Rhizobium populations in grassland acid soils of southwest Spain which nodulate Trifolium, Medicago, Ornithopus and Biserrula

Temprano F.J., Albareda M., Rodríguez-Navarro D.N.

in

Porqueddu C. (ed.), Tavares de Sousa M.M. (ed.).
Sustainable Mediterranean grasslands and their multi-functions

Zaragoza : CIHEAM / FAO / ENMP / SPPF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 79

2008
pages 127-130

Rhizobium populations in grassland acid soils of Southwest Spain which nodulate *Trifolium, Medicago, Ornithopus* and *Biserrula*

F.J. Temprano, M. Albareda and D.N. Rodríguez-Navarro
IFAPA-Centro Las Torres, Junta de Andalucía, 41200 Alcalá del Río (Sevilla), Spain

SUMMARY – Legumes are important components in grasslands because they can fix atmospheric nitrogen. Clovers (*Trifolium* sp.), annual medics (*Medicago* sp.), serradella (*Ornithopus* sp.) and biserrula (*Biserrula pelecinus*) are some of the important legumes in Mediterranean pastures, which are nodulated by specific rhizobia. 24 soils from Andalucía and Extremadura regions (SW-Spain) were analysed for the presence and effectiveness, in nitrogen fixation, of native rhizobia populations for those legumes. Specific rhizobia for clovers and serradella were present in high density, in all the soils tested, but rhizobia densities for medics or biserrulla were quite variable. Even some soils were devoid of *Sinorhizobium* for medics. Randomly selected strains from these populations were tested for their effectiveness in nitrogen fixation with their respective host legume and they showed high fixing capacity.

Keywords: *Rhizobium, Trifolium*, *Medicago*, *Ornithopus*, *Biserrula pelecinus.*

RESUME – *"Les populations de* Rhizobium *dans les sols acides des pâtures du SO de l'Espagne, qui nodulent* Trifolium*,* Medicago*,* Ornithopus *et* Biserrula*)". Les légumineuses sont des éléments importants dans les prairies, car elles peuvent fixer l'azote atmosphérique. Parmi les légumineuses importantes dans les pâturages méditerranéens se trouvent les trèfles (*Trifolium *sp.), les luzernes annuelles* (Medicago *sp.), serradella (*Ornithopus *sp.) et biserrula (*Biserrula pelecinus*) qui sont nodulées par des* Rhizobium *spécifiques. Vingt-quatre sols des régions de l'Andalousie et l'Estrémadure (SO Espagne) ont été analysés pour évaluer la présence et l'efficacité, dans la fixation de l'azote, des populations rhizobiennes qui nodulent ces légumineuses. Les* Rhizobium *spécifiques aux trèfles et serradella étaient présents, à une forte densité, dans tous les sols testés, mais les densités des* Rhizobium *pour les luzernes ou biserrulla étaient assez variables. Certains sols étaient même dépourvus de* Sinorhizobium *pour les luzernes annuelles. Des isolats prélevés au hasard à partir de ces populations ont été testés pour leur efficacité de fixation de l'azote avec leurs légumineuses hôtes respectives et ils montraient une grande capacité de fixation.*

Mots-clés : Rhizobium*,* Trifolium*,* Medicago*,* Ornithopus*,* Biserrula pelecinus.

Introduction

Legumes can fix atmospheric nitrogen in association with *Rhizobium* sp. bacteria. The fixed nitrogen is introduced into the ecosystem and consequently saves N-fertilizers. Clovers, annual medics, serradella and biserrula are among the more important annual legumes in Mediterranean pastures and they may contribute to maintain their productivity. Biological Nitrogen Fixation (BNF) is a less expensive source of N and more crop specific, in relation to N-fertilizers application, so that the use of *Rhizobium* inoculants is an agricultural practice recommended in sustainable agriculture (Vance, 1998).

