Effect of Inoculation and Growth Regulator on Soybean Yield and Photosynthetic Pigment Content

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Summary

The main objective of this study was to evaluate the influence of soybean inoculation with different *Bradyrhizobium japonicum* strains and the application of Bioalgeen-S90, as a growth regulator, on photosynthetic pigment content and soybean yield. Regarding the main objective, both greenhouse experiment and field trials were conducted. The positive effect of interaction between Bioalgeen-S90 and seed inoculation with *B. japonicum* strain D344 on photosynthetic pigment content was determined in greenhouse experiment. The results from field trials showed a significant influence of Bioalgeen-S90 on chlorophyll b and a+b content and soybean yield. Regardless of the *B. japonicum* strain used, inoculation had a significantly positive effect on chlorophyll a, b and a+b content. Compared to the non-inoculated control variants, inoculation with both *B. japonicum* strains, showed the statistically significant effect on soybean yield. The simultaneous application of Bioalgeen-S90 and *B. japonicum* strains caused the highest yield increase.

Key words

soybean; *Bradyrhizobium japonicum*; growth regulator; photosynthetic pigments; yield

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Introduction

One of the main postulates of sustainable agriculture is the improved management of environmental nitrogen pool as well as the reduction of nitrogen fertilizers input. The cost effective and ecologically acceptable legume production relies on benefits obtained from symbiotic nitrogen fixation (Redžepović, S. 1986). Special attention has been aimed at selection of high quality *B. japonicum* strains and finding the most efficient symbiotic association between the soybean cultivar and *B. japonicum* strain in order to increase the utilization of nitrogen fixation and enhance the production of soybeans (Redžepović, S. et al. 1990; 1991; 1992; 2001). Considering that symbiotic fixation depends on the photosynthetic capacity of the specific legume, special efforts have been done in finding patterns of increasing plants' photosynthetic capacity (Redžepović, S. et. al. 1999; 2004). Comprehensive literature source does not provide exact information on the relationship between photosynthetic capacity of soybean and the quantity of fixed nitrogen. Darlington et al. (1996), Dudeja and Kamlesh (2000), and Rajala A. (2003) state that the only way to improve photosynthetic capacity is to use plant growth regulators. The main objective of this study was to test effect of the different *B. japonicum* strains for soybean inoculation and the use of Bioalgeen-S90 as a growth regulator on the content of photosynthetic pigment and soybean yield in the agroecological conditions of eastern Croatia.

Materials and methods

The influence of Bioalgeen-S90 as growth regulator and inoculant *B. japonicum* strains was tested on soybean cultivar Tisa, created at the Agriculture Institute in Osijek. The growth regulator Bioalgeen-S90 (Schultze & Hermsen GmbH, Germany) is a natural product based on extracts of the brown algae *Ascophillum nodosum*. The soybean was inoculated with *B. japonicum* strains D344 (reference strain) and USDA 110 obtained from University of Minnesota, MN, USA. Both strains belong to genetically divergent groups of bradyrhizobia (Sikora and Redžepović, 2003).

Greenhouse experiment

The greenhouse pot experiment was set up in order to assess the effect of the growth regulator, seed inoculation and the pre-sowing fungicide treatment on soybean photosynthetic capacities. Three-factor (2x2x3) experiment was set up in a randomized complete block design (RCBD) with four replicates. The factors in experiment were as follows:

I) foliar application of Bioalgeen-S90: without application of Bioalgeen (NO) and with application of Bioalgeen (YES). Bioalgeen-S90 was used in a dosage of 2L/400L water per hectare and applied in following growth phases – (i) after the soybean germination and (ii) in the V2 phase.

II) inoculation of soybean seed: non-inoculated seeds (0) and seeds inoculated with the *B. japonicum* strain D344 (344);

III) fungicide seed treatment: untreated seeds (F_0), seeds treated with the fungicide APRON 35 SD (100 g a.m. Metalaxil/100 kg seeds) + TMTD (100g a.m./100 kg seeds) (F_1); and seeds treated with fungicide APRON 35 SD + TMTD + amino acids (Protifert, 0.6 L/100 kg seeds) (F_2). For binding to the seed, Sacrust (350 g/100 kg seed) was used.

