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The Effect of Mineral N Fertilization and *Bradyrhizobium japonicum* Seed Inoculation on Productivity of Soybean (*Glycine max* (L.) Merrill)

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Abstract: Soybean yields can be considerably improved by inoculation with selected *Bradyrhizobium japonicum* strains and fertilization. The aim of this study was to assess the productivity of two soybean cultivars depending on the applied N mineral fertilizers and seed inoculation with *B. japonicum*. The study showed that on average, for both cultivars, the soybean yield was most favorably affected by the combined use of inoculation and nitrogen fertilization (increase in seed yield by 42%, protein yield by about 28%). The application of mineral nitrogen at the dose of 30 or 60 kg·ha^{-1} allowed the increase in the seed yield by about 17% and protein content by about 14% compared to the control. Inoculation of soybean seeds with *B. japonicum* increased the yield of soybeans by about 20%, proteins by about 10% compared to the control, and inoculation of Hi®Stick Soy favored a better yield than Nitragina. Inoculation of seeds with Nitragina or Hi®Stick Soy and fertilization with mineral nitrogen increased the content of protein and fiber in seeds of both soybean cultivars, as well as reduced the amount of ash and fat. The seeds of cv. Aldana had a higher amount of protein and ash than cv. Annushka, but a similar amount of fat and fiber.

Keywords: *Glycine max.* (L.) Merril; *Bradyrhizobium japonicum*; N fertilization; inoculation; seed; yield

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1. Introduction

Soybean [*Glycine max* (L.) Merr.] is an important legume cultivated worldwide and due to the high biological value of protein it is considered the most important protein plant in the world [1–3]. Global soybean production in 2020 was reported to be 361 million tones [4]. Soybean covers about 29% of the world supply of consumer vegetable oil [5]. Oil and protein are the two major seed compositions that give soybean the potentiality for use in various applications [6,7]. The soybean seed contains 40–42% of protein and 18–22% of oil [8–13]. Moreover, soybean consists of carbohydrate, potassium, and sodium [6].

Soybean is a host for N_2, which is a fixing bacteria representing the *Rhizobium* group, and they can obtain up to 50% or more of their N needs through BNF [14–18]. The key bacteria species that are used as soybean inoculants are *B. japonicum* and *B. elkanii* [19]. Soybean inoculation is usually conducted by coating the seeds with bacteria cells before sowing [20]. *Bradyrhizobium* strains remain in areas with previous soybean cropping. In addition, the inoculation benefits may be influenced by the size of the population of soil bacteria that are capable of nodulating soybean, including naturalized strains [17,21,22]. Therefore, re-inoculation is recommended as it can result in increases in grain yield of soybean exceeding 10%, even in soils with high populations of soybean bradyrhizobia [23]. A few of the studies indicated that legume seed inoculation is a profitable practice providing financial benefits [24]. BNF made by the *Bradyrhizobium* genus decreased costs of production and promoted more sustainable soybean yields. This is possible due to the fact that BNF sufficiently contributes to a high soybean production, in addition to replacing the N mineral fertilization [15,16,25]. Although the relevance of biological N nutrition of soybean [*Glycine* *max* (L.) Merr.] is recognized worldwide, inoculation with *B. japonicum* shows variable results and the benefit needs to be validated under current crop production practices.

The aim of this study was to assess the productivity of two soybean cultivars depending on the applied N doses of mineral fertilizers and seed inoculation with *B. japonicum.*

2. Materials and Methods

2.1. Field Experiment and Cultivation Management

A two-factor factorial field experiment was carried out in the years 2017-2018 in split-plot design on a Luvisol soil with sandy loam texture classes [26] in four replications at the Agricultural Experimental Station in Grabów [51°21′18″ N; 21°40′09″ E] (Masovian Voivodeship, Poland) belonging to the Institute of Soil Science and Plant Cultivation—State Research Institute in Puławy. The first experimental factor was soybean cultivar: Aldana (000) early cultivar from the Polish breeding, and a very early one from the Ukrainian breeding, cv. Annushka (0000). The second factor was: The nitrogen fertilization dose ($N \cdot ha^{-1}$): 1—control—0 kg; 2—30 kg; 3—60 kg; 4—Hi®Stick + 0 kg; 5—Nitragina IUNG Puławy + 0 kg; 6—30 kg + Nitragina IUNG Puławy; 7—60 kg + Nitragina IUNG Puławy; 8—30 kg + Hi®Stick Soy; 9—60 kg + Hi®Stick Soy. Ammonium nitrate (NH_4NO_3) was used.

Seed material was inoculated with two inoculants—domestic Nitragina (IUNG-PIB, PL) or foreign HiStick® Soy (BASF Agricultural Specialities Limited, GB). Both Nitragina and HiStick® Soy contain *B. japonicum* strains specific for soybean, in which peat is a carrier. Both cultivars were fertilized with mineral N in doses: 0, 30, and 60 $kg \cdot ha^{-1}$ before planting by mixing with surface soil.

The plots for sowing were 36 m^2 in size and 32.3 m^2 for harvesting. At the beginning of the trial, at a depth of 0–25 cm, the soil layer contained phosphorus (P), potassium (K), and magnesium (Mg): 10.2–12.0; 13.6–19.8; 4.2–6.0 ($mg \cdot 100\ kg^{-1}$ soil), respectively. The mineral N content in the profile of 0–60 cm was 50–55 $kg \cdot ha^{-1}$. The soil pH was 5.6–6.4 (measured in 1 M of potassium chloride (KCl)). Phosphorus (P) and potassium (K) fertilization was applied in doses of 50 and 90 $kg \cdot ha^{-1}$, respectively. The forecrop was winter wheat.

