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Effect of Soybean Seed Inoculation with Symbiotic Bacteria

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ABSTRACT

Background: The main advantage of leguminous plants is the ability to self-supply atmospheric nitrogen through symbiosis with the bacterium *Bradyrhizobium japonicum*. Therefore, seed inoculation with microbiological preparations is often used in agricultural practice to increase nodulation on the roots.

Methods: A field experiment was conducted to examine the effect of commercial preparations or coated seeds containing *Bradyrhizobium japonicum* on soybean yield and quality. The experiment was performed in four replicates in a randomized block design. The seeds were inoculated using commercial preparations (HiStick® Soy, Nitragina®, TURBOSOY®, PRIMSEED® BIOM Soja, bi soya, RHIZOBIUM SOJA), which were mixed with the seeds on the day of sowing. Sowing coated seeds using the "Fix Fertig" technology did not require seed inoculation before sowing.

Result: It has been demonstrated that HiStick® Soy and TURBOSOY® exerted the most beneficial effect on nodulation, SPAD index, number of pods per plant, TSW and yield. The preparation RHIZOBIUM SOY was also highly effective. Sowing inoculated or coated seeds positively affected the protein content of the seeds but the fat content was the highest in the control seeds. It has been demonstrated that the weather conditions in individual years modified the investigated parameters.

Key words: Chemical composition, *Glycine max* (L.) Merr., Microbiological preparations, Yield.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr] is one of the most important crops in the world. This is due to the possibility of versatile utilization of seeds, especially for food and feed purposes (Jarecki and Migut, 2022). However, the most important advantage of this species is the symbiosis with rhizobia and the ability of plants to self-supply nitrogen from the air. Therefore, seed inoculation with microbiological preparations is often used in agricultural practice to increase nodulation on the roots (Kühling *et al.*, 2018). Santos *et al.* (2019) reminded that it has been more than a century since the first microbial inoculant was developed for plants. Impressive progress is now being recorded in the production, commercialization and use of inoculants. Previous studies (Jarecki 2020; Akley *et al.*, 2023) have shown that such treatment is necessary in some regions of soybean cultivation and recommended in others. Torres *et al.* (2022) showed that soybean seed inoculation resulted in an increase in seed yield from 13.3% to 17.3%, depending on the variant tested. However, the effectiveness of commercial microbial preparations is not always satisfactory, as noted by Kühling *et al.* (2018). Zimmer *et al.* (2016) proved that inoculation of soybean seeds at low temperatures resulted in lesser effects than expected. Other authors (Prusiński *et al.*, 2020; Nyzhnyk *et al.*, 2022) reported that deficiencies in atmospheric precipitation resulted in a decrease in nodule dry weight. Belyavskaya *et al.* (2022) and Martins *et al.* (2022) have concluded that many factors influence the process of biological nitrogen fixation (BNF), such as variety, soil composition, agronomic practices and environmental conditions. Therefore, the inoculation of soybean seeds does not always result in successful nodulation.

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Many authors (Wächter *et al.,* 2013; Belyavskaya *et al.,* 2022; Kumar *et al.,* 2022) believe that if soybean is cultivated for the first time in a given field, the dose of inoculant can be increased, even up to twice the recommended amount. It does not pose any threat to the environment and results in greater nodulation and yield. On the other hand, Carciochi *et al.* (2019) demonstrated that there was no need for additional inoculant application on soils where soybean was previously cultivated. They demonstrated that symbiotic bacteria could be applied to seeds, but also to soil or a combination of both approaches could be used. López-García *et al.* (2009) have shown that the use of commercial inoculants in soybean cultivation does not always yield the expected results. Therefore, scientists from this field recommend conducting studies in various environments.

