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Effects of bacterial seed inoculation on microbiological soil status and maize grain yield

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Abstract

Rhizosphere microorganisms are essential for plant growth and development. Many factors, including environmental conditions, genotype, seed inoculation with plant growth promoting rhizobacteria (PGPR) and plant growth stages, influence rhizosphere microbiology. In this study, the dynamics of nitrogen and microbiological soil status at different growth stages of two maize hybrids, as well as their grain yield were analyzed, under the influence of seed inoculation with inoculant containing *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* on during three experimental years. Higher nitrogen amount (NA), total number of microorganisms (TNM), number of azotobacters (NAZ), number of aminoheterotrophs (NAM), total number of sporogenic bacteria (NS), number of actinomycetes (NAC), number of oligotrophic (NO) and grain yield, as well as lower number of fungi (NF) were obtained under favorable environmental conditions. The hybrid NS 6010 had higher values of NA, TNM, NAZ, NAM, NO, and grain yield than hybrid Dijamant 6.

What is more, higher NA, TNM, NAZ, NAM, NO, and grain yield were obtained under inoculation treatment, demonstrating that the microorganisms improve maize production and microbial abundance in the rhizosphere. The highest TNM, NAM, NF and NO were determined at stage 6-7 leaves, while the highest NA and NAC were at silking stage and NAZ at wax ripeness stage. Accordingly, hybrid choice and seed inoculation could serve as a good management practice for an increase in maize grain yield, and improved microbial population and nitrogen amount in the rhizosphere.

Introduction

The mineral fertilizers use in maize crop is essential for achieving high yields. However, the unreasonable application of nitrogen fertilizers has a negative environmental impact via volatile and leaching losses. The reduction in the N fertilizer amounts is possible through including nitrogen-fixing bacteria. It is estimated that the biological nitrogen fixation supply 65% of N in agricultural systems (Matiru and Dakora 2004), which is about 50-70 million tones N year (Herridge et al., 2008). Biofertilizers contain plant growth promoting rhizobacteria (PGPR) that convert atmospheric nitrogen (N2) to ammonium ions (NH4+), which can be readily used by plants. The bacteria of the genus *Azotobacter* sp. and *Bacillus* sp. applied alone or in mixture with other PGPR strains, expressed a positive influence on phosphorus solubilization (Ahemad and Kibret, 2014). Also, PGPR produces various regulatory chemicals, antibacterial, and antifungal compounds that could improve plant growth and development (Wani et al., 2013). Moreira et al. (2010) reported that nitrogen fixation by PGPR in maize, wheat, and rice ranged from 25 to 50 kg ha^{-1} year^{-1}, which is equivalent to the approximately 17% of average N requirements.

On the other hand, Odoh (2017) noted that the seed inoculation with PGPR promotes the ecological balance, which positively reflects on the production and food safety. Many types of research showed that the inoculation of the seeds with PGPR increases soil fertility and maize yield. Thus, Mandić (2011) concluded that seed inoculation with *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* increases microbial populations in the soil, soil fertility, and grain yield and also reduces the total amount of applied mineral fertilizers thus reducing maize production costs. Similar results were verified by Jarak et al. (2011), who found that seeds inoculation with *Azotobacter chroococcum* increases grain yield, total bacterial number, and the number of azotobacter in the maize rhizosphere. Mandic et al. (2016) found that maize inoculation with biofertilizer increases the available nitrogen content and number of microorganisms, presumably azotobacter and aminoheterotrophs in the soil. Inoculation treatment of seeds with PGPR increased significantly nitrogen content in the soil, germination, grain yield, and its quality (Gholami et al., 2009). Grain yield could be increased by 15 to 35% when maize seeds were inoculated with Azotobacter (Baral and Adhikari, 2013). These authors indicate that Azotobacter fixes about 20 kg N ha^{-1} $year^{-1}$ and that their products such as thiamin, riboflavin, indoleacetic acid, and gibberellins improve seed germination and control plant diseases. Mrkovački et al. (2016) pointed out that maize inoculation with rhizobacteria increases the total number of microorganisms, as well as several Azotobacter and free-living nitrogen-fixing bacteria. What is more, Rojas-Tapias et al. (2012) reported that PGPR inoculation increases the stress resistance and Sharifi (2011) found that inoculation with different *Azotobacter* and *Azospirillum* strains significantly increases the dry matter accumulation and maize grain yield. This research aimed to examine the response of two maize hybrids to seed inoculation with PGPR mixture, through measurement of grain yield, nitrogen dynamics, and microbiological status in the rhizosphere during different growth stages.

