EFFECT OF BEAN INOCULATION WITH *Rhizobium phaseoli* ON SOIL MICROBIAL ACTIVITY

Jelena B. MARINKOVIC

Institute of Field and Vegetable Crops, 21000 Novi Sad, S&M

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Bacteria of the genus *Rhizobium* are symbiotic gram-negative soil microorganisms that affect the forming of bean root nodules. The objective of this study was to assess the effect of inoculation of different bean genotypes (cv. Belko, Sremac) with *Rhizobium phaseoli* on soil microbial activity. Experiments were conducted in 2002 and 2003 at the location Rimski Sancevi on a chernozem soil. Immediately before planting, bean seeds were inoculated with *Rhizobium phaseoli* strains. The control variant was not inoculated. The study included 6 variants: **1.** bean seeds inoculated with strain *Rhizobium phaseoli* 1; **2.** seeds inoculated with strain *Rhizobium phaseoli* 2; **3.** seeds inoculated with strain *Rhizobium phaseoli* 3; **4.** seeds inoculated with strain *Rhizobium phaseoli* 4; **5.** seeds inoculated with a mixture of four strains; **6.** control. Soil samples for microbiological analyses were taken at the flowering stage and at the end of bean growing season. The obtained results showed that the abundance of the microbial groups depended on bean genotype. The average two-year results indicated that inoculation significantly affected the number of microorganisms in the soil. With cv. Belko, the numbers of azotobacters, actinomycetes and the total number of microorganisms were higher in the inoculated variants than in the control. Cv. Sremac had increased numbers of azotobacters and free nitrogen-fixing bacteria. Inoculation had no effect on the numbers of fungi and ammonifiers, with the exception of cv. Sremac at the end of growing season, when the number of ammonifiers was increased. The examined strains exhibited various effects on soil microbiological activity.

Key words: inoculation, bean, microorganisms, *Rhizobium phaseoli*

INTRODUCTION

Soil is an ecological system which contains numerous and diverse microorganisms whose enzymes play an important role in the metabolic activity of soil. Soil microbiological processes are determined by organic matter content; fertility of a soil type is linked to the activity of its microflora (Milić *et al*. 1997, Milić 1999a). Soil bacteria, fungi and actinomycetes take part in the process of microbiological transformation of organic matter. Dominance of various groups of microorganisms guides the processes of synthesis and decomposition determining the quality of soil.

Bacteria from the genus *Rhizobium* are symbiotic, gram-negative soil microorganisms that affect the nodulation of bean roots. Although the bean (*Phaseolus vulgaris L.)* is capable of associating with N-fixing symbionts, its productivity in commercial production is frequently limited by N shortage (Rosas *et al*. 1998). Native strains of *Rhizobium phaseoli* typically improve bean nodulation but fix less N (Dowling and Brughton 1986, Moxly *et al*. 1986). To improve N-fixing capacity of bean genotypes it is necessaryto select effective strains of nodular bacteria. The objective of this study was to assess under field conditions the effect of bean inoculation on soil microbiological activity.

MATERIAL AND METHOD

The experiment was established in random blocks design at Rimski Šančevi experiment field of Institute of Field and Vegetable Crops in Novi Sad, on a chernozem soil. Two bean genotypes were tested, cv. Belko and Sremac, both developed at the Institute. Planting was performed in early May of 2002 and 2003 in 4 replications. Immediately before planting, bean seeds were inoculated with *Rhizobium phaseoli* strains. The study included 6 variants: 1. bean seeds inoculated with strain Rhizobium phaseoli 1; 2. seeds inoculated with strain Rhizobium phaseoli 2; 3. seeds inoculated with strain Rhizobium phaseoli 3; 4. seeds inoculated with strain Rhizobium phaseoli 4; 5. seeds inoculated with a mixture of four strains; 6. control. Sojevi *Rhizobium phaseoli* strains 1 and 2 were obtained from Faculty of Agriculture in Novi Sad, strains 3 and 4 from the Institute. Rhizosphere soil (0-20 cm) was sampled for microbiological analyses at the flowering stage and at the end of bean growing season.

Microbial number was determined by the plating dilution method of Pochon and Tardieux (1962): total microorganisms on soil agar, ammonifiers on meat peptone agar, azotobacters and free N-fixing bacteria on Feodorov medium, fungi on Chapek agar and actinomycetes on synthetic agar of Krasilnikov. Microbial number was calculated per gram of absolutely dry soil. The obtained

results were statistically processed by the analysis of variance and tested by the LSD method.

RESULTS AND DISCUSSION

At the flowering stage, on average for the two years (Table 1), inoculation effect was best demonstrated in the number of azotobacters, which showed highly significant increases in both genotypes. Inoculation also significantly increased the numbers of total microorganisms and actinomycetes in both genotypes plus the number of free N-fixing bacteria in the genotype Sremac. Inoculation showed no effect on the numbers of ammonifiers and fungi in either genotype.

Inoculation was most effective with the mixture of all four strains. The numbers of azotobacters, free N-fixing bacteria and actinomycetes were increased in the genotype Belko, the numbers of azotobacters, total microorganisms, free N-fixing bacteria and fungi in the genotype Sremac.

