agronomy

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Effect of Nitrogen Fertilization and Inoculation with *Bradyrhizobium japonicum* on Nodulation and Yielding of Soybean

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Abstract: Legumes' nutrition relies on two sources of nitrogen (N): mineral N from soil, and biological N fixation (BNF). The aim of this study was to verify the effect of bacterial inoculation, as well as to compare it with the effect of different mineral N fertilization on the main nodulation characteristics, yield components and seed yield of two soybean (*Glycine max* (L.) Merr.) cultivars in the conditions of south-eastern Poland. A randomized block design was used with four replications and combining the application rates of mineral N (0, 30 and 60 kg·ha^{-1}), and seed inoculation with *Bradyrhizobium japonicum* (HiStick® Soy and Nitragina) were applied for two soybean cultivars (Aldana, Annushka). It has been shown that inoculation of *B. japonicum* increases the nodulation on plant roots, yield components and seed yield, but no significant effect of the bacterial preparation used on the seed yield was observed. The application of 30 kg N·ha^{-1} did not result in a significant reduction in the number and weight of nodules, including on the main root and lateral roots, compared to seeds inoculated and not fertilized with N, as observed under a dose of 60 kg N·ha^{-1}, but resulted in an increase in the number of pods and the number and weight of seeds per plant. For both soybean cultivars, the best combination was nitrogen fertilization at 30 kg N·ha^{-1} and seed inoculation with *B. japonicum*, regardless of the bacterial preparation used.

Keywords: *Glycine max* (L.) Merr.; inoculation; nitrogen fertilization; nitrogen fixation; leaf area index; yield components; yield

Citation: Szpunar-Krok, E.; Bobrecka-Jamro, D.; Pikuła, W.; Jańczak-Pieniążek, M. Effect of Nitrogen Fertilization and Inoculation with *Bradyrhizobium japonicum* on Nodulation and Yielding of Soybean. *Agronomy* **2023**, *13*, 1341. https://doi.org/10.3390/agronomy13051341

Academic Editor: Robert P. Larkin

Received: 6 April 2023
Revised: 28 April 2023
Accepted: 9 May 2023
Published: 10 May 2023

1. Introduction

Legumes have exceptional nutritional properties and are of great economic importance [1]. Soybeans (*Glycine max* (L.) Merr.) belong to this group of plants. It is one of the most important plants grown in the world. Soybeans are a valuable source of protein and fat, which makes them useful for the production of food and animal feed [2–5]. In recent years, there has been a growing interest in soybean cultivation. The current area of cultivation of this plant in the world is 129.5 million ha, while global production is 371.7 million tons [6].

Nitrogen (N) is an essential component of numerous biomolecules, which include proteins, nucleic acids that condition the life of living organisms. This element is therefore responsible for many processes occurring in plants, such as the synthesis of chlorophyll or proteins [7]. Nitrogen is also important for the vegetative growth of soybeans and the production of optimal biomass [8]. Despite the presence of molecular N_2 in the atmosphere, the reduction of this component to forms that are easily absorbed by organisms is difficult [9]. It is estimated that in agricultural areas symbiotic associations are able to cover up to 80% of the demand for N [10]. Some plants are able to use the molecular N present in the atmosphere through the process of symbiosis with bacteria, binding and reducing it via the nitrogenase enzyme. This group of plants includes plants from the *Fabaceae* family [11,12].

Legumes develop root nodules, which contain *Rhizobium* bacteria. The leguminous plants provide carbohydrates to bacteria for all their physiological processes including nitrogen fixation [13]. The plant provides the bacteria with reduced carbohydrates, which are used as food and energy to stimulate the N_2-binding process. [14,15]. Concerning the nodules formation on soybean roots, direct contact of *Bradyrhizobium japonicum* bacteria with the roots of the plant is necessary. In order to ensure optimal colonization of the roots by bacteria, inoculation of soybeans before sowing is practiced [16]. The inoculation treatment is particularly important on soils where soybeans have not previously been grown and compatible populations of *Bradyrhizobium* are rarely available [17]. Since N binding by soybeans is enhanced in the growth phases R3–R5 (3.6–4.3 kg ha^{-1} d^{-1}) [18] and slows down in phases R5–R7 [19], this may result in N deficiency during seed filling and a consequently lower seed yield. It was confirmed that biologically fixed N (55%) contributes more to N accumulation in pods and seeds by the R7 growth stage compared to soil N (43%) [17].

Due to the increasing interest in biological methods of improving soil fertility in recent years, and thus increasing the size and quality of the crop, interest in the inoculation of soybeans with bacteria is growing [20]. The cultivation of leguminous crops is justified from the point of view of soil enrichment in sustainable agricultural systems, in particular those occurring in temperate climate zones [21]. According to Schweiger et al. [22], only 40–52% of the total N uptake comes from symbiotic N-binding, and the rest of this nutrient is derived from the soil. The inoculation of soybean seeds with *B. japonicum* bacteria in combination with the optimal level of fertilization results in the most effective biological N fixation (BNF) [16]. The effect of using N fertilization on nodule formation and BNF depends, e.g., on the amount of application and the timing of fertilization. High N concentrations can inhibit soy nodule formation and N fixation, while low N concentrations promote root nodules and BNF [23–26].

One of the challenges to further improve soybean seed yield is the high demand of N in comparison to cereals and oilseed crops. High-yielding soybean is related to high nutrient uptake [27]. In studies by Kakabouki et al. [28], the yield of soybeans was positively affected by N fertilization, and the most effective was the use of 80 kg N·ha^{-1}.

A higher application of N combined with early sowing is an effective way to increase soybean productivity, especially in dry years, by extending the period of effective N uptake [29]. Taking into account the yield and chemical composition of the seeds, in the Polish soil and climate conditions Głowacka et al. [30] recommends fertilizing soybeans with 60 kg N·ha^{-1} in two application rates: $\frac{1}{2}$ or $\frac{3}{4}$ before sowing and the remainder during the development of pods and seeds—in combination with sulfur application. The application of N can also modify the chemical composition of the seeds. Another experiment showed that pre-sowing soybean fertilization with nitrogen at a dose of 30 kg·ha^{-1} contributes to an increase in the content of C16:0, C16:1 and saturated fatty acids (SFA) in seeds while reducing the content of C18:0, C20:0 acids and unsaturated (UFA) and polyunsaturated fatty acids (PUFAs) [31], but seeds with a better balance of fatty acids for nutritional purposes were obtained at a dose of 60 kg N·ha^{-1} [32]. Lorenc-Kozik and Pisulewska [33], on the other hand, point to the different N requirements of soybean varieties. In their study, the most favorable dose for the yield of the cv. Aldana was 30 kg N·ha^{-1} (yield 1.9 t·ha^{-1}), resulting in an increase in seed yield by 22,7% compared to the control group, and for the cv. Nawiko 60 kg N·ha^{-1} (yield 2.3 t·ha^{-1}) causing an increase in yield compared to the control by 25%.

Since the excessive use of N fertilizers negatively affects the functioning of ecosystems and increases the carbon footprint [34,35], from the point of view of food security and the principles of sustainable agriculture, the priority should be to increase the productivity of plants, including soybean, through the implementation of more efficient cultivation technologies, taking into account the need to minimize the negative effects of N fertilization on the natural environment.

The aim of this study is to verify the effect of *B. japonicum* inoculation, mineral N fertilization rates on two soybean cultivars' main nodulation characteristics, yield components, and seed yield in the conditions of south-eastern Poland.

2. Materials and Methods

2.1. Experimental Design

A three-year field experiment (2017–2019) was conducted on the arable land at the Experiment Station for Cultivar Assessment in Przecław (50°11′ N, 21°29′ E; 189 m.a.s.l.; south-eastern Poland). The experiment was carried out in a field where soybean had not been grown before. The forecrop was winter wheat (*Triticum aestivum* L.).

