CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 59 (3-4) 339-345, 2010.
UDC: 63(497.1)(051)-"540.2" ISSN 0350-1205

UDC: 579:633.15

EFFECT OF PGPR ON THE EARLY GROWTH OF MAIZE AND MICROBIAL ABUNDANCE IN RHIZOSPHERE

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SUMMARY: The objective of this study was to investigate the microbial abundance in maize rhizosphere depending on the applied bacterial inoculants and the leaf stage of maize as well as to examine the effect of plant growth promoting rhizobacteria (PGPR) on the early growth of maize plants. In this experiment three bacterial species: Azotobacter chroococcum, Bacillus subtilis and Pseudomonas fluorescens applied individually and in mixture were used as inoculants. One hybrid of maize included in the tests: NS 6010 developed at the Institute of Field and Vegetable Crops in Novi Sad. Experiments were established in laboratory and in greenhouse. The number of the investigated microbial groups depended on the applied bacterial inoculants and the leaf stage of maize plants. Bacterial strains used in this study had a statistically significant influence on the number of microorganisms in maize rhizosphere and a positive effect on the early growth of maize. Bacterial inoculants increased plant height and weight of young maize plants.

Key words: *Azotobacter*, *Bacillus*, *maize, PGPR*, *Pseudomonas*.

INTRODUCTION

In agricultural soils, the action of soil microorganisms is a major determinant of efficient nutrient cycling and plant growth. The microbe-plant interaction can be beneficial, neutral, variable, or deleterious for plant growth (Husen, 2003). An important group of these microbial communities that exerts beneficial effects on plant growth were first defined by Joseph Kloepper and Milton Schroth and termed as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). PGPR are the group of bacteria that actively colonize rhizosphere and plant roots and increase plant growth and yield (Adesemoye et al., 2009). Bacteria of diverse genera such as *Azotobacter*,

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Original scientific paper / *Originalni naučni rad*

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Azospirillum, *Alcaligenes*, *Azoarcus*, *Enterobacter*, *Klebsiella*, *Bacillus*, *Pseudomonas*, *Beijerinckia* were identified as PGPR (Berg, 2009). PGPR can directly stimulate plant growth in several different ways. They can: fix atmospheric nitrogen (Mrkovački and Milić, 2001; Jensen and Hauggaard-Nielsen, 2003), synthesize several plant hormones (Dobbelaere et al., 2003), solubilize minerals (Rodrigez and Fraga, 1999; Cakmakci, 2006), synthesize enzymes that can modulate plant hormone levels (Ahmad et al., 2008). The indirect promotion of plant growth include: production siderophores that limit the available iron to the pathogen (Husen, 2003), production antibiotics that kill the pathogen and induction systemic resistance in plant (Ramamoorthy et al., 2001).

The use of PGPR has become a common practice in many regions of the world. Economic and environmental benefits can include increased income from high yields, reduced fertilizer costs and reduced emission of the greenhouse gas (Kennedy et al., 2004). Therefore, the objective of this study was to examine the effect of *Azotobacter chroococcum*, *Bacillus subtilis* and *Pseudomonas fluorescens* and their mixture on the microbial abundance in maize rhizosphere and the early growth of maize plants.

MATERIAL AND METHODS

In this experiment three bacterial species: *Azotobacter chroococcum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were used as inoculants. The effect of four different variants of inoculation has been tested, in three replication: I – *Azotobacter chroococcum*, II – *Bacillus subtilis*, III – *Pseudomonas fluorescens*, IV – *Azotobacter chroococcum* + *Bacillus subtilis* + *Pseudomonas fluorescens*. One hybrid of maize included in the tests: NS 6010 developed at the Institute of Field and Vegetable Crops in Novi Sad. Maize seeds were treated with bacterial inoculants. Inoculation was performed with a liquid culture of mentioned strains and with their mixture, with the concentration of 10^8 cells per ml. *Azotobacter chroococcum* was prepared in the liquid Fiodor medium, *Bacillus subtilis* in the nutrient agar and *Pseudomonas fluorescens* in the King B medium. No treated seeds were designed as control. The effect of PGPR on seed germination and early growth of maize were evaluated in laboratory and in greenhouse. In laboratory ten seeds were placed in Petri dishes on previously moist filter paper with strerile water. In greenhouse ten seeds were sown at 4 to 5 cm depth of soil in each Mitscherlich pot.