Soybean seeds were sown into sterile siliceous sand (grain size 0.5-2.5mm). During the vegetation, nutrient medium without nitrogen was used. In the full bloom phase, samples of physiologically youngest trefoils from main stem were taken in order to determine chlorophyll a and total chlorophyll (a+b) content (Jensen, 1978). Statistical analysis of all study traits was conducted using an analysis of variance (ANOVA) by the RCBD model.

Field experiments

In 2001, split plot experiments with and without application of Bioalgeen-S90 (YES/NO) as main plots and inoculation treatments (uninoculated, strain D344, USDA 110) as subplots, were conducted in a RCBD with four replicates, at the locations Karanac and Mirkovac. The experiment at Karanac was set up on eutric cambisol luvisol, with neutral reaction (pH 7.05), slightly humic (2.63%), and rich in physiological phosphorus and potassium (40.28 mg P_2O_5/100 g soil and 37.98 mg K_2O/100g soil). The experiment at the Mirkovac was set up on humogley soil, alkaline (pH 8.31), slightly humic (2.48%), and rich in physiological phosphorus (29.8 mg P_2O_5/100 g soil) and moderate to good supplied with physiologically active potassium (15.39 mg K_2O/100 g soil).

Pre-sowing fertilization at both locations was performed using 400 kg/ha NPK 8:26:26. The cultivar Tisa was inoculated with different *B. japonicum* strains directly into sowing rows. Foliar application of Bioalgeen-S90 followed emergence phase. The first treatment was conducted in the phase 2-3 trifoliate leaves and the second treatment in the V5 growth phase. In each growth phase, the application dosage of Bioalgeen-S90 was 2 L/400 L water per hectare. In the full bloom phase of soybean, 10 plants per plot were taken in order to determine chlorophyll a, chlorophyll b and chlorophyll a+b content.

Statistical analysis of all studied traits was conducted according to two models of analysis of variance (ANOVA) by the split plot method: a) Model 1 –combined analysis of experimental data at two locations and b) Model 2 – separate analysis for each location individually. All analyses were conducted using the statistical program SAS System for Win. Ver. 8.02 (SAS Inc. 1989).

Results

Greenhouse experiment

The compatibility between *B. japonicum* strain D344 and soybean cultivar Tisa was determined. In the greenhouse experiment, the influence of Bioalgeen-S90, coating of the seed with fungicide and seed inoculation with *B. japonicum* strain D344 on the photosynthetic capacities of soybean was assessed. All factors in experiment as well as their interactions showed significant influence on the chlorophyll a and a+b content with one exception (Table 1). The interaction between Bioalgeen-S90 and seed fungicide treatment on chlorophyll a content was not determined. Seed treatment with fungicide and amino acids negatively affected soybean photosynthetic capacity by reducing chlorophyll a and a+b content. Inoculation with strain D344 substantially increased the chlorophyll a and chlorophyll a+b content in comparison to non-inoculated variants (Table 1). Foliar application of Bioalgeen-S90 significantly increased chlorophyll a and a+b content in comparison to the control variants. The importance of the interaction strain x Bioalgeen was particularly stressed through the effect of Bioalgeen-S90 on the non-inoculated variants where significantly higher chlorophyll a+b content was determined in comparison with non-inoculated variants without Bioalgeen-S90 application. The effect of the interaction fungicide x Bioalgeen was revealed through the decreased content of chlorophyll a+b in the samples treated with fungicide (F_1). On the other hand, the application of Bioalgeen-S90 substantially increased the chlorophyll content both in plants not treated (F_0) and in those treated with a combination of fungicide + amino acid (F_2). The negative effect of fungicide treatment was determined in inoculated plants. Seed treatment with fungicides resulted in a lower chlorophyll a and a+b content in the inoculated variants while in non-inoculated variants reduction of chlorophyll content was less expressed(Fig.1). These results indicated that inoculated variants were more

