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Spontane nodulation of *Medicago sativa* with native rhizobia of the Central Valley of Catamarca

Summary

In soils of arid and semi-arid regions, nitrogen constitutes one of the most critical elements for plant nutrition. This makes it essential to look for mechanisms or tools that allow improving the nutrition of species of economic importance, without harming the environment. It is known that numerous species of bacteria participate in the biogeochemical cycle of nitrogen and promote its movement through the soil-plant system, thereby acquiring great relevance in plant nutrition. There are numerous antecedents of the response of alfalfa to inoculation with symbiotic fixing bacteria of atmospheric nitrogen, and this knowledge takes on greater importance in the organic production of this crop. The objective of the work was to isolate symbiotic fixing bacteria from atmospheric N_2 in alfalfa cultivated in the central Valley of the province of Catamarca, to be used as potential biofertilizers. In a plot of sandy loam soil with little nitrogen availability and no inoculation history in more than 40 years, a random sampling of 60-day alfalfa plants (type RR) with spontaneous nodulation was carried out. The presence of nodules was observed and from them the isolations were made in YEMA culture medium, incubating at 28°C and their cultural characteristics were determined. The preliminary results indicate that in the initial physiological states of the legume the existence of nodules, which manifests the presence of native rhizosphere colonizing rhizobia and with high competitiveness in infection. The cultural characteristics of the isolates obtained show the variability of nodular strains, creamy, fluid, translucent, pink, white colonies were observed, with development times between 24 and 48 hours of rapid growth. Conclusion: Medicago sativa may nodule with rhizobia native to the Central Valley area of the Province of Catamarca, given its compatibility and ecological and biological adaptation, a desirable aspect in the future selection of strains. Knowledge of these symbiotic relationships will allow their integration with other production practices and the development of environmentally safe management techniques, seeking to maximize the productive efficiency of crops.

Keywords: nursery, microcutting, genotype, propagation, survival

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Introduction

In soils of arid and semi-arid regions, nitrogen constitutes one of the most critical elements for plant nutrition. This makes it essential to look for mechanisms or tools that allow improving the nutrition of species of economic importance, without harming the environment. It is known that numerous species of bacteria participate in the biogeochemical cycle of nitrogen and promote its movement through the soil-plant system, thus acquiring great relevance in plant nutrition.[1] There are numerous antecedents of the response of alfalfa to inoculation with symbiotic fixing bacteria of atmospheric nitrogen and this knowledge becomes more important in the organic production of this crop.[2,3]

Alfalfa is an important crop given that it is a natural source of protein (up to 55%), amino acids, fiber (16%), vitamins (A, B, E, K) and minerals (calcium, iron, phosphorus, zinc, copper, selenium, and silica). In addition, its contribution to the landscape and its usefulness as a conservation crop for fauna and soil. At the same time, it reduces the energy involved in the symbiotic fixation of nitrogen for the crop itself and for the following crops, in the rotation of which it is a part. In addition, its cultivation provides elements of interest as a limiter and reducer of erosion.[4]

Rhizobia are Gram negative bacteria and common inhabitants of soil where legumes are present.[5] However, not all rhizobia can form nodules and/or fix nitrogen with all legumes. *Sinorhizobium meliloti* is specific for alfalfa. This allows differentiating rhizobia by their infectivity or nodulation capacity, there is specificity in the association or symbiotic pair.[3]

One of the most important attributes of legumes is their property of fixing atmospheric nitrogen when they live in symbiosis with specific bacteria.[1] Legumes are a broad group, which includes plants of economic importance such as soybeans, clover, alfalfa, vetch, chickpeas, and beans, which are characterized by having the seeds inside a pod. *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium,* and *Azorhizobium* are Gram-negative motile bacilli. Infection of the roots of a legume with the appropriate species of some of these bacterial genera leads to the formation of root nodules, which can convert gaseous nitrogen to combined nitrogen, in the process called atmospheric nitrogen fixation. This process has reached such great and basic importance for the construction of plant material as respiration and photosynthesis.[5–8] The importance of this process is demonstrated based on the current evaluation of the FAO where they assign to the annual symbiotic fixation in the world a deposit value to the soil of 10 times the annual production of fertilizers and commercial fertilizers. This phenomenon has promoted artificial inoculation practices.[9] As the native or naturalized bacterial strains that nodulate leguminous plants are the most efficient and effective fixers of atmospheric nitrogen compared to the commercial ones and are considered more promising in their selection due to local ecological adaptation, therefore their isolation and permanent study is of great importance.[2,10]

