is necessary to maintain or increase the nitrogen reserves in soil and sustain agricultural productivity. However, its excessive use has led to negative environmental impacts, such as eutrophication of water bodies, volatilization of compounds into the atmosphere, and changes in soil through alterations in organic matter content, microbial life, and acidity (Khan and Mohammad, 2014; Pan *et al.*, 2016; Singh, 2018).

In this context, it is important to consider sustainable strategies for soil fertility management. One alternative is the use of symbiotic nitrogen fixation by legumes, which fixes atmospheric N_2 to soil (Vanlauwe *et al.*, 2019). Legumes can enhance agroecosystem productivity by forming associations with soil bacteria, as biological nitrogen fixation has been reported to range from 16.0 to 389 kg ha^{-1}, depending on the region and legume species (Pereyra *et al.*, 2015; Kebede, 2021).

A promising example of nitrogen-fixing legume is *Leucaena leucocephala*, a widely distributed shrub in the tropics. It revitalizes monoculture-based animal production systems into competitive silvopastoral systems, offering an affordable protein source for livestock due to its nitrogen-fixing ability (Shelton and Dalzell, 2007; Murgueitio *et al.*, 2014). However, the effectiveness of nodulation in *Leucaena* spp. depends on the association with specific rhizobial strains, highlighting the need for systematic inoculation with selected strains (mostly of the genus *Rhizobium*) to maximize symbiotic efficiency (Wong *et al.*, 1989; Turk and Keyser, 1992; Bala and Giller, 2001).

Incorporating shrub legumes like *Leucaena* sp. can significantly enhance soil fertility due to their nitrogen-fixing ability in symbiosis with rhizobia bacteria, enhancing soil fertility with rates surpassing 250-500 kg N per ha annually in tropical regions (Casanova-Lugo *et al.*, 2014). Beyond soil benefits (such as increase of soil organic C and soil total N), legumes offer significant nutritional value, with *L. leucocephala*-grass pastures in Australia yielding more beef and profits than grass-only systems (Bueno y Camargo, 2015; Hopkins *et al.*, 2019); however, the successful introduction of legumes into new areas depends on the presence of compatible rhizobia in the soil (Clúa *et al.*, 2018).

In this sense, there is growing interest in using rhizobia as inoculants to enhance crop productivity. For legumes with specific symbiotic requirements, such as *Leucaena* sp., identifying and isolating effective rhizobial strains can be beneficial. Sourcing rhizobia from the legume's native region (such as Mexico) offers promising potential, given the rich diversity of strains found there, including some not reported elsewhere (Martínez-Romero and Caballero-Mellado, 1996).

Moreover, apart from facilitating nodulation and nitrogen fixation, certain rhizobia strains produce growth-promoting compounds, including phytohormones like indole acetic acid (IAA) and gibberellins; given IAA's role in plant functions, its production by rhizobia is noteworthy (Lugtenberg and Kamilova, 2009; Maithani *et al.*, 2023).

It has been reported that legumes often show greater compatibility with specific rhizobial strains; similarly, *L. leucocephala* exhibits selective associations, with nodulation occurring primarily when inoculated with its own species-specific rhizobia (Wong *et al.*, 1989; Turk and Keyser, 1992). This highlights the importance of identifying compatible

rhizobial strains for *L. leucocephala* to enhance symbiotic efficiency in regions where it is being introduced or promoted. Such is the case of *L. leucocephala* cv. Cunningham, which has been promoted in silvopastoral systems due to its high nutritional value (276.8 g crude protein per kg of dry matter) (Cuartas *et al.*, 2015). The aim was to evaluate the effect of native rhizobial strains on nodulation, growth, dry biomass production, total nitrogen (N) and carbon (C) content in plant tissue, as well as nitrogenase activity in seedlings of *Leucaena leucocephala*.

