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Optimizing the Amount of Nitrogen and Seed Inoculation to Improve the Quality and Yield of Soybean Grown in the Southeastern Baltic Region

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Abstract: The cultivation of soybeans, especially where this species has not been grown in large areas, requires the determination of the optimal mineral nitrogen fertilization and seed inoculation with *Bradyrhizobium japonicum*. The purpose of the study was to determine the optimal dose of mineral N fertilization and seed inoculation treatments with *B. japonicum* under field conditions in the southeastern Baltic region. The objective of this study was to achieve nitrogen supply and/or inoculation with *B. japonicum*: check-0 kg N ha^{-1}, 30 kg N ha^{-1}, 60 kg N ha^{-1}, HiStick® Soy + 0 kg N ha^{-1}, Nitroflora + 0 kg N ha^{-1}, HiStick® Soy + 30 kg N ha^{-1}, HiStick® Soy + 60 kg N ha^{-1}, Nitroflora + 30 kg N ha^{-1}, Nitroflora + 60 kg N ha^{-1}. Higher yields of seeds, protein and fat were found after application HiStick® Soy compared to Nitorflora. The inoculation with *B. japonicum* together with nitrogen fertilization improved crude protein content in seeds, biometrical features, yield components and especially the seed yield of 'Aldana' soybean. The highest seed yield was found after the application of HiStick® Soy and nitrogen fertilization in doses 30 kg N ha^{-1} or 60 kg N ha^{-1}. Compared to the control, combined *B. japonicum* inoculation and nitrogen fertilization in soybean cultivation proved to be a significant factor in improving the productivity of this species in southeastern Baltic conditions.

Keywords: soybean; inoculation; N fertilization; productivity; chemical composition

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Citation: Panasiewicz, K.; Faligowska, A.; Szymańska, G.; Ratajczak, K.; Sulewska, H. Optimizing the Amount of Nitrogen and Seed Inoculation to Improve the Quality and Yield of Soybean Grown in the Southeastern Baltic Region. *Agriculture* **2023**, *13*, 798. https://doi.org/10.3390/agriculture13040798

Academic Editors: Paulo Mazzafera and Peng Hou

Received: 13 February 2023
Revised: 27 March 2023
Accepted: 29 March 2023
Published: 30 March 2023

1. Introduction

Soybean (*Glycine max* L. Merril) has been one of the most important crops in the world for many years. This species is characterized by a valuable chemical composition, which is primarily caused by the content of oil (18–23%) with a high content of unsaturated fatty acids and protein (33–45%) and a well-balanced amino acid profile [1–7]. World soybean production in 2020–2021 amounted to 368 million tonnes [8]. In 2019, 2.8 million tonnes of soya were produced in the EU-27 [9]. In the EU, which includes Poland, an alternative to GMO feed is needed. Significant dependence on genetically modified soybean in Poland (annual import is about 2.4 million tons) and the EU (19 million tonnes), with a simultaneous ban on the cultivation of these forms in many countries, creates an urgent need to produce domestic non-GMO soya protein. EU countries use soybean meal as the primary source of plant protein in the feeding of poultry, pigs and less cattle. One of the possible ways to improve protein balance is to obtain protein from legumes [10–13]. Therefore, in many countries, there has been a gradual increase in the cultivation of these species, including soybean. In Poland, it is estimated that, in 2020, the cultivation area of soybean was 20,000 ha [9] and continues to increase. The cultivation of soybeans in Northern and Central Europe is still relatively new. Many breeders have taken steps to introduce varieties adapted to cooler conditions, but this takes time. Therefore, we still observe a lack of varieties adapted to regional growing conditions, especially early varieties.

Furthermore, soybean plays a significant role in cultivation, since as a legume, it enables the binding of nitrogen from the air as a result of symbiosis with nodule bacteria [14–16]. This makes soybean a desirable crop and should be considered in crop rotation [17]. The global trend towards sustainable agriculture is due to a reduction in the use of various inputs, especially chemical inputs, and its ultimate goal is to achieve yield stability and reduce adverse environmental effects. Legumes, including soybeans, provide nitrogen to the soil in the form of above-ground crop residues, roots and nodules that contain a significant amount of nitrogen from the atmosphere, which is the most economical way to enrich the soil with this component and an important factor in sustainable and ecological agriculture [18–22]. The amount of nitrogen biologically fixed by symbiotic bacteria depends on many factors, such as species, variety, development stage, health condition, light intensity, soil moisture and reaction, as well as fertilization with macro and microelements. As a result, it provides easily available nitrogen, which translates into reducing nitrogen fertilization. Unfortunately, the lack of prior cultivation of this species in European soils results in the need to inoculate the seeds before sowing. The seeds must be inoculated with *Bradyrhizobium* strains to fix nitrogen and then realize their yield potential [16]. However, there are not many agrotechnical recommendations for this species, especially with regard to varying regional conditions. Therefore, in our study, we hypothesized that the use of *B. japonicum* inoculation and/or nitrogen fertilization may modify soybean productivity in the conditions of the southeastern Baltic region, and to recognize this, the earliest domestic variety was used in the study. Moreover, the experiments were also conducted to show whether and to what extent the addition of mineral nitrogen would significantly increase the yield of soybean.

The goal of this research was to evaluate the possibility of optimizing soybean productivity depending on mineral nitrogen fertilization and seed inoculation with *B. japonicum*.

2. Materials and Methods

2.1. Site Description

The field experiment on soybean crop was conducted at the Gorzyń Experimental and Educational Station, Poznań University of Life Sciences (N—52.56692, E—015.90933, 69 m AMSL) at the Wielkopolska region, Poland. The field experiment was established on soils classified as typical luvisol soils formed from light loamy sands, and then deposited in a shallow layer on light loam according to Polish Soil Classification [23] and Haplic Luvisols according to FAO-WRB [24]. Total N content in the soil was 527 mg kg^{-1} soil, plant available phosphorus (P) was 13.9 mg kg^{-1} soil, and potassium (K) was 10.9 mg kg^{-1} soil, pH = 5.1 measured in 1 M KCL, 1.3 % organic matter.

