Improvement of Rhizobial Inoculants: A Key Process in Sustainable Soybean Production

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Summary

Symbiotic nitrogen fixation has important role in sustainable soybean production because of utilization of atmospheric nitrogen for soybean nutrition. Pre-sowing soybean seed inoculation with selected rhizobial strains is used to improve the amount of symbiotically fixed nitrogen. Besides strain selection, suitable inoculant formulation is important for the success of inoculant application. The aim of this research is the evaluation of symbiotic efficiency and compatibility of *Bradyrhizobium japonicum* strains with soybean cultivar as well as possibility of using different inoculant formulation in soybean production. During two years of field trials in eastern Slavonia, nodule dry weight, nitrogen content in plant, seed yield, 1000 seeds weight, protein and oil content in seed were determined. Results of this study indicate that inoculant formulation as well as the use of selected strains affects nodulation, symbiotic and agronomic properties of soybean. Despite the differences in results in both experimental years, it can be concluded that the strains used as well as inoculant formulations are suitable for soybean inoculation in agroecological conditions of eastern Slavonia.

Key words

pre-sowing inoculation, soybean, *Bradyrhizobium japonicum* strains, inoculant formulation

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Introduction

Sustainable agriculture, as part of the overall sustainable development, is a production system that combines ecological and economic production elements as well as human health concerns. One of the bases underlying this production is utilization of nitrogen from the environment and rational nitrogen fertilization (Bockman, 1997; Redžepović et al. 2007). This concept certainly encompasses also pre-sowing soybean seed inoculation and other economically important legumes, which has become a standard agricultural practice. The said agricultural practice enables a significant input of biologically fixed nitrogen, which contributes to the economical use of nitrogen fertilizers and has a positive effect on microbial abundance in soil; it also has both economic and ecological advantages in the production of leguminous plants (Strunjak and Redžepović, 1986; van Kessel and Hartley, 2000; Matoša et al. 2010; Blažinkov et al. 2010). The amount of nitrogen that is introduced into soil by biological fixation may be 300 kg/ha, which covers 94% of plant nitrogen requirements (Albareda et al. 2009, cit. Hungria et al. 2006). Therefore, seed inoculation with selected highly efficient *B. japonicum* stains has become a usual procedure in sustainable soybean production (Sikora et al. 2002) The effect of inoculation depends on a number of factors, such as ecological factors in soil, symbiotic efficiency compatibility of the soybean genotype with highly efficient strains and the genetic potential of the host plant (Hume and Blair, 1992; Fesenko et al., 1995; Herridge and Rose, 2000; Komesarović et al., 2007; Sikora et al., 2008; Blažinkov et al., 2010; Matoša et al., 2010). In the production of inoculants, exclusively competitive, efficient and adapted strains are used. Their symbiotic efficiency has to be tested in vegetation and field trials involving different soybean cultivars. Selected *B. japonicum* strains are grown in laboratory and then applied onto the material serving as a carrier (peat, compost, vermiculite) to obtain the product – inoculant for pre-sowing inoculation of legumes. Carrier selection is of the utmost importance for ensuring and maintaining inoculant quality during storage, transport and application (Kremer and Peterson, 1983; Smith, 1992). Inoculants differ in formulation: powdered inoculant, granular inoculant, liquid inoculant, pre-inoculated seed and lyophilized preparations (Khavazi et al., 2007). In our country, the most common method of inoculant application for pre-sowing inoculation is by applying powdered inoculant directly onto seed or into soil. Powdered inoculant based on a peat carrier has been the most popular formulation for legumes inoculation since 1895, while liquid and granular inoculants are alternative formulations available in limited quantities in America and Europe (Stephens and Rask, 2000; Khavazi et al., 2007). However, market demands and conditions for application of particular agricultural practices at family farms impose the need to introduce also other inoculant formulations (Marufu et al., 1995; Thao et al., 2002; Deaker et al., 2004; Khavazi et al., 2007). For this purpose, investigations with liquid inoculant formulation and different rhizobial strains were started. The aim of these investigations was to assess: *i*) the efficiency of different *B. japonicum* strains and their compatibility with a soybean cultivar *ii)* possible application of different inoculants formulations as well as their effect on the quality of soybean seed, soybean yield and nodulation capability of soybean root.