Materials and Methods

Plant material and experimental field

The commercial maize hybrids NS 6010 and Dijamant 6 of FAO 600 maturity group were tested. The field experiment was conducted under dry land farming at experimental station Putinci in Serbia (44° 59’ N Lat., 19° 58' E Long.) during the 2006-2008 period.

A four-level factorial experiment was set, with growing season as the first factor, hybrid as the second factor, seed inoculation as the third factor (control - non inoculation and inoculation) and maize growth phases as the fourth factor (time sampling was provided three times at the 6-7 leaves, silking and wax ripeness stage). Inoculation was carried out with a bacterial mixture of the following titre (CFU ml^{-1}): *Azotobacter chroococum* - 10^8, *Azotobacter vinelandii* - 10^8, *Bacillus megaterium* - 10^9 and *Bacillus licheniformis* - 10^9. The experimental design was a randomized block design with four replications and subplot area with 16.8 m^2 (2.8m x 6m). In all three years, the preceding crop was winter wheat, and maize was sown in the first decade of April with a density of 59.523 plants ha^{-1}. A mixture of bacteria was applied at a rate of 20 g per 100 g seeds immediately before sowing. A standard cultivation practice was applied. NPK fertilizer (10:30:20) was applied in autumn at a rate of 300 kg ha^{-1} and KAN (27% N) in spring, at a rate of 90 kg ha^{-1}.

Soil characteristics and meteorological conditions

The soil in the experimental field is calcareous chernozem type (Table 1). The soil pH was neutral to slightly alkaline, high in carbonate, low in humus, with medium P and high K content. Total nitrogen was high in the first year and medium in the other two research years.

Climate diagram according to Walter and Lieth (1967) showed that in the first year was moderate in rainfall level, while April and July in the second year and August in the third year were the driest months.

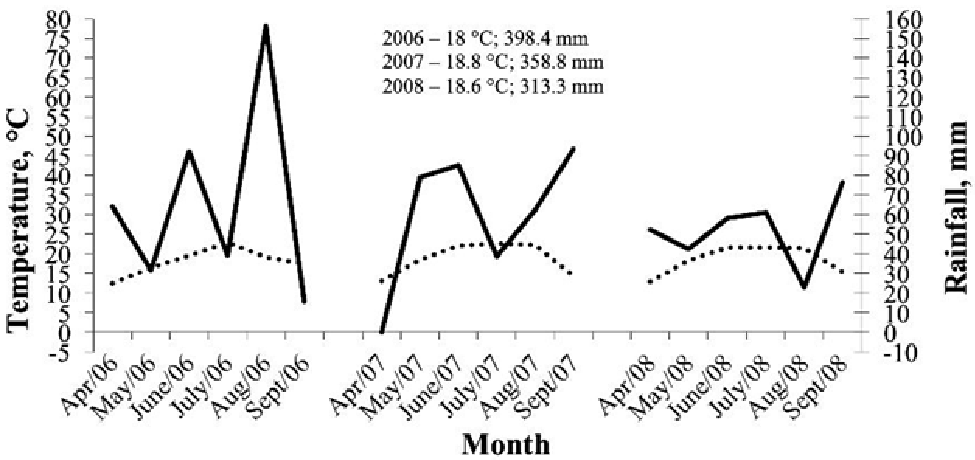

Figure 1 Climate diagram, according to Walter and Lieth (1967) for the experimental site.

