Short Communication

Improved Soybean Production after Inoculation with *Bradyrhizobium japonicum*

Lucrecia Brutti¹, Juan Cancio Pacheco Basurco¹, Hans Ljunggren² and Anna Mårtensson³*

¹Instituto de Suelos, CIRN-INTA, CC25 1712 Castelar, Buenos Aires, Argentina and Departments of Microbiology, P.O. Box 7025, and Soil Sciences, P.O. Box 7014, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden. ²Microbiology, P.O. Box 7025, and ³Soil Sciences, P.O. Box 7014, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden

Introduction

Inoculation of legumes with *Rhizobium* or *Bradyrhizobium* spp. is a common agricultural practice used to increase the yields of legumes without adding nitrogen (N) fertilizers. Rhizobial populations in the soil exist as saprophytes and are able to use a diverse range of energy sources including almost all organic compounds. The presence of the legume host plant stimulates the *Rhizobium* populations in the rhizosphere, where the inoculants have to compete for nutrients with the existent microbial community, including the native *Rhizobium* populations (McDermott et al., 1991). Therefore, it is not surprising that population densities have been positively correlated not only with the presence of legumes and vigour of legume growth but also with other biotic factors such as soil organic matter. Abiotic factors such as clay content, soil pH, base saturation, mineral N status, mean annual rainfall and irradiance also affect population densities (Bezdicek, 1972; Hitbolt et al., 1985; Hurse & Date, 1992; Bushby, 1993). Other studies, however, have not shown any simple relationship between population density and specific environmental parameters (George et al., 1987; Bottomley, 1992).

In order to investigate whether naturalized soil strains of *Bradyrhizobium japonicum* in Argentine soils can compete successfully for nodulation and make a better contribution to the crops than inoculated strains, a field experiment was set up using strains of *B. japonicum* of different origins.

Materials and methods

Soil conditions

Three 1-year experiments with soybean seed inoculations were conducted at different places in the same field situated at Castelar, in the western region of the Province of Buenos Aires, Argentina. Sowing methods and cultural operations for soybean cultivation followed normal farming practices in which fertilizers are not used. The climate is humid with a mean annual precipitation of 1000 mm. The mean annual temperature is 16.6°C. Initially, the soil was free from *B. japonicum*, as tested by the soil dilution plant infection technique (Somasegaran & Hoben, 1994). The soil had an organic matter content of 2% and a total N content of 0.11%. Over the years soil acidity decreased from pH 5.6 in year 1 to 4.9 in year 3. The soil was low in phosphorus (P) content, ranging between 6.4 and 9.5 ppm plant-available P. The experimental design was complete blocks at random with four replicates and six treatments. The plots were 6 m long with four rows of soybean separated...
Fig. 1. Nodule occupancy of soybeans inoculated with *Bradyrhizobium japonicum* strain E110 (selected for its high N efficiency) and strain E112 (representing the native populations) in three successive growing seasons. The strain combinations studied were: 4, soybean inoculated with E110 (50%) plus E112 (50%); 5, soybean inoculated with E110 (25%) plus E112 (75%); and 6, soybean inoculated with E110 (75%) plus E112 (25%). The SD of each treatment is represented by bars.

Plants and bacteria

Seeds of *Glycine max* cv. Asgrow 5308 were inoculated and then sown on the field site. Two strains of *B. japonicum* were used, E110 (original number 5019), obtained from MIRCEN (Microbiological Resource Centre), Brazil, and E112, isolated at the Institute of Microbiology and Agricultural Zoology, CICA, INTA, Argentina. Strain E110 was chosen since earlier observations indicated that the strain had superior N-fixation properties (Brutti et al., 1999). Strain E112 was chosen as a representative of native bradyrhizobial populations.

Treatments

The inoculant strains were used in varying proportions, including the following treatments: 1, soybean without inoculation; 2, soybean inoculated with E110; 3, soybean inoculated with E112; 4, soybean inoculated with E110 (50%) plus E112 (50%); 5, soybean inoculated with E110 (25%) plus E112 (75%); and 6, soybean inoculated with E110 (75%) plus E112 (25%). The inoculation dose was $1.2 \times 10^8$ viable bradyrhizobial cells per seed.

Dry weights and yields

For the determination of dry weights, samples of 10 randomly collected plants were dried to constant weight at 65°C. The grains from plants occupying an area of 7 m$^2$ were collected.

Results and discussion

Soil data indicated a P deficiency (6.4–9.5 ppm) together with a low pH (4.8–5.6), but with an organic matter content and total N averaged for Argentine soils. The low pH and P content could be of importance in the experimental soil since competition between strains for nodulation and persistence of
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Bradyrhizobium in the soil can be influenced by soil mineral status (Streeter, 1994).

The nodule occupancy studies showed 100% of the occupancy by E110 in treatment 2 and 100% by E112 in treatment 3, when the respective strain alone was inoculated on the seed. When both strains were inoculated in the same proportion on the seed (treatment 4), nodules were formed by both strains in similar proportions during experimental years 2 and 3 (Fig. 1). When the inoculation proportions were different, the strain with the higher dose added formed more nodules.

Nodulation was sufficient every year. Plants that were seed-inoculated appeared darker green than those without inoculation. Nodules were formed mainly on the main roots by strain E110, but if strain E112 was present nodules were more frequently distributed on lateral roots. This may be a consequence of delayed nodulation by strain E112, which nodulates later than E110 according to in vitro experiments (L. Brutti, in preparation).

The inoculated treatments increased the yield between 2 and 19% compared with the uninoculated plants \( (P = 0.05) \) (Fig. 2). When plants were inoculated with E110 alone (treatment 2), in equal proportion with E112 (treatment 4) or with a higher inoculation proportion of E110 (treatment 6), yields were higher than for the others.

The dry matter was not significantly different in the experiments for years 1 and 2 (Fig. 2). Year 2, however, showed significant differences \( (P = 0.05) \) between all the treatments; when the inoculant included E110 the highest values were obtained. The dry matter production was correlated with total N uptake during the whole field experiment (data not presented). E110 was found to increase plant growth (Brutti et al., 1999).

The data for nodule dry weight showed no differences between nodule dry weight of plants from seeds inoculated either with separate strains or with the strains together in different proportions (Fig. 3). The values were lower for the second year and this may be a consequence of the lower percentage of N in the shoot. From the present work it can be concluded that it is possible to increase the yield and the N content in the seed in this type of soil. Limitations arose mainly due to P availability and low pH, but it might be possible to obtain higher yields if these deficiencies were corrected.

In conclusion, the possibility for the two strains to form nodules correlated with the proportion of inoculant added to the seed. Inoculation with E110 generally increased yield. The strain isolated from the naturalized population of the Argentine soybean area (E112) was less effective than the selective strain (E110) used as inoculum.
Fig. 3. Nodule dry weights of soybean inoculated with *Bradyrhizobium japonicum* strain E110 (selected for its high N efficiency) and strain E112 (representing the native population) in different proportions during three successive growing seasons. The SD of each treatment is represented by bars.

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References


