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Response of New Yellow Lupin Varieties to Inoculation with *Bradyrhizobium* sp. *Lupinus* under Central European Conditions

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Abstract: The aim of a two-factorial field experiment was to determine how the inoculation of seeds/soil with preparations of *Bradyrhizobium* sp. *Lupinus* (Nitragina—seed inoculation, Nitroflora I—seed inoculation, Nitroflora II—soil inoculation, HiStick® Lupin—seed inoculation) affected plant development, seed chemical composition and yield of two yellow lupin varieties (Bursztyn, Puma). This experiment was carried out with four replications in 2018 and 2019 in Poland. Precipitation during both vegetation periods was similar to or lower than the long-term mean. Average seed yield of Puma was significantly greater than Bursztyn (by 0.22 t ha^{-1}). According to the correlation coefficients, seed yield was mainly related to plant height, dry mass of nodules per plant and mass of 1000 seeds. Our results suggest that legumes, such as lupin, should always be inoculated with *Bradyrhizobium*, especially if they are cultivated for the first time in a field. For optimal results, the highest-quality preparations should be used. In our study, the best results were observed after HiStick® Lupin inoculation, which resulted in the highest protein content, seed yield and protein yield across all treatments.

Keywords: tillage; legumes; BNF; macroelements; composition of seeds; seeds production

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1. Introduction

Legumes, depending on the species, are cultivated for food, fodder, green manure and even as ornamentals [1]. The introduction of post-harvest residues into the soil after legume cultivation determines the amount and timing of mineral forms of nitrogen, which are then available to subsequent crops [2–7]. This process causes an increase in the seed yield of crops grown after legumes [8–12]. According to Nemecek et al. [13], the introduction of grain legumes into intensive crop rotations, which are characterized by a high proportion of cereals and intensive N-fertilization, results in a reduction in energy demand, global warming potential, ozone formation and acidification, as well as reduced eco- and human toxicity per unit of cultivated area. Transformations of leguminous crop residues in the soil (as well as the proportion of nitrogen contained therein) for subsequent plants are determined by their chemical composition and the soil and climatic conditions in the area of their cultivation [14]. Lupin (*Lupinus* sp.) is one of the main grain legumes that is economically cultivated in Europe [15]. This crop is an important source of domestic protein in many countries, and is often considered as an alternative to soybeans, given its elevated and high-quality protein content, suitability for sustainable production and consumer acceptability [16]. In Central Europe, three lupin species are grown for agricultural use as dry seeds: white lupin (*L. albus* L.), yellow lupin (*L. luteus* L.) and narrow-leaved lupin (*L. angustifolius* L.). According to FAOSTAT (2021), the total harvested area of lupin in Europe is 251,253 ha (397,942 tonnes) and the harvested area of this species in Poland (139,120 ha) is greater than other legumes, such as pea (12,870 ha), soybean (9210 ha), broad beans and horse beans (35,920 ha).

Two specific processes are of paramount importance for the yield of leguminous plants [17]. First is the proper functioning of the root nodules, which is guaranteed by the occurrence of a symbiotic plant–bacteria relationship that enables atmospheric nitrogen (N_2) fixation. Second is the development and maintenance of flowers in the maternal plant and, consequently, the formation of protein-enriched pods [17]. Successful *Rhizobium*–legume symbioses have been shown to increase the level of biological nitrogen fixation (BNF) in soil ecosystems [18]. In practice, legume seeds should be inoculated before sowing to improve N_2 fixation by using organisms that can fix N_2. Using the optimal bacterial strain is critical to obtain maximum N_2 fixation in agricultural practice [19]. According to Prusiński et al. [20] and Księżak and Bojarszczuk [21], the inoculation of soybean seeds with *Bradyrhizobium japanicum* bacteria was found to stimulate development and yield. Moreover, the synergistic action between rhizobia and plant growth-promoting rhizobacteria has been documented in an experiment with pea [22]. Our hypothesis here is that the same effect will be achieved in yellow lupin after inoculation with *B.* sp. *Lupinus*. Therefore, the aim of this study was to determine the reaction of two new yellow lupin varieties to inoculation with a number of new preparations that are available on the market and are targeted at lupin but have not been studied to date.

2. Materials and Methods

2.1. Experimental Site and Growing Conditions

A two-factorial experiment was carried out in 2018–2019 at the Research and Education Center Gorzyń, Poland (52°34′ N, 15°54′ E). The experiments were conducted in four replicates in randomized plots. The first experimental factor was the yellow lupin variety: Bursztyn or Puma. The second factor was inoculant type: Nitragina—seed inoculant, Nitroflora I—seed inoculant, Nitroflora II—soil inoculation, HiStick® Lupin—seed inoculant.