Weather conditions in the years of the study during the vegetation period of soybean are presented using the hydrothermal index according to Sielianinov (Table 1). To calculate the value of K, the following formula was used: $K = (Mo \times 10)/(Dt \times days)$ where K is the hydrothermal coefficient for an individual month during the growing season, Mo is total monthly precipitation, and Dt is the mean daily temperature in a particular month.

The variability of weather conditions in the years of the study is reflected in the values of the index K, which combine temperature and precipitation. Lower values of this index according to multi-year average were recorded in March for the years 2016, 2017, 2019 and 2020; April (2016–2020); May (2016, 2018 and 2020); June, July and August (2018–2020); and September (2016, 2018 and 2020). More suitable moisture conditions for the period of soybean vegetation were observed in 2016 and 2017, than in the drier 2018 (May: K = 0.43, June: K = 0.41), 2019 (April: K = 0.23, June: K = 0.1), and 2020 (April: K = 0.28, June: K = 0.6) seasons, which resulted in lower yields obtained in these years.

Table 1. Hydrothermal index: Sielianinov index (K) in the growing season (March to September) in 2016–2020 (recorded at the Agrometeorological Observatory in Gorzyń, Poland).

Year	Months							Average
	III	IV	V	VI	VII	VIII	IX	
2016	2.95	0.93	1.11	1.71	1.56	1.15	0.13	1.37
2017	2.93	1.44	1.93	2.65	2.79	1.91	1.15	2.12
2018	6.01	1.45	0.43	0.41	1.32	0.14	1.04	1.54
2019	2.84	0.23	2.36	0.10	1.24	0.62	1.44	1.26
2020	2.12	0.28	1.39	0.60	1.25	1.17	1.04	1.12
1958–2015	3.86	2.00	1.42	1.21	1.36	1.10	1.09	1.72

Sielianinov Index (K): <0.5–drought, 0.5–1.0–semi-drought, 1.0–1.5–border of optimal moisture, >1.5–excessive moisture.

2.2. Experimental Design and Agronomic Management

The field experiment on soybean with early cultivar 'Aldana' (000) from Polish breeding was replicated at the same location every year over a 5-year period (2016–2020). The study was conducted in a randomized block design with four replications and 36 plots (plot size 14 m $\times$ 1.5 m). The experience factor was N supply (N fertilization and/or seed inoculation) with nine combinations: 1. check (0 dose of nitrogen and seeds without inoculation), 2. 30 kg N ha^{-1}, 3. 60 kg N ha^{-1}, 4. HiStick® Soy + 0 kg N ha^{-1}, 5. Nitroflora + 0 kg N ha^{-1}, 6. HiStick® Soy + 30 kg N ha^{-1}, 7. HiStick® Soy + 60 kg N ha^{-1}, 8. Nitroflora + 30 kg N ha^{-1}, 9. Nitroflora + 60 kg N ha^{-1}. The applied N mineral fertilization was used as an ammonium nitrate (NH_4NO_3) before seed sowing by mixing with surface soil. Prior to sowing, one seed combination was inoculated with a domestic inoculant called Nitroflora, and the other combination was inoculated with foreign HiStick® Soy (BASF Agricultiral Specialities Limited, Littlehampton West Sussex, UK). All inoculants contain *Bradyrhizobium japonicum* strains dedicated for soybean. The seeds were sown in the research year on 26 April, 24 April, 23 April, 25 April and 21 April. In each year, seed sowing was performed at row spacing—15 cm, sowing density—90 seeds per 1 m^2, and 3–4 cm sowing depth. The forecrop in each year of the study was winter rye. A pre-sowing fertilizer with phosphorus and potassium was applied at 100 kg P_2O_5 ha^{-1} (43.6 kg P ha^{-1}) and 100 kg K_2O (83 kg K ha^{-1}), respectively. Directly after sowing, weed control was carried out with pendimetalina (Stomp Aqua 455 CS) in a dose of 1.5 L ha^{-1} and additionally bentazon and imazamoks (Corum 502.4 SL) in dose of 1.4 L ha^{-1} and Olejan 85 EC (85% rape oil) in dose 1.5 L ha^{-1}. Seed harvesting was carried out at the full maturity stage of soybean from late August to early September, with a 1.5 m wide Wintersteiger plot combine harvester.

2.3. Data Collection

Each year, in the late flowering phase, 10 whole plants were randomly collected for nodulation measurements involving the nodule dry mass (laboratory drier, 80 °C/48 h). Before harvesting, after reaching full maturity, the biometric traits of 10 randomly selected plants and yield components of soybean yield: the number of pods, number of seeds per plant, number of seeds per nod, and 1000 seed weight were determined. Seed yield per ha was calculated at the 15% moisture level.

2.4. Chemical Analysis

In each year of the study, a random sample of soybean seeds from each plot was taken after harvesting for chemical analysis. The prepared samples were then stored in closed containers at a temperature of 4 °C. The seeds were grounded to pass through a 0.5 mm sieve. The chemical composition analysis included measurements of dry matter content (DM), crude protein (CP), and crude fibre (CF) content that were carried out according to standard AOAC procedures [25]. The nitrogen content of the seeds was determined using the Kjeldahl method [25] and expressed as total protein content (N × 6.25) [25] and crude fat (CF) using the Soxhlet method. The crude fibre was determined via the hydrolyzation of other components of the plant material, and crude ash (CA) was determined via incineration. Nitrogen-free extracts (NFE) were calculated as NFE = DM − (CP + CA + CF). Seed protein content was expressed on a dry weight basis (g kg^{-1}) and recalculated as protein yield (kg ha^{-1}).