The soil in the study location is a silt loam (SiL) [36] classified as a Fluvic Cambisol (CMfv) [37]. The soil before setting up the experiment, at a depth of 0–25 cm, was neutral (2017) or slightly acidic (2018, 2019). The N_{total} content in the soil in the years of research ranged from 0.1036% to 0.1294%. The soil was characterized by very high content of available forms of phosphorus and magnesium, and very high (2017) or medium (2018, 2019) potassium. No microelement deficiency was found.

Studied factors:

I. Soybean cultivars: Aldana belonged to the early maturity group (000+++) (Hodowla Roślin Strzelce Sp. z o.o. IHAR group, Strzelce, Poland) and Annushka (belonged to very early maturity group (0000) (Naukowo Badawcze Centrum Rozwoju Soi "AgeSoya" Sp. z o.o., Poland).
II. N fertilizer: 0, 30, 60 kg N·ha^{-1}.
III. Bacterial inoculant (with symbiotic bacteria *B. japonicum*): control (without bacterial inoculation), HiStick® Soy (BASF, Ludwigshafen, Germany), Nitragina (Institute of Soil Science and Plant Cultivation—State Research Institute, Puławy, Poland).

The experiment was conducted in a Randomized Complete Block design (RCBD) in a split-plot arrangement with three replications. The plot had an area of 19.5 m^2 (13.0 m × 1.5 m). Soybeans were sown in a planting capacity of 90 seeds per 1 m^2, with a row spacing of 15 cm, to a depth of about 3–4 cm. Seed inoculation with HiStick® Soy and Nitragina was performed immediately before sowing (according to the manufacturer's recommendations). The dates of soybean management are shown in Table 1.

Table 1. Agricultural practices in the experiment.

Cultivation Treatments	2017	2018	2019
Pre-winter plowing (depth of about 25 cm)	21 November (2016)	12 November (2017)	30 October (2019)
PK fertilization + Cultivation aggregate	28 April	12 April	7 March
Cultivation aggregate (depth approx. 8 cm)	2 May	24 May	24 April
Sowing N fertilization (selected variants)	2 May	24 April	25 April
Herbicide spraying	2 May Sencor Liquid (0.5 dm^3·ha^{-1})	24 April Boxer 800 EC (4.0 dm^3·ha^{-1})	26 April Boxer 800 EC (4.0 dm^3·ha^{-1})
Insecticide spraying	-	-	10 June Cyperkil Max 500 EC (1.5 dm^3·ha^{-1})
Harvest	30 August—Annushka 1 September—Aldana	7 September—Annushka 10 Septembe—Aldana	27 August—both cultivars

The mineral fertilizer rate was: 15.3 kg P·ha^{-1} (superphosphate 19%) and 78.9 kg K·ha^{-1} (potassium salt 60%), and 30 kg and 60 kg N·ha^{-1} (ammonium nitrate 34%). The detailed methodology of the field experiment is presented in the work of Szpunar-Krok et al. [31].

In the R 3 (beginning of pot development) phase, 10 plants were randomly dug up on each plot, to a depth of approx. 25 cm. The aboveground part of the plants (leaves and stem) is cut off on the surface of the soil, and the roots were washed out well, immersing them (along with the adjacent lump of earth) in a large bucket of water. All root samples were collected with minimal damage to the entire root system, including small roots. Nodules were torn off manually from the roots, and then their number and dry matter were determined separately for the main root and lateral roots.

Leaf area index (LAI) measurements were carried out three times during the growing season in phases R 2, R 3, and R 4 [38] in the morning hours. The LAI value measurement was made in each plot, by performing one measurement over the canopy and four in the canopy. This measurement was performed using LAI 2000 apparatus (LI-COR, Lincoln, NE, USA).

In order to determine the influence of factors on the formation of morphological characteristics of soybean, 20 plants were randomly taken from each plot of land and measured before harvesting. The yield of seeds and the weight of 1000 seeds are given in terms of 15% moisture.

2.2. Weather Conditions

Meteorological conditions during the soybean vegetation period are given according to the SDOO Meteorological Station in Przecław, Poland.

The weather conditions in the years of the study were diverse, especially in terms of humidity (Figure 1). In 2017 and 2019, 470.8 mm and 461.5 mm rainfall occurred during the growing season of soybeans, respectively, higher than the average for the years 1956–2012 by 19.4% and 17.1%, respectively. In turn, in 2018, the rainfall total was 371.1 mm and was lower than the average in many years by 5.9%; however, the distribution of precipitation in individual months of the growing season was more favorable for soybeans than in other years of research.