Soil chemical analyses were performed at the Regional Agricultural Chemical Station in Poznań, Poland. The soil at the field site was characterized as light soil with a granulometric composition of sandy clay, classified as a typical podzolic soil formed from light sandy clay sands, embedded in a shallow layer of light clay. The soil type, according to the World Reference Base (WRB), is an Albic Luvisol that overlies a gray–brown podzolic. Total N content in the soil was 527 mg kg^{-1} soil, available phosphorus (P) was 13.9 mg kg^{-1} soil and potassium (K) was 10.9 mg kg^{-1} soil. Precipitation and air temperature during the study period were recorded at the Meteorological Station in Gorzyń (Table 1).

Table 1. Weather conditions during the vegetation period 2018–2019 (Meteorological Station, Gorzyń, Poland). Long-term average (1957–2018) precipitation and air temperature values are also shown.

Month	Precipitation Sum (mm)			Air Temperature (°C)		
	Year			Year		
	2018	2019	Long-Term Average	2018	2019	Long-Term Average
April	55.5 **	6.5 **	47.8	12.8 *	9.6 *	8.2
May	22.3	86.3	33.8	16.6	12.1	14.4
Jun	23.8	6.9	61.5	19.4	22.0	17.1
July	84.2	71.8	78.0	20.5	19.1	18.8
Sum/Mean	185.8	171.5	221.2	17.3	15.7	14.6

* Mean monthly air temperature (°C); ** monthly rainfall sum (mm).

Monthly weather conditions differed during the study period. Although precipitation in July was similar in both years of the study, precipitation in the other months was more variable. During the study period, the highest rainfall occurred in May 2019 and the lowest in April of the same year. Moreover, June 2019 was also dry (6.9 mm). However, precipitation sums in 2018 and 2019 were very similar (185.8 mm and 171.5 mm, respectively). Mean air temperatures ranged from 15.7 °C in 2019 to 17.3 °C in 2018. Compared to the long-term average, precipitation in these two years was lower and mean air temperature values were higher.

2.2. Inoculant Treatments

The three preparations that contain live strains of Rhizobia bacteria (*Rhizobium*) available on the market were used in the experiment. The tested inoculants are available under the brand names Nitragina (produced by Institute of Soil Science and Fertilization—National Research Institute, IUNG-PIB, Puławy, Polska, PL), Nitroflora (produced by MYKOFLOR, Końskowola, Polska, PL) and HiStick® Lupin (produced by BASF Agricultural Specialities Ltd., Littlehampton West Sussex, United Kingdom, UK). According to the manufacturers' information, HiStick® Lupin contains >2 × 10^9 (>2 billion) *Rhizobium* bacteria (*Bradyrhizobium* sp. *Lupinus*), while Nitragina and Nitroflora contain 2–3 × 10^{10} of *Rhizobium* bacteria.

Immediately before sowing, the lupin seeds were inoculated with a prepared suspension of Rhizobia bacteria, and in the case of Nitroflora II, after seed planting directly on soil. The inoculation procedure was carried out according to the guidelines provided on the label. During inoculation, seeds were mixed for even coverage. We inoculated yellow lupin (*L. luteus* L.) varieties Bursztyn (registered in 2014: Poznańska Hodowla Roślin Ltd., Tulce, Polska, PL) and Puma (registered in 2017: HR Smolice Spółka z o.o. The IHAR Group, Kobylin, Polska, PL).

2.3. Agrotechnics

The agrotechnical and lupin cultivation treatments were carried out in accordance with the principles of good agricultural and experimental practice. Lupin seeds were sown at a row spacing of 18 cm, at a sowing rate of 100 germinating seeds per m^2 and at a sowing depth of 4 cm. Both lupin varieties were sown at the same time (beginning of April). The sowing date depended on weather conditions in each year of the study. The plots were 24 m^2 in size (plot area: 4 m width × 6 m length). All plots were drilled mechanically using a seeder (Great Plains, Solid Stand 100 equipped with a fluted coulter for residue cutting, a double disk for seed placement and a press wheel, 3 m wide, weight of the tractor 2885 kg). Each year in autumn, the soil was plowed after the forecrop (winter wheat) and harrowed in the spring before lupin sowing. At the beginning of the trial and in the early spring of each year of the study, P and K fertilization were applied. The P and K fertilization applications were 82.5 P_2O_5 and 80 kg K_2O_5 per ha^{-1}, respectively. During the growing season, recommended pesticides (fungicides, insecticides and herbicides) were used for particular target species. Lupin plants were harvested at the full maturity stage in July of each year of the study. The seed harvest was carried out once with a 1.5 m wide Wintersteiger plot combine. Lupin seeds were harvested from the whole plot area.

2.4. Plant Biometric Assessment

At the full plant maturity phase, 15 plants were randomly taken from each plot. The number of pods and seeds on each plant was determined, as were the number of seeds per pod and the height of the plants. During the flowering phase, 10 whole plants were randomly collected for nodulation measurements. A laboratory dryer (at 80 °C for 48 h) was used to determine the dry weight of the nodules. After harvest, seed yield ha^{-1} at 15% moisture content and 1000 seed weight (2 × 500 seeds were counted and weighed) were determined.