2.5. Statistical Analysis

The recorded results were statistically analyzed using an analysis of variance (ANOVA) in four replications with the SAS package [26]. The means were compared between the treatments via the least significant difference (LSD) test, where $p < 0.05$ and $p < 0.01$ levels were considered statistically significant using Tukey's multiple range test (post hoc Tukey HSD). In the heat map, Pearson's correlation matrix was used to analyze parameters for three the most different combinations: check object, 30 kg N ha^{-1}; HiStick® Soy + 30 kg N ha^{-1}; and Nitroflora + 30 kg N ha^{-1}.

3. Results

The weather conditions during the study years significantly influenced the yield of soybean (Table 2). The highest yields were found in 2016 (seed yield: 2.03 t ha^{-1}, protein yield: 628 kg ha^{-1}, fat yield: 361 kg ha^{-1}) and the lowest yields were found in 2018 (0.77, 238 and 138 respectively).

Table 2. The seed yield (t ha^{-1}), protein yield (kg ha^{-1}) and fat yield (kg ha^{-1}) in research years: 2016–2020.

Year	Specification		
	Seed Yield	Protein Yield	Fat Yield
2016	2.03 [a]	628 [a]	361 [a]
2017	1.91 [a]	594 [a]	337 [a]
2018	0.77 [c]	238 [c]	138 [c]
2019	1.03 [b]	318 [b]	183 [b]
2020	1.16 [b]	358 [b]	206 [b]
LSD value	0.188 **	57.7 **	33.5 **

Values followed by the same letters in the column are not significantly different ** $p < 0.01$.

Differences of 62% were found between the study years for seed, protein, and fat yields. The relationship between protein and fat yields during the study years was similar to that in seed yield because of the counting method. The factor evaluated in our research had a significant impact on the seed yield, protein yield and fat yield of soybean (Table 3). Compared to the controls, all combinations significantly increased the value of the discussed features.

The highest values were found after the application HiStick® Soy and nitrogen fertilization in doses 30 kg N ha^{-1} or 60 kg N ha^{-1}. These combinations, compared to seeds without fertilization and inoculation, caused increases in seed yield by 58.3% and 57.3%, protein yield by 82.9% and 80.5%, and fat yield by 49.2% and 49.7%, respectively. The supply of N mineral fertilizers and seed inoculation by B. japonicum modifies most biometrical features and yield components of soybean, except plant density and the number of seeds per pod (Table 4).

Table 3. The effect of N supply and inoculation on seed yield (t ha^{-1}), protein yield (kg ha^{-1}) and fat yield (kg ha^{-1}).

Specification	Seed Yield	Protein Yield	Fat Yield
Control	1.03 ^{d}	293 ^{e}	189 ^{e}
30 kg N ha^{-1}	1.17 ^{c}	338 ^{d}	217 ^{d}
60 kg N ha^{-1}	1.26 ^{c}	368 ^{cd}	232 ^{d}
HiStick® Soy	1.46 ^{b}	463 ^{b}	257 ^{c}
Nitroflora	1.27 ^{c}	389 ^{c}	226 ^{d}
HiStick® Soy + 30 kg N ha^{-1}	1.63 ^{a}	536 ^{a}	282 ^{ab}
HiStick® Soy + 60 kg N ha^{-1}	1.62 ^{a}	529 ^{a}	283 ^{a}
Nitroflora + 30 kg N ha^{-1}	1.46 ^{b}	460 ^{b}	260 ^{bc}
Nitroflora + 60 kg N ha^{-1}	1.47 ^{b}	469 ^{b}	258 ^{c}
LSD value	0.121 **	38.95 **	22.68 **

Values followed by the same letters in the column are not significantly different ** $p < 0.01$.

Table 4. The effect of N supply and inoculation on biometrical features and yield components.

Specification	PH	DMN	PD	NP	NS	NSP	TSW
Control	37.3 ^{c}	0.5 ^{d}	79.5	8.7 ^{d}	14.2 ^{c}	1.6	183.5 ^{f}
30 kg N ha^{-1}	41.2 ^{ab}	0.5 ^{d}	73.7	10.1 ^{cd}	15.6 ^{c}	1.5	187.4 ^{ef}
60 kg N ha^{-1}	43.4 ^{a}	0.7 ^{cd}	78.1	12.8 ^{a}	20.1 ^{ab}	1.5	188.8 ^{de}
HiStick® Soy	38.2 ^{b}	3.1 ^{a}	73.0	10.6 ^{bcd}	17.2 ^{bc}	1.6	200.5 ^{a}
Nitroflora	37.0 ^{c}	4.1 ^{a}	74.0	9.5 ^{d}	16.3 ^{bc}	1.7	198.7 ^{ab}
HiStick® Soy + 30 kg N ha^{-1}	40.6 ^{ab}	2.8 ^{ab}	77.1	12.4 ^{ab}	21.7 ^{a}	1.6	203.4 ^{a}
HiStick® Soy + 60 kg N ha^{-1}	42.6 ^{a}	2.7 ^{b}	70.7	11.5 ^{abc}	19.2 ^{ab}	1.5	200.1 ^{ab}
Nitroflora + 30 kg N ha^{-1}	40.9 ^{ab}	1.9 ^{bc}	75.1	10.0 ^{cd}	16.1 ^{c}	1.6	194.6 ^{bc}
Nitroflora + 60 kg N ha^{-1}	43.1 ^{a}	2.9 ^{ab}	78.7	12.9 ^{a}	20.5 ^{a}	1.5	193.1 ^{c}
LSD value	3.22 **	1.35 **	NS	1.95 **	3.09 **	NS	4.12 **

NS: not significant; Values followed by the same letters in the column are not significantly different ** $p < 0.01$. Specification: PH, plant height (cm); DMN, dry mass of nodules per plant (g); PD, plant density (no. m^2); NP, number of pods per plant; NS, number of seeds per plant; NSP, number of seeds per pod; TSW, 1000 seed weight (g).

