

MICROBIAL ABUNDANCE IN RHIZOSPHERE OF SUGARBEET IN DEPENDANCE OF FERTILIZATION AND INOCULATION WITH *AZOTOBACTER CHROOCOCCUM*

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Abstract: *The nonsymbiotic N fixing azotobacter is an obligate aerobe living in the soil, the rhizosphere and the plant root. The ability of various strains to fix atmospheric N positively affect plant growth and yields. Microbial interactions with roots may involve either endophytic or free living microorganisms and can be symbiotic, assosiative or casual in nature. Associative and free living microorganisms may also contribute to the nutrition of plants through a variety of mechanisms including direct effects on nutrient availability (N₂-fixation), enhancement of root growth (PGPR – plant growth promoting rhizobacteria) as antagonists of root pathogenes or as saprophytes that decompose soil detritus and subsequently increase nutrient availability through mineralization and microbial turnover. Therefore, the abundance of azotobacter, fungi, actinomycetes and the total number of microorganisms in inoculated and non-inoculated sugar beet rhizosphere were determine in this study. Simultaneously we studied the effects of application of nitrogen fertilizer, manure and harvest residues. Samples of rhizosphere soil were*

taken three times in the course of growing season (May, July, September). The experiment included two variants (inoculated with Azotobacter chroococcum and non-inoculated) at four fertilization levels (non-fertilized control, 50, 100, 150 and 200 kg N/ha), in five replication. Total number of microorganisms was determined in soil agar (dilution of 10⁶). Fungi were determined on Chapek agar (dilution of 10⁴), actinomycetes on a synthetic agar (dilution of 10⁴) and azotobacters on Fiodor substrate (dilution of 10²). The results of the study for all four fertilization types and all four levels of added N showed that azotobacter abundance was higher in inoculated treatments than in the non-inoculated ones. The highest increases of total microbial abundance and number of free N-fixing bacteria were recorded in the inoculated treatments with NPK fertilizer alone and in the inoculated treatments with 100 kg N/ha. The highest percentage increases of azotobacter were obtained in the inoculated treatments with manure and manure plus harvest residues, respectively and in the variant without N.

Key words: *microorganisms, azotobacter, rhizosphere, sugarbeet*

INTRODUCTION

The rhizosphere can be defined as the zone of soil around plant roots whereby soil properties are influenced by the presence and activity of the root. Changes of the physical, chemical and biological properties of rhizosphere soil has significant influence on the subsequent growth and health of plants (RICHARDSON et al., 2009). Microbial interactions with roots may involve either endophytic or free living microorganisms and can be symbiotic, assosiative or casual in nature. Associative and free living microorganisms may also contribute to the nutrition of plants through a variety of mechanisms including direct effects on nutrient availability (N₂-fixation), enhancement of root growth (PGPR – plant growth promoting rhizobacteria) as antagonists of root pathogenes (RAAJMAKORS et al., 2009) or as saprophytes that decompose soil detritus and subsequently increase nutrient availability through mineralization and microbial turnover.

The ability of various strains to fix atmospheric N positively affect plant growth and yields (HAMEEDA et al., 2008; EBRAHIMI et al., 2007; JENSEN AND NIELSEN, 2003; SHEHATA AND EL-KHAURES, 2003). Beside fixing nitrogen, N₂-fixing bacteria also produce various polysaccharides, vitamins and growth substances and thus create favorable living conditions for other microorganisms (JENSEN AND NIELSEN, 2003; PATRA et al., 2005, 2007).

Besides wheat and corn rhizospheres population dynamics of soil microorganisms was also studied in sugarbeet rhizospheres (SARIĆ, 1968; SARIĆ I RISTIĆ, 1982; MRKOVAČKI et al., 1997, 1998). However, these studies were not focused on relationships between soil microorganisms status and *Azotobacter* inoculation combined with different kinds of fertilizers.

Therefore, the objective of this study was to assess the number of the total number of microorganisms, fungi, actinomycetes and azotobacter in inoculated and noninoculated sugarbeet rhizosphere.