Table 1.
Mean values of chlorophyll a and a+b, and significance of their differences in greenhouse experiment§

Fungicide	Bioalgeen	Strain	Chlorophyll a ($mg\ g^{-1}$)		Chlorophyll a+b ($mg\ g^{-1}$)	
F_0	NO	0	2.495		2.888	
		344	3.335		3.848	
	mean NO		2.915	b	3.368	c
	YES	0	3.137		4.108	
		344	3.106		4.221	
	mean YES		3.122	a	4.165	a
F_1	NO	0	2.831		3.686	
		344	3.151		4.166	
	mean NO		2.991	ab	3.926	ab
	YES	0	3.398		4.166	
		344	2.797		3.349	
	mean YES		3.098	ab	3.758	b
F_2	NO	0	2.176		2.608	
		344	2.503		3.398	
	mean NO		2.340	d	3.003	d
	YES	0	2.186		2.792	
		344	3.234		4.081	
	mean YES		2.710	c	3.437	c
mean total		F_0	3.018	a	3.766	a
		F_1	3.044	a	3.842	a
		F_2	2.525	b	3.220	b
		NO	2.748	b	3.431	b
		YES	2.976	a	3.786	a
		0	2.704	b	3.375	b
		344	3.021	a	3.843	a

§ factor level means accompanied by various letters are significantly different with an error $p<0.05$

Figure 1.
Greenhouse experiment pointed out positive effect of inoculation with *B. japonicum* strain D344, application of Bioalgeen-S90 and their interaction on photosynthetic pigment content
1. without inoculation, without Bioalgeen-S90 treatment, and without fungicide treatment
2. without inoculation, with Bioalgeen-S90 treatment, and without fungicide treatment
3. with inoculation, without Bioalgeen-S90 treatment, and without fungicide treatment
4. with inoculation, with Bioalgeen-S90 treatment, and without fungicide treatment

susceptible to fungicide treatment in comparison with variants where inoculation was omitted.

Field experiment

The results obtained in the greenhouse experiment directed the further research towards the testing different *B. japonicum* strains for soybean inoculation and the use of Bioalgeen-S90 as a growth regulator on the content of photosynthetic pigment and soybean yield in the specific agro-ecological conditions. Field experiments were set up at two different soil types in eastern Croatia (locations Karanac and Mirkovac). The chlorophyll content followed the similar pattern on both locations indicating that soil type and other environmental conditions didn't have significant impact on chlorophyll a, b and a+b content. The influence of Bioalgeen-S90 on the chlorophyll b and chlorophyll (a+b) content was determined while that was not a case for chlorophyll a content. Significantly higher chlorophyll content was determined on those plots where Bioalgeen-S90 had been applied. Significantly positive effect of inoculation on the chlorophyll a, chlorophyll b and total chlorophyll (a+b) content was determined, irrespective of the strain used. Common influence of location, Bioalgeen-S90 application and *B. japonicum* strain on chlorophyll content was not revealed. Significant influence of Bioalgeen S90 on chlorophyll b and a+b content was determined. However, considering the separate location analyses (Tables 2) the influence of Bioalgeen-S90 on chlorophyll content was not detected. At the location Karanac, the significant effect of inoculation on chlorophyll a and a+b content was detected, irrespective of the strain used. The influence of inoculation on chlorophyll b content was not determined (Table 2). The field experiment at location Mirkovac gave similar results, except that at this location, the effect of inoculation on chlorophyll b content was also revealed (Table 2). Soybean yield was significantly affected by location, Bioalgeen-S90 application and *B. japonicum* strain. However, no interaction with location (location x Bioalgeen, location x strain, location x Bioalgeen x strain) could be considered as significant. Regarding the soybean yield, only the interaction between Bioalgeen-S90 and *B. japonicum* strain was significant (data not shown). Significantly higher values for soybean yield were detected at location Mirkovac. At both locations, the application of Bioalgeen-S90 caused significant yield increase. The seed inoculation with both *B. japonicum* strains resulted also in significantly higher seed yield. The common effect of Bioalgeen-S90 and *B. japonicum* strain positively reflected on seed yield. Therefore, in comparison with non-inoculated plots, the higher soybean yield was determined on plots where Bioalgeen-S90 and inoculation with both *B. japonicum* strains were applied.