MATERIALS AND METHODS

Experiment conditions: The experiment was conducted under semi-controlled conditions in a greenhouse at the Faculty of Veterinary Medicine and Zootechnics of the Universidad Autónoma de Yucatán (Mérida, México). The substrate employed was limestone rock powder, sterilized beforehand in an oven at 120 °C for 24 h to eliminate any present bacteria o fungi. Following sterilization, the substrate was placed into clean 5 L plastic bags.

L. leucocephala seeds were previously disinfected and scarified (with hot water at 80 °C for 2 min.), in each bag, three seeds were sown. After a week of post-germination, thinning was conducted to retain only one seedling per bag. Finally, irrigation was performed up to the field capacity of the substrate using the following nitrogen-free nutrient solution: 279 K_2SO_4; 493 $MgSO_4.7H_2O$; 0.23 KH_2PO_4; 145 K_2HPO_4; 371 $CaCl_2.2H_2O$; 1.43 H_3BO_3; 1.02 $MnSO_4$; 0.22 $ZnSO_4$; 0.08 $CuSO_4$; 0.05 Na_2MoO_4; 0.10 $CoCl_2.4H_2O$; 16.70 $FeCl_3.6H_2O$ (quantities are in mg L^{-1}).

Rhizobia isolation, inoculant preparation and inoculation: Six previously biochemical and molecular characterized native rhizobium strains were selected (Tzec-Gamboa *et al.*, 2020), chosen for their production of specific growth-promoting compounds (Table 1). Rhizobia strains were isolated from two localities of Yucatán, Caucel (21° 01' 13.2" N; 89° 42' 29.7" W) and Motul (21° 04' 07.2" N; 89° 16' 45.7" W).

The inoculum preparation of the rhizobial isolates was performed in 125 mL Erlenmeyer flasks with 50 mL of yeast extract-Mannitol (ELM) medium. The flasks were incubated at 30 °C under continuous stirring at 120 rpm until the exponential phase of each of the inoculum was reached. Cell growth was standardized with medium turbidity at a given optical density, relating it to a standard viable count curve, adjusting it to 10^7-10^9 cells mL^{-1}.

The cultivated rhizobia cells served as inoculum for *L. leucocephala* cv. Cunningham seeds. Prior to sowing, 1 mL of the desired inoculum in saline solution (10^7-10^9 cells mL^{-1}) was applied per seed (9 ml per bag). A week after snowing, a second inoculation of 3 mL of the inoculant was administered per bag.

Experimental design: Six native rhizobia strains (treatments) (Table 1) and one control (non-inoculated) were evaluated using a completely randomized design, with 15 experimental units (replicates) per treatment [7 treatments × 15 replicas = 105 *L. leucocephala* plants]. Three destructive samplings were conducted (at 45, 75, and 105 days after inoculation). For each sampling, five plants from each treatment were randomly selected.

Table 1. Native rhizobia strains used as inoculants in this study, including main characteristics and the site where obtained.

Rhizobia Strain	Genus	GPC produced	Site	DT (h^{-1})
26	*Sinorhizobium* sp.	IAA	Caucel	4.3
34b	*Sinorhizobium* sp.	IAA, SID	Caucel	3.5
40	*Sinorhizobium* sp.	IAA	Caucel	4.5
41(2)	*Ralstonia* sp.	IAA, SID	Caucel	4
46	*Sinorhizobium* sp.	IAA	Caucel	3.5
74	*Rhizobium* sp.	IAA	Motul	4.0

Note: GPC=Growth promoting compound; IAA=Indoleacetic Acid; SID=Siderophores; DT=Duplication time.

Evaluation of agronomic variables: For sampling and subsequent measurement of agronomic variables, each selected bag was placed on a sterile plastic tray. The bag was then laterally cut open, and the substrate was carefully removed to avoid damaging the roots of the plants. Once all the substrate was removed, the following measurements were conducted: 1) Nodule count per plant; 2) Plant height (from the base of the stem to the apex), the roots were cleaned, using a sterile scalpel, the above-ground and root portions were separated and measured using a ruler; and 3) Dry biomass, both segments of the plant were placed in paper bags and dried in an oven at 60 °C until a constant weight was achieved, and then weighed.

