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Effect of Inoculation on the Number of *Azotobacters* in Soil and Rhizosphere During Sugar beet Growing Season

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Abstract: The objective of the study was to investigate the abundance of *Azotobacter* in the soil and rhizosphere of four sugarbeet hybrids (Hy-11, Dana, Delta and Norma) inoculated with *Azotobacter chroococcum* strains 2, 3, 5, 8, 14 and 2/1. Samples have been taken on three different dates. The greatest number of *Azotobacters* was registered in August, the lowest in October. Inoculation with strains *Azotobacter chroococcum* 14, 5, and 2/1 produced the largest number of the bacteria.

Key words: *Azotobacter chroococcum*, inoculation, rhizosphere, soil, sugar beet

Introduction

Non-symbiotic N_2-fixation contributes to soil fertility and agricultural production. The beneficial effects of non-symbiotic soil microorganisms living in the rhizosphere have been studied for several crop species (Okon and Hadar, 1987).

By means of inoculation, it is possible to beget a population of microorganisms that promotes plant growth (O' Connell et al., 1996). Application of non-symbiotic N_2-fixers of the genus *Azotobacter* increases yields by 12 to 18% in some field crops (Jagnow, 1987; Maltseva et al., 1995). For a successful application of diazotrophs, namely *Azotobacter* to sugarbeets, it is of vital importance not only to isolate free living diazotrophs but also to determine the partner which in association with particular hybrid will be the most effective in N_2-fixation.

The objective of this study was to investigate the increases in the number of *Azotobacter*s during the growing season of four inoculated sugar beet genotypes.

Material and methods

A field trials estabilished at Rimski Šančevi on a chernozem soil involved four variety hybrids of sugar beet (Hy-11, Dana, Delta and Norma selected at the Institute of Field and Vegetable Crops in Novi Sad). In the course of seed processing, sugarbeet seeds were immerged into a liquid culture of selected *Azotobacter* strains. The strains of *Azotobacter* chroococcum (2, 3, 5, 8, 14 and 2/1) were isolated from the rhizospheres of the varietyies hibrids Dana and Hy-11. A randomized block design in five replications was used. Samples for microbiological analyses were taken on June 10 (1st sampling date), August 7 (2nd sampling date) and October 7 (3rd sampling date). The number of *Azotobacter*s was determined by means of the drop method, using Fjodorž s medium. The abundance of *Azotobacter* was determined by dilution and expressed per 1 gram of completely dry soil.

Results and dicussion

The number of *Azotobacter*s in the inoculated treatments varied with sampling date, variety hybrid and the strain applied.

Number of *Azotobacter*s in soil

Quantitative studies of the microflora of sugarbeet conducted by Sarić et al. (1968) showed that, at the beginning and the end of growing season, sugarbeets did not extend an inhibiting effect on the microorganisms, including *Azotobacter*.

Sarić and Rašović (1963) studied the dynamics of *Azotobacter* during the growing season of soybean, sunflower and sugarbeet in dry farming on a chernozem soil. The highest number of *Azotobacter* was found in June, whereupon it decreased. In sugarbeets, the number of the bacteria outside the rhizosphere was greater than in the other field crops under investigation.

In this study, the largest numbers of *Azotobacter*s were achived with Hy-11, Delta and Norma on first sampling date, but only with Dana on the second date (Fig. 1.). The results are in correlation with previous results (Mrkovački et al., 1997a). With all genotypes, the lowest numbers of *Azotobacter*s in the soil were registered in the third sampling date with strain 3, except Delta which achived the lowest number with strain 8.

The largest number of *Azotobacter*s in the soil was achived with strain 5 in the case of Dana and Delta, and with strains 2/1 and 14 in the case of the genotypes Hy-11 and Norma (Tab. 1).

Table 1. Number of Azotobacter in soil (10^2/g dry soil)

Strain	Variety - Hybrid												Average
	Hy-11			Dana			Delta			Norma			
	Time of sampling												
	I	II	III	I	II	III	I	II	III	I	II	III	
2	103,7	62,15	52,10	167,7	116,37	76,89	132,16	120,13	72,32	147,14	30,40	77,51	96,55
3	61,98	31,34	26,35	99,59	82,39	56,21	158,13	160,65	129,1	82,19	38,58	23,37	79,15
5	141,4	50,32	41,34	106,4	206,39	65,00	153,59	166,42	127,2	143,96	79,58	62,07	112,00
8	100,3	75,01	66,33	102,6	95,20	81,21	132,90	130,75	54,19	57,50	84,85	53,93	86,23
14	251,9	118,54	113,5	120,2	112,76	101,3	124,51	88,98	74,16	193,15	88,43	80,50	121,49
2/1	218,9	159,35	71,40	107,3	133,90	121.0	135,84	110,96	97,30	218,95	63,00	49,68	123,96
Average	96,44			108,50			120,51			87,49			