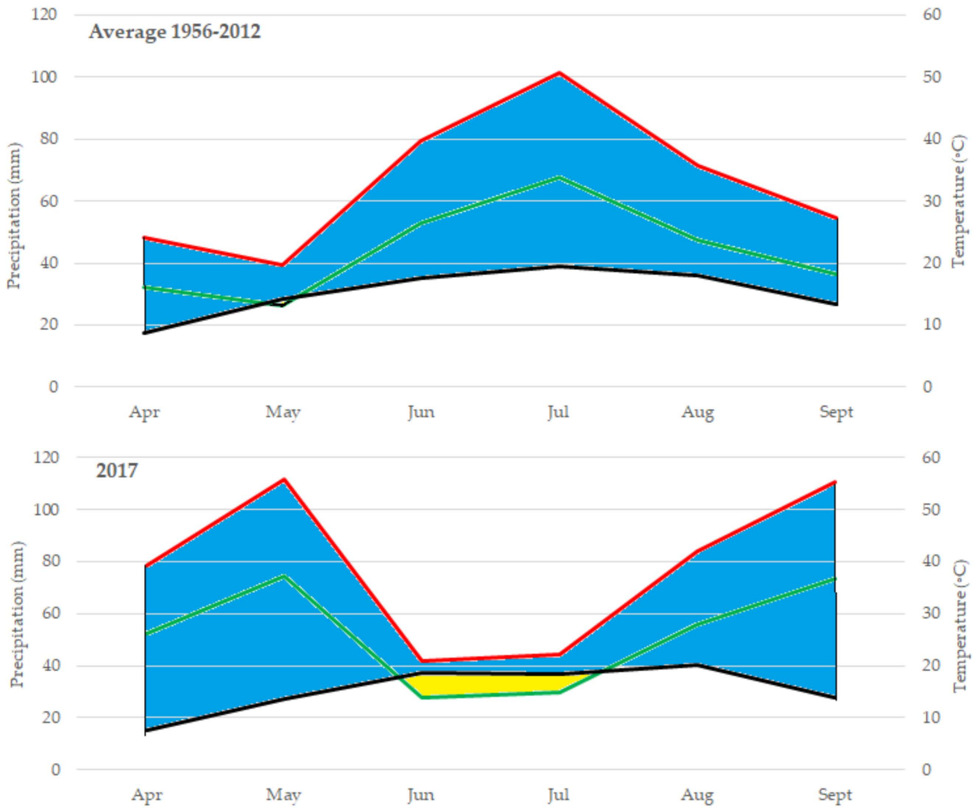

Figure 1. *Cont.*

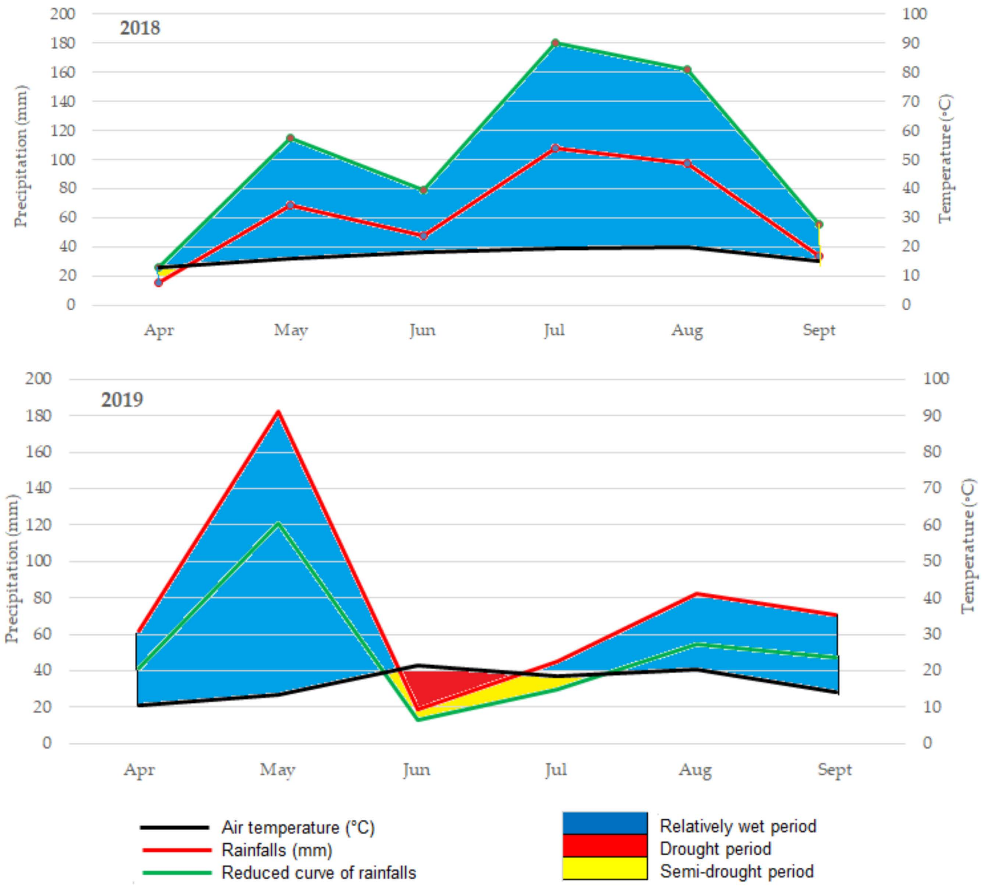

Figure 1. Climate graphs according to Walter [39] characterizing weather conditions in Przecław, Poland, during the soybean growing season in 2017–2019.

2.3. Data Statistical Analysis

All data were analyzed with the TIBCO Statistica 13.3.0 software (TIBCO Software Inc., Palo Alto, CA, USA). In the first stage of the statistical analysis, a three-way ANOVA was used to analyze the effects of cultivar (C), N fertilizer level (NF), seed inoculation with *B. japonicum* (I), and their bi-/tri-interactions on the yield of seeds, morphological features of plants and the number and weight of root nodules. Sampling years were treated as a random factor. Then, the Tukey test (HSD) was used as the criterion for comparing the means for all measured parameters. For the LAI parameter, a two-way ANOVA test with repeated measurements (with time as a factor) was also used. The statistical significance was calculated with the Tukey test at $\alpha \leq 0.05$. By using the same software, Pearson's correlations at $p = 0.05$ between the main parameters were also determined.

3. Results

3.1. Effect of N Fertilization and Seed Inoculation with B. japonicum on Seed Yield and Yield Attributes

In the 3-year research period (2017–2019), the amount of precipitation during the ripening period had a greater impact on the seed yield than the periodic deficiencies during flowering and setting pods (Table 2). Indeed, the highest seed yield was obtained in 2018, with the most favorable weather for soybean during the growing season (relatively wet period), and the lowest in 2017 (39.3% lower than in 2018), which was mainly influenced by heavy rainfall in September (110.6 mm). In turn, in 2019, despite the drought period in June and semi-drought period and rainfall during the ripening period of plants remaining at the level typical for perennials, a decrease in seed yield by 10.8% was achieved compared to 2018, but this yield was 24.2% higher than in 2017. On average, in the course of the research, soybean cvs Aldana and Annushka did not differ significantly in seed yield. The increase in N fertilization rates resulted in an increase in seed yield and seed fertility. Significant dependencies were shown in 2017 and 2019, while in 2018 only such a trend was observed. On average, for the years of research, the highest yield of soybean was obtained under the influence of fertilization with a dose of 60 kg N·ha^{-1}, significantly higher compared to the yield of plants fertilized with a dose of 30 kg N·ha^{-1}, and plants not fertilized with N by 2.3% and 5.6%, respectively. In all the years of the experiment, seed inoculation with *B. japonicum* bacteria resulted in a significant increase in soybean yield, but the type of bacterial preparation did not affect the yield of seeds.

Table 2. Seed yield and 1000 seed weight of soybean depending on cultivars, N and seed inoculation with *B. japonicum*.

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Seed Yield (t·ha^{-1})			
			2017	2018	2019	Mean
Aldana	0	Uninoculated	2.50 ± 0.04	4.53 ± 0.20	3.74 ± 0.19	3.59 ± 0.88
		HiStick® Soy	2.84 ± 0.28	4.86 ± 0.18	3.96 ± 0.19	3.89 ± 0.89
		Nitragina	2.81 ± 0.08	4.77 ± 0.08	3.95 ± 0.15	3.84 ± 0.84
	30	Uninoculated	2.49 ± 0.18	4.37 ± 0.23	3.74 ± 0.20	3.53 ± 0.84
		HiStick® Soy	2.93 ± 0.15	4.83 ± 0.25	4.28 ± 0.20	4.01 ± 0.85
		Nitragina	2.86 ± 0.37	4.65 ± 0.18	4.19 ± 0.20	3.90 ± 0.83
	60	Uninoculated	2.86 ± 0.11	4.57 ± 0.22	4.06 ± 0.21	3.83 ± 0.77
		HiStick® Soy	2.91 ± 0.19	4.90 ± 0.14	4.22 ± 0.18	4.01 ± 0.88
		Nitragina	3.10 ± 0.17	4.62 ± 0.02	4.22 ± 0.21	3.98 ± 0.69
Annushka	0	Uninoculated	3.02 ± 0.15	3.56 ± 0.05	3.55 ± 0.12	3.38 ± 0.29
		HiStick® Soy	3.66 ± 0.20	4.39 ± 0.41	3.73 ± 0.15	3.93 ± 0.43
		Nitragina	3.34 ± 0.07	4.32 ± 0.08	3.79 ± 0.12	3.82 ± 0.42
	30	Uninoculated	3.43 ± 0.12	3.77 ± 0.16	3.66 ± 0.13	3.62 ± 0.20
		HiStick® Soy	3.77 ± 0.30	4.38 ± 0.28	4.04 ± 0.22	4.06 ± 0.36
		Nitragina	3.63 ± 0.11	4.42 ± 0.33	4.09 ± 0.06	4.05 ± 0.38
	60	Uninoculated	3.48 ± 0.17	3.78 ± 0.37	3.81 ± 0.13	3.69 ± 0.27
		HiStick® Soy	3.78 ± 0.11	4.62 ± 0.10	4.02 ± 0.09	4.14 ± 0.38
		Nitragina	3.76 ± 0.13	4.33 ± 0.28	4.09 ± 0.11	4.06 ± 0.30
Average for factors						
C		Aldana	2.81 ± 0.26 a	4.68 ± 0.23 b	4.04 ± 0.26 b	3.84 ± 0.82 a
		Annushka	3.54 ± 0.28 b	4.18 ± 0.42 a	3.86 ± 0.22 a	3.86 ± 0.41 a
NF		0	3.03 ± 0.41 a	4.40 ± 0.47 a	3.79 ± 0.20 a	3.74 ± 0.68 a
		30	3.19 ± 0.51 b	4.40 ± 0.40 a	4.00 ± 0.28 b	3.86 ± 0.65 a
		60	3.31 ± 0.41 c	4.47 ± 0.41 a	4.07 ± 0.20 b	3.95 ± 0.59 a
I		Uninoculated	2.96 ± 0.42 a	4.10 ± 0.46 a	3.76 ± 0.22 a	3.61 ± 0.61 a
		HiStick® Soy	3.31 ± 0.48 b	4.66 ± 0.31 b	4.04 ± 0.24 b	4.01 ± 0.66 b
		Nitragina	3.25 ± 0.40 b	4.52 ± 0.25 b	4.05 ± 0.20 b	3.94 ± 0.60 b
Significance (F/p value)						
	C		290.13/0.0000	90.02/0.0000	20.29/0.0000	0.05/0.8300
	NF		14.89/0.0000	0.69/0.5054	18.80/0.0000	1.99/0.1388
	I		25.41/0000	41.02/0.0000	24.46/0.0000	8.16/0.0004
	C*NF		2.35/0.1050	1.48/0.2360	0.20/0.8209	0.29/0.7455
	C*I		1.88/0.1628	7.31/0.0016	0.55/0.5786	0.38/0.6869
	NF*I		1.75/0.1532	0.70/0.5969	2.01/0.1068	0.10/0.9808
	C*NF*I		0.71/0.5856	0.41/0.8017	0.24/0.9169	0.11/0.9793

Note: Means in the columns marked with different letters are significantly different at the level of p = 0.05.