Compared to the control, all combinations significantly increased plant height. The highest plants were observed after using 60 kg N ha^{-1} (43.4 cm) and using a combination of Nitroflora (43.1 cm) and HiStick® Soy (42.6 cm). Nitrogen fertilization without inoculant did not influence the dry mass of nodules per plant, but inoculation by B. japonicum affected the increase in dry mass of nodules per plant both with and without nitrogen fertilization. There was no significant difference between the applied inoculants, but a higher dry mass of nodules from the plant was found after using Nitroflora (4.1 g). A significantly higher number of pods and seeds per plant were observed after the application of 60 kg N ha^{-1}, HiStick® Soy + 30 kg N ha^{-1} or HiStick® Soy + 60 kg N ha^{-1} and Nitroflora + 60 kg N ha^{-1}. Compared to the control, inoculation with B. japonicum substantially affected 1000-seed weight, and the highest values were observed after HiStick® Soy inoculation without nitrogen fertilization (by 9.3%) and with 30 kg N ha^{-1} (by 10.8%) or 60 kg N ha^{-1} (by 9.0%). The chemical composition of the dry matter of soybean seeds is shown in Table 5.

Table 5. The effect of N supply and inoculation on organic components and ash contents in soybean seeds (g kg^{-1} DM).

Specification	Crude Protein	Crude Fat	Crude Fibre	Crude Ash	N-Free Extract
Control	332.6 ^{b}	215.0	79.5	64.0	308.7 ^{a}
30 kg N ha^{-1}	339.5 ^{ab}	218.7	85.8	64.7	291.1 ^{abc}
60 kg N ha^{-1}	341.9 ^{ab}	215.6	81.1	63.4	297.8 ^{ab}
HiStick® Soy	372.0 ^{ab}	206.4	83.8	62.9	274.6 ^{abc}
Nitroflora	359.1 ^{ab}	208.4	97.6	64.7	269.9 ^{bc}
HiStick® Soy + 30 kg N ha^{-1}	385.2 ^{a}	202.7	90.0	62.2	259.7 ^{c}
HiStick® Soy + 60 kg N ha^{-1}	381.8 ^{a}	204.6	95.0	61.2	257.1 ^{c}
Nitroflora + 30 kg N ha^{-1}	370.5 ^{ab}	209.3	99.2	61.5	259.3 ^{c}
Nitroflora + 60 kg N ha^{-1}	373.2 ^{ab}	205.8	94.6	61.1	265.0 ^{bc}
LSD value	48.18 **	NS	NS	NS	37.93 **

NS: not significant; Values followed by the same letters in the column are not significantly different ** $p < 0.01$.

Previous studies show significant differences in protein content depending on the doses of nitrogen and the applied inoculation. A significantly higher content of crude protein compared to control without fertilization and inoculation with B. japonicum was observed in seeds after the application of HiStick® Soy with 30 kg N ha^{-1} and HiStick® Soy with 60 kg N ha^{-1} and the differences were 52.6 g kg^{-1} (15.8%) and 49.2 g kg^{-1} (14.8%), respectively. Compared to the control, decrease in the value of N-free extract was noted in all other combinations. Significant differences were found for seeds inoculated only with Nitroflora and fertilized with nitrogen, as well as in soybean seeds where both HiStick® Soy inoculation and nitrogen fertilization were used. The experimental factor used in our study did not modify the crude fat, fibre and ash content in seed of soybean 'Aldana'.

The analysis of the correlation coefficients for the analyzed parameters depending on the selected combinations is presented in Figure 1. In our study, on the control plot, i.e., without seed inoculation and nitrogen fertilization, a strong correlation was observed between seed yield and nodule dry matter (r = 0.94), as well as a strong relationship between the number of pods per plant and the number of seeds per plant (r = 0.93).

On the combination where fertilization was applied at a dose of 30 kg N ha^{-1}, it was found that decreasing plant density was accompanied by a decrease in plant height (r = −0.98). In addition, a strong relationship was recorded between the number of pods per plant and the number of seeds per plant (r = 0.91).

On the object with HiStick® Soy inoculation and fertilization at a dose of 30 kg N ha^{-1}, a strong relationship between seed yield and the dry matter of nodules (r = 0.91) was found. Furthermore, the height of plants was observed, taking into consideration the 1000-seed weight (r = 0.92) and the practically functional relationship of the number of pods with the number of seeds per plant (r = 0.98). On the combination with Nitroflora and 30 kg N ha^{-1}, a strong relationship was found for the number of pods with the number of seeds per plant (r = 0.90), and it was also noted that a decrease in plant density was accompanied by a decrease in the number of pods per plant (r = −0.93). In addition, on this object, the weakest relationship between seed yield and nodule dry mass (r = 0.70) was found.

