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Investigation note

Mycorrhization of *Lupinus* spp. in nursery to improve the development of plants in ecological restoration strategies

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Abstract

The species of the genus *Lupinus* are considered to be of great ecological and nutritional importance due to their ability to fix atmospheric nitrogen to the soil and to the high content of proteins (seeds), fibers (leaves), sugars (leaves), and minerals (seeds). The present study aimed to evaluate the development of *Lupinus montanus* and *L. campestris* as a response to seed mycorrhization. Inoculation was performed with *Rhizophagus irregularis*-BIOFertilizante INIFAP®. The treatments were 400 inoculated plants (200 per species) and 400 without inoculation, which were considered as controls. The variables evaluated were growth in basal diameter (mm), total height (cm), and nodule production one year after establishment. The experiment was conducted in January 2022 at the nursery of the Faculty of Forestry Sciences of the Autonomous University of Nuevo León, in Linares, Nuevo León, Mexico. The average growth in plants with mycorrhization showed a difference of 20% in basal diameter and 14% in height in *L. montanus*, whereas in *L. campestris*, it was 5% in basal diameter and 4% in height. In nodule production, a difference of 12.5% was observed in *L. montanus* and 28% in *L. campestris* compared to uninoculated seeds (control). The mycorrhization of the seeds had a significant impact ($p < 0.001$) on the development of *Lupinus* and its growth characteristics one year after its establishment. Both *L. campestris* and *L. montanus* are ideal for use in ecological restoration strategies because they generate beneficial microhabitats for the associated species.

Keywords:

Rhizophagus irregularis, nitrogen fixation, nurse plants, symbiosis.

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The genus *Lupinus* stands out for the ability of its root system to fix atmospheric nitrogen and improve soil fertility (Weisany *et al*., 2013). This process occurs through the symbiosis between roots and native bacteria of the genus *Rhizobium* (Masson-Boivin and Sachs, 2018). In addition to this capacity, its high content of proteins, minerals, and vitamins stands out, mainly in its seeds (Sujak *et al*., 2006; Pablo-Pérez *et al*., 2013, 2015). Likewise, it contains secondary metabolites, mainly flavonoids and quinolizidine alkaloids, of utmost importance for their pharmacological and agricultural applications (Zamora-Natera *et al*., 2019).

More than 100 species of the genus *Lupinus* have been recorded in Mexico, which are distributed in temperate and cold zones as well as in humid or dry regions, from Baja California to Chiapas, along mountain ranges and at altitudes ranging from sea level to above 3 700 masl in subalpine forests (Bermúdez *et al*., 2000; Águila *et al*., 2018). Some species such as *Lupinus montanus* and *L. campestris* have a wide distribution in Mexico, mainly in lands disturbed by human activity, where they play an important role in the nitrogen cycle (Rzedowski and Rzedowski, 2005).

On the other hand, it has been proven that species of the genus *Lupinus* can be nurse species since, due to their rapid development, they provide shade, humidity, and nutrients, which increases the survival of species of ecological interest established under their protection (Ramírez-Contreras and Rodríguez-Trejo, 2009). Within the agricultural applications, *Lupinus* species have been used in association with various crops of economic importance to improve their yield, such as *L. mutabilis* Sweet with corn (Rodas *et al*., 2001) and *L. albus* L. and *L. angustifolius* L. with wheat (Barrientos *et al*., 2002). On the other hand, species such as *L. montanus* L. have been used with *Pinus hartwegii* Lindl. (Ramírez-Contreras and Rodríguez-Trejo, 2009).

Various studies have shown greater survival and development in plants inoculated with mycorrhizae, such as research carried out on pines (Montes-Rivera *et al*., 2001; Gómez-Romero *et al*., 2013) and legumes (Liriano *et al*., 2012; Trejo *et al*., 2021), so it is of utmost importance to deepen the knowledge of the potential of mycorrhizal associations to be used in ecological restoration programs (Carrillo-Saucedo *et al*., 2022).

Therefore, this study aimed to evaluate the development of *Lupinus montanus* and *L. campestris* as a response to inoculation with *Rhizophagus irregularis*-BIOFertilizante INIFAP® 12 months after its establishment. The hypothesis is that the mycorrhization of *Lupinus* contributed to the general development of the plant (basal diameter and total height) as well as to the production of nodules, in which the interaction between roots and atmospheric nitrogen-fixing bacteria occurs, for use in the ecological restoration of degraded forest ecosystems.

The experimental site was established in the nursery of the Faculty of Forestry Sciences of the Autonomous University of Nuevo León, located at km 145 of the national highway # 85, Linares, NL, between the geographical coordinates 24° 47’ north latitude and 99° 32’ west longitude, at an altitude of 379 m. The annual rainfall in Linares, NL, fluctuates between 500 and 1 100 mm, whereas the temperature varies from 14 to 24 °C.