2.5. Chemical Analyses

Analyses of the chemical composition of the yellow lupin seeds (milled into a fine powder) were performed using standard methods at the laboratory of the Agronomy Department, Poznań University of Life Sciences. Crude protein [23] and fiber content [24] were determined according to the Kjeldahl method, while lipid content was determined according to the Soxhlet method. Samples to determine the chemical composition were dried at 105 °C to a constant weight. The ash content was determined by ashing the samples to a constant weight at 925 °C [25]. Nitrogen-free extractives were determined by subtraction of the other components (i.e., crude protein, fat, water, ash, fiber) from the total. The chemical composition of the seeds was expressed on a dry weight basis. Protein content (in g kg^{-1}) was recalculated as protein yield (kg ha^{-1}).

Seed samples for analyses of macro- and micronutrients were collected at harvest time and were determined in the Chemical Laboratory at Poznań University of Life Science. Phosphorus content was determined by the vanadate–molybdate method in a Specol 11 spectrocolometer (spectrometric method in line with the standard PN-ISO 6491 (2000)). Magnesium (Mg) content was determined in a Flavo 4 apparatus. The remaining nutrients were determined by atomic absorption spectroscopy (FAAS) using a Hitachi Z-2000 apparatus.

2.6. Statistical Analysis

The results were statistically analyzed with the use of two-way analysis of variance (ANOVA). Statistical analysis was performed using Statistica v.12.0 software (StatSoft, Kraków, Poland). Tukey's multiple comparison test was used to compare the differences between the means for the inoculation treatment. The lowest significant difference (LSD) was considered at the level $\alpha = 0.05$ and $\alpha = 0.01$. The correlation coefficient (Pearson's procedure) with a level of significance $\alpha = 0.05$ was applied to show the strength of the relationship between the selected parameters.

3. Results

A number of yellow lupin biometrical features and yield components were differentiated by variety and inoculant (Table 2). The Puma variety had significantly greater plant density (by 16.3%), plant height (by 12.5%) and 1000 seed mass (by 6.1%). However, Bursztyn plants had a greater dry mass of nodules per plant (by 40%), number of pods per plant (by 26.2%) and number of seeds per plant (by 16.8%). The application of HiStick resulted in increased seed numbers (by 14.6% per plant) compared to the control. No significant differences were found between the control and the other treatments with regard to plant density and number of pods per plant, although the LSD values were relevant. The greatest plant density was found after Nitroflora I application, although a similar impact was also caused by HiStick. Moreover, plants harvested with the HiStick combination had the greatest number of pods per plant, and by comparison, 19% fewer pods were found after application of Nitroflora I. The use of HiStick also resulted in the greatest numbers of seeds per plant, and significantly lower values were found in the other treatments: Nitroflora I and II (by ~19%), and the control and Nitragina (by ~14%).

Significant differences in the chemical composition of the seeds were observed between the Bursztyn and Puma varieties (Table 3). Greater protein content (by 22.2 g kg^{-1} DM) was recorded in the Bursztyn seeds, although the crude lipid content was lower (by 6.3 g kg^{-1} DM). Although the inoculants resulted in significantly different seed protein contents, no differences between the control and other levels of this factor were observed. The greatest crude protein content was found after HiStick application and was significantly lower (by 4.7%) when the soil was inoculated with Nitroflora (II). In comparison to the control, the application of Nitragina caused an increase in the crude fiber content by an average of 19.6 g kg^{-1} DM. Crude fiber content was greatest after the application of Nitragina and was significantly lower (by 12.5%) after Nitroflora I application. In terms of macro- and micronutrient content, seeds from the Nitroflora II combination had the greatest P, Mg and manganese (Mn) contents in comparison to the control (Table 4). These seeds also contained the greatest content of calcium (Ca) and zinc (Zn), although the differences were not significant. The greatest K content was found in seeds harvested from the control treatment, and significantly lower K content (by 6.7%) was observed in seeds after HiStick inoculation.

Table 2. Influence of variety and inoculation type on biometrical features and yield components of yellow lupin.

Specification	PH	DMN	PD	NP	NS	NSP	MTS
				Variety			
Bursztyn	53.4	1.4	66.7	8.2	24.4	3.0	125.6
Puma	60.1	1.0	77.6	6.5	20.9	3.2	133.3
LSD value	4.36 **	0.38 *	1.49 **	1.35 *	3.29 *	NS	2.13 **
				Inoculant			
Control	56.1	1.2	71.6	7.5	22.6	3.0	129.1
Nitragina	56.7	1.2	72.1	7.2	22.3	3.1	128.4
Nitroflora I	57.3	1.1	74.0	6.6	21.0	3.2	130.6
Nitroflora II	57.4	1.3	69.0	7.2	21.3	3.0	128.9
HiStick	56.4	1.2	73.9	8.2	25.9	3.2	130.2
LSD value	NS	NS	3.09 *	1.03 *	2.96 *	NS	NS

NS: not significant; * $p < 0.05$ and ** $p < 0.01$. Specification: PH, plant height (cm); DMN, dry mass of nodules per plant (g); PD, plant density (no. m^2); NP, number of pods per plant; NS, number of seeds per plant; NSP, number of seeds per plant pod; MTS, mass of 1000 seeds (g).

