Journal of Environmental Protection and Ecology 17, No 3, 1003–1010 (2016)

Ecology

EFFECT OF BACTERIAL SEED INOCULATION ON NITROGEN DYNAMICS, NUMBER OF BACTERIA IN SOIL UNDER MAIZE, AND MAIZE YIELD

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Abstract. The aim of this study was to determine the effects of the inoculation of maize seeds with mixtures of bacteria (*Azotobacter chroococum*, *Azotobacter vinelandi*, *Bacillus megaterium, Bacillus licheniformis*) during different phenophases (6–7 leaves, silking and wax ripeness stage) and on the nitrogen dynamics, total number of microorganisms, number of azotobacter and aminoheterotrophs in Chernozem and grain yield of maize hybrid ZP 684 during 2006, 2007 and 2008. Nitrogen amount in soil was significantly higher in 2006, due to favourable meteorological conditions for microbial activity (higher total number of microorganisms and number of azotobacter), than in 2007 and 2008. The minimum amount of nitrogen was in the stage of wax ripeness although the total number of microorganisms, azotobacter and aminoheterotrophs were the largest. Seed inoculation of maize significantly increases the values of all studied parameters.

Keywords: N-fixing bacteria, nitrogen dynamics, maize, seed inoculation, yield.

AIMS AND BACKGROUND

Maize participates in crop structure in Serbia with 38% (1.2 million ha). Intensive maize production is based primarily on the use of mineral nitrogen fertilisers. The introduction and application of biofertilisers in maize production practice increase free-living nitrogen-fixing bacteria in the rhizosphere, increase soil fertility, grain yield and biomass, reduce the use of expensive nitrogen fertilisers and production costs[1]. Also, application of biofertilisers influences the maintenance of the ecological balance which is reflected on the safety of food and a favourable economic effect[2]. Microorganisms population are very useful as indicators of soil fertility[3]. Biofertilisers contain plant growth promoting rhizobacteria (PGPR). These are the

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numerous species of soil bacteria which flourish in the rhizosphere of plants and stimulate plant growth by a plethora of mechanisms[4]. PGPR can promote plant growth with several mechanisms: production of phytohormone (indole-3-acetic acid, gibberellic acid, cytokinins and ethylene), asimbiotic N_2 fixation, biocontrol of pathogenic microorganisms and solubilisation of mineral phosphate and other nutrients[5]. Kuzevski et al.[6] reported that that rhizobacteria have stimulating effect on the growth and development of plants by producing enzymes and antibiotics. Microorganisms, due to their enzyme system perform decomposition of organic and inorganic substances and preparing plant assimilative for plants requirements[7]. Nitrogen fixing bacteria in the process of biological nitrogen fixation to gradually converted atmospheric nitrogen to biologically useful NH_3 by an enzyme nitrogenase. According to Umarov[8], and Milosevic et al.[9], the nitrogen fixing bacteria in the soil of the temperate climatic zone can fix 30–90 kg N ha^{-1}, even 150 kg N ha^{-1}. Grain yield and microbial activity in the rhizosphere increase by inoculating maize seed with a mixture of bacteria *Azotobacter vinelandi*, *Azospirillum lipoferum*, *Bacillus megaterium* and *Bacillus suptilis*[10] and *Azotobacter chroococcum* and *Bacillus megaterium*[11] and bacteria *Azotobacter chroococcum*[12]. Contrary, Mandic et al.[13] showed that the strain of azotobacter in the fertilisation of maize did not increase grain yield and maize silage. The application of biofertiliser Bactofil increased the grain yield of NS 640 maize hybrid by 47% and number of azotobacter and other free fixing bacteria in the rhizosphere compared to control[14]. Inoculation of maize seeds with Plant Growth Promoting Rhizobacteria significantly increased the maize grain yield[15], maize biomass and nitrogen content in the soil[16] and significantly enhanced seed germination and seedling vigour of maize, plant height, 100 seed weight, number of seed per ear and leaf area[17].

The aim of this investigation was to estimate the effects of seed inoculation with mixtures of bacteria (*Azotobacter chroococum*, *Azotobacter vinelandi*, *Bacillus megaterium, Bacillus licheniformis*) on the amount of nitrogen, total number microorganisms, azotobacter and aminoheterotrophs in the rhizosphere and grain yield in maize hybrid ZP 684 (FAO 600 maturity group).

EXPERIMENTAL

The investigation was conducted on the experimental fields in southwest Vojvodina Province (Republic of Serbia) in region Srem (Putinci 44°59′31″ N lat., 19°58′19″ E long.) during 2006–2008. Maize hybrid ZP 684 of FAO maturity group 600 was used as material. Monthly meteorological data for the investigated period are presented in Table 1.