Data Collection

Rhizosphere samples were taken three times over the maize vegetation period. The total number of microorganisms (TNM) was obtained on soil-extract agar medium according to Hamaki et al. (2005), the number of azotobacters (NAZ) by fertile drops method on Fyodorov's medium (Anderson, 1965), the number of aminoheterotrophs (NAM) on meat-peptone agar according to Stamenov et al. (2012), the number of sporogenic bacteria was determined by heating the inoculum at 80°C for 10 min and then seeding on meat-peptone agar, while the number of fungi was determined on Czapek-Dox medium (Shalau Microbiology, 2000), the number of actinomycetes was determined on a synthetic Waksman-Carey agar (Shalau Microbiology, 2000) and the number of oligotrophs on medium without nitrogen developed by Fjodorov (Anderson and Domasch, 1958). The total number of microorganisms, aminoheterotrophs, sporogenic bacteria and oligotrophic were calculated as 10^5, azotobacter as 10^2, and fungi and actinomycetes as 10^4 number per g of absolutely dry soil and presented as CFU g^{-1} soil.

Statistical analysis

Maize was harvested manually in October. Grain yield was calculated on a 14% moisture basis. Data were processed by ANOVA using Statistica version 10, an RCBD, and Duncan's Multiple Range Test was used to compare differences among treatment means ($P \le 0.05$).

Results and Discussion

The effect of climatic conditions on microbial status and maize growth parameters

The present climatic conditions significantly affected the microbial status and grain yield (Table 2). The highest NA values (22.41 kg ha^{-1}), TNM (169.12 x 10^5 g^{-1} soil), NAZ (153.17 x 10^2 g^{-1} soil), NAM (102.58 x 10^5 g^{-1} soil), NS (25.26 x 10^5 g^{-1} soil), NAC (329.51 x 10^4 g^{-1} soil), NO (153.15 x 10^5 g^{-1} soil) and grain yield (14.20 t ha^{-1}), as well as the lowest NF (4.32 x 10^4 g^{-1} soil) were recorded in first year of investigation under the favorable environmental conditions. The favorable weather and soil conditions sustain high rates of the microbial population growth. Numerous and diverse soil microorganisms improve soil mineralization, contributing to better plant nutrition and performance (Jacoby et al., 2016). According to Mandic et al. (2016), soil water deficiency hurts the process of mineralization, thus reducing the nitrogen fixation in the maize rhizosphere.

Consequently, the optimal rainfall amount and distribution, in the first year, influenced greater bacteria reproduction, increasing mineralization process, and nitrogen fixation. This resulted in a substantially increased amount of nitrogen when compared to the other two years. The number of the investigated microorganisms groups was high in this research, and typical for fertile soils such as chernozem. As it was expected, the highest grain yield was recorded in the first experimental year. Climate diagram based on Walter and Lieth (1967) showed the absence of the arid period during the vegetation period of 2006. The lower grain yield was recorded in the second experimental year when the arid period was present in April and July, and the lowest in the third experimental year when the arid period was noticed in August. In July, maize develops reproductive organs. Reid et al. (2014) found that water stress in July causes delay in the anthesis-silking interval and estimates that the delay in this interval for 0.3, 0.6 and 0.9 days caused reduction from 5 to 36% in grain yield. In our study, the rainfall deficiency in July has not affected flowering and pollination time. Examined hybrids have a shorter anthesis-silking interval and higher grain weight (data not showed) and grain yield due to favorable weather conditions in August. Contrary, in August in the third experimental year, high temperatures and lower rainfall present during grain filling process resulted in shortening of this phase and reduction of weight of grain per ear and 1000-grain weight (data not showed) and hence grain yield.

The effect of maize genotype and seed inoculation on soil biogenicity

We demonstrated that different types of microbes efficiently colonized the roots of both maize hybrids. However, results showed that the soil biogenicity differed per genotype. In the rhizosphere of NS 6010 significantly higher NA (17.99 kg ha^{-1}), TNM (140.87 x

Table 1 Chemical soil properties of the experimental site

Parameters	pH (H_2O)	pH (KCl)	$CaCO_3$, %	Humus, %	Nitrogen, %	mg 100 g^{-1}	
						P2O5,	K2O,
First year	7.38	7.16	16.80	3.61	0.2342	17.18	28.20
Second year	7.67	7.46	10.08	2.79	0.1951	21.86	22.20
Third year	7.52	6.89	15.90	2.58	0.1706	16.37	21.40

Table 2 Effects of year, hybrid, seed inoculation, and phenophase on rhizosphere soil microbes and grain yield.