Material and methods

In the spring of 2011, a trial was established at the experimental field of the Agricultural Institute Osijek using the completely randomized block design with four replications. The trial was set on a hydroameliorated hypogley soil of the following chemical characteristics: pH 6.5, 2.2% humus, 0.13% total nitrogen, 18.5 mg P_2O_5/100 g soil and 27.3 mg K_2O/100 g soil. Sowing date was 5 May 2011 with the very early soybean cultivar Lucija (maturity group 00) created at the Agricultural Institute Osijek. The same trial was repeated in the following year. Sowing was performed on 9 May 2012 with the soybean cultivar Lucija in the soil of the following characteristics: pH 7.12, 3.3% humus, 0.16% total N, 24.9 mg P_2O_5/100 g soil and 30 mg K_2O/100 g soil. Trial factors were:

a) strains for pre-sowing soybean inoculation – different *B. japonicum* strains (two indigenous strains, 9 and 125, and the reference strain 344) from the strain collection of the Department of Microbiology, Faculty of Agriculture in Zagreb were used

b) different inoculant formulations for pre-sowing soybean inoculation – liquid and powdered. The main plot was sized 12.5 m^2. At the full flowering stage, plant samples were taken to determine the nodulation ability of strains as well as their compatibility with the soybean cultivar used. Ten plants were taken from each plot, nodules were removed from the roots and nodule dry weight per plant was determined. The total nitrogen content in aerial parts of plants was determined by the standard Kjeldahl procedures. At the end of the growing period, trial plots were combine-harvested and the weight of 1000 seeds (g) was determined, seed yield was measured and expressed at 13% moisture level. Seed protein and oil content was assessed from the average seed sample per plot using an Infratec1241 Analyzer and expressed as % of absolutely dry matter (% in AST). Field trial data were statistically processed throught standard analyses of variance (ANOVA).

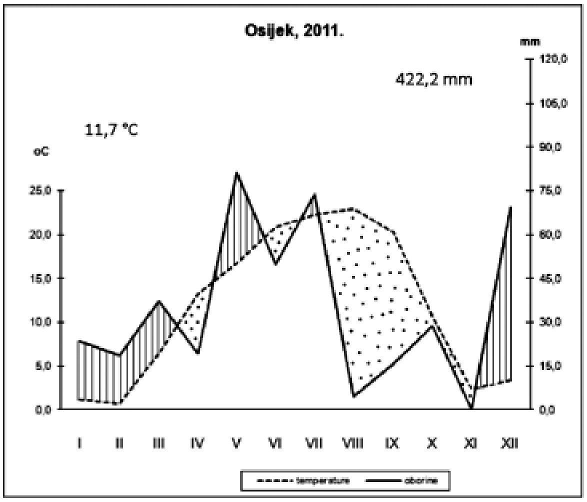
Climate conditions

Almost 230 mm lower precipitation than the multiyear average for 1961-1990 was recorded in Osijek in 2011 (Figure 1). Distribution of monthly precipitation allows the conclusion that April and June were months with significantly lower precipitation, and so was the whole period from August to November. At the same time, all mean monthly temperatures during the soybean growing period were higher compared to the corresponding average multiyear values of mean monthly temperatures (1961-1990), which particularly applies to August and September.

About 50 mm lower precipitation was recorded in 2012 compared to the average values for the period 1961-1990. Moreover, the period from June to September had less precipitation and much greater evapotranspiration needs considering the mean monthly temperatures in that period (Figure 1). In June, July and August of 2012, mean monthly air temperatures were by 3-4°C higher than the corresponding average monthly values for the period 1961-1990.