MATERIAL AND METHODS

A field trial was established on a chernozem soil at Rimski Šančevi experiment field of Institute of Field and Vegetable Crops in Novi Sad. The experimental object was the sugarbeet hybrid variety Sara developed at the Institute. The trial included two variants, inoculated and non-inoculated. Inoculation was performed with a mixture of *Azotobacter* strains, with the concentration of 10⁹ per ml, incorporated into the soil before planting. The trial was established in a block design with five replications, four levels of N fertilization (50, 100, 150 and 200 kg N/ha) and the nonfertilized control. Besides the effects of different levels of mineral N, we also examined the effects of application of manure, harvest residues and their combinations.

Total number of microorganisms was determined in soil agar (dilution of 10⁶). Fungi were determined on Chapek agar (dilution of 10⁴), actinomycetes on a synthetic agar and azotobacters on Fiodor substrate (dilution of 10²).

RESULTS AND DISCUSSIONS

The results for all four levels of added N and all four fertilization types showed that azotobacter abundance was higher in inoculated treatments than non-inoculated ones (Fig. 1, Fig. 2). Inoculation of soil in sugar beet field with sufficient inoculum consisting of selected, highly effective *Azotobacter chroococcum* strains increased the population size of azotobacter in the rhizosphere as a result of bacterial adaptation to the environment and ecological conditions (ARTE AND SHENDE, 1981; MRKOVAČKI AND MILIĆ, 2001; MRKOVAČKI AND MEZEI, 2003).

The highest number of azotobacter, in both treatments inoculated and non-inoculated, was registered in the variant without N and the smallest with 200 kg N/ha (Fig. 1). The highest percentage increases of azotobacter were obtained in the inoculated treatments in the variant without N. These results are in agreement with previous results (MRKOVAČKI et al. 2003, 2006, 2007).

The highest number of fungi was obtained with 100 kg N/ha and the smallest with 50 kg N/ha (Fig. 1). In our previous results (MRKOVAČKI et al., 2007) the smallest number of fungi was recorded with 150 kg N/ha. Studying the effect of several levels and combinations of NPK on biological value of soil under sugar beet (SARIĆ, 1972) concluded that the application of mineral fertilizers caused minor changes in the abundance of fungi in the soil.

The number of actinomycetes was higher in non-inoculated treatments, than inoculated. These results are in agreement with our previous results (MRKOVAČKI et al., 2008). The results showed that in inoculated and non-inoculated treatments the highest number of

actinomycetes and total microbial abundance were obtained with 150 kg N/ha and the lowest with 100 kg N/ha (Fig. 1).

In this experiment, inoculation tended to increase the abundance of all groups of examined microorganisms, except actinomycetes, in sugar beet rhizosphere. The average increases of azotobacter and total microbial abundance were 50.26 and 34.30% respectively.

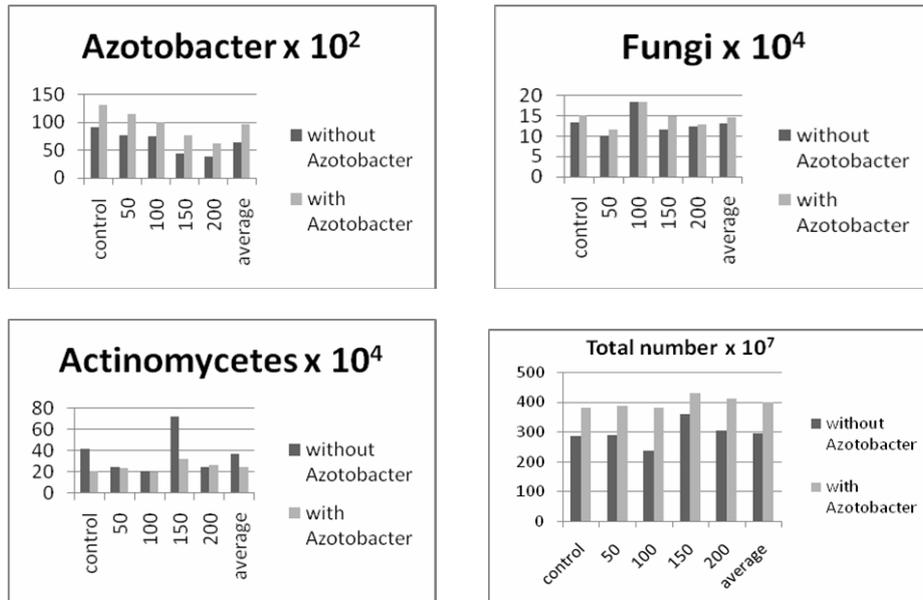


Figure 1: Abundance of microbes in sugar beet rhizosphere depending on inoculation and fertilization

