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Soybean Response to Seed Coating with Chitosan + Alginate/PEG and/or Inoculation

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Abstract: Inoculated or coated soybean seeds are often sown in agricultural practice. These treatments play a different role depending on the chemical composition of the preparation. The aim of the field experiment was to demonstrate the effectiveness of the developed coating (chitosan + alginate/PEG) and commercial inoculant (HiStick® Soy) applied alone or in combination to soybean seeds. Uncoated (control) seeds were sown for comparison. The research was carried out in 2018–2020 using the cultivar 'Mavka'. The experiment was located in Makowisko, Podkarpackie Province, Poland. Coating composition was developed in a laboratory belonging to the Łukasiewicz Research Network—Institute of Biopolymers and Chemical Fibers in Łódź, Poland. The main role of the coating is to protect soybean seeds from low temperatures. HiStick® Soy inoculant contains *Bradyrhizobium japonicum* bacteria which increase nodulation on the roots. The conducted research demonstrated that sowing only coated seeds was not very effective, because the suitable number of nodules had not developed on soybean roots. The application of the inoculant alone positively affected the assessed traits compared to control, however, plant population was lower than expected. The highest seed yield was obtained after sowing coated seeds in combination with the inoculant (4.32 t·ha^{-1}) and only inoculated seeds (4.23 t·ha^{-1}) compared to control (3.64 t·ha^{-1}). The test of the novel seed-coating agent showed that it had an good effect and efficacy, but only in combination with the inoculation procedure.

Keywords: *Glycine max* (L.) Merr.; seed coating; seed inoculation; nodulation; nitrogen-fixing bacteria; soil plant analysis development; leaf area index; stomatal conductance of leaves; yield; protein

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1. Introduction

Legumes account for 14.5% of arable land worldwide, but only 1.5% in European agriculture. Soybean is the most important species in the group of these plants, which results from high demand of agricultural markets for vegetable oil and protein [1]. In the EU, there is a growing interest in soybean cultivation, therefore increasingly better cultivars are transferred to agricultural practice and their agrotechnics are being improved [2]. Soybean is a thermophilic plant, therefore the seeds are sown in warm soil to avoid low temperature stress. It is assumed that soil temperature below 10 °C adversely affects seed germination [3]. In turn, excessively delayed sowing may result in a decrease in yields, especially when drought occurs [4]. An important procedure in soybean cultivation is seed inoculation with rhizobia, usually *Bradyrhizobium japonicum* [5–7]. Symbiotic nitrogen fixation (SNF) is of great importance for agriculture. However, nodulation is often limited by various abiotic and biotic stresses [8–10]. Seed inoculation is especially recommended when rhizobia are not present in the soil or exhibit low viability [2,9,11]. In turn, the effectiveness of inoculation is lower in areas where soybean is commonly grown. Then native rhizobia present in the soil may be competitive in establishing symbiosis in relation to commercial preparations [6,10,12]. Torres et al. [13] indicated the need for periodic evaluation of commercial inoculants to ensure their effectiveness in different soybean growing regions. Giongo et al. [14] showed a high genetic diversity of *Bradyrhizobium bacteria*, which were present in cultivated fields. This information is important in studies concerning new commercial preparations and their adaptation to local environmental conditions. Commercial offer includes coated soybean seeds containing *Bradyrhizobium japonicum*, e.g., the "Fix fertig" technology [15]. Commercial inoculants can also be purchased and subsequently applied to seeds [16,17] or soil [18]. Pereira et al. [19] reported that both liquid and solid inoculants for soybean seeds were effectively increasing nodulation. They also showed that the liquid preparation significantly increased dry weight of the shoots. The study of Flajšman et al. [15] showed that the inoculant "NS-Nitragin" increased soybean yield compared to control. Sowing seeds coated with the "Fix fertig" technology was less successful. Deaker et al. [20] demonstrated that increasing the dose of a seed inoculation preparation above the manufacturer's recommendations was not harmful and even caused a linear increase in nodulation and yield. Yamakawa and Fukushima [21], using an inoculant above the recommended dose, did not obtain a significant increase in soybean yield. The yield was more affected by the appropriately selected bacterial strain for inoculation and the method of its application to the seeds. Hungria et al. [22] reported that it was possible to spray the soil with diluted inoculant both during seeding and after plant emergence. They considered this to be a better solution than abandoning the inoculation procedure. With proper nodulation, soybean nitrogen demand is met in 40–57% [2] and even in 50–60% [23] by SNF. Soybean cultivation does not require nitrogen fertilization [5,24] or the application of a small dose of 30 to 60 N $kg \cdot ha^{-1}$ [25]. This has important economic [26] and ecological [12] aspects. Hungria et al. [22] demonstrated that symbiosis between the plant and rhizobia can be inhibited when preparations toxic to bacteria are used for seed dressing. Lacerda et al. [27] pointed out that soybean seed dressing should not be phytotoxic and should additionally stimulate seedling physiological activity. The best quality seeds should be sown in order to obtain a fast and correct soybean emergence. For this purpose, the seeds are cleaned after harvest, sorted, dried and enhanced with various substances [28]. Sarreta and de Castro Neto [29] have reported that pre-sowing laser seed irradiation is one of such methods, which accelerates the vigor and emergence of soybean and increases plant density. Hara [30] showed that tungsten and molybdenum compounds applied to soybean seeds improved plant health status in conditions of excess water in the soil. Many such solutions are already in practical use, others are still at the research stage. Coating is an advanced procedure, involving the application of layers of different chemical composition and purpose to the seeds. Afzal et al. [31] and Elshafie and Camele [32] stated that seed coating is a developing technology. Its aim is to protect the seeds after sowing and to stimulate the initial growth of the soybean plants. Many such developed coatings are already in use [31,33]. Zeng et al. [34] showed that seed coating was effective in controlling pests and it increased soybean yield. Avelar et al. [35] showed that a liquid polymer most effectively coated soybean seeds, however, it was not very effective. The best results were obtained after the combined use of a polymer with a fungicide. Soybean emergence was then fast and even. Ludwig et al. [36] compared two methods of polymer application to soybean seeds and found no differences in the tested parameters or water retention capacity of the seeds. However, they proved that large seeds had greater vigor. Pedrini et al. [37] concluded that coated seeds should be sown only in justified cases and coating composition should be ecological and adapted to local conditions. They believed that further research was needed in this area for the safe use of this technology. In this aspect, Poliserpi et al. [38] pointed out that some components of seed coatings could pose a risk to birds. Therefore, when authorizing new seed coating chemicals, their impact on the environment [39,40] and human health [41] should be carefully assessed. Gesch et al. [42] reported that in the case of early sowing dates, properly prepared coatings could increase soybean cold tolerance. This is especially important for simplified or no-tillage cultivation. Ma [43] argued that coated seeds were an effective means of reducing various biotic and abiotic stresses in agricultural practice. As a result, the plants grew better, were healthier and produced higher yield. Evangelista et al. [44] showed that polymer coating applied in conditions of excessive soil moisture decreased the vigor of soybean seeds. Such a relationship was not observed when moisture conditions were optimal. Zeng and Zhang [45] developed a coating that increased soybean yield by 18% compared to control. Additionally, it was achieved at a 26% lower cost compared to a commercial seed coating formulation. They demonstrated that coating composition was effective in protecting against diseases and pests and, additionally, it was safe for the environment. Therefore, research in this field is important both for science and agricultural practice.

The aim of the present study was to determine soybean reaction to sowing seeds with a coating (chitosan + alginate/PEG) and/or a commercial inoculant. The research hypothesis assumed that the composition of the developed coating would increase the development of symbiotic bacteria on soybean roots and would improve the yield and quality of the seed.

2. Materials and Methods

The field experiment was conducted in the years 2018–2020. It was located on a private farm in Makowisko (50°3′ N 22°47′ E), Podkarpackie Province, Poland. The tested factor was the comparison of the efficiency of sowing seeds with coatings and/or a commercial inoculant compared to uncoated seeds (control). The following abbreviations are used in the remainder of the article:

A—control (seeds without coating),
B—inoculated seeds: HiStick® Soy,
C—coated seeds: chitosan + alginate/PEG
D—B + C.

As a result of laboratory work (Łukasiewicz Research Network—Institute of Biopolymers and Chemical Fibers in Łódź, Poland), a two-layer coating was prepared, whose main role was to protect soybean seeds against low temperature after sowing. The seed coat begins to dissolve when the soil temperature is about 10 °C. The first layer was chitosan and the second layer was sodium alginate. As a result, the coating did not dissolve quickly after contact with water. Substances were introduced into the coating that ensured its variable decomposition depending on soil temperature. Additionally, the coating was composed of a mixture of polyethylene glycol PEG 400 and PEG 600. Polyethylene glycol (PEG) is supposed to increase the viscosity and preserve it with the seed coating.