Results and discussion

In the first trial year, analysis of variance revealed significant differences among the strains in nodule dry weight per plant and weight of 1000 seeds, while plant nitrogen content was



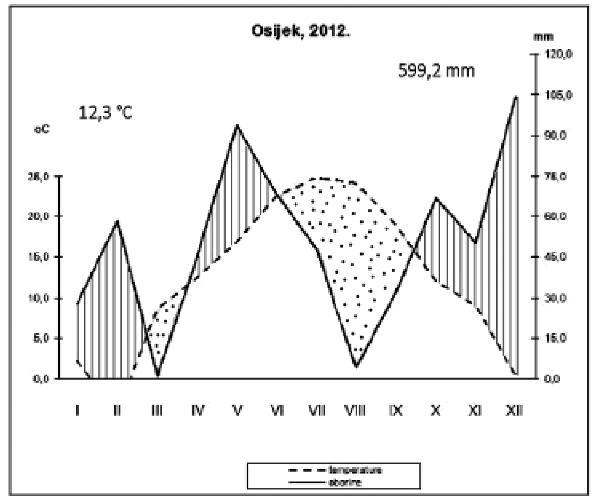


Figure 1. Climate diagrams for Osijek for years 2011 and 2012

Table 1a. The impact of trial factors on nodule dry weight/plant, 1000 seeds weight, and nitrogen content in aerial parts of plants in 2011

	Nodule	Dry (g)	Weight/plant	1000	Seeds (g)	Weight	Nitrogen parts of	Content plants	In aerial (%)
Strains/ formulation	Liquid	Powder	Average	Liquid	Powder	Average	Liquid	Powder	Average
Control	0.194	0.223	0.208ab	196.63	197.3	196.96b	3.06	2.78	2.92
Strain 344	0.20	0.115	0.158b	198.11	197.68	197.89b	2.92	2.905	2.91
Strain 9	0.205	0.193	0.199ab	201.35	199.70	200.53ab	3.15	2.93	3.04
Strain 125	0.214	0.273	0.244a	205.38	200.46	202.92a	2.96	2.835	2.90
Average	0.203ns	0.201	LSD5%=0.057	200.366ns	198.784	LSD5%=3.575	3.022*	2.862	ns

Table 1b. The impact of trial factors on soybean yield, protein, and oil content in seed in 2011

	Soybean	Yield	(t/ha)	Protein	Content (% ATS)	In seed	Oil	Content (% ATS)	In seed
Strains/ formulation	Liquid	Powder	Average	Liquid	Powder	Average	Liquid	Powder	Average
Control	3.12	3.14	3.13d	36.94	36.915	36.93c	23.23	23.175	23.20c
Strain 344	3.49	3.24	3.37c	37.975	37.76	37.87b	23.61	23.33	23.47b
Strain 9	3.71	3.315	3.51b	38.51	38.23	38.37a	23.68	23.43	23.56ab
Strain 125	3.98	3.53	3.76a	37.86	37.65	37.76b	23.755	23.55	23.65a
Average	3.575**	3.308	LSD5%=0.131	37.82**	37.64	LSD5%=0.171	23.57**	23.37	LSD5%=0.114

significantly influenced by the inoculant formulation (Tables 1a). Significantly higher plant nitrogen content was obtained in treatments in which liquid inoculant was applied, regardless of the *B. japonicum* strain used. It is well known that *B. japonicum* strains differ significantly in symbiotic efficiency, compatibility with the cultivar and response to ecological conditions. Selection of the most suitable strain in soybean production enhances the process of nitrogen fixation and expression of the cultivar's genetic potential (Komesarović et al. 2007; Sikora et al. 2008; Matoša et al. 2010), which was demonstrated also in this trial. Selection of the strain for pre-sowing soybean inoculation influenced the nodule dry weight per plant. The highest nodule dry weight was recorded in plants inoculated with the indigenous strain *B.japonicum* 125 in comparison with the reference strain 344. Inoculation with strain 125 affected the size of soybean seed as well. Namely, plants inoculated with this indigenous strain had higher weight of 1000 seeds compared to plants that were untreated as well as those inoculated with the reference strain *B. japonicum* 344. Results of this study showed significant differences in yield, protein and oil content in soybean seed between different rhizobial strains and inoculant formulations (Table 1b). Liquid inoculant formulation had a significant effect on the increase in the protein and oil content in soybean seed compared to the powdered inoculant formulation. In all