A significantly higher plant density after emergence was found for cv. Annushka compared to cv. Aldana (Table 3). Plants of the cv. Annushka also developed a greater number of pods (in total, with and without seeds) and more seeds compared to the cv. Aldana. Fertilization in doses 30 and 60 kg N·ha^{-1} and inoculation of seeds with *B. japonicum* significantly affected the increase in the number of total and full pods, and did not affect the number of plants after emergence, the number of pods without seeds and the number of seeds in the pod.

Table 3. Yield attributes of soybean depending on cultivars, N fertilization and seed inoculation with *B. japonicum*.

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Plant·m^{-2} (after Emergence)	Pods Plant^{-1}			Seeds Pod^{-1}
				Total	With Seeds	Without Seeds	
Aldana	0	Uninoculated	77.5 ± 6.2	16.9 ± 3.3	16.1 ± 3.1	0.8 ± 0.5	1.65 ± 0.17
		HiStick® Soy	79.8 ± 4.4	18.4 ± 2.3	17.8 ± 2.2	0.7 ± 0.2	1.56 ± 0.16
		Nitragina	81.0 ± 3.9	18.0 ± 3.0	17.3 ± 2.8	0.7 ± 0.4	1.64 ± 0.20
	30	Uninoculated	79.1 ± 4.0	17.9 ± 3.2	17.1 ± 3.0	0.7 ± 0.4	1.53 ± 0.22
		HiStick® Soy	79.6 ± 4.0	23.3 ± 3.4	22.5 ± 3.4	0.8 ± 0.4	1.38 ± 0.20
		Nitragina	80.7 ± 4.9	21.5 ± 3.7	20.6 ± 3.3	0.9 ± 0.5	1.44 ± 0.24
	60	Uninoculated	79.5 ± 4.0	20.7 ± 3.9	19.8 ± 3.7	0.8 ± 0.4	1.43 ± 0.25
		HiStick® Soy	78.4 ± 4.7	21.6 ± 1.9	20.4 ± 1.8	1.2 ± 0.4	1.45 ± 0.20
		Nitragina	79.0 ± 3.8	20.9 ± 2.9	20.2 ± 2.8	0.7 ± 0.3	1.47 ± 0.36
Annushka	0	Uninoculated	84.0 ± 4.5	20.1 ± 2.3	19.1 ± 2.1	1.0 ± 0.2	1.62 ± 0.22
		HiStick® Soy	82.5 ± 3.0	23.7 ± 2.4	22.4 ± 2.1	1.3 ± 0.9	1.78 ± 0.17
		Nitragina	82.7 ± 3.2	24.1 ± 3.7	22.9 ± 3.8	1.2 ± 0.5	1.64 ± 0.23
	30	Uninoculated	83.7 ± 6.3	21.4 ± 2.4	20.1 ± 2.4	1.3 ± 0.5	1.80 ± 0.18
		HiStick® Soy	81.6 ± 3.3	24.3 ± 3.0	23.1 ± 2.7	1.2 ± 0.9	1.82 ± 0.29
		Nitragina	82.7 ± 3.2	23.9 ± 2.6	22.3 ± 2.6	1.5 ± 0.7	1.78 ± 0.28
	60	Uninoculated	83.4 ± 3.4	22.5 ± 3.9	21.5 ± 3.4	1.0 ± 0.8	1.75 ± 0.28
		HiStick® Soy	81.7 ± 3.0	24.0 ± 2.7	22.7 ± 2.4	1.3 ± 0.8	1.78 ± 0.31
		Nitragina	82.6 ± 4.1	23.4 ± 2.3	22.2 ± 2.2	1.3 ± 0.7	1.76 ± 0.37
Average for factors							
C		Aldana	79.4 ± 4.5 a	19.9 ± 3.6 a	19.1 ± 3.5 a	0.8 ± 0.4 a	1.51 ± 0.24 a
		Annushka	82.8 ± 3.8 b	23.0 ± 3.1 b	21.8 ± 2.9 b	1.2 ± 0.7 b	1.75 ± 0.26 b
NF		0	81.0 ± 4.7 a	20.2 ± 3.9 a	19.3 ± 3.7 a	1.0 ± 0.5 a	1.65 ± 0.20 a
		30	81.2 ± 4.6 a	22.0 ± 3.7 b	20.9 ± 3.5 b	1.1 ± 0.6 a	1.62 ± 0.29 a
		60	80.8 ± 4.2 a	22.2 ± 3.2 b	21.1 ± 2.9 b	1.0 ± 0.6 a	1.61 ± 0.33 a
I		Uninoculated	81.2 ± 5.4 a	19.9 ± 3.7 a	18.9 ± 3.4 a	1.0 ± 0.5 a	1.63 ± 0.25 a
		HiStick® Soy	80.6 ± 3.9 a	22.5 ± 3.3 b	21.5 ± 3.0 b	1.1 ± 0.7a	1.63 ± 0.28 a
		Nitragina	81.4 ± 4.0 a	22.0 ± 3.6 b	20.9 ± 3.4 b	1.0 ± 0.6 a	1.62 ± 0.31 a
Significance (F/*p* value)							
C			34.28/0.0000	59.27/0.0000	49.52/0.0000	32.55/0.0000	106.6/0.0000
NF			0.28/0.7570	9.58/0.0001	9.62/0.0001	1.03/0.3590	0.99/0.3752
I			0.77/0.4647	15.40/0.0000	16.16/0.0000	0.54/0.5844	0.05/0.9526
C*NF			0.19/0.8303	4.28/0.152	4.90/0.0084	0.94/0.3934	15.03/0.0000
C*I			2.08/0.1272	0.39/0.6765	0.24/0.7877	0.88/0.4179	3.56/0.0306
NF*I			0.37/0.8264	1.84/0.1217	2.37/0.0538	1.40/0.2357	0.74/0.5641
C*NF*I			0.50/0.7345	1.09/0.3602	1.49/0.3636	0.68/0.6097	0.94/0.4398

Note: Means in the columns marked with different letters are significantly different at the level of $p = 0.05$.

In the conducted studies, Annushka plants developed significantly more seeds (by 34.0%) compared to Aldana plants, but a smaller weight per plant and a weight of 1000 seeds were found in Annushka (by 7.8 and 44.3%, respectively) (Table 4).

Table 4. Number and weight of seeds per soybean plant and 1000 seed weight depending on cultivars, N fertilization and seed inoculation with *B. japonicum*.