In soils devoid of specific rhizobia populations for the legume crop to be sown and with low soil N-levels, inoculation will be always recommended, and significant yield responses can be recorded over non-inoculated plots. Application of commercial inoculants into soils that already contain effective rhizobial populations can lead to different responses, a significant yield increase can be recorded, overall if the population size falls bellow 10^2 rhizobia/g of soil (Thies *et al*., 1991); or sometimes, yields are not increased by inoculation, but N concentration in seed or plant parts may be increased over that of non-inoculated plants. Thus, one way to assess the need of inoculation is to quantify the specific natural populations of rhizobia for the legume to be sown by the most probable number (MPN) method or others. High levels of specific rhizobia (>10^4 rhizobia/g soil) might preclude the use of commercial inoculants, due the competition problem between the naturalized rhizobia and the inoculant's strains, that could be very difficult to overcome. In such situations, special strategies must to be adopted, as the selection of highly competitive strains.

The objective of this work was the evaluation of the density of native *Rhizobium* populations of *Rhizobium leguminosarum* bv. *trifolii*, *Sinorhizobium meliloti/medicae, Bradyrhizobium* sp. and *Mesorhizobium* sp. present in pasture soils from Andalucía and Extremadura regions (SW-Spain). The effectiveness in nitrogen fixation of these populations with *T. glomeratum, M. polymorpha, O. compressus and B. pelecinus* was also estimated at the aim of recommending the use of highly effective inoculants.

Material and methods

24 samples from 30 cm top soil were taken from different pasture locations in Extremadura and Andalucía regions in SW Spain. The samples were analysed and different parameters like pH, texture, calcium carbonate content, organic matter, total nitrogen and available phosphorus and potassium were determined. Soil density of *R. leguminosarum* bv. *trifolii*, *S. medicae*, *Bradyrhizobium* sp. and *Mesorhizobium* sp. populations were evaluated using the most probable number (MPN) method (Brockwell, 1982), using *T. glomeratum*, *M. polymorpha*, *O. compressus* and *Biserrula pelecinus* as trap-host plants.

Rhizobial strains were isolated from nodulated plants (Vincent, 1970) and were assayed under controlled conditions in greenhouse for nitrogen fixing efficiency. 96 *R. leguminosarum* bv. *trifolii*, 36 *S. medicae*, 47 *Bradyrhizobium* sp. and 65 *Mesorhizobium* sp. isolates were obtained, representing the rhizobial populations of these soils. Plant-tests were carried out in test tubes (20 x 200 mm) with N-free nutrient solution (Rigaud and Puppo, 1975). Non-inoculated plants and highly effective strains were used as controls. The nitrogen fixing capacity of the strains was determined by the dry weight of plants, after 1 month of growing.

Results and discussion

All of the soils were mild acidic (pH 5.6-6.6), coarse textured (sandy, loam-sandy or loam textures predominated), did not contain carbonates, and had a variable organic matter content (2-5.7%). Total nitrogen content was in the range 0.1-0.3%, and available phosphorus and potassium values had medium to high scores.

The density of the *Rhizobium* populations in the soils is shown in Fig. 1. All of the soils tested had a high content in *R. leguminosarum* bv. *trifolii* and *Bradyrhizobium* sp., having 10^4-10^6 bacteria/g in most of the samples. *Mesorhizobium* sp., (the specific rhizobia-nodulating *B. pelecinus*) are in variable densities in the studied soils, ($<10^2$ to more 10^5 bacteria/g); whereas *S. medicae* strains are not present or are scarce (less than 10 bacteria/g) in half of the analyzed soils. The remaining soils had a variable content of this rhizobia species, from about 10 to more than 10^4 bacteria/g.

Different clovers species *(T. subterraneum*, *T. glomeratum*, *T. striatum*, *T. hirtum*, *T. cherleri* and others) and serradella (*O. compressus*, *O. sativus*) are common legumes in Mediterranean pastures of SW Spain and Portugal and they are usually nodulated by native rhizobia. *Biserrula* is a rare species in SW Spain and it can be found only in certain limited areas. It establishes symbiosis with *Mesorhizobium* sp. and little is known about this rhizobial group and the range of legumes that can nodulates. Annual medics are scarcely found in pastures growing on acid soils and *Sinorhizobium* species are also not well adapted to this type of soils. Thus, *S.meliloti*/*medicae* populations are not found or have a low density in some soils in the area. There is not relation between the density of *Sinorhizobium* and the pH of the soils studied.