The individual strains differed in their effect on microbial number in dependence of the genotype. Strains 2 and 3 were most effective, followed by strains 4 and 1. All strains caused significant increases in the number of azotobacters in both genotypes. Additionally, they increased the number of actinomycetes in the genotype Belko. Strain 2 caused significant increases in the number of total microorganisms in the genotype Belko and the numbers of free N-fixing bacteria and fungi in the genotype Sremac. Strains 3 and 4 caused significant increases in the numbers of azotobacters and free N-fixing bacteria in both genotypes and the number of actinomycetes in the genotype Belko. Additionally, strain 3 increased the total number of microorganisms in both genotypes, while strain 4 increased the total number of microorganisms only in the genotype Belko. Strain 1 increased the number of azotobacters in both genotypes, the number of actinomycetes in the genotype Belko and the number of free N-fixing bacteria in the genotype Sremac.

On average for the two years, the numbers of the studied microorganisms decreased at the end of the growing season (Table 2).

Strain 3 had the highest effect on the number of soil microorganisms. It caused significant increases in the numbers of azotobacters and ammonifiers in both genotypes. Also, strain 3 increased the numbers of total microorganisms and actinomycetes in the genotype Belko and the numbers of free N-fixing bacteria and fungi in the genotype Sremac. Strain 2 caused significant increases in the numbers of azotobacters, ammonifiers and free N-fixing bacteria in the genotype Sremac and it increased the number of actinomycetes in the genotype Belko. Strains 1 and 4 caused significant increases in the number of azotobacters in both genotypes, while strain 1 also increased the numbers of ammonifiers and free N-fixing bacteria in the genotype Sremac.

The mixture of the four strains increased significantly the number of fungi in both genotypes, the number of ammonifiers in the genotype Sremac and the number of total microorganisms in the genotype Belko.

It was evident on the basis of the obtained results that the microbiological activity of soil under beans inoculated with *Rhizobium phaseoli* strains depended on the applied strains as well as on the bean genotypes. These results were in good agreement with those of Somasegaron *et al*. (1991).

According to Jarak *et al*. (1994), inoculation increases the number of azotobacters in bean rhizosphere, which is supported by our results. The total number of microorganisms was lower at the end of the growing season than at the flowering stage, indicating that the number is affected by exudates of bean roots. Similar results were reported by Milić *et al*. (1999b).

According to Plancquaret (1999), the bean requires 300 kg N/ha which are mostly provided by symbiotic N fixation. Increase in mineral nitrogen fertilizer reduces the number of nodules formed by the strains used for inoculation while increasing the number of nodules formed by native strains (Vargas *et al*. 2000). These results emphasize the necessity of further study of applicability of *Rhizobium leguminosarum bv. phaseoli* strains for seed inoculation because these microorganisms not only reduce the use of mineral nitrogen fertilizers but also increase soil quality, i.e., soil fertility, by provoking an increased production of plant hormones and enzymes.

CONCLUSION

It was concluded on the basis of the obtained results that the microbiological activity of soil under beans inoculated with *Rhizobium phaseoli* strains depends on the applied bacterial strains as well as on the bean genotypes.

At the flowering stage, inoculation effect was best demonstrated in the numbers of azotobacters and free N-fixing bacteria which were increased in both genotypes. The highest inoculation effect was registered in variant 5, i.e., with the mixture of all four strains. The individual strains differed in their effect on microbial number. Strains 2 and 3 were most effective, followed by strain 4. Strain 1 was least effective.

On average for the two years, the number of soil microorganisms decreased at the end of the growing season. The mixture of all four strains caused highly significant increases in both bean genotypes only in the number of fungi. Strain 3 caused the largest increase in the number of soil microorganisms.

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UTICAJ INOKULACIJE PASULJA SA *Rhizobium phaseoli* NA MIKROBIOLOŠKU AKTIVNOST ZEMLJIŠTA

Jelena B. Marinković
Naučni Institut za ratarstvo i povrtarstvo, 21000 Novi Sad, SCG

Summary

Bakterije roda *Rhizobium phaseoli* su simbiotski Gram-negativni mikroorganizmi zemljišta koji utiču na formiranje kvržica na korenu pasulja. Cilj rada bio je da se ispita uticaj inokulacije različitih genotipova pasulja (Belko, Sremac) na mikrobiološku aktivnost zemljišta. Ogled je postavljen na zemljištu tipa černozem na lokalitetu Rimski Šancevi u toku 2002. i 2003 godine. Neposredno pred setvu seme je inokulisano sojevima *Rhizobium phaseoli*, a kontrolna varijanta nije inokulisana. Ogled je postavljen u 6 varijanti: **1.** seme inokulisano sojem *Rhizobium phaseoli* 1; **2.** seme inokulisano sojem *Rhizobium phaseoli* 2; **3.** seme inokulisano sojem *Rhizobium phaseoli* 3; **4.** seme inokulisano sojem *Rhizobium phaseoli* 4; **5.** seme inokulisano smešom četiri soja; **6.** kontrola. Mikrobiološke analize zemljišta urađene su u fazi cvetanja pasulja i na kraju vegetacije. Istaživanja su pokazala da postoji zavisnost između ispitivanih genotipova pasulja i zastupljenosti ispitivanih grupa mikroorganizama. Prosečni rezultati za obe godine ukazuju da je i inokulacija značajno uticala na brojnost mikroorganizama u zemljištu. Kod sorte Belko broj azotobaktera, aktinomiceta kao i ukupan broj mikroorganizama veći je kod inokulisanih varijanti u odnosu na kontrolu, dok je kod sorte Sremac veći broj azotobaktera i oligonitrofila. Inokulacija nije uticala na brojnost gljiva i amonifikatora (osim kod sorte Sremac na kraju vegetacije, gde je broj amonifikatora povećan). Sojevi su neujednačeno uticali na mikrobiološku aktivnost zemljišta.

Ključne reči:inokulacija, pasulj, mikroorganizmi, Rhizobium phaseoli