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EVALUATION OF RHIZOBIUM STRAINS COLLECTED IN TUNGURAHUA AND COTOPAXI ON THE GROWTH OF LUPINUS MUTABILIS, UNDER SEMI-CONTROLLED CONDITIONS

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Abstract: Two Rhizobium strains collected from chocho (Lupinus mutabilis, Sweet) plantations in the provinces of Tungurahua and Cotopaxi, Ecuador, were isolated and characterized in order to determine the effect of their inoculation on the vegetative development of Andean chocho, under semi-controlled conditions, under agricultural plastic cover. Molecular identification was performed by partial sequencing of the 16S rRNA gene, and the contrast with the GenBank database for Rhizobium was positive (99.7% certainty). The strains obtained were suspended in a sodium alginate matrix, both polymerized in alginate beads in a 0.1 M CaCl2 solution and in suspension, to be inoculated on selected chochocho seeds. The effect of strain origin, inoculation method and substrates on plant vegetative development was evaluated. Vegetative development was influenced by the substrate used for germination. The black Andean soil allowed the migration of the bacteroids towards the roots, taking advantage of the retained capillary water. The clay loam soil determined the lower vegetative development of the plant, as it was more susceptible to dehydration under canopy conditions. Strain origin also influenced vegetative development, although it was not evident in nodulation. The strain originating in Tungurahua produced more vigorous plants, especially in interaction with the Andean black soil substrate. Nodulation was significantly influenced by the substrates sand and Andean black soil, resulting in a higher number of nodules.
Keywords: 16S rRNA gene. Molecular identification. Lupinus mutabilis. Rhizobia sp. Genomic sequencing. GeneBank.

INTRODUCTION

Lupines (Lupinus mutabilis), commonly called lupins in Europe, Andean lupine, tarwi, or chocho in South America, are a genus of legumes that belongs to the Genisteae tribe of the Faboideae subfamily and encompasses more than 280 species of annual herbs and shrubs. herbaceous and woody perennials distributed mainly in southern and western North America, the Andes, the Mediterranean regions and Africa (Durán Wendt et al., 2013). Chochos are highly resistant to low temperatures with some species able to resist down to -9 °C. They are also tolerant of salinity to different degrees depending on the species (Atchison et al., 2016). Lupine can also be used to remediate contaminated soils in phytoremediation and phytoextraction processes (Nigussie, 2012). Since chocho has the ability to grow in poor soils, its cultivation is of great importance for production in marginal soils of the Ecuadorian highlands, contributing with a low-cost protein source to the food security of farmers (Atchison et al., 2016).

Members of the genus Lupinus have the ability to establish a symbiotic association with rhizobia and effectively fix atmospheric nitrogen in root nodules. These soil bacteria belong to the genera Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium, and Sinorhizobium. The chochos are mainly nodulated by soil bacteria classified in the genus Bradyrhizobium, although some other fast-growing strains have also been identified (Beligala, 2015). Rhizobium lupini is considered a basonym for the proposed reclassification as Bradyrhizobium lupini comb. Nov (Peix et al., 2015). For consistency with the reviewed studies and identification techniques, the bacteroid under study will be referred to as Rhizobium lupini.

Despite the marked advantages of the association of chochos and rhizobia, the native population of rhizobia is generally insufficient to achieve a beneficial relationship with the legume, so the increase in production

depends on the use of synthetic fertilizers (Cabrera Romero et al, 2017). The excessive use of synthetic nitrogenous fertilizers has caused soil contamination and eutrophication of surface and subsurface waters (Gonzales et al., 2019). The globalization of the fertilizer market has increased the vulnerability of local producers, so it is necessary to explore and take advantage of sustainable sources of nitrogen (De Vriese et al., 2021). For this, it is important the use of an infective (capacity to nodulate) and effective (efficient for N2 fixation) Rhizobium in the legume, which implies the need to identify and inoculate strains of different origin (Fernández-Pascual et al., 2007). The inoculation of lupine roots with different strains of Rhizobium improves the levels and availability of nitrogen and carbon in the soils and therefore the vegetative development of the plant (Taco & Zuñiga, 2017). The objective of this research was to evaluate the effect of the inoculation of two Rhizobium spp. isolated and characterized, on the vegetative development of Lupinus mutabilis lupine under semi-controlled conditions. Rhizobium strains were isolated from root nodules of Lupinus mutabilis cultivated in Tungurahua and Cotopaxi provinces.

METHODOLOGY STUDY LOCATION

The study was carried out in the Ambato canton, Tungurahua province, Ecuador, at the coordinates UTM17S 762413.17 E, 985683.013 N. The collection of roots infected with Rhizobium was carried out in two commercial crops in the Tungurahua and Cotopaxi provinces, the first in the province of Tungurahua (762289.59 E, 9856984.48N) and the second in the province of Cotopaxi (UTM17S 770397.45 E 9898067.25 S) (Figure 1).

The isolation and genetic sequencing of Rhizobium was carried out in the biotechnology laboratories of PlantSphere Laboratories, Ecuador.