Table 3. Influence of variety and inoculation type on the chemical composition of yellow lupin seeds (g kg^{-1} DM).

Specification	Crude Protein	Crude Lipid	Crude Fiber	Crude Ash	N-Free Extract
			Variety		
Bursztyn	466.8	49.5	169.9	47.3	266.2
Puma	444.6	55.8	172.8	46.9	279.6
LSD value	3.97 **	4.32 *	NS	NS	NS
			Inoculant		
Control	451.7	49.1	164.0	48.4	286.6
Nitragina	451.2	53.8	183.6	47.1	264.0
Nitroflora I	459.1	53.6	160.6	47.0	279.5
Nitroflora II	447.1	54.8	175.6	46.8	275.4
HiStick	469.4	52.1	173.0	46.2	259.1
LSD value	19.88 **	NS	15.0 *	NS	NS

NS: not significant; * $p < 0.05$ and ** $p < 0.01$. Specification: DM, dry matter.

Table 4. Influence of inoculation on macro- and micronutrient content in yellow lupin seeds (g kg^{-1} DM and *** mg kg^{-1} DM).

	Phosphorus	Potassium	Magnesium	Calcium	Zinc ***	Manganese ***
Control	0.3	18.1	1.1	6.5	22.0	79.4
Nitragina	0.3	17.0	1.3	6.3	19.0	72.4
Nitroflora I	0.4	17.6	1.4	6.3	20.6	83.1
Nitroflora II	0.5	17.1	1.5	6.6	22.3	95.0
HiStick	0.4	16.8	1.3	6.1	18.6	82.6
LSD value	0.13 *	1.19 **	0.15 **	NS	NS	12.89 *

NS: not significant; * $p < 0.05$ and ** $p < 0.01$. Specification: DM, dry matter.

During the study, the weather conditions between years were very similar and did not influence yellow lupin seed yield; no significant interaction was observed between year and variety (Figure 1). However, the average seed yield of Puma was significantly greater (by 18.8%) than that of Bursztyn. Seed yield was strongly positively correlated with precipitation sum during the vegetation period (r = 0.87) (Figure 2). Precipitation was very similar between years (Table 1). Air temperatures in 2018 and 2019 were greater compared to the long-term average, while precipitation during the two vegetation periods was lower than the long-term average. Correlation coefficients showed that seed yield was strongly dependent on plant height, dry mass of nodules per plant and mass of 1000 seeds (Figure 2).

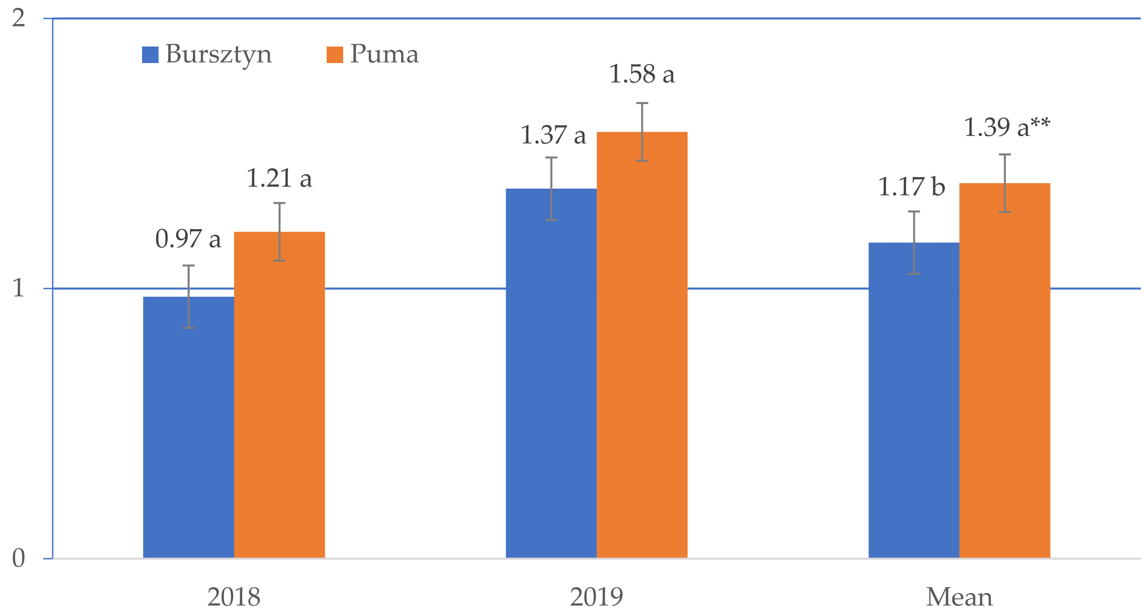

Figure 1. Seed yield (t ha^{-1}) of yellow lupin varieties in 2018 and 2019. (t ha^{-1}). Specification: Values followed by the same letters are not significantly different; ** $p < 0.01$.

