Physiological Response of Soybean Plants to Seed Coating and Inoculation under Pot Experiment Conditions

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Abstract: Improved seeds are increasingly being sown in agricultural practice. Such treatments play different roles depending on the substances used. They most often protect seeds and sprouts from abiotic and biotic stresses, but not only. Coating technology is one of the methods of seed improvement, requiring the selection of appropriate components. The purpose of the pot experiment was to test the efficacy of two coatings (C and D) and a commercial inoculant (B) applied to soybean seeds (cultivar Mavka). It was shown that the best option was the combined use of coating and inoculation (C + B or D + B). A significantly higher number of germinated seeds, nodulation, green fodder mass, green fodder protein content, and some physiological parameters of plants were obtained compared to control (A). Applying only the tested coatings (C or D) resulted in the lack of nodulation on roots and slight changes in plant physiological parameters. Sowing seeds with inoculant (B) or control seeds (A) accelerated plant emergence but reduced the number of properly formed sprouts compared to coated seeds. The results confirmed that the tested soybean seed coatings were effective, but in combination with inoculation.

Keywords: Glycine max (L.) Merr.; Bradyrhizobium japonicum; seed sowing; seed germination; seed treatment; inoculation; nodulation; green forage; physiological measurements of plants; protein

1. Introduction

Legumes can take up nitrogen from the soil, fertilizers, and air. In the latter case, it is known as the so-called biological nitrogen fixation (BNF) process. Each of these nitrogen sources has an important role in plant growth and development [1]. However, biological nitrogen fixation is the most significant process that allows for obtaining high and good-quality legume crops [2–4]. Masciarelli et al. [5] showed that the new PGPR strain isolated from soybean seeds can be utilized as bio-inoculant for improving plant growth and nodulation of different legumes. The co-inoculation technique is attractive for use in commercial inoculant formulations following proper field evaluation.

For example, the yielding potential of new soybean cultivars is over 7 t·ha⁻¹, with a high protein content in the seeds (approx. 40% DW). Therefore, further research should be pursued to characterize in more detail the interplay between N fixation and soil, plant, and environmental factors [6,7]. Soybean has a high nitrogen (N) requirement, as it needs to take up 80 kg N per tonne of grain produced. On average, 50–60% or even 90% of this N is supplied through biological N fixation (BNF) by symbiotic soil bacteria, mainly Bradyrhizobium japonicum [8]. Santachiara et al. [9] showed significant differences in nitrogen uptake by soybean cultivars, resulting in yield variability. The proportion of nitrogen uptake from BNF ranged from 60% to 78% for high-yielding cultivars. Córdova et al. [10] showed that BNF contributed between 23% and 65% to nitrogen accumulation in soybean plants, which depended on experiment location, year, and sowing date. In addition, they concluded that nitrogen experiments should take into account soil organic carbon content to obtain the correct N:C ratio. This is especially important for the soil environment and crop vegetation.
In agricultural practice, inoculants are used to increase nodulation on legume roots. Bogino et al. [11] reported that viable rhizobia can be introduced into the agricultural ecosystem via “on-seed inoculation”, or by direct application into the soil, termed “in-furrow inoculation”. Ntambo et al. [12] reported that inoculation of soybean seeds reduced the nitrogen dose to 50 kg·ha⁻¹, which had important economic and ecological implications. The amount of nitrogen fixed each year by the legume–rhizobia symbiosis is approx. 70 million tonnes. Assuming that at least 70% of this amount is utilized in agricultural and agroforestry systems, thus replacing commercial N fertilizer, it can be estimated that around 50 million tonnes of fixed nitrogen are made available to support agricultural production [13,14]. Mpepereki et al. [15] demonstrated that a properly selected variety and inoculant caused a significant increase in the size and quality of soybean seed yield. Asei et al. [16] have reported that symbiotic bacteria suitable for soybean are usually absent or low virulent in cultivated soils. In such cases, inoculation is a necessary treatment to increase nodulation and atmospheric nitrogen fixation. In agricultural practice, many commercial formulations do not provide satisfactory results, which is due to many abiotic and biotic factors. Yates et al. [17] and Kanonge-Mafaune et al. [18] reported that sometimes ineffective soil rhizobia formed nodules, which reduced the effectiveness of commercial inoculants. Lindström et al. [19] and Jaiswal et al. [20] concluded that Bradyrhizobial inoculants for soybean were very diverse, yet classification and characterization of strains have long been difficult. Recent genetic characterization methods permit more reliable identification and improve our knowledge of local populations. Carciochi et al. [8] indicated that the use of inoculation in areas where soybean is commonly grown has a smaller effect on the size and quality of seed yield. Hence, research results on inoculants depend on the local habitat conditions. Pedrozo et al. [21] showed that soybean seed inoculation was cost-effective but in combination with Ca + B fertilization, which increased the number of pods per plant, number of seeds per pod, and seed yield by 10% compared to control. Jarecki et al. [22] demonstrated that soybean seed inoculation significantly increased the number of pods per plant, thousand seed weight, yield, and seed protein content. In addition, the abovementioned features and parameters varied between the cultivars and years of research. Sheteiwy et al. [23,24] suggested that application of biofertilizers to soybean plants is a promising approach to alleviate drought stress effects on growth performance of soybean plants. The integrated application of biofertilizers may help to obtain improved resilience of the agroecosystems to adverse impacts of climate change and help to improve soil fertility and plant growth under drought stress. Sheteiwy et al. [25] report that seed priming with JA, foliar application of JA, and/or their combination significantly improved many properties of soybean plant under salinity stress compared with the untreated seedlings. These methods could efficiently protect early seedlings and alleviate salt stress damage and provide possibilities for use in improving soybean growth and inducing tolerance against excessive soil salinity [26].