SELECTION OF ROOT NODULES, ISOLATION AND IDENTIFICATION OF RIZHOBIUM

The nodule isolation process in Lupinus mutabilis was carried out under the 882 PSL-RHIZOBIUM protocol from PlantSphere Laboratories (Falconí, nd).

The prospecting and collection of root nodules was carried out in the predetermined locations in commercial crops. Two young and vigorous flowering plants were selected for each sampling point, with a total of 10 plants per lot. The sampling was carried out in a stratified way and recovering the intact root system.

For the processing of the nodules in the laboratory and primary isolation of Rhizobium, the sequence detailed in (Figure 2). Typical Rhizobium spp colonies were picked every 24 hours and subjected to a second isolation for Rhizobium differentiation. The identification of strains of Rhizobium sp. it was carried out from the colonies of the secondary isolation, by means of seeding by depletion in YMA + RC medium (Yeast Mannitol Agar + Congo Red), and based on the absorption of Congo red. For their conservation, the strains were cultivated in YMA + AB on an inclined plane and in Mannitol Yeast broth, then they were refrigerated at 4 - 5°C. Viability tests were performed every 30 days, by depletion seeding on YMA plates.

MOLECULAR IDENTIFICATION OF RHIZOBIUM ISOLATES

EXTRACTION OF DNA

The DNA of each strain was extracted by the phenol-chloroform method. The bacterial strains grown in YEM broth were centrifuged and the pellet was resuspended in 400 µl of TE

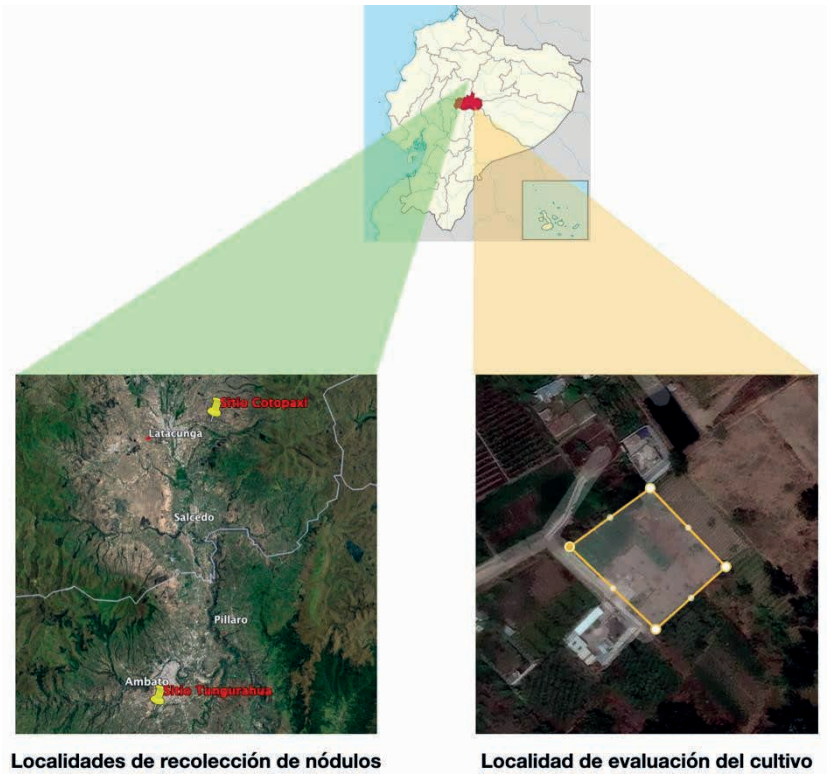

Figure 1 Geographical location of the study site

Source: Adapted from Directorate of Planning. GADP Tungurahua. Google Earth Engine.

