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ORIGINAL PAPER

The effect of mineral N fertilization and seed inoculation with *Bradyrhizobium japonicum* on soybean development and yield in north-eastern Poland*

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Abstract

Soybean [*Glycine max* (L.) Merr.] is a strategic crop with a high content of protein and fat that plays an important role in human and animal nutrition. The area under soybean fields continues to increase in Poland. Soybean yields are determined by a number of factors, including nitrogen (N) fertilization and the use of *Rhizobium* bacteria and weather conditions. In the present study, the effect of various N doses and seed inoculation with *Bradyrhizobium japonicum* bacteria on the yield, protein yield of seeds, yield components (number of pods per plant, number of seeds per pod, thousand seed weight) and gas exchange parameters (photosynthetic rate and transpiration rate) was determined in soybean grown in north-eastern Poland. Soybean seed yields increased by 40.3% in response to seed inoculation with the HiStick®Soy preparation, by 43.2% in response to seed inoculation with HiStick®Soy and the application of 30 kg N ha^{-1}, and by 41.9% in response to seed inoculation with HiStick®Soy and the application of 60 kg N ha^{-1} relative to the unfertilized control treatment. The application of 60 kg N ha^{-1} as well as 60 kg N ha^{-1} + HiStick®Soy also positively affected yield components. In both soybean cultivars (Aldana and Annushka), seed yield peaked in 2018, which was characterized by the most beneficial weather conditions for the growth and development of soybean plants. Nitrogen fertilization and seed inoculation exerted varied effects on the examined parameters. The leaf photosynthetic rate was higher at the beginning of flowering than during full flowering.

Keywords: soybean, nitrogen fertilization, *Bradyrhizobium japonicum*, yield, yield components, photosynthesis, transpiration

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INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is an important legume crop cultivated worldwide owing to the high biological value of its protein, which plays a key role in human and animal nutrition (Stein et al. 2008, Sudarić et al. 2019). The soybean seed contains 40-42% of protein and 18-22% of oil (Medić et al. 2014, Patil et al. 2018). The growing demand for feed protein has contributed to an increase in the area under soybean cultivation in the European Union, including Poland (Niwińska et al. 2020). Despite this increase, soybean production does not fully meet the protein requirements of animals (Jarecki, Bobrecka-Jamro 2021). The main factor that limits soybean production in Poland is climate (Cober, Morrison 2010, Serafin-Andrzejewska et al. 2021), in particular temperature (Janas et al. 2002). Research has shown that soybean can be grown in various Polish regions, including in north-eastern Poland (Prusiński et al. 2020, Serafin-Andrzejewska et al. 2021, Szpunar-Krok et al. 2021, Księżak, Bojarszczuk 2022, Fordoński et al. 2023). Since soybean cultivation has been gaining increasing interest in Europe and Poland, further research is needed to determine the effects of mineral nitrogen (N) fertilization, seed inoculation with *Bradyrhizobium* root nodule bacteria, particularly in areas where soybean had not been previously grown (Zimmer et al. 2016), and climate change (Ray et al. 2013) on soybean yields. Wright and Lenssen (2013) observed that *B. japonicum* inoculants should be used in soybean production in the USA. Their study demonstrated that root nodule bacteria should be applied in fields where soybeans had not been cultivated in the last five years and in areas subjected to strong environmental stress, such as prolonged flooding or drought. In turn, Griebsch et al. (2020) found that *B. japonicum* persists in arable soils in Central Europe (eastern Germany and Lower Silesia in Poland) and enters into symbiotic interactions with soybeans for up to seven years. Prusiński et al. (2020) noted that despite the growing interest in soybean production in Poland, the efficacy of N fertilization and seed inoculation with *B. japonicum* bacteria remains insufficiently investigated. Soybean plants derive nitrogen mainly through biological N fixation (BNF). Soil bacteria of the genus *Rhizobium* fix N symbiotically by entering into mutualistic relationships with plants (Salvagiotti et al. 2008, Zimmer et al. 2016, Albuquerque et al. 2017). Soybeans can establish symbiotic associations with rhizobium bacteria to fix atmospheric N through root nodule formation (Mayhood, Mirza 2021). Igiehon and Babalola (2021) and Bi et al. (2024) reported that inoculation with *Rhizobium* spp. promoted soybean growth relative to non-inoculated plants. Inoculation also significantly increased the number of pods, the number of seeds per pod, and seed dry weight. Previous studies have demonstrated that inoculation with *Rhizobium* contributed to an increase in dry matter accumulation, N content, the harvest index, and yield in soybean production (Sogut 2006). Other researchers found that seed inoculation combined with different N doses had a positive effect on the morphological traits and yield of various soybean cultivars

(Yang et al. 2018, Cordeiro, Echer 2019, Prusiński et al. 2020, Kaiężak, Bojarszczuk 2022). Księżak and Bojaszczurk (2022) observed that inoculation with *B. japonicum* and N fertilization induced the greatest increase in soybean yield (by 42%) and protein yield (by around 28%). Therefore, the aim of the present study was to evaluate the effect of different doses of mineral N and inoculants containing *B. japonicum* bacteria on the morphological traits, yield components, gas exchange parameters, seed yield, and protein yield in soybeans grown in north-eastern Poland.

MATERIALS AND METHODS

Soybeans were grown in a small-area field experiment established in the Agricultural Experiment Station (AES) in Bałcyny (53°35′46.4″ N, 19°51′19.5″ E, elevation 137 m, north-eastern Poland) between 2016 and 2018. The AES is part of the University of Warmia and Mazury in Olsztyn. The three-factor experiment had a split-plot design with three replications. The experimental variables were: (i) cultivar: Aldana (000), early cultivar developed in Poland, and Annushka (0000), very early cultivar developed in Ukraine, (ii) dose of N fertilizer (N ha^{-1}): 1) control – 0 kg, 2) 30 kg, 3) 60 kg, 4) HiStick®Soy + 0 kg, 5) Nitragina IUNG Puławy + 0 kg, 6) 30 kg + Nitragina IUNG Puławy, 7) 60 kg + Nitragina IUNG Puławy, 8) 30 kg + HiStick®Soy, 9) 60 kg + HiStick®Soy, (iii) year – 2016, 2017, and 2018. The field experiment was established on high-quality soil suitable for wheat cultivation (Polish soil quality classes 1 and 2) with a slightly acidic to neutral pH. Each year, the chemical properties of soil were determined in soil samples collected from every plot at a depth of 0-30 cm before the first agricultural treatments. Soil samples were analyzed at the Chemical and Agricultural Station in Olsztyn, and the results are presented in Table 1. The content of phosphorus was determined with the vanadate-molybdate

Table 1

Chemical properties of soil

Year	pH	P	K	Mg
	(mol dm^{-3})	(mg kg^{-1} soil)		
2016	6.6	179.4	190.8	94
2017	6.3	83.8	147.7	103
2018	5.9	101.8	134.0	71.2

reagent by atomic emission spectrometry (AES). The content of magnesium was determined by atomic absorption spectrometry (AAS). Soil pH was measured in 1 M KCl. Winter wheat was the preceding crop. Soil treatments were applied in line with good agricultural practice. In spring, soil was harrowed and tilled with a cultivation unit before sowing.

Nitrogen was applied at different doses, from 0 kg ha^{-1} (control) to 60 kg ha^{-1}. Phosphorus and potassium fertilizers were applied at a constant dose, and soybean seeds were inoculated (before sowing) with two different preparations containing *B. japonicum* root nodule bacteria (Nitragina – Puławy, HiStick®Soy – BSF Agricultural Solutions). Before sowing, mineral fertilizers were applied at: P_2O_5 – 60 kg ha^{-1} (Fos Dar 40 enriched superphosphate, 40%), K_2O – 120 kg ha^{-1} (potash salt, 60%), and N – at the doses specified above (ammonium nitrate, 34%). Fertilizers were incorporated into the soil. Soybean seeds were sown on 11 May 2016, 15 May 2017, and 11 May 2018, at 90 live seeds m^{-2}, to a depth of 3-4 cm, with 12.5 cm spacing. Weeds, pests, and pathogens were controlled according to the Integrated Crop Protection guidelines. In 2016 and 2017, weeds were managed by spraying the Stomp Aqua 455 CS herbicide at 1.5 L ha^{-1} directly after sowing (BBCH 00-01). In 2018, herbicides were applied twice: Stomp Aqua 455 CS was applied at 1.5 L ha^{-1} in mid-May and Corum 502.4 SL (1.25 L ha^{-1}) + Dash HC (1 L ha^{-1}) were applied in the first ten days of June (BBCH 25). In 2016 and 2017, the Gwarant 500 SC fungicide was additionally applied at 2 L ha^{-1} (BBCH 55-61). The harvested plot area was 15 m^2. During the growing season, the photosynthetic rate and the transpiration rate were measured on the youngest, fully developed leaf at BBCH 61 and BBCH 65. Gas exchange parameters were measured with a LI-COR 6400 portable photosynthesis system (DMP AG SA LTD) at a constant concentration of CO_2 (400 ppm) and light intensity of 1000 μmol m^{-2} s^{-1}. The light source was an LED lamp with the main peak centered at 670 nm and the secondary peak centered at 465 nm. Before harvest, 20 soybean plants were sampled randomly from each plot to determine yield components: number of pods, number of seeds per pod, and thousand seed weight. Soybean plants were harvested with a plot harvester in the fully ripe stage on 14 September 2016, 18 September 2017, and 10 September 2018. After harvest, seed yield per plot was determined by weighing, the results were adjusted to a moisture content of 15%, and expressed per 1 ha.

Statistical analysis

Data were processed statistically with the use of the Statistica software system v. 13.3 (Tibco Software Inc., Palo Alto, CA, USA, 2016). The normality of distribution was checked with the Shapiro-Wilk test. For normally distributed data, the significance of differences between means was determined with the Tukey's HSD test ($p \leq 0.05$).