Sharma et al. [27] indicated that various ingredients are applied for seed coating. In the case of chemical substances, special attention should be paid to their effects on the soil or aquatic environment [28,29]. Calvo-Agudo et al. [30] proved that the insecticide (neonicotinoids) contained in the seed dressing remained in the environment for a long time and posed a threat to beneficial insects. Poliserpi et al. [31] demonstrated the harmfulness of certain chemical treatments to small birds. For that reason, future research may be more focused on advanced physical (microwave, ultrasound, ozone treatment) and biological (biopriming, SMP) methods of treating seeds, as an alternative to chemical seed improvements. Therefore, coating seeds with natural substances is a promising method. Afzal et al. [32] reported that natural substances were effective, facilitated sowing, protected against pests, and increased the vigor of sprouts. There are different seed coating techniques both in terms of the equipment and components used. Depending on this, seed weight can increase from several to several dozen percent. Zeng and Zhang [33] showed that sowing coated seeds increased soybean yield by 17.95% relative to control. In addition, their seed coating composition was not harmful to the environment and the cost of application was lower than a commercial formulation. Santos et al. [34] pointed out that coating
of soybean seeds had a different effect depending on study site (laboratory, greenhouse, field). Therefore, the results obtained in laboratory or greenhouse experiments have not always been confirmed in field experiments [35]. Montanha et al. [36] demonstrated that the efficiency of seed enhancement in agricultural practice was about 70%. Jamilah et al. [37] reported that nodulation, plant growth, and development were better when coated seeds were sown with micronutrients. This was due to better supply of nutrients to the plants from the germination stage. Similar conclusions were reached by Montanha et al. [38], but the obtained coating effects depended on the form of the element applied—zinc in the form of ZnO turned out to be better than ZnSO₄. Ludwig et al. [39] proved that the addition of polymer to the coating prevented the loss of 20% of the active ingredient in comparison to control seeds (without polymer). Moreover, they showed that the coating of large seeds resulted in better sprout vigor compared to small seeds. Kuchlan et al. [40] and Tang et al. [41] reported that seed coating reduced disease development, which in turn increased the size and quality of soybean yields. Soares et al. [42] also confirmed the effectiveness of sowing coated seeds in agricultural practice. This was due to the greater plant density after emergence as compared to sowing seeds without the coating. Zhou et al. [43] applied a coating for soybean seeds to reduce the harmfulness of nematodes. Its advantage was that the composition did not include substances hazardous to the environment and it was not expensive. Ambika et al. [44] also showed that seed coating with natural components was effective, safe for the environment, improved plant emergence, and reduced the effects of abiotic stresses. Tripathi et al. [45] proved the effectiveness of coatings based on natural substances. Their innovative coatings allowed long storage of seeds and, after sowing, improved plant germination and emergence. Jeyabal et al. [46] confirmed the effectiveness of a coating containing natural substances, including bacteria. The research was carried out in three different soils, which modified the results. Faqir et al. [47] showed that chitosan, as well as plant growth promoters, played important roles in improving soil fertility and plant growth. Chitosan can have a key function in modern agriculture production and can be a valuable source promoting agricultural ecosystem sustainability. Li et al. [48] demonstrated that the combined use of physcion and chitosan-oligosaccharide as a seed coating synergistically improved the antioxidant potential of maize, resulting in plants with high stress resistance. Fu et al. [49] showed that alginate coatings reduced bacteria on alfalfa seeds and sprouts. Therefore, this compound may also be useful as an ingredient in soybean seed coatings. Other substances such as jojoba oil (Simmondsia chinensis) [50] or polyethylene glycol (PEG) [51] can also be used as a coating component.