Laboratory analysis: For the determination of total Carbon and Nitrogen in both the root and aerial parts of the plants; after drying, they were ground; and then, an elemental analysis was conducted using a Carbon/Nitrogen element analyzer (FLASH 2000® Series Organic Elemental Analyzers from Thermo Scientific).

Atmospheric nitrogen fixation was verified by the activity of the nitrogenase with the technique of reduction of acetylene to ethylene described by Peoples *et al.* (2009). The technique consisted of sectioning the nodules with a little of the root of each plant, the excess soil was removed and placed in vials with serological caps on the lid, 10% v/v of the air contained in the vial was removed, and the same amount of high purity acetylene was injected in an amount equivalent to 10% of the total volume of the vial, it was injected into the hermetically sealed container. The roots with the nodules were incubated at room temperature for one hour.

After incubation, two samples were taken from each container with 1 ml syringes, which were analyzed on a Hewlett Packard 5890 (HP; Palo Alto, CA, USA) gas chromatograph equipped with a flame ionization detector (FID) and a column HP-PLOT/Q column (50 m × 0.23 mm internal diameter). Helium was used as carrier gas (1 ml min^{-1}), hydrogen (45 ml min^{-1}), nitrogen (10 ml min^{-1}), and air (450 ml min^{-1}). The run temperatures were injector 110 °C, oven 60 °C and detector 160 °C. Acetylene and ethylene were used as reference standards (Hardy *et al.*, 1973; Vessey, 1994).

Statistical analysis: Data from the experiment were first subjected to test of normality and homogeneity of variance for each variable and then to analysis of variance (ANOVA),

using a significance level of $P<0.05$. Treatment means were compared using Tukey HSD significance test (SAS for Windows).

RESULTS AND DISCUSSION

Nodulation

Nodules were observed in all treatments; however, in some treatments, nodules could not be identified at the first sampling (45 days). Nodulation at 45 days was scarce and was only observed in the treatments inoculated with strains (40, 46 and 74). The other inoculated treatments with strains 26, 41-2, 34b and 74 showed formation of nodules after 75 days, while control plants that were not inoculated did not develop nodules (Table 2). The nodules were oval, brown in color and of variable dimensions between 2-3 mm in diameter (Figure 1).

The treatments inoculated with strains 40, 41-2 and 46, had the greatest number of nodules in the shortest time (75 days), with an average of 15.3, 9.6 and 16.6 nodules per plant respectively, at 105 days a decrease was observed in the nodulation of these treatments. In the other treatments, the number of nodules increased gradually reaching a maximum average at 105 days, strain 26 had the highest average value with 17.6 nodules per plant at 105 days and was also the highest average among all treatments (Table 2).

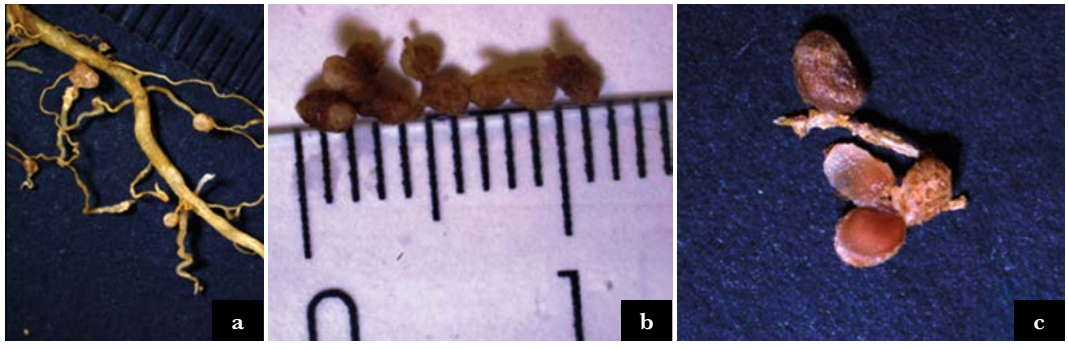

Figure 1. Main characteristics of the nodules found during the development of the experiment. (a) Distribution of nodules in the root system of plants, (b) Measurement of nodules (c) Active nodules, a longitudinal section of the nodule is shown, and the reddish coloration of the interior is observed, as an indicator of the presence of the enzyme nitrogenase.

