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Reaction of soybean [*Glycine max* (L.) Merr.] to seed inoculation with *Bradyrhizobium japonicum* bacteria

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Abstract: The aim of the study was to assess soybean response to sowing material inoculation with HiStick® Soy preparation, containing *Bradyrhizobium japonicum*. Based on the obtained results, it was found that the inoculation significantly increased the number and dry weight of nodules on soybean roots compared to control. The bacterial preparation significantly increased the number of pods per plant. As a result, a significant increase in seed yield (0.58 t/ha) was obtained compared to control. HiStick® Soy increased total protein content in seeds. Protein and fat yield was higher after seed inoculation by 318 kg/ha and 101 kg/ha, respectively, compared to control.

Keywords: nodulation; cultivar; yield components; symbiotic bacteria; chemical composition

In soybean cultivation, inoculation of sowing material with bacteria fixing free nitrogen from the air is an especially important procedure (Iturralde et al. 2019). This treatment is particularly recommended when native strains of symbiotic bacteria are not present in the soil, or there are too few of them (Zimmer et al. 2016). A good solution, in this case, may be the purchase for sowing material industrially inoculated with symbiotic bacteria (Flajšman et al. 2019) or the purchase of a commercial inoculant and its application on seeds (Jarecki et al. 2016, Pannecoucque et al. 2018, Jarecki and Bobrecka-Jamro 2019). Althabegoiti et al. (2008) reported that commercial soybean inoculant could be used on seeds or in the soil, in which they would be sown. These authors obtained better results in the latter case.

Numerous studies (Althabegoiti et al. 2008, Abou-Shanab et al. 2017) have demonstrated that the occurrence of *Rhizobium* bacteria in soil is common in some regions. In such a case, inoculation with commercial preparations may be less effective because native *Rhizobium* strains are competitive in establishing symbiosis (Wongphatcharachai et al. 2015, Iturralde et al. 2019). For these reasons, Pannecoucque et al. (2018) considered it important to carry out tests determining the efficiency of individual bacterial strains in N_2 fixing and, consequently, their effect on soybean yield. With proper nodulation, soybean plant demand for nitrogen is met in 40–57% (Zimmer et al. 2016) and even up to 60% (Salvagiotti et al. 2008) by biological N_2 fixing. Soybean cultivation does not require mineral nitrogen fertilisation (Kaschuk et al. 2016) or only a small dose of 20 to 60 N kg/ha (Bobrecka-Jamro et al. 2018). This has an important economic (Leggett et al. 2017) and environmental (Wongphatcharachai et al. 2015) aspect.

Many reports (Zimmer et al. 2016, Leggett et al. 2017, Adjetey 2019) showed that the use of seed inoculation significantly increased soybean yield compared to controls. In contrast, Abou-Shanab et al. (2017) reported that inoculation did not always increase soybean seed yield. Kaschuk et al. (2016) found that nodule formation on soybean roots was limited by high total nitrogen content in the soil. Duzan et al. (2004) added that nodulation could be inhibited by abiotic stress, such as low soil pH, low soil temperature or high soil salinity. Narożna et al. (2015) presented

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interesting studies on the survival of *Rhizobium* strains in the soil in the successive years following soybean cultivation. Therefore, experiments in this field should be considered as particularly important.

The aim of the study was to assess soybean plant response to sowing material inoculation with HiStick® Soy preparation, containing *Bradyrhizobium japonicum*. The research hypothesis assumed that HiStick® Soy would have a positive effect on the tested parameters as well as the quantity and quality of seed yield as compared to control.

MATERIAL AND METHODS

A strict field experiment was carried out in the years 2017–2019. It was located on an individual farm in Makowisko (50°3'N, 22°47'E), Podkarpackie Voivodeship, Poland. The first factor in the experiment was the HiStick® Soy biological preparation containing *Bradyrhizobium japonicum* and a control object (without inoculation). HiStick® Soy was applied according to the manufacturer's label (BASF, Ludwigshafen, Germany) on the day of sowing. One package (400 g) was used for 100 kg of seeds. The second factor was soybean cultivars: Annushka, Lajma, Madlen, Violetta, Atlanta and Smuglyanka. The breeder of cultivars was the Scientific Research Center of Soya Development, AgeSoya Sp. z o.o., Poland. The experiment was carried out in four replications in a split-plot design (n = 48).

Weather conditions are given according to meteorological data of the Experimental Stations for Variety Testing in Skołoszów. The experiment was established on sandy loam soil, Haplic Luvisol (FAO 2015). The soil was slightly acidic. The content of available phosphorus, potassium and magnesium was very high or high (Table 1). Soil sample analysis was carried out at the District Chemical-Agricultural Station in Rzeszów, according to Polish standards.