Wiatrak [52] found that polymeric coatings could stimulate soybean growth during drought. Under these conditions, the NDVI measurement of plants improved by an average of 10.5% 8 weeks after sowing the coated seeds. Vollmann et al. [53] have indicated that plant physiological measurements are becoming increasingly easier to perform, also at various developmental stages. For example, chlorophyll content measurements in leaves can provide information about the proper course of nodulation or BNF. Thompson et al. [54] showed that the SPAD measurement was useful for the assessment of plants' nutritional status both in laboratory and field experiments. They proved that the SPAD index was significantly correlated with 1000 seed weight, as well as protein and oil contents in seeds. Yu et al. [55] indicated that the physiological measurements of plants are more accurate in laboratory experiments, but they also obtained reliable results under field conditions. This concerned, for example, leaf stomatal conductance (Gs).

The purpose of the present pot experiment was to investigate how coating and/or inoculation of soybean seeds would affect the selected parameters and physiological measurements of plants. This would allow the selection and introduction of the optimal variant to the field experiments.
2. Materials and Methods

2.1. Plant Material and the Course of the Pot Experiment

The pot experiment was conducted at the University of Rzeszów, Poland. Soybean seeds were purchased from the Scientific Research Center of Soya Development, “AgeSoya” Sp. Z o.o., Poland. The seeds were sown in plastic pots (diameter—25 cm) containing 5 kg of soil qualified as loamy sand [56]. Soil was collected from the field belonging to the Experimental Station of the University of Rzeszów in Krasne, near Rzeszów. Soybean was not cultivated at the sampling site. The soil was slightly acidic (pH KCl 6.15) and phosphorus and potassium contents were very high (P 145.6 mg/kg soil DW and K 231.2 mg/kg soil DW). Soil analysis (at a depth of 30 cm) was performed in the accredited laboratory of the Regional Agricultural Chemical Station in Rzeszów, according to Polish standards [57].

The experiment was carried out as one-factor approach in the following variants:

- A—control;
- B—bioinoculant;
- C—coated seeds: chitosan + alginate/jojoba oil/E;
- D—coated seeds: chitosan + alginate/PEG;
- C + B;
- D + B.

The experiments were carried out in four replicates of 8 pots per variant (n = 48) in growth chambers (Model GC-300/1000; JEIO Tech Co., Ltd., Seoul, Korea) at 8 ± 1 °C for 7 days, subsequently increased temperature to 12 ± 1 °C, 60 ± 3% RH humidity, 16/8 h (L/D) photoperiod and maximum light intensity of about 300 µmol m⁻² s⁻¹ during the day. Substrate moisture was maintained at 60% of the field water capacity. Ten seeds were sown in each pot and the days from sowing to emergence were recorded. Plant developmental stages were given according to the BBCH scale (Biologische Bundesanstalt, Bundessortenamtund CHemische Industrie) [58]. After emergence (BBCH 10), the pots were transferred to a room with natural daylight and a temperature of about 20 °C. The pots settings were changed randomly every 5 days. Properly developed plants were counted in each pot. After the full emergence stage (BBCH 11), the plant density was adjusted to five plants per pot. The experiment was conducted till the full flowering stage (BBCH 65). Then, the aerial plant parts were harvested and fresh weight determined. The plants were weighed and subsequently air-dried. The dry weight of the plants was converted to a constant moisture of 10%. Total protein content was determined as N × 6.25 using the Kjeldahl procedure [59]. The roots were rinsed on sieves. The nodules were subsequently counted and allowed to air-dry to determine their dry weight.

2.2. Seed Coating

As a result of laboratory work (Łukasiewicz Research Network—Institute of Biopolymers and Chemical Fibers, Poland), two coatings with different chemical compositions were prepared. These were two-layer coatings, first: chitosan (average molecular weight: $\overline{M}_w = 235$ kDa, and deacetylation degree: SD = 87%, in the form of a lactate salt pH = 6.2) and second: sodium alginate. As a result, the weight of a single seed increased by about 20%. The thus obtained coating did not dissolve quickly upon contact with water. Substances were introduced into the coating that ensured its differential decomposition depending on soil temperature. In the first variant (C), jojoba oil was introduced in the form of an olive oil-based emulsion with the addition of a detergent, and in the second variant (D), the coating consisted of a mixture of polyethylene glycol PEG 400. The coating is intended to protect the seeds from abiotic and biotic stresses after sowing the seeds in low soil temperature (about 8 °C). This will allow early sowing of soybean and optimum planting density at emergence. The individual coating layers were prepared in the form of solutions or emulsions and sprayed on the seeds using a laboratory device (Figure 1).
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 ingredient application was adjusted to prevent mechanical damage or seed swelling due to moisture.