Table 2. Average number of nodules obtained in *Leucaena leucocephala* plants on different growth days.

Rhizobia Strain	Nodule number (average per plant)		
	45 days	75 days	105 days
26	0	5	18
34b	0	3	10
40	2	15	6
41-2	0	10	10
46	4	17	11
74	4	6	16
Control	0	0	0

Biomass

Plants inoculated with strain 26 had the highest average dry biomass (0.11±0.03 g plant^{-1}) at 45 days, this was significantly different from the other treatments. The average dry biomass of the other treatments was all very similar. However, treatments inoculated with strains 46 and 34b had the lowest dry biomass (Table 3).

At 75 days, the plants inoculated with strain 74 had the highest average dry biomass, the difference was significant compared to other treatments (0.45±0.24 g plant^{-1}). The treatment inoculated with strain 26 had the lowest average dry biomass. At 105 days, plants inoculated with strain 74 had a significantly higher average dry biomass. The other treatments had similar dry biomass between them, no significant difference was observed. At 45 days, strain 26 had the highest average dry biomass despite not having developed nodules. However, at 75 days this strain 26 was the treatment with lowest dry biomass.

Dry biomass of the control, at 45 and 75 days, was the same as the treatments inoculated with strains 41-2 and 40. At 75 days, it was the same as the treatments inoculated with strains 40, 46, and 34b. At 105 days the dry biomass was equal to the treatments except for the treatment inoculated with the 74 strain that was greater with significant differences over all the treatments including the control.

Plant growth

Plant growth was assessed by measuring the length of roots and shoots (Figure 2). At 45 days, the plants with the highest growth were those inoculated with the 74 and 41-2 strains, the plants with the lowest growth were those inoculated with the strains 40, strains 34b and 46 had a similar growth characteristic (without significant differences) (Table 4).

At 75 days, the plants inoculated with the 74 strains were the ones with the longest shoots, these were significantly different ($P<0.01$), the plants inoculated with the strains 26, 34b and 46 had shorter shoots, similar to the control. At 105 days, plants inoculated with strains 74, 40 and 34b were the ones with the highest growth and these differences were significant. The plants that were not inoculated (control) had similar length shoots to those of the treatments during the first two samplings (45 and 75 days), but at 105 days the growth of the control plants was significantly reduced compared to the other treatments.

Table 3. Dry biomass (average g plant^{-1} of DM ± Standart error) of *L. leucocephala* plants on different growth days.

Rhizobia Strain	45 days	75 days	105 days
26	0.11±0.03a	0.21±0.03c	0.28±0.08b
34b	0.04±0.09e	0.33±0.09b	0.33±0.09b
40	0.07±0.01bc	0.29±0.07b	0.29±0.06b
41-2	0.08±0.02bc	0.26±0.09bc	0.26±0.09b
46	0.05±0.01de	0.30±0.07b	0.30±0.07b
74	0.07±0.02cd	0.45±0.24a	0.45±0.23a
Control	0.08±0.02b	0.31±0.09b	0.31±0.09b

Note: DM=Dry matter. Values with the same letter are not significantly different, $P<0.01$).

Figure 2. Measuring plants during the experiment. (a) *L. leucocephala* plants at 105 days, (b) *Leucaena* shoot (c) *Leucaena* root.

Table 4. Plants height (cm) of *L. leucocephala* plants on different growth days.

