Editura AcademicPres

Available online: www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2017, 9(2):274-279. DOI: 10.15835/nsb9210081

Notulae Scientia Biologicae

Original Article

The Enhancement of Soybean Growth and Yield in a Field Trial through Introduction of Mixtures of *Bradyrhizobium japonicum*, *Bacillus* sp. and *Pseudomonas chlororaphis*

Renata M. ILIČIĆ[1], Radmila N. PIVIĆ[2], Zoran S. DINIĆ[2], Dragana S. LATKOVIĆ[1], Slobodan A. VLAJIĆ[1], Dragana Lj. JOŠIĆ[2]*

[1]*University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, Novi Sad, Serbia; renatailicic@gmail.com, dragana@polj.uns.ac.rs, svlajic89@gmail.com*

[2]*Institute of Soil Science, Teodora Drajzera 7, Belgrade, Serbia; drradmila@pivic.com, dinicszoran@gmail.com, dragana.josic@yahoo.com (*corresponding author)*

Abstract

The effects of plant growth promoting (PGP) bacteria on soybean growth and yield were tested in field conditions using four treatments: (A) *Bradyrhizobium japonicum* strain 526, combined with cell-free supernatants (CFS) of *Bacillus* sp. strain Q10 and *Pseudomonas chlororaphis* strain Q16; (B) *B. japonicum* 526 + *Bacillus* sp. Q10; (C) *B. japonicum* 526 + *P. chlororaphis* Q16 and (D) commercial fertilizer containing *B. japonicum*, which served as a control. The average values of dry weight per nodule and shoot dry weight had the maximum values in the B treatment. In dry shoots collected at the flowering stage, nitrogen and carbon content was similar across all treatments, while that of sulphur decreased in treatment A. Relative to the control (D), all treatments showed positive effects on pods number and grain mass per plant, with the best results yielded by treatment A. Nitrogen and sulphur content in grain were significantly higher in treatment C, whereas maximum carbon content was measured in treatment B. In the control, it was obtained the yield of 4,000 kg/ha^{-1}, which was in accordance with data reported by the seed producer for the same growing conditions (the maximum value). The yields of 4,229, as well as 4,286 and 4,400 kg ha^{-1} were measured for variants C, B and A, respectively, which were statistically significant higher (5.73 to 10%) than the commercial fertilizer (D). The improvement in soybean growth and yield in the field trial achieved by addition of PGP strains *Bacillus* sp. Q10 and *P. chlororaphis* Q16 to *B. japonicum* 526 can result in more productive agricultural practices.

Keywords: co-inoculation, humogley, plant growth promoting rhizobacteria, soybean

Introduction

Plant Growth Promoting Rhizobacteria (PGPR) increase plant growth under some conditions and have beneficial effects on plants. PGPR are a very small portion of rhizobacteria (2-5%) (Antoun, 2013). They affect plant metabolism (by fixing atmospheric nitrogen, solubilizing phosphorus and iron, and producing plant hormones), improve the plant tolerance to stress and prevent the deleterious effects of phytopathogenic microorganisms, thus increasing nutrient availability in the rhizosphere, as well as positively influencing root growth and morphology, and promoting other beneficial plant-microbe symbioses (Vessey 2003; Lugtenberg and Kamilova, 2009; Solomon *et al.*, 2012). Direct plant growth-promoting rhizobacteria enhance plant growth in the absence of pathogens. PGPR include representatives of the following genera: *Acinetobacter, Agrobacterium, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, Rhizobium, Serratia,* and *Thiobacillus*. However, *Pseudomonas* and *Bacillus* species, as well as *Streptomyces* species, are the bacteria most often found in the rhizosphere of many leguminous and nonleguminous crops (Bouizgarne, 2013). In recent years, interest in the use of PGPR to promote plant growth has increased. Bacteria that belong to *Pseudomonas* and *Bacillus* genera can stimulate plant growth and provide protection against pathogens. Mechanisms involved in *Bacillus* eliciting plant growth promotion include auxin production, increased phosphorus uptake availability, biocontrol abilities and induction of systemic resistance (Idris *et al.*, 2004; Bouizgarne, 2013). Lugtenberg and Kamilova (2009) and Figueiredo *et al.* (2010) stated that PGPR promote plant growth because they can reduce damage caused by pathogens and therefore act as biopesticides.