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Seeds Plant^{-1}	Seed Weight Plant^{-1} (g)	1000 Seed Weight (g)
Aldana	0	Uninoculated	26.3 ± 4.1	4.88 ±0.79	187 ± 30
		HiStick® Soy	27.6 ± 2.2	5.33 ± 0.79	194 ± 28
		Nitragina	27.9 ± 3.0	5.17 ± 0.65	186 ± 28
	30	Uninoculated	25.7 ± 3.1	4.82 ± 0.75	189 ± 29
		HiStick® Soy	30.7 ± 4.9	5.65 ± 0.82	187 ± 30
		Nitragina	29.3 ± 4.7	5.44 ± 0.95	187 ± 27
	60	Uninoculated	27.7 ± 4.0	5.14 ± 0.68	187 ± 28
		HiStick® Soy	29.5 ± 4.0	5.61 ± 0.90	191 ± 28
		Nitragina	29.0 ± 3.3	5.47 ± 0.71	190 ± 28
Annushka	0	Uninoculated	30.8 ± 5.7	3.97 ± 0.44	131 ± 23
		HiStick® Soy	39.8 ± 2.5	4.98 ± 0.50	126 ± 16
		Nitragina	36.8 ± 2.6	4.78 ± 0.73	130 ± 17
	30	Uninoculated	36.0 ± 4.9	4.48 ± 0.36	126 ± 12
		HiStick® Soy	41.5 ± 4.3	5.30 ± 0.56	129 ± 21
		Nitragina	39.5 ± 5.4	5.22 ± 0.53	134 ± 21
	60	Uninoculated	37.2 ± 5.6	4.80 ± 0.36	131 ± 18
		HiStick® Soy	39.8 ± 3.7	5.30 ± 0.60	134 ± 21
		Nitragina	38.5 ± 5.4	5.24 ± 0.40	139 ± 21
Average for factors					
C		Aldana	28.2 ± 4.0 a	5.28 ± 0.81 b	189 ± 28 b
		Annushka	37.8 ± 5.3 b	4.90 ± 0.65 a	131 ± 19 a
NF		0	31.5 ± 6.1 a	4.85 ± 0.78 a	159 ± 38 a
		30	33.8 ± 7.2 b	5.15 ± 0.78 b	159 ± 38 a
		60	33.6 ± 6.6 b	5.26 ± 0.66 b	162 ± 36 a
I		Uninoculated	30.6 ± 6.4 a	4.68 ± 0.68 a	158 ± 38 a
		HiStick® Soy	33.8 ± 7.2 b	5.36 ± 0.72 c	160 ± 39 a
		Nitragina	33.5 ± 6.3 b	5.22 ± 0.70 b	161 ± 36 a
Significance (F/*p* value)					
C			14.36/0.0002	58.68/0.0000	302.7/0.0000
NF			0.93/0.3963	15.60/0.0000	0.46/0.06339
I			0.01/0.9941	19.96/0.0000	0.18/0.8339
C*NF			1.33/0.2663	3.30/0.0390	0.21/0.8110
C*I			1.25/0.2891	0.50/0.6077	0.39/0.6795
NF*I			2.09/0.0841	0.07/0.9912	0.13/0.9720
C*NF*I			0.70/0.5956	0.64/0.6352	0.21/0.9314

Note: Means in the columns marked with different letters are significantly different at the level of $p = 0.05$.

The obtained results showed that N fertilization affects the formation of the values of the analyzed parameters. An increase in the mean values of the number and weight of seeds per plant was observed as a result of applying both doses 30 and 60 kg N ha^{-1}. Inoculation of seeds resulted in a higher number of seeds and weight of seeds per plant compared to uninoculated seeds. The use of HiStick® Soy resulted in an increase in the number and weight of seeds per plant by 13.7% and 14.5%, respectively, and those inoculated with Nitragina by 3.9% and 2.7%, respectively, compared to uninoculated seeds. However, there was no statistically significant effect of inoculation on the value of the obtained mass of 1000 seeds.

3.2. Effect of N Fertilisation and Seed Inoculation of B. japonicum on Root Nodulation

The experiment showed that the number and weight of nodules on the plant depended significantly on C, NF and I (Table 5). The number of nodules total and on the lateral root were significantly differentiated by the C*I interaction. In addition, the number of nodules per plant also significantly depended on the interaction of NF*I.

Table 5. Number of root nodules per soybean plant (in total, including main root and lateral roots) depending on cultivars, N fertilization and seed inoculation with *B. japonicum*.

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Number of Root Nodules from a Plant		
			Total	Main Root	Lateral Roots
Aldana	0	Uninoculated	1.9 ± 0.6	0.9 ± 0.5	0.7 ± 0.3
		HiStick® Soy	17.0 ± 4.6	5.2 ± 2.2	7.3 ± 4.0
		Nitragina	17.1 ± 6.6	5.8 ± 1.4	9.2 ± 3.2
	30	Uninoculated	2.4 ± 0.8	0.8 ± 0.4	1.9 ± 0.7
		HiStick® Soy	17.6 ± 7.1	5.8 ± 2.6	6.8 ± 4.9
		Nitragina	15.1 ± 3.2	5.3 ± 2.4	10.0 ± 3.1
	60	Uninoculated	2.1 ± 0.6	0.8 ± 0.6	1.3 ± 0.3
		HiStick® Soy	15.3 ± 9.0	5.5 ± 2.3	4.7 ± 0.7
		Nitragina	9.8 ± 2.7	3.1 ± 1.4	5.6 ± 1.0
Annushka	0	Uninoculated	2.3 ± 0.3	0.8 ± 0.2	1.6 ± 0.3
		HiStick® Soy	12.4 ± 5.2	4.7 ± 2.8	6.8 ± 1.2
		Nitragina	12.8 ± 3.5	3.5 ± 1.5	6.1 ± 1.6
	30	Uninoculated	1.8 ± 0.7	0.7 ± 0.4	0.8 ± 0.2
		HiStick® Soy	13.0 ± 4.1	4.6 ± 1.6	6.3 ± 1.1
		Nitragina	13.0 ± 4.9	4.6 ± 2.5	7.1 ± 3.7
	60	Uninoculated	1.9 ± 0.9	0.8 ± 0.3	0.6 ± 0.1
		HiStick® Soy	10.2 ± 2.5	4.0 ± 1.7	6.3 ± 1.0
		Nitragina	9.0 ± 3.1	3.1 ± 1.1	3.9 ± 1.4
Average for factors					
C		Aldana	10.9 ± 5.7 b	3.7 ± 2.7 b	7.1 ± 6.1 b
		Annushka	8.5 ± 8.1 a	3.0 ± 2.3 a	5.7 ± 4.3 a
NF		0	10.6 ± 7.5 b	3.5 ± 2.6 b	6.9 ± 5.5 b
		30	10.5 ± 7.3 b	3.6 ± 2.8 b	7.0 ± 5.5 b
		60	8.0 ± 6.2 a	2.9 ± 2.2 a	5.2 ± 4.8 a
I		Uninoculated	2.1 ± 0.7 a	0.8 ± 0.4 a	1.3 ± 0.5 a
		HiStick® Soy	14.2 ± 6.2 b	5.0 ± 2.2 c	9.1 ± 5.2 b
		Nitragina	12.8 ± 4.9 b	4.2 ± 2.0 b	8.8 ± 4.3 b
Significance (F/*p* value)					
C			18.01/0.0000	9.82/0.0020	8.20/0.0000
NF			8.67/0.0000	4.03/0.0193	5.82/0.0035
NF			183.31/0.0000	128.01/0.0000	101.67/0.0000

Table 5. *Cont.*

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Number of Root Nodules from a Plant		
			Total	Main Root	Lateral Roots
	C*NF		0.17/0.8459	0.28/0.7525	0.01/0.9823
	C*I		5.61/0.0043	1.96/0.1437	3.67/0.0273
	NF*I		3.11/0.0165	2.30/0.0601	2.22/0.0685
	C*NF*I		0.51/0.7236	1.68/0.1563	0.24/0.9156

Note: Means in the columns marked with different letters are significantly different at the level of p = 0.05.

The cv. Aldana developed a higher total number of nodules (by 28.2%), including on the main root (by 23.3%) and on the lateral roots (by 24.6%), compared to the cv. Annushka. Fertilization with the highest dose of N (60 kg N ha^{-1}) resulted in the lowest number of nodules (total, on main root and lateral roots). Inoculation of the seed with *B. japonicum* resulted in a significant increase in the total number of nodules on the plant, including the main root and lateral roots. As a result of the use of HiStick® Soy, a greater number of nodules on the lateral roots were obtained than after Nitragina inoculation.