Sowing of seeds was carried out in the first days of May. The sowing standard was 60 germinating seeds per m^2. The row spacing was 45 cm, and the sowing depth was 4 cm. The single plot area was 15 m^2. Winter wheat served as the forecrop. No soybean was previously grown in the experimental field. Phosphorus and potassium fertilisation amounted to 17.44 P kg/ha and 49.8 K kg/ha, respectively. Nitrogen fertilisation was not applied. To control weeds, Afalon Dispersive 450 S.C. (linuron) was used at a dose of 1.5 L/ha. Diseases and pests were not controlled due to their sporadic occurrence.

In the flowering stage (BBCH 65), 20 roots were randomly collected from one plot and then the number and dry weight of the nodules were determined. At the technical maturity stage, 20 plants were collected for measuring the number of pods per plant and the number of seeds in the pod. Thousand seed weight was determined on a scale to the nearest 0.1 g. The seeds were harvested at full maturity (BBCH 89). Seed yield obtained from the plots was converted into a yield per 1 ha with a moisture content of 15%.

The content of protein and fat in seeds was determined with the near-infrared spectroscopy (NIRS) method using an FT NIR MPA spectrometer (Bruker, Billerica, USA). Protein (PN-EN ISO 20483: 2014-02) and fat (PN-EN ISO 11085: 2015-10) yields were calculated from seed yield and the percentage of a given component in seeds.

The results were statistically analysed (Stat Soft, Inc., Tulsa, USA) using analysis of variance. The significance of differences between trait values was assessed based on Tukey's confidence intervals, at the significance level of P = 0.05. Results of interaction are not included in the tables due to the lack of dependence between the factors studied.

RESULTS AND DISCUSSION

Weather conditions varied during the years of research (Table 2). The air temperature was below the long-term average in May 2017 and 2019 during seed sowing and plant emergence. The high average temperature was recorded in June 2019. In subsequent months, temperatures did not differ significantly from the long-term average. Rainfall varied in individual years and months. In 2017, high precipitation was recorded in September. In 2018, the highest precipitation was in June. In turn, in 2019, May was the wettest month.

HiStick® Soy significantly increased the number and dry weight of nodules on the roots compared

Table 1. Properties of the soil profile (0–60 cm)

Year	Soil pH (1 mol/L KCl)	C_{org} (%)	N_{min}	P	K	Mg
			(mg/kg DM of soil)			
2017	6.24	1.06	14.0	123.8	287.2	44
2018	5.80	0.96	12.7	98.1	186.8	25
2019	5.90	1.42	14.5	145.6	228.3	54

C_{org} – organic carbon; N_{min} – mineral nitrogen; DM – dry matter

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Table 2. Weather conditions

Measurement	Year	IV	V	VI	VII	VIII	IX	X
Mean temperature (° C)	2017	6.4	12.6	17.3	18.0	18.4	12.5	8.3
	2018	10.9	14.9	17.0	18.5	18.4	12.9	7.3
	2019	7.3	11.8	19.5	17.9	17.7	12.7	8.1
	multi-year	9.0	14.1	16.6	18.5	18.1	13.4	8.8
Precipitation (mm)	2017	42.7	68.4	48.7	43.0	21.2	102.5	58.4
	2018	24.3	47.0	104.7	98.1	84.3	34.6	40.1
	2019	46.7	158.6	25.4	60.2	101.9	33.7	37.9
	multi-year	46.0	71.6	79.2	94.3	63.0	62.5	47.7

to control (Table 3). On average, 20.3 nodules were found on a single soy root after inoculation, and 0.55 nodules on a control. The analysed cultivars significantly differed in both nodule number and their dry weight. The highest number of nodules and their highest dry weight were obtained in cvs. Lajma and Atlanta, while the lowest in the cv. Violetta. The number and dry weight of nodules varied over the years of the study. In 2018, an average of 14.1 nodules was recorded per root. The fewest nodules (6.5 on average) and their lowest dry weight were obtained in 2017. Weather conditions had a significant impact on nodulation. Zerpa et al. (2013) obtained the highest number of nodules on soybean roots (17.5 on average) after combined use of two commercial bacterial strain. Nodulation was not found in the control object. Adjetey (2019) demonstrated the efficacy of commercial inoculates and observed few nodules in control plants (without inoculation). Abou-Shanab et al. (2017) concluded that the presence of native strains of *Rhizobium* bacteria in the soil also caused nodulation on non-inoculated roots (control). The obtained results of soybean seed inoculation by the authors mentioned above vary depending on the bacterial strain, soil nitrogen content, cultivar and study region. Wongphatcharachai et al. (2015) also confirmed that the number and size of nodules on soybean roots depended on many factors, including

