Effects of *Rhizobium* strains on growth and nitrogen fixation of annual medics

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Abstract

Continuous grain cropping in many areas of the world has reduced soil N levels to a degree such that agricultural production is now largely dependent upon nitrogen fertilizer. Identification of highly effective legume and *Rhizobium* strain combinations, increased production of protein- rich livestock forage and also improved the soil structure. Four species of annual medics (*Medicago truncatula, M. littoralis, M. polymorpha, M. rigidula)* were grown in all combinations with four various strains of *Rhizobium meliloti* (Locally, Hoomand, R_{95} and S_{13}). There was also an uninoculated check for each species. Control treatment (uninoculated) included mineral nitrogen (2.5 mMol nitrate). All combinations of medics and *Rhizobium* were grown without nitrate. The control treatment was wetted with nutrient solution contained 2.5 mMol nitrate. Growth chamber experiment was conducted for 90 days to evaluate shoot dry matter and total shoot N. Medic lines varied in their ability to form an effective symbiosis with the rhizobia. *M. truncatula* has higher symbiotic association with the strains Locally, Hoomand and R_{95}. *M. littoralis* also in four inoculated treatment has %N near to control treatment. *M. rigidula* had the highest symbiosis with R_{95} and then with S_{13}. *M.polymorpha* had low symbiosis with all the strains. *M. trumcatula* and *M. littoralis* exhibited similar response to all strains. *M. rigidula* required specific strains of *Rhizobium meliloti* in order to fix nitrogen. . *M. polymorpha* has no effective symbiosis with any one of the strains, this implies that this plant requires specific strains.

Media summary

Symbiotic performance with *Rhizobium meliloti* indicated that *Medicago truncatula and M. littoralis* had effective symbiosis with all the strains and *M. rigidula* and *M.polymorpha* required specific strains.

Key Words

Medicago, Nitrogen fixation, *Rhizobium meliloti*, Symbiosis.

Introduction

It is the current practice in cereal-producing areas of West Asia and North Africa to grow barley or wheat in rotation with fallow. It may beneficial to replace that fallow with self-regenerating clovers or annual medics (McWilliam 1982). This integrated system, known as ley-farming, is well developed in those regions of southern Australia that have a Mediterranean-type climate (Cocks et al. 1980). Annual medics have been valued for their capacity to provide N needs through biological nitrogen fixation (BNF). Effectively nodulated medics may fix 60 kg N/ha/yr (Koocheki and Astarayi 1996). In addition to supplying N for the current medic crop, BNF has also been valued for providing N to the succeeding rotating crops. Two characteristics of annual medics – reducing fertilizer needs and improving soil structure- are of particular importance to developing countries where agricultural production is often limited by an economical supply of N fertilizer (Burton 1981). There are about 25 million hectares (Mha) fallow lands in Iran, Iraq, Syria and Turkey, 19 Mha of them are suitable for cultivation of annual medics. According to Carter (1981) there are about seven Mha fallow lands in Iran, those lands are often allotted to cultivation of legume.

Annual medics are found in almost all region of Iran indicating that these plants are appropriate for cultivation in Iran. It was observed during the work that annual medics especially *M. rigidula* required specific strains of *Rhizobium meliloti* in order to fix N. Indeed some soils in Iran, climatically and edaphically suitable for the plant, contain no appropriate rhizobia. Our objectives were to define the degree of symbiotic specificity within the *Medicago-Rhizobium meliloti* association and to identify highly effective strains of rhizobia that might be used for inoculating seeds of medicago.