Rhizobia Strain	45 days	75 days	105 days
	Above-ground height (cm)		
26	17.70±1.07ab	19.29±0.67a	20.03±6.34ab
34b	14.94±0.57b	19.34±0.85a	18.56±5.87ab
40	17.41±0.63 ab	20.81±0.79a	19.14±6.05ab
41-2	19.43±1.49a	19.91±1.05a	17.94±5.67b
46	17.46±0.86 ab	18.46±0.84a	16.37±5.18ab
74	18.30±0.96 ab	18.96±0.79a	22.12±6.99a
Control	18.42±1.02 ab	17.32±0.76a	18.22±5.76ab
	Root length (cm)		
26	6.73±0.27a	13.09±1.76a	21.27±2.31a
34b	5.97±0.57a	14.98±1.40a	23.92±3.28a
40	5.64±0.41a	14.92±6.45a	22.70±3.37a
41-2	6.32±0.63a	23.44±2.53a	19.89±2.97a
46	4.97±0.69a	17.64±2.81a	15.12±2.97a
74	7.88±2.04a	18.44±2.45a	23.53±4.54a
Control	7.88±2.04a	11.99±1.62a	7.94±0.89b

Regarding root length, at 45 and 75 days, no significant differences were observed among any of the treatments, including the control. However, by 105 days, all strains exhibited significant differences compared to the control. Strains 34b and 74 showed the most substantial root development, though without significant differences between them or with any of the other strains (Table 4).

Nitrogen and carbon content

At 45 days, the plants inoculated with strain 34 had the highest average total nitrogen content, which was significantly different to all other treatments and the treatment with the lowest average nitrogen content was inoculated with strain 40 (Table 5). However, at the

Table 5. Nitrogen content (%) in *L. leucocephala* plants on different growth days.

Rhizobia Strain	45 days	75 days	105 days
26	5.3±0.01c	3.1±0.13d	3.6±0.00a
34b	5.8±0.11a	3.9±0.05b	3.8±0.15a
40	4.5±0.01e	3.2±0.18d	2.9±0.45c
41-2	4.7±0.12d	3.1±0.07d	3.2±0.17bc
46	5.4±0.03bc	3.8±0.07c	3.9±0.06a
74	4.7±0.09d	3.9±0.11bc	3.5±0.09ab
Control	5.5±0.15b	4.6±0.00a	3.8±0.09a

next sampling opportunity at 75 days a decrease in nitrogen content in plants was observed in all treatments. The control treatment maintained a higher nitrogen content with respect to the other treatments and this was significantly higher than other treatments.

At 105 days, the treatments inoculated with strains 26, 34b, and 46 together with the control, are those that stand out in their nitrogen content. However, it is noteworthy that in the course of time, in most treatments, a decrease in their nitrogen content was observed. However, the nitrogen content of the plants inoculated with strains 46, 26 and 41-2, which showed a decrease in N content between the first and second samples, were the only treatments that were able to increase their nitrogen content at 105 days. The carbon content was similar between the treatments and remained so until the end of the experiment (day 105), there were no significant differences (Table 6).

Nitrogenase activity

Nitrogen fixation by rhizobia was confirmed by the reduction of acetylene to ethylene by the enzyme nitrogenase present in the nodules. The activity of the nitrogenase enzyme was confirmed in all treatments except the control (Figure 3). However, response varied widely among treatments. The nodules of the strain 26 recorded the highest enzyme nitrogenase activity, as well as the highest concentration of ethylene (8214 μM C_2H_4 mg^{-1} nodules h^{-1}). Compared with these plants inoculated with strains 41-2 and 74 had the lowest values of enzyme activity with 989 and 581 μM C_2H_4 mg^{-1} nodules h^{-1}, respectively. The uninoculated treatment did not develop nodules, so it was not included in this analysis.

Table 6. Carbon content (%) in *L. leucocephala* plants on different growth days.