The germplasm was collected in pine-oak forests in the state of Oaxaca. Specimens of *Lupinus campestris* were collected in the community of Santiago Laxopa (north latitude, 17° 13.038’, west longitude 96° 18.882’, 1 854 masl.) and specimens of *Lupinus montanus* in Teococuilco de Marcos Pérez (north latitude 17° 19.890’, west longitude 96° 398.688’, 2 127 masl). The collection was carried out in November and December 2021 through the selection of individuals with adequate phenotypic characteristics, such as larger diameter and height, separated at a minimum distance of 10 m (Figure 1).

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Figure 1. A). Specimens of *Lupinus montanus*; and B) *L. campestris*.

The collected pods were placed in kraft paper bags, labeled and classified by species, then exposed to the sun for gradual opening (Sujak *et al*., 2006; Pablo-Pérez *et al*., 2013). Seeds of similar size and color were selected by removing the seeds that did not reach their full maturity to guarantee greater germination.

Experimental units of 50 plants were established, randomly distributed with four replications. The treatments were 400 inoculated plants (200 per species) and 400 without inoculation, which were considered as controls. The inoculation process consisted of impregnating the seeds with the adhesive mixture and applying 5 g of powdered mycorrhizae (*Rhizophagus irregularis*-BIOFertilizante INIFAP®) to each seed. The sowing was carried out on January 10, 2022, using germination trays and they were placed in the nursery with a 40% shade cloth.

After 30 days, transplantation was carried out in black polyethylene bags of 23 x 30 cm, using a mixture of peat moss, sand, silt, and clay was used as substrate, and they bags were placed in the open field in the nursery facilities. The variables evaluated were growth in basal diameter (mm), total height (cm), and nodule production 12 months after establishment. The variables of growth in diameter and height were evaluated with a digital vernier and a tape measure, whereas nodule counting was performed manually in each individual.

The data were subjected to an analysis of variance and a comparison of means using Tukey's test with a confidence level of 95% in the R-project Software (R Core Team, 2023). The dataset was normal according to the Shapiro-Wilk test. *Lupinus montanus* seedlings began to emerge at six days, with a total germination of 85% at 20 days; for its part, *L. campestris* did so at eight days, with a germination of 92% at 20 days of establishment.

At 12 months, *L. montanus* showed an average growth of 6.9 mm in basal diameter and 69 cm in total height while its control reached 5.5 mm and 59 cm, respectively. On the other hand, *L. campestris* had an average of 6.0 mm in basal diameter and 56 cm in total height while its control had 5.7 mm and 54 cm in the corresponding variables (Figure 2).

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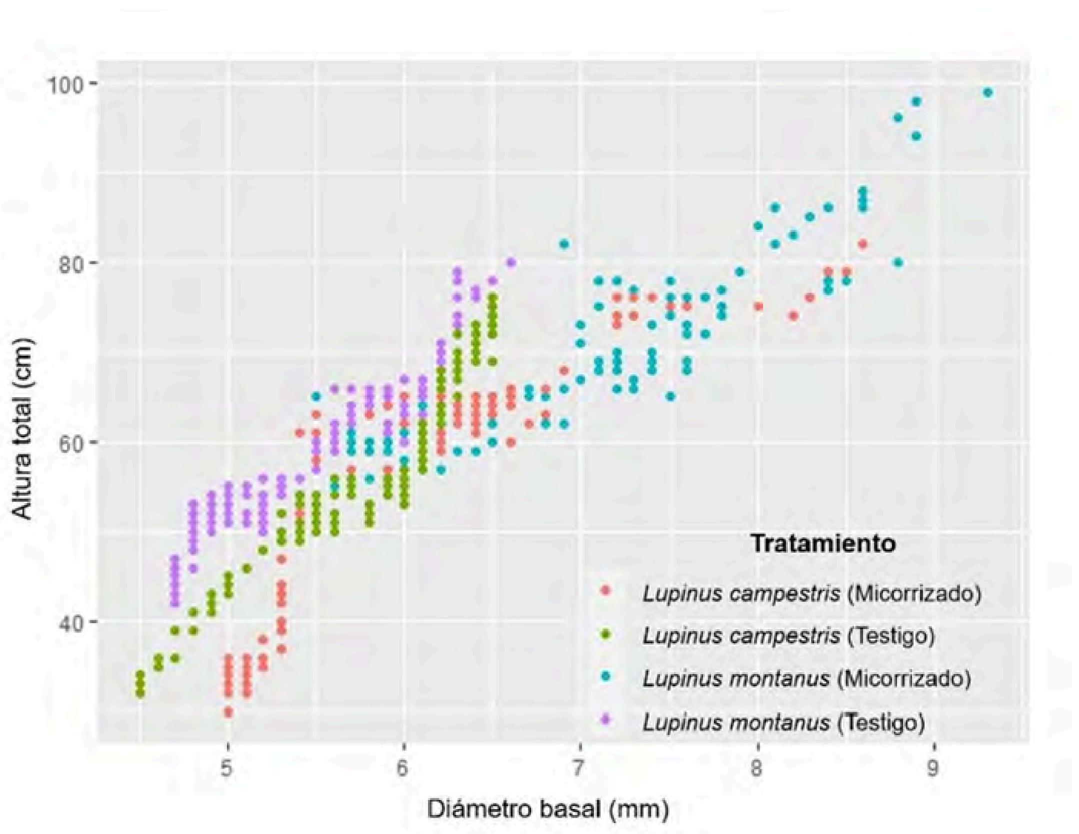
Figure 2. Growth in diameter and height (cm) of *Lupinus montanus* and *L. campestris*.