Table 2a. The impact of trial factors on nodule dry weight/plant, 1000 seeds weight, and nitrogen content in aerial part of plants in 2012

	Nodule	Dry (g)	Weight/plant	1000	Seeds (g)	Weight	Nitrogen parts of	Content plants	In aerial (%)
Strains/ formulation	Liquid	Powder	Average	Liquid	Powder	Average	Liquid	Powder	Average
Control	0.077	0.041	0.059b	134.8	142.58	138.69c	2.262	2.393	2.328b
Strain 344	0.152	0.079	0.115a	165.71	158.14	161.93a	2.48	2.488	2.484a
Strain 9	0.116	0.092	0.104ab	152.55	168.16	160.36a	2.428	2.537	2.482a
Strain 125	0.119	0.152	0.135a	147.5	148.11	147.81b	2.35	2.428	2.389b
Average	0.116ns	0.091	LSD5%=0.0465	150.14	154.247 ns	LSD5%=8.542	2.38	2.461*	LSD5%=0.066

Table 2b. The impact of trial factors on soybean yield, protein and oil content in seeds in 2012

	Soybean	Yield	(t/ha)	Protein	Content (% ATS)	In seed	Oil	Content (% ATS)	In seed
Strains/ formulation	Liquid	Powder	Average	Liquid	Powder	Average	Liquid	Powder	Average
Control	1.867	2.185	2.026c	38.88	38.78	38.83c	23.04	22.83	22.93b
Strain 344	2.47	2.418	2.444a	39.45	39.85	39.65ab	23.53	23.67	23.45a
Strain 9	2.34	2.695	2.517a	39.49	40.49	39.99a	23.33	23.31	23.32a
Strain 125	2.175	2.34	2.258b	39.22	39.68	39.44b	23.36	23.19	23.27a
Average	2.213	2.409*	LSD5%=0.136	39.26	39.69*	LSD5%=0.54	23.31	23.17ns	LSD5%=0.197

untreated plots lower protein and oil contents were determined, but differences in contents were obtained by inoculation with *B. japonicum* strains used. Inoculation with the indigenous strain *B. japonicum* 9 increased significantly the protein content in soybean seed compared to inoculation with the indigenous strain 125, reference strain and untreated plants. In the case of seed oil content, inoculation with indigenous strains contributed to higher seed oil content compared to the seed oil content in untreated plants. Plants inoculated with the indigenous strain 125 had higher seed oil content than plants inoculated with the reference strain. Both trial factors studied as well as their interaction had an impact on soybean yield. Plants inoculated with liquid inoculant gave significantly higher yield of soybean seeds compared to plants inoculated with powdered inoculant. Inoculation with the indigenous strain *B. japonicum* 125 resulted in significantly higher yield of soybean seeds compared to inoculation with the reference strain, indigenous strain *B. japonicum* 9 and untreated plants. Namely, a combination of liquid inoculant for pre-sowing inoculation with the indigenous strain *B. japonicum* 125 resulted in higher soybean yields compared to other combinations of the trial factors studied. The effect of this combination may be associated with the weather conditions prevailing in 2011, that is, the year with lower precipitation, especially in the period of soybean emergence. Application of the indigenous strain was proven to be justified, since this strain can adapt and achieve a high fixation potential under unfavourable soil conditions.