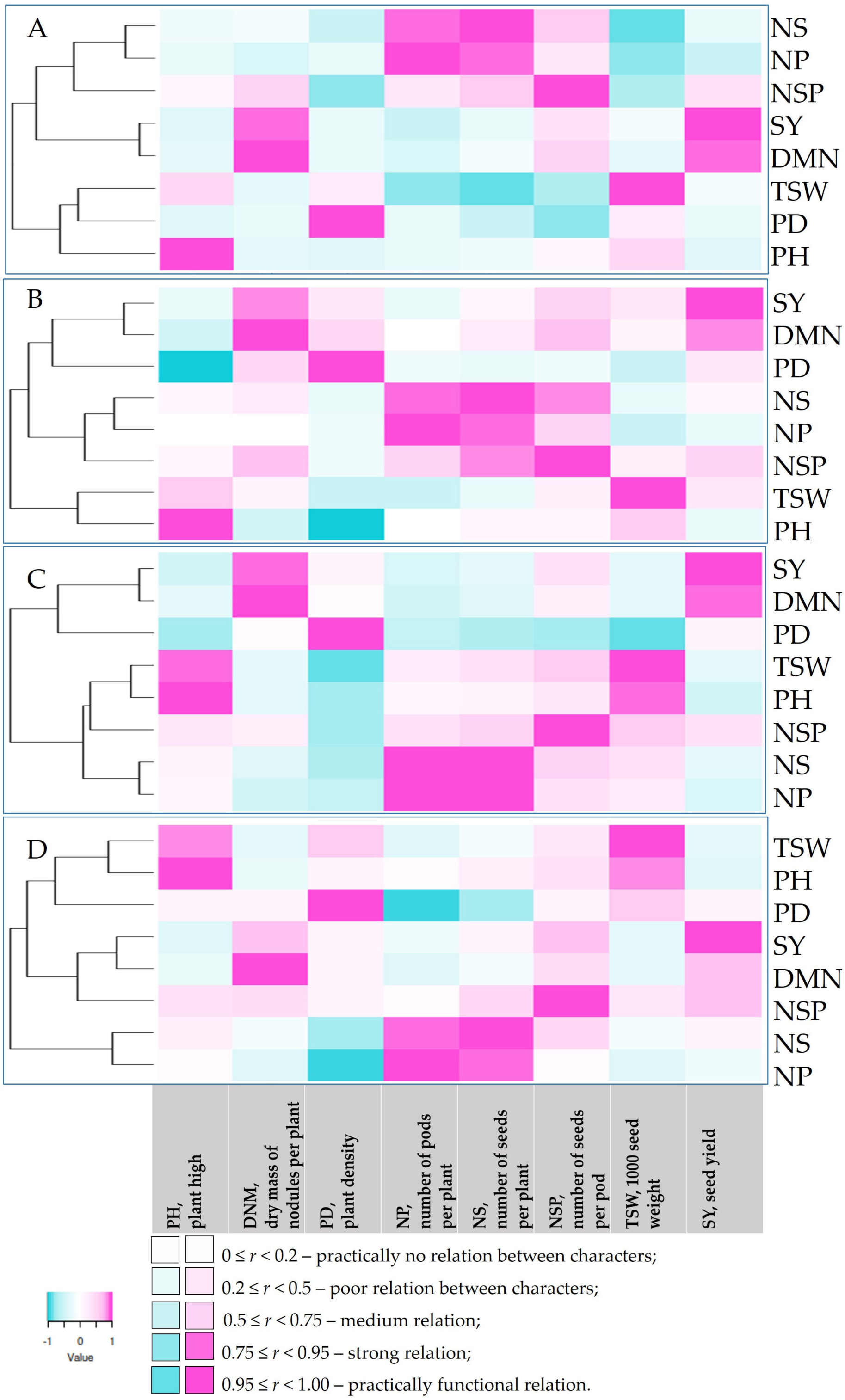

Figure 1. Correlation coefficients between features analyzed for (**A**) Control, (**B**) 30 kg N ha^{-1}, (**C**) HiStick® Soy + 30 kg N ha^{-1}, and (**D**) Nitroflora + 30 kg N ha^{-1}. Features compared: 1. PH, plant height (cm); 2. DMN, dry mass of nodules per plant (g); 3. PD, plant density (no. m^2); 4. NP, number of pods per plant; 5. NS, number of seeds per plant; 6. NSP, number of seeds per pod; 7. TSW, 1000 seed weight (g); 8. SY, seed yield (t ha^{-1}). Interpretation of Pearson's linear correlation coefficient: $0 \leq p < 0.2$—practically no relation between characters; $0.2 \leq p < 0.5$—poor relation between characters; $0.5 \leq p < 0.75$—medium relation; $0.75 \leq p < 0.95$—strong relation; $0.95 \leq p < 1.00$—practically functional relation.

4. Discussion

Many studies focus on the possibility of expanding regions for the cultivation of legumes, especially soybeans [27–31]. This is because increasing animal production in Europe results in increased demand for protein feeds. Therefore, the selection of varieties is especially important. Breeding programs and the introduction of new varieties adapted to specific habitat conditions provides the opportunity to produce protein for nutritional and feed purposes, which strengthens the protein safety of the region [16].

In addition to genetic factors, weather conditions during plant growth and development are important for soybean productivity [32,33]. Our research shows that, in favourable years, the seed yield was at a level of 2.0 t·ha^{-1}, but under unfavourable conditions the seed yield fell below 1.0 t·ha^{-1} (0.77 t·ha^{-1}). Dolijanowic et al., [34] Basal and Szabó [35], and Księżak and Bojarszczuk [36] observed that the most limiting seed yield among weather factors is precipitation. Particularly unfavourable conditions for soybean development were recorded when rainfall deficiencies occurred in May and June or during the sowing period, i.e., at the beginning of vegetation (April), and then during the flowering period (June). Similar observations for weather conditions were reported by Dolijanowic et al. [34], whereas Stojmenova and Alexieva [37] and Ohyama et al. [38] suggested the seed yield of soybean mainly depends on the total rainfall in May, July and August.

