Original scientific paper

Effect of inoculation with *Azotobacter chroococcum* on dynamics of the number of microorganisms in the rhizosphere of maize

Nastasija Mrkovački[1], Dragana Bjelić[1], Ivica Đalović[1], Srđan Šeremešić[2], Dragiša Milošev[1], Đorđe Jocković[1], Irena Jug[3]

[1]Institute of Field and Vegetable Crops, Maxim Gorki 30, 21 000 Novi Sad, Serbia
(ivica.djalovic@nsseme.com; maizescience@yahoo.com)
[2]Department of Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad
Sq. D. Obradovića 8, 21000 Novi Sad, Serbia
[3]J. J. Strossmayer University, Faculty of Agriculture Osijek, Croatia

Abstract
Successful production in monoculture requires intensive cropping, especially intensive tillage and fertilization with organic and mineral fertilizers. Microbial community composition can also respond differently to agricultural management practices. Monitoring changes in microbial communities induced by long-term fertilization regimes is suggested to be an important step towards sustainable agriculture. The aim of this study was to investigate dynamics of the number of microorganisms in the rhizosphere of maize in long–term monoculture. A two-level factorial split–plot experiment was set where the first factor was the fertilization (mineral and organic fertilizers) and the second factor was the inoculation with *Azotobacter chroococcum*. The inoculation with *Azotobacter chroococcum* increased the number of azotobacters, the total number of microorganisms and the number of P-mobilizers in maize rhizosphere. The highest increase (%) in the abundance of all examined microbial groups was obtained on variants NPK and NPK + manure. The best effects of inoculation were achieved in the second and third date of sampling.

Key words: *Azotobacter chroococcum*, microorganisms, rhizosphere, maize, yield

Introduction

In modern agricultural production growing crops in monoculture is widely present practice because it allows the maximum harvest with minimal labour. Successful production in monoculture requires intensive cropping, especially intensive tillage and fertilization with organic and mineral fertilizers. However, long periods of growing crops in monoculture reduces soil quality in terms of its physical and chemical properties, but also affect the diversity and structure of microbial communities as well as presence of certain soil borne pathogens (Nannipieri et al., 2003).

Soil beneficial rhizobacteria have been used in crop production for decades because of the role they play in supply nutrients to crops, stimulate plant growth through the production of plant hormones, control or inhabit the activity of plant pathogens, improve soil structure, and bioaccumulation or microbial leaching of inorganics (Hayat et al., 2010). The dynamics of soil microorganisms is affected by many abiotic and biotic factors: soil type, plant species, soil cultivation, application of organic and mineral fertilizers, irrigation, use of pesticides, etc.

Microbial community composition can also respond differently to agricultural management practices. Monitoring changes in microbial communities induced by long-term fertilization regimes is suggested to be an important step towards sustainable agriculture (Wu et al., 2011).

Complex relations that exist among the plant, the soil microorganisms and the soil itself are the determinants of plant health and soil fertility and can have a significant impact on plant growth and its yield. Knowledge of these relations is essential for the development of a cropping system which is based on the application of biofertilizers, phytostymulators and biopesticides that allows reduced use of chemicals in crop production, especially in long-term monoculture (Mrkovački et al., 2012).

The aim of this study was to investigate dynamics of the number of microorganisms in the rhizosphere of maize in long–term monoculture.

Material and Methods

The study was conducted in the multi–year stationary field experiment at the Institute of Field and Vegetable Crops, Novi Sad at Rimski Šančevi. The field is located on a calcareous chernozem on loess terrace. The experimental design was a randomized, complete block design (split-plot design experiment) with four replications. A two-level factorial split-plot experiment was set where the first factor was the fertilization (mineral and organic fertilizers) and the second factor was the inoculation with *Azotobacter chroococcum*. The study treatments were: Ø – control variant (maize in monoculture without fertilizer or organic fertilizers); Monoculture: NPK – maize in monoculture, fertilized only with mineral fertilizers; Monoculture: NPK + manure – maize grown in monoculture, with application of manure and mineral fertilizers; Monoculture: NPK + crop residue – maize grown in monoculture, with plowing crop residues (maize) and the application of mineral fertilizers; Treatments included two variants, inoculated and non–inoculated. Inoculation was performed with a mixture of *Azotobacter* strains, with the concentration of 10^9 per ml, incorporated into the soil before planting. The experiment was grown with maize hybrid NS 6010. Soil samples were taken for microbiological analyses at three dates (20^{th} May, 7^{th} June and 2^{nd} August). Total number of microorganisms was determined by the dilution method on agarized soil extract (dilution 10^7). Nitrogen–free medium and the method of fertile drops was used for number of azotobacters (dilution 10^2). The number of P-mobilizing bacteria was done in glucose-asparagine agar (dilution 10^5). All microbiological analyses were performed in three replications and the average number of microorganisms was calculated at 1.0 g absolutely dry soil (Jarak and Đurić, 2004).

Results and Discussion

Effects of the different types of fertilization and maize inoculation are presented in Tables 1, 2 and 3. Table 1 shows the number of azotobacters, Table 2 the total number of microorganisms and Table 3 the number of P–mobilizers in maize rhizosphere.

The number of azotobacters was increased by inoculation from 26.60% to 36.43% in the first and second sampling date. The largest increase was obtained on variant NPK + crop residue (88.15 and 82.30 x 10^2 g^{-1}). In the third sampling date number of azotobacters was increased on all variants of fertilization. Significantly higher increase over the control was obtained on variant NPK + manure (190.65 x 10^2 g^{-1}) (Table 1).