Rhizobia Strain	45 days	75 days	105 days
26	39.38±0.11	37.90±0.56	38.79±0.22
34b	39.42±0.16	38.02±0.16	37.41±0.46
40	38.36±0.08	37.95±0.90	40.32±7.05
41-2	38.96±0.45	38.20±0.26	37.65±0.53
46	39.82±0.37	39.15±0.10	38.13±0.25
74	39.49±0.16	38.96±0.34	38.03±0.60
Control	39.26±0.06	38.21±0.18	38.53±0.22

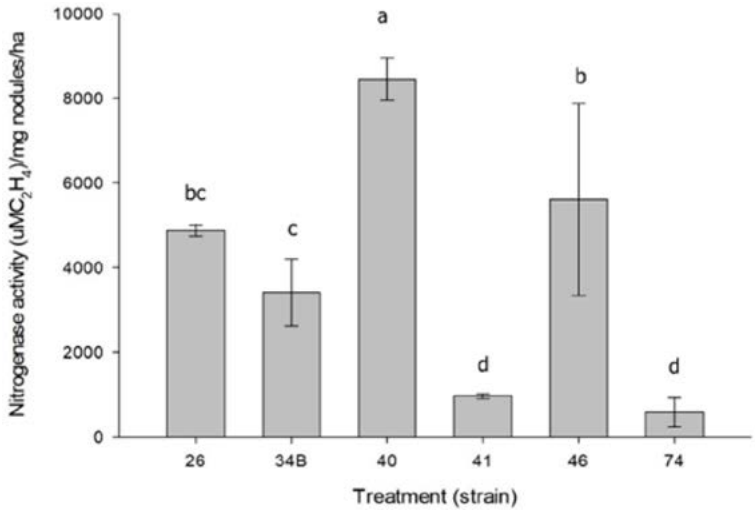

Figure 3. Nitrogenase enzyme activity in active nodules of *Leucaena leucocephala* seedlings inoculated with different native strains of rhizobia. Note: Standard error is presented in parentheses.

Generally, the average number of nodules per plant increased with the age of the plant, with a maximum of 18 and 16 nodules per plant from strains 26 and 74, respectively at 105 days. These findings are similar with those of Crespo-Flores *et al.* (2022), who discovered more nodulation in rhizobia-inoculated plants, although nodulation was observed in all treatments, there was variability among the strains, including the control (unlike in the present study, where the control showed no nodules). This might be caused by various factors, including the specificity between the host plant and the species/strain of rhizobia as well as the natural genetic variation of both (Borges, 2006; Duran *et al.*, 2013; Menezes *et al.*, 2017; Torres-Gutiérrez *et al.*, 2017).

In the present study, most treatments (26, 34b, 41-2, and the control) showed no nodulation at 45 days; similarly, Bueno and Camargo (2015) reported no nodulation during the first seven weeks (49 days), highlighting the effectiveness of the strains that did manage to induce nodulation (40, 46, and 74). Additionally, in the same study the nodulation peak was reached at 18 weeks (126 days), with an average of 25 nodules per plant, while in this study it was reach at 105 days; although the study ended at 105 days, a decrease in the number of nodules could not be observed in all treatments, which could mean that in some of the treatments the peak could have been reached days later.

Three of the treatments evaluated established effective symbiosis by day 45 through the formation of nodules. However, by day 105, some strains showed a decrease in nodule numbers, which is likely due to nodule senescence. This decline is likely due to nodule senescence, although pinpointing the exact causes, whether genetic factors of the strains or abiotic factors (*v.g.* drought or darkness) (Zhou *et al.*, 2021) is challenging at this stage.