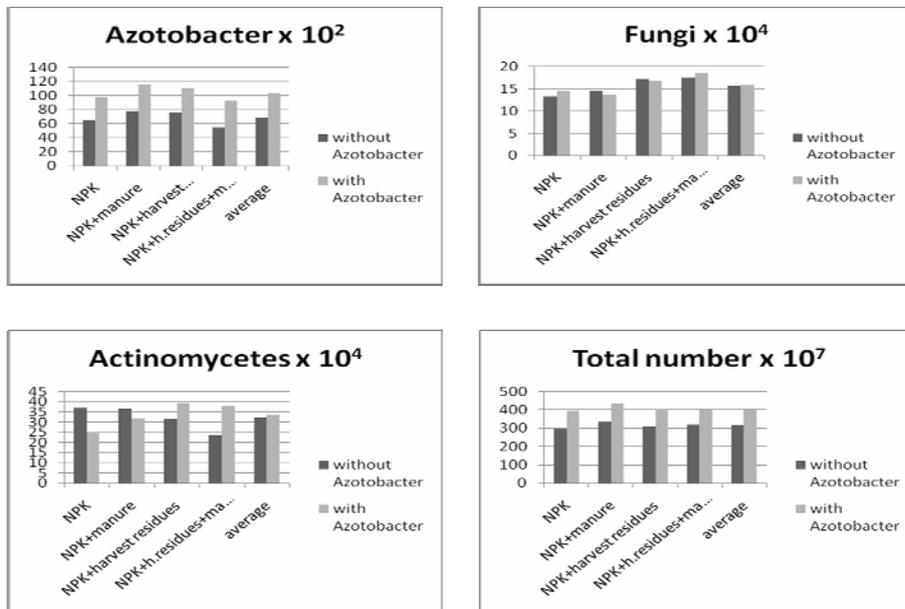


Figure 2: Abundance of microbes in sugar beet rhizosphere depending on inoculation and fertilization

Fig. 2 shows that the largest number of azotobacter and total microbial abundance were registered in the variant with NPK plus manure, while the largest number of fungi was observed in the variant with NPK plus harvest residues and manure (in both treatments non-inoculated and inoculated). In the inoculated treatment, the largest number of actinomycetes was found with NPK plus harvest residues, and in the non-inoculated treatment the largest number was with NPK alone (Fig. 2).

On average for the four kinds of fertilization, inoculation increased the number of azotobacter and the total number of microorganisms by 51.94% and 30.05% respectively. These results are in agreement with earlier investigation (MRKOVAČKI et al., 2007, 2008).

CONCLUSIONS

The inoculation with *Azotobacter chroococcum* increased total number of microorganisms and number of azotobacter in sugar beet rhizosphere.

In inoculated treatments the highest number of azotobacter was obtained in the variant without N. The highest number of actinomycetes and total microbial abundance were obtained with 150 kg N/ha.

The largest number of azotobacter and total microbial abundance were registered in the variant with NPK plus manure, while the largest number of fungi was observed in the variant with NPK plus harvest residues and manure.

