

## EFFECT OF DIFFERENT SEED INOCULATION METHODS TO NODULATION, YIELD AND QUALITY OF SOYBEAN CULTIVARS

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**Abstract.** Soybean is one of the most important high-quality protein source for human and animal nutrition in worldwide. With the soil bacteria *Bradyrhizobium japonicum* symbiotically colonizing the plant's roots, soybean are able to fix atmospheric nitrogen. Because this bacteria is not native to Hungarian soils, soybean seeds need to inoculated with *Bradyrhizobium* strains before sowing. There are a number of different seed and soil inoculation products which contain *Bradyrhizobium* strains on the market but few data are available on their efficacy and plantspecific utility. The aim of this study was: (i) to test the ability of two commercially available inoculants (inoculant 1 and inoculant 2), one arbuscular mycorrhiza (AM) product, one seed dressing product and their combination to maximize soybean yield, protein content and Pro-Fat value, (ii) to study the interaction of different inoculants with three different registered Hungarian soybean varieties (Aires, Bahia, Pannonia kincse). Small plot field trials were performed at the Szeged Research Station of Hungarian University of Agricultural and Life Sciences in 2018. Effective inoculation with *Bradyrhizobium* inoculant 1 and its combinations with AM product and seed dressing product significantly increased grain yield, protein content and Pro-Fat value compared with inoculant 2 and controls. Interaction between the soybean varieties and different technologies were significant for yield, protein content and Pro-Fat value. Applying the obtained results in practice can contribute to the efficiency of soybean production.

**Keywords:** soybean, *Bradyrhizobium*, arbuscular mycorrhiza, seed inoculation, yield, quality

### INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is an important source of food, protein and oil. It is the fifth in the world production of major crops after wheat, corn, rice and potato. Today, the annual demand for soybeans is 500-600 thousand tons in Hungary, but 90% of this is imported from South America (KÖVICS ET AL. 2020). The domestic and international market would also require GMO free soybean seed and meal. The Hungarian soybean production area is the sixth (58230 ha in 2019) after Italy, France, Romania, Croatia and Austria in the European Union (FAOSTAT 2019). The local climatic conditions make it possible to increase the soybean production area in Hungary, however, successful soybean production depends on many factors. After the choosing of appropriate soybean variety, the most important factor is the crop production technology. Cultivation technology includes inoculation of soil or seed with *Bradyrhizobium japonicum*, timely application of herbicides against weeds, and nutrient-fertilization (DORDEVIC ET AL. 2020). Regarding the fertilization, it is worth noting that soybean like other nutrient-intensive crops, requires that nutrients be available in adequate amounts and proportions during the growing season. Based on well-known nitrogen fixation mechanism in legumes, soybean plants absorb 50% of the essential nitrogen through root nodules. The nitrogen fixation of soybean root can only occur in the presence of compatible bacterial strains, namely *Bradyrhizobium japonicum*. Because soybean is originated from Asia, field test suggested that the *B. japonicum* not occur natively in Hungarian soils and the absence may limit decrease the yield (BÍRÓ ET AL. 1993). Therefore to develop symbiosis between the

plants and bacteria, the seeds or soils must be treated with inoculation product containing *B. japonicum* (SOÓS AND KÓNYA 1978; MULDOON ET AL. 1980).

Today the use of arbuscular mycorrhizal (AM) fungi in crop production is becoming more widespread to help absorbing nutrients from the soil. Their best-known positive effect on plants is to enhance the vitality of the host plant, thereby increasing their tolerance to biotic and abiotic stresses. The presence of AM fungi also has a beneficial effect on the physical properties of soils. Although the relationship between AM fungi and their host plant is not species-specific, there may be significant differences in the compatibility of individual plant and fungal species (CHALK ET AL. 2006).

More and more microbiological products are on the market in order to achieve higher yields and better quality, however, little data is available on their efficacy and plant variety-specific use. The study aimed to investigate the effects of two different *B. japonicum* seed inoculation products alone and combination with one arbuscular mycorrhiza product and one fungicide seed dressing product on nodulation, yield and yield quality of soybean genotypes.

## MATERIAL AND METHODS

The experiment of this study was conducted at the National Agricultural Research and Innovation Centre, Department of Field Crop Research Szeged-Öthalom, Hungary (now Hungarian University of Agriculture and Life Sciences) in the growing season 2018. The topography of the research area is flat. The soil was deeply salt meadow chernozem with organic matter of 2,8-3,2 %, pH of soil paste 7,2, 14,2 mg kg<sup>-1</sup> total N, 267 mg kg<sup>-1</sup> available P and 326 mg kg<sup>-1</sup> available K in the top 20 cm depth. The meteorological data of the experimental period was collected and presented in Figure 1.