RESULTS

Weather conditions

Weather conditions varied across the experimental years (Table 2). In 2018, mean daily temperature during the growing season (May to August) was 16.6-20.5°C, and it was around 2.3°C higher than the long-term average. Higher temperatures were noted in June and August of 2018, whereas mean daily temperatures in May and July were below the long-term average. Con-

Table 2

Weather conditions in Bałcyny (Region of Warmia and Mazury) in 2016-2018

Year/ Month		2016	2017	2018	1981-2015+
Precipitation (mm)					
April	1-10	4.8	7.7	2.5	
	11-20	19.2	30.8	10.0	
	21-30/31	9.1	13.6	15.6	
Monthly total		33.1	52.1	28.6	30.0
May	1-10	7.5	27.5	6.0	
	11-20	53.7	2.3	29.4	
	21-30/31	9.6	4.2	5.6	
Monthly total		70.8	34.0	41.0	59.0
June	1-10	12.1	16.6	13.0	
	11-20	28.6	29.7	7.8	
	21-30/31	26.1	63.6	43.9	
Monthly total		66.3	109.9	64.7	72.0
July	1-10	39.6	29.4	15.7	
	11-20	44.0	20.7	81.3	
	21-30/31	65.0	56.0	43.7	
Monthly total		138.6	106.1	140.7	85.0
August	1-10	54.5	31.6	16.3	
	11-20	10.4	11.7	8.0	
	21-30/31	7.0	11.5	6.9	
Monthly total		71.9	54.8	31.2	66.0
September	1-10	7.5	97.5	2.2	
	11-20	1.2	110.6	5.4	
	21-30/31	8.4	3.0	21.5	
Monthly total		17.1	211.6	29.1	55.0
Temperature (°C)					
April	1-10	10.3	9.8	9.5	
	11-20	9.1	4.3	13.5	
	21-30/31	6.9	6.1	12.7	
Daily average		8.8	6.7	11.9	7.8

cont. Table 2

Year/ Month		2016	2017	2018	1981-2015+
May	1-10	14.1	8.5	14.9	
	11-20	11.8	14.9	15.8	
	21-30/31	18.5	15.7	18.7	
Daily average		14.8	13	16.5	13.3
June	1-10	8.5	15.5	19.3	
	11-20	14.9	17.4	18.5	
	21-30/31	15.7	17.2	15.9	
Daily average		13.0	16.7	17.9	15.9
July	1-10	17.5	16.0	17.6	
	11-20	18.1	17.2	19.7	
	21-30/31	19.9	18.5	22.3	
Daily average		18.5	17.2	19.9	18.3
August	1-10	17.7	20.8	23.3	
	11-20	15.6	19.2	17.8	
	21-30/31	19.3	16.3	17.8	
Daily average		17.5	18.8	20.5	17.9
September	1-10	17.5	14.6	17.1	
	11-20	15.1	13.2	17.3	
	21-30/31	17.1	12.8	11.4	
Daily average		14.8	13.3	15.3	13.1

+ long-term average (1981-2015)

siderable differences in the distribution of mean daily temperatures were observed in 2016. Precipitation levels in the Region of Warmia and Mazury were high in 2017, particularly in June (109.9 mm), July (106.1 mm), and September (211.6 mm), which delayed seed harvest. Rainfall distribution was optimal in June, July, and August of 2016 and 2018.

Seed yield protein and protein yield of soybean seeds

Multiple ANOVA revealed that seed yield and the protein yield of soybean seeds were determined by treatment, year of the study, and treatment x cultivar and treatment x year interactions, but not by cultivar. The above factors significantly influenced yield components, the photosynthetic rate, and the transpiration rate. Tree-way ANOVA (treatment x cultivar x year) show significant effects of all factors (Table 3).

It was shown that total nitrogen content was highest in the sites where soybean seeds were inoculated with HiStick®Soy – data not significant at $p \leq 0.05$, mean from years of investigation (Table 4).

The seed yield of the examined soybean cultivars was significantly influenced by the applied N dose, seed inoculation with *B. japonicum* bacteria, year of the study, and weather conditions. In both soybean cultivars, seed yield

Table 3

The effects of the main factors (treatment, cultivar and year) and their interactions on the selected soybean parameters by tree-way ANOVA

Parameter	Treatment (T)	Cultivar (C)	Year (Y)	T x C	T x Y	C x Y	T x C x Y
Number of pods per plant	*	*	*	*	*	*	*
Number of seeds per pod	*	*	*	*	*	*	*
1000 seed weight	*	*	*	*	*	*	*
Seed yield	*	ns	*	*	*	ns	*
Protein yield	*	ns	*	*	*	*	*
Photosynthetic rate	*	*	*	*	*	*	*
Transpiration rate	*	*	*	*	*	*	*

* significant at $p \leq 0.05$, ns – not significant

Table 4

Total nitrogen content of soybean seeds cvs. Aldana and Annushka in 2016–2018

Treatment	Total nitrogen content (%)			
	2016	2017	2018	mean
Mean values for N fertilization and seed inoculation				
Control	4.62*b*	4.61*b*	4.32*b*	4.52*a**
30 kg N ha^{-1}	4.43*b*	4.49*b*	4.45*b*	4.46*a*
60 kg N ha^{-1}	4.59*b*	4.43*b*	4.33*b*	4.45*a*
HiStick®Soy	4.88*ab*	4.80*ab*	5.50*a*	5.06*a*
Nitragina	4.52*b*	4.78*ab*	4.97*ab*	4.76*a*
30 kg N ha^{-1} + HiStick®Soy	4.80*ab*	4.68*b*	5.29*a*	4.92*a*
30 kg N ha^{-1} + Nitragina	4.54*b*	4.61*b*	4.96*ab*	4.70*a*
60 kg N ha^{-1} + HiStick®Soy	4.84*ab*	4.70*b*	4.24*b*	4.59*a*
60 kg N ha^{-1} + Nitragina	4.65*b*	4.56*b*	5.02*ab*	4.74*a*
Mean values for cultivars				
Aldana		Annushka		
4.86a		4.59a		
Mean values for years	2016	2017	2018	
	4.65*a*	4.64*a*	4.91*a*	

a, *b*, *c* – in rows indicate statistically insignificant $p \leq 0.05$ differences based on the Tukey's post-hoc test

peaked in 2018 in response to seed inoculation with HiStick®Soy and the application of 30 kg N ha^{-1} + HiStick®Soy, at 4.50 t and 4.25 t in cv. Annushka, respectively, and at 4.23 t in cv. Aldana in both treatments (Figure 1*a*,*b*). Seed yield was influenced by treatment, year and interactions between factors. In 2016, the highest seed yield was noted in plants supplied with

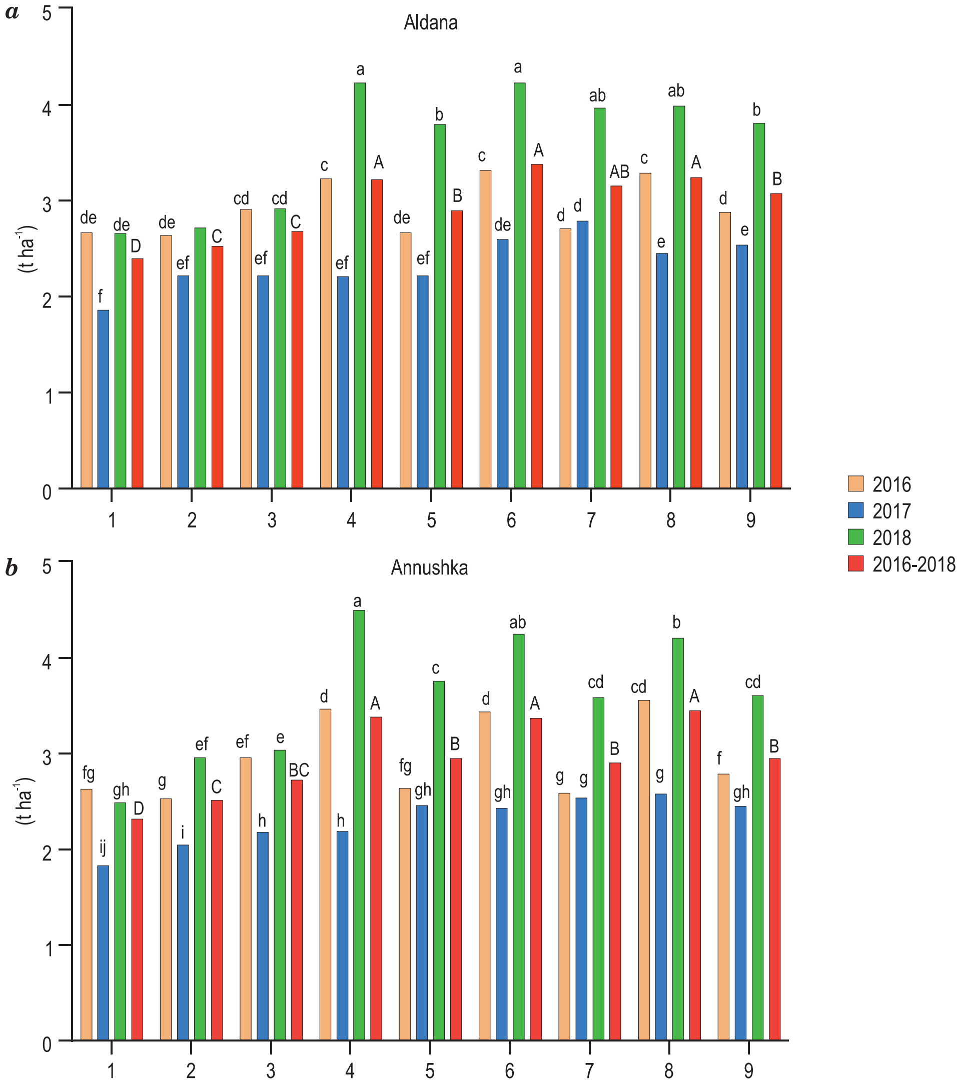

Fig. 1. Seed yield of soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*,b,c… – mean from year vs. combination and A**,B,C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

