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EFFICIENCY OF INOCULATION WITH *AZOTOBACTER CHROOCOCCUM* ON AGRONOMIC CHARACTERISTICS AND YIELD OF MAIZE AND SUGARBEET

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Abstract

The aim of this study was to determine the effect of inoculation with *Azotobacter chroococcum* strains on the root yield and white sugar yield of sugarbeet, as well as on the yield of maize, total microbial number and number of azotobacters in maize and sugarbeet rhizosphere. The effect of inoculation on microbial abundance in sugarbeet rhizosphere was evaluated in 2007 and 2008 at the locations of Pančevo and Rimski Šančevi. The experimental design was a randomized, complete block with four replications. The experimental object was the sugarbeet, cultivar Drena developed at Institute of Field and Vegetable Crops in Novi Sad. Five strains of *Azotobacter chroococcum* (1, 5, 8, 10 and 14) were used as microbiological fertilizer for sugarbeet. The strains are from NS Collection of Nitrogen fixing bacteria registered in WFCC World Data Center on Microoorganisms (registration number is 754 with the acronym (“NSCNFB”). The effect of inoculation on microbial abundance in maize rhizosphere was evaluated at the location of Rimski Šančevi. Three maize hybrids were used in the experiment: NS 444 ultra (FAO 400), NS 5010 (FAO 500) and Tisa (FAO 700) developed at Institute of Field and Vegetable Crops, Novi Sad. The microbial abundance in rhizosphere was determined during the growing season of investigated plants. Positive effect of inoculation with *Azotobacter chroococcum* was on total microbial number and number of azotobacters in the rhizosphere. Inoculation significantly increased maize yield, root yield and crystal sugar yield.

Key words: *Microbial abundance, rhizosphere, sugarbeet, maize, yield.*

Introduction

Microorganisms are important for agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers. Beneficial plant–microbe interactions in the rhizosphere are the determinants of plant health and soil fertility. In the era of sustainable agricultural production, the interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization, etc. from a limited nutrient pool in the soil and subsequent uptake of essential plant nutrients by the crop plants to realize full genetic potential of the crop (Mrkovački and Milić, 2001; Jeffries et al., 2003; Dey et al., 2004).

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield. Plant growth promoting bacteria (PGPR) may be important for plant nutrition by increasing N and P uptake by the plants, and playing a significant role as PGPR in the biofertilization of crops (Cakmakci et al., 2005). Bacterial inoculants are able to increases in germination rate, root growth, plant growth, yield, leaf area, chlorophyll content, nitrogen content, protein content, tolerance to drought, shoot and root weight, and delayed leaf senescence (Lugtenber et al., 2002; Dobbelaere et al., 2003). Successful examples of inoculation of maize, canola, wheat and other crops with PGPR species *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas* and *Enterobacter* have been

achieved both in laboratory and field trials (Glick et al., 1997; Sharma and Johri, 2003; Egamberdiyeva, 2007). Plant growth responses were variable and dependent upon the inoculant strain, soil organic matter content, growing stage, harvest date and growth parameters evaluated (Cakmakci et al., 2007). Inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of cereals (Bashan et al., 2004). Intensive research on associations between nitrogen–fixing bacteria and cereal roots began as early as the 1970s. In the production of field and vegetables crops, *Azotobacter* is the one which is most frequently applied. Inoculation of wheat and maize with *Azotobacter* strains increased the mass of the above–ground plant parts by 26–50% and yields by 19–30% (Jagnow, 1987). Numerous studies have established a significant impact of *Azotobacter chroococcum* on production and technological properties of sugar beet (Čačić et al., 2003; Mrkovački et al., 2009). Also, in several studies have reported that maize is able to support free–living N_2 fixers in its rhizosphere (Naureen et al., 2005; Perin et al., 2006; Mehnaz et al., 2007) and maximal nitrogenase activity has been reported to be dependent to the maize genotype (Picard et al., 2008). For example, Pandey et al. (1998) have reported that improvements in yield and plant growth resulted in part from the stimulation of N_2–fixing bacteria in the rhizosphere of maize after the bio–inoculation by two PGPR strains.
The aim of this study was to determine the effect of inoculation with *Azotobacter chroococcum* strains on the root yield and white sugar yield of sugarbeet, as well as on the yield of maize, total microbial count and number of azotobacters in maize and sugarbeet rhizosphere.