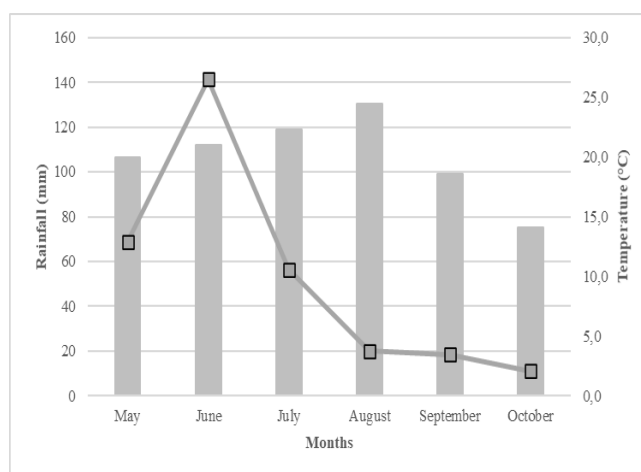


Figure 1. Meteorological data of growing season 2018

Seeds of three soybean genotypes (Aires, Bahia, Pannónia kincse) were obtained from Cereal Research Non-Profit Company Szeged, Hungary. The experiment was performed in 4 repeats, on 10 square meter randomized complete block design (RCBD). Treatments consists of 2 *Bradyrhizobium japonicum* seed inoculants (O1 and O2) combined with one arbuscular mycorrhiza (AM) product and one seed dressing product (F) and control without any seed adjustments (Table 1).

Table 1

Treatments of soybean varieties		
	Treatment	Sign
1.	Control	K
2.	<i>Bradyrhizobium japonicum</i> seed inoculant 1	O1
3.	<i>Bradyrhizobium japonicum</i> seed inoculant 1 + fungicide seed dressing product	O1F
4.	<i>Bradyrhizobium japonicum</i> seed inoculant 1 + arbuscular mycorrhiza product	O1AM
5.	<i>Bradyrhizobium japonicum</i> seed inoculant 2	O2
6.	<i>Bradyrhizobium japonicum</i> seed inoculant 2 + fungicide seed dressing product	O2F
7.	<i>Bradyrhizobium japonicum</i> seed inoculant 2 + arbuscular mycorrhiza product	O2AM

Two weeks before harvesting, 5 plants per plot, from the inner two ridges, were dug out carefully to avoid root injury. The roots were then washed carefully with tap water to remove soil particles and examined for the presence of nodules and counted. Before harvesting, 10 plants in each plot were randomly selected to measure the plant height by measuring stick. Harvesting was performed with a Wintersteiger type plot combine during the period of full ripening. Yield per plot was measured and applied to a moisture content of 9%. A sample from the harvested seed of each plot was used to determine oil and protein content by Foss Infracac 1241 with calibration of soybean. The Pro-Fat value (%) was calculated based on the oil content and protein content of the seed samples per plots.

Analysis of variance (ANOVA) appropriate for randomized complete block design was used according to SVÁB (1981). Means separation was carried out using the least significance difference (LSD) for the different characters.

**RESULTS AND DISCUSSION**

Different numbers of nodules were observed between soybean varieties and treatments but the results were lower than expected. Nodules were not formed on control plots and O2F treatments. Generally, O1 was more effective alone and also in combinations (O1F and O1AM) than O2. We found a significant difference in the average of the cultivars compared to the control between O1 (5.67 pcs) and O1AM (4.33 pcs) treatments. It was also observed that in the average of the three cultivars, significantly less nodules were formed during the combined use of O1 and seed dressing product (Fig. 2a). Among the cultivars, the number of nodules formed of Aires and Pannónia kincse exceeded those of Bahia, but the differences were not significant (Fig. 2b).

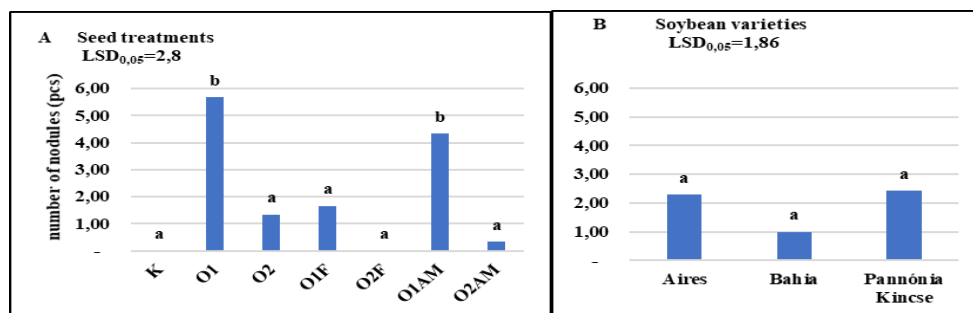


Figure 2. Effect of different seed treatments on mean number of nodules per plants

Plant height was influenced by the genotypes, but not influenced by inoculation treatments as well as no interaction between genotypes and inoculations. Pannónia kincse was the soybean genotype that had the highest plant height (115,12 cm) (Fig. 3 A, B).