In the conducted experiment, the dry mass of the nodules on the plant root depended significantly on the NF and I (Table 6). The dry mass of the total nodules and nodules in lateral roots also significantly depended on the interaction NF*I.

Table 6. Dry mass of root nodules per soybean plant (in total, including main root and lateral roots) depending on cultivars, N fertilization and seed inoculation with *B. japonicum*.

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Dry Mass of Root Nodules from a Plant (g)		
			Total	Main Root	Lateral Roots
Aldana	0	Uninoculated	0.044 ± 0.016	0.024 ± 0.012	0.020 ± 0.004
		HiStick® Soy	0.242 ± 0.082	0.122 ± 0.070	0.173 ± 0.024
		Nitragina	0.270 ± 0.121	0.110 ± 0.037	0.258 ± 0.127
	30	Uninoculated	0.048 ± 0.028	0.023 ± 0.017	0.037 ± 0.023
		HiStick® Soy	0.220 ± 0.087	0.108 ± 0.062	0.172 ± 0.012
		Nitragina	0.251 ± 0.104	0.099 ± 0.048	0.252 ± 0.046
	60	Uninoculated	0.028 ± 0.012	0.015 ± 0.009	0.018 ± 0.005
		HiStick® Soy	0.189 ± 0.102	0.086 ± 0.020	0.215 ± 0.080
		Nitragina	0.149 ± 0.112	0.072 ± 0.069	0.140 ± 0.017
Annushka	0	Uninoculated	0.043 ± 0.014	0.018 ± 0.010	0.030 ± 0.006
		HiStick® Soy	0.221 ± 0.100	0.109 ± 0.060	0.178 ± 0.036
		Nitragina	0.243 ± 0.080	0.105 ± 0.044	0.163 ± 0.093
	30	Uninoculated	0.031 ± 0.017	0.013 ± 0.009	0.029 ± 0.015
		HiStick® Soy	0.189 ± 0.052	0.087 ± 0.034	0.099 ± 0.024
		Nitragina	0.233 ± 0.102	0.105 ± 0.054	0.107 ± 0.055
	60	Uninoculated	0.032 ± 0.021	0.015 ± 0.009	0.022 ± 0.008
		HiStick® Soy	0.141 ± 0.054	0.079 ± 0.036	0.040 ± 0.009
		Nitragina	0.148 ± 0.078	0.078 ± 0.049	0.035 ± 0.027
Average for factors					
	C	Aldana	0.160 ± 0.122 a	0.073 ± 0.059 a	0.087 ± 0.081 a
		Annushka	0.142 ± 0.104 a	0.068 ± 0.054 a	0.075 ± 0.067 a
	NF	0	0.177 ± 0.123 b	0.081 ± 0.061 b	0.096 ± 0.081 b
		30	0.162 ±0.114 ab	0.072 ± 0.057 b	0.090 ± 0.077 b
		60	0.115 ± 0.094 a	0.057 ± 0.048 a	0.057 ± 0.060 a

Table 6. *Cont.*

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Dry Mass of Root Nodules from a Plant (g)		
			Total	Main Root	Lateral Roots
	I	Uninoculated	0.038 ± 0.019 a	0.018 ± 0.012 a	0.020 ± 0.012 a
		HiStick® Soy	0.200 ± 0.085 b	0.098 ± 0.051 b	0.102 ± 0.062 b
		Nitragina	0.216 ± 0.109 b	0.095 ± 0.051 b	0.121 ± 0.084 b
		Significance (F/*p* value)			
	C		2.975/0.0866	0.94/0.3343	5.80/0.1233
	NF		13.46/0.0000	5.95/0.0031	9.32/0.0001
	NF		122.1/0.0000	85.00/0.0000	61.53/0.0000
	C*NF		0.04/0.960	0.22/0.8003	0.05/0.9542
	C*I		0.66/0.5194	0.64/0.5299	0.69/0.5023
	NF*I		2.94/0.0216	1.00/0.4096	2.59/0.0383
	C*NF*I		0.22/0.9295	0.08//0.9879	0.38/0.8209

Note: Means in the columns marked with different letters are significantly different at the level of $p = 0.05$.

The tested cultivars did not significantly differ in the mass of the nodules formed on the plant roots. Fertilization at a dose of 60 kg N ha^{-1} resulted in a significantly lower nodule weight on soybean roots compared to the variant without N fertilization, and in the case of nodule weight on the main and lateral roots, it was also lower than after applying a dose of 30 kg N ha^{-1}. Inoculation of HiStick® Soy and Nitragina seeds resulted in a significant increase in the weight of nodules compared to the uninoculated variant.

3.3. Effect of N Fertilization and Seed Inoculation of B. japonicum on Leaf Area Index (LAI)

The three-way ANOVA showed that in the R 2 and R 3 developmental phases, the experimental factors studied had no significant effect on the LAI value (Table 7). In the R 4 phase, however, a significant effect of C and I on the examined parameter of the canopy architecture was found. It has been shown that in the R 4 phase of development, Aldana plants have an average higher LAI value of 7.7% compared to Annushka. The Nitragina bacterial inoculation increased the LAI value compared to the uninoculated variant. If HiStick® Soy preparation was used, LAI was higher compared to uninoculated seeds, but the difference was not statistically significant. Indeed, the highest LAI values in both cultivars were obtained in the R 3 phase.

Table 7. Changes in the leaf area index (LAI; m^2 m^{-2}) at the characteristic growth stages of soybean depending on cultivars, N fertilization and seed inoculation with *B. japonicum* in R 2, R 3 and R 4 phases of development.

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Phases of Development		
			R 2	R 3	R 4
Aldana	0	Uninoculated	3.28 ± 1.80 aA	4.57 ± 1.74 aB	4.44 ± 1.32 aB
		HiStick® Soy	3.48 ± 1.79 aA	4.84 ± 1.45 aB	4.73 ± 0.98 aB
		Nitragina	3.65 ± 1.77 aA	5.10 ± 1.28 aB	4.84 ± 1.00 aB
	30	Uninoculated	3.40 ± 1.83 aA	4.81 ± 1.61 aC	4.31 ± 1.10 aB
		HiStick® Soy	3.53 ± 1.72 aA	5.09 ± 1.30 aC	4.66 ± 0.88 aB
		Nitragina	3.41 ± 1.54 aA	5.24 ± 1.36 aB	5.06 ± 1.02 aB
	60	Uninoculated	3.55 ± 1.75 aA	5.20 ± 1.66 aC	4.63 ± 1.00 aB
		HiStick® Soy	3.74 ± 1.81 aA	5.11 ± 1.29 aB	4.95 ± 0.74 aB
		Nitragina	3.41 ± 1.72 aA	5.05 ± 1.49 aB	4.93 ± 0.62 aB