Seeds were sown in row spacing of 24 cm, sowing rate of 80 germinating seeds per m^2, and the sowing depth of 3–4 cm. Both cultivars were sown on 9 May 2017 and 25 April 2018.

Directly after sowing, Stomp 330 EC [3.5 $L \cdot ha^{-1}$] was applied into the soil, and after emergence, Corum 502.4 SL [1.25 $L \cdot ha^{-1}$] by KFMR Krukowiak sprayer was used to control the annual weeds.

Plants were harvested at the full maturity stage (R8) of soybean in the second 10 days of September.

2.2. Data Collection

Before harvesting, after reaching full maturity, the seed yield was determined, as well as the most important morphological traits of 10 plants and structural components of soybean yield: number of pods, number and weight of seeds per plant, number of seeds per nod. Moreover, the number and fresh weight of root nodules per plant and the plant height and the first pod height were determined. After harvesting, the seed yield, seed humidity, and 1000 seed weight (in 14% of moisture) were determined.

2.3. Chemical Analysis of Soybean Seeds

The N content (nitrogen %) in the dry weight of seeds (DWS) was determined by the flow analysis (FA) and spectrometric detection, the total protein by the Kjeldahl distillation method after mineralization in sulfuric acid, as well as the fat and ash content by the Soxhlet method.

2.4. Statistical Analysis

The results were statistically analyzed with the use of the variance analysis using Statistica v.10.0 software (StatSoft, Kraków, Poland). Tukey's multiple comparison test was used to compare the differences between the means for the cropping method, while confidence intervals for the means of LSD (α = 0.05) were used.

2.5. Weather Conditions

In both years of the study, the total sum of precipitation was similar in the vegetation season, but it was less by about 30 mm than the mean from the long-term average. In 2017, at the end of the second 10 days of April, strong cooling has occurred which delayed the sowing of soybean (sowing was in the first 10 days of May) (Figure 1). At the beginning of the third 10 days of April and the first 10 days of May, the high amount of precipitation was noted (exceeded by 77% of the long-term average), which made it difficult to make the mechanical treatment in the field of soybean crop. In June and in the first 10 days of July, a small amount of rainfall was recorded, compared to the long-term average, which had an unfavorable effect on the development of soybean crop. In the first 10 days of August, there were very small amounts of rainfall (0.9 mm), which caused premature soybean maturity. In 2018, the amount of precipitation in May and July exceeded the average from multi-years by 70.9 and 41.1%, respectively, which favored the yielding of soybean.

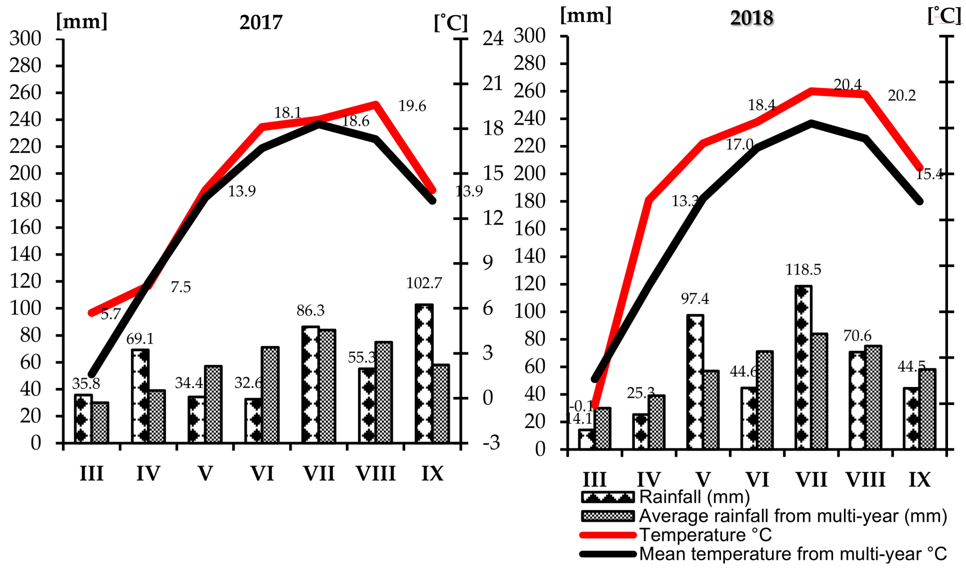

Figure 1. Monthly rainfall and temperature during the growing period in 2017 and 2018, and the long-term (1871–2000) average.

3. Results

The application of N mineral fertilizers and seed inoculation by *B. japonicum* or both the inoculation and N mineral fertilization and course of weather conditions during the growing season significantly affected the yielding of tested soybean cultivar.

In both years of the study, the amount of rainfall during the growing season was similar, but the delayed sowing date and the low amount of rainfall in June and the first 10 days of July in 2017 resulted in that the seed yield this year was lower by 35%, and the protein yield by about 39% than in 2018 (Table 1). On average, during both years of the study, for both cultivars, the N use at a dose of 30 or 60 kg·ha^{-1} increased the seed yield in relation to non-fertilized plants by about 17%, and protein by about 14%. The use of seed inoculation with Nitragina or Hi®Stick Soy, containing bacteria that increase the production of nodules on the soybean root system, positively influenced the level of soybean yielding, and the difference in the seed yield in relation to the control was about 20%, and the increase in protein yield was lower and amounted to about 10%. A more beneficial effect of increasing the N dose from 30 to 60 kg·ha^{-1} and the Hi®Stick Soy than the Nitragina on the soybean yield was also observed. The combined application of inoculation and nitrogen fertilization most favorably increased the seed yields in relation to the control by an average of 42%, and proteins by about 28%. The evaluated cultivars Aldana and Annushka yielded at a similar level in agroecological conditions. Moreover, during all the years of the study, the seeds of both cultivars were characterized by similar humidity during harvesting, regardless of the tested factors.