Increase the yield potential and acreage of soybean, especially in regions where the species has thus far not or rarely been grown, depends largely on the availability of commercial inoculants [16]. On average, for the five-year period of the research, it was noted that the application of *Bradyrhizobium* strain combined with nitrogen fertilization had a positive effect on the yield of 'Aldana' soybean seeds. Compared to the control, a significant increase in seed yield was obtained after using all combinations, but the highest value was found after application of HiStick® Soy and nitrogen fertilization in doses of 30 kg N ha^{-1} or 60 kg N ha^{-1}. The lack of a significant difference in yield, as well as higher fertilization costs between nitrogen doses, indicates that the optimal dose for soybean cultivation in the described region is 30 kg N ha^{-1}. Similarly, Prusiński et al. [39], for both assessed cultivars (Aldana and Annushka), observed an increase in seed yield after seed inoculation with HiStick® Soy with an application of 30 and 60 kg N ha^{-1}, as well as with Nitragina at 60 kg N ha^{-1}. According to Bassal and Szabó [35], under moderate drought conditions, inoculation with *Bradyrhizobium* strains can improve plant physiological properties and soybean seed yield.

In our study, the applied N fertilization and inoculation significantly increased the dry weight of nodules, but the highest value of this feature was primarily noted where only seed inoculation was performed. According to Franco and Munns [40] and Hungaria et al. [41], the use of nitrogen fertilization to optimize growth and yield appears to be controversial in the case of legumes, as the use of sufficient mineral nitrogen to stimulate growth can also discourage root nodule formation. Księżak and Bojarszczuk [36] evaluating the inoculation of soybean seeds of the 'Aldana', found a significant effect of the applied HiStick® Soy inoculation on the number and fresh weight of nodules.

The seed yield is regulated by yield structure and its components [34]. Our research showed that the applied nitrogen fertilization and/or inoculation had a significant effect on the plant height, the number of pods per plant, number of seeds per plant and 1000 seed weight. The highest value of these was observed at the highest of the tested nitrogen doses (60 kg N ha^{-1}), but also for HiStick® Soy at 30 kg N ha^{-1}, and Nitroflora at 60 kg N ha^{-1}. The highest effect on 1000-seed weight was found after HiStick® Soy inoculation and the combined application of this inoculation with the tested doses of nitrogen. Similarly, Księżak and Bojarszczuk [36] reported a higher 1000-seed weight, number of pods and number of seeds per soybean plant, where nitrogen and inoculants were applied, while Prusiński et. al., [39] evaluating plant height in two cultivars, 'Aldana' and 'Annushka', noted a significant effect of nitrogen fertilization only in the case of 'Annushka'. Research by Jarecki and Bobrecka-Jamro [29] shows that a higher 1000-seed weight value was obtained after nitrogen fertilization than after Nitragina and control. Furthermore, the number of seeds per pod was significantly increased by the nitrogen dose, but only compared to the control.

The results of our study indicate that the supply of mineral nitrogen and seed inoculation by *B. japonicum* modify the content of protein and N-free extract. The highest protein content was recorded after application of HiStick® Soy with 30 kg N ha^{-1} and HiStick® Soy with 60 kg N ha^{-1}. Moreover, our study shows that the use of inoculation and N fertilization did not have a significant effect on fat, fibre, and ash content. As reported by Prusiński et al. [39], the protein content in 'Aldana' seeds was significantly higher after the application of HiStick® Soy without N and with both rates of N. Księżak and Bojarszczuk [36] observed that nitrogen application at a dose of 60 kg·ha^{-1} significantly increased the protein content in seeds, and inoculation with Nitragina or Hi®Stick Soy had a positive effect. However, it simultaneously increased the fibre content and limited the amount of ash and fat. Similarly, Jarecki and Bobrecka-Jamro [29] reported an increase in protein content after bacterial inoculation.

The correlation analysis in our research showed that, in all of the evaluated objects, the relationship between the seed yield and the dry weight of the nodule was found, but with varying strength.

5. Conclusions

The agrometeorological conditions had a significant impact on the effects of the applied N mineral fertilization and inoculation with *B. japonicum*, which directly affected the development of soybean, and consequently the seed yield, protein yield, and fat yield. Limited precipitation has caused a significant decrease in yields of soybeans grown in the southeastern Baltic region. Inoculation with *B. japonicum* together with nitrogen fertilization had a positive effect on protein accumulation in seeds, biometrical features, yield components and finally on the yields of soybean 'Aldana'. The best conditions for soybean yield in our region, as indicated by a very strong positive correlation between nodule dry matter and yield and its components, occurred after using Hi®Stick Soy + 30 kg N ha^{-1}. This indicates the need for breeders to introduce more adapted varieties and to continue researching them.

Author Contributions: Conceptualization, K.P.; methodology, K.P. and A.F.; software, G.S.; validation, K.P., A.F., G.S. and H.S.; formal analysis, K.P. and G.S.; investigation, K.P., A.F. and G.S.; resources, K.P. and A.F.; data curation, K.P. and K.R.; writing—original draft preparation, K.P.; writing—review and editing, K.P.; visualization, K.R.; supervision, A.F. and H.S.; project administration, K.P. All authors have read and agreed to the published version of the manuscript.

Funding: The studies have been supported by the Ministry of Agriculture and Rural Development in Poland within the Multiannual Programme 2016–2020 IUNG-PIB: "Increasing the use of domestic feed protein for the production of high-quality animal products under sustainable conditions".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Anwar, F.; Kamal, G.M.; Nadeemb, F.; Shabir, G. Variations of quality characteristics among oils of different soybean varieties. *J. King Saud Univ. Sci.* **2016**, *28*, 332–338. [CrossRef]
2. Carrera, C.S.; Dardanelli, J.L. Water deficit modulates the relationship between temperature and unsaturated fatty acid profile in soybean seed oil. *Crop Sci.* **2017**, *57*, 3179–3189. [CrossRef]
3. James, A.T.; Yang, A. Interactions of protein content and globulin subunit composition of soybean proteins in relation to tofu gel properties. *Food Chem.* **2016**, *194*, 284–289. [CrossRef] [PubMed]
4. Medic, J.; Atkinson, C.; Hurburgh, C.R. Current knowledge in soybean composition. *J. Am. Oil Chem. Soc.* **2014**, *91*, 363–384. [CrossRef]