Panasiewicz *et al.* (2023) tested commercial soybean seed inoculants and found that they had varying effectiveness. These authors obtained the highest seed yield when HiStick® Soy was applied together with nitrogen fertilization at doses of 30 kg N ha^{-1} or 60 kg N ha^{-1}. Księżak and Bojarszczuk (2022) also demonstrated that the most beneficial effect on soybean yield was achieved through the combined use of inoculant and nitrogen fertilizer, resulting in a 42% increase in seed yield and approximately 28% increase in protein yield. Zilli *et al.* (2021) pointed out, however, that excessive nitrogen application had a negative impact on nodulation and did not increase soybean seed yield. In contrast, Kaschuk *et al.* (2016) argued that nitrogen fertilization of soybean was unnecessary with the development of proper nodulation, while Mirriam *et al.* (2022) proved that soybean seed inoculation was more effective with appropriate phosphorus fertilization (dose of 15 kg P ha^{-1}). Savala *et al.* (2021) confirmed that the course of nodulation in soybean was strongly affected by mineral fertilization, especially with nitrogen and phosphorus. Ilangumaran *et al.* (2021) demonstrated that inoculation of soybean seeds was beneficial treatment because it alleviated environmental stress, such as soil salinity.

Currently, an advanced and progressive technology involves coating soybean seeds with various substances (Korbecka-Glinka *et al.*, 2021; Jarecki 2022). For example, there are commercially available seeds coated with appropriate strains of symbiotic bacteria. Such seeds can be sown without the need for inoculation on the farm, which is a time-consuming procedure (Pedrini *et al.*, 2017). Laktionov *et al.* (2019) reported that the use of certain polymers for seed coating increased the survival rate of rhizobia more than 10-fold, which had significant practical implications.

The aim of the study was to determine the response of soybean to seed inoculation with commercial inoculants or sowing coated seeds containing symbiotic bacteria. The research hypothesis assumed that the effectiveness of the inoculants would vary.

MATERIALS AND METHODS

The experiment was carried out in 2020-2022 on a field of the Podkarpackie Agricultural Advisory Centre PODR in Boguchwała (21°57′ E, 49°59′ N), Podkarpackie Province, Poland. The experiment was performed in four replicates in a randomized block design. The factors tested were commercial inoculants for soybean seeds containing *Bradyrhizobium japonicum*. Below are the designations of the individual inoculants:

A - Control (without inoculation).
B - HiStick® Soy (BASF SE, Ludwigshafen am Rhein, Germany).
C - Nitragina® (BIOFOOD S.C., Wałcz, Poland).
D - TURBOSOY® (Saatbau, Leonding, Austria).
E - PRIMSEED® BIOM Soja (INTERMAG sp. z o.o., Olkusz, Poland).
F - Bi soya (Agrarius, Sp. z o.o., Przemyśl, Poland).
G - RHIZOBIUM SOJA (BIO-GEN Sp. z o.o., Łódź, Poland).
H - Coated seeds ready for sowing (seeds inoculated by Saatbau Polska Sp. z o.o., Środa Śląska, Poland) .

The experiment was conducted on the variety Abelina, recommended for cultivation in the research area. The seeds were inoculated using commercial preparations, which were mixed with the seeds on the day of sowing. The inoculation procedure was carried out according to the information provided on respective preparations. Only in Variant H the seeds were pre coated with *B. japonicum* bacteria (Fix Fertig technology from Saatbau Polska Sp. z o.o.), which did not require additional inoculation. No chemical seed treatment was applied.

The seeds were sown on the following dates: April 15, 2020, April 21, 2021 and April 19, 2022. The plot area was 15 m^2 with 1.5 m^2 isolation strips. The experimental field had no previous history of soybean cultivation. The fore crop was winter wheat. Sixty germinating seeds were sown per square meter. The row spacing was 45 cm and the sowing depth was 3.5 cm. Mineral fertilization consisted of 40 kg ha^{-1} P_2O_5 (superphosphate 19%) and 60 kg ha^{-1} K_2O (potassium salt 60%). Mineral fertilization with nitrogen was not applied. Mandryl 500 SC (metobromuron) and Corum 502,4 SL (bentazon, imazamoks) were used for weed control. Insecticides and fungicides were not applied.