An alternative to maintain the level of agricultural production, with less use of agrochemicals and sustainable management, is the inoculation of beneficial microorganisms associated with the cultivated species.[1,11–13] However, it is essential to take care of the proper selection of microorganisms to be used as inoculants, considering

their biofertilizing potential, their adaptability, permanence in the site of interest, and their ease of handling. Therefore, this work focused on generating a sustainable technology, based on native strains of rhizobia potentially useful as biofertilizers. Therefore, the objective of this work was to isolate symbiotic fixing bacteria of atmospheric N_2 in alfalfa grown in the central Valley of the Province of Catamarca, to be studied as potential biofertilizers.

Materials and methods

An alfalfa farm, cultivated in a lot of sandy loam soil with little nitrogen availability and without a history of inoculation in more than 40 years, located in the Central Valley of the Province of Catamarca (Argentina), a random sampling of alfalfa plants was carried out. (*Medicago sativa*) type RR of 60 days of age with spontaneous nodulation. The presence of nodules was observed and from them the isolations were made in YEMA culture medium, incubating at 28 °C[14] and their cultural characteristics were determined (Figure 1).

Figure 1 60-day-old alfalfa (*Medicago sativa*) type RR seedlings with spontaneous nodulation and with nodules of different shapes.

Results and discussion

Preliminary results indicate that in the initial physiological states of the legume under study the existence of nodules, which shows the presence of native rhizobia colonizers of the rhizosphere and with high competitiveness and infection capacity in alfalfa seedlings.

The cultural characteristics of the isolates obtained show the variability of nodulating strains, eleven different colonial types were obtained, among which creamy, fluid, translucent, pink, white colonies were observed, with development times between 24 and 48 hours, growth fast. This biodiversity indicates the potential in the capacity of several *Rhizobium* strains to promote the development and growth of different host plants, which has been widely studied in recent years to verify if nitrogen fixation is feasible in non-leguminous plants.[15]

The generation of a sustainable development strategy for alfalfa cultivation in the Central Valley of the Province of Catamarca requires the design of inoculants that allow covering different nutritional deficiencies of the soil and the adaptation and survival of bacterial strains under different schemes of agricultural practices (Figure 2).

Conclusion

Medicago sativa can nodulate with rhizobia native to the Central Valley area of the Catamarca Province, given its compatibility and ecological and biological adaptation, a desirable aspect in the future selection of strains.

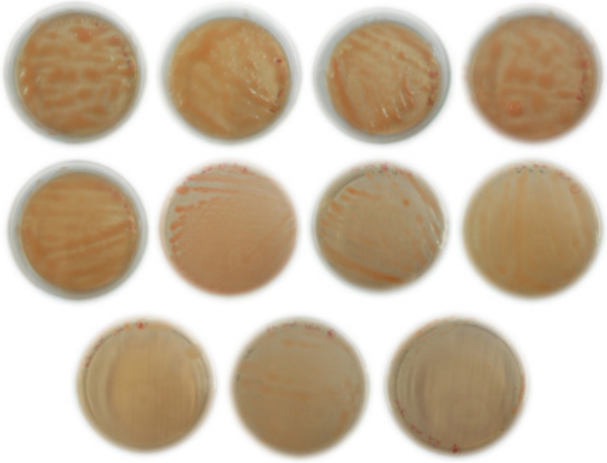

Figure 2 Different colonial characteristics of rhizobia isolates from alfalfa (*Medicago sativa*) nodules.

The knowledge of these symbiotic relationships will allow its integration with other production practices and the development of environmentally safe management techniques seeking to maximize the productive efficiency of crops.

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Conflicts of interest

There are no conflicts of interest presented or declared by the authors in this research.

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