60 kg N ha^{-1} + HiStick®Soy (3.42 t ha^{-1}), 30 kg N ha^{-1} + HiStick®Soy (3.38 t ha^{-1}), and HiStick®Soy (3.35 t ha^{-1}), whereas the lowest seed yield was observed in plants supplied with 30 kg N ha^{-1} (2.58 t ha^{-1}). In 2017, seed yield was highest in plants supplied with 30 kg N ha^{-1} + Nitragina (2.66 t ha^{-1}) and

lowest in the control treatment (1.84 t ha^{-1}). Soybean yields were higher in 2018, and seed yield peaked in response to the HiStick®Soy treatment (4.37 t ha^{-1}), whereas the lowest seed yield was noted in the control treatment (2.58 t ha^{-1}) – Table 5. Seed yield was comparable in both soybean cultivars. The analyzed parameter was highest in the third year and lowest in the second year of the study (Table 5). The third year of the study (2018) was characterized by the most supportive weather conditions for the growth and development of soybean plants (Table 2). Ground frost in 2017 delayed seedling emergence and successive phenological stages relative to the remaining years of the experiment (data not shown). As a result, the second year of the study was characterized by the lowest number of pods per plant (Table 6), the lowest number of seeds per pod (Table 6), and the lowest seed yield (Figure 1, Table 5). In the control treatment, the average seed yield in the entire three-year experiment reached 2.36 t ha^{-1}. The N dose of 30 kg N ha^{-1} increased seed yield by 6.8% relative to the unfertilized control treatment. An increase in the initial N dose to 60 kg N ha^{-1} increased seed yield by 14.7% in comparison with the unfertilized control treatment.

Seed inoculation with *B. japonicum* contributed to higher seed yield. The application of Nitragina increased seed yield by 24.1% (0.56 t ha^{-1}), and seed inoculation with HiStick®Soy increased seed yield by 40.3% (0.95 t ha^{-1}) relative to the control treatment (Table 5). The application of Nitragina + + 30 kg N ha^{-1} increased seed yield by 24.1% (0.67 t ha^{-1}), and HiStick®Soy + + 30 kg N ha^{-1} increased seed yield by 43.2% (1.02 t ha^{-1}). Seed yield increased by 41.9% (0.98 t ha^{-1}) in response to 60 kg N ha^{-1} + HiStick®Soy and by 27.7% (0.65 t ha^{-1}) in response to 60 kg N ha^{-1} + Nitragina relative to the control treatment (Table 5). Total protein yield in the entire three-year study was highest in treatments where seeds were inoculated with HiStick®Soy (1061 kg ha^{-1}) and lowest in the control treatment (659 kg ha^{-1}). On average, total protein yield was higher in cv. Aldana (912 kg ha^{-1}) than in cv. Annushka (853 kg ha^{-1}), although the difference was not significant. This parameter was highest in the third year (1119 kg ha^{-1}) and lowest in the second year of the study (672 kg ha^{-1}) – Table 5. The highest total protein yields were noted in 2018 in cv. Aldana inoculated with HiStick®Soy, 30 kg N ha^{-1} + HiStick®Soy and in 2018 in cv. Annushka supplied with HiStick®Soy, 30 kg N ha^{-1} + HiStick®Soy and 60 kg N ha^{-1} + HiStick®Soy (Figure 2*a*,*b*). Total protein yield peaked in response to seed inoculation with HiStick®Soy in all years of the study (Table 5).

Yield components

The number of pods per plant was determined by the experimental factors (N fertilization and seed inoculation with *B. japonicum*), and it also differed across years of the study and soybean cultivars. In cv. Annushka, the number of pods per plant peaked in 2016 in treatments with HiStick®Soy: 60 kg N ha^{-1} + HiStick®Soy (28.5 pods), 30 kg N ha^{-1} + HiStick®Soy

Table 5

Seed yield and protein yield of soybean plants in 2016–2018

Treatment	Seed yield (t ha^{-1})				Protein yield (kg ha^{-1})			
	2016	2017	2018	mean	2016	2017	2018	mean
Mean values for N fertilization and seed inoculation								
Control	2.65*b*	1.84*c*	2.58*c*	2.36*cd*	751*c*	530*d*	696*d*	659*cd*
30 kg N ha^{-1}	2.58*b*	2.13*bc*	2.84*c*	2.52*c*	717*c*	599*c*	791*c*	702*c*
60kg N ha^{-1}	2.93*ab*	2.20*b*	2.98*c*	2.70*c*	844*b*	609*c*	807*c*	753*c*
HiStick®Soy	3.35*a*	2.20*b*	4.37*a*	3.31*a*	1023*a*	660*b*	1499*a*	1061*a*
Nitragina	2.65*b*	2.34*b*	3.78*b*	2.92*b*	753*c*	670*b*	1176*b*	866*bc*
30 kg N ha^{-1} + HiStick®Soy	3.38*a*	2.51*ab*	4.24*a*	3.38*a*	1015*a*	735*a*	1405*a*	1052*a*
30 kg N ha^{-1} + Nitragina	2.65*b*	2.66*a*	3.78*b*	3.03*b*	805*bc*	766*a*	1179*b*	920*b*
60 kg N ha^{-1} + HiStick®Soy	3.42*a*	2.51*ab*	4.10*a*	3.34*a*	974*ab*	738*a*	1345*ab*	1019*a*
60 kg N ha^{-1} + Nitragina	2.83*ab*	2.49*ab*	3.71*b*	3.01*b*	825*b*	710*ab*	1169*b*	901*b*
Mean values for cultivars								
	Aldana		**Annushka**		**Aldana**		**Annushka**	
	2.95*a*		2.99*a*		912*a*		853*a*	
Mean values for years	**2016**	**2017**	**2018**		**2016**	**2017**	**2018**	
	3.00*b*	2.32*c*	3.60*a*		856*b*	672*c*	1119*a*	

*a**, *b*, *c*... – in the rows indicate statistically insignificant $p \leq 0.05$ differences based on the Tukey's post-hoc test

Table 6

Yield components in soybeans grown in 2016-2018

Treatment	Number of pods per plant (pods)				Number of seeds per pod (seeds)				1000 seed weight (g)			
	2016	2017	2018	mean	2016	2017	2018	mean	2016	2017	2018	mean
Mean values for N fertilization and seed inoculation												
control	19.6*c*	12.7*c*	11.7*d*	14.7*d*	1.8*a*	1.8*a*	1.9*a*	1.8*a*	157*a*	173*ab*	157*bc*	162*c*
30 kg N ha^{-1}	20.4*bc*	14.0*bc*	14.9*c*	16.4*c*	1.8*a*	1.9*a*	1.9*a*	1.9*a*	153*a*	170*b*	165*b*	163*c*
60 kg N ha^{-1}	20.5*bc*	14.4*bc*	15.0*c*	16.6*c*	1.9*a*	1.7*a*	1.9*a*	1.8*a*	155*a*	170*b*	158*bc*	161*c*
HiStick®Soy	27.4*a*	16.2*b*	21.8*a*	19.5*ab*	2.0*a*	1.9*a*	2.0*a*	2.0*a*	169*a*	181*a*	191*a*	180*a*
Nitragina	19.8*c*	14.8*bc*	18.8*b*	17.8*b*	1.9*a*	1.8*a*	2.0*a*	1.9*a*	153*a*	179*a*	183*ab*	172*ab*
30 kg N ha^{-1} + HiStick®Soy	27.7*a*	15.5*b*	21.1*a*	21.1*a*	2.0*a*	1.7*a*	1.9*a*	1.9*a*	169*a*	172*ab*	183*ab*	175*a*
30 kg N ha^{-1} + Nitragina	23.4*b*	16.2*b*	19.2*b*	19.6*ab*	1.9*a*	1.6*a*	1.8*a*	1.8*a*	153*a*	175*a*	185*ab*	171*ab*
60 kg N ha^{-1} + HiStick®Soy	28.4*a*	20.0*a*	20.2*a*	20.9*a*	2.0*a*	1.8*a*	2.0*a*	1.9*a*	162*a*	169*b*	189*a*	173*ab*
60 kg N ha^{-1} + Nitragina	22.6*b*	18.4*ab*	19.1*b*	20.0*ab*	1.8*a*	1.7*a*	2.0*a*	1.8*a*	152*a*	169*b*	182*ab*	168*b*
Mean values for cultivars												
	Aldana		Annushka		Aldana		Annushka		Aldana		Annushka	
	17.1*b*		20.9*a*		1.93*a*		1.80*a*		192*a*		147*b*	
Mean values for years	2016	2017	2018		2016	2017	2018		2016	2017	2018	
	23.4*a*	15.8*bc*	18.0*b*		1.90	1.75	1.95		158*b*	173*a*	177*a*	

a*, b, c… – above the bars indicate statistically insignificant $p \leqslant 0.05$ differences based on the Tukey's post-hoc test