2.3. Seed Inoculation

Commercial HiStick® Soy preparation (BASF Agricultural Specialties Limited, Littlehampton, UK) was used for seed inoculation. This preparation contained at least 2 billion ($2 \times 10^9$) viable Rhizobium (*Bradyrhizobium japonicum*) cells per gram of peat substrate. In addition, it contained a natural polymer in its composition to ensure adhesion to the seeds. The dosage recommended by the manufacturer was 0.4 kg of the preparation per 100 kg of seeds. According to the experimental setup, the measured amount of inoculant was thoroughly mixed with the seeds (dry) on the day of sowing. This allowed the testing of the effectiveness of the inoculum (B) and the effect of application to the coated seeds (C + B or D + B). For comparison of the obtained results, control seeds (A) were sown without inoculation and without coating. Chemical seed treatment was not used.

2.4. Measurement Gs and SPAD

The measurement of stomatal conductance of leaves (Gs) was performed using a Meter Porometer SC-1 apparatus (Pullman, Washington, USA). The plant nutritional status (SPAD—soil plant analysis development) was measured with a SPAD 502P chlorophyllometer (Konica Minolta, Inc., Tokyo, Japan). Determination of physiological parameters (Gs and SPAD) was carried out three times (in five replicates per pot) on fully developed leaves: unfolded trifoliate leaf on the 3rd node (BBCH 13), first bud (BBCH 51), and full flowering (BBCH 65). A similar experiment was carried out by Alotaibi et al. [60].

2.5. Chlorophyll Fluorescence

Chlorophyll fluorescence measurements in leaves were performed with a Pocket PEA apparatus (Hansatech Instruments, King’s Lynn, Norfolk, UK), equipped with black shading clips applied to the leaf lamina away from the leaf nerve. The following parameters were measured: maximum quantum yield of photosystem II (PSII) (Fv/Fm), maximum quantum yield of primary photochemistry (Fv/F0), and photosynthesis yield index (PI). Five measurements of chlorophyll fluorescence were made per pot (BBCH 65). The maximum available intensity was 3500 µmol (photon), which was applied for 1 s at a peak wavelength of 627 nm. Fully developed leaves were dark-adapted for 30 min using leaf clips applied to the adaxial leaf blades. A similar experiment was carried out by Hamada AbdElgawad et al. [61].

2.6. Measurement of Gas Exchange

A Portable Photosynthesis Measurement System LCpro-SD (ADC BioScientific Ltd., Hoddesdon, UK) was used to determine the gas exchange parameters: net photosynthetic...
rate (PN), transpiration rate (E), and intercellular CO$_2$ concentration (Ci). The plant leaf photosynthesis chamber of LCpro-SD had a flow accuracy of 2% of its range. During measurements, light intensity was 300 mmol m$^{-2}$ s$^{-1}$ and leaf chamber temperature was 21 ºC. Five measurements of gas exchange were made per pot (BBCH 65). A similar experiment was carried out by Hamada AbdElgawad et al. [61].

2.7. Statistical Analysis

The obtained results were statistically analyzed using the Statistica 13.3.0 program (TIBCO Software Inc., Palo Alto, CA, USA). One-way ANOVA was used. Tukey’s HSD post-hoc test ($p = 0.05$) was used to determine the differences between the mean values of the analyzed parameters.

3. Results

3.1. Plant Emergence and Nodulation

The earliest emergence of plants was recorded in control (A) and inoculated seeds (B). Seed coating (regardless of variant) delayed the emergence stage by about 3 days (Table 1). The number of plants after emergence was higher when coated seeds were sown compared to control. A better plant density was obtained in coated variants D and D + B than after inoculation (B).

Table 1. The emergence of soybean plants.

| Factor | Emergence (Days from the Date of Sowing) | Number of Plants after Emergence (Plants·pot$^{-1}$) |
|--------|----------------------------------------|----------------------------------------------------|
| A      | 14.50 $^b$                             | 7.50 $^c$                                           |
| B      | 14.50 $^b$                             | 7.70 $^{bc}$                                        |
| C      | 18.00 $^a$                             | 8.80 $^{ab}$                                        |
| D      | 17.25 $^a$                             | 9.10 $^a$                                           |
| C + B  | 18.00 $^a$                             | 8.80 $^{ab}$                                        |
| D + B  | 17.25 $^a$                             | 8.90 $^a$                                           |

A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.

The inoculant significantly increased the number and weight of nodules on roots. Sowing of control seeds resulted in the lack of nodulation. It was proven that the chemical composition of coating C or D had no negative impact on the effectiveness of inoculation. In variants C + B and D + C, the number and weight of nodules were similar to those in variant B (Table 2).