Table 1. Effect of mineral nitrogen fertilization and inoculation on seed and protein yields in two soybean cultivars in 2017 and 2018.

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Seed Yield (t ha^{-1})			Protein Yield (kg ha^{-1})		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
	0	1.43 ± 0.023 *	2.01 ± 0.072	1.72	402.2 ± 25.20	800.2 ± 35.62	601.2
	30	1.73 ± 0.127	2.12 ± 0.086	1.93	530.0 ± 29.30	750.4 ± 36.50	640.2
	60	1.66 ± 0.079	2.26 ± 0.138	1.96	508.4 ± 23.60	780.9 ± 36.60	644.6
	Hi Stick + 0	1.63 ± 0.124	2.60 ± 0.464	2.12	499.3 ± 18.90	789.3 ± 35.60	644.3
Aldana	Hi Stick + 30	1.74 ± 0.136	3.13 ± 0.276	2.43	543.7 ± 55.00	932.6 ± 68.20	738.2
	Hi Stick + 60	1.72 ± 0.124	3.39 ± 0.275	2.56	580.1 ± 59.80	1008.3 ± 72.20	794.2
	Nitragina + 0	1.52 ± 0.104	2.42 ± 0.268	1.97	456.0 ± 29.80	753.2 ± 45.60	604.6
	Nitragina + 30	1.71 ± 0.092	2.90 ± 0.187	2.31	534.4 ± 32.20	864.6 ± 36.90	699.5
	Nitragina + 60	1.74 ± 0.072	3.11 ± 0.266	2.42	576.5 ± 56.20	917.9 ± 65.20	747.2
mean		1.65	2.66	-	514.6	844.2	-
	0	1.36 ± 0.206	2.03 ± 0.157	1.69	357.0 ± 18.50	751.6 ± 25.50	554.3
	30	1.65 ± 0.068	2.29 ± 0.093	1.97	464.1 ± 19.20	814.4 ± 55.60	639.3
	60	1.86 ± 0.108	2.42 ± 0.231	2.14	558.0 ± 56.60	856.6 ± 65.20	707.3
	Hi Stick + 0	1.78 ± 0.059	2.51 ± 0.064	2.14	522.8 ± 35.60	817.8 ± 55.80	670.3
Annushka	Hi Stick + 30	1.98 ± 0.121	2.87 ± 0.168	2.43	594.4 ± 22.20	876.3 ± 30.40	735.4
	Hi Stick + 60	1.89 ± 0.125	3.14 ± 0.425	2.51	602.3 ± 25.70	954.4 ± 45.90	778.4
	Nitragina + 0	1.56 ± 0.065	2.48 ± 0.384	2.02	448.5 ± 32.20	813.4 ± 45.20	630.9
	Nitragina + 30	1.75 ± 0.041	2.76 ± 0.166	2.26	513.9 ± 33.50	852.6 ± 39.90	683.2
	Nitragina + 60	2.08 ± 0.179	3.00 ± 0.462	2.54	624.0 ± 48.20	897.3 ± 40.10	760.6
mean	-	1.77	2.61	-	520.5	848.3	-
Mean for cultivar	-	1.71	2.63	-	517.5	846.2	-
LSD (α = 0.05):			n.s.			n.s.	
Cultivar (A)		n.s. **			2.802		
Treatment (B)	-	0.233	0.523	-	5.493	47.69	-
B/A		n.s.	n.s.		7.769	67.45	
A/B		n.s.	n.s.		5.102	43.85	

* Mean ± standard error; ** significant at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test; n.s.: Non-significant.

The favorable distribution of rainfall in 2018 significantly affected the growth and development of soybean, and the plants were characterized by a more favorable structure, which also resulted in a higher yield level. Soybean plants in cultivation with seed inoculation and different nitrogen fertilization rates were characterized by larger seeds, and set more pods and seeds on the plant than plants without fertilization and inoculation (Tables 2–4). In both years of the study, the Annushka cultivar was characterized by a greater number of pods and seeds, as well as weight per plant, but less 1000 seeds weight. Moreover, these factors only slightly changed the height of the first pod and the height of the soybean plants (Table 5). Unfavorable soil moisture conditions (drought) occurring during the growing season resulted in poor nodule fixation on the soybean root system. The formation of nodules took place only after significant rainfall at the turn of July and August. The number of nodules as well as their weight were small, although more were recorded on the root system of plants that received the Hi®Stick Soy (Table 6). In addition, the cv. Annushka set slightly more nodules than the cv. Aldana.

Table 2. Weight of 1000 seeds and humidity of seeds depending on the mineral N fertilization applied and the inoculation.