Methods

In order to evaluate the effects of *Rhizobium* strains on N fixation by medics, an experiment was conducted in Iranian Research Institute of Forest and Rangelands. Four strains of *Rhizobium meliloti* (Locally, Hoomand, R_{95} and S_{13}), collected from different regions of Iran, were grown on yeast manitol agar (YMA) medium culture (Vincent 1970). Seeds of Four species of annual medics (*Medicago truncatula* (Barrel)*, M. littoralis* (Strand)*, M. polymorpha* (Burr)*, M. rigidula)* were planted in pots containing quartz, and then were watered with sterile distilled water. When cotyledon leaves appeared, the plants were inoculated with 1 ml of cultures suspended in quarter-strenght solution (McKnight 1949). Inoculated treatments were wetted with a nitrogen-free nutrient solution (McKnight 1949). The control treatment (uninoculated) was wetted with mineral nitrogen (2.5 mM nitrate solution) (Heidari 1994). Plants were grown in a growth chamber (GRR SET 10000G) on a 12 hours day with a light density of 450 µmol/m^2/s and 25/20 ^{0C} day/night temperature regime. The experiment was set up in a completely randomized block design with 4 replications.

Plants were harvested 90 days after planting. Shoots were dried at 75^0 C for 24 hours and weighted. For determination of total shoot N, shoots were analyzed by Kjeldahl digestion (AOAC 1970). Shoot dry matter was transformed to square root before analysis of variance was conducted. Means were compared with the least significance difference (LSD) test.

Results

Effect of strains, species of medics and interactions of them were statistically significant on shoot N (%) and shoot dry matter ($p<0.05$). Medic lines varied in their ability to form an effective symbiosis with the rhizobia. *M. truncatula* had higher symbiosis with strains Locally, Hoomand and R_{95}. Likewise, %N was similar to control treatment. *M. littoralis* had %N near to control treatment in four inoculated treatments. *M. rigidula* had the highest symbiosis with R_{95} and then with S_{13}. *M.polymorpha* had low symbiosis with all the strains and the amount of its shoot N in inoculated treatment was half of control treatment (Figure 1).

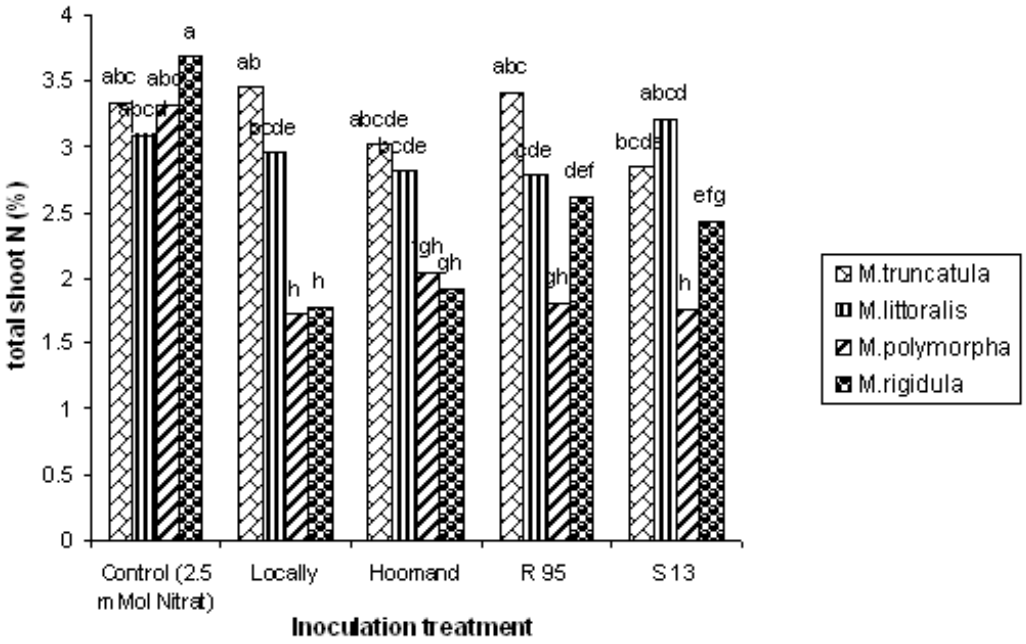

Figure 1. Effects of Rhizobium strains on shoot N (%)

M. truncatula had the highest shoot dry matter (SDM) with the locally strain. *M. littoralis* had the highest SDM with Locally strain among inoculated treatments. The SDM of *M. littoralis* and *M. truncatula* in inoculated treatments were higher than two other lines while in control treatment SDM of *M. polymorpha* was the highest. The SDM of *M. rigidula* was least in all treatments expect the strain Locally (Figure 2).