Table 2. Nodulation on the roots.

| Factor | Number of Nodules from the Plant | DW of Nodules from the Plant (g) |
|--------|----------------------------------|----------------------------------|
| A      | -                                | -                                |
| B      | 25.8 $^a$                        | 0.38 $^a$                        |
| C      | -                                | -                                |
| D      | -                                | -                                |
| C + B  | 23.8 $^a$                        | 0.33 $^a$                        |
| D + B  | 24.4 $^a$                        | 0.35 $^a$                        |

A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.

3.2. Green Mass

The highest fresh and dry weight of green fodder was obtained after sowing inoculated seeds (B) and seeds with coating D + B. Significantly lower results were recorded in control (A) and after sowing seeds with coating C or D (Figure 2).
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![Figure 2. The mass of forage from one plant. A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.](image)

3.3. Physiological Measurements

The SPAD measurement in plants at BBCH 13 was not significantly modified. Another measurement at BBCH 51 showed that inoculation (B) and variants C + B or D + B significantly increased SPAD readings. Significantly lower results were obtained after sowing seeds with coating C. The last SPAD reading (BBCH 65) confirmed that seed inoculation had a positive effect on the plant nutritional status. Significantly lower results were obtained for control and after sowing seeds with coatings C or D (Table 3).

| Factor | Developmental Phase on the BBCH Scale |
|--------|---------------------------------------|
|        | 13 BBCH | 51 BBCH | 65 BBCH |
| A      | 27.3 a   | 32.6 ab | 33.2 b  |
| B      | 27.6 a   | 35.4 a  | 36.2 a  |
| C      | 26.9 a   | 31.5 b  | 32.5 b  |
| D      | 27.1 a   | 32.4 ab | 33.0 b  |
| C + B  | 27.5 a   | 34.6 a  | 35.8 a  |
| D + B  | 27.7 a   | 35.1 a  | 36.0 a  |

A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.

Measurements of leaf stomatal conductance (Gs) showed that chitosan + alginate/PEG coating (variant D) worsened the results. Significantly higher readings were recorded in control after inoculant application and in variant C + B. The lowest Gs readings in the second measurement were obtained in control. At the BBCH stage 65, the inoculant exerted the most positive effect on the Gs index. Significantly lower results were obtained for control after coating C and variant C + B (Table 4).
Table 4. Stomatal conductance Gs (mmol m$^{-2}$ s$^{-1}$).

| Factor | Developmental Phase on the BBCH Scale |
|--------|---------------------------------------|
|        | 13 BBCH | 51 BBCH | 65 BBCH |
| A      | 329.3$^a$ | 332.5$^b$ | 295.5$^d$ |
| B      | 325.6$^a$ | 411.8$^a$ | 434.1$^a$ |
| C      | 315.3$^{ab}$ | 380.3$^a$ | 383.0$^c$ |
| D      | 285.5$^b$ | 400.3$^a$ | 409.5$^{abc}$ |
| C + B  | 324.8$^a$ | 391.5$^a$ | 395.5$^b_c$ |
| D + B  | 296.2$^{ab}$ | 414.6$^a$ | 427.2$^{ab}$ |

A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.

The measurement of net photosynthesis (PN) was the highest after seed inoculation application. Significantly lower readings were obtained after sowing seeds with coatings C or D. The transpiration rate (E) was not modified. Intercellular CO$_2$ concentration (Ci) was found to be the highest in control. A significantly lower measurement was obtained when inoculant and coatings C + B were applied to the seeds (Table 5).

Table 5. Impact inoculation and/or coating on soybean gas exchange parameters.

| Factor | Intensity of Photosynthesis Net (PN) (µmol(CO$_2$)·m$^{-2}$·s$^{-1}$) | Transpiration Rate (E) (mmol(H$_2$O)·m$^{-2}$·s$^{-1}$) | Intercellular CO$_2$ Concentration (Ci) (mmol·L$^{-1}$) |
|--------|-------------------------------------------------------------|-------------------------------------------------|-----------------------------------------------|
| A      | 16.3$^{ab}$                                                   | 3.13$^a$                                        | 68.5$^a$                                      |
| B      | 17.6$^a$                                                      | 3.31$^a$                                        | 52.3$^b$                                      |
| C      | 16.1$^b$                                                      | 3.08$^a$                                        | 63.2$^{ab}$                                   |
| D      | 15.9$^b$                                                      | 3.11$^a$                                        | 64.6$^{ab}$                                   |
| C + B  | 17.1$^{ab}$                                                   | 3.26$^a$                                        | 55.3$^b$                                      |
| D + B  | 17.3$^{ab}$                                                   | 3.29$^a$                                        | 57.3$^{bc}$                                   |

A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.