Table 7. *Cont.*

Cultivars (C)	Nitrogen Fertilization (kg N·ha^{-1}) (NF)	Inoculation (I)	Phases of Development		
			R 2	R 3	R 4
Annushka	0	Uninoculated	3.16 ± 1.09 aA	4.30 ± 0.31 aC	4.03 ± 0.52 aB
		HiStick® Soy	3.52 ± 1.05 aA	4.77 ± 0.42 aC	4.23 ± 0.62 aB
		Nitragina	3.02 ± 1.32 aA	4.48 ± 0.60 aB	4.19 ± 0.61 aB
	30	Uninoculated	3.38 ± 1.04 aA	4.80 ± 0.45 aC	4.32 ± 0.60 aB
		HiStick® Soy	3.61 ± 0.89 aA	5.33 ± 0.73 aC	4.55 ± 0.57 aB
		Nitragina	3.39 ± 0.97 aA	5.01 ± 0.57 aC	4.57 ± 0.50 aB
	60	Uninoculated	3.41 ± 0.92 aA	5.01 ± 0.42 aC	4.37 ± 0.50 aB
		HiStick® Soy	3.50 ± 0.92 aA	5.10 ± 0.65 aC	4.68 ± 0.56 aB
		Nitragina	3.60 ± 1.04 aA	4.95 ± 0.48 aC	4.59 ± 0.66 aB
Average for factors					
	C	Aldana	3.49 ± 1.69 aA	5.00 ± 1.43 aC	4.73 ± 0.97 bB
		Annushka	3.40 ± 1.01 aA	4.86 ± 0.59 aC	4.39 ± 0.59 aB
	NF	0	3.35 ± 1.47 aA	4.68 ± 1.10 aC	4.41 ± 0.90 aB
		30	3.45 ± 1.34 aA	5.05 ± 1.08 aC	4.58 ± 0.82 aB
		60	3.53 ± 1.37 aA	5.07 ± 1.08 aC	4.69 ± 0.70 aB
	I	Uninoculated	3.36 ± 1.41 aA	4.78 ± 1.21 aC	4.35 ± 0.88 aB
		HiStick® Soy	3.56 ± 1.38 aA	5.04 ± 1.03 aC	4.63 ± 0.75 abB
		Nitragina	3.41 ± 1.39 aA	4.97 ± 1.04 aC	4.69 ± 0.79 bB
Significance (F/*p* value)					
C			0.10/0.0991	0.81/0.3685	9.33/0.0026
NF			3.344/0.7113	2.75/0.0666	2.16/0.1175
NF			0.21/0.8081	0.99/0.3737	3.64/0.0279
C*NF			0.18/0.8328	0.38/0.6835	0.65/0.5230
C*I			0.04/0.9633	0.48/0.6165	0.51/0.6035
NF*I			0.02/0.9995	0.36/0.8347	0.20/0.9401
C*NF*I			0.23/0.9201	0.09/0.9856	0.10/0.9820

Note: Means in the columns marked with different letters are significantly different at the level of $p = 0.05$.

3.4. Correlations between Features

In the experiment, soybean yield was positively correlated with plant density after emergence (r = 0.263), by weight of 1000 seeds (r = 0.530), seed mass per plant (r = 0.872), total number of nodules (r = 0.157) and on the main root (r = 0.265) and LAI values in BBCH phases 61, 70 and 79 (r = 0.615, r = 0.779 and r = 0.665, respectively), and negatively correlated with the number without seeds pods from the plant (r = −0.239) (Table 8). The mass of 1000 seeds was positively correlated with the mass of seeds from the plant (r = 0.529), weight of nodules in total and on lateral roots (r = 0.138 and r = 0.169, respectively) and LAI values in BBCH phases 61, 70 and 79 (r = 0.169, r = 0.213 and r = 0.381, respectively), and strongly negatively correlated with the number of seeds per plant (r = −0.741). For this variable, a negative correlation was also found with the number of pods (in total, with and without seeds) on the plant (r = −0.466, r = −0.417, r = −0.466, respectively) and the number of seeds in the pod (r = −0.444). A positive correlation was found between the number of seeds per plant and the number of seeds in the pod (r = 0.612), the number of pods on the plant and LAI in the R 2 and 79 phases (r = 0.288 and r = 0.145, respectively). The seed mass from the plant was strongly positively correlated with the LAI at each measurement date. The experiment also showed a positive correlation between the total dry weight of nodules and the number and dry mass of nodules on the lateral roots and the LAI index in the R 2 and 70 phases. The LAI index was weakly negatively correlated with the number of pods without seeds.

Table 8. Correlation coefficients (r) between the studied features.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.000																	
2	0.263	1.000																
3	0.530	−0.054	1.000															
4	0.090	0.010	−0.741	1.000														
5	0.872	−0.137	0.529	0.153	1.000													
6	0.047	0.058	−0.444	0.612	0.042	1.000												
7	0.029	−0.041	−0.466	0.573	0.092	−0.277	1.000											
8	0.073	−0.049	−0.417	0.555	0.148	−0.306	0.988	1.000										
9	−0.239	0.029	−0.466	0.334	−0.290	0.057	0.471	0.330	1.000									
10	0.157	−0.028	0.031	0.065	0.148	−0.137	0.225	0.229	0.063	1.000								
11	0.265	−0.010	0.083	0.076	0.266	−0.102	0.201	0.206	0.053	0.770	1.000							
12	0.066	−0.039	0.032	0.077	0.054	−0.129	0.232	0.232	0.093	0.943	0.597	1.000						
13	0.018	−0.047	0.138	0.172	0.030	−0.067	0.291	0.282	0.173	0.858	0.723	0.827	1.000					
14	0.169	−0.013	0.055	0.182	0.176	0.003	0.236	0.222	0.174	0.677	0.772	0.544	0.825	1.000				
15	0.100	−0.061	0.169	0.125	−0.088	−0.105	0.266	0.262	0.133	0.800	0.522	0.852	0.905	0.505	1.000			
16	0.615	0.014	0.213	0.288	0.655	0.369	−0.072	−0.035	−0.239	0.177	0.099	0.199	0.214	0.144	0.218	1.000		
17	0.779	0.179	0.381	0.124	0.707	0.130	−0.001	0.028	−0.167	0.120	0.006	0.176	0.193	0.037	0.267	0.754	1.000	
18	0.665	−0.004	0.344	0.145	0.711	0.155	−0.014	0.031	−0.268	0.013	0.099	0.039	0.051	0.037	0.105	0.820	0.758	1.000

(1) Seed yield ($t{\cdot}ha^{-1}$)
(2) $Plant{\cdot}m^{-2}$ (after emergence)
(3) 1000 seed weight
(4) Seeds $plant^{-1}$
(5) Seed weight $plant^{-1}$
(6) Seeds pod^{-1}
(7) Pods $plant^{-1}$—total
(8) Pods $plant^{-1}$—with seeds
(9) Pods $plant^{-1}$—without seeds
(10) No nodules $plant^{-1}$—total
(11) No nodules $plant^{-1}$—main root
(12) No nodules $plant^{-1}$—Lateral roots
(13) Nodule dry weight—total
(14) Nodule dry weight—main root
(15) Nodule dry weight—Lateral roots
(16) LAI in R 2 phase
(17) LAI in R 3 phase
(18) LAI in R 4 phase

Significant at $p < 0.05$. Correlations: $0.0 < |r| \leq 0.1$—slight correlation; $0.1 < |r| \leq 0.3$—weak correlation; $0.3 < |r| \leq 0.5$—medium correlation; $0.5 < |r| \leq 0.7$—high correlation; $0.7 < |r| \leq 0.9$—very high correlation; $0.9 < |r| < 1.0$—almost full correlation; $|r| = 1$—full correlation.

4. Discussion

Due to their wide use in many industries, soybeans are classified as strategic crops [40]. Therefore, it is particularly important to improve the agrotechnics of this plant aimed at increasing the yield of seeds; on the other hand, it is necessary to take into account the need to minimize the negative effects of agricultural practices, including N fertilization, on the natural environment. The soybean yield is the result of the interaction of agricultural practices and environmental factors [29,41–43].

Nitrogen is one of the most important nutrients for plants. The source of this element for leguminous plants is BNF, soil organic matter and N fertilization used during sowing [3,7]. Bean plants tend to take mineral N from the soil rather than bind it from the atmosphere. N binding through symbiosis with bacteria is associated with high costs for plants: direct costs of energy production, costs of reducing N_2 to NH_3, as well as protein synthesis and transport to the aerial part, and indirect costs are associated with the formation and maintenance of root nodules [44]. It has been estimated that BNF requires 6–7 g C g^{-1} N, while the assimilation of mineral N requires 4 g C g^{-1} N. Soybeans meet the cost of BNF by a reduction in seed yield, particularly in stressful environments, and a secondary contribution of reduced seed oil concentration. Nitrogen fertilization reduces BNF and increases seed yield by enhancing C allocation to seeds as reviewed by Tamagno et al. [45]. Kakabouki et al. [28] and Nget et al. [46] suggested a positive correlation between the yield and the number of nodules. The results of the present study confirm this dependency. The seed yield was positively correlated with the number of nodules in total and on the main root and with the mass of nodules on the main root. It has also been shown that the mineral N inhibits the formation of nodules (total, on main root and lateral roots) and binding N.