Individual coating layers were sprayed onto the seeds using a laboratory device. The device is a prototype for coating seeds in laboratory conditions. It was produced by Scientific Research Center of Soya Development, "AgeSoya" Ltd. (Huta Krzeszowska, Poland). The seeds moved in a stream of warm air introduced from below. The air temperature did not exceed 40 °C, so as not to reduce seed germination energy, and the rate of component application was adjusted so not to inflict any mechanical damage or seed swelling due to moisture.

The HiStick® Soy commercial preparation (BASF, Ludwigshafen am Rhein, Germany), for the inoculation of soybean seeds, was used with or without the coating. It allowed to assess the influence of coating components on nodulation. The preparation contained *Bradyrhizobium japonicum* bacteria (2×10^9 cells per gram of peat). The content of the sachet (400 g) is sufficient for the inoculation of 100 kg of seeds. It was applied dry and thoroughly mixed with the seeds directly before sowing.

The research was carried out on the cultivar 'Mavka'. It is a mid-early cultivar (non GMO) recommended for cultivation in the research area. It was a single factor experiment. Each treatment was replicated four times in a randomized block block design.

The experiment was established on sandy loam soil, Haplic Luvisol. The soil was slightly acidic from 5.8 to 6.1 1 mol/L KCl. The content of available phosphorus (P from 98 to 146 mg/kg DM of soil), potassium (K from 187 to 231 mg/kg DM of soil) and magnesium (Mg from 25 to 54 mg/kg DM of soil) was very high or high. The soil samples analysis was carried out at the District Chemical-Agricultural Station in Rzeszów, according to the Polish standards.

Weather conditions were given according to the readings of the Experimental Station for Variety Testing in Skołoszów, Poland. Distance from the experimental field about 10 km.

Seed sowing in individual years was performed on: 12 April 2018, 16 April 2019 and 15 April 2020. The sowing area was 16.2 m^2 (1.8 m × 9 m) for the harvest of 15 m^2. Soybean was not grown in the experimental field before and winter wheat (cv. RGT Kilimanjaro) was the forecrop. Sixty germinating seeds were sown per 1 m^2. Row spacing was 45 cm (4 rows) and sowing depth was 3.5 cm. Mineral fertilization was as follows: 30 N (34% ammonium nitrate), 40 P_2O_5 (19% superphosphate) and 60 K_2O (60% potassium salt) kg·ha^{-1}.

Afalon Dyspersacyjny 450 S.C.—linuron (ADAMA Polska, Ltd., Warsaw, Poland) at a dose of 1.5 L/ha was used for weed control. Plant development stages were given according to Fehr et al. [46].

Plant population after emergence and before harvest was counted per 1 m^2. The number of bacterial nodules were counted and dry weight recorded at the beginning of flowering (R1 stage). For this purpose 10 roots were randomly collected from each plot, in four replications. The roots were thoroughly cleaned of soil and subsequently rinsed in the laboratory on sieves.

Soil plant analysis development (SPAD is estimating leaf chlorophyll) and leaf stomatal conductance (Gs) measurements were performed in the V3 stage. Leaf area index (LAI) was analyzed at the R1 stage. A SPAD 502P chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan) was used for SPAD index measurements. Leaf area index (LAI) measurements were performed using an AccuPAR LP-80 apparatus (Meter Group, Inc., Pullman, WA, USA). Gs measurements were performed with a Porometr SC-1 apparatus (Meter Group, Inc.).

Biometric measurements (number of pods per plant, number of seeds per pod) were made using 20 plants collected from plots at the beginning maturity stage (R7). Thousand-seed weight (TSW) was determined. Soybean was harvested at the full maturity stage, including in the results the plants collected for biometric measurements. Seed yield from the plots was calculated per 1 ha taking into account 13% moisture.

The obtained results were combined across years and statistically analysed with the analysis of variance (ANOVA). The significance of differences between the characteristic values was found based on Tuckey's half-confidence intervals. Statistical analysis was performed using TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

3.1. Weather Conditions

The seeds were sown in the second decade of April. This is the earliest recommended sowing date for soybean in the study area. In 2018, soil temperature in this period was 10.3 °C. The soil in 2019 and 2020 was colder, 6.1 °C and 7.6 °C, respectively (Table 1). Soil temperature in the third decade of April increased in each of the analyzed years.

Table 1. The soil temperature (°C) in April at a depth of 3.5 cm.

Years	Decade of the Month (Days of the Month)		
	I (1–10)	II (11–20)	III (21–30)
2018	5.7	10.3	11.5
2019	6.0	6.1	10.3
2020	5.5	7.6	10.1

Higher air temperatures were recorded in April and May 2018 compared to long-term data. On the other hand, June was the warmest in 2019 and 2020. July temperatures in each analyzed year were close to the long-term average. In 2020, high temperatures were recorded in August and September. Intensive rainfall was recorded in May 2019 and 2020. Compared to the long-term total, June in 2019 and July in 2020 were dry months (Figure 1).

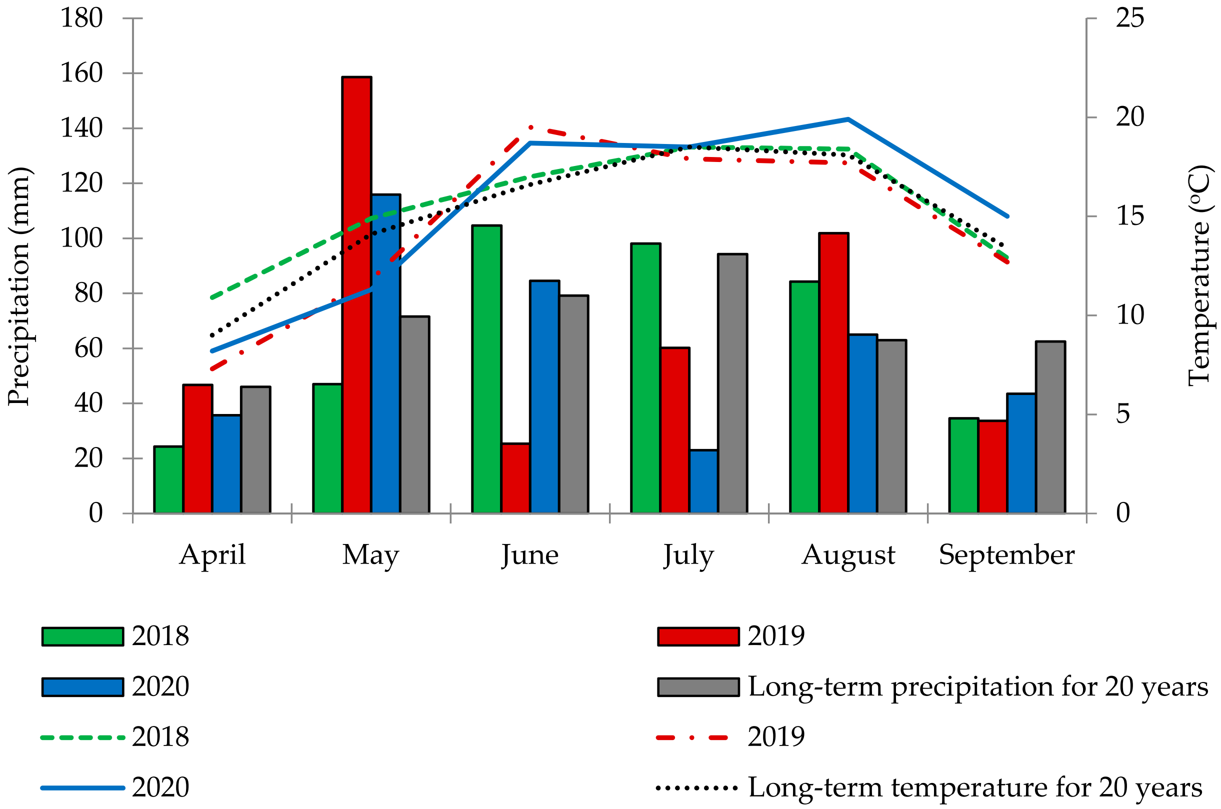

Figure 1. Seasonal weather conditions in the study years.

3.2. Field and Biometric Measurements

Sowing of coated seeds (variant C and D) resulted in a significant delay in emergence as compared to control and inoculated seeds. Differences in the date of emergence were also noted in the study years. The discussed phase in 2018 and 2020 occurred earlier than in 2019. The highest plant population per 1 m^2 and field emergence capacity were recorded on the plots where coated seeds were sown. Significantly lower results were obtained for control seeds and those treated with the inoculant alone. In 2018, plant population per 1 m^2 and field emergence capacity were the highest. Lower results for both traits were obtained in 2020, and the lowest in 2019 (Table 2). Weather conditions significantly modified the emergence of soybean plants.