The effectiveness of the rhizobia populations able to nodulate with clovers, annual medics, serradella and biserrula seems to be high. Plants of *T. glomeratum* and *M. polymorpha* inoculated with native isolates of *R. leguminosarum* bv. *trifolii* and *S. medicae* significantly have a higher dry weight than those of non inoculated controls (Fig. 2). Also the effectiveness of these isolates was in the same range than the reference strains previously selected by their nitrogen fixing capacity. The *Bradyrhizobium* sp. and *Mesorhizobium* sp. isolates showed a similar pattern in nitrogen fixing capacity with *O. compressus* and *B. pelecinus*, respectively, than those in Fig. 2 (data not shown).

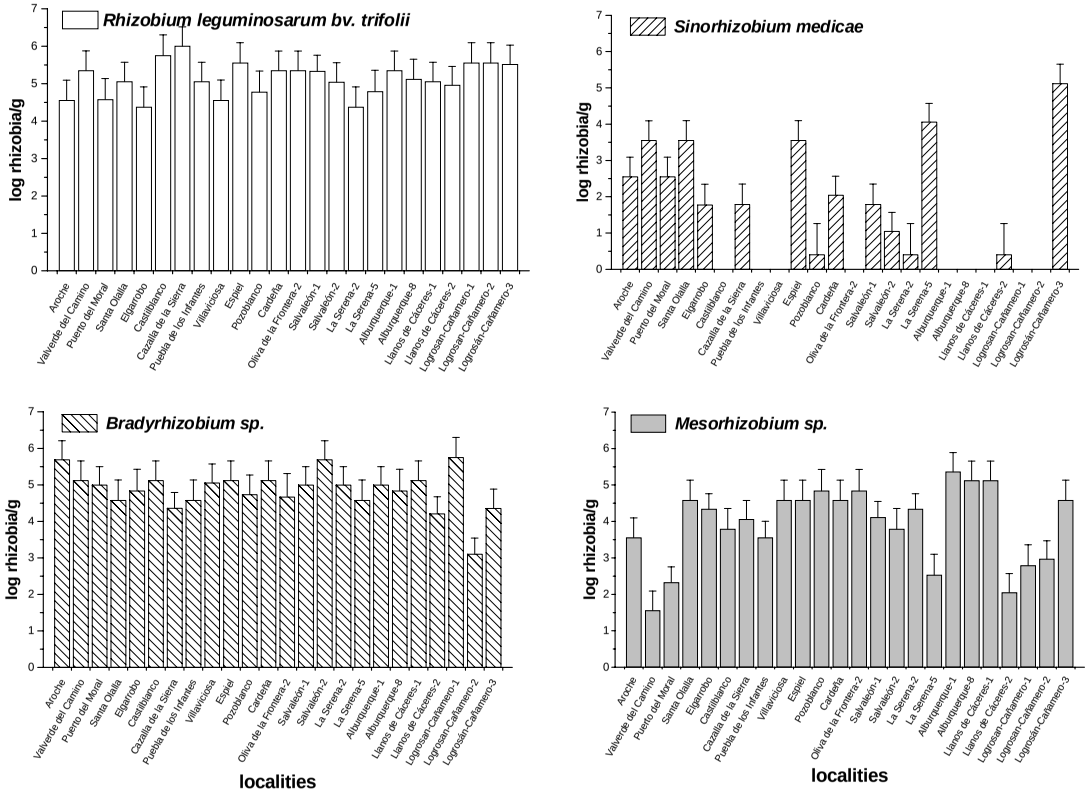

Fig. 1. MPN of the native *Rhizobium* populations which nodulate *Trifolium* (*R. leguminosarum* bv. *trifolii*), *Medicago (S. medicae), Ornithopus (Bradyrhizobium sp.)* and *Biserrula* (*Mesorhizobium* sp.) in 24 pasture soils from different localities of Southwest Spain.