The experiment was established on sandy loam soil, Haplic Luvisol. The soil was slightly acidic from 5.8 to 6.3 1 mol/L KCl. The content of available phosphorus (P_2O_5 16.9 8.8 mg 100 g^{-1} soil) and potassium (K_2O 19.8 21.7 mg 100 g^{-1} soil) was very high or high (Table 1). Soil samples analysis was carried out at the District Chemical-Agricultural Station in Rzeszów, according to the Polish standards.

Weather conditions were given according to data of the meteorological station of the University of Rzeszów located in the Rzeszów Zalesie municipal district at a 5 km distance from the experimental field. High rainfall was recorded in June 2020, August 2021 and September 2022. The lowest precipitation occurred from May to August 2022, additionally at high air temperatures. July 2021 was also characterized by high air temperatures (Table 2).

Table 1: Chemical analysis of soil (30 cm).

Parameter	Unit	Year		
		2020	2021	2022
pH in 1 mol/L KCl	-	6.3	6.1	5.8
N_{min}	kg·ha^{-1}	68	74	71
Humus	%	1.4	1.1	1.2
K_2O	mg·100 g^{-1}	21.7	20.2	19.8
P_2O_5	Soil	18.8	17.5	16.9

At the end of the flowering stage, 10 roots were dug up from each plot and washed on sieves. This allowed to calculate the number of nodules on the roots and after drying (at 20°C), the dry weight of the nodules was measured.

The soil plant analysis development (SPAD) measurements were conducted during the flowering stage. A SPAD 502P chlorophyll meter (Konica Minolta, Inc. Japan) was used for the measurements. Biometric measurements (number of pods per plant, number of seeds per pod) were performed on 20 plants collected from plots before harvest. Thousand seed weight (TSW) was determined to one decimal place using an analytical balance. Plant density before harvest was calculated per square meter. Soybean was harvested at full maturity in the second or third decade of September. The obtained seed weight from the plots was converted into yield from 1 ha, taking into account the moisture content of 14%.

The obtained results were subjected to statistical analysis using analysis of variance (ANOVA). The significance of differences between the characteristic values was determined using Tukey's half-confidence intervals. Statistical analysis was performed using TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Plants treated with HiStick® Soy and TURBOSOY® preparations had the highest number of nodules on their roots. A high number of nodules was also obtained using the preparation RHIZOBIUM SOJA. The remaining commercial inoculants, including the sowing of coated seeds, yielded lower results compared to HiStick® Soy, TURBOSOY® and RHIZOBIUM SOJA preparations. However, they still showed significant improvements in nodule development compared to the control. The dry weight of papillae on the roots was the highest in variants B and D (Table 3). The dry weight of nodules from the sowing of coated seeds (variant H) was relatively low, but the obtained result was significantly higher compared to the control. Martins *et al.* (2022) reported that inoculated soybean increased nodulation, plant biomass, BNF and yield components compared to uninoculated soybean. In addition, the latter authors demonstrated a strong correlation between nodule dry weight and pod dry weight. Vorobey *et al.* (2022) reported that, on average, the number of nodules formed on a single soybean root after seed inoculation ranged from 14 to 45. Other study demonstrated that the weight of nodules on a single soybean

Table 2: Weather conditions.

Month	Sum of precipitation (mm)				Temperature (°C)			
	2020	2021	2022	Multi-Years	2020	2021	2022	Multi-years
April	10.0	49.4	41.8	46.0	9.2	6.5	7.3	8.7
May	83.3	63.9	2.6	77.1	11.3	12.8	15.5	13.7
June	162.9	47.3	0.9	80.2	18.1	18.8	20.3	17.1
July	18.9	55.0	11.1	95.4	18.8	21.6	20.6	19.0
August	7.3	107.4	10.0	65.0	19.9	17.5	21.1	18.4
September	43.5	85.8	116.1	62.5	15.0	13.1	12.9	13.6

Table 3: Root nodulation measurement, SPAD index and plant density before harvest.