The measurement of the SPAD index performed at the V3 stage showed that seed inoculation had a positive effect on plant nutritional status. Significantly lower readings were obtained when only coated or control seeds were sown because sowing seeds without inoculation resulted in poor nodulation. Different SPAD index values were recorded over the years of the study. In 2018, the so-called "leaf greenness" was significantly higher (good condition of the plants) in comparison to the results obtained in the following two years. Sowing seeds only with inoculant had a positive effect on the measurement of leaf stomatal conductance (Gs). Significantly lower index values were obtained after sowing seeds with coatings alone. Coating composition resulted in a slight stress of the plants, which was eliminated when the inoculant was applied to the coating (variant D). The LAI values was not modified by the experimental factor, but it varied over the years of the study. Both Gs and LAI measurements had higher values in 2018, lower in 2020 and the lowest in 2019 (Table 3). It should be noted that both too low and high LAI values are unfavorable. Optimum, depending on the soybean variety, is about 3.5 m^2 per m^2.

Table 2. Observations of emergence and plant population measurements.

Treatment (T)	Emergence (Days from the Date of Sowing)	Plant Population after Emergence (Plants·m^{-2})	Field Emergence (%)	Plant Population before Harvest (Plants·m^{-2})
		Tested factor—TF		
A	15.0 ^{b}	47.5 ^{b}	79.2 ^{b}	43.4 ^{b}
B	14.8 ^{b}	47.3 ^{b}	78.9 ^{b}	42.9 ^{b}
C	16.3 ^{a}	50.8 ^{a}	84.6 ^{a}	46.4 ^{a}
D	16.8 ^{a}	50.0 ^{a}	83.3 ^{a}	45.7 ^{a}
		Years—Y		
2018	15.2 ^{b}	54.6 ^{a}	90.9 ^{a}	49.9 ^{a}
2019	16.6 ^{a}	44.1 ^{c}	73.5 ^{c}	40.2 ^{c}
2020	15.4 ^{b}	48.0 ^{b}	80.0 ^{b}	43.7 ^{b}
		ANOVA *p* value		
TF	≤0.001	≤0.001	≤0.001	≤0.001
Y	≤0.001	≤0.001	≤0.001	≤0.001
TFxY	n.s.	n.s.	n.s.	n.s.

n.s.—non-significant, according to Tukey's honestly significant difference (HSD) test. Mean values with different letters (a–c) in columns are statistically different. A—control (seeds without coating), B—inoculated seeds: HiStick® Soy, C—coated seeds: chitosan + alginate/PEG, D—B + C.

Table 3. Field measurements of leaves and plants.

Treatment (T)	SPAD	Gs (mmol m^{-2} s^{-1})	LAI
	Tested factor—TF		
A	39.4 ^{b}	367.7 ^{ab}	3.29 ^{a}
B	43.1 ^{a}	371.5 ^{a}	3.39 ^{a}
C	39.4 ^{b}	360.1 ^{b}	3.30 ^{a}
D	42.6 ^{a}	363.1 ^{ab}	3.39 ^{a}
	Years—Y		
2018	42.9 ^{a}	381.5 ^{a}	3.56 ^{a}
2019	40.0 ^{b}	352.6 ^{c}	3.06 ^{c}
2020	40.4 ^{b}	362.8 ^{b}	3.41 ^{b}
	ANOVA *p* value		
TF	≤0.001	≤0.01	≤0.05
Y	≤0.001	≤0.001	≤0.001
TFxY	n.s.	n.s.	n.s.

n.s.—non-significant, according to Tukey's honestly significant difference (HSD) test. Mean values with different letters (a–c) in columns are statistically different. A—control (seeds without coating), B—inoculated seeds: HiStick® Soy, C—coated seeds: chitosan + alginate/PEG, D—B + C. SPAD—soil plant analysis development. Gs—leaf stomatal conductance, LAI—leaf area index.

A significant interaction of the examined factor with years (TFxY) was demonstrated for the number and dry weight of nodules. The highest number and dry mass of nodules was obtained after sowing inoculated seeds. However, the results obtained depended on the years of research (Figures 2 and 3).

The lowest number and dry mass of nodules was recorded in 2018. The effectiveness of inoculation might have been limited by low precipitation and high temperatures in April and May. Sowing uncoated seeds (control) or only coated seeds resulted in low nodulation. This was expected since soybean had not been previously cultivated in the experimental field.

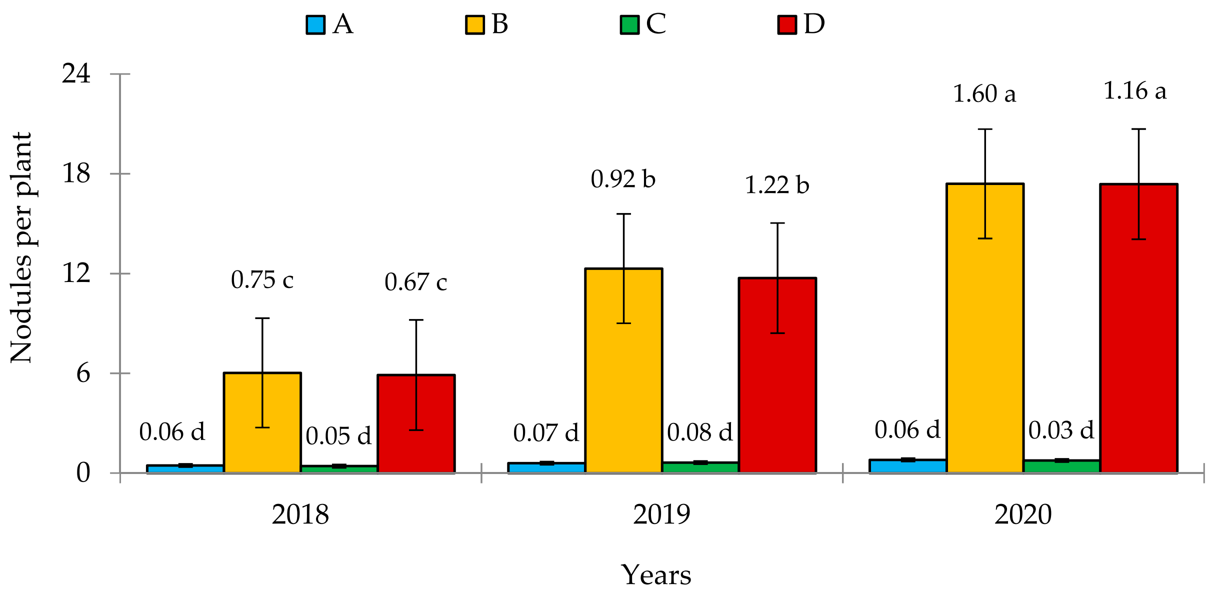

Figure 2. Number of nodules during the flowering stage. A—control (seeds without coating), B—inoculated seeds: HiStick® Soy, C—coated seeds: chitosan + alginate/PEG, D—B + C. Mean values with different letters (a–d) are statistically different at $p < 0.001$. The numbers on the bars indicate the standard error (SE).

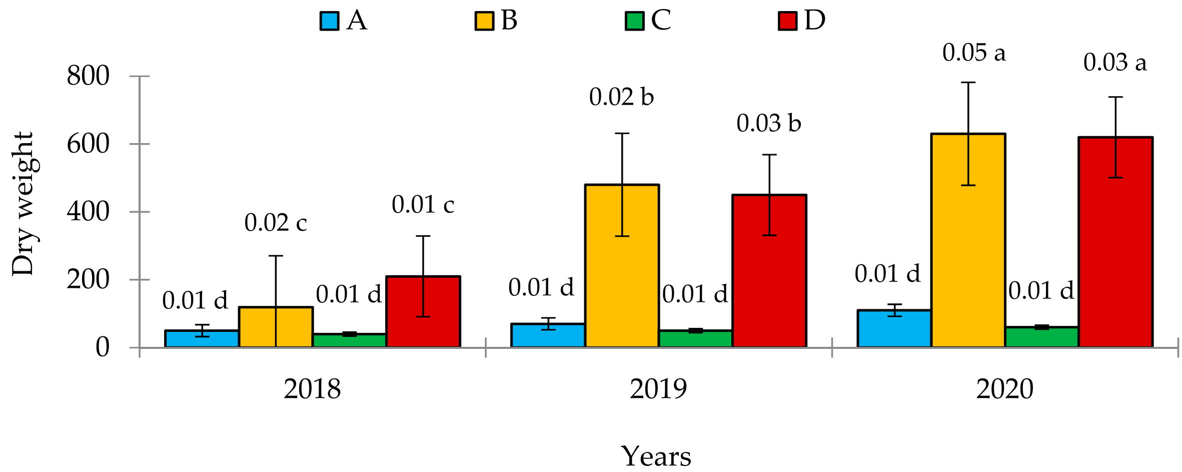

Figure 3. Dry weight of nodules (mg) during the flowering stage. A—control (seeds without coating), B—inoculated seeds: HiStick® Soy, C—coated seeds: chitosan + alginate/PEG, D—B + C. Mean values with different letters (a–d) are statistically different at $p < 0.001$. The numbers on the bars indicate the standard error (SE).