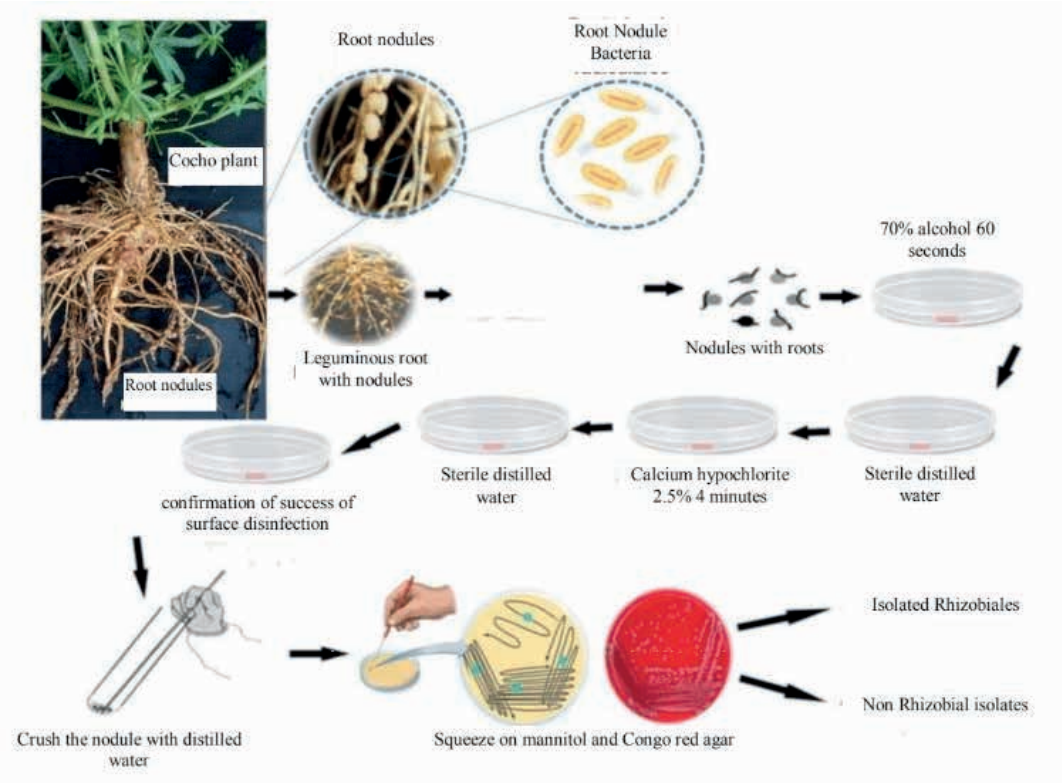

Figure 2 Isolation and Identification of Rizhobium Strains

Note. Adapted from Etesami (2022)

buffer, 5 µl of 20 mg/ml proteinase K and 40 µl of 10% SDS were added and incubated at 560C for 45 min, 400 µl of tris-saturated phenol was added and centrifuged for 10 min. The aqueous phase was transferred to a new tube. To the mixture were added 200µl of saturated tris phenol and 200µl of chloroform:isoamyl alcohol (24:1). The supernatant after centrifugation was taken and 0.1 volume of 3M sodium acetate and 2 volumes of chilled absolute ethanol were added and incubated again for 2 hours at 20C. After centrifugation, the pellet was washed with 70% ethanol and resuspended in 50 µl TE buffer (Girija et al., 2020).

POLYMERASE CHAIN REACTION AND IDENTIFICATION OF RHIZOBIUM SP ISOLATE

The partial sequence of the 16S rRNA gene was amplified using the universal primers, 616V: 5′-AGA GTT TGA TYM TGG CTC AG -3′, 699R: 5′-RGG GTT GCG CTC GTT -3. Amplification conditions were set at 940C for 90 s (denaturation), 550C for 40 s (annealing), and 720C for 90 s (extension). PCR was carried out for 34 cycles, plus a final extension at 720C for 10 minutes. DNA bands separated by electrophoresis were observed under a UV illuminator. The PCR product was purified and sequenced according to Scigenom Pvt. Cochin (Girija et al., 2020). The primers for PCR amplification and sequencing were the 16S rRNA bacterial sequences from the EMBL/GenBank Data Library (Huasasquiche, 2018). Sequence identity of 16S rRNA was established with the National Center for Biotechnology Information NCBI GenBank database by neighbor joining method using MEGA 7 software.

MULTIPLICATION OF RHIZOBIUM SP.

The Rhizobium isolate to be multiplied was taken from the preserved samples. The quantity of the isolate was increased by successive passages in flasks of increasing volumes until reaching a number of Rhizobium of approximately 106 bacteria/g of inoculant.

INOCULATION TECHNIQUES

A sodium alginate solution (Sigma-Aldrich, Inc. St Louis, USA) of 20 g L-1 was prepared by solubilization in deionized water at 60°C. The mixture was kept under constant stirring until the complete solubilization of the alginate. The solution was sterilized in an autoclave for 15 minutes at 121°C. A 100 ml volume of the cultures of the two Rhizobium strains were concentrated by centrifugation at 3500 rpm for 15 min. The cells were resuspended in 100 ml of the previously prepared sodium alginate solution at room temperature. The alginate matrix with the bacteria in suspension was polymerized in a 0.1 M CaCl2 solution dropwise into a CaCl2 solution under constant stirring. The CaCl2 was kept under constant stirring for 30 minutes. The beads were recovered on a sieve and rinsed with sterile distilled water. The immersion of preselected and sterilized lupine seeds were submerged in the suspension of Rhizobium strains. The seeds were recovered with a sieve.

FIELD EVALUATION OF LUPINE SEEDS INOCULATED WITH RHIZOBIUM SP.

The field evaluation of the two Rhizobium strains (A1 Tungurahua and A2 Cotopaxi) in the growth of Lupinus mutabilis was carried out in the Huachi Chico parish (UTM17S 762413.17 E, 985683.013 N), Ambato canton, Tungurahua province. A factorial design with 3 factors (Table 1). The cultivation conditions were under plastic cover, providing protection to the physical climatic elements (temperature,

precipitation and wind), which were partially controlled. Statistical analysis was performed in R Studio.