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Weight of 1000 Seeds (g)		
		Year		Mean
		2017	2018	
Aldana	0	146.6 ± 3.192 *	166.3 ± 6.218	156.4
	30	153.9 ± 6.350	169.2 ± 2.607	161.6
	60	150.3 ± 4.024	167.4 ± 12.958	158.9
	Hi Stick + 0	154.7 ± 3.556	175.6 ± 4.170	165.2
	Hi Stick + 30	154.6 ± 2.010	180.3 ± 9.127	167.5
	Hi Stick + 60	154.1 ± 7.800	180.4 ± 5.406	167.3
	Nitragina + 0	148.8 ± 7.005	187.1 ± 11.165	168.0
	Nitragina + 30	148.6 ± 8.361	177.6 ± 4.195	163.1
	Nitragina + 60	148.3 ± 11.237	179.2 ± 4.565	163.8
mean		151.1	175.9	-
Annushka	0	115.3 ± 3.456	119.9 ± 11.173	117.6
	30	114.5 ± 1.979	130.5 ± 4.161	122.5
	60	116.2 ± 6.313	127.3 ± 7.093	121.8
	Hi Stick + 0	118.3 ± 5.347	132.4 ± 7.068	125.4
	Hi Stick + 30	118.5 ± 0.829	135.1 ± 10.401	126.8
	Hi Stick + 60	116.3 ± 5.874	138.2 ± 11.258	127.3
	Nitragina + 0	111.6 ± 5.354	131.5 ± 3.583	121.6
	Nitragina + 30	115.7 ± 5.325	134.6 ± 4.710	125.2
	Nitragina + 60	110.9 ± 3.566	133.5 ± 14.679	122.2
mean	-	115.3	131.7	-
Mean for cultivar	-	133.2	153.8	-
LSD (α = 0.05):			0.278	
Cultivar (A)		0.897 **		
Treatment (B)		1.661	0.641	-
B/A		2.350	0.906	
A/B		1.565	0.576	

* Mean ± standard error; ** significant at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test.

Table 3. Pod number per plant and seed number per pod depending on the mineral N fertilization applied and the inoculation.

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Pod Number per Plant			Seed Number per Pod		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
Aldana	0	8.20 ± 0.406 *	12.08 ± 2.143	10.14	1.63 ± 0.138	1.85 ± 0.109	1.74
	30	8.13 ± 0.163	12.18 ± 1.883	10.16	1.55 ± 0.172	1.92 ± 0.065	1.74
	60	8.03 ± 1.101	12.80 ± 2.253	10.41	1.58 ± 0.141	1.82 ± 0.157	1.70
	Hi Stick + 0	8.40 ± 1.387	13.04 ± 2.258	10.72	1.88 ± 0.143	1.86 ± 0.010	1.87
	Hi Stick + 30	8.33 ± 0.352	14.25 ± 2.693	11.29	1.64 ± 0.140	1.82 ± 0.089	1.73
	Hi Stick + 60	8.48 ± 0.991	14.40 ± 2.577	11.44	1.64 ± 0.098	1.83 ± 0.145	1.74
	Nitragina + 0	8.10 ± 0.831	13.43 ± 2.144	10.76	1.58 ± 0.162	1.88 ± 0.045	1.73
	Nitragina + 30	8.07 ± 0.452	13.32 ± 2.568	10.69	1.60 ± 0.131	1.79 ± 0.137	1.69
	Nitragina + 60	8.09 ± 0.786	13.70 ± 2.395	10.89	1.57 ± 0.113	1.84 ± 0.063	1.70
mean		8.20	13.24	-	1.63	1.85	-
Annushka	0	10.10 ± 1.471	16.50 ± 2.028	13.30	1.76 ± 0.058	2.00 ± 0.227	1.88
	30	10.53 ± 1.482	17.68 ± 2.228	14.11	1.69 ± 0.095	1.92 ± 0.150	1.81
	60	10.36 ± 1.810	17.68 ± 1.310	14.02	1.75 ± 0.116	1.85 ± 0.016	1.80
	Hi Stick + 0	10.70 ± 2.089	17.53 ± 1.941	14.11	1.79 ± 0.073	1.89 ± 0.329	1.84
	Hi Stick + 30	10.73 ± 0.676	18.30 ± 2.239	14.52	1.79 ± 0.159	1.94 ± 0.099	1.86
	Hi Stick + 60	11.35 ± 2.263	18.50 ± 3.473	14.93	1.74 ± 0.174	2.02 ± 0.086	1.88

Table 3. *Cont.*

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Pod Number per Plant			Seed Number per Pod		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
	Nitragina + 0	9.89 ± 1.150	17.60 ± 2.424	13.74	1.69 ± 0.150	1.99 ± 0.150	1.84
	Nitragina + 30	9.80 ± 1.922	18.20 ± 4.024	14.00	1.64 ± 0.112	2.02 ± 0.121	1.83
	Nitragina + 60	10.00 ± 1.437	18.10 ± 3.004	14.05	1.59 ± 0.245	2.01 ± 0.101	1.80
mean	-	10.38	17.79	-	1.71	1.96	-
Mean for cultivar	-	9.29	15.51	-	1.67	1.90	-
LSD (α = 0.05): Cultivar							
(A)	-	0.377 **	0.020	-	n.s.	0.033	-
(B)		0.289	0.348		0.096	0.037	
B/A		0.409	0.493		0.135	0.052	
A/B		0.416	0.285		0.111	0.042	

* Mean ± standard error; ** significant at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test; n.s.: Non-significant.

Table 4. Seed number and weight per plant depending on the mineral N fertilization applied and the inoculation.