Table 1. Monthly meteorological data in 2006, 2007 and 2008

Year	Month							$\Sigma / \bar{x}$ (IV–IX)
	X–III	IV	V	VI	VII	VIII	IX	
	Rainfall (mm)							
2006	211.8	63.9	31.4	92.3	39.0	156.2	15.6	398.4
2007	254.8	0.0	79.0	85.2	38.7	62.5	93.4	358.8
2008	333.6	52.4	42.4	58.1	61.0	22.7	76.7	313.3
	Temperature (°C)							
2006	–	12.5	16.4	19.6	22.8	19.1	17.5	18.0
2007	–	13.0	18.5	22.0	22.6	22.3	14.3	18.8
2008	–	12.9	18.3	21.7	21.7	21.5	15.4	18.6

Two variants of inoculation seed were tested: control (non-inoculation) and inoculation with a mixture of bacteria. Active substance consists of the following bacteria titre (CFU/ml): *Azotobacter chroococum* – 10^8, *Azotobacter vinelandi* – 10^8, *Bacillus megaterium* – 10^9 and *Bacillus licheniformis* – 10^9. Seeds were inoculated just before sowing (20 ml per 100 g seeds). Sub-plot area was 4.2 m^2 (4 rows, inter-row distance 0.7 m, and length of 6 m). Plot was set up in a randomised block design with 4 replicates. The sowing dates were April. Plant density was 59.523 plants ha^{-1}. Preceding crop was winter wheat in three seasons. A standard cultivation practice was applied. Plots fertilised in autumn with NPK fertiliser 10:30:20 at the rate of 300 kg ha^{-1} and in spring with KAN (27% N) at the rate of 90 kg ha^{-1}.

Conducted is preliminarily observation of the soil by standard methods[18]. The total number of microorganisms, azotobacter and aminoheterotrophs in the rhizosphere under maize crops in the vegetation season were estimated three times at the phase 6–7 leaves, silking and wax ripeness. The total number of microorganisms was determined on soil-extract agar medium[19], the number of azotobacter by fertile drops method on the Fyodorov medium[20] and aminoheterotrophs on meat-peptone agar[21].

Maize harvest was performed manually. Grain yield is calculated on a 14% moisture basis. Data were processed by ANOVA using Statistica version 10, a RCBD and Duncan Multiple Range Test was used to compare differences among treatment means ($P \leq 0.05$).

RESULTS AND DISCUSSION

The main chemical properties of the Chernozem soil are presented in Table 2.

The soil is characterised by neutral to weakly alkaline reaction, high available calcareous and potassium and medium available humus and phosphorus. Soil was high available total nitrogen in 2006 and medium available in 2007 and 2008.

Results of ANOVA in Table 3 indicated that year had highly significant effect on amount of nitrogen in soil, total number microorganisms and azotobacter.

Table 2. Chemical parameters of Chernozem soil before sowing during the 2006–2008 period

Year	Depth (cm)	pH		$CaCO_3$	Humus	Total N	Amount of nitrogen (kg ha^{-1})	P_2O_5	K_2O
		H_2O	n/1 KCl	(%)				(mg/100 g soil)	
2006	0–30	7.38	7.16	16.80	3.61	0.2342	23.29	17.18	28.20
2007	0–30	7.67	7.46	10.08	2.79	0.1951	17.98	21.86	22.20
2008	0–30	7.52	6.89	15.90	2.58	0.1706	14.31	16.37	21.40

Table 3. Effects of year, time sampling and seed inoculation on nitrogen dynamics, total number microorganisms, azotobacter and aminoheterotrophs, and grain yield

Factor	Amount of nitrogen (kg ha^{-1})	Total number microorganisms (10^{-5} g^{-1} soil)	Number of azotobacter (10^2 g^{-1} soil)	Number of aminoheterotrophs (10^{-5} g^{-1} soil)
	Year effects (A)			
2006	23.04^a	197.07^a	138.15^a	114.92
2007	17.69^b	103.73^b	56.69^b	113.21
2008	14.01^c	80.52^c	63.18^b	104.59
F test	**	**	**	ns
	Time sampling effects (B): TS1 – Stage 6–7 leaves; TS2 – silk; TS3 – wax ripeness			
TS1	18.29^b	108.14^b	90.34^a	108.91^b
TS2	19.12^a	118.52^b	77.20^b	87.64^c
TS3	17.34^c	154.66^a	90.48^a	136.16^a
F test	**	**	*	**
	Inoculation seed effects (C): Co – Control; SI – Seed inoculation			
Co	11.41^b	109.40^b	78.83^b	97.21^b
SI	25.09^a	144.82^a	93.18^a	124.60^a
F test	**	**	**	**
	Interactions (*F* test)			
AB	**	**	**	**
AC	**	**	**	**
BC	**	**	**	**
ABC	**	**	**	**
M	18.25	127.10	86.01	110.91

Means followed by the same letter within a column are not significantly different by the Duncan Multiple Range Test at the $p \leq 0.05$ level; *, ** significant at the 0.05 and 0.01 probability levels, respectively; ns – non-significant.