Sowing only inoculated seeds significantly increased the number of pods per plant compared to only coated and control seeds. The combined application of the coating and inoculant had a positive effect on the number of pods in relation to seeds with the coating alone. In 2020, plants developed the highest number of pods. Significantly lower results were obtained in 2019 and the lowest in 2018.

The number of seeds in a pod was significantly increased by the inoculant compared to coating and control. The combined application of the coating and inoculant had a positive effect on the number of seeds per pod, but the differences were insignificant compared to the other variants.

The highest TSW was obtained as a result of sowing inoculated seeds both without and with coating. The obtained difference, compared to the control, was 6.1 g and 7.3 g, respectively. In 2018, test weight was the highest, in 2020 it was lower and in 2019 the lowest.

The study showed that sowing inoculated seeds with or without the coating significantly increased soybean yield in comparison to control. The obtained difference was 0.68 and 0.59 t·ha^{-1}, respectively. The use of the coating alone did not significantly affect the yield, which could be explained by too weak nodulation on the roots (Table 4).

Table 4. Yield components and seed yield.

Treatment (T)	Number of Pods per Plant	Number of Seeds in the Pod	TSW (g)	Seed Yield (t·ha^{-1})
		Tested factor—TF		
A	31.6 ^{bc}	1.97 ^{b}	138.9 ^{b}	3.64 ^{b}
B	34.2 ^{a}	2.05 ^{a}	145.0 ^{a}	4.23 ^{a}
C	30.1 ^{c}	1.98 ^{b}	140.0 ^{b}	3.76 ^{b}
D	33.0 ^{ab}	2.02 ^{ab}	146.2 ^{a}	4.32 ^{a}
		Years—Y		
2018	27.7 ^{c}	2.00 ^{a}	149.3 ^{a}	4.04 ^{b}
2019	33.4 ^{b}	2.02 ^{a}	135.9 ^{c}	3.61 ^{c}
2020	35.6 ^{a}	1.99 ^{a}	142.3 ^{b}	4.32 ^{a}
		ANOVA *p* value		
TF	≤0.001	≤0.01	≤0.01	≤0.001
Y	≤0.001	n.s.	≤0.001	≤0.001
TFxY	n.s.	n.s.	n.s.	n.s.

n.s.—non-significant, according to Tukey's honestly significant difference (HSD) test. Mean values with different letters (a–c) in columns are statistically different. A—control (seeds without coating), B—inoculated seeds: HiStick® Soy, C—coated seeds: chitosan + alginate/PEG, D—B + C.

3.3. Protein Content

The applied inoculant increased protein content in harvested seeds. Seed concentration of protein in treatment A and C was significantly lower. It was shown that protein content in seeds differed over the years of the study. The highest percentage of the discussed component in seeds was in 2020, and the lowest in 2018 (Figure 4). No interaction between the studied factor and the years was statistically found.

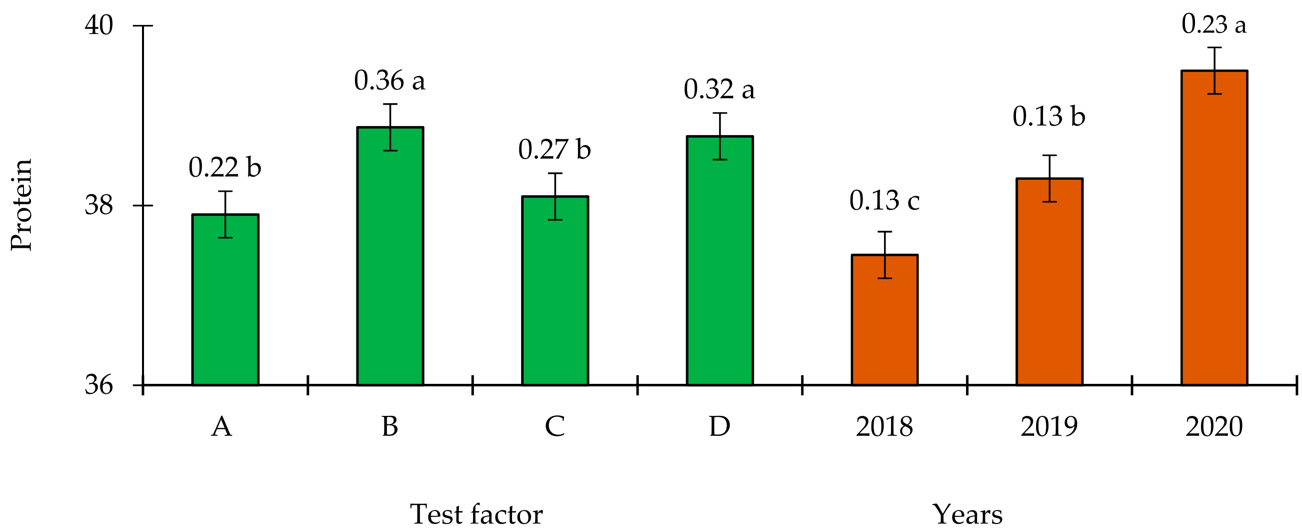

Figure 4. Total protein content (%) in the dry matter of seeds. A—control (seeds without coating), B—inoculated seeds: HiStick® Soy, C—coated seeds: chitosan + alginate/PEG, D—B + C. Mean values with different letters (a–c) within treatment or year are statistically different at $p < 0.001$. The numbers on the bars indicate the standard error (SE).

4. Discussion

4.1. Influence of Weather Conditions

Soybean is highly sensitive to unfavorable weather conditions, which causes yield variability. For this reason, Kuchlan et al. [47] considered research concerning the protection of plants against abiotic and biotic stresses as particularly important. Hungria et al. [22] reported that weather conditions also determined the proper course of soybean symbiosis with rhizobia. They showed that nodulation was significantly limited by drought and high temperature, which was noticed in the present study. In 2018, the number and dry weight of nodules (Figures 2 and 3) were the lowest, which may have been due to the high temperature and low rainfall in April and May compared to the long-term data (Figure 1). The introduction of soybean to a wider cultivation in Poland still faces many difficulties related to the climatic conditions. Therefore, research in this field is up-to-date.

4.2. Inoculation and Nodulation

The condition for symbiosis is the presence of *Bradyrhizobium japonicum* bacteria, which under natural conditions do not occur in Polish soils and therefore soybean seeds must be inoculated with a bacterial preparation. Zerpa et al. [48] considered it justified in soybean cultivation to inoculate seeds in order to increase nodulation. The highest number of nodules on soybean roots (an average of 17.5 nodules) was obtained after the combined application of two commercial bacterial inoculant. They did not find nodulation on the control. Adjetey and Mbotho [49] also confirmed the effectiveness of commercial inoculants, with only a few root nodules observed in control (without the inoculant). Abou-Shanab et al. [50] reported that soil may contain native strains of rhizobia that are capable of symbiosis. They showed that the results of soybean seed inoculation depended on the bacterial strain, nitrogen content in the soil, cultivar or study area. In our experiment, soybean seed inoculation was a beneficial procedure. The effectiveness of soybean seed inoculation varied over the study years, which is presented in Figures 2 and 3. Sowing seeds without inoculum resulted in practically no nodulation. It should be noted that advantage of inoculant is the savings on nitrogen fertilizer and the associated reduction in environmental pollution.

Althabegoiti et al. [18] reported that commercial inoculants can be applied to seeds or applied to the soil in which they will be sown. Interestingly, they obtained better results in the latter case. Coskan and Dogan [51] reported that the nodules on soybean roots were round and large, and had a reddish tinge inside when proper nodulation occurred. Salvagiotti et al. [23] have argued that soybean seed yield is largely determined by the availability of symbiotic nitrogen, which affects the growth and development of a single plant. Many reports [2,26,49] showed that inoculation of soybean seeds resulted in a significant increase in the yield as compared to control. In my experiment, soybean seed inoculation was also needed. This treatment increased the tested parameters, including the seed yield (Table 4). Importantly production of 1 t of soybeans requires twice as much of nitrogen than, for example, peas.

Abou-Shanab et al. [50] obtained a higher dry weight of plants after seed inoculation in relation to control, while seed yield did not differ. Zerpa et al. [48] recorded an increase in soybean leaf area after the combined application of two commercial products containing *Bradyrhizobium japonicum*. A single application of inoculants was less effective, which was observed for most measurements taken. Adjetey and Mbotho [49] reported a higher number of leaves after seed inoculation compared to controls; the uptake of nutrients by plants was also increased. Jarecki et al. [52] proved that nitrogen availability for plants increased their assimilation surface. However, they found no interaction between LAI measurements and the yield of soybean seeds. Ambrosini et al. [53] found that soybean seed inoculation did not always increase the yield. The effects of using commercial biological products depend on many factors, including habitat [49,54], so the study of agrotechnical and environmental interactions requires conducting a series of experiments with cultivars in many environments, i.e., in years and/or habitats or under different conditions of cultivation technologies.