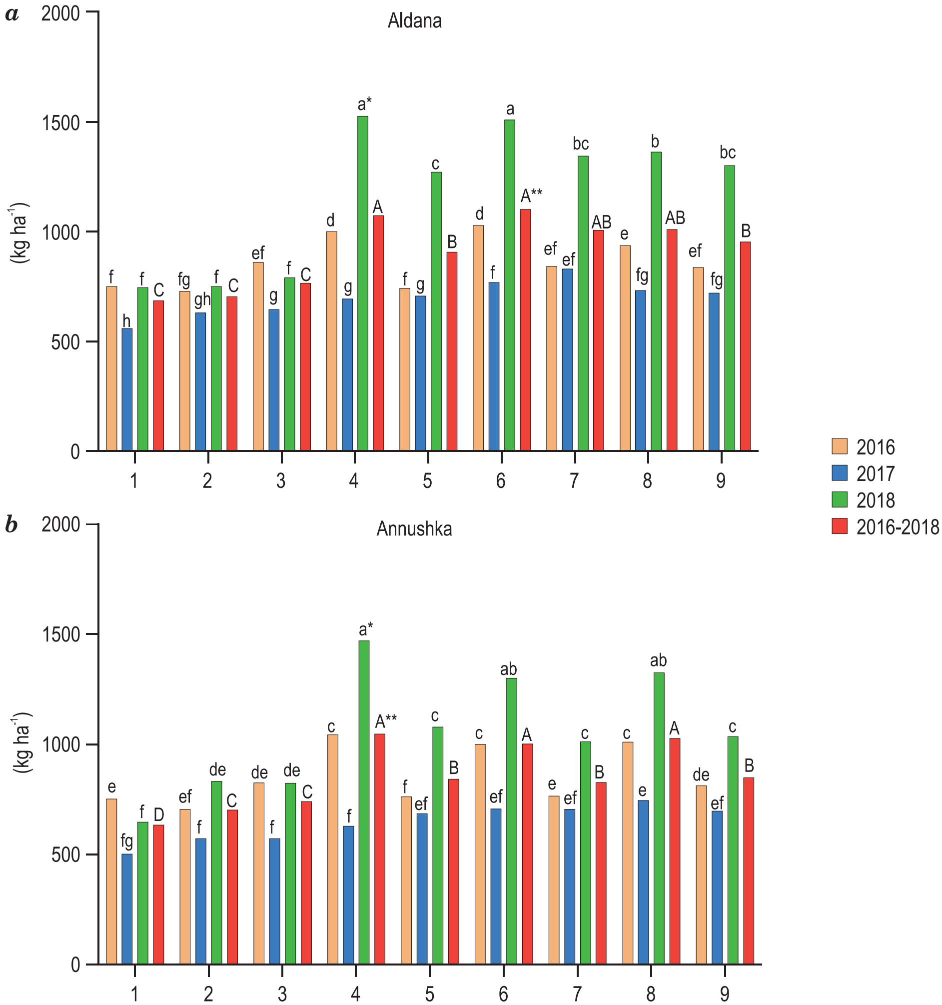

Fig. 2. Protein yield of seeds in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*,b,c… – mean from year vs. combination and A**,B,C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

(28 pods), HiStick®Soy (27.8 pods), and 30 kg N ha^{-1} + Nitragina (27.4 pods). An increase in the number of pods per plant was also observed in cv. Aldana in response to seed inoculation with HiStick®Soy and different N doses (Figure 3*a*,*b*, Table 6). Combined treatments induced a similar increase in the number of pods per plant in both soybean cultivars in 2018. In 2016, the analyzed parameter was highest in response to 60 kg N ha^{-1} + HiStick®Soy (28.4 pods), 30 kg N ha^{-1} + HiStick®Soy (27.7 pods), and HiStick®Soy (27.4 pods),

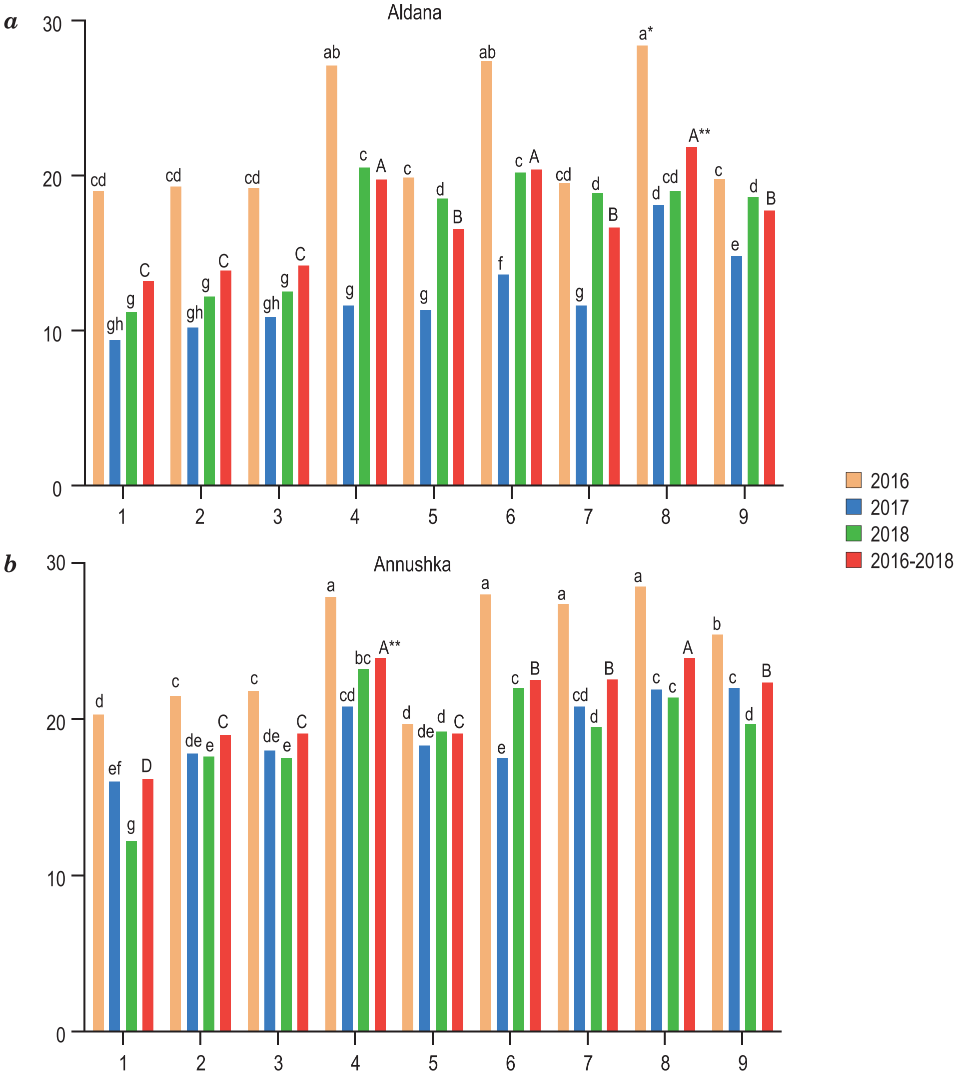

Fig. 3. Number of pods per plant in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*, b, c... – mean from year vs. combination and A**,B,C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

and lowest in the control treatment (19.6 pods) – Table 5). In 2017, the number of pods per plant was highest in the treatment supplied with 60 kg N ha^{-1} + HiStick®Soy (20.0 pods) and lowest in the control treatment (12.7 pods) – Table 6. In 2018, the examined parameter was highest in treatments supplied with HiStick®Soy (21.8 pods) and 30 kg N ha^{-1} + HiStick®Soy (21.1 seeds), and lowest in the control treatment (11.7 pods) – Table 6. The applied treatments induced significant differences in the number of pods per plant during the entire three-year experiment. The number of pods per plant was highest in the first year of the study (2016) and lowest in the second year (2017), which was characterized by less favorable weather conditions for soybean growth. The number of pods per plant was 22.2% higher in cv. Annushka (Table 6). Seed inoculation with HiStick®Soy and the treatment combining HiStick®Soy with 30 and 60 kg N ha^{-1} induced the greatest increase in the number of pods per plant (Figure 3*a*,*b*, Table 6).

The number of seeds per pod peaked in cv. Aldana treated with 60 kg N ha^{-1} + Nitragina and 60 kg N ha^{-1} + HiStick®Soy in 2018 and Nitragina and 30 kg N ha^{-1} in 2017 (Figure 4*a*). However, in cv. Annushka in the variants: control, HiStick®Soy, Nitragina, 30 kg N ha^{-1} + HiStick®Soy in 2018 and HiStick®Soy, 30 kg N ha^{-1} + HiStick®Soy, 60 kg N ha^{-1} + HiStick®Soy in 2016 (Figure 4*b*). In 2016, the number of seeds per pod was highest in treatments supplied with 30 kg N ha^{-1} + HiStick®Soy, 60 kg N ha^{-1} + HiStick®Soy, and HiStick®Soy alone (2.0 seeds). In 2017, the analyzed parameter was highest when soybean plants were treated with 30 kg N ha^{-1} and HiStick®Soy (1.9 seeds), and it was lowest when plants were supplied with 30 kg N ha^{-1} + Nitragina (1.6 seeds). In 2018, the analyzed parameter was lowest in the treatment supplied with 30 kg N ha^{-1} + Nitragina (1.8 seeds) – Table 6. The number of seeds per pod was significantly higher in cv. Aldana than cv. Annushka (Table 5). In the entire three-year experiment, the lowest value of this parameter was noted in 2017. In all years, the average number of seeds per pod peaked in response to seed inoculation with HiStick®Soy and the application of 60 kg N ha^{-1} + HiStick®Soy (Figure 4*a*,*b*, Table 6).