Fig. 1. Number of Azotobacter in soil in dependance of variety

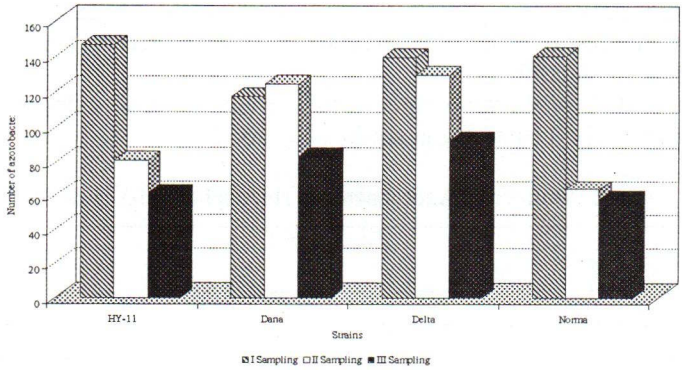
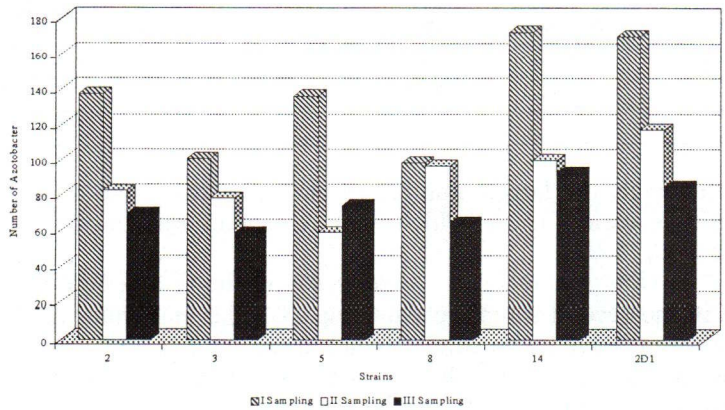


Fig. 2. Number of Azotobacter in soil in dependance of strain



On average, the largest number of *Azotobacters* in the soil was achived with the varieties Delta and Dana, the lowest with Norma (Tab. 1). The maximum number of *Azotobacters* was obtained with the variety Hy-11 in the first samling date (Fig. 1), the maximum number of *Azotobacters* in the soil was obtained with strain 14 in the first date (Fig. 2)

Number of *Azotobacters* in sugarbeet rhizosphere

The largest number of *Azotobacters* was registered on the second sampling date with all varieties under study, which is in accordance with our previous results (Mrkovački et al., 1997b). Since rhizosphere provides optimum conditions for the growth of *Azotobacters*, it is natural that their number is greater in it than in the surrounding soil. The results of Sarić and Ristić (1982) indicate that, very soon after emergence, the root of sugar beet becomes colonized by a large number of heterotrophic bacteria which prevent *Azotobacter* from reaching the root throughout the growing season. In our study, however, when the seeds were inoculated with *Azotobacter*, the bacterium managed to survive on the roots of the studied variety hybrids of sugarbeet.

The smallest number of *Azotobacters* was achived on the third sampling date as well as in the soil.

The best *Azotobacter* partner of the variety Delta was strain 5, of Norma strain 2/1, and of Hy-11 and Dana strain 14 (Tab. 2).

Table 2. Number of Azotobacter in rhizosphere (10^2/g dry soil)

Strain	Variety - Hybrid												Average
	Hy-11			Dana			Delta			Norma			
	Time of sampling												
	I	II	III	I	II	III	I	II	III	I	II	III	
2	38,21	121,97	36,28	53,81	105,44	67,60	74,89	149,48	74,10	42,48	152,73	35,56	79,41
3	50,07	110,86	58,14	53,69	93,64	23,84	128,80	158,19	70,15	47,19	118,60	47,43	80,05
5	58,07	123,17	38,70	42,25	111,51	28,26	165,91	132,40	62,46	102,46	139,63	45,67	87,54
8	40,53	130,59	26,90	86,81	108,52	59,57	148,38	133,13	58,51	73,30	155,29	45,70	78,32
14	73,69	162,07	36,51	67,36	136,57	45,36	107,75	164,83	62,02	103,27	110,52	36,73	92,22
2/1	78,78	124,51	27,71	64,03	117,17	23,13	113,57	162,75	35,71	78,66	198,86	32,97	88,15
Average	74,29			71,58			111,28			87,06			

On average, the largest number of *Azotobacters* in the rhizosphere was achived with the variety Delta, which is in agreement with our previous results (Mrkovački et al., 1998).

The maximum number of *Azotobacters* in the rhizosphere was obtained with Delta in the second sampling date (Fig. 3). The maximum number in the rhizosphere was achieved with strain 2/1 in the second date (Fig. 4).

The increase in the number of *Azotobacters* at the time of the second sampling shows that the conditions for their reproduction were favorable, i.e.

that the roots of the investigated hybrids provided sufficient amounts of nutrients needed for their growth.