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Seed Number per Plant			Seed Weight (g plant^{-1})		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
Aldana	0	13.41 * ± 0.901	22.35 ± 4.049	17.88	1.98 ± 0.161	3.72 ± 0.735	2.85
	30	12.70 ± 2.750	23.28 ± 3.478	17.99	1.96 ± 0.475	3.95 ± 0.607	2.96
	60	12.74 ± 2.818	23.05 ± 2.797	17.89	1.90 ± 0.407	4.10 ± 0.497	3.00
	Hi Stick + 0	15.83 ± 2.207	24.25 ± 5.455	20.04	2.35 ± 0.399	4.48 ± 1.041	3.41
	Hi Stick + 30	13.72 ± 2.829	25.85 ± 4.671	19.78	2.20 ± 0.465	5.09 ± 0.761	3.64
	Hi Stick + 60	13.98 ± 1.299	26.35 ± 7.334	20.16	2.19 ± 0.274	5.14 ± 2.020	3.66
	Nitragina + 0	12.90 ± 2.247	25.20 ± 4.014	19.05	1.91 ± 0.336	4.76 ± 1.013	3.33
	Nitragina + 30	12.99 ± 3.178	23.81 ± 4.379	18.04	1.90 ± 0.532	4.90 ± 0.551	3.40
	Nitragina + 60	12.82 ± 1.483	25.21 ± 3.860	19.01	2.00 ± 0.221	4.88 ± 0.521	3.44
mean		13.45	24.36	-	2.04	4.56	-
Annushka	0	17.87 ± 2.770	32.65 ± 7.795	25.26	2.18 ± 0.219	3.85 ± 0.765	3.02
	30	17.80 ± 3.007	33.55 ± 2.403	25.67	2.22 ± 0.399	4.01 ± 0.282	3.11
	60	18.19 ± 7.295	32.71 ± 2.331	25.45	2.20 ± 1.050	4.14 ± 0.265	3.17
	Hi Stick + 0	19.21 ± 3.506	33.13 ± 5.141	26.17	2.39 ± 0.373	4.40 ± 0.977	3.39
	Hi Stick + 30	19.27 ± 2.257	35.50 ± 7.109	27.38	2.28 ± 0.241	4.97 ± 0.885	3.62
	Hi Stick + 60	19.80 ± 2.408	37.37 ± 5.836	28.58	2.39 ± 0.476	5.15 ± 0.734	3.77
	Nitragina + 0	16.78 ± 2.021	35.00 ± 4.185	25.89	2.15 ± 0.372	4.48 ± 0.612	3.31
	Nitragina + 30	16.23 ± 3.805	36.76 ± 5.717	26.49	2.15 ± 0.661	4.88 ± 0.561	3.51
	Nitragina + 60	15.99 ± 3.883	46.38 ± 5.588	31.18	2.13 ± 0.531	4.93 ± 0.534	3.53
mean	-	17.90	35.89	-	2.23	4.53	-
Mean for cultivar	-			-	2.14	4.55	-
LSD (α = 0.05):							
Cultivar (A)	-	0.060 **	0.236	-	0.053	n.s.	-
Treatment (B)		0.277	0.601		0.091	0.561	
B/A		0.392	0.849		0.128	n.s	
A/B		0.232	0.531		0.087	n.s.	

* Mean ± standard error; ** significant at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test; n.s.: Non-significant.

Table 5. Morphological features depending on the mineral N fertilization applied and the inoculation.

Treatment Nitrogen Rates (kg ha^{-1})	2017				2018			
	Aldana		Annushka		Aldana		Annushka	
	First Pod Height (cm)	Plant Height (cm)	First Pod Height (cm)	Plant Height (cm)	First Pod Height (cm)	Plant Height (cm)	First Pod Height (cm)	Plant Height (cm)
0	14.0 ± 1.971 *	47.8 ± 4.071	19.7 ± 2.632	55.6 ± 5.089	10.0 ± 0.884	45.2 ± 3.899	13.0 ± 0.810	55.4 ± 7.693
30	14.7 ± 1.756	49.5 ± 2.921	21.6 ± 2.952	57.4 ± 2.574	12.1 ± 1.003	47.5 ± 0.978	13.2 ± 1.209	57.3 ± 2.222
60	16.6 ± 2.285	50.1 ± 0.805	18.9 ± 4.458	61.5 ± 1.979	9.9 ± 1.186	46.9 ± 1.847	14.1 ± 1.731	55.9 ± 1.965
Hi Stick + 0	13.6 ± 1.556	51.7 ± 5.137	21.9 ± 1.803	59.2 ± 2.097	10.8 ± 1.596	46.7 ± 2.804	13.8 ± 2.395	55.8 ± 1.105
Hi Stick + 30	13.6 ± 1.728	52.9 ± 2.962	20.9 ± 3.031	57.9 ± 5.018	9.8 ± 0.991	50.2 ± 4.335	13.4 ± 0.804	62.1 ± 2.239
Hi Stick + 60	13.4 ± 1.416	59.0 ± 2.129	19.6 ± 1.725	61.5 ± 6.492	9.9 ± 1.390	52.3 ± 2.834	14.2 ± 2.745	59.8 ± 6.717
Nitragina + 0	16.4 ± 1.859	49.3 ± 2.023	21.5 ± 2.788	57.8 ± 2.812	9.9 ± 0.460	46.4 ± 3.383	14.2 ± 1.424	58.4 ± 3.688
Nitragina + 30	14.3 ± 3.146	53.7 ± 2.612	21.8 ± 2.481	58.2 ± 7.372	9.9 ± 1.665	50.1 ± 2.794	11.9 ± 1.653	60.1 ± 4.033
Nitragina + 60	16.6 ± 2.022	53.1 ± 2.859	22.5 ± 3.161	59.9 ± 7.744	10.9 ± 0.865	48.3 ± 3.593	13.3 ± 2.951	60.0 ± 4.004
mean	14.8	51.9	20.9	58.8	10.4	48.2	10.4	58.3

* Mean ± standard error.

Table 6. Number and fresh matter of nodules per plant depending on the mineral N fertilization applied and the inoculation.