Cigelske et al. [47] also observed a reduction in root nodulation (from 31.8 to 23.7 nodules per plant) and nodule size under N fertilization.

Under Polish conditions, symbiotic binding of N_2 by *Fabaceae* plants does not fully cover their nutritional needs, therefore fertilization up to 60 kg N·ha^{-1} is often recommended, especially on soils less rich in N [48]. Soybean yield largely depends on the availability of N affecting plant growth and development [24,29,49]. The availability of N for plants affects the supply of sucrose to different organs and may affect yield traits in different ways [50]. In the studies conducted by Kulig and Klimek-Kopyra [29] under conditions of Polish fertilization of mineral N at a dose of 60 kg N·ha^{-1} resulted in an increase in soybean yields compared to a dose of 30 kg N·ha^{-1} by an average of 0.2 t·ha^{-1}. In this research, the highest yield of soybeans was obtained under the influence of fertilization with a dose of 60 kg N·ha^{-1}, but it was higher by only 2.3% (by 0.09 t ha^{-1}) compared to plants fertilized with a dose of 30 kg N·ha^{-1} and 5.6% (by 0.21 t ha^{-1}) lower than in the absence of fertilization with this element. Therefore, the analysis of the data indicates that in the conditions of south-eastern Poland, the use of high doses of N in soybean cultivation is not justified, while grafting seeds with symbiotic bacteria should be considered a more yield-generating treatment. Mourtzinis et al. [51], based on synthesis-analysis (207 environments for 5991 N-treated soybean yields), found that N fertilization has a small and inconsistent effect on soybean yield. In these authors' studies of the total yield variability, <1% was attributable to each N variable and 68% was associated with the effect of the environment. In our study, after inoculation of *B. japonicum* and lack of N fertilization, cv. Aldana had an average increase in seed yield over the research period by 7.1% (0.28 t·ha^{-1}), and in the case of cv. Annushka—by 14.6% (0.50 t·ha^{-1}). It has also been shown that both bacterial preparations have a similar effect on increasing the yield potential of the tested soybean cultivars.

Previous studies have shown that high doses of N fertilization inhibit the development of root nodules and BNF. Soybean cultivation therefore does not require the application of mineral N in large quantities, which is particularly important from an economic and environmental point of view [52]. Mineral N fertilization should be ensured in the event of a deficiency of fixed N_2 bound by symbiosis [8]. In Poland, under natural conditions, *B. japonicum* bacteria is not present in soils, so for symbiosis to occur, it is necessary to inoculate seeds with bacterial preparations, and this treatment is considered an effective way to increase the yield of soybeans [53]. In soybean cultivation, seed inoculation with *B. japonicium* bacteria, which bind free N from the air, is therefore a particularly important procedure [54]. Many authors [55–58] have observed an increase in nodule formation as a result of the use of bacterial preparations compared to seeds without inoculation. In studies conducted by Novytska et al. [59], inoculation had a differentiating effect on the development of yield parameters, while the effect on seed yield was the same in different cultivars. The effectiveness of inoculation is largely influenced by N fertilization. Poor effectiveness of nodule formation may be caused by a high dose of N applied before sowing [60]. Significant decreases in nodule dry weight, nodule count and nitrogenase activity due to an increase in N concentration have been confirmed by Lyu et al. [23]. In the study of Abdel-Wahab and Abd-Alla [61], the application of N fertilization with low doses of N (16–32 kg N ha^{-1}) significantly increased the number of nodules on the plant. In our own studies, under the influence of N fertilization at a dose of 60 kg N·ha^{-1}, the number of nodules was slightly reduced compared to inoculated objects and fertilization with a dose of 30 kg N·ha^{-1}; however, under the influence of each dose of Na, a decrease in their mass was observed both on the main root and on the lateral roots.

According to Sincik et al. [62], the growth dynamics of the LAI value is closely related to the yield of soybeans. It is assumed that an LAI value in the range of 3.5 to 4.0 is the soybean yielding measure [63]. In the conducted experiment, similar or higher LAI values were obtained, and its highest values were obtained in the R3 phase. The decrease in LAI may result from the water deficit in the seed filling phase [3], which was observed in 2017 when the least favorable weather conditions occurred (rainfall shortages in June and July).

It can also result from the effect of plant shading, which causes a decrease in the number of pods and the number and weight of seeds per plant [64]. In this experiment, an increase in LAI values in the R2–R4 phases was observed, and not only a tendency of a decrease in the number of pods per plant, but an increase in the number and weight of seeds from the plant and the weight of 1000 seeds was also observed. The appropriate value of the LAI leaf area index allows capturing the radiation and converting light energy into chemical energy (carbohydrates), which results in high seed yields. Papillary bacteria require a continuous supply of carbohydrates to produce the required energy and to capture atmospheric N_2. Therefore, during flowering, N fertilization is justified when root nodules age or if there are few of them. In addition, in conditions of N deficiency, leaves and pods are significant competitors for carbohydrates for nodules [61]. The increase in the LAI value is also influenced by N fertilization. According to multiple authors' studies [28,65–67], there was a positive relation between N fertilization and LAI. A similar relationship was also confirmed in this experiment. With the increase in N fertilization and higher N fixation caused by inoculation of seeds with *B. japonicum* bacteria, an increase in the value of this parameter of the field architecture was observed. This can be explained by the stimulating effect of N on the elongation and multiplication of cells. Greater accumulation of this element in leaves induces the course of biological processes, which results in the production of wider leaves, thus affecting the increase in the value of the LAI [68].

Although the increase in soybean yields in recent decades has been significant, soybean growers are constantly looking for opportunities to optimize crop management and increase seed yields, including the use of N fertilizers.

5. Conclusions

Current research shows the effect of the use of differentiated N fertilization and bacterial inoculation on nodule formation and yielding of soybean of Aldana and Annushka cultivars. Examining the effectiveness of inoculation of *Bradyrhizobium japonicum* in soybean, it was shown that it consistently increases the nodulation on plant roots, has a positive effect on the yield components (number of pods, number and weight of seeds per plant) and seed yield, while no effect of the type of bacterial preparation on the yield was found. On average, for the years of the study, N fertilization had no effect on the seed yield, but the application of a dose of 30 kg $N{\cdot}ha^{-1}$ did not result in a significant reduction in the number and weight of nodules, including on the main root and lateral roots, compared to seeds inoculated and not fertilized with N, as observed under a dose of 60 kg $N{\cdot}ha^{-1}$, but resulted in an increase in the number of pods and the number and weight of seeds per plant. Based on the results of that study, it was found that for both soybean cultivars tested, the best combination was nitrogen fertilization at 30 kg $N{\cdot}ha^{-1}$ and seed inoculation with *B. japonicum*, regardless of the bacterial preparation used (HiStick® Soy or Nitragina).

Considering the increasing pressure to increase food production while minimizing environmental impacts, it is advisable to conduct further research into the efficiency of soybean N uptake by plants under different environmental conditions and cultivation systems.

Author Contributions: Conceptualization, E.S.-K. and D.B.-J.; methodology, E.S.-K.; formal analysis, E.S.-K.; investigation, E.S.-K., M.J.-P. and W.P.; writing—original draft preparation, E.S.-K.; writing—E.S.-K. and M.J.-P.; visualization, E.S.-K.; supervision, E.S.-K. and D.B.-J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Polish Ministry of Agriculture and Rural Development, Project: Improving domestic sources of plant protein, their production, trading and use in animal feed, grant No. HOR 3.6/2016–2020.

Data Availability Statement: The datasets used and/or analyzed in the current study are available from the author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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