Factor	levels	Description of the levels	
Origin of the Isolates	2	Tungurahua.	(A1)
		Cotopaxi	(A2)
Method ofinoculation	2	Immersion	(I1)
		encapsulation in alginate	(I2)
Guy of substratum	3	Sand	(S1)
		clay loam soil	(S2)
		andean black soil	(S3)

Table 1 Factors under study

Planting was carried out in plastic nursery bags, which were previously disinfected with 10% fifth-generation quaternary ammonium. The substrates were sterilized in an autoclave at 121oC for 30 minutes. The cultural labors were limited to weeding the exposed substrate and irrigation.

VARIABLES EVALUATED

Data was collected for the following variables:

days to emergency. The days elapsed from sowing to the emergence of 50% of sown seeds that had cotyledonous leaves breaking the soil surface were counted. The emerged plant was considered to be the one whose cotyledonous leaves could be seen on the surface of the substrate.

vegetative development. The growth of the plant was defined as the volume of a cone, formed as the base by the radius of the stem, taken over the root neck, and the height of the stem, taken from the root neck to the apex of the plant.

Stem height was recorded in cm at 15, 30 and 45 days after sowing (DAS). The measurement was made from the neck of the root to the apex of the plant. Stem diameter in cm was determined with a Vernier caliper, at 15, 30 and 45 days after sowing (DAS). The measurement was made on the neck of the root of the plant.

Number of nodules per plant. They were counted at 45 days, in each plant of the experimental unit and reported an average per treatment.

RESULTS

ISOLATION AND IDENTIFICATION OF RHIZOBIUM STRAINS FROM CHOCHO ROOT NODULES

ISOLATION AND CHARACTERIZATION OF RHIZOBIUM

The viability tests of the bacteroides present in the nodules were performed with Gram staining, observing the presence of viable Gram-negative rhizobia (Figure 3**Mistake! Reference source not found.**). Typical colony morphology, and cell growth was observed by Gram stain. The strains that presented growth in the second isolate were coded to allow typical Rhizobium differentiation.

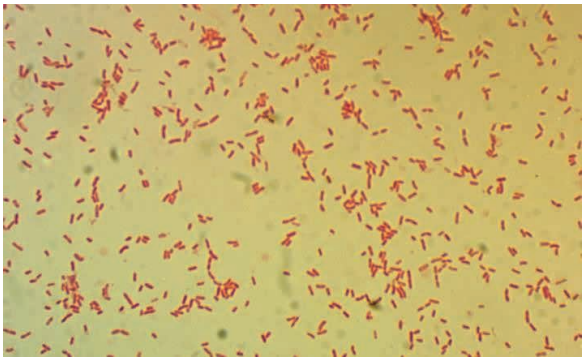

Figure 3 Viable Rhizobium bacteroides, viewed under the light microscope (1000x)

The morphology of the bacterial colonies of each site showed consistent and homogeneous characteristics, for which it was decided to pool the isolates by location. The description of the morphotype of the two isolates is detailed in the Table 2

Molecular identification of Rhizobium

Horizontal electrophoresis shows distinct bands of DNA fractions, between 1000 and 1500 base pairs (Figure 4**Mistake! not found Origin of the reference.**). The sample DNA fragment is observed in lane 2.

SANGER sequencing was performed for the template and complementary DNA strands. The obtained sequences were cleaned and assembled using the bioinformatics programs FinchTV v1.5.0 and Geneious v11.1.5. After a satisfactory DNA extraction, the 16S barcode of the MB00 sample was amplified and a species of the genus Rhizobium (Table 3).

BOARD 3

MOLECULAR IDENTIFICATION OF BACTEROIDES

BioSin Code	Length (bp)	Quality (%)	barcode	Organism	Identity(%)	No Access-GenBank
MB00	1307	97.4	16S	Rhizobia sp.	99.77	MT271224.1

VEGETATIVE DEVELOPMENT VARIABLES

DAYS TO EMERGENCY

Highly significant differences were determined for the substrates factor (p=0.001). The times to emergence corresponding to the sand (S1) and clayey loam (S2) substrates were significantly longer (Tukey a=0.05). The substrate with Andean black soil substrate (S2), show a significant delay in the emergence of seedlings (Figure 5**Mistake! The self-reference to the bookmark is invalid.**).

VEGETATIVE DEVELOPMENT

The statistical analysis of the factorial demonstrated a significant effect of the substrates factor (S) at all evaluation times. When comparing the growth of the plants 15 days after sowing between the different treatments, it is observed that the plants of those treatments that include the substrate black páramo soil (S3) are significantly more developed (Figure 6TO).

After 30 days of evaluation, the plants of the treatments that included clay loam soil showed less development (Figure 6B). The limitations of the initial development were reflected up to this stage, being consistent with the moisture retention conditions of the substrate. When analyzing the development of the plants 45 days after sowing, a clear trend of the interactions and control with Andean black soil is observed to facilitate a better vegetative development (Figure 6C). The greater contribution and retention of humidity of the organic matter in the Andean soil provides better conditions for the growth of the plant as well as for the better functioning of the nitrifying symbionts.