Specification	Number of nodules per plant	Dry weight of nodules per plant (g)	SPAD	Plant density before harvest (pcs·m^{-2})
		Factor (F)		
A	1.08c	0.03d	38.6c	48.6
B	18.6a	0.45a	45.3a	49.3
C	14.4b	0.35b	42.7b	47.5
D	18.9a	0.46a	44.6a	48.9
E	14.3b	0.33bc	42.6b	47.6
F	14.6b	0.36b	42.9b	47.3
G	15.3ab	0.38b	43.6ab	49.1
H	12.6b	0.29c	41.9b	47.6
		Years (Y)		
2020	12.4b	0.29b	43.4a	47.8ab
2021	18.1a	0.44a	45.3a	50.7a
2022	10.7b	0.26b	39.6b	46.3b
		Interaction		
F × Y	r.n.	r.n.	r.n.	r.n.

A-H - Variant of the tested factor. Different letters in the same column indicate significant differences (p<0.05) according to the analysis of variance (ANOVA).

root ranged from 0.22 to 0.24 g, depending on the experimental soil conditions (Miljaković *et al.*, 2022).

The measurement of the SPAD index indicated that the plants inoculated with HiStick® Soy and TURBOSOY® preparations showed the most optimal nutritional status. Higher SPAD measurements were also obtained when the preparation RHIZOBIUM SOJA was used compared to the control (Table 3). Kühling *et al.* (2018) showed that SPAD measurements were significantly higher after seed inoculation with symbiotic bacteria compared to the control; however, the differences were significant only during the early seed filling stage.

The number of plants before harvest was not significantly different between the experimental variants. The interaction of the studied factor with years (F × Y) was not significant. It has been only demonstrated that the weather conditions in individual years modified the investigated parameters (Table 3). Miljaković *et al.* (2022) proved that soybean seed inoculation significantly improved germination energy, seedling vigor and other parameters compared to the control. As a result, this contributed to the desired plant density per unit area.

The inoculation of seeds in the present resulted in a significant increase in the number of pods per plant compared to the control. The smallest effect was observed after sowing coated seeds (variant H), but it was significantly better than in the control. The number of seeds per pod was not significantly different. The most beneficial effect on TSW and seed yield was observed for the inoculation with HiStick® Soy and TURBOSOY® preparations and to a lesser extent with RHIZOBIUM SOJA. For the other formulations tested, the effects were less pronounced, but still significantly higher compared to the control (Table 4). The resulting difference in seed yield after inoculation with HiStick® Soy and TURBOSOY® was more than 40%. The interaction of the studied factor with years (F × Y) was not significant. It has only been found that the weather conditions in individual years modified the investigated parameters. Our results were consistent with findings of Akley *et al.* (2023), who confirmed that commercial inoculants varied in their effectiveness. The most effective allowed for an increase in soybean yield by 20-30% compared to the control. Similarly, Zimmer *et al.* (2016) demonstrated that both the quantity and quality of soybean yields varied and depended on the type of commercial inoculant. In a study conducted by Panasiewicz *et al.* (2023), higher seed yields were obtained using the HiStick® Soy preparation compared to Nitorflora. However, the most optimal results were obtained when *B. japonicum* inoculant and nitrogen fertilization were applied jointly. A study by Prusiński *et al.* (2020) confirmed that soybean responded with increased seed yield when inoculated with HiStick® Soy or Nitragina in combination with nitrogen fertilization. However, the results obtained varied over the years. According to Kumar *et al.* (2022), the application of inoculants had a positive impact on the soybean yield, as well as the accumulation of organic matter and nitrogen in the soil. Zilli *et al.* (2021) reported that soybean seed inoculation resulted in an increase in seed yield from 12% to 18%, depending on the variant tested. However, the differences were not always statistically significant. Lopez-Garcia *et al.* (2009) did not obtain in their study the expected effects with soybean seed inoculation. Wächter *et al.* (2013) proved that the effectiveness of sowing coated seeds using the Fix Fertig technology was lower compared to traditional seed inoculation. Namozov *et al.* (2022) in turn demonstrated that yield components, *i.e.* the number of pods and 1000 seed weight, were significantly higher after inoculation, with the best results observed after applying a double dose.