In separate analysis of locations, the significant effect of Bioalgeen-S90 and *B. japonicum* strain was detected on both locations. The results indicated that inoculation with different strains caused higher influence on seed yield. At location Karanac, significant common effect of Bioalgeen-S90 application and *B. japonicum* strain on seed yield was revealed. Common application of Bioalgeen-S90 and *B. japonicum* strain D344, as well as strain USDA 110, resulted in significantly higher yield. However, at location Mirkovac, Bioalgeen-S90 application and *B. japonicum* strain interaction was not significant. At both locations, the inoculation with strain D344 caused slightly higher values for seed yield although the differences between *B. japonicum* strain D344 and USDA110 were not significant.

Discussion

During the past decade, special attention has been paid to the energy side on mutual activities of photosynthesis and biological nitrogen fixation. Harper J.E. (1999) stated that there was a strong dependence of nitrogen fixation and the dry matter yield upon the photosynthetic capacity of a plant. This was considered to be the key problem in advancing this physiological parameter. The main objective of this study was to test different *B. japonicum* strains and to assess the possibility of Bioalgeen S90 application as well as its impacts on the symbiotic potential in the integrated relationship soil-microorganism-plant. The final objective was to obtain higher soybean yield and to increase the protein level by enhancing the photosynthetic and nitrogen fixing capacity of soybean.

The preliminary greenhouse experiment confirmed the importance of soybean inoculation and growth regulators, as both treatments lead to increased chlorophyll a and a+b content. The results obtained in greenhouse experiment revealed the negative effect of the fungicides Apron 35SD and TMTD. The field experiments were designed to evaluate the effect of the Bioalgeen-S90 application and inoculation of the soybean seed with different *B. japonicum* strains. In field experiments, two *B. japonicum* strains were used for soybean inoculation in order to study their interactions with the Bioalgeen-S90 growth regulator and their symbiotic efficiency under various ecological conditions. The obtained results, indicated that *B. japonicum* strains used, D344 and USDA 110, increased chlorophyll a, b and a+b content in the leaves of soybean cultivar Tisa. These results were in agreement with the results of other authors who found that the symbiotic relationship of the nitrogen fixing bacteria and the legumes achieved a positive impact on the photosynthetic potential of the legumes. Jakovljeva

Table 2.
Mean values for chlorophyll a, b and a+b, and yield and the significance of the factor effects[§] from both models of statistical analysis (Model 1 and 2)

Location	Bioalgeen	Strain	Chlorophyll			Yield (t ha^{-1})
			A (mg g^{-1})	B (mg g^{-1})	a+b (mg g^{-1})	
Karanac	NO	0	2.792	1.141	3.933	2.118
		344	3.601	1.128	4.729	2.483
		110	3.581	1.258	4.839	2.608
	mean NO		3.324	1.176	4.500	2.403 b
	YES	0	2.945	1.288	4.233	2.235
		344	3.562	1.550	5.112	2.900
		110	3.439	1.326	4.764	2.763
	mean YES		3.315	1.388	4.703	2.633 a
	mean	0	2.869 b	1.214	4.083 b	2.176 b
		344	3.582 a	1.339	4.921 a	2.691 a
		110	3.510 a	1.292	4.802 a	2.685 a
	mean Karanac		3.320	1.282	4.602	2.518 b
Mirkovac	NO	0	2.611	1.174	3.785	2.223
		344	3.488	1.565	5.052	2.680
		110	3.676	1.621	5.298	2.645
	mean NO		3.258	1.453	4.711	2.516 b
	YES	0	2.931	1.342	4.273	2.438
		344	3.520	1.586	5.106	3.065
		110	3.649	1.855	5.503	2.995
	mean YES		3.367	1.594	4.961	2.833 a
	mean	0	2.771 b	1.258 b	4.029 b	2.330 b
		344	3.504 a	1.575 a	5.079 a	2.873 a
		110	3.662 a	1.738 a	5.400 a	2.820 a
	mean Mirkovac		3.312	1.524	4.836	2.674 a
mean total		NO	3.291	1.315 b	4.606 b	2.459 b
		YES	3.341	1.491 a	4.832 a	2.733 a
mean total		0	2.820 b	1.236 b	4.056 b	2.253 b
		344	3.543 a	1.457 a	5.000 a	2.782 a
		110	3.586 a	1.515 a	5.101 a	2.753 a
mean total			3.316	1.403	4.719	2.596