When evaluating the strains collected at the beginning of plant growth, the one from Cotopaxi (A2) favored the initial development of the plant. Apparently, the Tungurahua strain (A1) had a better adaptation to local conditions and, based on its better infection capacity and nodular development, allowed the nitrogen provided by the symbiont to allow better development at 45 days. The A1 strain from Tungurahua has consistently shown a better response in the evaluation of vegetative development, at the end of the study, with a significantly higher mean, according to Tukey, 0.05 (Figure 7).

NUMBER OF NODULES PER PLANT

For this variable, the statistical factorial analysis found significance at 5% for the substrates factor in the number of nodules per plant. The effect of the remaining factors was not significant. The sand (S1) and Andean black soil (S2) substrates allowed a statistically higher number of nodules (Figure 8). Once the bacteroides are released from the alginate capsule or pellicle created by immersion, they move to infect the roots, where they multiply

Characteristics	Isolated A1 Tungurahua	Isolated A2 Cotopaxi
coloniesDiameter(mm) Colour:		
Shape:	2 -2.5	1.8 -2.0
Edge: Elevation: Consistency:	Round Brilliant Translucent Smooth Pulvinada Mucilaginous	Beige Round Smooth Powdered Soft
bacteria		
Shape:	Rod cells elongated 2.0m-2.5 µ	Spheroidal cells 0.9m-1.0m
Long:	1.0µ	0.9m-1.0m
Broad:	Genus: Rhizobia	Genus: Rhizobia
Taxonomic determination: Additional remarks:	Cells showing transverse septa stained positively with Gram stain. Time of incubation for 5 to 7 days.	Incubation time 6 days