5. Ortez, O.A.; Salvagiotti, F.; Enrico, J.M.; Prasad, P.V.V.; Armstrong, P.; Ciampitti, I.A. Exploring nitrogen limitation for historical and modern soybean genotypes. *Agron. J.* **2018**, *110*, 2080–2090. [CrossRef]
6. Patil, G.; Vuong, T.D.; Kale, S.; Valliyodan, B.; Deshmukh, R.; Zhu, C.; Wu, X.; Bai, Y.; Yungbluth, D.; Lu, F.; et al. Dissecting genomic hotspots underlying seed protein. oil. and sucrose content in an interspecific mapping population of soybean using high-density linkage mapping. *Plant Biotechnol. J.* **2018**, *16*, 1939–1953. [CrossRef]
7. Wilk, M.; Ród, S.Ź.; Cennych, E.M.; Adników, S.K. Soya as a source of valuable nutrients/Soja źródłem cennych składników żywieniowych. *Żywność Nauka Technol. Jakość* **2017**, *2*, 16–25. (In Polish)
8. Soystats. International: World Soybean Production (soystats.com). 2022. The American Soybean Association. Available online: http://soystats.com/international-world-soybean-production/ (accessed on 12 February 2023).
9. Eurostat. *Agriculture, Forestry and Fishery Statistics—2020*; Edition 2020, ss. 234.; Publications Office of the European Union: Luxembourg, 2020.
10. Jańczak-Pieniążek, M.; Buczek, J.; Bobrecka-Jamro, D.; Szpunar-Krok, E.; Tobiasz-Salach, R.; Jarecki, W. Morphophysiology, productivity and quality of soybean (*Glycine max* (L.) Merr.) cv. Merlin in response to row spacing and seeding systems. *Agronomy* **2021**, *11*, 403. [CrossRef]
11. Panasiewicz, K. Chemical Composition of Lupin (*Lupinus* spp.) as Influenced by variety and tillage system. *Agriculture* **2022**, *12*, 263. [CrossRef]
12. Sulewska, H.; Niewiadomska, A.; Ratajczak, K.; Budka, A.; Panasiewicz, K.; Faligowska, A.; Wolna-Maruwka, A.; Dryjański, L. Changes in *Pisum sativum* L. plants and in soil as a result of application of selected foliar fertilizers and biostimulators. *Agronomy* **2020**, *10*, 1558.
13. Szpunar-Krok, E.; Wondołowska-Grabowska, A.; Bobrecka-Jamro, D.; Jańczak-Pieniążek, M.; Kotecki, A.; Kozak, M. Effect of nitrogen fertilisation and inoculation with *Bradyrhizobium japonicum* on the fatty acid profile of soybean (*Glycine max* (L.) Merrill) seeds. *Agronomy* **2021**, *11*, 941. [CrossRef]
14. Natarajan, S. Analysis of soybean seed proteins using proteomics. *J. Data Min. Genom. Proteom.* **2014**, *5*, 10–12. [CrossRef]
15. Zhang, J.; Wang, X.; Lu, Y.; Bhusal, S.J.; Song, Q.; Cregan, P.B.; Yen, Y.; Brown, M.; Jiang, G.L. Genome-wide scan for seed composition provides insights into soybean quality Improvement and the impacts of domestication and breeding. *Mol. Plant* **2016**, *11*, 460–472. [CrossRef]
16. Zimmer, S.; Messmer, M.; Haase, T.; Piepho, H.P.; Mindermann, A.; Schulz, H.; Habekuß, A.; Ordon, F.; Wilbois, K.-P.; Heß, J. Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. *Eur. J. Agron.* **2016**, *72*, 38–46. [CrossRef]
17. Miladinovic, J.; Kurosaki, H.; Burton, J.W.; Hrustic, M.; Miladinovic, D. The adaptability of short season soybean genotypes to varying longitudinal regions. *Eur. J. Agron.* **2006**, *25*, 243–249.
18. Klepa, S.M.; Ferraz Helene, L.C.; O'Hara, G.; Hungaria, M. *Bradyrhizobium cenepequi* sp. nov., *Bradyrhizobium semiaridum* sp. nov., *Bradyrhizobium hereditatis* sp. nov. and *Bradyrhizobium australafricanum* sp. nov., symbionts of different leguminous plants of Western Australia and South Africa and definition of three novel symbiovars. *Int. J. Syst. Evol. Microbiol.* **2022**, *72*, 005446.
19. Legget, M.; Diaz-Zorita, M.; Koivunen, M.; Bowman, R.; Pesek, R.; Stevenson, C.; Leister, T. Soybean response to inoculation with *Bradyrhizobium japonicum* in the United States and Artentina. *Agron. J.* **2017**, *109*, 1031–1038.
20. Popovic, V.; Tatic, M.; Spalevic, V.; Rajicic, V.; Filipovic, V.; Todosijevic, L.S.; Stevanovic, P. Effect of nitrogen fertilization on soybean plant height in arid year. In Proceedings of the 2nd International and 14th National Congress of Soil Science Society of Serbia—Solutions and Projections for Sustainable Soil Management, Conference Soil and Food, Novi Sad, Serbia, 25–28 September 2017; pp. 65–73.
21. Zilli, J.E.; Alves, B.J.R.; Rouws, L.F.M.; Simões-Araujo, J.L.; Soares, L.H.B.; Cassa'n, F.; Castellanos, M.O.; O'Hara, G. The importance of denitrification performed by nitrogen-fixing bacteria used as inoculants in South America. *Plant Soil* **2020**, *451*, 5–24. [CrossRef]
22. Zilli, J.E.; Pacheco, R.S.; Gianluppi, V.; Smiderle, O.J.; Urquiaga, S.; Hungria, M. Biological N2 fixation and yield performance of soybean inoculated with *Bradyrhizobium*. *Nutr. Cycl. Agroecosystems* **2021**, *119*, 323–336. [CrossRef]
23. Kabała, C.; Charzyński, P.; Chodorowski, J.; Drewnik, M.; Glina, B.; Greinert, A.; Hulisz, P.; Jankowski, M.; Jonczak, J.; Łabaz, B.; et al. Polish Soil Classification, 6th edition—Principles, classification scheme and correlations. *Soil Sci. Annu.* **2019**, *70*, 71–97. [CrossRef]
24. IUSS Working Group WRB. *World Reference Base for Soil Resources 2014, International Soil Classification System for Naming Soils and Creating Legendsfor Soil Maps*; Update 2015; FAO: Rome, Italy, 2014; p. 182.
25. Horwitz, W.; Latimer, G.W., Jr. (Eds.) *AOAC Official Methods of Analysis of AOAC International*, 18th ed.; Revision 4; AOAC International: Gaithersburg, MD, USA, 2011.
26. SAS Institute. *SAS/STAT User's Guide;* 7th ed.; SAS Campus Drive: Cary, NC, USA, 1999.
27. Căpățână, N.; Bolohan, C.; Marin, D.I. Research regarding the influence of mineral fertilization along with *Bradyrhizobium japonicum* on soybean grain yield (*Glycine max* (L.) Merrill), under the conditions of south-east Romania. *Sci. Papers. Ser. A Agron.* **2017**, *60*, 207–214.
28. Fogelberg, F. Soybean (*Glycine max*) cropping in Sweden—Influence of row distance, seeding date and suitable cultivars. *Acta Agric. Scand. Sect. B—Soil Plant Sci.* **2021**, *71*, 311–317. [CrossRef]