Materials and Methods

The effect of inoculation with *Azotobacter chroococcum* on sugarbeet and microbial abundance in sugarbeet rhizosphere was evaluated in 2007 and 2008 at the locations of Pančevo and Rimski Šančevi (Serbia). The experimental design was a randomized, complete block with four replications. The length of the experimental unit was 10 meters, the width was 2 meters. The experimental object was the sugarbeet, cultivar *Drena* developed at Institute of Field and Vegetable Crops in Novi Sad. Five strains of *Azotobacter chroococcum* (1, 5, 8, 10 and 14) were used as microbiological fertilizer for sugarbeet. The strains are from NS Collection of Nitrogen fixing bacteria registered in WFCC World Data Center on Microoorganisms (registration number is 754 with the acronym ("NSCNFB"). The strains were grown on Fiodorov medium in liquid culture. The soil was treated with 2 l ha^{-1} of inoculum (cell density of 10^9 in 1 ml) added to 300 l ha^{-1} of water. No treated soil were designed as control. Three maize hybrids were used in the experiment: NS 444 ultra (FAO 400), NS 5010 (FAO 500) and Tisa (FAO 700) developed at Institute of Field and Vegetable Crops, Novi Sad. Before seeding the maize, a mixture of *Azotobacter* strains (5, 8, 14) was introduced into one half of the experimental plot. One liter of inoculum with the cell density of 10^9 in 1 ml was dilluted in 300 l of water and sprayed into the soil. The microbial abundance in rhizosphere was determined during the growing season of investigated plants. The total microbial count was done in soil agar (dilution 10^6), the number of azotobacters in Fiodorov medium (dilution 10^2) (Jarak and Đurić, 2006). After harvesting, the grain yield (with 14% grain humidity) was measured in t ha^{-1}. The data were processed by analysis of variance and the significance was expressed by LSD test.

Results and Discussion

Effect of *Azotobacter* on sugarbeet: root and white sugar yield

The average yield of sugarbeet in both locations, achieved in this study in consequence to *Azotobacter* application, was 68.92 t ha^{-1} or 3.10 t ha^{-1} over the control. All *Azotobacter* strains exhibited positive effects on sugarbeet yield. The maximum increase in root yield – 2.8

t ha^{-1} was achieved by strain 8. For Pančevo location average yield increase was 4.11 t ha^{-1}. The highest root yield on location Rimski Šančevi were obtained with strains 10 and 8 (2.64 and 2.25 t ha^{-1} over control). In Pančevo the highest effect on root yield were with strains 1 and 5 (4.63 t ha and 3.40 t ha^{-1} over control) (tab.1).

Table 1. Effect of *Azotobacter chroococcum* strains on root and white sugar yield of sugarbeet (t ha^{-1})

Root yield of sugarbeet (t ha^{-1})								
Location	Control	Strain 1	Strain 5	Strain 8	Strain 10	Strain 14	Average	Increase (t ha^{-1})
R. Šančevi	68.55	69.32	69.28	70.80	71.19	70.71	69.98	2.09
Pančevo	65.18	69.81	68.58	68.53	67.56	67.52	67.86	4.11
Average	**66.86**	**69.56**	**68.93**	**69.66**	**69.37**	**69.11**	**68.92**	**3.10**
White sugar yield								
R. Šančevi	9.71	9.89	9.78	10.11	10.21	10.04	9.96	0.25
Pančevo	8.58	8.79	8.74	8.77	8.46	8.69	8.67	0.09
Average	**9.14**	**9.34**	**9.26**	**9.44**	**9.33**	**9.36**	**9.31**	**0.17**

LSD		
0.05	3.61	0.51
0.01	4.78	0.68
CV (%)	9.22	10.33

The average yield of sugar obtained in both locations by *Azotobacter* application was 9.31 t ha^{-1}. A positive effect on calculated sugar yield was achieved by applying all five *Azotobacter* strains while the biggest increase was recorded in the case of strain 8 (9.44 t ha^{-1} or 0.3 t ha^{-1} over the control. On location Rimski Šančevi the highest yield of white sugar were with strains 10 and 8 (with increase of 0.5 and 0.4 t ha^{-1} over control). In Pančevo the highest increase in white sugar were with strains 1 and 8, 0.21 and 0.19 t ha^{-1} over control (tab. 1).