Table 3. Impact of seed inoculation on nodulation

Specification	The number of nodules per plant	The dry weight of nodules (g) per plant
Inoculation		
Control	0.55^{b}	0.05^{b}
HiStick® Soy	20.3^{a}	0.73^{a}
Cultivar		
Annushka	10.9^{ab}	0.43^{ab}
Lajma	12.7^{a}	0.55^{a}
Madlen	8.9^{bc}	0.26^{c}
Violetta	7.8^{c}	0.23^{c}
Atlanta	12.6^{a}	0.53^{a}
Smuglanka	9.7^{bc}	0.36^{bc}
Year		
2017	6.50^{c}	0.23^{c}
2018	14.1^{a}	0.55^{a}
2019	10.7^{b}	0.39^{b}

Values with the same letters are not significantly different at $P < 0.05$

Table 4. Yield components

Specification	The number of pods per plant	The number of seeds per pod	Thousand grains weight (g)
Inoculation			
Control	30.3^{b}	1.88^{a}	148.8^{a}
HiStick® Soy	35.3^{a}	1.90^{a}	152.3^{a}
Cultivar			
Annushka	32.9^{ab}	1.79^{b}	145.6^{b}
Lajma	31.6^{b}	1.81^{b}	147.8^{b}
Madlen	32.1^{b}	1.78^{b}	161.8^{a}
Violetta	31.9^{b}	1.95^{ab}	145.9^{b}
Atlanta	34.0^{a}	1.94^{ab}	155.4^{ab}
Smuglanka	34.3^{a}	2.08^{a}	146.8^{b}
Year			
2017	32.8^{b}	1.83^{ab}	142.1^{b}
2018	37.9^{a}	1.77^{b}	158.4^{a}
2019	27.8^{c}	2.09^{a}	151.2^{a}

Values with the same letters are not significantly different at $P < 0.05$

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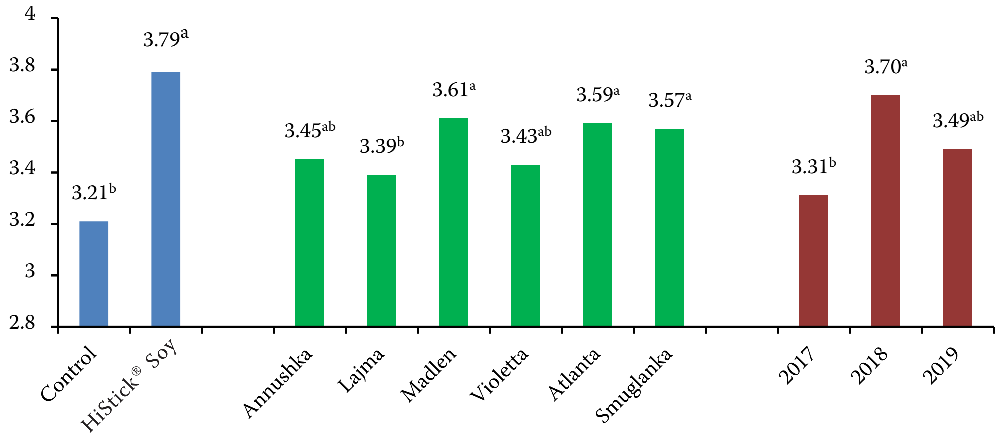

Figure 1. Soybean yield (t/ha), the average for factors. Values with the same letters are not significantly different at $P < 0.05$

the location of the plantation and bacterial strain. Iturralde et al. (2019) also obtained a varied number and dry weight of nodules on soybean roots depending on the strain of symbiotic bacteria. This provided the basis for selecting the best one for the production of commercial preparations.

HiStick® Soy has significantly increased the number of pods per plant (Table 4). As a result, a significant increase in seed yield (0.58 t/ha) was obtained compared to control (Figure 1). Madlen, Atlanta and Smuglanka were high yielding cultivars. The cv. Lajma produced a significantly lower seed yield. In the years of research, elements of yield structure and seed yield varied significantly. Seed yield in 2018 was 3.7 t/ha, while in 2017 – 3.31 t/ha. Therefore, the obtained difference amounted to 0.39 t/ha. No significant relationships were found between the examined factors (Table 5).