Kaschuk et al. [24] showed that nodulation of soybean roots was limited by high nitrogen content in soil. Therefore, they considered fertilizing soybean with nitrogen redundant, even in the cultivation of high-yielding varieties. Duzan et al. [55] added that nodulation could be inhibited by abiotic stresses, such as low pH, low temperature or high soil salinity. Suzuki et al. [56] studied *Bradyrizobium japonicum* and *Bradyrhizobium elkanii* and demonstrated that the former were more effective at lower temperatures. Albareda et al. [57] and Narożna et al. [58] presented studies on the survival of rhizobia in soil in the successive years following soybean cultivation. Experiments in this field should be considered important in terms of recommendations for seed inoculation. The need for inoculation often depends on the interaction of genetic, environmental, climatic and agrotechnical factors. In my area, inoculation should be a recommended element of soybean agrotechnics. There are marked differences between products that use the same or similar strains of *Bradyrhizobium japonicum*. Peat-based products (e.g., HiStick® Soy, Nitragina) are regarded as standard inoculant products. Inoculant is relatively inexpensive and several new products have entered the market, creating a renewed interest in seed inoculation even on fields that have a history of soybean production.

Wongphatcharachai et al. [12] found that the number and size of nodules on soybean roots depended on many factors, including field location or bacterial strain. Solomon et al. [11] reported that the number and dry weight of nodules on soybean roots depended on the interaction of the cultivar with the strain of *Bradyrhizobium japonicum*. Iturralde et al. [6] also obtained a different number and dry weight of nodules on soybean roots, which was related to the applied strain of symbiotic bacteria. These works provide the basis for selecting the best strain for the production of commercial products. Studies [16,49,52] showed that commercial inoculants containing *Bradyrhizobium japonicum* significantly increased yield components and seed yield. Argav [59] showed that soybean seed inoculation was effective, especially in late maturing cultivars. Căpățână et al. [60] achieved an increase in soybean yield (by 3.8%) after seed inoculation as compared to control. Mineral nitrogen fertilization turned out to be more effective, as yield was increased by 30.2%. Solomon et al. [11] and Thuita et al. [61] confirmed that not every strain of *Bradyrhizobium japonicum* brought the expected results. They showed that soybean yield was mainly determined by the selection of the appropriate soybean variety for local conditions. López-García et al. [62] presented experiments which demonstrated that increased nodulation in soybean had little effect on the yield, and seed nitrogen content did not change. Carciochi et al. [63] indicated that none of the inoculation methods applied modified the yield and chemical composition of soybean seeds under favorable soil conditions especially if these plants had been previously cultivated in a given field. In my field experiment, the effects of seed inoculation were significant compared to the control. This was because soybeans were grown in the field for the first time.

Ludwig et al. [64] demonstrated high efficiency of the spraying method of polymer application to soybean seeds. However, they noted that in order to obtain an optimal coating, the equipment should be well adjusted when the preparation composition and/or seeds are changed. In this aspect Abou-Shanab et al. [50] indicated that legume seed inoculation does not always significantly increase seed yield. Hence, recommendations regarding the use of biological preparations should be related to local conditions. This was confirmed by the experiments carried out by Leggett et al. [26], who showed that inoculation effects of soybean seeds with *Bradyrhizobium japonicum* varied depending on the years of research and specific characteristics of a given region. In the present study, the highest soybean yield was obtained in 2020, when nodulation was also the greatest. It should be emphasized, however, that despite the weakest nodulation in 2018, the obtained seed yield was satisfactory (Table 4).

Averitt et al. [65] showed that the efficacy of products applied to soybean seeds varied over the years. On average, the authors did not show the influence of dressed or coated seeds on soybean yield during the research period. Other studies [52,66,67] proved that the inoculation of soybean seeds was justified, but on the condition of proper availability of all necessary nutrients to plants. The absence or excess of even one nutrient can significantly reduce nodulation in soybean plants. Stecca et al. [68] showed that sowing seeds with a coating and an inoculant was effective even in acidic soil (pH 5.3). As a result of such treatment, they obtained satisfactory nodulation and an increase in seed yield by 10.8% compared to control. Pereira et al. [19] demonstrated that treating soybean seeds with a fungicide together or separately with a polymer did not differentiate nodulation on the roots. The time of application of the above-mentioned products also did not modify the development of nodules. In the current study, no negative influence was found of coating components on nodulation, as shown in Figures 2 and 3. It should be noted that sowing coated and inoculated seeds had a similar effect as sowing only inoculated seeds (Table 4).

4.3. Effect of Inoculation on Seed Quality

Pannecoucque et al. [16] reported that commercial products effectively increased nodulation in soybean, resulting in a higher protein content in seeds compared to control. Flajšman et al. [15] also obtained a beneficial effect of inoculation treatment on the increase of protein content in soybean seeds and oil yield. Zimmer et al. [2] concluded that the application of commercial bacterial inoculants was justified in soybean cultivation. However, these authors noted that the increase in protein yield due to inoculation depended on the location of the experiment. Cafaro La Menza et al. [69] concluded that obtaining high soybean yields, including protein yields, required adequate supply of plants with nitrogen. However, they recorded a decrease in seed oil content with high nitrogen availability. In the present study, inoculation had a positive effect on the quality of soybean seeds, as it increased protein content (Figure 4). The protein content in seeds was also modified in the years of research.

4.4. Effect of Seed Coating

Apart from inoculation or dressing, soybean seeds can be covered with various types of coatings. Soybean yield in the study of Wiatrak [70] increased from 8.1% to 14.0% after sowing seeds with a polymer containing microelements compared to control. Gesch et al. [42] also obtained a beneficial effect of coated seeds on soybean yield, but only in one study year and at a very early sowing date. They showed that the difference in seed yield between 2005 and 2006 was on average 1.0 $t \cdot ha^{-1}$. Rocha et al. [71] concluded that the effects of using coated seeds depended on many factors, including plant species or habitat conditions. Nevertheless, they believed that seed coating is gaining popularity in agricultural practice and therefore requires further research. Santos et al. [72] showed that sowing soybean seeds provided different results depending on the location of the experiment: a laboratory, a greenhouse or an arable field. The lowest differentiation of the examined features, and thus the results from sowing coated seeds, was obtained by the authors in field conditions. Chachalis and Smith [73] showed that a hydrophobic polymer applied to soybean seeds was effective in regulating water uptake, which improved plant germination and emergence, especially when soil moisture was unfavorable after sowing. Ambika et al. [74] confirmed that soybean coating contributed to a better tolerance of plants to water stress. As a result of using a polymer (Quick), the plants emerged evenly and grew and yielded better. Sharratt and Gesch [75], demonstrated that temperature-activated coatings (10–12 °C) were useful when sowing soybean seeds in cold and wet soil. The temperature-activated polymer coatings delayed germination and emergence but improved plant density compared to control. Gesch et al. [42], after sowing coated soybean seeds at an early date, obtained a higher plant density compared to control. However, this result varied over the study years. When coated seeds were sown at a normal or late date, fewer plants emerged compared to the control, especially during spring drought. Tripathi et al. [76] found that polymer coatings based on natural substances effectively improved soybean seed germination. This has an important practical aspect. The present experiment showed that sowing coated seeds delayed soybean emergence, but at the same time increased plant population in comparison to control. However a high capacity of soybean to compensate the yield when exposed to a lower plant population affected reduced seed coating effect.

Jeyabal et al. [77] showed that coating soybean seeds with organic or inorganic substances increased the number of pods per plant. As a result, seed yield increased from 29.6% to 37.2% compared to control. The obtained effects were different depending on the type of soil. Macák and Candráková [78] reported that the number of pods per plant, thousand seed weight and seed yield of soybean varied over the years, while the number of seeds in a pod was usually stable. In addition, it was shown that yield components were significantly increased by the inoculant in the uncoated variant (B) compared to control and seeds only with coating. Jarecki and Wietecha [79] showed that the coatings did not differentiate seed yield in 2018 due to favourable weather conditions. The use of coating D (chitosan + alginate/PEG) in the following years increased seed yield by 0.46 t/ha in 2019 and by 0.51 t/ha in 2020 compared to control. This shows advantages of the treatments in which the polimer coating is used, which was also shown in this experiment (Table 4).