BIBLIOGRAPHY

1. EBRAHIMI, S., NEJAD, H.I., RAD, A.H.S., AKBARI, G.A., AMIRY, R., SANAVY, S.A.M.M. (2007): Effect of *Azotobacter chroococcum* Application on Quantity and Quality Forage of Rapeseed Cultivars, *Pakistan Journal of Biological Sciences*, Vol 10(18), 3126-3130.
2. HAMEEDA, B., HARINI, G., RUPELA, O.P., WANI, S.P., REDDY, G. (2008): Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna, *Microbiological Research*, Vol 163, 234-242.
3. JENSEN, E.S., HAUGGAARD-NELSEN, H. (2003): How can increased use of biological N₂ fixation in agriculture benefit the environment? *Plant Soil*, Vol 252, 177-186.
4. MARINKOVIĆ, J., MRKOVAČKI, N., ČAČIĆ, N. (2007): The effect of inoculation and fertilization on microbial abundance in sugar beet rhizosphere, 9th International Symposium Interdisciplinary Research (Hungary – Romania - Serbia) Abstract book. Section 4 – Natural Resources in the region including Agriculture.p31.
5. MRKOVAČKI, N., MEZEI, S., KOVAČEV, L., ČAČIĆ, N., SKLENAR, P. (1997): The effect of inoculation of sugar beet with *Azotobacter chroococcum* on their number in rhizosphere, *Proceedings of the 60th IIRB Congress*, Cambridge, 521-525.
6. MRKOVAČKI, N., MEZEI, S., KOVAČEV, L., ČAČIĆ, N., SKLENAR, P. (1998): The effect of inoculation of sugar beet with various strains of *Azotobacter chroococcum* on the bacterial number of the root and in the rhizosphere, *Archive of Biological Sciences*, Vol 50, 189 - 193.
7. MRKOVAČKI, N., MEZEI, S., ČAČIĆ, N. (2003): Population dynamics of *Azotobacter chroococcum* in sugarbeet rhizosphere depending on mineral nutrition, *Zbornik Matice srpske za prirodne nauke*, Vol 104, pp.91 – 97.
8. MRKOVAČKI, N., MEZEI, S. (2006): Quantitative characteristics of rhizosphere microflora of sugarbeet depending of fertilization, *Zemljište i biljka*, Vol 55(1), 67-72.
9. MRKOVAČKI, N., MARINKOVIĆ, J., ČAČIĆ, N. (2007): Effect of fertilization on microbial abundance in sugarbeet rhizosphere, *Scientific Papers Faculty of Agriculture XXXIX Partea a II-a*, *Lucrari stiintifice facultatea de agricultura XXXIX*, 269-272.
10. MRKOVAČKI, N., ČAČIĆ, N., MEZEI, S., KOVAČEV, L., NAGL, N. (2008): Biogenost rizosfere šećerne repe inokulisane sa *Azotobacter chroococcum*, *Institut za ratarstvo I povrtarstvo*, Vol 45(2), 241-246.
11. PATRA, A.K., LE ROUX, X., ABBADIE, L., CLAYS-JOSSERAND, A., POLY, F., LOISEAU, P., LOUAULT, F. (2007): Effect of Microbial Activity and nitrogen Mineralization on Free-living

- Nitrogen Fixation in Permanent Grassland Soils, *J. Agronomy & Crop Science*, Vol 193, 153-156.
12. RAAIJMAKERS, J.M., PAULITZ, T.C., STEINBERG, C., ALABOUVETTE, C., MOENNE-LOCCOZ, Y. (2009): The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* doi: 10.1007/s11104-008-9568-6.
 13. RICHARDSON, A.E., BAREA, J.M., MCNEILL, A.M., PRIGENT-COMBARET, C. (2009): Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms, *Plant Soil*, Vol 321, 305-339.
 14. SARIĆ, Z. (1968): Uticaj nekih vrsta zemljišnog ekstrakta na zastupljenost pojedinih mikroorganizama u rizosferi soje i šećerne repe, *Savremena poljoprivreda*, Vol 6, 525-534.
 15. SARIĆ, Z., RISTIĆ, O. (1982): Kvalitativna i kvantitativna zastupljenost rizosferne mikroflore u toku vegetacije šećerne repe, *Zemljište i biljka*, Vol 31 (3), 385-393.
 16. SARIĆ, Z. (1972): Uticaj različitih doza i kombinacija NPK na biogenost černozema pod šećernom repom, *Agrohemija*, Vol 9-10, 509-521.
 17. SHEHATA, M.M, EL-KHAWAS, S.A. (2003): Effect of Two Biofertilizers on Growth Parameters, Yield Characters, Nitrogenous Components, Nucleic Acids Content, minerals, Oil Content, Protein Profiles and DNA Banding Pattern of Sunflower (*Helianthus annus L. Cv. Vedock*) Yield, *Pakistan Journal of Biological Sciences*, Vol 6 (14), 1257-1268.