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Number of Nodules per Plant			Fresh Matter of Nodules (g· Plant^{-1})		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
Aldana	0	0.10 *	0.27	0.18	0.04	0.13	0.08
	30	0.05	0.30	1.17	0.02	0.12	0.07
	60	0.02	0.33	0.17	0.04	0.12	0.08
	Hi Stick + 0	0.61	5.60	3.10	0.12	1.40	0.76
	Hi Stick + 30	0.52	6.11	3.31	0.07	2.30	1.18
	Hi Stick + 60	0.59	7.13	3.86	0.02	2.40	1.21
	Nitragina + 0	0.10	6.27	3.18	0.02	3.49	1.76
	Nitragina + 30	0.35	5.47	2.91	0.06	2.23	1.14
	Nitragina + 60	0.10	5.18	2.64	0.02	1.93	0.97
mean		0.27	4.07	-	0.04	1.57	-
Annushka	0	0.11	4.20	2.16	0.05	1.24	0.64
	30	0.10	2.27	1.18	0.05	1.03	0.54
	60	0.11	2.60	1.36	0.05	0.99	0.52
	Hi Stick + 0	1.15	6.13	3.64	0.30	1.37	0.83
	Hi Stick + 30	1.35	5.93	3.64	0.23	1.34	0.78
	Hi Stick + 60	0.92	6.12	3.52	0.19	1.60	0.89
	Nitragina + 0	0.40	9.80	5.10	0.14	3.29	1.71
	Nitragina + 30	0.15	6.20	3.17	0.04	1.73	0.88
	Nitragina + 60	0.20	9.00	4.60	0.03	2.63	1.33
mean	-	0.49	5.81	-	0.12	1.69	-
Mean for cultivar	-	0.38	4.93	-	0.083	1.63	-
LSD (α = 0.05):							
Cultivar (A)		0.091 **	0.192		0.021	n.s.	
Treatment (B)	-	0.123	0.421	-	0.014	0.032	-
B/A		n.s.	n.s.		n.s.	n.s.	
A/B		n.s.	n.s.		n.s.	n.s	

* Mean; ** significant at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test; n.s.: Non-significant.

A more even distribution of rainfall during the growing season in 2018 favored the accumulation of protein in soybeans, as well as caused a reduction in fat content, but had a slight effect on the amount of ash and fiber (Tables 7 and 8). The seeds of cv. Aldana were characterized by higher protein and ash content, a similar amount of fat and fiber, although a slightly higher amount of fat and fiber was observed in the seeds of cv. Annushka than cv. Aldana. A significantly higher protein content was found in the seeds of both soybean cultivars, in which 60 kg·ha^{-1} nitrogen was used and seeds were inoculated with Nitragina or Hi®Stick Soy. The use of seed inoculation and fertilization with mineral nitrogen had a positive effect on the accumulation of protein in the seeds of both soybean cultivars. However, at the same time, it increased the fiber content. In addition, the studied factors limited the amount of ash and fat.

Table 7. Total protein and fat content in dry matter of the soybean seeds depending on the mineral N fertilization applied and the inoculation.

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Protein Concentration (mg·g DW^{-1})			Fat Concentration (mg·g DW^{-1})		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
Aldana	0	281.3 *	271.7	276.5	270.2	251.2	260.7
	30	306.3	282.5	294.4	263.3	253.1	258.2
	60	306.3	289.4	297.8	270.1	259.4	264.7
	Hi Stick + 0	306.3	329.4	317.8	272.2	257.5	264.8
	Hi Stick + 30	312.5	335.6	324.1	264.4	247.3	255.8
	Hi Stick + 60	337.5	336.2	336.8	263.3	246.4	254.8
	Nitragina + 0	300.0	321.3	310.6	266.9	254.3	260.6
	Nitragina + 30	312.5	335.4	323.9	267.7	242.5	255.1
	Nitragina + 60	331.3	338.8	335.1	269.2	244.4	256.8
mean		310.4	315.6	-	268.3	250.7	-
Annushka	0	262.5	270.1	266.3	270.1	252.3	261.2
	30	281.3	281.2	281.3	267.4	262.4	264.9
	60	300.0	282.5	291.3	260.3	265.5	262.9
	Hi Stick + 0	293.7	306.9	300.3	266.6	259.4	263.0
	Hi Stick + 30	300.0	327.5	313.7	266.3	244.6	255.4
	Hi Stick + 60	318.7	329.0	323.8	264.2	243.8	254.0
	Nitragina + 0	287.5	304.9	296.2	268.1	263.2	265.6
	Nitragina + 30	293.7	323.7	308.7	267.4	248.6	258.0
	Nitragina + 60	300.0	334.3	317.2	265.5	246.3	255.9
mean	-	293.0	306.7	-	266.2	254.0	-
Mean for cultivar	-	301.7	311.1	-	267.1	252.3	-
LSD (α = 0.05):							
Cultivar (A)		2.30 **	0.82		n.s.	0.25	
Treatment (B)	-	6.56	6.82	-	4.11	2.18	-
B/A		9.28	9.65		5.81	3.09	
A/B		5.64	5.62		4.74	1.80	

* Mean at $p \leq 0.05$; ** significant at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test; n.s.: Non-significant.

Table 8. Ash and fiber content in dry matter of the soybean seeds depending on the mineral N fertilization applied and the inoculation.