The measurement of the maximal photochemical efficiency of PSII (Fv/Fm) was not significantly different (Table 6). It was demonstrated that the maximum quantum yield of primary photochemistry (Fv/F0) and performance index (PI) were the highest after applying inoculation and variants C + B or D + B. Significantly lower results were obtained in control and after the use of coatings C or D.

Table 6. Impact of inoculation and/or coating on chlorophyll fluorescence parameters in the soybean leaves.

| Factor | Maximal Photochemical Efficiency of PSII (Fv/Fm) | Maximum Quantum Yield of Primary Photochemistry (Fv/F0) | Performance Index (PI) |
|--------|-------------------------------------------------|-------------------------------------------------|------------------------|
| A      | 0.731$^a$                                       | 2.28$^b$                                        | 3.48$^b$               |
| B      | 0.768$^a$                                       | 3.05$^a$                                        | 4.29$^a$               |
| C      | 0.742$^a$                                       | 2.33$^b$                                        | 3.35$^b$               |
| D      | 0.746$^a$                                       | 2.37$^b$                                        | 3.41$^b$               |
| C + B  | 0.745$^a$                                       | 2.88$^a$                                        | 4.17$^a$               |
| D + B  | 0.753$^a$                                       | 2.99$^a$                                        | 4.23$^a$               |

A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.

3.4. Protein Content

Protein content in dry matter of green fodder differed significantly under the influence of the variants tested. The application of inoculant and coatings C + B or D + B increased protein concentration in green fodder. Significantly lower contents of the discussed component were determined in green fodder obtained from the control and after the application of
coatings C or D (Figure 3). Bioinoculant had significant effects on protein, with or without seed coatings, but seed coatings per se did not have any significant effect on protein.

### Figure 3. Total protein content in the dry matter of forage. A, B, C, D, C + B, D + B—see Section 2. Mean values with different letters are statistically different.

#### 4. Discussion

The extent of biological nitrogen fixation (BNF) resulting from the symbiosis between legumes and their root bacteria amounts to at least 70 million tonnes per year. Therefore, the promotion of legume cultivation, improvement of their agriculture practices, and increasing the efficiency of BNF are currently of great importance for the development of sustainable agriculture [13,62]. The observed climate changes have become a serious global problem not only for agriculture [63]. Hence, new solutions are sought in plant cultivation technologies in order to provide food for humans and feed for animals. This goal should be achieved, but in such a way that environmental risks are minimized (Kulkarni et al. [64]). Poliserpi et al. [31] demonstrated that some seed dressings contain active substances harmful for birds. This requires an urgent change and the search for other alternative solutions in seed protection. Lentola et al. [65] reported that the new methods allow the accurate determination of the toxicity of all active substances to living organisms. On the basis of such results, permits should be granted for the commercial sale of chemical plant protection preparations. Lentola et al. [66] confirmed that certain seed dressings contain dangerous insecticides. They are effective in pest control, but also dangerous for beneficial animals. Han et al. [67] presented interesting studies on the effects of pesticide production on employees’ health. As regards seed coating, it was necessary to ensure the safety of people exposed to direct contact with toxic substances. Pedrini et al. [68] argued that chemical preparations should not be used indiscriminately due to the novelty, but should have proven benefits for agricultural practice. Rocha et al. [69] have indicated that there are many microbial preparations intended for crops that can reduce the use of agrochemicals and increase plant nutrition or tolerance to environmental stresses. One such solution is coating the seeds with natural substances. Depending on the ingredients used, coatings plays a different role, including protection against abiotic and biotic stresses. Ehsanfar and Modarres-Sanavy [70] wrote that the coating technique was first used in 1930. A layer of pesticide was applied to cereal grains, which was supposed to reduce pathogen harmfulness.

In the current study, two innovative coatings were tested, which were intended to protect seeds sown in moist and cold soil and to improve germination and physiological parameters of plants after emergence. The experiments demonstrated that seed coating increased plant density, but delayed the emergence phase by about 3 days compared to control (Table 1). Chachalis and Smith [71] reported that coating seeds with polymer could
reduce water absorption, but it did not affect plant density after emergence under optimal soil conditions. In the study of Sharratt and Gesch [72], germination of maize and soybean after sowing coated seeds was delayed by 6 and 11 days, respectively, as compared to uncoated seeds. This delay was due to the use of a polymer that dissolved depending on the temperature. As a result, early sowing of seeds into cold and moist soil allowed uniform emergence. Jarecki [51] confirmed that sowing coated seeds delayed emergence, but at the same time increased plant density per 1 m². The work of Schogo-lev and Raievski [73] demonstrated that inoculation of soybean seeds decreased the mass of the root system. In contrast, nitrogen deficiency in control plants stimulated root development, which was an important aspect of research on soybean cultivation.