Results obtained in the second trial year differ significantly in the impact of strain selection and inoculant formulation upon the traits studied. Significantly higher nitrogen content in plant was obtained in treatments in which powdered inoculant was applied compared to treatments involving liquid inoculant (Table 2a). Application of powdered inoculant resulted in higher weight of 1000 seeds however, the differences from liquid inoculant were not significant. The two-year trial results indicate that both inoculant formulations are applicable under the described agroecological conditions. Namely, the mentioned inoculant formulations are applied in pre-sowing inoculation of legumes and are a subject of numerous investigations of the quality and optimization aimed at improving the process of biological nitrogen fixation (Kremer and Peterson, 1983; Hume and Blair, 1992; Brockwell and Bottomley, 1995; Graham and Vance, 2000; Loupwayi et al., 2000; Thao et al., 2002). Some authors maintain that liquid inoculants are easier to handle and equally efficient (Hynes et al., 1995), the others list their limitations with respect to storage (Stephens and Rask, 2000). Still, what is the most important is that the formulation should suit the agricultural producer who uses it and be applicable to the existing legume sowing system. Analysis of variance revealed significant differences between the strains used in weight of 1000 seeds, plant nitrogen content, yield of soybean seeds and seed protein, and oil content. No differences between the strains were detected for nodulation capability and compatibility with the cultivar Lucija. Significantly lowest nodule dry weight per plant were recorded for untreated plots in comparison with plants inoculated with strains *B. japonicum* 125 and 344 (Table 2a). Selection of the strain for soybean inoculation affected the seed yield. Significantly highest soybean yields as well as plant nitrogen contents were found in plants inoculated with strains *B. japonicum* 344 and the indigenous strain 9 compared to untreated plants and plants inoculated with the indigenous strain 125 (Table 2b). The said indigenous strain and the reference strain affected the size of soybean seed as well. Namely, plants inoculated with the mentioned strains had higher weight of 1000 seeds compared to untreated plants and those inoculated with the indigenous strain *B. japonicum* 125 (Table 2a). Regarding seed protein content, the highest percentage was achieved in plants inoculated with strain 9 (Table 2b). Differences in protein content were significant in comparison with inoculation with strain 125 as well as with untreated plants. Significantly lowest seed oil content was obtained in untreated treatments compared to inoculated treatments, regardless of the strain used. The applied strains had a significant effect upon all the traits studied and can be regarded suitable for pre-sowing inoculation in the described soybean growing region.

Conclusion

The results of field trials revealed different impact of inoculant formulation and rhizobial strains depending on the year of research. In the first trial year, the liquid formulation showed a significant influence on increase in yield and the oil and protein seed content. In the second trial year better results were obtained with the powdered inoculant formulation, which led to larger seed size, higher plant nitrogen content and soybean yield. Also, these results confirmed the importance of strain selection for soybean inoculation. It was found that the selected indigenous as well as reference strain of *B. japonicum* had a significant effect on soybean yield, seed quality, and plant nitrogen content. In spite of the differences between strains in both trial years, it can be concluded that the strains and inoculant formulations used are fully suitable for soybean inoculation under the described agroecological conditions.

References

Albareda, M., Rodriguez-Navarro, D. N., Temprano, F. J. (2009). Soybean inoculation: Dose, N fertilizer supplementation and rhozobia persistence in soil. Field Crop Research 113: 352-356.

Blažinkov, M., Vrbanac, D., Huić Babić, K., Sikora, S. (2010). Fenotipska karakterizacija autohtonih sojeva *Sinorhizobium meliloti*. Agronomski glasnik. 4-5: 191-203.

Bockman, O. C. (1997). Fertilizers and biological nitrogen fixation as sources of plant nutrients: perspectives for future agriculture. Plant and Soil 194: 11-14.

Brockwell, J., Bottomley, P. J. (1995). Recent advances in inoculant technology and prospects for the future. Soil Biol. Biochem. 27(4/5): 683-697.

Deaker, R., Roughley, R. J., Kennedy, I. R. (2004). Legume seed inoculation technology – a review. Soil Biology and Biochemistry 36: 1275-1288.