Thousand seed weight (TSW) in the analyzed soybean cultivars was influenced by year of the study and weather conditions. The highest values of TSW were noted in 2017 in cv. Aldana inoculated with Nitragina (216 g) and in 2018 in cv. Annushka supplied with HiStick®Soy (172 g) and with 60 kg N ha^{-1} + HiStick®Soy (172 g) – Figure 5*a*,*b*). In 2016, TSW peaked in response to 30 kg N ha^{-1} + HiStick®Soy and HiStick®Soy alone (169 g), and it was lowest in the treatment supplied with 60 kg N ha^{-1} + Nitragina (152 g). In 2017, TSW was highest in the treatment where seeds were inoculated with HiStick®Soy (181 g), and lowest in the treatments with 60 kg N ha^{-1} + Nitragina and 60 kg N ha^{-1} + HiStick®Soy (169 g). The last year of the study (2018) was characterized by favorable conditions for soybean cultivation, and TSW was highest in the treatment with HiStick®Soy (191 g) and lowest in the control treatment (157 g) – Table 6. The applied treatments induced significant variations in TSW in the three-year study. The analyzed parameter was 45 g higher in cv. Aldana than in cv. Annushka. Thousand seed weight was lowest in the first year of the experiment. This parameter was

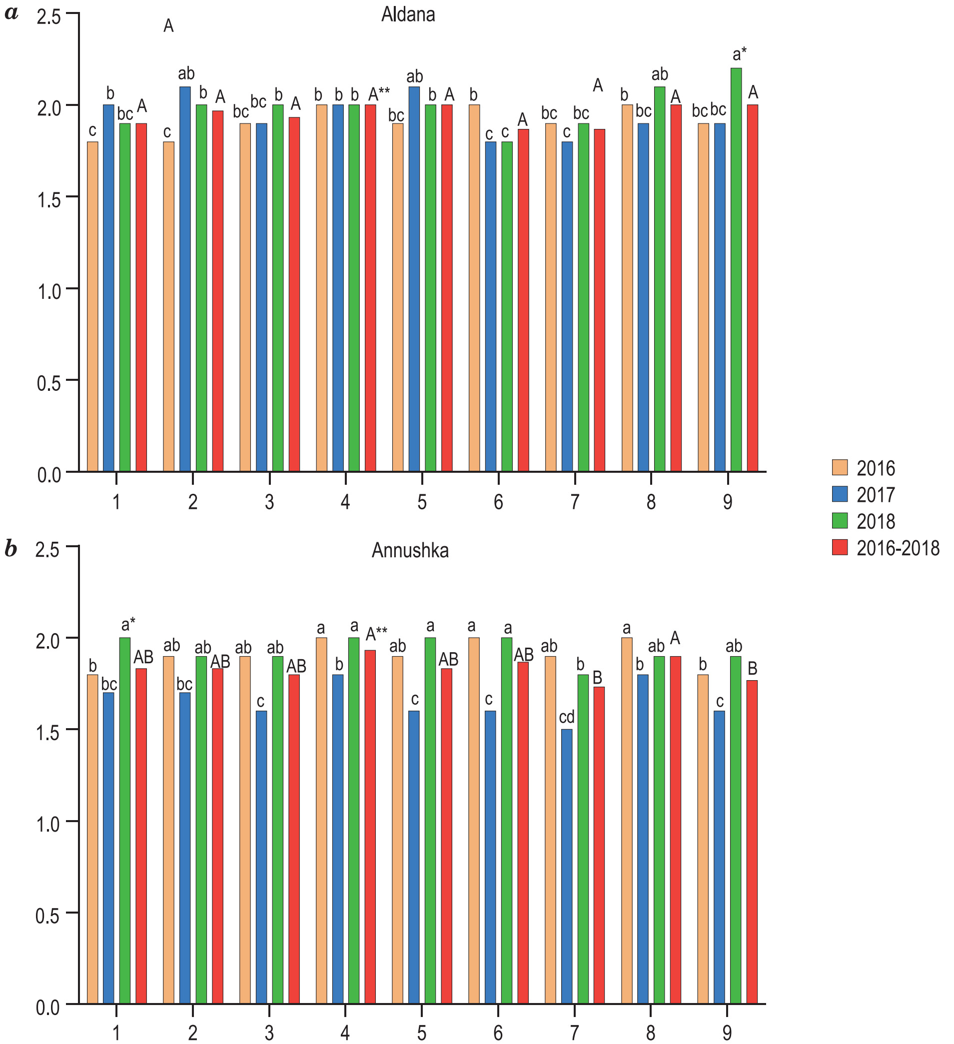

Fig. 4. Number of seeds per pod in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*,b,c... – mean from year vs. combination and A**,B,C... – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

significantly higher in treatments where N fertilization was combined with seed inoculation with *B. japonicum* bacteria. Thousand seed weight peaked in response to inoculation with HiStick®Soy, as well as the application of 30 and 60 kg N ha^{-1} combined with HiStick®Soy (Table 6).

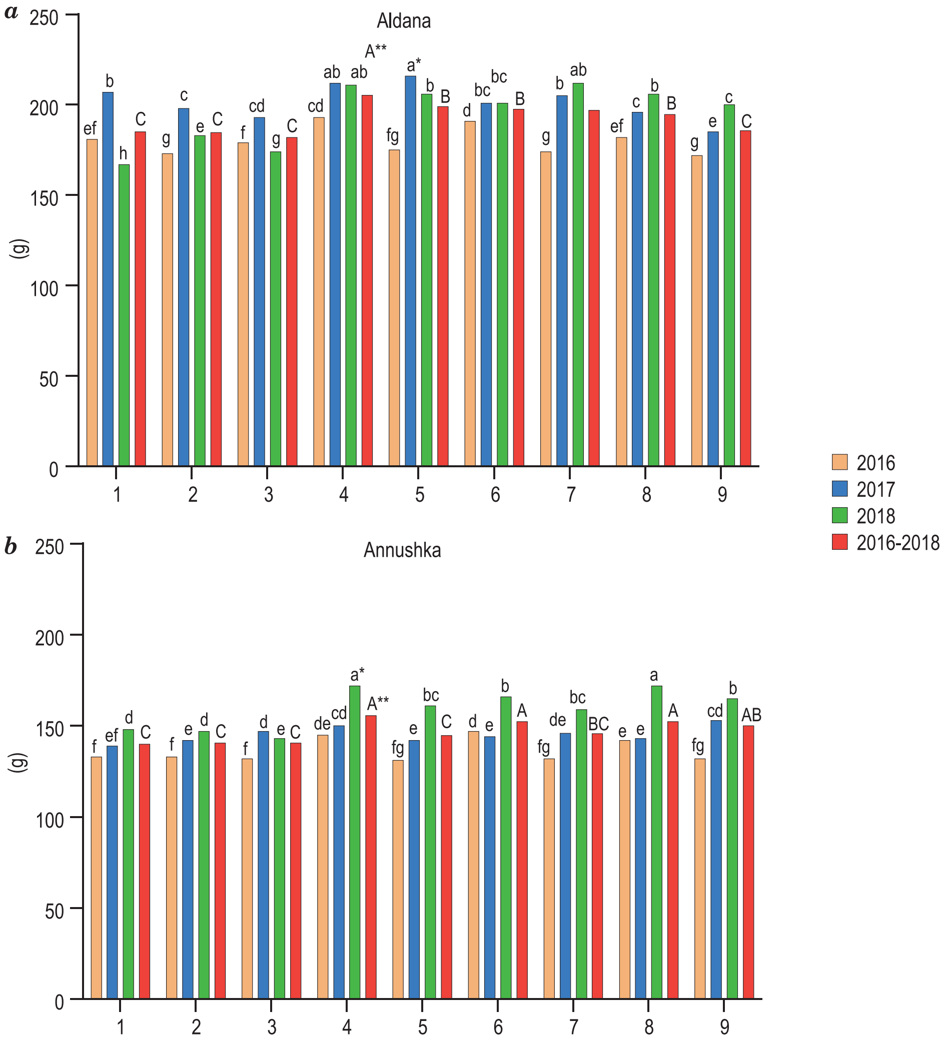

Fig. 5. Thousand seed weight (TSW) in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*, b, c... – mean from year vs. combination and A**,B,C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

Number of root nodules

The number of root nodules in the analyzed soybean cultivars was significantly influenced by seed inoculation with *B. japonicum* and different N doses across years of the study. The average number of root nodules was highest in 2017, when it reached 5.2 in cv. Aldana and 5.0 in cv. Annushka.

This parameter was lower in 2018 at 4.14 and 3.69, respectively, and it was lowest in 2016 at 2.51 and 2.48, respectively. In cv. Aldana, the number of nodules peaked in 2017 and 2018 in response to seed inoculation with Nitragina. In cv. Annushka, the highest value of this parameter was noted in 2018 in response to seed inoculation with HiStick®Soy (Figure 6*a*,*b*).