Table 2 Morphological description of the colonies and isolated bacteria

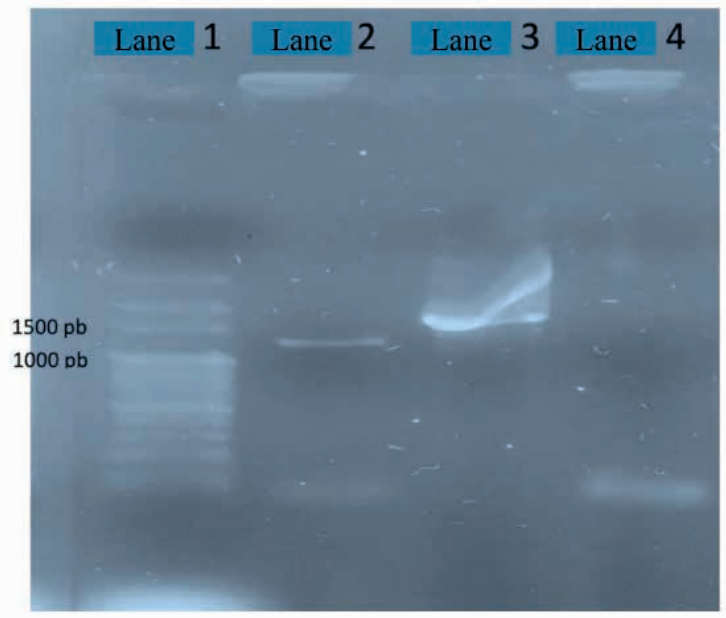

Figure 4 Horizontal electrophoresis of conventional PCR products

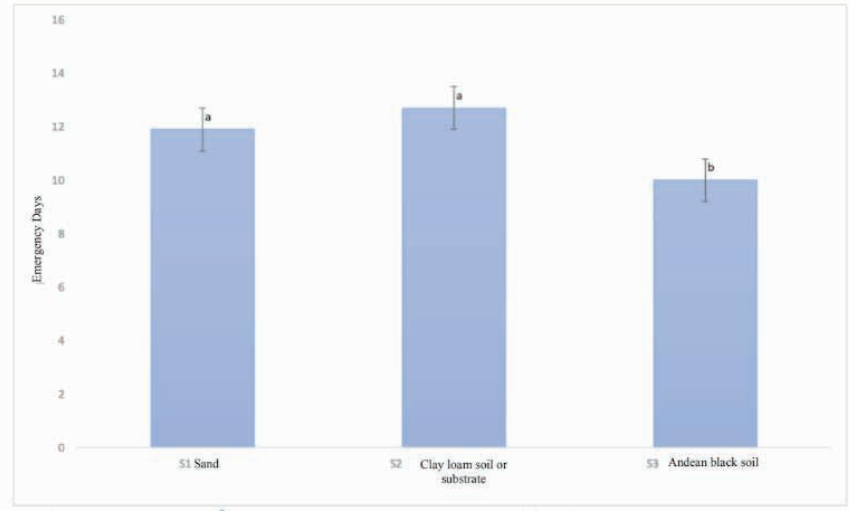

Figure 5 Tukey test at 5% for treatments in the variable days to emergency

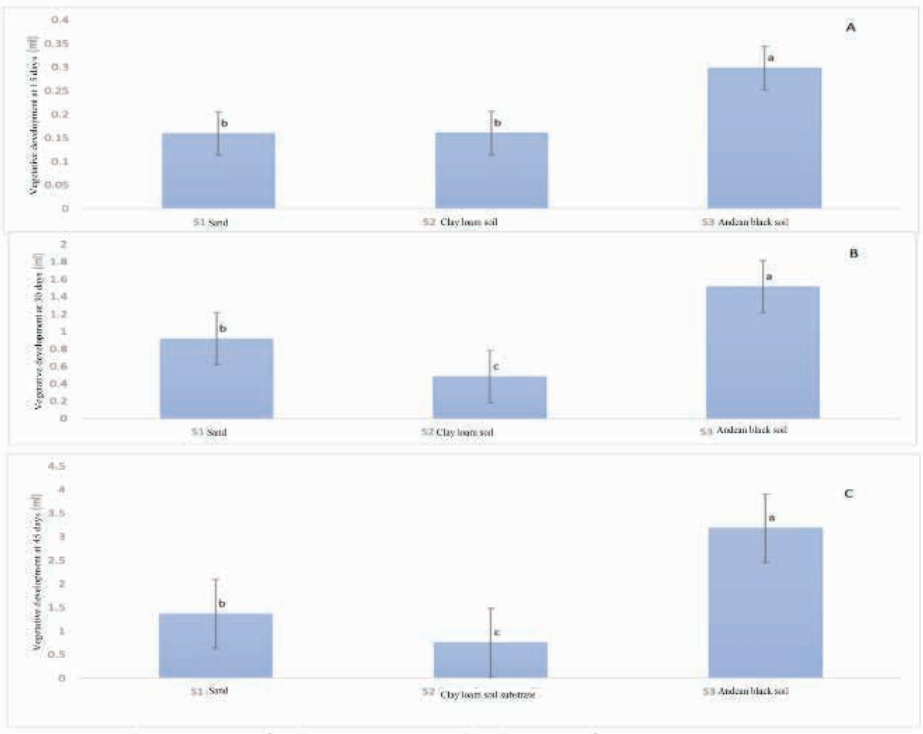

Figure 6 Tukey test at 5% for lupine vegetative development after sowing

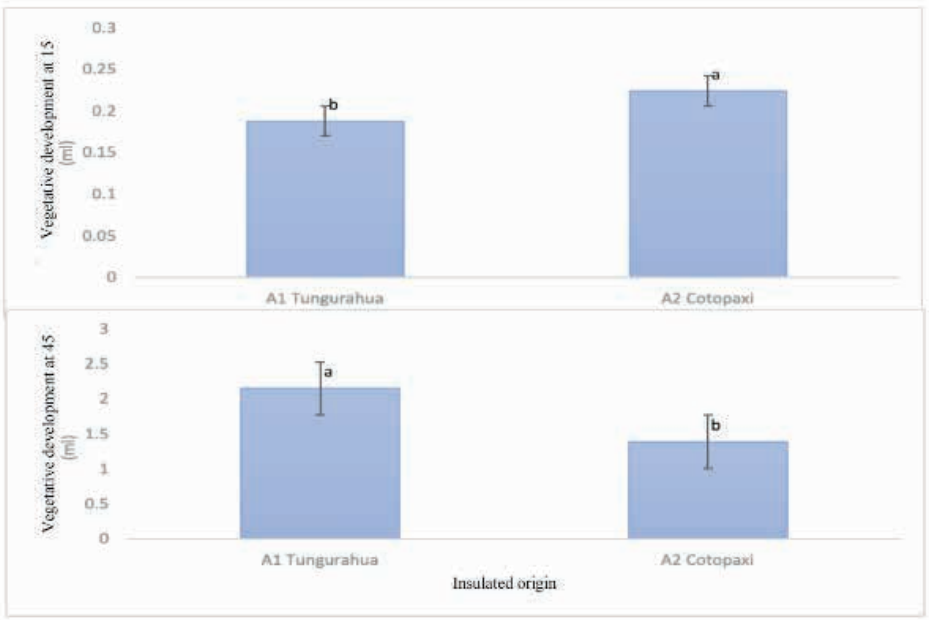

Figure 7 Vegetative development of lupine for the Origin of the Isolate factor

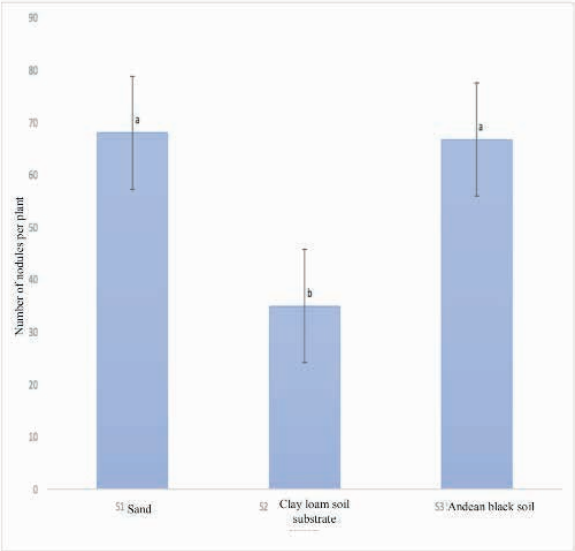

Figure 8 Tukey test at 5% for substrate in the variable number of nodules per plant

in the cells of the growing host nodule at a certain density.