Factor[2/]	NA[1/]	TNM	NAZ	NAM	NS	NF	NAC	NO	GY
	Year effects (A)								
2006	22.41 a	169.12 a	153.17 a	102.58 a	25.26 a	4.32 c	329.51 a	153.15 a	14.20 a
2007	17.19 b	108.40 b	95.97 b	85.76 c	15.58 c	6.05 b	197.72 c	93.56 b	12.81 b
2008	13.78 c	106.36 c	61.49 c	93.30 b	21.92 b	11.14 a	256.45 b	140.54 a	10.85 c
F test	**	**	**	**	**	**	**	**	**
	Hybrid effects (B)								
NS 6010	17.99 a	140.87 a	107.51 a	98.03 a	21.88	7.52	269.34	135.32 a	13.08 a
Dijamant6	17.60 b	115.05 b	99.58 b	89.74 b	19.96	6.81	253.11	122.85 b	12.16 b
F test	**	**	**	**	ns	ns	ns	*	*
	Inoculation seed effects (C): Co - Control; SI - Seed inoculation								
Co	11.09 b	105.64 b	99.17 b	86.10 b	21.30	8.84 a	264.05	116.65 b	11.57 b
SI	24.50 a	150.27 a	107.92 a	101.67 a	20.54	5.49 b	258.40	141.52 a	13.67 a
F test	**	**	**	**	ns	**	ns	**	**
	Maize phenophase effects (D): TS1 - Stage 6-7 leaves; TS2 – silking stage; TS3 - wax ripeness								
TS1	18.15 b	140.44 a	97.96 b	110.62 a	21.55	8.60 a	174.33 c	152.36 a	-
TS2	18.54 a	114.37 c	92.74 c	84.55 c	20.94	6.85 b	319.10 a	121.19 b	-
TS3	16.69 c	129.06 b	119.94 a	86.48 b	20.28	6.06 b	290.24 b	113.71 b	-
F test	**	**	**	**	ns	**	**	**	-
M	17.80	127.96	103.55	93.88	20.92	7.17	261.23	129.09	12.89

[1/] nitrogen amount - NA (kg ha^{-1}), total number microorganisms - TNM (10^5 g^{-1} soil), number of azotobacters - NAZ (10^2 g^{-1} soil), number of aminoheterotrophs - NAM (10^5 g^{-1} soil), total number of sporogenic bacteria - NS (10^5 g^{-1} soil), number of fungi - NF (10^4 g^{-1} soil), number of actinomycetes - NAC (10^4 g^{-1} soil), number of oligotrophic - NO (10^5 g^{-1} soil); grain yield - GY (t ha^{-1}).

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

10^5 g^{-1} soil), NAZ (107.51 x 10^2 g^{-1} soil), NAM (98.03 x 10^5 g^{-1} soil) and NO (135.32 x 10^5 g^{-1} soil) were observed, together with higher grain yield (13.08 t ha^{-1}), when compared to hybrid Dijamant 6 (17.60 kg ha^{-1}, 115.05 x 10^5 g^{-1} soil, 99.58 x 10^2 g^{-1} soil, 89.74 x 10^5 g^{-1} soil, 122.85 x 10^5 g^{-1} soil and 12.16 t ha^{-1}, respectively). Our results confirm previously reported studies that maize genotype expresses diverse effects on numbers of different groups of microorganisms (Frąc et al., 2012; Obid et al., 2016; Gałązka et al., 2017; Walters et al., 2018). Plants can alter soil microbiota in the rhizosphere by secreting root exudates, which is under host-genetic control (Bulgarelli et al., 2013). Thus we can assume that the metabolites secreted by root of hybrid NS 6010 promoted the reproduction of microorganisms resulting in the increased nitrogen absorption and production of higher grain yield. Also, this hybrid left a larger residue of available nitrogen in the soil. This indicated that the majority of this nitrogen could serve later for the next crop in the rotation. Also, the studies of Mandić (2011) and Mrkovački et al. (2012) suggested that higher amount of microorganisms in the soil implies greater microbial activity, organic matter mineralization and the creation release of nutrients in relatively larger amounts available to the plants.