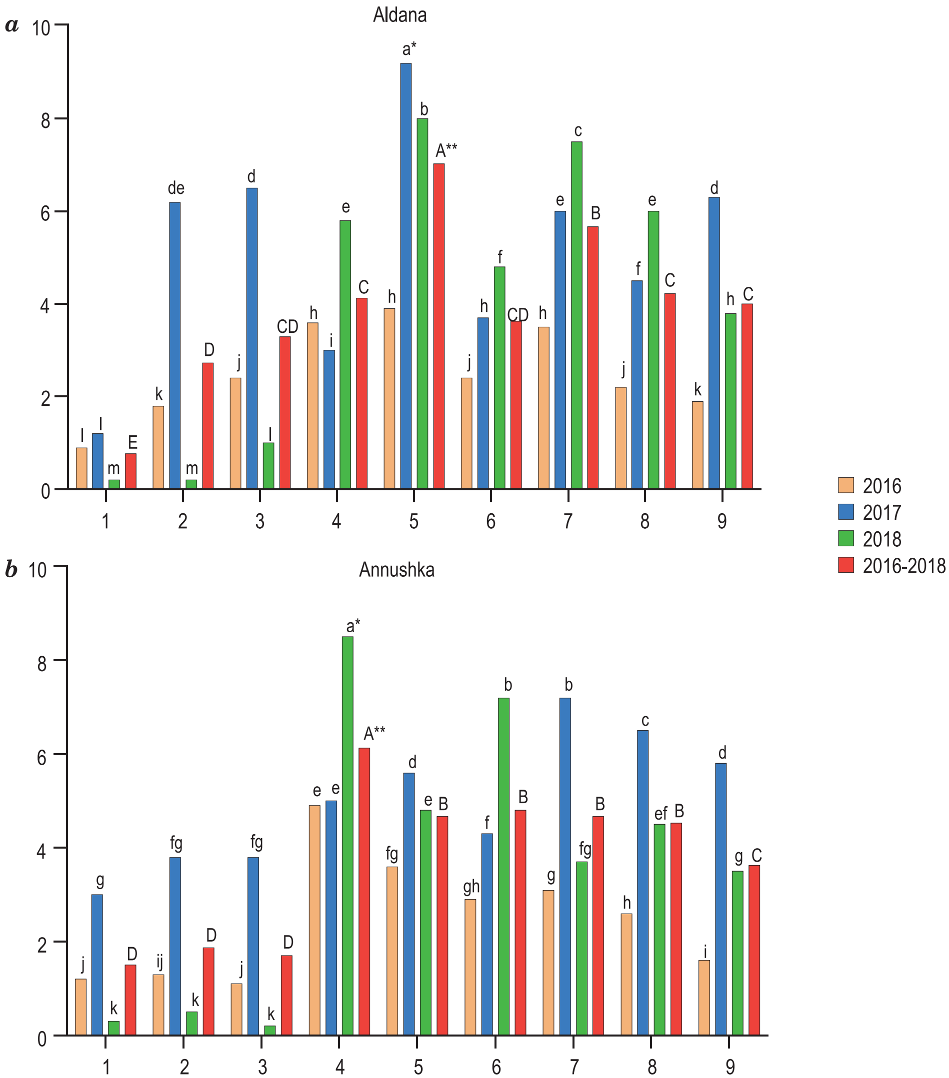

Fig. 6. Number of root nodules in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*, b, c... – mean from year vs. combination and A**,B,C... – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

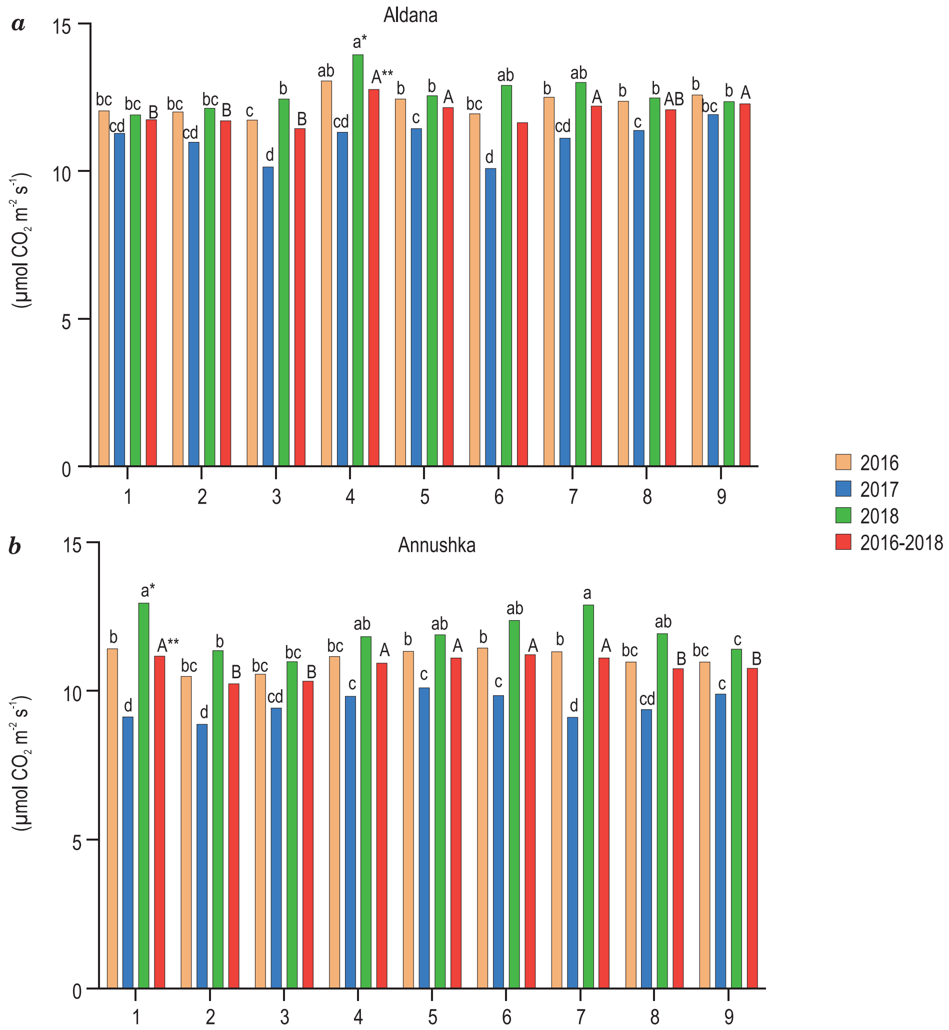

Fig. 7. Photosynthetic rate at the beginning of flowering in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*, b, c... – mean from year vs. combination and A**, B, C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

Photosynthesis and transpiration

In each year of the study, the photosynthetic rate and the transpiration rate were measured twice at the beginning of flowering (BBCH 61) and during full flowering (BBCH 65). The rate of photosynthesis was higher at the beginning of flowering than during full flowering (Figures 7, 8). This parameter peaked in 2018 in both cultivars: in cv. Aldana – in response to seed

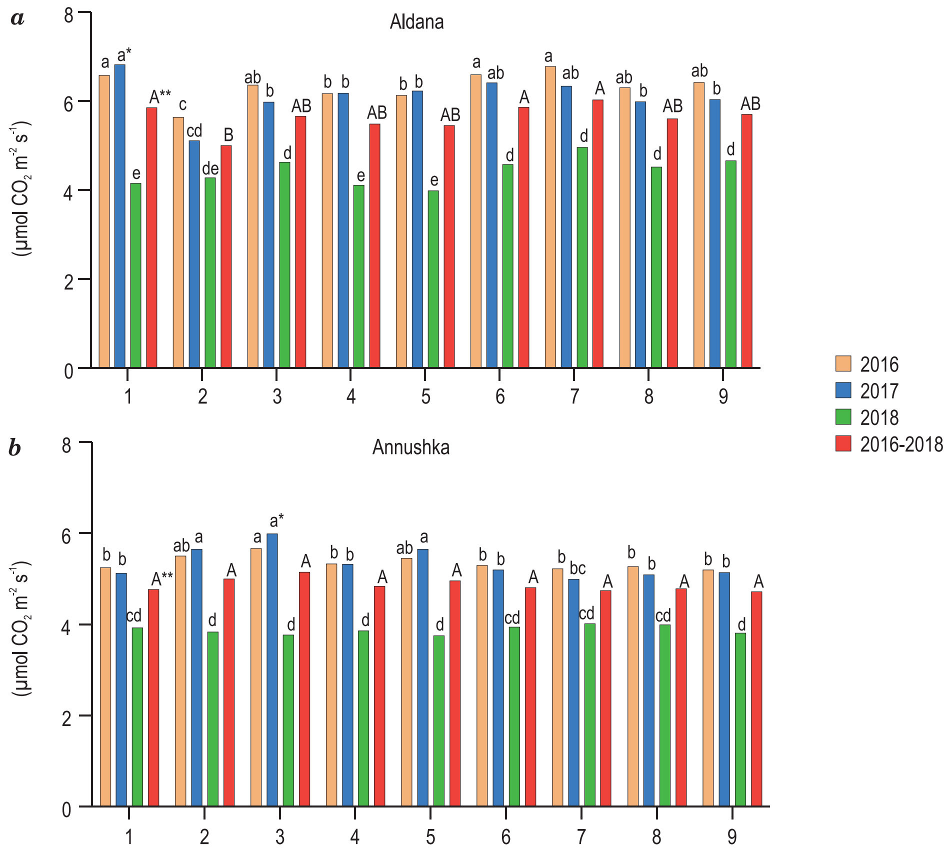

Fig. 8. Photosynthetic rate during full flowering in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*, b, c… – mean from year vs. combination and A**, B, C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

inoculation with HiStick®Soy (13.94 μmol CO_2 m^{-2} s^{-1}), and in cv. Annushka – in the control treatment (12.95 μmol CO_2 m^{-2} s^{-1}) and in the treatment with 30 kg N ha^{-1} + Nitragina (12.89 μmol CO_2 m^{-2} s^{-1}) – Figure 7*a*,*b*). The photosynthetic rate was higher in cv. Aldana (Figures 7, 8). In 2018, the photosynthetic rate was highest at BBCH 61 and lowest at BBCH 65 (Figures 6, 7). The tested N doses and seed inoculation with *B. japonicum* exerted differential effects on the net photosynthetic rate.