Elshafie and Camele [74] have reported that coating is often used to protect seeds after sowing and to improve the vigor of sprouts. Coating compositions include many various substances, e.g., growth regulators, pesticides, fertilizers, and microorganisms. Korbecka-Glinka et al. [75] showed that natural polymers of animal or plant origin could be used for seed improvement. Many such biopolymers have a bioactive effect, stimulate sprout growth, or provide additional protection for young plants against harmful environmental factors. Biodegradability, non-toxicity, and small amounts applied to seeds make such preparations environmentally friendly, and thus they can be used for seed improvement in organic farming. Kintl et al. [76] showed that coating seeds with various PEG concentrations might be advisable, but only in moderate drought conditions. With a water deficit, the germination capacity of coated seeds drops significantly compared to control seeds. Sarrocco et al. [77] used, among others, sodium alginate for seed coating. The method is simple as CaCl₂ is applied to dry seeds followed by sodium alginate. Such seeds store better and are well protected after sowing. Zeng et al. [78] demonstrated that chitosan coating reduced pest occurrence, increased seed germination, and stimulated plant growth and soybean yield. The application of chitosan for seed coating can be a good alternative to many toxic substances. Aboalfayah and Samara [79] have shown that many essential oils, including jojoba oils, reduced pest pressure. Ludwig et al. [39] reported that the use of a polymer for coating prevented the loss of about 20% of the active ingredient compared to coatings without polymer. They found that the method of polymer application had no effect on seed physiological quality, but larger seeds exhibited a greater viability. Evangelista et al. [80] demonstrated that polymer coatings improved the emergence of plants under optimal laboratory conditions. However, coating decreased the seed vigor under stressful conditions (excess water).

Seed inoculation in soybean cultivation is an important procedure, which has been confirmed by many authors [8,11,12,16,22,51,73,81]. The present study showed that coating seeds with a preparation containing Bradyrhizobium japonicum had a positive effect on nodulation (Table 2) and selected plant physiological measurements (Tables 3 and 5). Asei et al. [16] indicated that the application of a seed inoculation preparation (Legumefix), significantly increased dry weight of nodules. Additionally, they demonstrated the benefits of combining inoculation with a molybdenum fertilizer (Teprosyn Mo). Their results, however, depended on the location of the experiments. Stecca et al. [82] proved that seed inoculation increased the number and dry weight of nodules even in acidic soil (pH KCl 5.3). Procházka et al. [83] showed that inoculants increased nodulation and plant biomass. As a result, they achieved a significant increase in seed yield compared to control. In another study, Procházka et al. [84] confirmed that complex seed treatment increased soybean yield and, additionally, seed oil content. Kasper et al. [85] indicated that the application of appropriate inoculants did not always result in proper nodulation on soybean roots. This situation is influenced by many different abiotic and biotic factors that need to be identified.

Coating seeds with C or D coating together with the inoculant did not have a negative effect on the number and weight of nodules (Table 2). However, the highest fresh and dry weight of green fodder was obtained after sowing only inoculated seeds (B) and seeds in variant D + B (Figure 2). Ortez et al. [86] showed that BNF determined the proper growth and development of soybean plants. When nodulation was weak or not observed, it
always negatively influenced the vegetative and generative development of plants. Santos et al. [34] confirmed that the sowing of improved seeds was favorable relative to control. However, this was demonstrated in laboratory or greenhouse experiments. When they conducted field experiments, the effects were not always statistically confirmed, especially with respect to physiological measurements of plants or seed yield.

Gesch et al. [87] demonstrated that in the case of zero-tillage, soil slowly warms up in spring and then dries out rapidly. It is not conducive to soybean seed germination with early sowing. In turn, the delay in sowing creates a risk of drought and the necessity of sowing early cultivars. In such a situation, temperature-activated polymer coatings may enable seed sowing in unheated soil and selection of late cultivars. Studies of Sharratt and Gesch [72] and Gesch et al. [87] demonstrated that the effects of sowing coated seeds depended on the sowing date and years of research. Early sowing of coated seeds resulted in an increase in plant density after emergence. On the other hand, sowing at the optimal date, but into dry soil, caused the opposite effect.

Meghvansi et al. [88] stated that knowledge of rhizobia found in soils in individual regions is particularly important for the successful use of commercial inoculants. In agricultural practice, it happens that ineffective soil bacteria develop nodules, which reduces BNF. This translates into lower nitrogen availability for legumes.