The superiority in height growth of *Lupinus montanus* is related to its adaptation to microclimatic conditions because it is a species that is distributed from 2 500 masl in *Quercus*-*Pinus* forests to 4 100 masl in alpine meadows (Rzedowski and Rzedowski, 2005) whereas *L. campestris* is found from 2 400 to 3 200 masl (Pablo-Pérez *et al*., 2013).

The Anova analysis (α= 0.05) indicates that *Lupinus montanus* with mycorrhization differs significantly from the rest of the treatments in height growth (F= 32.782, p< 0.001). Regarding basal diameter, *L. montanus* differs from the rest of the treatments; however, there is no significant difference between *L. montanus* control with *L. campestris* with mycorrhization and *L. campestris* control (F= 70.0387, p< 0.001). As for the production of nodules, there is a difference between *L. montanus* and the other treatments (F= 27.774, p< 0.000); nonetheless, there is no significant difference between *L. campestris* with mycorrhization and *L. montanus* control (Table 1).

Table 1. Statistical parameters of treatments.

Species	Treatments	# Nodules	Model f= y_0+a*x+b*x^2	R^2	Shapiro-Wilk
L. montanus	Mycorrhization	32	y_0 = 96.619 a = -17.286 b = 1.881	0.818	0.9749
L. campestris	Mycorrhization	25	y_0 = -230.288 a = 77.3723 b = -4.8704	0.8906	0.9069
L. montanus	Control	28	y_0 = 69.1809 a = -19.2035 b = 3.1288	0.9225	0.9887

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Species	Treatments	# Nodules	Model f= y_0+a*x+b*x^2	R^2	Shapiro-Wilk
L. campestris	Control	18	y_0 = 79.3889 a = -28.6283 b = 4.2373	0.9391	0.9805

Rapid growth is closely related to an efficient symbiosis between roots and bacterial nodules during the early stages of growth (Zamora-Natera *et al*., 2019). *Lupinus campestris* had a better response to seed mycorrhization with a 28% increase compared to *L. montanus*, which presented a 12.5% increase in nodule production. These results are consistent with what was reported by Águila *et al*. (2018), who obtained, as a result, a high nitrogen fixation capacity in *L. campestris* compared to *Pisum sativum* L. and *Trifolium* spp.

The atmospheric nitrogen fixed by *L. montanus* and *L. campestris* significantly benefits species that develop in association with them because nitrogen is an essential element for plants as part of proteins, nucleic acids, and chlorophyll, which are mainly required in growing plant tissues (Ferrari and Wall, 2004), as documented by Zamora-Natera *et al*. (2019) in their analysis with *Lupinus exaltatus* Zucc., *L. mexicanus* Cerv. ex Lag., and *L. rotundiflorus* M.E. Jones, where they conclude that the nitrogen contribution was higher compared to other legumes, which is closely related to the production of nitrogen-fixing nodules.

According to Ferrari and Wall (2004), the advantage of this plant-fungus symbiosis is that *L. montanus* and *L. campestris* can be self-sufficient in nitrogen, which considerably increases their protein content and nitrogen contribution to an associated crop, as well as the possibility of leaving nitrogen available in the soil by incorporating dry matter.

The implementation of *L. montanus* and *L. campestris* as nurse plants will allow a decrease in water stress and solar radiation due to the rapid development of the leaf area, and this contributes to the survival of species of ecological interest established under their protection (Ramírez-Contreras and Rodríguez-Trejo, 2009).

Nurse plants develop microhabitats, which can be demonstrated by work carried out by Aerts *et al*. (2007) with seedlings of *Olea europaea* L., which showed a higher survival under shrub cover compared to those established in patches of bare soil and work conducted by Mendoza-Hernández *et al*. (2013), who found that plant density and species richness was significantly higher under the canopies of *Buddleja cordata*, *Ageratina glabrata* (Spreng.) R.M. King & H. Rob., and *Sedum oxypetalum* Kunth compared to open sites.

Conclusions

The application of mycorrhizae (*Rhizophagus irregularis*-BIOFertilizante INIFAP®) in seeds of *L. montanus* and *L. campestris* contribute significantly to the growth in height and diameter as well as to the production of nodules, in which the interaction between roots and atmospheric nitrogen-fixing bacteria occurs. The symbiosis between fungus, root, and bacteria contributes significantly to the development of these two species due to the growth (diameter and height) and production of roots (production of nodules), which allow efficient use of the water and nutrients available in the soil, whereas bacteria fix atmospheric nitrogen to improve soil fertility; therefore, they can be used as nurse plants in ecological restoration strategies.

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