Received: 27 Apr 2017. Received in revised form: 07 Jun 2017. Accepted: 15 Jun 2017. Published online: 30 Jun 2017.

In the production of leguminous crops, it is necessary to apply the appropriate rhizobial culture for elemental atmospheric nitrogen fixation (Solomon *et al*., 2012). *Bradyrhizobium* is capable of forming root nodules on leguminous crops, as well as fixing atmospheric nitrogen, reducing it to ammonia. In the case of soybean (*Glycine max* L.), it is important to inoculate seeds with relevant strains of *Bradyrhizobium japonicum*. According to Figueiredo *et al*. (2010), co-inoculation studies with PGPR and rhizobia have shown increased plant nodulation and N fixation.

Although bacteria from genera *Pseudomonas* and *Bacillus* exert a positive influence on the growth of cultivated plants and *Bradyrhizobium* is important for nitrogen fixation, the aim of the present investigation was to assess the enhancement of soybean growth and yield in the field achieved by addition of *Bacillus* sp. Q10 and *P. chlororaphis* Q16 to *B. japonicum* 526.

Materials and Methods

Soil properties

The field experiment was conducted in 2014 in the field Vajska (lat. 45° 24' N, long. 19° 06' E) AP Vojvodina, Serbia, on soybean cv. Angela. Previously, the fields were used for maize cultivation (*Zea mays* L.). The present study was performed on soil type Humogley (WRB, 2014).

Soil samples were obtained from the surface soil layer (0–0.3 m) in a disturbed condition. Composite soil samples were carried to the laboratory, dried, and passed through a 2 mm sieve (SRPS ISO 11464: 2004). Particle size distribution was determined by sieving and sedimentation (ISO 11277: 2009). pH in water and 1M KCl was analyzed potentiometrically using glass electrode (SRPS ISO 10390: 2007). Carbonate content was determined using Schiebler's calcimeter (SRPS ISO 10693: 2005), while total N was determined by employing elemental CNS analyzer Vario EL III (Nelson and Sommers, 1996). The humus content (SOM) was determined on the basis of total N content. Available P_2O_5 and K_2O were analyzed by AL method according to Egner-Riehm (Riehm, 1958), using 0.1 M lactate (pH = 3.7) as an extract. After the extraction, K was determined by flame emission photometry, and P by spectrophotometer after color development with ammonium-molybdate and $SnCl_2$.

As can be seen in the Table 1, according to the soil texture, the soil used belonged to sandy clay loam soil. The results show that the soil is medium heavy in terms of its mechanical properties, with total technical and colloidal clay fraction of about 51.7% and 31%, respectively. In this table the main fertility parameters of the studied soil were presented. The soil was weakly acidic in reaction (pH in 1M KCl = 4.98), and has high humus (SOM) content and low content of available phosphorus and potassium.

Meteorological condition during soybean growing season 2014 in the area western Serbia is shown in Table 2.

Bacterial strains and growth conditions

Bacterial strains used in the present investigation were already tested for PGP activities in earlier studies. The indigenous *Bacillus* sp. strain Q10 and *P. chlororaphis* strain Q16 were isolated recently as a part of III46007 Project and their respective PGP activities are described elsewhere (Jošić *et al*., 2012; Poštić *et al*., 2013; Pivić *et al*., 2015; Jošić *et al*., 2015). Different *Bradyrhizobium japonicum* strains have been in use in Serbia for more than 60 years in several commercial fertilizers. One of them is *B. japonicum* strain 526 used in this investigation.

B. japonicum 526 was grown on YMB (Yeast Manitol Broth), *Bacillus* sp. Q10 on TSB (Tripton Soy Broth) and *P. chlororaphis* Q16 on King B liquid medium at 26 °C for 72 h (*B. japonicum* 526) or 48 h, before being optimized to 10^8 CFU mL^{-1}. The cell-free supernatant (CFS) was maintained by centrifugation at 12000 rpm for 15 min and filtration through 0.22 μm filters (Merck Millipore Ltd.).