Previous studies (Pannecoucque et al. 2018, Adjetey 2019) showed that commercial inoculants containing *Bradyrhizobium japonicum* significantly increased yield components and seed yield. Căpățână et al. (2017) obtained an increase in soybean seed yield after inoculation, but only by 3.76% compared to control. Interesting results were presented by López-García et al. (2009), which showed that the improvement of soybean nodulation resulted in a low, insignificant increase in yield, and nitrogen content in seeds did not change. Abou-Shanab et al. (2017) concluded that inoculation of legumes with nitrogen-fixing bacteria did not always result in a significant increase in seed yield. Therefore, recommendations for the use of biological preparations should be related to regional habitat conditions. This was also confirmed by the experiments carried out by Leggett et al. (2017), who showed that the effects of soybean seed inoculation with *Bradyrhizobium japonicum* varied depending on the years of research and the specificity of a given area.

The applied bacterial strain significantly increased protein content in seeds and total protein yield. Crude fat content was not modified, but the fat yield was significantly higher after HiStick® Soy application compared to control (Table 6). The tested cultivars had significantly different content of total protein and crude fat in seeds and the yield of both of these

Table 5. Soybean yield (t/ha)

Year	Inoculant	Cultivar					
		Annushka	Lajma	Madlen	Violetta	Atlanta	Smuglyanka
2017	control	2.97 ± 0.21	2.98 ± 0.18	3.26 ± 0.22	3.19 ± 0.12	3.05 ± 0.19	3.10 ± 0.17
	HiStick® Soy	3.62 ± 0.13	3.63 ± 0.15	3.50 ± 0.20	3.48 ± 0.09	3.43 ± 0.17	3.52 ± 0.16
2018	control	3.22 ± 0.08	3.00 ± 0.13	3.50 ± 0.18	3.21 ± 0.08	3.60 ± 0.11	3.58 ± 0.17
	HiStick® Soy	3.95 ± 0.11	3.84 ± 0.15	4.23 ± 0.16	3.76 ± 0.07	4.29 ± 0.09	4.27 ± 0.18
2019	control	3.12 ± 0.15	3.02 ± 0.11	3.29 ± 0.18	3.19 ± 0.12	3.33 ± 0.16	3.19 ± 0.14
	HiStick® Soy	3.79 ± 0.13	3.84 ± 0.08	3.86 ± 0.21	3.72 ± 0.10	3.81 ± 0.14	3.73 ± 0.09

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Table 6. Chemical seed composition and protein and fat yield

	Specification	Protein total (% DM)	Protein yield (kg/ha)	Crude fat (% DM)	Fat yield (kg/ha)
Inoculation	Control	37.2[b]	1 194[b]	20.8[a]	668[b]
	HiStick® Soy	39.9[a]	1 512[a]	20.3[a]	769[a]
Cultivar	Annushka	38.2[ab]	1 318[ab]	20.7[ab]	714[ab]
	Lajma	37.8[b]	1 281[b]	21.2[a]	719[ab]
	Madlen	38.7[ab]	1 397[a]	21.1[a]	762[a]
	Violetta	39.6[a]	1 358[ab]	20.4[ab]	700[b]
	Atlanta	38.8[ab]	1 393[a]	19.9[b]	714[ab]
	Smuglanka	38.2[ab]	1 364[ab]	20.0[b]	714[ab]
Year	2017	37.6[b]	1 245[b]	21.5[a]	712[b]
	2018	39.4[a]	1 458[a]	19.3[b]	714[b]
	2019	38.7[ab]	1 351[ab]	20.9[ab]	729[a]

Values with the same letters are not significantly different at $P < 0.05$. DM – dry matter

components. The highest total protein content was determined in seeds of the cv. Violetta and crude fat in the seeds of the cvs. Lajma and Madlen. Cvs. Madlen and Atlanta produced the highest protein yield and cv. Madlen the highest crude fat content. Pannecoucque et al. (2018) reported that commercial preparations effectively increased soybean nodulation, which resulted in an increase in protein content in seeds and protein yield compared to control. Flajšman et al. (2019) also obtained a beneficial effect of inoculation on the increase of total protein content in soybean seeds and seed, protein and fat yield. Zimmer et al. (2016) stated that the use of commercial bacterial strain in soybean cultivation was justified. At the same time, they obtained various effects in the form of an increase in protein yield depending on the study area.

The results of this study indicate that inoculation of soybean seeds with a commercial preparation containing *Bradyrhizobium japonicum* bacteria has had beneficial effects. This treatment is particularly recommended when native strains of symbiotic bacteria are not present in the soil, or there are too few of them. Therefore, inoculation of soybean seeds with nitrogen-fixing bacteria should be applied in the study area. However, the effects will be modified by weather conditions.

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