4.5. Measurements of the Condition of the Plants

Leaf analysis may identify nutrient deficiency or excess in plant tissue. Plant tests performed with optical instruments can be a significant simplification of the methods applied to attain precise determination of the nutritional status of plants during a groing season. Wiatrak [70] showed that the result of sowing coated seeds was an increase in the Normalized Difference Vegetation Index (NDVI) and LAI measurements, but only in certain stages of development. The author also demonstrated that coating with polymer did not significantly affect the LAI values. Santos et al. [72] showed slight differences in plant physiological measurements under the influence of sowing improved seeds, especially in field conditions. Thompson et al. [80] confirmed the usefulness of SPAD measurements in assessing chlorophyll content in soybean leaves, and the obtained results were significantly modified by environmental conditions. Fritschi and Ray [81] argued that SPAD readings were useful for assessing the nutritional status of soybean plants, but should be supplemented with other measuring techniques. Kühling et al. [54] reported that the SPAD value was higher after the application of inoculation treatment, but only at the beginning of filling of soybean seeds. The study of Jarecki et al. [52] showed that better nourished soybean plants (higher SPAD) were characterized by greater yields, which was confirmed by a strong correlation (r = 0.83). The SPAD test is a technique useful in agricultural practice owing to the ease of performing non-invasive measurements of the chlorophyll content. SPAD measurements can therefore be useful for predicting soybean seed yield.

Vollmann et al. [82] added that the assessment of chlorophyll content in the leaves also provided information about the condition of root nodules and nitrogen bound by them from the air. It also allows to predict quality parameters of soybean seeds. Measurements in the present study confirmed that seed inoculation, regardless of the coating, increased the SPAD values. The nutritional status of the plants was therefore the best. It was also shown that the SPAD, LAI and Gs indices varied over the study years. The highest measurements of the mentioned indices were recorded in 2018 (Table 3).

Yu et al. [83] reported that the results of leaf stomatal conductance (Gs) were more accurate when conducted under controlled laboratory conditions compared to field conditions. In the latter case, it resulted from changing weather conditions at the time of taking the measurements. Leaf stomatal conductance (Gs) is used as an indicator of gas-exchange capacity. Maximum stomatal conductance is controlled mainly by stomatal size and density, two parameters that change with environmental conditions.

5. Conclusions

Weather conditions, including soil temperature, had a modifying effect on the effectiveness of the applied HiStick® Soy product. The highest number and dry weight of nodules was obtained after inoculant application, regardless of whether the seeds were coated or not. Seed inoculation had a positive effect on the measurement of the so-called leaf greenness—SPAD—and stomatal leaf conductance—Gs (V3 stage). Sowing seeds only with coating or with coating and inoculant resulted in delayed emergence but increased plant population per 1 m^2 and higher field emergence. The coating composition (variant C) caused a slight stress in the plants, which was eliminated when the inoculant was additionally applied to the seeds (variant D). The combined application of the coating and inoculant had a more favorable effect on the number of formed pods per plant in relation to the variant with coating alone. The number of seeds in a pod and TSW was significantly increased above all as a result of seed inoculation. The study showed that the developed coating can be applied to soybean seeds together with the inoculant. This treatment significantly improved the yield and quality of soybean seeds compared to control. Sowing only coated seeds did not increase soybean yield, which could be explained by insufficient nodulation on the roots. The application of only the inoculant positively influenced most of the assessed parameters, but plant population was lower than expected. I conclude that test of the novel seed-coating agent has a good effect and efficacy, but only in combination with the inoculation procedure. However a high capacity of soybean to compensate the yield when exposed to an excessively low plant population affected a lower seed coating effect.

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Abbreviations

Gs	leaf stomatal conductance
LAI	leaf area index
NDVI	normalized difference vegetation index
PEG	polyethylene glycol
SE	standard error
SPAD	soil plant analysis development
TSW	thousand-seed weight

References

1. Watson, C.A.; Reckling, M.; Preissel, S.; Bachinger, J.; Bergkvist, G.; Kuhlman, T.; Lindström, K.; Nemecek, T.; Topp, C.F.E.; Vanhatalo, A.; et al. Chapter four—Grain legume production and use in European agricultural systems. *Adv. Agron.* **2017**, *144*, 235–303. [CrossRef]
2. 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]
3. Bandara, A.Y.; Weerasooriya, D.K.; Bell, T.H.; Esker, P.D. Prospects of alleviating early planting-associated cold susceptibility of soybean using microbes: New insights from microbiome analysis. *J. Agron. Crop Sci.* **2021**, *207*, 171–185. [CrossRef]
4. Madias, A.; Di Mauro, G.; Vitantonio-Mazzini, L.N.; Gambin, B.L.; Borrás, L. Environment quality, sowing date, and genotype determine soybean yields in the Argentinean Gran Chaco. *Eur. J. Agron.* **2021**, *123*, 126217. [CrossRef]
5. Gwata, E.T.; Wofford, D.S.; Pfahler, P.L.; Boote, K.J. Genetics of promiscuous nodulation in soybean: Nodule dry weight and leaf color score. *J. Hered.* **2004**, *95*, 154–157. [CrossRef]
6. Iturralde, E.T.; Covelli, J.M.; Álvarez, F.; Pérez-Giménez, J.; Arrese-Igor, C.; Lodeiro, A.R. Soybean-nodulating strains with low intrinsic competitiveness for nodulation, good symbiotic performance, and stress-tolerance isolated from soybean-cropped soils in Argentina. *Front. Microbiol.* **2019**, *10*, 1061. [CrossRef]

7. Vargas-Díaz, A.A.; Ferrera-Cerrato, R.; Silva-Rojas, H.V.; Alarcón, A. Isolation and evaluation of endophytic bacteria from root nodules of *Glycine max* L. (Merr.) and their potential use as biofertilizers. *Span. J. Agric. Res.* **2019**, *17*, e1103. [CrossRef]
8. Dwivedi, S.L.; Sahrawat, K.L.; Upadhyaya, H.D.; Mengoni, A.; Galardini, M.; Bazzicalupo, M.; Biondi, E.G.; Hungria, M.; Kaschuk, G.; Blair, M.W.; et al. Chapter one—Advances in host plant and Rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. *Adv. Agron.* **2015**, *129*, 1–116. [CrossRef]
9. Marinković, J.B.; Bjelić, D.Đ.; Tintor, B.B.; Ignjatov, M.V.; Nikolić, Z.T.; Đukić, V.H.; Balešević-Tubić, S.N. Molecular identification of *Bradyrhizobium japonicum* strains isolated from root nodules of soybean (*Glycine max* L.). *Matica Srp. J. Nat. Sci.* **2017**, *132*, 49–56. [CrossRef]
10. Thilakarathna, M.S.; Raizada, M.N. A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions. *Soil Biol. Biochem.* **2017**, *105*, 177–196. [CrossRef]
11. Solomon, T.; Pant, L.M.; Angaw, T. Effects of inoculation by *Bradyrhizobium japonicum* strains on nodulation, nitrogen fixation, and yield of soybean (*Glycine max* L. Merill) varieties on Nitisols of Bako, western Ethiopia. *Int. Sch. Res. Not.* **2012**, *2012*, 261475. [CrossRef]
12. Wongphatcharachai, M.; Staley, C.; Wang, P.; Moncada, K.M.; Sheaffer, C.C.; Sadowsky, M.J. Predominant populations of indigenous soy-bean nodulating *Bradyrhizobium japonicum* strains obtained from organic farming systems in Minnesota. *J. Appl. Microbiol.* **2015**, *118*, 1152–1164. [CrossRef] [PubMed]
13. Torres, A.R.; Kaschuk, G.; Saridakis, G.P.; Hungria, M. Genetic variability in *Bradyrhizobium japonicum* strains nodulating soybean [*Glycine max* (L.) Merrill]. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1831–1835. [CrossRef] [PubMed]
14. Giongo, A.; Ambrosini, A.; Vargas, L.K.; Freire, J.R.J.; Bodanese-Zanettini, M.H.; Passaglia, L.M.P. Evaluation of genetic diversity of bradyrhizobia strains nodulating soybean [*Glycine max* (L.) Merrill] isolated from South Brazilian fields. *Appl. Soil Ecol.* **2008**, *38*, 261–269. [CrossRef]
15. Flajšman, M.; Šantavec, I.; Kolmanič, A.; Kocjan Ačko, D. Bacterial seed inoculation and row spacing affect the nutritional composition and agronomic performance of soybean. *Int. J. Plant Prod.* **2019**, *13*, 183–192. [CrossRef]
16. 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]
17. Jarecki, W.; Bobrecka-Jamro, D. Influence of seed inoculation with commercial bacterial inoculants (*Bradyrhizobium japonicum*) on growth and yield of soybean. *Legume Res.* **2019**, *42*, 688–693. [CrossRef]
18. Althabegoiti, M.J.; López-García, S.L.; Piccinetti, C.; Mongiardini, E.J.; Perez-Gimenez, J.; Quelas, J.I.; Perticari, A.; Lodeiro, A.R. Strain selection for improvement of *Bradyrhizobium japonicum* competitiveness for nodulation of soybean. *FEMS Microbiol. Lett.* **2008**, *282*, 115–123. [CrossRef]
19. Pereira, C.E.; de Souza Moreira, F.M.; Oliveira, J.A.; Caldeira, C.M. Compatibility among fungicide treatments on soybean seeds through film coating and inoculation with Bradyrhizobium strains. *Acta Sci. Agron.* **2010**, *32*, 585–589. [CrossRef]
20. Deaker, R.; Roughley, R.J.; Kennedy, I.R. Legume seed inoculation technology—A review. *Soil Biol. Biochem.* **2004**, *36*, 1275–1288. [CrossRef]
21. Yamakawa, T.; Fukushima, Y. Low inoculum densities of *Bradyrhizobium japonicum* USDA 110 is effective on production of soybean (*Glycine max* L. Merr.) cultivar Fukuyutaka. *J. Fac. Agric. Kyushu Univ.* **2014**, *59*, 45–53. [CrossRef]
22. Hungria, M.; Nogueira, M.A.; Araujo, R.S. Alternative methods of soybean inoculation to overcome adverse conditions at sowing. *Afr. J. Agric. Res.* **2015**, *10*, 2329–2338. [CrossRef]
23. Salvagiotti, F.; Cassman, K.G.; Specht, J.E.; Walters, D.T.; Weiss, A.; Dobermann, A. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crop. Res.* **2008**, *108*, 1–13. [CrossRef]
24. Kaschuk, G.; Nogueira, M.A.; de Luca, M.J.; Hungria, M. Response of determinate and indeterminate soybean cultivars to basal and topdressing N fertilization compared to sole inoculation with *Bradyrhizobium*. *Field Crop. Res.* **2016**, *195*, 21–27. [CrossRef]
25. 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]
26. Leggett, M.; Diaz-Zorita, M.; Koivunen, M.; Bowman, R.; Pesek, R.; Stevenson, C.; Leister, T. Soybean response to inoculation with in the United States and Argentina. *Agron. J.* **2017**, *109*, 1031–1038. [CrossRef]
27. Lacerda, M.P.; Umburanas, R.C.; Martins, K.V.; Rodrigues, M.A.T.; Reichardt, K.; Dourado-Neto, D. Vigor and oxidation reactions in soybean seedlings submitted to different seed chemical treatments. *J. Seed Sci.* **2021**, *43*, e202143012. [CrossRef]
28. Korbecka-Glinka, G.; Wiśniewska-Wrona, M.; Kopania, E. The use of natural polymers for treatments enhancing sowing material. *Polimery* **2021**, *66*, 11–20. [CrossRef]
29. Sarreta, Y.; de Castro Neto, J.C. Effects of 660 nm laser irradiation of soybean seeds on germination, emergence and seedling growth. *Acta Agroph.* **2021**, *28*, 5–18. [CrossRef]
30. Hara, Y. Comparison of the effects of seed coating with tungsten and molybdenum compounds on seedling establishment rates of rice, wheat, barley, and soybean under flooded conditions. *Plant Prod. Sci.* **2017**, *20*, 406–411. [CrossRef]
31. Afzal, I.; Javed, T.; Amirkhani, M.; Taylor, A.G. Modern seed technology: Seed coating delivery systems for enhancing seed and crop performance. *Agriculture* **2020**, *10*, 526. [CrossRef]
32. Elshafie, H.S.; Camele, I. Applications of absorbent polymers for sustainable plant protection and crop yield. *Sustainability* **2021**, *13*, 3253. [CrossRef]