The current experiments showed no differences in the SPAD measurement at BBCH 13, because the nodules were at the setting stage. An increase in SPAD readings was recorded only in BBCH stage 51, in the following variants: inoculant (B) and coatings C + B or D + B. The last SPAD measurement in BBCH 65 confirmed that seed inoculation had a positive effect on the plant nutritional status (Table 3). Previous studies [51] showed that soybean seed inoculation resulted in a significant increase in SPAD readings compared to controls. Jarecki et al. [22] and Jarecki [51] showed that a commercial preparation containing *Bradyrhizobium japonicum* increased the number and dry weight of nodules on the roots and SPAD measurements. Fritschi and Ray [89] did not confirm a significant relationship between chlorophyll and nitrogen content in soybean leaves. Therefore, they concluded that SPAD measurements were useful, but should be complemented by other plant physiological analyses.

The chitosan + alginate/PEG coating (variant D) reduced the Gs measurements in BBCH 13. This was not confirmed when measurements were taken at BBCH stages 51 and 65, and the lowest Gs readings were obtained in control. Thus, coating components did not have a negative effect on leaf stomatal conductance in the later stages of plant development (Table 4). Ma [90] argued that seed coating could effectively reduce biotic and abiotic stresses in plants. Therefore, the development of this technology is promising. It will depend on the soil conditions of a given region (droughts, salinity, and low temperatures), cultivation systems (conventional, ecological) and the cultivated plant. Jarecki [51] proved that soybean seed inoculation had a positive effect on the measurement of leaf stomatal conductance (stage V3) in comparison to control. On the other hand, sowing coated seeds caused stress in young plants, as indicated by the Gs measurement. This effect was not observed when the inoculant was applied to polymer coatings. Jarecki and Wietecha [50] demonstrated that changing weather conditions modified the effects of sowing coated seeds. Seed coating did not provide the expected results under favorable moisture and thermal conditions. On the other hand, it allowed for better emergence and planting density in unfavorable conditions (decrease in soil temperature after sowing) compared to control.

The results of the present research showed differences in the physiological measurements of plants. Net photosynthesis (PN) was the highest after seed inoculation. Significantly lower PN readings were obtained after sowing seeds with coatings C and D. The measurement of intercellular CO₂ concentration (Ci) was the highest in control. Significantly lower results were recorded after sowing inoculated seeds and in variant C + B (Table 5). Kaschuk et al. [91] reported that seed inoculation with bacteria and/or fungi improved plant physiological parameters and leaves died later than in control.
It was demonstrated that the maximum quantum yield of primary photochemistry (Fv/F0) and performance index (PI) were the highest after applying inoculation and coating variants C + B or D + B (Table 6). Avelar et al. [92] assessed the physiological response of soybean plants to seed coating in the laboratory and under field conditions. They showed that the use of a liquid polymer with a fungicide increased plant emergence and vigor, which translated into the obtained seed yield. Jańczak-Pieniżek et al. [93] confirmed that the plant physiological measurements in a field experiment allowed the assessment of plant responses to various agronomic treatments. As a result, it enables the implementation of the best solutions for agricultural practice.

The application of inoculant (b) or inoculant with coatings C + B or D + B increased protein concentration in green fodder. Significantly lower contents of the discussed component were determined in green fodder obtained from control and after the application of coatings C or D (Figure 3). Meghvansi et al. [90] reported that seed inoculation with symbiotic bacteria in soybean cultivation is an important procedure that increases nodulation and BNF. Rhizobia are frequent rhizosphere colonizers of a wide range of plants and may also inhabit non-leguminous plants endophytically. In these rhizospheric and endophytic habitats, they may exhibit several plant growth-promoting effects, such as hormone production, phosphate solubilization, and pathogen suppression [72,94]. Pedrini et al. [95] argued that coating of seeds with various natural substances is an advanced and perspective technology. Such a product is sought on the market; it is profitable and already offered by private companies. However, they emphasize that these are scientific institutions that are of key importance in the further development of this technology. This will help meet the challenges posed by modern agriculture.

5. Conclusions

Seed improvement is a well-known practice in agriculture, with great prospects for the development of seed coating technology. The pot experiment carried out in this study demonstrated the usefulness of two innovative coatings (C and D) and a commercial inoculant (B) for application to soybean seeds (cultivar Mavka). The use of variants C + B or D + B resulted in obtaining significantly higher parameters for such features as the number of germinated seeds, nodulation, green fodder mass, and protein content in green fodder. Some plant physiological parameters were also increased compared to control (A). The lack of seed inoculation resulted in the absence of nodulation. It was shown that sowing coated seeds delayed plant emergence but increased the number of properly formed sprouts compared to control.

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