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Ash Content (%)			Fiber Content (%)		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
Aldana	0	6.20 *	6.31	6.26	5.41	5.30	5.36
	30	5.94	6.02	5.98	5.52	5.46	5.49
	60	6.11	6.03	6.07	5.58	5.48	5.53
	Hi Stick + 0	5.85	5.95	5.90	5.61	5.53	5.57
	Hi Stick + 30	5.91	5.91	5.91	5.35	5.22	5.28
	Hi Stick + 60	5.92	5.96	5.94	5.61	5.53	5.57
	Nitragina + 0	6.11	6.12	6.11	5.98	6.02	6.00
	Nitragina + 30	6.05	5.95	6.00	5.62	5.56	5.59
	Nitragina + 60	6.01	5.86	5.94	6.00	6.16	6.08
mean		6.01	6.01	-	5.63	5.58	-

Table 8. *Cont.*

Cultivar	Treatment Nitrogen Rates (kg ha^{-1})	Ash Content (%)			Fiber Content (%)		
		Year		Mean	Year		Mean
		2017	2018		2017	2018	
Annushka	0	6.01	6.12	6.06	6.32	6.24	6.28
	30	5.90	6.14	6.02	6.51	6.42	6.46
	60	5.95	6.03	5.99	6.48	6.60	6.54
	Hi Stick + 0	6.01	5.96	5.98	6.57	6.62	6.60
	Hi Stick + 30	5.74	5.82	5.78	6.61	6.68	6.64
	Hi Stick + 60	5.86	5.78	5.82	6.44	6.39	6.41
	Nitragina + 0	5.93	5.85	5.89	6.76	6.89	6.82
	Nitragina + 30	5.82	5.74	5.78	6.43	6.38	6.40
	Nitragina + 60	5.71	5.76	5.73	6.62	6.73	6.67
mean	-	5.88	5.91	-	6.53	6.55	-
Mean for cultivar	-	5.95	5.96	-	6.08	6.07	-
LSD (α = 0.05):							
Cultivar (A)		0.127 **	0.017		0.153	0.152	
Treatment (B)	-	0.085	0.105	-	0.112	0.170	-
B/A		0.121	0.148		0.158	0.241	
A/B		0.136	0.087		0.167	0.193	

* Mean at $p \leq 0.05$; ** significant at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test.

4. Discussion

In Europe, soybean needs approximately 500 mm of precipitation in the growing season, including at least 300 mm at the flowering and fruit formation stage [27].

The yield of soybean is determined by the climate conditions: Air temperature, total rainfall, and its distribution [28–35]. A negative influence of high air temperature was recorded on the yielding of both cultivars of soybean. The average air temperature at the main development stage was as follows: Flowering and pod formation stage was much lower than 25 °C, although higher than the long-term mean in Poland. Montanez et al. [36] considered this as most favorable for the soybean yield and biological N fixation. The seed yield of soybean mostly depends on the total rainfall in May, July, and August, as soybean plants assimilate approximately 20% N since the beginning of flowering, and 80% during generative development [30,31].

The study by Seneviratne et al. [37] has shown that the inoculation and N fertilizer use promote plant growth and increase yields of soybean. Incorporating 23 kg N·ha^{-1} as the primary fertilizer application and adding 23 kg N·ha^{-1} at the end of flowering stage does not inhibit soybean nodulation. Mourtzinis et al. [38] recorded an increase in yield by 120 kg ha^{-1}, La Menza et al. [39] by up to 11%, while Capatana et al. [40] by up to 30%. Kaschuk et al. [18] stated that mineral N fertilization of soybean caused a decrease in the number of nodules and in the seed yield, but it also depends on the N dose. Prusiński et al. [35] found that in some cases N fertilization in combination with inoculation excels the inoculation alone. Capatana et al. [40] stated that an increase in the seed yield in soybean only after inoculation was increased by 3.7%, while the mineral fertilizer with inoculation increased by about 30%. Albareda et al. [41] found that the seed inoculation resulted in a significantly higher grain yield and nodulation than in the controls. The effect of the mineral N fertilization and the seed inoculation on the soybean yield, result in highly diversified findings [35]. In our own study, the average of 2-year studies, the highest seed yields were recorded after applying HiStick with 60 kg N·ha^{-1} in both cultivars.

In the study by Jarecki et al. [42], using Nitragina, Mikrokomplex, and Nitragina + Mikrokomplex resulted in a significant increase in soybean yield and a change in the chemical composition of seeds. Debela et al. [43] found that the combined application of 2 t VC·ha^{-1} (vermicompost prepared from raw materials, such as soybean straw, wood ash, and animal manure) and 75 kg NPS·ha^{-1} (blended fertilizer used as a source of nitrogen, phosphorus, and sulfur: 19% N, 38% P_2O_5, and 7% S) inoculated with *Rhizobium* strain had resulted in a better and optimum yield of 3870 kg ha^{-1}, which is environmentally sound for soil management and is tentatively recommended for use. Zilli et al. [25] in their studies found that re-inoculation provided average increases in grain yield of 12 to 18% compared to the non-inoculated control. Studies by Saturno et al. [44] showed that the N-fertilizer impaired nodulation and N_2 fixation and did not improve grain production [18,45]. Zilli et al. [25,46] stated that soybean, brings additional economic benefits due to BNF by the bacteria *Bradyrhizobium japonicum*, and BNF allows high soybean yields at a low cost. According to Zuffo et al. [47], the application of 50 kg N ha^{-1} of mineral fertilizer associated with the *Bradyrhizobium* spp. inoculation enhanced the physiological quality of soybean seeds, resulting in higher seed germination percentage and higher emergence and seedling emergence speed index. Zerpa et al. [48] found that the inoculants combination treatment resulted as more favorable to shoot height, dry weight and leaf area, nodule number, as well as dry weight. Leggett et al. [24] showed a significant increase in soybean grain yield due to the inoculation with *B. japonicum*.

Under the favorable humidity in the period from June to August in the second year of the study (2018), a plant response to inoculation and mineral N fertilization was significantly different than in the first year (2017), with the significantly lower precipitation in the above period. According to Korsak-Adamowicz et al. [49], neither drought nor high air temperature is favorable for the symbiosis of soybean and *B. japonicum*.

