EFFECT OF INOCULATION ON *Azotobacter* POPULATION SIZE IN SUGARBEET RHIZOSPHERE DEPENDING ON FERTILIZATION

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ABSTRACT: The objective of this study was to assess the effect of inoculation on Azotobacter population size in dependence of fertilizer dose and fertilization method. Differences were registered in Azotobacter population size which depended on both, nitrogen dose and fertilization method. On average, the highest percentage of increase in Azotobacter population size, in relation to the non-inoculated variant, was registered in the variant with nitrogen, liquid manure and harvest residues. The largest increase in Azotobacter population size was obtained in the inoculation variants.

Key words: *Azotobacter chroococcum*, inoculation, mineral fertilizers, rhizosphere

INTRODUCTION

The major sources of nitrogen for agricultural soil are mineral fertilizers and biological nitrogen fixation. One approach to improving the nitrogen economy of crops has been to apply diazotrophic bacteria to non-leguminous crops in rotation in the expectation that they would fix atmospheric nitrogen and so provide combined nitrogen to the plant for enhanced crop production. (Sloger and Van Berkum, 1992; Sturz et al., 2000; Mrkovački and Milić, 2001). *Azotobacter* is a free N-fixing bacterium which, in addition to the capacity to fix nitrogen, has a number of characteristics which positively affect plant growth (production of growth regulators, antibiotics, vitamins, siderophores). Inoculation of soil in sugarbeet field with sufficient inoculum consisting of selected, highly effective *Azotobacter chroococcum* strains increased the population size of *Azotobacter chroococcum* in the rhizosphere as a result of bacterial adaptation to the environment and ecological conditions (Arte and Shende, 1981; Mrkovački and Mezei, 2003).

The objective of this study was to assess the effect of inoculation on *Azotobacter* population size in sugarbeet rhizosphere depending on fertilizer dose and fertilization method.

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MATERIAL AND METHODS

A field trial was conducted at Rimski Šančevi experiment field in 2003, on a chernozem soil, with the sugarbeet hybrid variety Sara developed at Institute of Field and Vegetable Crops in Novi Sad. Inoculation of sugarbeet seeds was performed with a culture of *Azotobacter* strains in the concentration of 109/ml (5, 8, 14), which was incorporated into soil immediately before planting. The strains had been isolated from the rhizospheres of the varieties Dana and Hy-11.

The trial was replicated five times in a block design. Seeds in the control variant were left non-inoculated. The trial included five variants of fertilization: 1. no fertilizer; 2. 50 kg N/ha; 3. 100 kg N/ha; 4. 150 kg N/ha; 5. 200 kg N/ha. In addition to the different N levels, we assessed also the effects of application of liquid manure and harvest residues.

Samples for microbiological analyses of rhizospheric soil were taken three times, in May, July and October. *Azotobacter* population size was analyzed by the drop method on Fodorov's medium, preceeded by the dilution method, expressed per 1 g of dry soil.

RESULTS AND DISCUSSION

The population size of *Azotobacter* in the rhizosphere of the cultivar Sara is shown in Table 1. Differences were registered in *Azotobacter* population size which depended on both, nitrogen dose and fertilization method. On average, the highest percentage of increase in *Azotobacter* population size (57.3%), in relation to the non-inoculated variant, was registered in the variant with nitrogen, liquid manure and harvest residues. The second highest percentage of increase (50.2%) was registered in the variant with nitrogen alone.

Regarding the effect of nitrogen dose, the largest increase in *Azotobacter* population size was obtained in the variant with 100 kg N/ha (55%), followed by the variant without nitrogen (52.9%). It could be noticed that *Azotobacter* population size decreased with the increase in nitrogen dose in both inoculated and non-inoculated variants. This was an indication of inhibitory effect of mineral fertilizers, especially of high nitrogen doses, on *Azotobacter* population size. These results confirm our 2002 results on the inhibitory effects of 150 and 200 kg N/ha on the number of *Azotobacters* in sugarbeet rhizosphere (Mrkovački *et al*., 2003a).

Soil nitrogen level evidently affects plant response to inoculation. Interactions are sometimes observed between inoculation and nitrogen application. The application of medium doses of nitrogen, from 30 to 80 kg/ha, increased the response of sarghum (Smith *et al*., 1984), corn (Meshram and Shende, 1982) and wheat (Kapulnik *et al*., 1983). In another experiment, wheat responded to the application of 160 kg N/ha (Dart, 1986).

Evidently, it is necessary to determine experimentally the level of nitrogen fertilizer that has to be added. This level is in correlation with response to inoculation exhibited by the crop as well as with location.

Table 1. Azotobacter population size in the rhizosphere of Sara depending on fertilization method and dose

Fertilization method	kg N/ha (dose)							
	Inocul -ation	Ó	50	100	150	200	Averag e	% of increase due to inoculation
NPK	-	76.34	90.95	74.84	43.63	38.75	64.90	50.2
	+	132.47	115.61	99.72	62.50	77.31	97.52	
NPK + liquid manure	-	97.33	89.56	68.72	77.96	55.42	77.79	48.8
	+	144.55	120.72	118.51	113.04	82.20	115.80	
NPK + harvest residues	-	95.16	94.51	65.04	70.88	57.82	76.68	43.8
	+	121.31	140.54	110.92	87.41	91.31	110.30	
NPK + liquid manure + harvest residues	-	67.87	54.12	57.62	49.96	56.35	57.18	57.3
	+	116.65	101.65	84.15	92.30	55.11	89.97	
Average	-	84.17	82.28	66.55	60.60	52.08		
	+	128.74	119.63	103.32	88.81	76.48		
% of increase	+	52.9	45.3	55.2	46.5	46.8		

CONCLUSIONS

- Inoculation increased the *Azotobacter* population size in the rhizosphere of the variety Sara in all four fertilization methods.
- *Azotobacter* population size was largest in the inoculated variant without N application (control) and in the variant with 50 kg N/ha. The lowest population size was registered in the variant with 200 kg N/ha.
- The largest increase in *Azotobacter* population size was obtained in the variant in which nitrogen, liquid manure and harvest residues were applied.

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