The effects of PGP bacteria were tested using four treatments:

A - *B. japonicum* 526 with CFS of *Bacillus* sp. Q10 and *P. chlororaphis* Q16 (2:1:1)

B - *B. japonicum* strain 526 and *Bacillus* sp. strain Q10 (1:1)

C - *B. japonicum* strain 526 and *P. chlororaphis* strain Q16 (1:1)

D - commercial fertilizer containing *B. japonicum,* which served as a control.

100 mL of each treatment was used for all treatments and mixed with sterile solid carrier (peat coal) before being applied to the seed just before planting.

Table 1. Physical properties and chemical characteristics of the studied soil prior to conducting the experiment (mean ± standard deviation)

Physical properties		Chemical characteristics	
Fraction	Humogley Granulometric composition (%)	Property/composition	Humogley
		pH in H_2O	6.50 ± 0.02
Coarse sand, 2-0.2 mm	2.0	pH in 1M KCl	5.85 ± 0.01
Fine sand, 0.02-0.002 mm	46.3	Total N (%)	0.34 ± 0.005
Silt, 0.02-0.002 mm	22.1	SOM (%)	6.51 ± 0.02
Clay, <0.002 mm	31.0	$CaCO_3$ (%)	below the detection limit
Total sand,>0.02 mm	48.3	Available P_2O_5 (mg 100 g^{-1})	9.83 ± 0.56
Silt + Clay, < 0.02 mm	51.7	Available K_2O (mg 100 g^{-1})	9.31 ± 0.93

Table 2. Meteorological condition during soybean growing season 2014 in the area western Serbia

Month	May	June	July	August	September	October	Average/Total
Temperature (°C)	16.3	20.5	21.9	20.9	17.2	13.3	18.35
Rainfall (mm)	202.1	38.2	141.1	78.7	84.3	64.1	101.4

Field trial and laboratory testing of plant materials

This experiment was conducted on Vajska locality. Each variant was performed in four replicates and every plot (length 97 m; width 9 m) consisted 18 rows. The total area under variant was 0.35 ha. 25 plants per replicate were selected for vegetative characters. One application of herbicides (Oxon 75WG + Pulsar 40 + Harmony 75 WG) and inter-row cultivation was performed without irrigation throughout the growing season.

The plant materials were collected in two vegetative soybean periods - flowering and maturity. At the flowering stage, plant height, trifoliate leaf number, root weight, root length, nodule number and nodule dry weight were measured, as along with nitrogen, carbon and sulphur content in dry shoot. At the maturity stage, plant height, trifoliate leaf number, pod number per plant, and grain mass per plant, along with nitrogen, carbon and sulphur content in grain, were measured. Most importantly, the yield was measured and compared to the value of 4,000 kg ha^{-1} pertaining to the control treatment, which was in accordance with the maximum value for the same growing conditions reported by the seed producer (Raiffeisen Agro, 2014). The contents of total forms of carbon, nitrogen and sulphur in soybean plant and grain were determined by elemental analysis using CNS analyzer Vario EL III (Benton Jones, 2001).

For statistical analysis, Duncan's Multiple Range Tests were performed to determine the level of significance, which was accepted at $p < 0.05$ (Statistica ver. 12 StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and Discussion

Soybean plant growth and yield enhancement

The co-inoculated soybean plants showed very similar development to plants inoculated with a commercial fertilizer (D) (Table 3). Trifoliate leaf numbers, root length and nodule numbers per root were not statistically significantly different at the flowering stage. The differences between nodule dry weight values at the flowering stage were statistically significant for all treatments. The shoot dry weight of plants co-inoculated with *B. japonicum* 526 and *Bacillus* sp. Q10 (B) was statistically significantly higher than that measured for plants inoculated with commercial fertilizer, as well as those in treatment A and C.

Significant plant development was observed during vegetation and maximum plant height during maturity stage was observed in treatment A, while the trifoliate leaf number and pod number per plant were higher in treatment A and B relative to C and D (Table 4). All mixtures of *B. japonicum* 526 and *Bacillus* sp. Q10 or *P. chlororaphis* Q16 resulted in a statistically significant increase in grain mass per plant; however, the maximum value was obtained for *B. japonicum* 526 with extracellular metabolites of both PGP strains. Maximum yield of 4,400 kg ha^{-1} in the field trial was achieved for treatment A, whereas all co-inoculation variants were more efficient than the commercial fertilizer, with the yield increasing from 5.73 to 10%. Statistically content of total forms of N, C and S in soybean plants at the flowering stage and in grain is shown in Table 5.