33. Pedrini, S.; Merritt, D.J.; Stevens, J.; Dixon, K. Seed coating: Science or marketing spin? *Trends Plant Sci.* **2017**, *22*, 106–116. [CrossRef]
34. Zeng, D.; Luo, X.; Tu, R. Application of bioactive coatings based on chitosan for soybean seed protection. *Int. J. Carbohydr. Chem.* **2012**, *2012*, 104565. [CrossRef]
35. Avelar, S.A.G.; Baudet, L.; de Oliveira, S.; Ludwig, M.P.; Crizel, R.L.; Rigo, G.A. Soybean seed treatment and coating with liquid and powdered polymer. *Interciencia* **2015**, *40*, 133–137.
36. Ludwig, E.J.; Nunes, U.R.; Prestes, O.D.; Fagundes, L.K.; Fernandes, T.S.; Saibt, N. Polymer coating in soybean seed treatment and their relation to leaching of chemicals. *Rev. Ambient. Água* **2020**, *15*, e2602. [CrossRef]
37. Pedrini, S.; Balestrazzi, A.; Madsen, M.D.; Bhalsing, K.; Hardegree, S.P.; Dixon, K.W.; Kildisheva, O.A. Seed enhancement: Getting seeds restoration ready. *Restor. Ecol.* **2020**, *28*, 266–275. [CrossRef]
38. Poliserpi, M.B.; Cristos, D.S.; Brodeur, J.C. Imidacloprid seed coating poses a risk of acute toxicity to small farmland birds: A weight-of-evidence analysis using data from the grayish baywing *Agelaioides badius*. *Sci. Total Environ.* **2021**, *763*, 142957. [CrossRef]
39. Lentola, A.; Giorio, C.; Petrucco Toffolo, E.; Girolami, V.; Tapparo, A. A new method to assess the acute toxicity toward honeybees of the abrasion particles generated from seeds coated with insecticides. *Environ. Sci. Eur.* **2020**, *32*, 93. [CrossRef]
40. Lentola, A.; Giorio, C.; Bogialli, S.; Roverso, M.; Marzaro, M.; Girolami, V.; Tapparo, A. Methiocarb metabolites are systemically distributed throughout corn plants grown from coated seeds. *Environ. Chem. Lett.* **2021**, *19*, 1887–1892. [CrossRef]
41. Han, R.; Wu, Z.; Huang, Z.; Man, X.; Teng, L.; Wang, T.; Liu, P.; Wang, W.; Zhao, X.; Hao, J.; et al. Tracking pesticide exposure to operating workers for risk assessment in seed coating with tebuconazole and carbofuran. *Pest Manag. Sci.* **2021**, *77*, 2820–2825. [CrossRef]
42. Gesch, R.W.; Archer, D.W.; Spokas, K. Can using polymer-coated seed reduce the risk of poor soybean emergence in no-tillage soil? *Field Crops Res.* **2012**, *125*, 109–116. [CrossRef]
43. Ma, Y. Seed coating with beneficial microorganisms for precision agriculture. *Biotechnol. Adv.* **2019**, *37*, 107423. [CrossRef] [PubMed]
44. Evangelista, J.R.E.; Oliveira, J.A.; Botelho, F.J.E.; Oliveira, R.M.E.; Pereira, C.E. Performance of film coated soybean seeds in soil different water contents. *Ciênc. Agrotecnologia* **2007**, *31*, 994–999. [CrossRef]
45. Zeng, D.-F.; Zhang, L. A novel environmentally friendly soybean seed-coating agent. *Acta Agric. Scand. B Soil Plant Sci.* **2010**, *60*, 545–551. [CrossRef]
46. Fehr, W.R.; Caviness, C.E.; Burmood, D.T.; Pennington, J.S. Stage of development descriptions for soybeans, *Glycine Max* (L.) Merrill. *Crop Sci.* **1971**, *11*, 929–931. [CrossRef]
47. Kuchlan, P.; Kuchlan, M.K.; Ansari, M.M. Efficient application of Trichoderma viride on soybean [*Glycine max* (L.) Merrill] seed using thin layer polymer coating. *Legume Res.* **2019**, *42*, 250–259. [CrossRef]
48. Zerpa, M.; Mayz, J.; Mendez, J. Effects of *Bradyrhizobium japonicum* inoculants on soybean (*Glycine max* (L.) Merr.) growth and nodulation. *Ann. Biol. Res.* **2013**, *4*, 193–199.
49. Adjetey, J.A.; Mbotho, K. Evaluation of *Bradyrhizobium* formulations on performance of soybean grown on soil without a long-term history of the crop. *Bots. J. Agric. Appl. Sci.* **2019**, *13*, 66–70. [CrossRef]
50. Abou-Shanab, R.A.I.; Wongphatcharachai, M.; Sheaffer, C.C.; Orf, J.C.; Sadowsky, M.J. Competition between introduced *Bradyrhizobium japonicum* strains and indigenous bradyrhizobia in Minnesota organic farming systems. *Symbiosis* **2017**, *73*, 155–163. [CrossRef]
51. Coskan, A.; Dogan, K. Symbiotic nitrogen fixation in soybean. In *Soybean Physiology and Biochemistry*; El-Shemy, H., Ed.; InTech: London, UK, 2011; Volume 307, pp. 167–182. [CrossRef]
52. Jarecki, W.; Buczek, J.; Bobrecka-Jamro, D. Response of soybean (*Glycine max* (L.) Merr.) to bacterial soil inoculants and foliar fertilization. *Plant Soil Environ.* **2016**, *62*, 422–427. [CrossRef]
53. Ambrosini, V.G.; Fontoura, S.M.V.; de Moraes, R.P.; Tamagno, S.; Ciampitti, I.A.; Bayer, C. Soybean yield response to *Bradyrhizobium* strains in fields with inoculation history in Southern Brazil. *J. Plant Nutr.* **2019**, *42*, 1941–1951. [CrossRef]
54. Kühling, I.; Hüsing, B.; Bome, N.; Trautz, D. Soybeans in high latitudes: Effects of *Bradyrhizobium* inoculation in northwest Germany and southern west Siberia. *Org. Agric.* **2018**, *8*, 159–171. [CrossRef]
55. Duzan, H.M.; Zhou, X.; Souleimanov, A.; Smith, D.L. Perception of *Bradyrhizobium japonicum* Nod factor by soybean [*Glycine max* (L.) Merr.] root hairs under abiotic stress conditions. *J. Exp. Bot.* **2004**, *55*, 2641–2646. [CrossRef]
56. Suzuki, Y.; Adhikari, D.; Itoh, K.; Suyama, K. Effects of temperature on competition and relative dominance of *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* in the process of soybean nodulation. *Plant Soil.* **2014**, *374*, 915–924. [CrossRef]
57. Albareda, M.; Rodriguea-Navarro, D.N.; Temprano, F.J. Soybean inoculation: Dose, N fertilizer supplementation and rhizobia persistence in soil. *Field Crops Res.* **2009**, *113*, 352–356. [CrossRef]
58. Narożna, D.; Pudełko, K.; Króliczek, J.; Golińska, B.; Sugawara, M.; Mądrzak, C.J.; Sadowsky, M.J. Survival and competitiveness of *Bradyrhizobium japonicum* strains 20 years after introduction into field locations in Poland. *Appl. Environ. Microbiol.* **2015**, *81*, 5552–5559. [CrossRef] [PubMed]
59. Argaw, A. Symbiotic effectiveness of inoculation with *Bradyrhizobium* isolates on soybean [*Glycine max* (L.) Merrill] genotypes with different maturities. *SpringerPlus* **2014**, *3*, 753. [CrossRef]