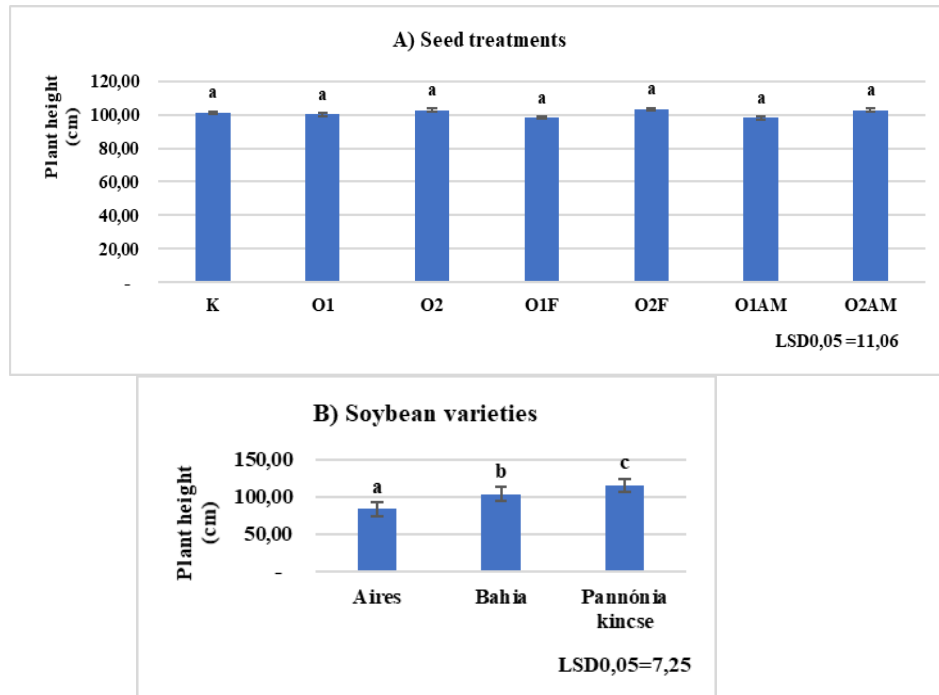


Figure 3. Plant height of soybean varieties

Table 2

Effect of seed inoculation on yield of soybean varieties

Treatment	Aires		Bahia		Pannónia kincse		Average of treatments	
	t/ha	%	t/ha	%	t/ha	%	t/ha	%
<b>K</b>	2,65	100,00	2,95	100,00	2,69	100,00	2,76	100,00
<b>O1</b>	3,24	122,24	3,21	109,08	3,50	130,45	3,32	120,22
<b>O2</b>	2,65	99,72	3,10	105,35	2,80	104,19	2,85	103,17
<b>O1F</b>	3,08	116,12	3,52	119,52	3,19	118,90	3,26	118,23
<b>O2F</b>	2,46	92,84	3,07	104,16	2,93	109,12	2,82	102,14
<b>O1AM</b>	3,12	117,62	3,41	115,79	3,17	117,97	3,23	117,08
<b>O2AM</b>	2,81	106,03	3,26	110,53	3,00	111,64	3,02	109,45
<b>average of genotypes</b>	2,89	109,10	3,26	110,74	3,10	115,38		

LSD<sub>0,05</sub>(genotypes)=0,16; LSD<sub>0,05</sub>(treatments)=0,78

The smallest grain yield was 2.46 t/ha (Aires O2F) and the highest grain yield was 3.52 t/ha (Bahia O1F). The examined seed treatment combinations resulted 11.72% more yield

compared to control plots. O1 alone and also in combination significantly increased seed yield relative to uninoculated control and O2 inoculated plants (Table 2). LAMPTEY ET AL. 2014, ULZEN ET AL. 2016, ZIMMER ET AL. 2016 reported 12-21% yield increasing as a result of seed inoculation with *Bradyrhizobium japonicum*. In Hungary, Soós and Kónya (1978) and KÖVICS et al (2020) observed an increase in yield of 18-32% and 10-15 % depending on the inoculant.