Measurement	SP	PH	DMN	PD	NP	NS	NSP	MTS	SY
PS	1.00								
PH	0.85	1.00							
DMN	0.87	0.99	1.00						
PD	–0.99	–0.78	–0.80	1.00					
NP	0.24	0.72	0.69	–0.13	1.00				
NS	0.53	0.91	0.89	–0.43	0.95	1.00			
NSP	0.98	0.71	0.74	–0.99	0.02	0.34	1.00		
MTS	0.65	0.96	0.94	–0.56	0.89	0.99	0.47	1.00	
SY	0.87	0.99	1.00	–0.81	0.69	0.88	0.74	0.94	1.00

r = 1.0 full correlation
0.9 < r < 1.0 almost full correlation
0.7 < r < 0.9 very high correlation
0.5 < r < 0.7 high correlation
0.3 < r < 0.5 medium correlation
0.1 < r < 0.3 weak correlation
0.0 < r < 0.1 slight correlation
0.0 < r < –0.1 slight correlation
–0.1 < r < –0.3 weak correlation
–0.3 < r < –0.5 medium correlation
–0.5 < r < –0.7 high correlation
–0.7 < r < –0.9 very high correlation
–0.9 < r < –1.0 almost full correlation
r = 1.0 full correlation

Figure 2. Correlation coefficients (r) between precipitation sum, biometrical features and seed yield. Specification: PS, precipitation sum; PH, plant height; DMN, dry mass of nodules per plant; PD, plant density; NP, number of pods per plant; NS, number of seeds per plant; NSP, number of seeds per plant pod; MTS, mass of 1000 seeds; SY, seed yield.

In 2019, the seed yields of both varieties were greater than in 2018 (Figure 1), as was protein yield (Figure 3). The difference in protein yield between years was significant: 158 kg ha^{-1} (by 41%) in Bursztyn and 138 kg ha^{-1} (by 30.2%) in Puma. On average, Puma protein yield was 62 kg ha^{-1} (13.4%) greater than Bursztyn yield.

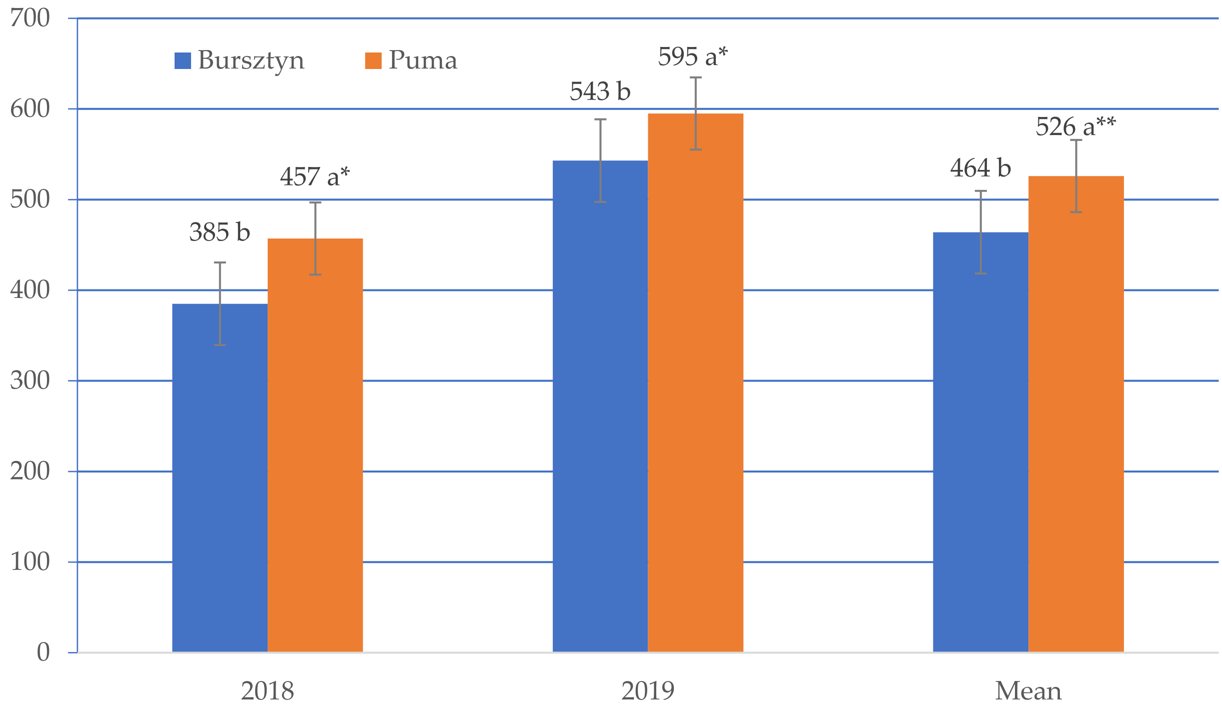

Figure 3. Protein yield (kg ha^{-1}) of yellow lupin varieties in 2018 and 2019. Specification: Values followed by the same letters are not significantly different; * $p < 0.05$ and ** $p < 0.01$.