Effect of *Azotobacter* on sugarbeet: total microbial and *Azotobacter* count

Microbial populations in both locations were more numerous in the treated variants than in the control. The average total bacterial count was 223.16 which was 34.3% higher than the control. The biggest effect on the total number of microorganisms was achieved in the case of strain 14, and the largest increase in Pancevo location – 56.02%. The highest effect on total microbial number was achieved with strain 1 on location Rimski Šančevi (27.9% over control) and with strain 14 on location Pančevo (99.1% over control). The number of *Azotobacters* was increased by all tested strains. The actual increases ranged from 34.3 to 53.1% compared with the control. The highest increase in the *Azotobacter* count was obtained in case of strain 8 (tab. 2).

Table 2. Effect of *Azotobacter chroococcum* strains on microbial abundance in sugarbeet rhizosphere

Total microbial count								
Location	Control	Strain 1	Strain 5	Strain 8	Strain 10	Strain 14	Average	Increase (%)
R. Šančevi	185.57	237.43	219.07	202.63	225.97	234.35	217.50	17.2
Pančevo	146.66	228.06	255.23	225.29	225.68	292.02	228.82	56.0
Average	**166.11**	**232.74**	**237.15**	**213.96**	**225.82**	**263.18**	**223.16**	**34.3**
***Azotobacter* count**								
R. Šančevi	23.03	36.40	39.27	39.26	32.85	40.73	35.26	53.1
Pančevo	58.84	83.53	83.38	84.98	82.40	80.81	78.99	34.2
Average	**40.93**	**59.96**	**61.32**	**62.12**	**57.62**	**60.77**	**57.12**	**39.6**

The highest number of *Azotobacter* was obtained with strain 14 on location Rimski Šančevi (76.8% over control) and with strain 8 on location Pančevo (44.2% over control) (tab. 2).

Effect of *Azotobacter* on maize: yield of maize

In all three hybrids, the grain yield was significantly increased in the variants with *Azotobacter* compared to the control. The increase in yield in NS 5010 was highly significant. Grain yield was higher by about half a ton per hectare, in relation to the control (tab. 3).

Table 3. Effect of *Azotobacter chroococcum* strains on maize yield (t ha^{-1})

Hybrid (A)	**NS 444 ultra**	**NS 5010**	**Tisa**	**Average**
Ø (B)	9.898	9.008	9.496	9.467
A. chroococcum (B)	10.294	9.649	9.889	9.944
A.c.- Ø	0.396	0.641	0.393	0.477

LSD		
0.05	0.484	0.395
0.01	0.669	0.546

Effect of *Azotobacter* on maize: total microbial and Azotobacter count

Microbial population with three examined hybrids, were numerous in the treated variant than in the control. The average total bacterial count was 120.5 which was much higher than the control. The biggest effect on total microbial count was achieved with hybrid NS 5010. The number of *Azotobacters* was increased with inoculation. The highest increase in the *Azotobacter* count was obtained in case of NS 444 ultra. The number of the examined microorganisms was also dependant upon the maize hybrid (table 4).

Table 4. Effect of *Azotobacter chroococcum* strains on microbial abundance in maize rhizosphere

Total microbial count				
Hybrid (A)	**Tisa**	**NS 5010**	**NS 444 ultra**	**Average**
Ø (B)	9.3	5.3	11.9	8.8
A.chroococcum (B)	23.4	252.5	85.5	120.5
A.c.- Ø	14.1	247.2	73.6	111.6
***Azotobacter* count**				
Ø (B)	42.2	29.5	40.5	37.4
A.chroococcum (B)	60.1	44.7	79.7	61.5
A.c.- Ø	17.9	15.2	39.2	24.1