Although the treatments inoculated with strains 26 and 34 did not develop nodules at 45 days, they excelled in total dry biomass and nitrogen content; while no correlation was observed between nodulation, dry biomass and nitrogen content at 45 days, this could be explained that during the first days of development, the seedlings take the necessary

nutrients for the development of the plantlets from the seeds (Salisbury and Ross 1992; Sánchez *et al.*, 2011). Another explanation is that the strains used are producers of growth promoting compounds such as IAA; since in a previous study we reported that these strains produce these compounds (Tzec-Gamboa *et al.*, 2020), and it has been shown that the effect of multiple hormones produced by rhizobia strains affects not only nodulation, but also other processes such as germination and plant growth (Santillana *et al.*, 2005).

Strains 40 and 46, belonging to the genus *Sinorhizobium* had the highest average number of nodules at 75 days, they showed high dry biomass production and plant height. Symbiosis with *Sinorhizobium*, was particularly effective, since all strains tested were able to nodulate at 105 days, as did strain 74 (*Rhizobium*), *Sinorhizobium* is a genus of bacteria, which has been reported as nodulant of *L. leucocephala*, however, not all strains belonging to this genus are able to do it (Wang *et al.*, 2002). Regarding *Rhizobium*, several studies highlight it as a common nodulating genus for *L. leucocephala*; however, it is important to emphasize that this symbiosis exhibits a high degree of specificity, even within the *Rhizobium* genus itself (Trinick, 1968; Moawad and Bohlool, 1984; Chen *et al.*, 2021; Ríos-Ruiz *et al.*, 2024).

Furthermore, treatment with strain 74 showed the highest dry biomass and best growth at 75 days. However, nodulation peaked at 105 days (16 nodules), with nodulation showing a positive correlation with time.

Perhaps the compounds produced by the rhizobia during plant establishment initially aid plants in more efficiently utilizing nutrients, rather than promoting nodule development. At 75 days, apart from the treatment inoculated with strain 74, all other treatments had a similar response, and no correlation was observed between nodulation and dry biomass.

As for nitrogen content, there were no significant differences between most of the strains and the control (105 days). However, in previous studies, it has been suggested that plant growth is independent of nodule growth, since although the N content in plants decreases over time, the total amino acid content does not change (Pereyra *et al.*, 2015). Similarly, Singleton and Tavares (1986) found a similar behavior where the nitrogen content in shoots was lower compared to the roots, attributing it to the concentration of the *Rhizobium* population.

Values for nitrogenase activity are shown in Figure 3, the amount of nitrogen fixed in this experiment compared favorably to previously published values, although it was not anywhere near as high as the values of 22,069 μM C_2H_4 mg nodules^{-1} H^{-1} reported in works performed in peanut inoculated with *Bradyrhizobium* (which is another promising nitrogen fixing genus) and a series of combinations of PGPR's (Badawi *et al.*, 2011). The selected inoculants showed a positive effect on growth, nodulation and nitrogen fixation. Plants inoculated with strain 26, which had the highest activity of the enzyme at 105 days, was the only treatment that increased plant growth by 16%, compared to the growth it showed at 75 days.

Although only root sections containing the nodules were used in the trial, the values obtained at 105 days were higher than those reported by Anthraper and Dubois (2003) in *L. Leucocephala* when using the complete root system, where levels of 10 Methylene plant^{-1} H^{-1}, were obtained. However, observed a 100% increase in enzyme activity after 147 days, due to an increase in the number of nodules.

CONCLUSIONS

All the treatments showed nodulation, suggesting that the strains are able to form a symbiosis with *L. leucocephala*. But among the strains, the plant response and nitrogenase activity varied, indicating that some strains have more potential for nitrogen fixation as well as for help in seedling development and growth. For their quantity of nodules generated and nitrogenase activity, for instance, strains 26 and 74 stood out; generally showing better performance in dry biomass generation and shoot growth. These results suggest that this interaction might foster positive effects on the establishment of *L. leucocephala* in low nitrogen soils. To evaluate the effectiveness of the strains, it is imperative to verify the observed effects under field conditions. Furthermore, treatment inoculated with strain 74 showed the highest dry biomass and best growth at 75 days. However, nodulation peaked at 105 days, with nodulation showing a positive correlation with time.

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