On average, there were no significant differences between the control and the other treatments in terms of seed yield and protein yield (Table 5). However, the lowest values were observed after Nitragina application (mean seed yield: 1.24 t ha^{-1}, mean protein yield: 475 kg ha^{-1}) and the greatest after HiStick application (mean seed yield: 1.39 t ha^{-1}, mean protein yield: 515 kg ha^{-1}). In interaction, HiStick caused a significant increase (by 11%) compared to Nitragina in the seed yield of Bursztyn. The HiStick application also resulted in a significant increase in the protein yield of Bursztyn in comparison to Nitragina (on average by 8.4%).

Table 5. Influence of variety and inoculation type on yellow lupin seed yield (t ha^{-1}) and protein yield (kg ha^{-1}).

Variety	Inoculant				
	Control	**Nitragina**	**Nitroflora I**	**Nitroflora II**	**HiStick**
			Seed Yield		
Bursztyn	1.18	1.09	1.16	1.20	1.21
Puma	1.40	1.39	1.40	1.41	1.37
Mean	1.29	1.24	1.28	1.30	1.39
LSD value	I—NS; V × I—0.100 *				
			Protein Yield		
Bursztyn	455	444	467	461	491
Puma	533	503	529	524	538
Mean	494	475	498	492	515
LSD value	I –27.5 *; V × I—38.8 *				

NS: not significant; * $p < 0.05$.

Protein yield after HiStick application was strongly correlated with plant height (r = 0.83), number of pods per plant (r = 0.86), seed yield (r = 0.84) and number of seeds per plant (r = 0.92) (Figure 4). However, protein yield after HiStick application was fully determined by seed yield (r = 1.0).

Measurement	PH	DMN	PD	NP	NS	NSP	MTS	SY	CP	PY
PH	1.00									
DMN	–0.73	1.00								
PD	–0.95	0.52	1.00							
NP	0.86	–0.48	–0.77	1.00						
NS	0.92	–0.69	–0.79	0.97	1.00					
NSP	–0.88	0.36	0.88	–0.96	–0.89	1.00				
MTS	–0.55	–0.17	0.69	–0.72	–0.54	0.86	1.00			
SY	0.84	–0.59	–0.92	0.47	0.56	–0.61	–0.41	1.00		
PC	–0.19	0.29	0.33	0.33	0.19	–0.16	–0.22	–0.67	1.00	
PY	0.83	–0.58	–0.91	0.47	0.55	–0.61	–0.41	1.00	–0.68	1.00

$r = 1.0$ full correlation
$0.9 < r < 1.0$ almost full correlation
$0.7 < r < 0.9$ very high correlation
$0.5 < r < 0.7$ high correlation
$0.3 < r < 0.5$ medium correlation
$0.1 < r < 0.3$ weak correlation
$0.0 < r < 0.1$ slight correlation
$0.0 < r < –0.1$ slight correlation
$–0.1 < r < –0.3$ weak correlation
$–0.3 < r < –0.5$ medium correlation
$–0.5 < r < –0.7$ high correlation
$–0.7 < r < –0.9$ very high correlation
$–0.9 < r < –1.0$ almost full correlation
$r = 1.0$ full correlation

Figure 4. Correlation coefficients (r) between biometrical features, seed yield, crude protein content in the seeds and protein yield in the HiStick inoculant treatment. Specification: PH, plant height; DMN, dry mass of nodules per plant; PD, plant density; NP, number of pods per plant; NS, number of seeds per plant; NSP, number of seeds per plant pod; MTS, mass of 1000 seeds; SY, seed yield; PC, crude protein content in seeds; PY, protein yield.

Positive and negative correlations were found between the individual components (Figure 5). There were positive correlations between crude lipid content and Mg content (r = 0.91), and between N-free extract and K (r = 0.92), P and Mn (0.97), and Ca and Zn (0.92). Very high positive correlations were found between seed yield and Mn content, protein yield and crude protein content, crude ash and K, crude ash and N-free extract, N-free extract and Zn, P and Mg content, and Mg and Mn. Negative relationships were also observed, particularly between crude protein and Ca content and between crude fiber content and N-free extract/K content ($-0.7 < r < -0.9$ very high correlation).