Cell, they adapt to endosymbiotic living conditions and microaerobic conditions, maturing into functional nitrogen-fixing symbionts.

DISCUSSION

ISOLATION AND MOLECULAR IDENTIFICATION OF RHIZOBIUM STRAINS FROM ROOT NODULES OF LUPINUS MUTABILIS

The characterization of microorganisms is of vital importance for microbial ecology studies. The classification, identification and differentiation of microorganisms has traditionally been based on phenotypic characteristics. However, molecular methods based on the polymerase chain reaction (PCR) have become the faster, more reliable and easier alternatives to characterize and differentiate microorganisms. When typing biological material, the goal is to reveal the diversity within taxa. Taxa or taxonomic units are groups arranged on the basis of similarities or defined relationships that distinguish them from other organisms in a process called classification. Identification refers to assigning unknown isolates to a distinct taxonomic unit and naming it accordingly. Classification, nomenclature and identification make up the taxonomy with the 'species' as the central taxonomic unit (Etesami, 2022). As a complement to taxonomy, typing allows the differentiation of isolates within species or subspecies, that is, at the strain level. A variety of phenotypic and genotypic methods are used for microbial typing, identification, and classification (Huasasquiche, 2018). The applied method presents its advantages and limitations, in terms of ease of application, reproducibility, need for sophisticated equipment, mode of action, and level of phylogenetic and taxonomic resolution. DNA-based typing may involve specific or non-specific PCR amplification, restriction enzyme digestion, and fragment length analysis. PCR-based typing methods allow exploration of part or all of the Rhizobium genomic structure.

VEGETATIVE DEVELOPMENT VARIABLES

The emergence of the lupine vegetative structures may be due to the fact that the lupine has an "epigeal" emergence. As the chocho is an epigeal seedling, it develops with its cotyledons above the ground. While still underground, the apex of the hypocotyl bends to form a hook. Obstructing seedling growth (for example, by superficial crusting on the soil) keeps the hook closed and promotes lateral expansion of the stem to strengthen the emerging shoot. The hypocotyl is the first part to emerge from the ground, followed by the cotyledons. (Walker et al., 2011). The substrate with Andean black soil (S3), due to its high content of organic matter, tends to generate hardening of the surface.

It is known that the elongation of the main stem of lupine plants is largely controlled by the accumulated temperature (thermal time). Internodes elongate by 0.1–0.2 mm per degree-day, depending on their location on the stem. All stem internodes begin and end their growth in sequence, from the base of the stem to the top of the plant (apex). The Andean black soil substrate (S3) proved to have a great moisture retention capacity, which increases the thermal mass and therefore, the temperature of the substrate that directly influences the early development of the plant (Wolko et al., 2011). Although the lupine adapts to poor soils, the lower availability of water in the sandy substrate may be the reason for the lower initial development of the plant. Despite the fact that the lupine is a species that tolerates water scarcity, it is important

that there is moisture at planting for good germination and seedling emergence (Peralta et al., 2012). These answers are tried to explain considering that soils are a key component of the carbon cycle and Andean black soils represent the largest organic carbon sink. Environmental and biological factors are determining factors in the control of carbon dynamics in the soil, rather than the molecular structure of the soil or its physical parameters. This change in the paradigm emphasizes the need to understand how environmental conditions and microorganisms interact to modify the dynamics of Carbon, Nitrogen and Phosphorus in the soil (Camenzind et al., 2018). It is important that there is moisture at planting for good germination and seedling emergence (Peralta et al., 2012). These answers are tried to explain considering that soils are a key component of the carbon cycle and Andean black soils represent the largest organic carbon sink. Environmental and biological factors are determining factors in the control of carbon dynamics in the soil, rather than the molecular structure of the soil or its physical parameters. This change in the paradigm emphasizes the need to understand how environmental conditions and microorganisms interact to modify the dynamics of Carbon, Nitrogen and Phosphorus in the soil (Camenzind et al., 2018). It is important that there is moisture at planting for good germination and seedling emergence (Peralta et al., 2012). These answers are tried to explain considering that soils are a key component of the carbon cycle and Andean black soils represent the largest organic carbon sink. Environmental and biological factors are determining factors in the control of carbon dynamics in the soil, rather than the molecular structure of the soil or its physical parameters. This change in the paradigm emphasizes the need to understand how environmental conditions and microorganisms interact to modify the dynamics of Carbon, Nitrogen and Phosphorus in the soil (Camenzind et al., 2018). These answers are tried to explain considering that soils are a key component of the carbon cycle and Andean black soils represent the largest organic carbon sink. Environmental and biological factors are determining factors in the control of carbon dynamics in the soil, rather than the molecular structure of the soil or its physical parameters. This change in the paradigm emphasizes the need to understand how environmental conditions and microorganisms interact to modify the dynamics of Carbon, Nitrogen and Phosphorus in the soil (Camenzind et al., 2018). These answers are tried to explain considering that soils are a key component of the carbon cycle and Andean black soils represent the largest organic carbon sink. Environmental and biological factors are determining factors in the control of carbon dynamics in the soil, rather than the molecular structure of the soil or its physical parameters. This change in the paradigm emphasizes the need to understand how environmental conditions and microorganisms interact to modify the dynamics of Carbon, Nitrogen and Phosphorus in the soil (Camenzind et al., 2018). rather than the molecular structure of the soil or its physical parameters. This change in the paradigm emphasizes the need to understand how environmental conditions and microorganisms interact to modify the dynamics of Carbon, Nitrogen and Phosphorus in the soil (Camenzind et al., 2018). rather than the molecular structure of the soil or its physical parameters. This change in the paradigm emphasizes the need to understand how environmental conditions and microorganisms interact to modify the dynamics of Carbon, Nitrogen and Phosphorus in the soil (Camenzind et al., 2018).