Plant growth-promoting bacteria stimulate plant growth and some strains have the ability to fix biological nitrogen. In this work, the effects of *Bacillus* sp., *P. chlororaphis* and *B. japonicum* on soybean growth and yield enhancement in field conditions were tested. According to the obtained results, the average values of dry weight per nodule and shoot dry weight at the flowering stage were significantly higher in plants treated with the mixture of *B. japonicum* 526 and *Bacillus* sp. Q10 (B). The root weight, root length and nodule number were not statistically significantly different from those measured for the control, while maximum plant height was obtained in treatment A. According to Vacheron *et al.* (2013), PGPR modify root functioning, improve plant nutrition, influence vegetative growth and physiology of the whole plant, and play an important role in the plant hormonal network.

In the present study, the nitrogen and carbon content in dry shoots were similar in all treatments, while sulphur content was lower in treatment A (Table 5). Carbon content of 44.02-44.78% in the analyzed soybean dry shoots was similar to 43.6-45.2% reported by Al-Kaisi *et al.* (2005), but was higher than 41% measured in earlier work of Collins *et al.* (1997).

Nitrogen content was 4.85-5.08% and was significantly higher than or equal to the values reported by Sholihah *et al.* (2012). Araujo and Hungria (1999) demonstrated that *B. subtilis* introduction increases the contribution of the biological nitrogen fixation process in co-inoculated soybean seeds with crude or formulated metabolites. Sulphur content (1.17-1.52%) was significantly higher in our experiment compared to the levels obtained for the application of sulphur for fertilization, which ranged from 0.181 for soybean without S fertilization to 0.237% obtained when 60 kg ha^{-1} of sulphur was added (Dhage *et al.*, 2014).

During the maturity stage, statistically significant increase in plant height and grain mass per plant was observed in treatment A, as well as in the trifoliate leaf number and pod number per plant in treatment A and B. All applied PGP strains improved soybean growth and yield relative to that obtained when commercial fertilizer was used. The higher yields of 4,229, 4,286 and 4,400 kg ha^{-1} were measured for variants C, B and A, respectively. These results correspond to those obtained by other authors, who concluded that the application of PGP increased yield (Gholami *et al.*, 2009; Morel *et al.*, 2012). Morel *et al.* (2012) posited that co-inoculation results in a marked increase in legume yield when compared with single inoculation in gnotobiotic laboratory,

Table 3. Response of soybean ('Angela' cultivar) to inoculation with PGPR strains during the flowering stage

Treatment	Plant height (cm)	Trifoliate leaf number	Root length (cm)	Nodule number root^{-1}	Nodule dry weight root^{-1} (mg)	Dry weight nodule^{-1} (mg)	Shoot dry weight plant^{-1} (mg)
A*	69.07^{a}	10.33	20.99	20.1	1376^{d}	68.71^{c}	7796^{c}
B	67.44^{ab}	9.77	19.5	21.11	1799^{b}	85.22^{a}	9638^{a}
C	65.77^{b}	10.44	20.6	25.1	1527^{c}	60.83^{d}	7543^{d}
D	67.33^{ab}	10.77	18.88	25.11	1889^{a}	75.22^{b}	8546^{b}

Note: * A – *B. japonicum* 526 + CFS (Q10 and Q16), B – *B. japonicum* 526 + *Bacillus* sp. Q10, C-*B. japonicum* 526 + *P. chlororaphis* Q16, D – commercial fertilizer *B. japonicum*. Within columns means followed by the same letter are not significantly different (Duncan, p<0.05)

Table 4. Response of soybean ('Angela' cultivar) to inoculation with PGPR strains during the maturity stage

Treatment	Plant height (cm)	Trifoliate leaf number	Pod number $plant^{-1}$	Grain mass $plant^{-1}$ (g)	Yield (kg/ha^{-1})	Yield increase compared to control (%)
A*	106.88^a	17.99^a	38.8^a	20.96^a	$4{,}400^a$	10
B	94.33^b	16.99^a	35.99^a	17.14^b	$4{,}286^b$	7.15
C	91.21^b	14.55^b	34.1^a	17.77^b	$4{,}229^b$	5.73
D	88.32^b	14.77^b	27.1^b	12.56^c	$4{,}000^c$	-