Values of NA, TNM, NAZ, NAM, NO, and grain yield were significantly higher while NF was lower in treatment with seed inoculation compared to the control. After inoculation, microorganisms reproduced quickly in the rhizosphere, intensifying mineralization of the organically-bound nutrients and promoted their availability for plants. The maize seed inoculation with diazotrophic bacteria enhances grain yield (Hungria et al., 2010; Mehnaz et al., 2010) since nitrogenase enzyme of N2-fixing bacteria converges atmospheric N2 into ammonia (Santi et al., 2013). Earlier research of García et al. (1996) showed that the biologically fixed N represents about 1/3 of the total plant N content. Cvijanović et al. (2007) concluded that the seed inoculation with a mixture of *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Azospirillum lipoferum*, *Bacillus megatherium*, and *Bacillus subtilis* increased the number of microorganisms, particularly azotobacter and phosphorus-cycle bacteria, as well as maize grain yield. Also, maize seed inoculation with biofertilizers containing *Azotobacter* and *Azospirillum* increased grain yield when compared to control (Naserirad et al., 2011). Hajnal-Jafari (2010) find that seed inoculation increases the number of nitrogen-fixing bacteria in the rhizosphere and maize grain yield.

this point, the interactions between studied factors were highly significant for TNM, NAZ, and NAM.

The interaction of year and hybrid expressed significant effect on TNM, NAZ, NAM, NS, NF, NAC,

Table 3 Interactions between year (factor A), hybrid (factor B), seed inoculation (factor C) and maize phenophase (factor D) on rhizosphere soil microbes, and grain yield.

Factor	NA1/	TNM	NAZ	NAM	NS	NF	NAC	NO	GY
A × B	ns	**	**	**	**	**	**	**	ns
A × C	**	**	**	**	ns	**	**	**	ns
A × D	**	**	**	**	**	**	**	**	-
B × C	**	**	**	**	ns	ns	**	**	ns
B × D	**	**	**	**	ns	**	**	**	-
C × D	**	**	**	**	**	**	ns	ns	-
A × B × C	**	**	**	**	ns	ns	*	ns	ns
A × B × D	**	**	**	**	ns	**	**	ns	-
A × C × D	**	**	**	**	**	**	**	**	-
B × C × D	**	**	**	**	ns	*	**	ns	-
A×B×C×D	**	**	**	**	**	*	**	**	-

n1/ nitrogen amount - NA total number microorganisms - TNM number of azotobacters - NAZ number of aminoheterotrophs - NAM total number of sporogenic bacteria - NS number of fungi - NF number of actinomycetes - NAC number of oligotrophic - NO grain yield - GY.

*, ** significant at the 0.05 and 0.01 probability levels, respectively; ns – non-significant.

Variations in soil biogenicity during different maize phenophases

The NA, TNM, NAZ, NAM, NF, NAC, and NO varied significantly at different phenophases. The highest NA and NAC were in the silking stage, TNM, NAM, NF and NO in the stage 6-7 leaves and NAZ in wax ripeness stage. In maize hybrids, approximately 60% of the total N uptake and 40% of the total dry matter accumulation occurred at the silking stage, while 40% of total N uptake was between V7 and VT (Holland and Schepers, 2010). Therefore, N management programs should correspond with the seed inoculation to ensure proper N availability during the later stages of grain filling. In maize, the most significant N uptake occurs at the silking growth stage, when the nitrogen amount in the plants was highest. This can be explained by the mutual effect of fertilizer and increased number of microorganisms in the soil that resulted in better exploitation of biological nitrogen and the present amount of mineral nitrogen in the soil. Moreover, Broeckling et al. (2008) and Mandic et al. (2016) concluded that the composition of rhizosphere microbial communities strongly dependent on plant genotype and developmental stage

Interactions between climatic conditions, maize hybrid and phenophase on soil biogenicity and grain yield