The soybean seed yield depends mainly on the number of pods per plant [27,30], which states that with the high number of pods per plant, the number of seeds increase to two units. In our own research, a positive effect of the inoculants and mineral N fertilization was found on the morphological features: Pod number, as well as number and weight of seeds in both cultivars, but higher values of those traits were found in Annushka cv. which was lower than in cv. Aldana. The 1000 seed weight was higher in Aldana cv. than in Annushka cv. In the study by Prusiński et al. [35], no significant effect of the inoculants and mineral N fertilization was found on the pod number and the 1000 seeds weight in the tested cultivars. Jarecki and Bobrecka-Jamro [50] found that the starting N rate of 25 kg N·ha^{-1} facilitates producing significantly more seeds per pod than as the result of Nitragina inoculation. Boros [51] stated that when the purpose of selecting legumes is to increase the yield, both the number of pods, as well as the number of seeds per plant and the 1000 seeds weight value must be taken into account simultaneously, especially in soybeans. In our research, the mineral N and inoculation had little effect on plant height. Additionally, in both cultivars in all years of the study, higher plants were observed after the application of Nitragina + 60 kg N·ha^{-1} than after Nitragina alone. In the study by Prusiński et al. [35], the favorable effect of mineral N on plant height was confirmed only in cv. Annushka.

BNF in soybean plants is a sensitive process, dependent mostly on the weather pattern, that directly affects the development of *B. japonicum* bacteria, nodulation, and N fixation [49]. In our research, factors of the study (mineral N and inoculation) had little effect on the number of root nodules and dry weight. The quality of root nodules and their weight was low. However, the more nodule amount on the root system was noted after the application of inoculants Hi®Stick Soy. A higher count of nodules was noted in cv. Annushka than Aldana. Jarecki and Bobrecka-Jamro [50], after the application of inoculants, observed an increase in the number of root nodules and dry weight, as well as the higher count of *B. japonicum.* Kaschuk et al. [18] and Saturno et al. [44] stated that mineral N fertilization of soybean can negatively affect the nodules number. Mrkovacki and Morinkovic [52] stated that the low doses of mineral N are able to increase the nodules number and their dry weight, which, however, was not noted in our research. Results of Debela et al. [43] studies showed that the combination of VC 2 tons ha^{-1} with *Rhizobium* inoculation (TAL-379) gave the highest number of effective nodules per plant. According to those authors, three factors of interaction of *Rhizobium* inoculation, VC, and NPS rates significantly influenced the number of primary branches (NPB), number of pod per plant, seed yield, and harvest index.

Martyniuk [53] drew attention to the need for selecting an appropriate seed dressing if there was a plan to inoculate the seeds of legumes with nitragine. In turn, Korsak-Adamowicz et al. [49] reported that the symbiosis of nodule bacteria with the crop is not conducive to drought and high temperature. According to De Bruin et al. [54], Mason et al. [55], Hungria et al. [21], and Ambrosini et al. [22], the benefit from inoculation of the soybean with *Bradyrhizobium* spp. strains is affected by the soil cropping in the first year, but with smaller effects in the next seasons.

In our study, a significantly higher protein content was found in the seeds of both soybean cultivars when mineral nitrogen in a dose of 60 kg·ha^{-1} was applied and seeds were inoculated with Nitragina or Hi®Stick Soy. The use of seed inoculation and fertilization with mineral nitrogen increased the fiber content. It was confirmed by Jarecki et al. [42] who found a similar effect on protein content in the seeds, which was significantly higher in bacterial inoculant Nitragina (N) (36.4% dry matter) and Nitragina+foliar fertilization with Mikrokompleks (N+M) (36.3% dry matter) treatments as compared with foliar fertilization with Mikrokompleks treatment (M) and the control (C). Shahid et al. [56] and Afzal et al. [57] also used the bacterial inoculant to obtain an increase in protein content. Vratarić et al. [58] stated that foliar fertilization differentiates the chemical composition of soybean seeds, affecting an increase in the protein and fat contents. In the study by Jarecki et al. [42], Nitragina resulted in the significant change in the chemical composition of seeds. Zuffo et al. [47] reported that the mineral nitrogen fertilization increased the crude protein content of seeds.

Herein, the studied factors limited the amount of ash in seeds. While Jarecki and Bobrecka-Jamro [59] using the bacterial inoculant stated an increase in ash content in soybean seeds. Considering a widespread interest in soybean cultivation in Europe, it seems justifiable, also in Poland, to continue further studies in various regions.

5. Conclusions

In conclusion, agroecological conditions have affected the effectiveness of inoculation and mineral N fertilization and directly impacted the soybean development, which also modifies the yield. In worse agroecological conditions, inoculation and N fertilization are advisable.

The soybean yield was most favorably affected by the combined use of inoculation and nitrogen fertilization (increase in seed yield by 42%, protein yield by about 28%). The application of mineral nitrogen at the dose of 30 or 60 kg·ha^{-1} allowed the increase of the seed yield and protein yield compared to the control. The inoculation of soybean seeds with *B. japonicum* increased the yield of soybeans. In addition, the protein yield compared to the control and inoculation of Hi®Stick Soy favored a better yield than Nitragina.

The seed inoculation and differentiated doses of nitrogen fertilization had a positive effect on the morphological features of both soybean cultivars. The small amount of rainfall during significant periods of growth and development of soybean strongly limited nodule binding on the root system, and their numbers and weights were small. Hi®Stick Soy has a better effect on soybean nodulation, and the cv. Annushka nodded slightly more than the cv. Aldana.

The inoculation of seeds with Nitragina or Hi®Stick Soy and fertilization with mineral nitrogen increased the content of protein and fiber in seeds of both soybean cultivars and reduced the amount of ash and fat.

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