According to the results of grain quality test the average protein content of the varieties and treatments also exceeded 30%. The lowest protein content was identified in the control of Pannónia kincse (31,7 %) while the highest was measured in the O1 treatment of Aires (42,8 %). O1 alone and also combinations significantly increased the mean seed protein content compared to uninoculant control plants (Table 3). Similarly, MORSHED ET AL (2008) AND ZIMMER ET AL. (2016) reported increase in protein content of soybean following seed inoculation of *Rhizobium japonicum*. In our experiment the oil content of the seeds are the opposite of the degree of protein content (Table 4): O1 significantly decreased the seed oil content. This could be due to the fact that the oil content decreases during protein synthesis, so there is a strong negative correlation between the two values (FILHO ET AL. 2001).

Table 3

Effect of seed treatments for protein content (%) of soybean varieties

Treatments	Protein content (%)			
	Aires	Bahia	Pannónia kincse	Average of treatments
<b>K</b>	30,20	26,35	24,70	27,08
<b>O1</b>	35,80	31,20	33,35	33,45
<b>O2</b>	31,10	26,35	27,00	28,15
<b>O1F</b>	35,15	29,70	31,20	32,02
<b>O2F</b>	31,25	27,00	26,45	28,23
<b>O1AM</b>	35,50	30,50	33,30	33,10
<b>O2AM</b>	29,85	26,40	28,70	28,32
<b>average of genotypes</b>	32,69	28,21	29,24	

LSD<sub>0,05</sub>(genotypes)=1,12; LSD<sub>0,05</sub>(treatments)=1,71

Table 4

Effect of seed treatments for oil content (%) of soybean varieties

Treatments	Oil content (%)			
	Aires	Bahia	Pannónia kincse	Average of treatments
<b>K</b>	20,65	23,00	23,75	22,47
<b>O1</b>	18,75	20,00	18,90	19,22
<b>O2</b>	21,50	22,80	21,80	22,03
<b>O1F</b>	19,10	20,85	20,00	19,98
<b>O2F</b>	21,60	22,10	22,45	22,05
<b>O1AM</b>	19,15	20,50	19,15	19,60
<b>O2AM</b>	22,25	22,85	22,35	22,48
<b>average of genotypes</b>	20,43	21,73	21,20	

LSD<sub>0,05</sub>(genotypes)=0,71; LSD<sub>0,05</sub>(treatments)=1,08

The prominent role of soybean in animal feeding is related to its high protein and oil content. Soybean oil is often added separately to soybean meal during feed production, which significantly increases prime cost. However, there are several types of soybean based feed on the market, the best known are full-fat soybean (FFS) and low-fat soybean (SBM) (Smidt et al. 2014). At the FFS the oil is not extracted during processing, so the combined indicator of protein content and oil content determines the feed value (Pro-Fat value). From this point of view, the lowest category is 50 and the most valuable is 52-54 Pro-Fat value. In our study the highest average of Pro-Fat value was for the Aires cultivar (53.12%) (Table 5). Among the treatments, we found a significant difference between untreated control, O1 and its combinations (O1F, O1AM) and O2AM.

Table 5

Treatments	Pro-Fat value (%) of soybean genotypes			
	Aires	Bahia	Pannónia kincse	Average of treatments
<b>K</b>	50,85	49,35	48,45	49,55
<b>O1</b>	54,55	51,20	52,25	52,67
<b>O2</b>	52,60	49,15	48,80	50,18
<b>O1F</b>	54,25	50,55	51,20	52,00
<b>O2F</b>	52,85	49,10	48,90	50,28
<b>O1AM</b>	54,65	51,00	52,45	52,70
<b>O2AM</b>	52,10	49,25	51,05	50,80
<b>average of genotypes</b>	53,12	49,94	50,44	

LSD<sub>0,05</sub>(genotypes)=0,78; LSD<sub>0,05</sub>(treatments)=1,18

## CONCLUSIONS

The application of rhizobium inoculation alone and in combinations with arbuscular mycorrhiza and fungicide seed dressing significantly increased grain yield of the three studied soybean varieties. However, in our study O1 was shown to be more effective than O2. We also found that differences could be observed between the studied varieties according to the applied technology. However, the results did not support that the seed inoculation with the examined arbuscular mycorrhiza product can further increase the yield (t/ha) and the protein content (%). In the future, as a result of stricter rules on the use of plant protection products and the withdrawal of active substances, there is need to develop alternative growing technologies that can help maintain yield stability.

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