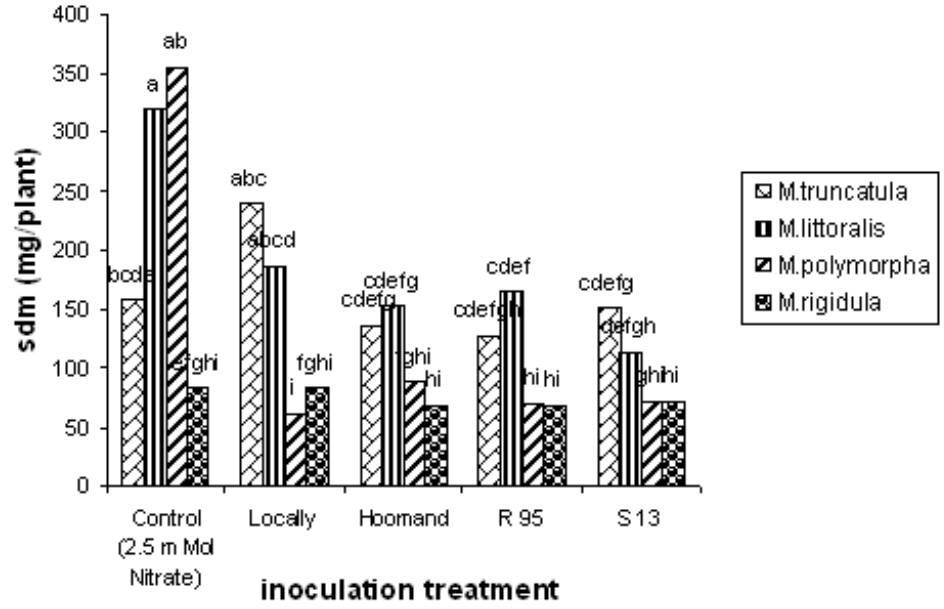

Figure 2. Effects of Rhizobium strains on shoot dry matter (mg/plant)

Conclusion

High symbiosis of *M. truncatula* and *M. littoralis* with all the four strains indicated no specific response of these medics. High symbiotic performances of these medics with bacteria may be related to genetic ability in N fixation with any strain type. High %N in *M. rigidula* in symbiosis with R_{95} and S_{13} strains showed that this plant required specific strains of *Rhizobium meliloti* in order to fix N (Brockwell et al. 1988). The R_{95} and S_{13} strains were obtained from cold regions of country (Hamadan and Zanjan) and *M. rigidula* is tolerant to sever frosts (Cocks and Ehrman 1987) and is suitable for cool region of Iran. High degree of symbiosis in these cases could be attributed to the presence of cold region strains and this specific action was due to adaptation with climatic conditions. *M. plymorpha* had no effective symbiosis with any strains which means this line required specific strain. Other scientists (Ballard and Charman 2000) observed that *M. polymorpha* had low symbiotic performance. With regard to high SDM of *M. trumcatula* in symbiosis with Locally and S_{13} strains, this plant could be recommended for cultivation in Zanjan and Karaj for forage production, and use *M. littoralis* in Hoomand and Hamadan. Because of high symbiosis of *M. trumcatula* and *M. littoralis* with various strains, these plants could be used for improving soils of dry lands. If plant species are to be matched to localities, based on their efficiency of N fixation, regional maps detailing the symbiotic performance of key medic species with soil rhizobia will need to be developed. This information would clearly be used in assisting with pasture legume recommendations and could be used to optimize the symbiosis in the field.

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