Measurement	SY	PY	CP	CL	CF	CA	NFE	P	K	Mg	Ca	Zn	Mn
SY	1.00												
PY	0.69	1.00											
CP	0.13	0.80	1.00										
CL	–0.21	–0.27	–0.18	1.00									
CF	–0.53	–0.47	–0.24	0.47	1.00								
CA	–0.03	–0.40	–0.54	–0.68	–0.38	1.00							
NFE	0.38	–0.15	–0.51	–0.40	–0.71	0.79	1.00						
P	0.67	0.21	–0.26	0.53	0.03	–0.38	0.11	1.00					
K	0.18	–0.14	–0.33	–0.63	–0.74	0.89	0.92	–0.28	1.00				
Mg	0.20	–0.02	–0.17	0.91	0.18	–0.63	–0.15	0.80	–0.48	1.00			
Ca	0.41	–0.37	–0.85	0.07	–0.03	0.46	0.64	0.60	0.36	0.27	1.00		
Zn	0.61	–0.12	–0.66	–0.15	–0.42	0.56	0.85	0.54	0.61	0.16	0.92	1.00	
Mn	0.81	0.37	–0.16	0.37	–0.18	–0.31	0.22	0.97	–0.13	0.71	0.58	0.61	1.00

$r = 1.0$ full correlation
$0.9 < r < 1.0$ almost full correlation
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$–0.5 < r < –0.7$ high correlation
$–0.7 < r < –0.9$ very high correlation
$–0.9 < r < –1.0$ almost full correlation
$r = 1.0$ full correlation

Figure 5. Correlation coefficients (r) between seed yield, protein yield and chemical composition of the yellow lupin seeds. Specification: SY, seed yield; PY, protein yield; CP, crude protein; CL, crude lipids; CF, crude fiber; CA, crude ash; NFE, N-free extract; P, phosphorus; K, potassium; Mg, magnesium; Ca, calcium; Zn, zinc; Mn, manganese.

4. Discussion

The most common practices to integrate legumes and their associated BNF into agricultural systems are crop rotation, simultaneous intercropping, improved fallow, green manuring and alley cropping. However, maximizing the utilization of N_2 fixation requires improvement of the systems, such as the selection of appropriate legume genotypes, inoculation with effective rhizobia species and the use of appropriate agronomic practices and cropping systems [26]. Key research areas of interest include innovative phenotypic and genome-based selection procedures for crop yield, tolerance to abiotic and biotic stresses enhanced by a changing climate, intercropping, and emerging crop quality traits [27]. In the current study, a comparison of two yellow lupin cultivars was carried out over a two-year period, and strong relationships were found between seed yield and plant height, dry mass of nodules per plant and mass of 1000 seeds. The Puma variety was characterized by significantly greater plant height (by 6.7 cm) and 1000 seed mass value (by 7.7 g) compared to Bursztyn, and average Puma seed yield was greater by 0.22 t ha^{-1}. In the study of Księżak and Bojarszczuk [28], the two yellow lupin cultivars examined (Bursztyn and Perkoz) had the same yield. As in our study, the protein content in Bursztyn seeds was greater and the lipid content was lower, although the protein yield was significantly greater in only one year of the experiment. In this current study, protein yield differed between years, with the yields of both varieties greater in 2019 than in 2018. On average, the protein yield of Puma was greater than that of Bursztyn, as also noted for seed yield.

Seed yield was strongly positively correlated with precipitation sum during the vegetation period. Precipitation during the vegetation periods was lower than the long-term average value and may have influenced the low yields of both Bursztyn and Puma (1.17 and 1.39 t ha^{-1} on average, respectively). Księżak and Bojarszczuk [28] conducted an experiment in 2016–2018 and also recorded low seed yields for two yellow lupin cultivars: 0.85–1.55 t ha^{-1} for Bursztyn and 1.0–1.66 t ha^{-1} for Perkoz. The negative effect of the precipitation deficit is dependent on the developmental phase of lupin and the genotype [26]. Podleśna et al. [29] reported that a long period of soil water deficit occurred from the beginning of flowering (BBCH 60) to pod setting (BBCH 75), which had a considerable effect on morphological features and yellow lupin yield. In their study, unsuitable weather in this period resulted in a decrease in plant height, a reduction in leaf area, inhibition of the relative growth rate and a reduction in pods and seed number per plant. In Poland, lupin is in the flowering and pod-setting phases in June. In 2018 and 2019, precipitation in this month was much lower than the long-term average and undoubtedly had an influence on yellow lupin yield, which was shown by Pearson's correlation coefficients. In turn, seed yield and protein yield had an influence on the chemical composition of the seeds, and the correlations indicated a strong positive relationship between seed yield and Mn content, as well as between protein yield and crude protein content. Moreover, protein yield was strongly positively correlated with crude protein content in the seeds, while the relationship between seed yield and protein yield was weaker ($r = 0.69$). In contrast, Csajbók et al. [30] noted that protein yield in soybeans was determined by seed yield rather than by protein content, while Jarecki et al. [1] showed that seed yield was very strongly positively correlated with protein yield. Seed protein content was strongly negatively correlated with lipid content but was strongly positively correlated with K content and highly correlated with Mg content.