The rhizosphere soil for microbiological analysis was sampled at the three- and six leaf stage of maize. At the two stages in greenhouse as well as after 10 days in laboratory conditions the growth parameters like plant height and plant weight were recorded.

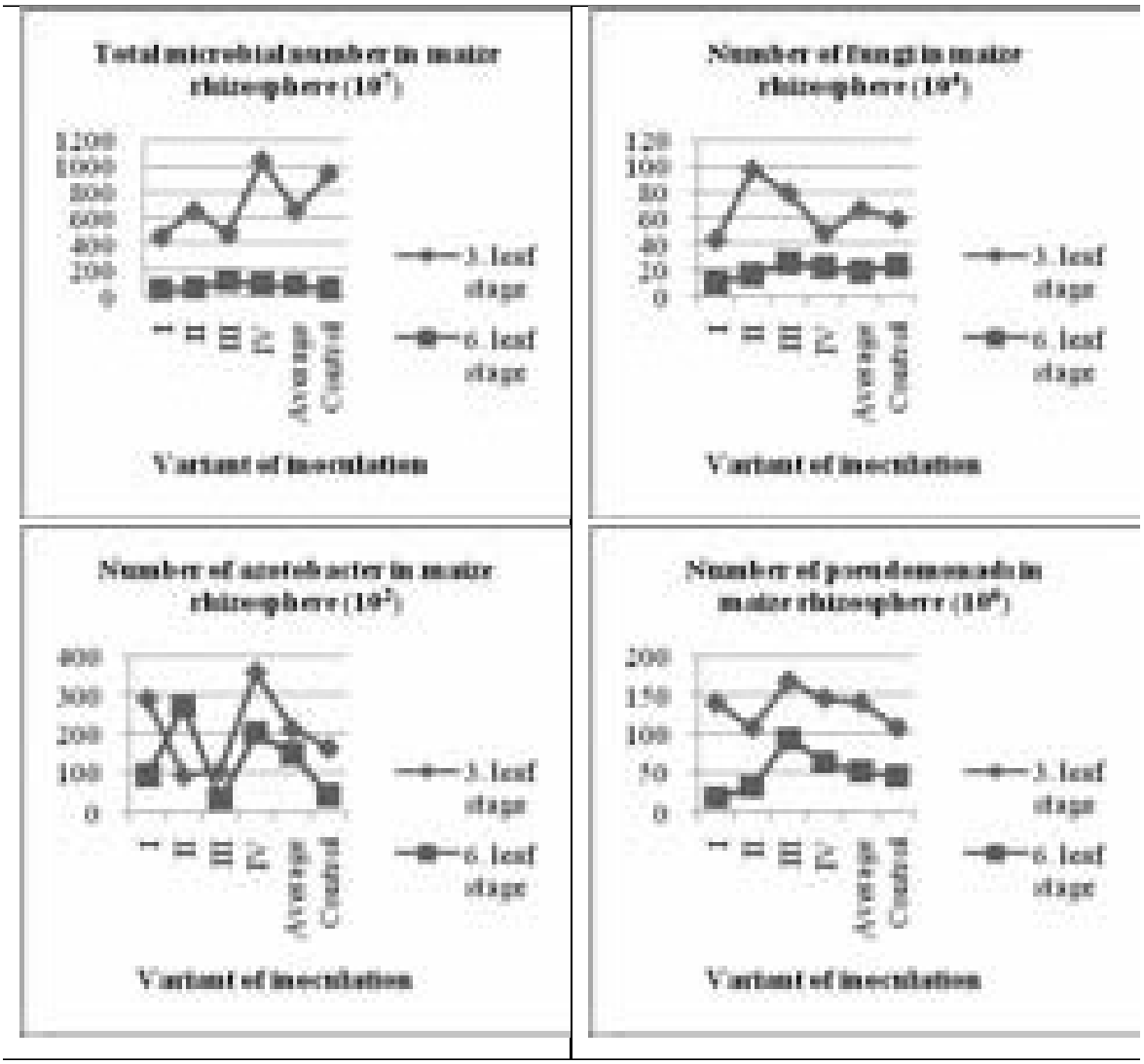
Total number of microorganisms was determined on soil agar (dilution 10^{-7}). Fungi were determined on potato dextrose agar (dilution 10^{-4}), azotobacter on Fiodor substrate (dilution 10^{-2}) (Jarak and Đurić, 2004) and pseudomonads on King B medium (dilution 10^{-6}) (Jošić et al., 2010). Statistical analyses (LSD–test) were performed according Mead et al. (1996).