Note:* A – *B. japonicum* 526 + CFS (Q10 and Q16), B – *B. japonicum* 526 + *Bacillus* sp. Q10, C – *B. japonicum* 526 + *P. chlororaphis* Q16, D – commercial fertilizer *B. japonicum*. Within columns means followed by the same letter are not significantly different (Duncan, $p < 0.05$)

Table 5. Content (%) of total forms of N, C and S in soybean plant and grain

Treatment	Dry shoots			Grain		
	N	C	S	N	C	S
A*	4.85	44.02	1.17^b	6.66^b	53.03^{ab}	0.32^{ab}
B	4.87	44.60	1.26^{ab}	6.53^b	53.15^a	0.31^b
C	4.87	44.65	1.45^{ab}	7.24^a	52.75^b	0.33^a
D	5.08	44.78	1.52^a	6.56^b	52.80^{ab}	0.28^c

Note: * A – *B. japonicum* 526 + CFS (Q10 and Q16), B – *B. japonicum* 526 + *Bacillus* sp. Q10, C – *B. japonicum* 526 + *P. chlororaphis* Q16, D – commercial fertilizer *B. japonicum*. Within columns means followed by the same letter are not significantly different (Duncan, $p < 0.05$)

hydroponics, greenhouse and field conditions. According to the same authors, inoculation and co-inoculation experiments must be performed in field conditions in order to allow for a realistic assessment in practical farming settings. In the field, application of *Bradyrhizobium* co-inoculated with PGPR, including *Pseudomonas* spp. and *Azospirillum* spp., significantly improved yield and yield components of soybean when compared with the sole application of some of applied strains (Son *et al*., 2006; Seyed Sharifi, 2016). Masciarelli *et al.* (2014) co-inoculated *B. amyloliquefaciens* from soybean seeds with *B. japonicum*, a natural symbiont of soybean and reported enhanced plant growth parameters and significantly improved nodulation.

In the present study, the carbon content in grain ranged from 52.75 to 53.15%, and the maximum value obtained for B treatment was significantly higher than in the remaining treatments. Carbon content in grain was below 54% reported by Connor *et al.* (2011). *Bacillus* sp. Q10 included in treatment B was already described as wheat growth promoting strain by Pivić *et al.* (2015).

Maximum nitrogen and sulphur content in grain was measured in this study in treatment C, whereas *P. chlororaphis* Q16 promoted not only yield, but soybean seed quality as well. Based on the analysis of 289 grain samples, Salvagiotti *et al.* (2008) determined the minimum, maximum, median, as well as 25 and 75 percentile value of N. Values obtained in our experiment (6.53-7.24%) exceeded 75%. The S content in grain (0.28-0.33%) measured in our study was at the level reported by Dhage *et al.* (2014) for grain under fertilization with 20 kg ha^{-1} S. All treatments were effective in improving sulphur content in soybean grain, suggesting presence of high levels of sulphur-rich amino acids methionine and cysteine. Grain yield and quality increase in treatment C containing *P. chlororaphis* Q16 may be due to multiple PGP traits, including phosphosolubilization ability, production of indole acetic acid, siderophores, HCN, acyl homoserine lactones, enzymes (protease, chitinase, urease, phosphatase, gelatinase, lipase, pectinase, cellulase, amylase), and phenazine antibiotics, as shown in our earlier investigations (Jošić *et al*., 2012; Poštić *et al*., 2013; Jošić *et al*., 2015). Some PGP traits are responsible for phytostimulation, since production of antibiotics and lytic enzymes is involved in suppression of phytopathogens. *P. chlororaphis* Q16 have been used for yield improvement of potato, cardoon and wheat, as well as in biological control of phytopathogenic fungi affecting cardoon and wheat (Jošić *et al*., 2012; Poštić *et al*., 2013; Pivić *et al*., 2015). Cattelan *et al.* (1999) demonstrated that isolates positive for production of 1-aminocyclopropane-1-carboxylate deaminase, siderophore and β-1,3-glucanase, and for P solubilization enhanced at least one aspect of early soybean growth. Hernandez *et al.* (2004) proposed the use of *P. chlororaphis* PCL1391 strain, which is capable of releasing soluble iron from insoluble ferric oxides, suggesting that phenazines might contribute to iron mobilization in soils. Field trials of pseudomonad strains as seed inoculants can result in growth promotion and have great influence on legume yields (Saharan and Nehra, 2011).