Fesenko, A. N., Provorov, N. A., Orlova, I. F., Orlov, V. P., Simarov, B. V. (1995). Selection of *Rhizobium leguminosarum* bv. *viciae* strains for inoculation of Pisum sativum L. cultivars: analysis of symbiotic efficiency and nodulation competitiveness. Plant Soil. 172: 189-198.

Graham, P. H., Vance, C. P. (2000). Nitrogen fixation in perspective: an overview of research and extension needs. Field Crop Research 65: 93-106.

Herridge D. F., Rose, I. (2000). Breeding for enhanced nitrogen fixation in crop legumes, Field Crops Research 65: 229-248

Hume, D. J., Blair, D. H. (1992). Effect of numbers of *Bradyrhizobium japonicum* applied in commercial inoculants on soybean seed yield in Ontario. Can. J. Microbiol. 38: 588-593.

Hynes, R. K., Craig, K. A., Covart, D., Smith, R. S., Rennie, R. J. (1995). Liquid rhizobial inoculants for lentil and field pea. J. Prod. Agric., 8: 547-552.

Khavazi, K., Rejali, F., Seguin, P., Miransari, M. (2007). Effect of carrier, sterilisation method and incubation on survival of *Bradyrhizobium japonicum* in soybean (*Glycine max* L.) inoculants. Enzyme and Microbial Technology 41: 780-784.

Kremer, R. J., Peterson, H. L. (1983). Effect of inoculant carrier on survival of *Rhizobium* on inoculated seed. Soil Sci. 134: 117-124.

Komesarović, B., Redžepović, S., Blažinkov, M., Sudarić, A., Uher, D., Sikora, S. (2007). Simbiozna učinkovitost selekcioniranih autohtonih sojeva *Bradyrhizobium japonicum*. Mljekarstvo 57 (4): 289-302

Loupwayi, N. Z., Olsen, P. E., Sande, E. S., Kayser, H. H., Collins, M. M., Singleton, P. W., Rice, W. A. (2000). Inoculant quality and its evaluation. Field Crops Res. 65, 259-270.

Marufu, L., Karanja, N., Ryder, M. (1995). Legume inoculant production and use in east and southern Africa. Soil Biol. Biochem. 27: 735-738.

Matoša, M., Sudarić, A., Vratarić, M., Volenik, M., Redžepović, S. (2010). Simbiozna fiksacija dušika - alternativa održivog uzgoja soje. Proceedings & abstracts: 3rd international scientific/professional conference Agriculture in nature and environment protection. Osijek, pp. 145-150.

Redžepović, S., Sikora, S., Čolo, J., Blažinkov, M., Pecina, M. (2007). Influence of plant growth regulator and rhizobial inoculation on nodulation and soybean nitrogen content. Cereal Research Communication. 35; 2 Part 2: 993-996.

Sikora, S., Redžepović, S., Bradić, M. (2002). Genomic fingerprinting of *Bradyrhizobium japonicum* isolates by RAPD and rep-PCR. Microbiological Research. 157, 3: 213-219.

Sikora, S., Blažinkov, M., Babić, K., Sudarić, A., Redžepović, S. (2008). Symbiotic nitrogen fixation and sustainable soybean production. Cereal Research Communications 36, S5: 1483-1486.

Smith, R. S. (1992). Legume inoculant formulation and application. Can. J. Microbiol. 38: 485-492.

Stephens J. H. G., Rask H. M. (2000). Inoculant production and formulation. Field Crop. Res. 65 (2-3): 249-258.

Strunjak, R., Redžepović, S. (1986). Bakterizacija leguminoza – agrotehnička mjera u službi štednje energije. Poljoprivredno znanstvena smotra 72: 109-115

Van Kessel, C., Hartley, C. (2000). Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? Field Crops Research 65: 165-181.

Thao, T. Y., Singleton, P. W., Herridge, D. (2002). Inoculation Responses of Soybean and Liquid Inoculants as an Alternative to Peat-Based Inoculants. Inoculants and Nitrogen Fixation of Legumes in Vietnam. ACIAR Proceedings pp. 67-74

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