29. Jarecki, W.; Bobrecka-Jamro, D. Reaction of soybean plants to the vaccination of seeds with nitragina and initial nitrogen fertilization. *Nauka Przyr. Technol.* **2016**, *10*, 12. Available online: http://www.npt.up-poznan.net (accessed on 15 January 2022). (In Polish)
30. Pannecoucque, J.; Goormachtigh, S.; Ceusters, J.; Debode, J.; Van Waes, C.; Van Waes, J. Temperature as a key factor for successful inoculation of soybean with *Bradyrhizobium* spp. under cool growing conditions in Belgium. *J. Agric. Sci.* **2018**, *156*, 493–503. [CrossRef]
31. Richard, D.; Leimbrock-Rosch, L.; Keßler, S.; Zimmer, S.; Stoll, E. Impact of different mechanical weed control methods on weed communities in organic soybean cultivation in Luxembourg. *Org. Agric.* **2020**, *10* (Suppl. S1), 79–92. [CrossRef]
32. Lu, W.; Misselbrook, T.H.; Feng, L.; Wu, L. Assessment of nitrogen uptake and biological nitrogen fixation responses of soybean to nitrogen fertiliser with SPACSYS. *Sustainability* **2020**, *12*, 5921.
33. Toleikiene, M.; Slepetys, J.; Sarunaite, L.; Lazauskas, S.; Deveikyte, I.; Kadziuliene, Z. Soybean development and productivity in response to organic management above the Northern Boundary of soybean distribution in Europe. *Agronomy* **2021**, *11*, 214. [CrossRef]
34. Dolijanowic, Z.; Kovacevic, D.; Oliaca, S.; Jovovic, Z.; Stipesevic, B.; Jug, D. The multi-year soybean grain yield depending on weather conditions. In Proceedings of the Međunarodni Simpozij Agronoma, Dubrovnik, Croatia, 17–22 October 2013; pp. 422–477.
35. Basal, O.; Szabó, A. Inoculation enhances soybean physiology and yield under moderate drought. *Life Int. J. Health Life Sci.* **2019**, *5*, 1–13. [CrossRef]
36. Księżak, J.; Bojarszczuk, J. The Effect of Mineral N Fertilization and *Bradyrhizobium japonicum* Seed Inoculation on Productivity of Soybean (*Glycine max* (L.) Merrill). *Agriculture* **2022**, *12*, 11. [CrossRef]
37. Stojmenova, L.; Alexieva, S. Impacts of climate condition on soybean yield. Pochvoznanie. *Agrokhimiya Ekol.* **2009**, *43*, 10–14.
38. Ohyama, T.; Minagawa, R.; Ishikawa, S.; Yamamoto, M.; Hung, N.V.P.; Ohtake, N.; Sueyoshi, K.; Sato, T.; Nagumo, Y.; Takahashi, Y. Soybean eed production and nitrogen nutrition. In *A Comprehensive Survey of International Soybean Research—Genetics, Physiology, Agronomy and Nitrogen Relationships*; IntechOpen: London, UK, 2013; pp. 115–157.
39. Prusiński, J.; Baturo-Cieśniewska, A.; Borowska, M. Response of Soybean (*Glycine max* (L.) Merrill) to Mineral Nitrogen Fertilization and *Bradyrhizobium japonicum* Seed Inoculation. *Agronomy* **2020**, *10*, 1300. [CrossRef]
40. Franco, A.A.; Munns, D.N. Nodulation and growth of *Phaseolus vulgaris* in solution culture. *Plant Soil* **1982**, *66*, 149–160.
41. Hungria, M.; Barradas, C.A.A.; Wallsgrove, R.M. Nitrogen fixation, assimilation and transport during the initial growth stage of *Phaseolus vulgaris* L. *J. Exp. Bot.* **1991**, *42*, 839–844.