Conclusions

The economic yield and grain quality increases due to co-inoculation observed in this field trial were influenced by all treatments using *Bacillus* sp. Q10 or *P. chlororaphis* Q16. The maximum yield of 4400 kg ha^{-1} was recorded in treatment A, comprising of *B. japonicum* 526 supplemented with CFS of both *Bacillus* sp. Q10 and *P. chlororaphis* Q16, representing 10 % increase relative to commercial fertilizer (D). Additional use of PGP *Bacillus* sp. Q10 and *P. chlororaphis* Q16 as a *B. japonicum* co-inoculant can be recommended for further trial to evaluate their suitability for wide soybean production.

Acknowledgements

This research was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project III46007).

References

Al-Kaisi MM, Yin X, Licht MA (2005). Soil carbon and nitrogen changes as affected by tillage system and crop biomass in a corn–soybean rotation. Applied Soil Ecology 30:174-191.

Antoun H (2013). Plant-Growth-Promoting Rhizobacteria Brenner's Encyclopedia of Genetics. 2nd ed, Volume 5 http://www.bashanfoundation.org/Members/Antoun-

H/hanipgpr.pdf doi:10.1016/B978-0-12-374984-0.01169-4

Araujo FF, Hungria M (1999). Nodulacao e rendimento de soja co-infectada com *Bacillus subtilis* e *Bradyrhizobium japonicum* / *Bradyrhizobium elkanii*. Pesquisa Agropecuária Brasileira 34:1633-1643.

Benton Jones J (2001). Laboratory guide for conducting soil tests and plant analysis. CRC Press LLC.

Bouizgarne B (2013). Bacteria for plant growth promotion and disease management. In: Maheshwari DK (Ed). Bacteria in Agrobiology: Disease Management. Springer-Verlag Berlin Heidelberg.

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Science Society of America Journal 63:1670-1680.

Collins HP, Paul EA, Paustian K, Elliot ET (1997). Characterization of soil organic carbon relative to its stability and turnover. In: Paul EA, Paustian K, Elliot ET, Cole CV (Eds). Soil organic matter in temperate agroecosystems. CRC Press, Inc pp 51-102.

Connor DJ, Loomis RS, Cassman KG (2011). Crop ecology: productivity and management in agricultural systems. 2nd ed. Cambridge University Press.

Dhage SJ, Patil VD, Patange MJ (2014). Effect of various levels of phosphorus and sulphur on yield, plant nutrient content, uptake and availability of nutrients at harvest stages of soybean [*Glycine max* (L.)]. International Journal of Current Microbiology and Applied Sciences 3(12):833-844.

Figueiredo MVB, Seldin L, Fernando de Araujo F, de Lima Ramos Mariano R (2010). Plant growth promoting rhizobacteria: fundamentals and applications. In: Maheshwari DK (Ed). Plant Growth and Health Promoting Bacteria Microbiology, Monographs 18, Springer-Verlag Berlin Heidelberg.

Gholami A, Shahsavani S, Nezarat S (2009). The effect of plant growth promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Academy of Science, Engineering and Technology 49:19-24.

Hernandez ME, Kappler A, Newman DK (2004). Phenazines and other redox-active antibiotics promote microbial mineral reduction. Applied Environmental Microbiology 70:921-928.

Idris EES, Bochow H, Ross H, Borriss R (2004). Use of *Bacillus subtilis* as biocontrol agent. Phytohormone like action of culture filtrates prepared from plant growth-promoting *Bacillus amyloliquefaciens* FZB24, FZB42, FZB45 and *Bacillus subtilis* FZB37. Journal of Plant Diseases and Protection 111:583-597.

ISO 11277 (2009). Soil quality- determination of particale size distribution in mineral soil material-method by sieving and sedimentation.