[§] factor level means accompanied by various letters are significantly different with an error of $p<0.05$

et al. (1973, cited in Beresteckij et al. 1984) claimed that inoculated soybean caused a 28% increase in chlorophyll content over the control. The results also showed that the combination of Bioalgeen-S90 and the inoculation with different *B. japonicum* strains positively affected the increase of chlorophyll a content. At the location Karanac, the combination of Bioalgeen-S90 with the strain D344 resulted in a 27.7% increase in chlorophyll a content, while increases at location Mirkovac were even higher (34%) in comparison to the control (Table 2). A similar trend was determined with chlorophyll b content, at location Karanac. Chlorophyll b content was increased by 36% and 35% at location Mirkovac compared to the control. Very similar trends in the increase in both chlorophylls (a+b) were confirmed on the experimental plots at both locations, such that the combination of Bioalgeen-S90 and *B. japonicum* strains was much more positive in increasing the quantity of chlorophyll a+b in growing soybean Tisa cultivar at location Mirkovac. In comparison to the control, the most positive effect was the combination of Bioalgeen-S90 and strain USDA 110 at location Mirkovac (increase of 45%), while at location Karanac, the combination of Bioalgeen-S90 and strain D344 led to a 30% increase of chlorophyll a+b content over the control (Table 2). All the above data confirm the increased photosynthetic capacity of the soybean cultivar Tisa after the application of inoculation and Bioalgeen-S90. However, the most important data were concerning soybean yield. The results suggested that the soil characteristics significantly affect the efficiency of the symbiotic associations between *B. japonicum* strains and the soybean cultivar. The statistical analysis of all results clearly showed the positive effect of the inoculation with different *B. japonicum* strains on yield of the cultivar Tisa. However, the most superior effect on soybean yield was revealed with the combination of the use of Bioalgeen-S90 and the *B. japonicum* strains in comparison to the control variants.

At the location Karanac, the combination of Bioalgeen-S90 and strain D344 increased soybean yield by about 37%, while the combination Bioalgeen-S90 and strain USDA 110 increased yield by 30% compared to controls. Similar results were found at the location Mirkovac. The combination of Bioalgeen-S90 and strain D344 increased soybean yield about 38% while with strain USDA 110 by about 35% compared to controls. All the obtained results, expressed in terms of soybean yield, give an answer on the justification of this procedure in advancing soybean production, for soybean yield are the best indicator of the successful application of agricultural operations and the proper selection of cultivars for a specific agro-ecological area (Pospišil et al. 2002). The obtained differences in soybean yield, indicated that Bioalgeen-S90, as a growth regulator, together with *B. japonicum* strains significantly influenced the yield increase.

Conclusions

Further research is needed in order to determine the most suitable symbiotic association of rhizobial strain and soybean cultivar for particular agro-ecological region. If the results of this study can be confirmed in other regions, than more efficient soybean production could be achieved with lower investments, the risks associated with high inputs of mineral nitrogen would be reduced, less fossil fuel energy sources (oil, gas) would be used, biological diversity of the soil would be preserved and therefore, basic postulates of sustainable soil management would be easier to realize.

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