Fig. 3. Number of Azotobacter in rhizosphere in dependence of variety

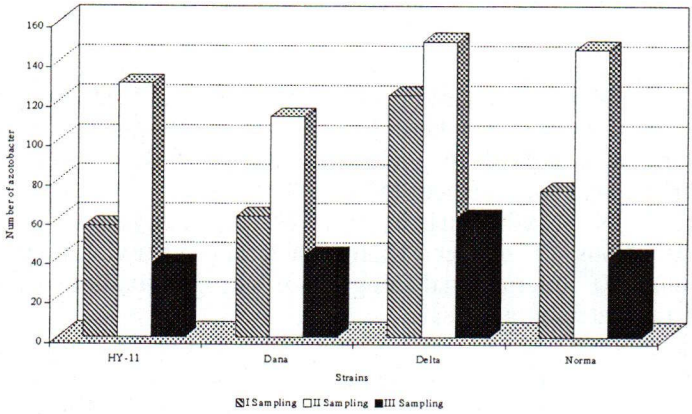
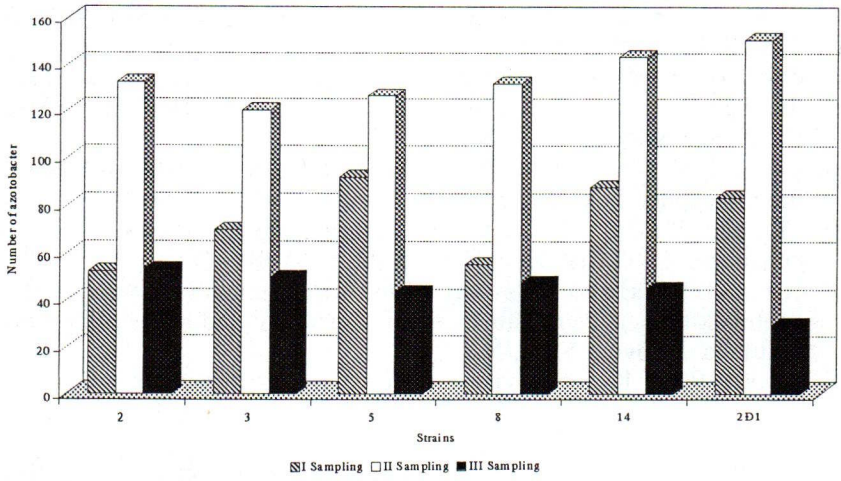


Fig. 4. Number of Azotobacter in rhizosphere in dependance of strain



Conclusions

Maximum numbers of *Azotobacter*s in the soil were registered in the 1st and 2nd sampling, in the rhizosphere only in the 2nd sampling.

Maximum numbers of *Azotobacter*s in the soil were were achieved with the varieties Delta and Dana, in the rhizosphere with the varieties Delta and Norma.

The largest numbers of *Azotobacters* in the soil and rhizosphere were produced by strains 2/1, 14 and 5.

The smallest numbers of *Azotobacters* in the soil and rhizosphere were registered at the time of the 3rd sampling.

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BROJ *Azotobactera* U ZEMLJIŠTU I RIZOSFERI U TOKU VEGETACIJE ŠEĆERNE REPE U ZAVISNOSTI OD INOKULACIJE

-originalni naučni rad-

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Rezime

U cilju primene diazotrofa, *Azotobactera* kod šećerne repe, veoma je važno determinisati soj koji se u asocijaciji sa određenim hibridom biti najefektivniji u azotofiksaciji. Stoga je cilj ovoga rada bio da se ispita broj *Azotobactera* u toku vegetacije šećerne repe. Ogled je postavljen na Rimskim Šančevima na zemljištu tipa černozem sa 4 hibridne sorte šećerne repe (Hy-11, Dana, Delta i Norma). Seme je pre setve inokulisano sojevima *Azotobacter chroococcum* (2, 3, 5, 8, 14 i 2/1). Ogled je postavljen po slučajnom blok sistemu u 5 ponavljanja. Uzorci za mikrobiološke analize uzimani su sa više mesta od svake varijante, u tri roka (10.06 - I rok; 7.08 - II rok; 7.10 - III rok). Brojnost *Azotobactera* određivana je metodom kapi na podlozi Fjodora. Najveći broj *Azotobactera* ostvaren je u zemljištu u I i II roku uzimanja uzoraka, a u rizosferi samo u II roku. Najveći broj *Azotobactera* u zemljištu dobijen je kod sorata Delta i Dana, a u rizosferi kod Delte i Norme. Najveći broj *Azotobactera* i u zemljištu i u rizosferi ostvaren je sa sojevima 2/1, 14 i 5. Najmanji broj *Azotobactera* i u zemljištu i u rizosferi ostvaren je u III roku uzimanja uzoraka.