The interactions between examined factors were significant for microbial status in the rhizosphere. From

and NO. The interaction of year and seed inoculation had a significant effect on NA, TNM, NAZ, NAM, NF, NAC, and NO. The interaction of year and maize phenophase had a significant effect on NA, TNM, NAZ, NAM, NS, NF, NAC, and NO. The interaction of hybrid and seed inoculation also expressed the highly significant effect on NA, TNM, NAZ, NAM, NAC, and NO. The interaction of hybrid and phenophase had a highly significant effect on NA, TNM, NAZ, NAM, NF, NAC, and NO. The interaction of seed inoculation and phenophase had a highly significant effect on NA, TNM, NAZ, NAM, NS, and NF. The interaction of year, hybrid, and seed inoculation had a significant effect on NA, TNM, NAZ, NAM, and NAC. The interaction of year, hybrid, and maize phenophase had a highly significant effect on NA, TNM, NAZ, NAM, NF, and NAC. The interaction of year, seed inoculation, and phenophase expressed a highly significant effect on NA, TNM, NAZ, NAM, NS, NF, NAC, and NO. The interaction of hybrid, seed inoculation, and phenophase had a significant effect on NA, TNM, NAZ, NAM, NF, and NAC. Also, the interaction of year, hybrid, seed inoculation, and phenophase had a significant effect on NA, TNM, NAZ, NAM, NS, NF, NAC, and NO.

It is evident that the microbial number and their diversity in the rhizosphere dependent on the climatic factors, hybrids, and phenophase. The climatic conditions affect the microbial population in the rhizosphere. The lower temperature and higher water availability influenced the higher number of microorganisms. This study provides evidence that PGPR inoculation of maize seeds improves the soil fertility because it increases the content of available nitrogen in the soil. This measure has improved soil microbiota due to the increased number of beneficial microorganisms for the plants and decreased number of the fungi.

What is more, treatment with PGPR inoculation was reflected positively on the grain yield increase. The results demonstrated that the inoculation of the seeds with beneficial bacteria could also be a useful tool for maize production improvement. Besides, the farmers who would use this practice may also realize savings, by reducing the amount of nitrogen fertilizer for the next crop in rotation. Accordingly, seed inoculation should be incorporated into regular agricultural practices.

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References

Ahemad M, Kibret M, 2014. Mechanisms and applications of plant growth promoting rhizobacteria: a current perspective. J King Saud Univ – Sci 26:1-20.

Anderson GR, 1965. Ecology of Azotobacter in the soil of the Palouse region, I: Occurrence. Soil Sci 86:57-65.

Anderson JPE, Domasch KH, 1958. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biol Biochem 10:215-221.

Baral BR, Adhikari P, Parbati A, 2013. Effect of Azotobacter on growth and yield of maize. SAARC J Agric 11:141-147.

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM, 2008. Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738.

Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P, 2013. Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807-838.

Cvijanović G, Milošević N, Jarak M, 2007. The importance of diazotrophs as biofertilizers in the maize and soybean production. Genetika, 39, 395-404.

Frąc M, Oszust K, Lipiec J, 2012. Community-level physiological profiles (CLPP), characterization, and microbial activity of soil amended with dairy sewage sludge. Sensors 12:3253-3268.

García de Salamone IE, Döbereiner J, 1996. Maize genotypes effects on the response to *Azospirillum* inoculation. Biol Fert Soils 21:193-196.

Gałązka A, Gawryjołek K, Grządziel J, Frąc M, Księżak J, 2017. Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques. Plant Soil Environ 63: 264-270.

Gholami A, Shahsavani S, Nezarat S, 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth, and yield of maize. Intern J Biol Life Sci 5:35-40.

Hajnal-Jafari T, 2010. The effect of inoculation on the maize yield and microbial activity in the soil (In Serbian). (Doctoral dissertation), Faculty of Agriculture, University of Novi Sad, Serbia.

Hamaki T, Suzuki M, Fudou R, Jojima Y, Kajiura T, Tabuchi A, Sen K, Shibai H. 2005. Isolation of novel bacteria and Actinomycetes using soil-extract agar medium. J Biosci Bioeng 99:485-492.

Herridge DF, Peoples MB, Boddey RM, 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1-18.

Holland KH, Schepers JS, 2010. Derivation of a variable rate nitrogen application model for in-season fertilization of corn. Agron J 102:1415-1424.

Hungria M, Campo RJ, Souza EM, Pedrosa FO, 2010. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331:413-425.

Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S, 2017. The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. Front Plant Sci 8:1617.