In the present study, sowing inoculated or coated seeds positively affected the protein content in the seeds compared

Table 4: Yield components and seed yield.

Specification	Number of pods per plant	Number of seeds per pod	Thousand grains weight (g)	Yield (t ha^{-1})
		Factor (F)		
A	20.6c	2.01	145.3c	2.92c
B	26.3a	1.98	160.3a	4.12a
C	24.9ab	2.02	151.5b	3.62b
D	26.5a	1.99	162.4a	4.19a
E	24.6ab	2.02	152.3b	3.60b
F	24.4ab	2.01	151.9b	3.52b
G	25.3a	2.00	155.7ab	3.87ab
H	22.8b	2.03	150.3b	3.31b
		Years (Y)		
2020	26.3a	1.98ab	152.1b	3.79a
2021	21.6b	2.17a	168.3a	4.00a
2022	25.4a	1.87b	140.7c	3.09b
		Interaction		
F x Y	r.n.	r.n.	r.n.	r.n.

A-H - variant of the tested factor. Different letters in the same column indicate significant differences ($p < 0.05$) according to the analysis of variance (ANOVA).

Table 5: Protein and fat content and yield of both components.

Specification	Protein total(% DM)	Crude fat(% DM)	Protein yield (t1.ha^{-1})	Oil yield (t1.ha^{-1})
		Factor (F)		
A	33.6c	20.8a	0.98d	0.61d
B	37.6a	19.3b	1.55a	0.80a
C	35.6ab	19.5b	1.29b	0.71b
D	37.5a	19.1b	1.57a	0.80a
E	35.9ab	19.6b	1.29b	0.71b
F	35.8ab	19.7b	1.26b	0.69bc
G	36.6a	19.4b	1.42ab	0.75ab
H	34.8b	19.9b	1.15b	0.66cd
		Years (Y)		
2020	35.6b	20.6a	1.35a	0.78a
2021	34.9b	20.1a	1.40a	0.80a
2022	37.3a	18.3b	1.15b	0.57b
		Interaction		
F x Y	r.n.	r.n.	r.n.	r.n.

A-H - variant of the tested factor. Different letters in the same column indicate significant differences ($p < 0.05$) according to the analysis of variance (ANOVA).

to the control. However, the fat content was significantly higher in the control seeds compared to the variants with inoculants and coating. The protein and fat yield per hectare was the highest after seed inoculation with HiStick® Soy and TURBOSOY® preparations. Good results were also recorded after seed inoculation with the preparation RHIZOBIUM SOJA. The chemical composition of the seeds and protein and fat yields were influenced by the weather conditions during the years of the study (Table 5). Kühling *et al.* (2018) reported that the protein content in soybean seeds was significantly higher after seed inoculation in the experiment conducted in Germany, but this effect was observed only once in Russia. The protein yield was significantly higher after inoculation but only in the warmest habitat. Legget *et al.* (2017) demonstrated that the use of inoculants resulted in higher yield increases in Argentina (190 kg ha^{-1}) compared to the United States (60 kg ha^{-1}). Księżak and Bojarszczuk (2022) applied inoculation with Nitragina or Hi®Stick Soy preparations in combination with nitrogen fertilization and obtained increased the protein and fiber content in soybean seeds, but reduced the ash and fat content. Nimnoi *et al.* (2014) reported that all applied inoculation combinations significantly increased the content of nutrients in soybean plants.

CONCLUSION

Seed inoculation is an important treatment in soybean agronomy, especially if the species has not been previously cultivated in a given field. The conducted experiment demonstrated that all commercial seed inoculation products, including coated seeds, significantly increased soybean yield compared to the control group. However, the best results were obtained after using the HiStick® Soy and TURBOSOY® preparations. The difference in seed yields after using these inoculants was 1.2 and 1.3 t ha^{-1}, respectively, compared to the control. Sowing of inoculated or coated seeds positively affected the protein content in the seeds, while the fat content was the highest in the control seeds. It has been documented that the weather conditions modified the investigated parameters in individual years of the study.

Conflict of interest: None.

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