The potential use of *Azotobacter* as biofertilizer has been rewieved by Mrkovački & Milić (2001) who concluded that inoculation with these microorganisms occasionally promote plant growth probably by mechanisms other than biological N fixation. *Azotobacter* has been used as a potential fertilizer to increase sugarbeet yields (Stainberga et al., 1996; Antipcuk et al., 1997). The results obtained in production fields in the Vojvodina Province inoculated with a mixture of *Azotobacter chroococcum* strains (NS *Betafixin*), showed that the sugarbeet yield was increased by 3.08 t ha $^{-1}$ in 2007 and by 6.18 t ha^{-1} in 2008 (Mrkovački et al., 2009). In previous studies, the increases in the root yield of three sugarbeet cultivars treated with *Azotobacter* strains were from 0.65 to 3.7 t ha^{-1} respectively, as compared with the control (Čačić et al., 2003). Antipcuk et. al. (1997) obtained increase of 2.6–12.7% relative to the control plot, in two year study of *Azotobacter* influence on sugarbeet yield with no nitrogen mineral fertilizer additions. The introduction of *Azotobacter* into the soil increased the sugarbeet yield 17–24% in relation to the control (Steinberga et al., 1996). Sahin et al. (2009) conclude that inoculation significantly increased root and sugar yield of sugarbeet over the control. Single inoculation with N_2–fixing bacteria increased sugarbeet root yield by 5.6–11%. Increases of white sugar yield by 680 kg ha^{-1} and by 1050 kg ha^{-1} were achieved in production plots in the Vojvodina Province in 2007 and 2008, respectively (Mrkovački et al., 2009). Rodelas et al. (1999) concluded that the yield of sugarbeet, carrot and cabbage was increased by 10% in the case of *Azotobacter* application. Some researcers have shown negative or no effects of EMC (effective microorganism culture) application on crop growth and yield (Kinjo et al., 2000; Formowitz et al., 2007; Daiss et al., 2008). Our previous studies (Mrkovački et al., 2002, 2008; Mrkovački and Mezei, 2006) showed that the total microbial count in the rhizosphere of inoculated sugarbeet was increased by 45.7 % or from 35 to 118 % in relation to control. According to results of Govedarica et al. (2004), with introduction of *Azotobacter*, biological activity in soil increases and yield of maize depends on hybrids and applied strains. The increase in yield can be due to the influence of *Azotobacter chroococcum* which fixes up to 90 kg N ha^{-1} a year which increases the nitrogen pool and biological activity of soil (Irissarri and Reinhold–Hurek, 2001; Hajnal et al., 2005; Raimam et al., 2007). Using *Azospirillum* as PGPR bacteria, Okon and Labandera-Gonzales (1994) obtained an increase in maize yield by 15–25%, and by 40% when inoculation was combined with fertilization. They were also reported a constant increase of the yield in medium–fertility soil and observed possibility of replacing 35–40% nitrogen fertilizer by using biofertilizers. Inoculation of maize increased shoot dry weight from 63–115% (Biari et al., 2008). Plants secrete through the root different organic and mineral substances which are used by rhizospheric microorganisms for nutrition. Even though the plant is the same, the number of microorganisms in rhizospheric soil also depends on hybrids and varieties within the same sort (Walker et al., 2003). A large number of rhizospheric microorganisms, including *azotobacter*, produce growth substances such as auxins, gibberellins etc. Biofertilizer PGPR inoculation significantly increased maize growth, seed maize yield as compared to treatment without inoculation (Yazdani et al., 2009). Important characteristic of *Azotobacter* assosiated with plant improvement is excretion of ammonia in the rhizosphere in the presence of root exudates which could explain why the inoculation resulted in a slightly higher total N content in soil (Wu et al., 2005). Egamberdiyeva (2007) examined the effect of PGPR on the growth and adsorption of nutrients of maize in two different soils and concluded that inoculation had a better stimulating effect on plant growth in soil with lower nutrient content than in rich.

Conclusions

The inoculation increased yield of the studied sugarbeet variety and maize hybrids. The total microbial count and azotobacter count in the rhizosphere of sugarbeet and maize were increased by inoculation. Long-term positive effect of PGPR may result in improvements in plant growth and sustainable food production with a positive relation toward the environment and economy of production. Overall, the results suggest that inoculants could be used to allow reductions in the current high rates of fertilizer and the resulting environmental problems without compromising plant productivity. However, it should be noted that no microbial inoculant can be universal for all systems as the effectiveness may be affected by plant type, soil type, and some other factors. Further greenhouse and field studies should provide more definitive information about the movement and uptake of macroelements (N and P) to plants with the impacts of PGPR–based inoculants.

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