Apparently the inoculation methodologies

determined the development behavior of the seedlings. The inoculation by encapsulation provides a better distribution of bacteroides in the root zone, and its active migration in the capillaries of the substrate. A more effective nodulation determines the supply of nitrogen to the plant for its early development. It has been estimated that only 10 to 15% of the cells in a Rhizobium culture are capable of adhering to the roots, and less than a quarter of these cells manifest irreversible adhesion. The largest adherence surface and the best distribution of the inoculum in the encapsulation process define the observed behavior, with the potential for nodulation and early provision of nutrients to the developing plant being higher (Lodeiro, 2015).

Great variability in infection capacity, atmospheric N fixation rate, and resistance to particular soil conditions has been reported in Rhizobium strains from contiguous regions, even in different sections of the same field. In rhizobia, their wide geographical distribution and the different hosts and niches have also been proposed.

Currently, it is recognized that rhizobia are aquatic and endophytic in addition to being soil bacteria. It seems that some nodule occupants may be favored by environmental conditions or even root depth (Sessitsch et al., 2002). The strains recovered in Cotopaxi come from arid zones, with lower rainfall regimes than those observed in the Tungurahua collection site.

Nodulation is influenced by the composition of the substrate. Once the bacteroides are released from the alginate capsule or pellicle created by immersion, they move to infect the roots, where they multiply in the cells of the growing host nodule at a certain cell density, adapting to the style of They live endosymbiotically and under microaerobic conditions and mature into nitrogen-fixing bacteroides (Sessitsch et al., 2002). The terminal differentiation of bacteroides is controlled by the host, evolved into multiple branches of the family Leguminosae, indicating host advantage and likely increased symbiotic performance. The higher number of nodules in the sand substrate can be explained by the response of the plants to both local and systemic soil nitrate.

The terminal differentiation of bacteroides is controlled by the host, evolved into multiple branches of the family Leguminosae, indicating host advantage and likely increased symbiotic performance. The higher number of nodules in the sand substrate can be explained by the response of the plants to both local and systemic soil nitrate. Nitrate transporters have an important role in nitrate sensing and nitrogen demand signaling in legumes affects the nitrate-dependent regulation of primary root growth through abscisic acid signaling, suggesting their role as a nitrate sensor (Chaulagain & Frugoli, 2021).

CONCLUSIONS

Molecular identification of local Rhizobium strains is essential to ensure inoculation into lupine seeds and potentially other legumes. The partial sequencing of the 16S rRNA gene, and the contrast with the GenBank database for *rhizobia*it was positive (99.7%) for Rhizobium sp. Having several Rhizobium accessions ensures the effectiveness of the inoculation, and symbiotic interactions are more likely given the potential genetic variability available. Inoculation is necessary, especially in soils that are poor in organic matter, since native strains have difficulties with infection due to the low bacterial load that occurs in such conditions.

The origin of the Rhizobium strains does not define the greatest vegetative development, rather it is evident that inoculation with strains from different locations ensures

the infectibility of nitrifying bacteria. The incorporation of genetic variability of strains from other origins increases the presence of nodule and makes the nitrogen fixation process more efficient.

The best vegetative development of the chocho can be extended until the maturation of the plant with an adequate symbiotic infection. The yields given by the greater availability of nitrogen in inoculated crops, ensures that the producer has a greater availability of seeds, both for consumption as well as to ensure high-quality germplasm for the preservation and expansion of the crop.

The technological complexity and the high dependence on highly specialized equipment and information, determines that the identification of Rhizobium is done systematically, creating an information network on the presence and availability of local strains of symbionts, and that they may be available to the local producers in a systematic way.

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