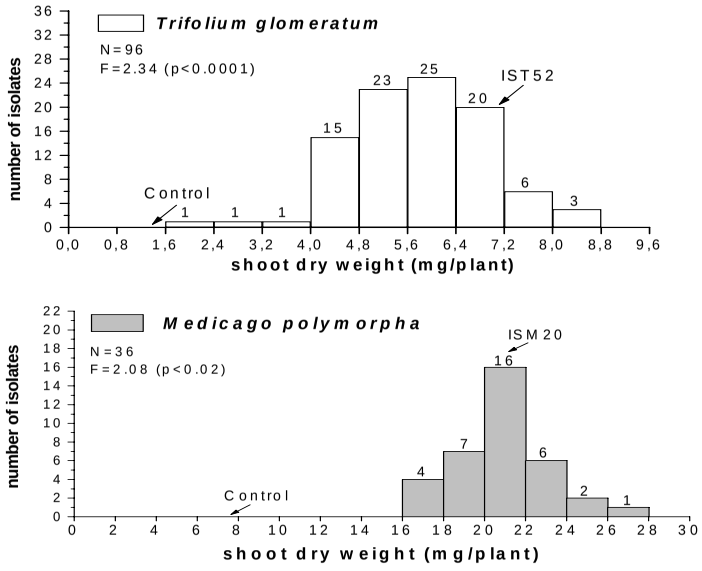

Fig. 2. Frequency histograms of *R. leguminosarum* bv. *trifolii* and *S. medicae* in relation to their effectiveness on *T. glomeratun* and *M. polymorpha* (IST52 and ISM20 are reference strains.

The soils from the Mediterranean pastures in SW Spain are rich in effective rhizobia capable of nodulate *Trifolium* and *Ornithopus.* So that, inoculation of these plants is probably not necessary when they are sown in pastures. When *Biserrula* species are introduced in pastures, in some soils seed inoculation might improve nitrogen fixation of the plants. Finally, *M. polymorpha* and other annual medics would need to be inoculated with *S. medicae* in many areas of acid soils from SW. Spain before sowing these legumes. These *Sinorhizobium* strains must be highly effective in nitrogen fixation and acid tolerant to colonize and survive in this kind of soils.

There is a strong interaction between the nitrogen fixation capacity of *Rhizobium* strains and the different species -or even cultivars- of clovers or annual medics (Howieson *et al.,* 2000). This probably also occurs for *Ornithopus* and *Biserrula*. So, the selected *Rhizobium* strains for the inoculants should be effective with the different species of their host legume present in the pastures.

Conclusions

The density of native populations of *R. leguminosarum* bv. *trifolii* and *Mesorhizobium* sp. in pasture soils from SW-Spain is high. Nevertheless, *Mesorhizobium* sp. (*Biserrula*) and *S. medicae* populations are in low densities or even are not present in those soils.

The isolates from those populations show a high nitrogen fixing capacity with their host legumes, *T. glomeratum, M. polymorpha, O. compressus* and *B. pelecinus*.

Inoculation of seeds is recommended when *M. polymorpha* or eventually *B. pelecinus* are going to be introduced in pastures in those areas.

References

Brockwell, J. (1982). Plant-infection counts of rhizobia in soils. In: *Nitrogen Fixation in Legumes*. Vincent, J.M. (ed.). Academic Press, Australia, p. 41-58.

Howieson, J.G., O´Hara, G.W., Loi, A. (2000). The Legume-Rhizobia relationship in the Mediterranean basin. In: Legumes for the Mediterranean forage crops, pastures and alternative uses. In: *10th Meeting of the Mediterranean Sub-network of the FAO-CIHEAM Inter-regional Cooperative Research and Development Network on Pastures and Fodder Crops*. Sassari (Italy), April 2000, Sulas, L. (ed.). *Cahiers Options Méditerranéennes*, vol. 45: 305-314.

Rigaud, J. and Puppo, A. (1975). Indole-3-acetic acid catabolism by soybean bacteroids. *Journal of General Microbiology*, 88: 223-228.

Thies, J.E., Singleton, P.W. and Bohlool, B.B. (1991a). Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Applied and Environmental Microbiology,* 57: 19-28.

Vance, C.P. (1998). Legume symbiotic nitrogen fixation: Agronomic aspects. In: *The Rhizobiaceae*. Spaink, H.P., Kondorosi, A. and Hooykaas, P.J.J. (eds). Kluwer Academic Publishers, Dordrecht, The Netherlands, p. 509-530.

Vincent, J.M. (1970). A manual for the practical study of the root-nodule bacteria. *International Biological Programme Handbook No. 15*. Blackwell Scientific Publications, Oxford.