Jošić D, Ćirić A, Soković M, Stanojković-Sebić A, Pivić R, Lepšanović Z, Glamočlija J (2015). Antifungal activities of indigenous plant growth promoting *Pseudomonas* spp. from alfalfa and clover rhizosphere. Frontiers in Life Science 8(2):131-138.

Jošić D, Protolipac K, Starović M, Stojanović S, Pavlović S, Miladinović M, Radović S (2012). Phenazines producing *Pseudomonas* isolates decrease *Alternaria tenuissima* growth, pathogenicity and disease incidence on cardoon. Archives of Biological Sciences 64:1495-1503.

Lugtenberg B, Kamilova F (2009). Plant-growth-promoting rhizobacteria. Annual Review of Microbiology 63:541-556.

Masciarelli O, Llanes A, Luna V (2014). A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation Microbiological Research 169(7-8):609-615.

Morel MA, Braña V, Castro-Sowinski S (2012). Legume crops, importance and use of bacterial inoculation to increase production. Crop Plant 12:218-240.

Nelson DW, Sommers LE (1996). Total carbon, organic carbon, and organic matter. In: Sparks DL (Ed) Methods of soil analysis, Part 3. SSSA, Madison, Wisconsin pp 961-1010.

Pivić R, Starović M, Delić D, Rasulić N, Kuzmanović Dj, Poštić D, Jošić D (2015). Bacterial antagonists *Bacillus* sp. Q3 and *Pseudomonas chlororaphis* Q16 capable to control wheat powdery mildew in wheat. Romanian Biotechnological Letters 20(3):10448-10460.

Poštić D, Starović M, Popović T, Bosnić P, Stanojković-Sebić A, Pivić R, Jošić D (2013). Selection and RAPD analysis of *Pseudomonas* spp. isolates able to improve biological viability of potato seed tubers. Genetika 45(1):237-249.

Raiffeisen Agro (2014). Seeds catalogue 2014, Corn, Sunflower, Soybean (*in Serbian:* Katalog semena 2014, Kukuruz, Suncokret, Soja).

Riehm H (1958). Die Ammoniumlaktatessigsaure-Methode zur Bestimmung der leichtloslichen Phosphorsure in Karbonathaltigen Boden. Agrochimica 3:49-65.

Saharan BS, Nehra V (2011). Plant growth promoting rhizobacteria: A critical review. Life Sciences and Medicine Research, Volume 2011:LSMR.

Salvagiotti F, Cassman KG, Specht JE, Walters DT, Weiss A, Dobermann A (2008). Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field Crops Research 108:1-13.

Seyed Sharifi R (2016). Application of biofertilizers and zinc increases yield, nodulation and unsaturated fatty acids of soybean. Zemdirbyste-Agriculture 103(3):251-258.

Sholihah A, Prijono S, Utami S, Handayanto E (2012). N mineralization from residues of crops grown with varying supply. Journal of Agricultural Science 4(8):117-123.

Solomon T, Pant LM, Angaw T (2012). Effects of inoculation by *Bradyrhizobium japonicum* strains on nodulation, nitrogen fixation, and yield of soybean (*Glycine max* L. Merill) varieties on nitisols of Bako, Western Ethiopia. International Scholarly Research Network ISRN, Agronomy Volume Article ID 261475, 1-8.

Son TTN, Diep CN, Giang TT (2006). Effect of *Bradyrhizobia* and phosphate solubilizing bacteria application on soybean in rotational system in the Mekong Delta. Omonrice 14:48-57.

SRPS ISO 10390 (2007). Soil quality-determination of pH, Institute for Standardization of Serbia, Belgrade.

SRPS ISO 10693 (2005). Soil quality-determination of carbonate content, Institute for Standardization of Serbia, Belgrade.

SRPS ISO 11464 (2004). Soil quality-pretreatment of samples for physico-chemical analyses, Institute for Standardization of Serbia, Belgrade.

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C (2013). Plant growth-promoting rhizobacteria and root system functioning. Front Plant Science 4:356.

Vessey JK (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255(2):571-586.

WRB (2014). World Reference Base for Soil Resources-International soil classification system for naming soils and creating legends for soil maps. Food and Agriculture Organization of the United Nations, Rome, http://www.fao.org/3/a-i3794e.pdf.