60. 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. Pap. Ser. A Agron.* **2017**, *60*, 207–214.
61. Thuita, M.; Pypers, P.; Herrmann, L.; Okalebo, R.J.; Othieno, C.; Muema, E.; Lesueur, D. Commercial rhizobial inoculants significantly enhance growth and nitrogen fixation of a promiscuous soybean variety in Kenyan soils. *Biol. Fertil. Soils* **2012**, *48*, 87–96. [CrossRef]
62. López-García, S.L.; Perticari, A.; Piccinetti, C.; Ventimiglia, L.; Arias, N.; De Battista, J.J.; Althabegoiti, M.J.; Mongiardini, E.J.; Pérez-Giménez, J.; Quelas, J.I.; et al. In-Furrow inoculation and selection for higher motility enhances the efficacy of *Bradyrhizobium japonicum* nodulation. *Agron. J.* **2009**, *101*, 357–363. [CrossRef]
63. Carciochi, W.D.; Rosso, L.H.M.; Secchi, M.A.; Torres, A.R.; Naeve, S.; Casteel, S.N.; Kovács, P.; Davidson, D.; Purcell, L.C.; Archontoulis, S.; et al. Soybean yield, biological N_2 fixation and seed composition responses to additional inoculation in the United States. *Sci. Rep.* **2019**, *9*, 19908. [CrossRef]
64. Ludwig, M.P.; Filho, O.A.L.; Baudet, L.; Dutra, L.M.C.; Avelar, S.A.G.; Crizel, R.L.; de Oliveira, S. Coating efficiency of soybean seeds in equipment with spray system. *Ciência Rural* **2011**, *41*, 557–563. [CrossRef]
65. Averitt, B.J.; Welbaum, G.E.; Li, X.; Prenger, E.; Qin, J.; Zhang, B. Evaluating genotypes and seed treatments to increase field emergence of low phytic acid soybeans. *Agriculture* **2020**, *10*, 516. [CrossRef]
66. Thuita, M.; Vanlauwe, B.; Mutegi, E.; Masso, C. Reducing spatial variability of soybean response to rhizobia inoculants in farms of variable soil fertility in Siaya County of western Kenya. *Agric. Ecosyst. Environ.* **2018**, *261*, 153–160. [CrossRef] [PubMed]
67. Egamberdieva, D.; Jabborova, D.; Wirth, S.J.; Alam, P.; Alyemeni, M.N.; Ahmad, P. Interactive effects of nutrients and *Bradyrhizobium japonicum* on the growth and root architecture of soybean (*Glycine Max,* L.). *Front. Microbiol.* **2018**, *9*, 1000. [CrossRef]
68. Stecca, J.D.L.; Martin, T.N.; Dall'Coll Lúcio, A.; Deak, E.A.; Fipke, G.M.; Bruning, L.A. Inoculation of soybean seeds coated with osmoprotector in diferents soil pH's. Acta Scientiarum. *Agronomy* **2019**, *41*, e39482. [CrossRef]
69. Cafaro La Menza, N.; Monzon, J.P.; Specht, J.E.; Grassini, P. Is soybean yield limited by nitrogen supply? *Field Crop. Res.* **2017**, *213*, 204–212. [CrossRef]
70. Wiatrak, P. Effect of polymer seed coating with micronutrients on soybeans in Southeastern Coastal Plains. *Am. J. Agric. Biol. Sci.* **2013**, *8*, 302–308. [CrossRef]
71. Rocha, I.D.S.; Ma, Y.; Souza-Alonso, P.; Vosátka, M.; Freitas, H.; Oliveira, R.S. Seed coating: A tool for delivering beneficial microbes to agricultural crops. *Fron. Plant Sci.* **2019**, *10*, 1357. [CrossRef]
72. Santos, V.M.; Oliveira, T.C.; Mendes, M.G.; Yamanaka, C.H.; Macedo, W.R. Soybean seed chemical treatment associated with inoculants: Physiological and agronomical analyses. *Plant Physiol. Rep.* **2021**, *26*, 247–255. [CrossRef]
73. Chachalis, D.; Smith, M.L. Hydrophobic-polymer application reduces imbibition rate and partially improves germination of emergence of soybean seedlings. *Seed Sci. Technol.* **2001**, *29*, 91–98.
74. Ambika, S.; Manonmani, V.; Bhaskaran, M.; Deepika, S. Influence of polymer coated KSL 441 (op) soybean seed on productivity under moisture stress conditions. *Legume Res.* **2017**, *40*, 150–154. [CrossRef]
75. Sharratt, B.S.; Gesch, R.W. Emergence of polymer-coated corn and soybean influenced by tillage and sowing date. *Agron. J.* **2008**, *100*, 585–590. [CrossRef]
76. Tripathi, B.; Pandey, A.; Bhatia, R.; Walia, S.; Yadav, A.K. Improving soybean seed performance with natural colorant-based novel seed-coats. *J. Crop Improv.* **2015**, *29*, 301–318. [CrossRef]
77. Jeyabal, A.; Kuppuswamy, G.; Lakshmanan, A. Effect of seed coating on yield attributes and yield of soybean (*Glycine max* L.). *J. Agron. Crop Sci.* **1992**, *169*, 145–150. [CrossRef]
78. Macák, M.; Candráková, E. The effect of fertilization on yield components and quality parameters of soybeans [(*Glycine max* (L.) Merr.)] seeds. *J. Cent. Eur. Agric.* **2013**, *14*, 1232–1242. [CrossRef]
79. Jarecki, W.; Wietecha, J. Effect of seed coating on the yield of soybean *Glycine max* (L.) Merr. *Plant Soil Environ.* **2021**, *67*, 468–473. [CrossRef]
80. Thompson, J.A.; Schweitzer, L.E.; Nelson, R.L. Association of specific leaf weight, an estimate of chlorophyll, and chlorophyll concentration with apparent photosynthesis in soybean. *Photosynth. Res.* **1996**, *49*, 1–10. [CrossRef] [PubMed]
81. Fritschi, F.B.; Ray, J.D. Soybean leaf nitrogen, chlorophyll content, and chlorophyll a/b ratio. *Photosynthetica* **2007**, *45*, 92–98. [CrossRef]
82. Vollmann, J.; Walter, H.; Sato, T.; Schweiger, P. Digital image analysis and chlorophyll metering for phenotyping the effects of nodulation in soybean. *Comput. Electron. Agric.* **2011**, *75*, 190–195. [CrossRef]
83. Yu, M.; Ding, G.; Gao, G.; Zhao, Y.; Sai, K. Leaf temperature fluctuations of typical psammophytic plants and their application to stomatal conductance estimation. *Forests* **2018**, *9*, 313. [CrossRef]