RESULTS AND DISSCUSION

Effects of the different variants of maize inoculation are presented in Graph 1, Tables 1 and 2. Graph 1 shows the total number of microorganisms, the number of fungi, the number of pseudomonads and the number of azotobacter in maize rhizosphere.

The largest effects of inoculation on the total number of microorganisms, at the three leaf stage, were obtained with mixture of strains (IV) and in the variant with *Pseudomonas fluorescens* (III), at the six leaf stage.

The highest number of fungi was recorded in the variants with *Bacillus subtilis* (II), at the three leaf stage, and with *Pseudomonas fluorescens* (III), at the six leaf stage of maize plants.



Graf. 1. Efekat inokulacije na broj mikroorganizama u rizosferi kukuruza

Graph. 1. Effect of inoculation on the number of microorganisms in maize rhizosphere

The best effect of inoculation on the number of azotobacter, at the three leaf stage, was obtained in the variant with mixture of strains (IV). At the six leaf stage the biggest effect was achieved in the variant with *Bacillus subtilis* (II). At both leaf stages the highest influence on the number of pseudomonads was recorded in the variant with *Pseudomonas fluorescens* (III).

On average for all variants it has been shown that inoculation significantly increased the total microbial number, the number of fungi and azotobacter at the three leaf stage in relation to six leaf stage of maize plants.

The effectiveness of the application of microorganisms in plant production depends on plant species, species of microorganisms, quantities of fertilizers, time and place of sampling (Govedarica, 1986; Cvijanović, 2002). Bacterial stains used in this study had a positive effect on the growth parameters of maize under laboratory conditions as well as in pot experiment. Inoculation was positively affected on the plant height and weight of young maize plants respectively. Results of these studies are presented in Tables 1 and 2.

Tabela 1. Efekat inokulacije na visinu nadzemnog dela kukuruza (cm)
Table 1. Effect of inoculation on the height of maize plants (cm)

Variant of inoculation	Plant height of maize seedlings (cm)	Plant height at the three leaf stage (cm)	Plant height at the six leaf stage (cm)
I	7,36	18,03*	37,68*
II	6,71	17,18	38,64**
III	6,41	18,82**	38,66**
IV	6,41	16,30	39,07**
Average	6,72	17,58	38,51
Control	6,18	15,52	32,89
LSD $_{0.05}$	2.57	2.43	3,71
LSD $_{0.01}$	3.55	3.27	4,92

On average, all three examined strains as well as their mixture (6,72 cm) in relation to the control (6,18 cm) showed an increase in the plant height of maize seedlings. The biggest effect on the plant height under laboratory conditions was obtained in the variant with *Azotobacter chrococcum* (Table 1).

The biggest effect on the height of plants in the pots at the three leaf stage was registrered in the variant with *Pseudomonas fluorescens* (III) and it increase was statistically significant. The greatest effect on the plant height at the six leaf stage was obtained in the variant with mixture of all three strains (IV) (Table 1). On average for the two stage all the investigated variants (17,58 and 38,51 cm) increased plant height in relation to the non-inoculated variants (15,52 and 32,89 cm).