The amount of nitrogen (23.04 kg ha^{-1}), total number microorganisms (197.07 × 10^{-5} g^{-1} soil) and number of azotobacter (138.15 × 10^2 g^{-1} soil) were

higher in 2006 than in 2007 (17.69 kg ha^{-1}, 103.73 × 10^{-5} g^{-1} soil and 56.69 × 10^{2} g^{-1} soil, respectively) and 2008 (14.01 kg ha^{-1}, 80.52 × 10^{-5} g^{-1} soil and 63.18 × 10^{2} g^{-1} soil, respectively). Optimum weather conditions in year 2006 had positive impact on soil conditions, better reproduction of bacteria (largest total number of microorganisms and number of azotobacter) and mineralisation process resulting in the significantly higher content of mineral nitrogen than in 2007 and 2008. Water deficiency in the soil, especially in June and August 2008, slowed down the process of mineralisation and reduced the amount of mineral nitrogen in the rhizosphere. Favourable soil moisture intensifies the processes of mineralisation and increases the content of mineral nitrogen[1]. Larger numbers of microorganisms implies greater microbial activity, mineralisation of organic matter and the creation of large quantities of accessible plant nutrients[1,22].

The time sampling had significant effect on amount of nitrogen, total number microorganisms, azotobacter and aminoheterotrophs. The highest amount of nitrogen was in silk stage (19.12 kg ha^{-1}). The highest total number microorganisms (154.66 × 10^{-5} g^{-1} soil), azotobacter (90.48 × 10^{2} g^{-1} soil) and aminoheterotrophs (136.16 × 10^{-5} g^{-1} soil) were in wax ripeness stage. Maize plants acquire nitrogen more intensively to silking stage, and the most intensive uptake of nitrogen is in the stage of 9–11 leaves and fertilisation (30–35 days). Since maize was treated with KAN fertiliser in stage 4–6 leaves and the number of microorganisms and nitrogen fertilising bacteria significantly increased during the growing period, that resulted in better exploitation of biological nitrogen and the amount of mineral nitrogen was high in the moments when the uptake of nitrogen by plants was the most intensive. The major factors determining the composition of rhizosphere microbial communities are plant species, plant developmental stage and soil type[23].

Seed inoculation significantly increases the amount of nitrogen, number of microorganisms, azotobacter and aminoheterotrophs in the rhizosphere. The results are consistent with research of Hajnal-Jafari[24]. After inoculation, microorganisms in the rhizosphere multiply and intensify a microbiological process which accelerates mineralisation and creates a greater amount of plant nutrients. The total number of microorganisms, azotobacter and phosphorus cycle bacteria, and maize grain yield were significantly increased with inoculation seeds with the strains of *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Azospirillum lipoferum*, *Bacillus megatherium*, *Bacillus subtilis*, etc.[25] The application of the mixture cultures of *Azotobacter chroococcum*, *A. vinelandi*, *Derxia* sp., *Bacillus megatherium*, *B. licheniformis* and *B. subtilis* increases soil biogenity (total number of microorganisms and oligonitrophiles) and the number of the total number of microorganisms, number of oligonitrophiles, actinomycetes, azotobacter and fungi during the plant growing season[26]. Number and diversity of microorganisms in soil under maize depends on the physicochemical properties of soil, climatic factors, growing system,

methods of cultivation, hybrids and composition of root exudates[22]. Interactions of all parameters were significant.

Grain yield was dependent on the year and seed inoculation (Table 4). Grain yield was significantly higher in 2006 (13.77 t ha^{-1}) than in 2007 (11.34 t ha^{-1}) and 2008 (9.52 t ha^{-1}). Grain yield was significantly higher in variant seed inoculation (12.19 t ha^{-1}) than non-inoculation (10.89 t ha^{-1}). Interaction between factors was not found.

Table 4. Effect of year, genotype and seed inoculation on grain yield (t ha^{-1})

Year effects (A)			Seed inoculation effects (B):	
2006		13.77^{a}	control	10.89^{b}
2007		11.34^{b}	seed inoculation	12.19^{a}
2008		9.52^{c}		
F test	A	B	AxB	
	**	**	ns	

Means followed by the same letter within a column are not significantly different by the Duncan Multiple Range Test at the $p \leq 0.05$ level; *, ** significant at the 0.05 and 0.01 probability levels, respectively: ns – non-significant.

Grain yield was highest in 2006, when there was no critical period for maize in regard to water supply. In 2007 and 2008 dry periods in the silking stage (ASI) and the stage of grain filling, respectively, resulted in a significant reduction in grain yield. Hajnal-Jafari[24] has received a significantly higher grain yield of hybrid NS 640 in variant with seed inoculation (11.56 t ha^{-1}) compared to control (8.69 t ha^{-1}).

CONCLUSIONS

Inoculation of maize seed with mixtures of bacteria (*Azotobacter chroococum*, *Azotobacter vinelandi*, *Bacillus megaterium, Bacillus licheniformis*) can be used to increase the content of available nitrogen in the rhizosphere and grain yield. Also, seed inoculation increases soil biogenity. This result could be useful for farmers in applying biofertilisers in organic agriculture or reducing the use of expensive mineral fertilisers. Due to improved soil fertility and the biological value is expected that the production of the next crop in the rotation may be based on the use of smaller amounts of nitrogen fertiliser and thus decrease the cost of production.

Acknowledgements. This study was financially supported by the Ministry of Education and Science of Republic of Serbia, Project TR-31053 and TR-31037.

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Received 17 April 2016
Revised 13 June 2016