In all years of the study, the transpiration rate was higher at the beginning of flowering in both soybean cultivars (Figures 9, 10). In cv. Aldana, the transpiration rate was highest in 2018 in treatments supplied with Nitragina, 60 kg N ha^{-1} + HiStick®Soy, and 30 kg N ha^{-1}. In cv. Annushka, the analyzed parameter also peaked in 2018 in response to the application

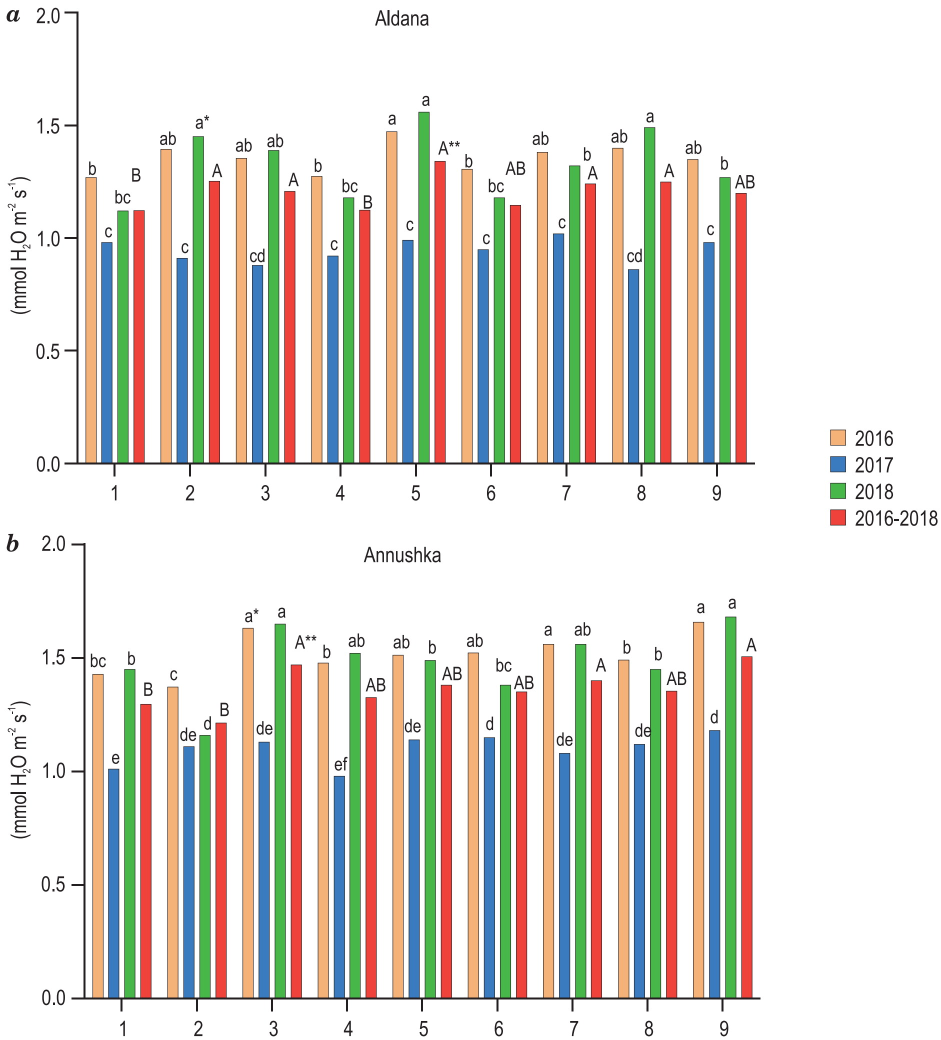

Fig. 9. Transpiration rate at the beginning of flowering in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*, b, c... – mean from year vs. combination and A**, B, C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

of 60 kg N ha^{-1} and 60 kg N ha^{-1} + Nitragina. At BBCH 65, the transpiration rate in cv. Aldana differed across years of the study, whereas in cv. Annushka, this parameter was higher in 2017 and 2018, in particular in the control treatment and in treatments supplied with Nitragina and with 60 kg N ha^{-1} + Nitragina (Figures 9, 10). The transpiration rate at BBCH 61 was influenced by the N dose and seed inoculation with *Rhizobium* bacteria.

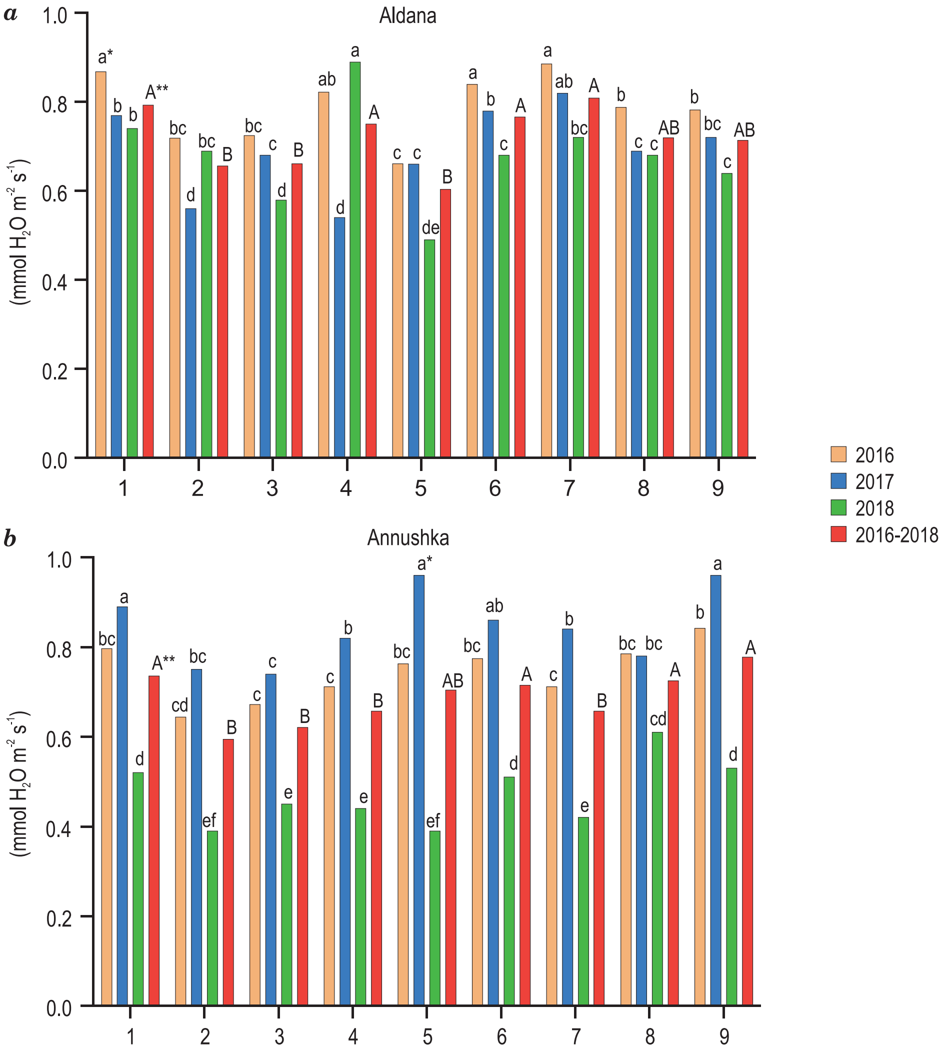

Fig. 10. Transpiration rate during full flowering in soybean cvs. Aldana and Annushka in 2016-2018 in response to different nitrogen doses and seed inoculation: 1 – control, 2 – 30 kg N ha^{-1}, 3 – 60 kg N ha^{-1}, 4 – HiStick®Soy, 5 – Nitragina, 6 – 30 kg N ha^{-1} + HiStick®Soy, 7 – 30 kg N ha^{-1} + Nitragina, 8 – 60 kg N ha^{-1} + HiStick®Soy, 9 – 60 kg N ha^{-1} + Nitragina. The same letters (a*, b, c... – mean from year vs. combination and A**, B, C… – mean from years vs. combination separately) above the bars indicate statistically insignificant $p \leq 0.05$ differences based on two-way ANOVA and the Tukey's post-hoc test

DISCUSSION

Due to the growing interest in soybean (*Glycine max.* (L.) Merrill.) cultivation in Europe and Poland, the current study was undertaken to determine the effect of mineral N fertilization and seed inoculation with *Rhizobium* bacteria on soybean yield. *Rhizobium* bacteria enter into symbiotic interactions with soybean plants, particularly in fields where this crop species had not been previously cultivated (Zimmer et al 2016, Prusiński et al. 2020, Bi et al. 2024). Soybean yield is also significantly affected by climate change (Ray et al. 2013). The study demonstrated that N doses of 30 kg ha^{-1} and 60 kg ha^{-1}, as well as seed inoculation with Nitragina and/or HiStick®Soy increased soybean yield by 6.8% to 40.3% relative to the control treatment (without N fertilization and seed inoculation with *B. japonicum*). Higher N doses combined with seed inoculation increased yield by 41.9% (60 kg N ha^{-1} + HiStick®Soy) and 27.7% (60 kg N ha^{-1} + Nitragina), compared with the control treatment. Similar results were reported by Księżak and Bojarszczuk (2022), who found that seed inoculation with *B. japonicum* combined with N fertilization induced the greatest increase in soybean yield (seed yield increased by 42% and protein yield increased by approx. 28%). Mineral N applied at 30 and 60 kg ha^{-1} enhanced both seed yield and protein yield. In the work of Capatana et al. (2017), seed yield increased by 3.7% after inoculation, and by around 30% in response to seed inoculation combined with mineral fertilization. Panasiewicz et al. (2023) also reported the greatest increase in seed yield in treatments supplied with HiStick® Soy and 30 kg N ha^{-1} or 60 kg N ha^{-1}. Seed inoculation with *B. japonicum* combined with N fertilization significantly improved soybean yield in the south-eastern Baltic Region. Jarecki and Bobrecka-Jamro (2019) also demonstrated that soybean yield peaked in response to HiStick®Soy (3.31 t ha^{-1}) and Nitrazon (3.37 t ha^{-1}) treatments in 2017, and HiStick®Soy (3.92 t ha^{-1}) and Nitragina (3.87 t ha^{-1}) treatments in 2018. In turn, Szpunar-Krok et al. (2023) found that the N dose of 30 kg ha^{-1} combined with seed inoculation with *B. japonicum*, regardless of the bacterial preparation, induced the greatest increase in soybean yield. Corderio and Echer (2019) evaluated the effect of different N doses and biological N fixation efficiency on soybean crops cultivated in unfavorable environments. Soybeans were grown on a degraded pasture and in an area previously cropped with soybeans. The cited authors reported that moderate N doses combined with high inoculant doses increased soybean yield in a degraded pasture despite adverse edaphoclimatic conditions (Corderio, Echer 2019). In a study by Bi et al. (2024), soybean yield was higher on average in treatments inoculated with *Rhizobium* bacteria than in uninoculated treatments. According to Jarecki (2023), seed coating with *B. japonicum* combined with the foliar fertilization of molybdenum increases yield and can be recommended for agricultural practice. In the work of Głowacka et al. (2023), N and sulfur fertilization significantly increased soybean yield. Seed yield peaked in treatments where the N dose of 60 kg ha^{-1} was divided into two portions: 30 kg ha^{-1} before sowing and 30 kg ha^{-1} after emergence, and 45 kg ha^{-1} before sowing and 15 kg ha^{-1} after emergence. In these treatments, seed yield was 8-10 dt ha^{-1} higher than in the unfertilized control treatment. Sulfur fertilization had no significant effect on soybean yield.