On average, all strains showed a positive influence on the plant weight (0,20 and 0,65 g) of young maize plants in relation to the control (0,15 and 0,48 g) (Table 2). The biggest effect on the plant weight at the two stage was obtained in the variant with all three strains (IV). Inoculation with all applied bacterial inoculants significantly increased the plant weight at the three- and six leaf stage of maize plants.

Tabela 2. Efekat inokulavije na masu suve materije kukuruza (g)
Table 2. Effect of inoculation on the weight of young maize plants (g)

	Variant of inoculation					
Leaf stage	I	II	III	IV	Average	Control
3	0,20**	0,18**	0,19**	0,22**	0,20	0,15
6	0,65**	0,57**	0,58**	0,79**	0,65	0,48
	LSD $_{0.05}$ 0,01 $_{0.01}$ 0,02		LSD $_{0.05}$ 0,02 $_{0.01}$ 0.03			

It has been shown that inoculation with *Azotobacter*, *Bacillus* and *Pseudomonas* strains could increase maize yield, seed germination and seedling growth, root and shoot elongation of maize (Govedarica et al., 2001; Cvijanović et al., 2007; Egamberdiyeva, 2007; Gholami, 2009).

Gholami (2009) examined six bacterial strains PGPR on germination, seedling growth and yield of maize. All bacteria except *Azospirillum lipoferum* increased seed germination up to 18,5% over nontreated control.

Brown (1982) reviewed that use of *Azotobacter spp.* as biofertilizers and concluded that inoculation with these microorganisms occasionally promote growth by mechanism other than biological N fixation. Lalande et al. (1989) suggested that PGPR stimulate plant growth with production of phytohormone. Similarly, Dey et al. (2004); Kloepper et al. (1988) suggested that PGPR enhance the growth and seed emergence. Shaukat et al. (2006) reported that *Azotobacter sp.* inoculation increased the wheat seed germination percentage to about 58,6%. He also reported that *Azotobacter sp.* inoculation significantly enhanced the plant height.

CONCLUSION

The number of the investigated microbial groups depended on the applied bacterial inoculants and the leaf stage of maize plants. On average for all variants it has been shown that inoculation significantly increased the total microbial number, the number of fungi and azotobacter at the three leaf stage in relation to six leaf stage of maize plants.

On average, all three examined strains showed an increase in the height and weight of maize plants in relation to the control. The biggest effect on the plant weight was obtained in the variant with mixture of all three strains.

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EFEKAT RIZOBAKTERIJA (PGPR) NA POČETNI RAST KUKURUZA I BROJNOST MIKROORGANIZAMA U RIZOSFERI

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Izvod

Cilj ovih istraživanja bio je da se odredi brojnost mikroorganizama u rizosferi kukuruza u zavisnosti od primenjenih bakterijskih inokulanata i faze rasta kukuruza kao i da se ispita efekat rizobakterija - promotora biljnog rasta (PGPR) na početni rast kukuruza. Kao inokulanti korišćene su tri vrste bakterija: *Azotobacter chroococcum*, *Bacillus subtilis* i *Pseudomonas fluorescens* primenjene pojedinačno i u smeši. U istraživanjima je korišćen jedan hibrid kukuruza: NS 6010 stvoren u Institutu za ratarstvo i povrtarstvo u Novom Sadu. Ogledi su postavljeni u laboratoriji i u žičari. Broj ispitivanih grupa mikroorganizama zavisio je od primenjenih bakterijskih inokulanata i faze rasta kukuruza. Sojevi bakterija ispoljili su statistički značajan uticaj na brojnost mikroorganizama u rizosferi kukuruza i imali su pozitivan efekat na rast biljaka kukuruza. Inokulacijom je povećana visina i masa biljaka kukuruza.

Ključne reči: Azotobacter, Bacillus, maize, PGPR, Pseudomonas.

Received / *Primljen*: 11.10.2010.
Accepted / *Prihvaćen*: 23.11.2010.