Table 1. Number of azotobacters in maize rhizosphere depending on fertilization treatment and inoculation (x 10^2 g^{-1})

Sampling	Inoculation	Fertilization Treatment					Increase (%)
		NPK	NPK + M	NPK + CR	Control	Average	
I	-	5.80	6.00	107.80*	21.80	35.35	36.43
	+	27.20	7.90	68.50	89.30	48.23	
	Average	16.50	6.95	88.15	55.55	41.79	
II	-	3.80	17.20	102.30*	21.50	36.20	26.60
	+	11.70	13.70	62.30	95.60	45.83	
	Average	7.75	15.45	82.30*	58.55	41.01	
III	-	109.90	229.10*	108.70	84.70	133.10	-0.75
	+	139.60	152.20	140.70	95.90	132.10	
	Average	124.75	190.65*	124.70	90.30	132.60	

LSD $_{0.05}$		I	II	III
	I	25.41	15.86	35.23
	T	35.94	22.42	49.82
	I x T	50.83	31.71	70.45

The total number of microorganisms on average was increased by inoculation in all sampling dates. The biggest effect of inoculation was achieved in the second sampling (64.31%). In the second and third sampling date, significant increase in the total microbial number was obtained on variants NPK and NPK + manure (202.80 and 154.60 x 10^7 g^{-1}) (Table 2).

Table 2. Total number of microorganisms in maize rhizosphere depending on fertilization treatment and inoculation (x 10^7 g^{-1})

Sampling	Inoculation	Fertilization Treatment					Increase (%)
		NPK	NPK + M	NPK + CR	Control	Average	
I	-	198.00	246.00	155.00	221.00	205.00	9.27
	+	229.00	218.00	196.00	253.00	224.00	
	Average	213.50	232.00	175.50	237.00	214.50	
II	-	184.40*	115.10	92.60	100.50	123.15	64.31
	+	203.40*	290.50*	168.20	147.30	202.35*	
	Average	193.90*	202.80*	130.40	123.90	162.75	
III	-	128.50*	162.30*	111.10	63.00	116.23	2.24
	+	122.90	146.90	106.50	99.00	118.83	
	Average	125.70*	154.60*	108.80	81.00	117.53	

LSD $_{0.05}$		I	II	III
	I	47.2	25.83	26.75
	T	66.7	36.54	37.83
	I x T	94.3	51.67	53.51

Table 3. Number of P-mobilizers in maize rhizosphere depending on fertilization treatment and inoculation (x 10^5 g^{-1})

Sampling	Inoculation	Fertilization Treatment					Increase (%)
		NPK	NPK + M	NPK + CR	Control	Average	
I	-	243.20*	158.30	137.10	157.20	173.95	-1.75
	+	229.60*	163.80	127.10	163.30	170.95	
	Average	236.40*	161.05	132.10	160.25	172.45	
II	-	184.00	161.00	91.00	156.00	148.00	6.59
	+	171.00	199.00	131.00	130.00	157.75	
	Average	177.50	180.00	111.00	143.00	152.88	
III	-	331.00	277.00	316.00	307.00	307.75	27.86
	+	439.00	312.00	372.00	451.00	393.50*	
	Average	385.00	294.50	344.00	379.00	350.63	

		I	II	III
LSD $_{0.05}$	I	28.34	44.6	71.7
	T	40.08	63.1	101.4
	I x T	56.68	89.2	143.4

The number of P-mobilizing bacteria was increased by inoculation in second and third date of sampling (6.59 and 27.86%). The significant increase in the number of P-mobilizers in the first sampling on average for both variants of inoculation, was obtained on NPK variant and amounted to 236.40 x 10^5 g^{-1} i.e. 47.5%. On this variant of fertilization the best effect in the third sampling was recorded (385.00 x 10^5 g^{-1}), while at second sampling equally good results were ontained on variants NPK and NPK + manure (Table 3).

Mandić et al. (2005) showed that the use of *Azotobacter* in the maize mineral nutrition did not significantly increase the yield of maize silage and grain. The results of their study showed that lower doses of nitrogen and solid manure during the growing season stimulated number of azotobacters in the soil, while liquid manure showed a smaller effect. High doses of fertilizers repressed number of investigated micobial groups, especially at the beginning of growing season, as well as during low soil moisture and drought period, which is in agreement with our results. Many authors state that the total number of microorganisms varies depending on the time of sampling. Mandić (2011) recorded that the total number of microorganisms on average for year, hybrids and methods of biofertilization, was the highest in samples taken at the stage of waxy ripeness. Regarding the fertilization treatment, the smallest total number of microorganisms was obtained in the control variant and the highest in variant of seed inoculation. The number of azotobacters was a statistically significantly higher (P < 0.01) in samples taken at the stage of waxy ripeness than in the silking stage. In the three–year period, the highest number of azotobacters was achived in the case of hybrid NS 6010 than in the case of hybrids ZP 684 and Dijamant–6.

Conclusions

The inoculation with *Azotobacter chroococcum* increased the number of azotobacters, the total number of microorganisms and the number of P-mobilizers in maize rhizosphere. The highest increase (%) in the abundance of all examined microbial groups was obtained on variants NPK and NPK + manure. The best effects of inoculation were achieved in the second and third date of sampling.

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