In this study, inoculation type was found to influence the morphological features, seed quality and yield in both varieties. The strongest impact was noticed after HiStick application, which resulted in greater plant density and the greatest number of pods and seeds per plant. The numbers of pods and seeds per plant were most dependent on plant height in this combination, and in turn, influenced seed yield and protein yield. On average, seed yield and protein yield were greatest after HiStick inoculation. Results from Egypt [31] would suggest that improvement through the development of combined inoculation strains could be possible and would offer security for nodulation. In that study, high yields associated with seed inoculation were reported, particularly when lupin was cultivated for the first time and when indigenous populations of rhizobia in the soil were low. All tested combinations of *Rhizobium* strains enhanced the growth and total N accumulation in the lupin cultivars and inoculation increased both seed and straw yield compared with the non-inoculated control. In Tunisia, significant increases were recorded in inoculated plants for leaf chlorophyll content (+9–11%), plant height (+16–18%), shoot dry weight (+38–44%) and root dry weight (+51–61%) compared to unfertilized control plants [31]. More recently, experiments have been conducted with soybean inoculation in Central Europe [32]. Soybean is a new crop that can now be cultivated in this region because of a changing climate. Księżak and Bojaszczuk [21] found that inoculation of soybean seeds with *B. japonicum* increased the seed yield. For protein yield, inoculation of soybean by HiStick resulted in a better yield than that from the control and Nitragina inoculation. Prusiński et al. [20] found that both cultivars of soybean in their study responded with increased seed yield after seed inoculation with HiStick.

The results of other experiments have shown that inoculation can also modify the chemical composition of the seeds [33,34]. In our current study, the application of Nitroflora II caused an increase in the P, Mg and Mn content in yellow lupin seeds. There were also greater Ca and Zn contents, although the differences were not significant. In contrast, Zetochová et al. [35] studied the influence of an inoculant on the content of biogenic elements in ten varieties of white lupin (*L. albus*) and three varieties of grass pea (*Lathyrus sativus* L.). They found that inoculation did not significantly affect the chemical composition of the selected legume species.

According to Zveushe et al. [36], the efficiency of BNF depends on factors, such as bacterial species and strain, crop cultivar, symbiosis specificity and prevailing environment [37–39]. Moreover, location with respect to the plant roots was determined to be the main factor that controls the bacterial community, followed by developmental stage and soil type. Plant genotype plays only a minor role [40]. In addition, studies have found that some other microorganisms in the soil can affect the vitality of the nitrifying bacteria, which in turn affects its N_2 fixation efficiency [41,42]. The poor effect of yellow lupin nodulation in our study may be caused by the poor Bradyrhizobium competitiveness against non-nodulating root colonizers, such as Rhizobium. Hence, inoculant strain selection of symbionts should be based not only on their N_2 fixation potential but, more importantly, on their competitiveness in agricultural soils [40]. So, of key importance is the quality of the preparation that is used for inoculation. In our study, the best results were noted after HiStick® Lupin inoculation, which contains >2 × 10^9 (>2 billion) *Rhizobium* bacteria (*Bradyrhizobium* sp. *Lupinus*) and resulted in the highest protein seed content, seed yield and protein yield of yellow lupin across all treatments.

Grain legumes could emerge as a suitable group of crops in the future [43]. Among the different species of lupins, significantly greater levels of crude protein have been observed in *L. luteus* (34.2%, on average) [44]. Lupin holds an important place among legumes, and the utilization of lupin as a dietary protein source is an excellent environmentally friendly alternative to animal-based products for human nutrition [45]. The increase in domestic production of GMO-free plant proteins represents a way to reduce dependence on feedstuff imports. Among legumes, lupin appears to be an interesting and promising crop [44]. White, yellow and narrow-leafed lupins are native European legumes that can become true alternatives to soybean, given their elevated and high-quality protein content, potential health benefits, suitability for sustainable production and acceptability to consumers [46].

5. Conclusions

In our current study, the final effect of inoculation was not confirmed, and further research is required. However, our study clearly showed that the greatest protein content, seed yield and protein yield of yellow lupin were found after HiStick application. Soil inoculation by *B.* sp. *Lupinus* can modify the chemical composition of yellow lupin seeds. The application of Nitroflora to the soil (Nitroflora II combination) caused an increase in P, Mg and Mn contents in yellow lupin seeds (in comparison to the control). Farmers should know that seed inoculation is a quick way to achieve good and healthy crop production. Independent of world region, many soil conditions require inoculating legume crops to obtain maximum yields. The choice of the seed or soil inoculation method depends on legume species, climate and soil conditions.

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