Jarak M, Jelicic Z, Kuzevski J., Mrkovacki N, Djuric S. 2011. The use of Azotobacter in maize production: the effect on the microbiological activity of the soil, early plant growth, and grain yield. Contemp Agri 60:80-85.

Mandić V, 2011. The genotypic response of stay green maize hybrids on increased crop density. (Doctoral dissertation), Faculty of Agriculture, University of Belgrade, Serbia.

Mandic V, Dordevic S, Stanojevic D, Bijelic Z, Krnjaja V, Tomic Z, Dragicevic V, 2016. Effect of bacterial seed inoculation on nitrogen dynamics, the number of bacteria in soil under maize, and maize yield. J Environ Prot Ecol 17:1003-1010.

Matiru VN, Dakora FD, 2004. The potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. Afr J Biotechnol 3:1-7.

Mehnaz S, Kowalik T, Reynolds B, Lazarovits G, 2010. Growth promoting effects of corn (*Zea mays*) bacterial isolates under greenhouse and field conditions. Soil Biol Biochem 42:1848-1856.

Odoh CK, 2017. Plant growth promoting rhizobacteria (PGPR): A bioprotectant bioinoculant for sustainable agrobiology. Intern J Adv Res Biol Sci 4:123-142.

Mrkovački N, Jarak M, Đalović I, Jocković Đ, 2012. Importance of PGPR application and its effect on microbial activity in the maize rhizosphere. Ratar Povrt / Field Veg Crop Res 49: 335-344.

Mrkovački, N., Đalović, I., Jošić, D., Bjelić, D., & Brdar-Jokanović, M. (2016). The effect of PGPR strains on the microbial abundance in maize rhizosphere in field conditions. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research, 53, 15-19.

Naserirad H, Soleymanifard A, Naseri R, 2011. Effect of integrated application of bio-fertilizer on grain yield, yield components, and associated traits of maize cultivars. Am Eur J Agric Environ Sci 10:271-277.

Obid SA, Idris AE, Ahmed BE, Abdelgadir M, 2016. Effect of bio-fertilizer on growth and yield of two maize (*Zea mays* L.) cultivars at shambat, Sudan scholars. J Agric Vet Sci 3: 313-317.

Reid A, Gonzalez V, Sikkema PH, Lee EA, Lukens L, Swanton CJ, 2014. Delaying weed control lengthens the anthesis-silking interval in maize. Weed Sci 62:326-337.

Rojas-Tapias D, Moreno-Galv́an A, Pardo-Dıaz S, Obando M, Rivera D, Bonilla R, 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). App Soil Ecol 61:264-272.

Santi C, Bogusz D, Franche C, 2013. Biological nitrogen fixation in non-legume plants. Ann Bot 111:743-767.

Sharifi RS, Khavazi K, Gholipouri A, 2011. Effect of seed priming with plant growth promoting rhizobacteria (PGPR) on dry matter accumulation and yield of maize (*Zea mays* L.) hybrids. J Food Agric Environ 9:393-397.

Moreira FMS, Silva K, Nóbrega RSA, Carvalho F, 2010. Diazotrophic associative bacteria: diversity, ecology and potential applications. Computat Sci 1:74-99.

Shalau Microbiology, (2000). Handbook of microbiological culture media. 87.

Stamenov D, Jarak M, Đurić S, Hajnal-Jafari T, Anđelković S, 2012. The effect of Azotobacter and Actinomycetes on the growth of English ryegrass and microbiological activity in its rhizosphere. Res J Agric Sci 44:93-99.

Walter H, Lieth H, 1967. Klimadiagram-Weltatlas. VEB Gustav Fischer Verlag, Jena, Germany.

Walters WA, Jin Z, Youngblut N, Wallace JG, Sutter J, Zhang W, González-Peña A, Peiffer J, Koren O, Shi Q, Knigh, R, Glavina Del Rio T, Tringe SG, Buckler ES, Dangl JL, Ley RE, 2018. A large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proc Nat Acad Sci 115:7368-7373.

Wani SA, Chand S, Ali T, 2013. The potential use of *Azotobacter chroococcum* in crop production: an overview. Curr Agric Res 1:35-38.