In the current study, seed yield was similar in both soybean cultivars. Seed yield was highest in the third year (2018) and lowest in the second year of the experiment (2017). The third year was characterized by the most favorable weather conditions for the growth and development of soybean plants. In 2018, minimum daily temperatures in May, June, July, and August were higher, which had a positive effect on seedling emergence and the growth and development of plants. Total precipitation in June, July, and, August of 2018 also promoted soybean growth. In 2017, ground frost delayed seedling emergence and successive phenological stages, which negatively affected soybean yield. Weather conditions (temperature, total precipitation, and rainfall distribution) also significantly influenced soybean yield in other studies (Prusiński et al 2020, Wu et al. 2020, Toleikiene et al. 2021, Księżak, Bojarszczuk 2022, Jarecki 2023). Staniak et al. (2021) analyzed the impact of cold stress during flowering on plant morphology, seed yield, and the chemical composition of seeds in 15 soybean cultivars: early (EC), medium-early (MC), and late (LC). They found that cold stress exerted a negative effect on the morphological traits of soybean plants (plant height, number of nodes per shoot, stem dry mass, number and weight of pods per plant, number and weight of seeds per plant), and decreased seed yield by 24% on average relative to the control treatment. In a study conducted in Ghana (2022), soybean biomass and yield were drastically reduced by the combined effect of high temperature and drought as compared to the combined effect of temperature and abundant water supply. The cited authors concluded that rising temperatures and uneven rainfall distribution associated with climate change pose a potential threat to soybean production in Ghana. Księżak and Bojarszczuk (2022) also found that high temperature decreased seed yield in soybean cvs. Aldana and Annushka.

The total protein yield of soybean seeds was higher in cv. Aldana than in cv. Annushka, but the difference was not statistically significant. Protein yield was highest in the third year (1119 kg ha^{-1}) and lowest in the second year of the study (672 kg ha^{-1}). Total protein yield peaked in response to seed inoculation with HiStick®Soy. In the work of Mandić et al. (2020), year and N dose induced the greatest differences in crude protein yield (CPY) and crude oil yield (COY), which peaked in response to 60 kg N ha^{-1}. However, no significant differences in CPY were noted in treatments fertilized with 60 and 90 kg N ha^{-1}.

Panasiewicz et al. (2023) also reported a greater increase in the protein and fat content of soybean seeds after the application of HiStick®Soy than the Nitroflora preparation. Protein yield peaked in response to HiStick®Soy + 30 kg N ha^{-1} and HiStick®Soy + 60 kg N ha^{-1}. Seed inoculation with *B. japonicum* combined with N fertilization increased the crude protein content of seeds, biometric traits, and yield components, in particular seed yield in cv. Aldana. Nitrogen fertilization and seed inoculation with *B. japonicum* also enhanced protein yield in the experiments conducted by Prusiński et al. (2020) and Księżak and Bojarszczuk (2022), where the greatest increase in protein yield was reported in cv. Aldana. Staniak et al. (2021) found that cold stress during flowering induced a significant increase in the content of protein (by 4.1% on average) and ash (by 3.8%), and a decrease in the fat content (by 6.9%) of soybean seeds. In the cited study, protein, fat, and ash content was influenced by genotype (cultivar). In turn, Kozak et al. (2008) observed that weather conditions exerted a more profound influence on the chemical composition of soybean seeds than cultivar did.

In the present study, the number of pods per plant was 22.2% higher in cv. Annushka, which corroborates the findings of Księżak and Bojarszczuk (2022). The number of pods per plant was influenced mainly by seed inoculation with HiStick®Soy, as well as the application of HiStick®Soy combined with 30 and 60 kg N ha^{-1}. Głowacka et al. (2023) also reported a significant increase in the number of pods per plant under the influence of various N doses in comparison to control. In the current experiment, the number of seeds per pod was significantly higher in cv. Aldana than cv. Annushka. This parameter was lowest in 2017. In the entire three-year experiment, the average number of seeds per pod peaked in response to seed inoculation with HiStick®Soy, as well as the application of 60 kg N ha^{-1} + HiStick®Soy. The applied treatments induced significant differences in TSW. Thousand seed weight was 45 g higher in cv. Aldana than cv. Annushka. This parameter was lowest in the first year of the study. Thousand seed weight was significantly higher in treatments where N fertilization was combined with seed inoculation with *B. japonicum*. In a study by Księżak and Bojarszczuk (2022), cv. Annushka was characterized by a higher number of pods and a higher number and weight of seeds per plant, but lower TSW. They found that N and inoculants exerted a beneficial effect on TSW and the number of pods and seeds per plant. Mandić et al. (2020) reported that the N dose significantly influenced morphological parameters, yield components, and rain use efficiency (RUE), and the highest values of these parameters were achieved when N was applied at 60 kg ha^{-1}. However, no significant differences in first pod height were noted between treatments fertilized with 60 and 90 kg N ha^{-1}, or in seed weight per plant between treatments supplied with 30 and 60 kg N ha^{-1}. The greatest improvement in the quantitative and qualitative parameters of soybean plants was observed in response to 60 kg N ha $^{-1}$, and this N dose can be recommended for optimum soybean production in rain-fed conditions. In turn, Bi et al. (2024) demonstrated that seed inoculation with *Rhizobium* bacteria increased the average number of seeds per plant and seed weight per plant, but it did not induce significant changes in the number of nodes, number of branches, number of pods, or 100-seed weight. In the work of Panasiewicz et al. (2023), N fertilization and/or seed inoculation significantly affected plant height, the number of pods per plant, the number of seeds per plant, and TSW. The highest values of these parameters were reported in treatments supplied with the highest N dose (60 kg ha^{-1}), HiStick® Soy + 30 kg N ha^{-1}, and Nitroflora + 60 kg N ha^{-1}. The greatest increase in TSW was observed in response to seed inoculation with HiStick® Soy, as well as HiStick® Soy in combination with the tested N doses. According to Israilov et al. (2023), the fertilizer dose of $N_{30}P_{90}K_{60}$ combined with seed inoculation with *B. japonicum* can be recommended for improving soybean yields and seed quality as the key components of sustainable agricultural production under salt-stressed field conditions. On average for two growing seasons, soybean yield was higher by 20.4%, 19.0%, 34.1%, and 6.1% in inoculated treatments without fertilization and in treatments supplied with $N_0P_{90}K_{60}$, $N_{30}P_{90}K_{60}$, $N_{60}P_{90}K_{60}$, respectively, than in the corresponding fertilization treatments without seed inoculation.

The number of root nodules was highest in cv. Aldana in 2017 and 2018 in response to seed inoculation with Nitragina, and in cv. Annushka in 2018 in response to seed inoculation with HiStick® Soy. Szpunar-Krok et al. (2023) found that seed inoculation with *B. japonicum* increased the number of root nodules and seed yield in soybean production. In the work of Księżak and Bojarszczuk (2022), a dry spell during the growing season led to poor nodule fixation on soybean roots. Nodules were formed only after heavy rainfall at the turn July and August. The number of nodules and nodule weight were low, but more nodules were set on roots in treatments supplied with HiStick®Soy. In addition, the number of nodules was somewhat higher in cv. Annushka than cv. Aldana (Księżak, Bojarszczuk 2022).

The influence of the experimental factors on photosynthesis and transpiration was also analyzed in the current study. The net photosynthetic rate was higher at the beginning of flowering than during full flowering. Cultivar Aldana was characterized by a higher photosynthetic rate. The maximum value of the analyzed parameter was noted in 2018 at BBCH 61. The tested N doses and seed inoculation with *B. japonicum* exerted varied effects on the net photosynthetic rate. The transpiration rate at BBCH 61 was enhanced by N fertilization and inoculation with *Rhizobium* bacteria. In the work of Gai et al. (2017), the photosynthetic rate at BBCH 65 reached 22.1-25.9 μmol CO_2 m^{-2} s^{-1} in response to the N dose of 25-75 kg N ha^{-1}. The cited authors also reported a positive correlation between N fertilization and the photosynthetic rate, but only up to the N dose of 50 kg ha^{-1}. The tested N doses exerted varied effects on leaf area index. In turn, Zhang et al. (2013) found that N fertilization and seed inoculation contributed to an increase in the value of this parameter in soybean plants.

CONCLUSIONS

Mineral N and seed inoculation with *B. japonicum* bacteria significantly influenced the growth, development, and yield of soybean plants. In north-eastern Poland, seed inoculation with the HiStick®Soy preparation, as well as HiStick®Soy inoculation combined with N fertilization led to optimal soybean yield. Seed yield was comparable in the tested soybean cultivars. However, in the case of some yield components, cv. Annushka achieved higher values for the number of pods per plant, but cv. Aldana had a higher 1000 seed weight. The highest seed yield was noted in the third year of the study, characterized by the most favorable weather conditions (optimal temperature and rainfall distribution) during the growing season, which promoted the growth and development of soybean plants. The study demonstrated that a starter dose of mineral N promotes N assimilation before the optimal number of root nodules are formed in soybeans, which optimizes plant growth and seed yield. Both N doses of 30 kg ha^{-1} and 60 kg ha^{-1} positively influenced soybean yield and yield components, but the differences were not statistically significant. Therefore, for soybean production under the conditions of north-eastern Poland, N doses of 30 kg ha^{-1} to ensure good yields. However, further research is needed, involving soybean genotypes that are tolerant to short-term temperature drops in the studied region.

Author contributions

Conceptualization – A.P. and G.D; methodology – G.D., A.O., J.O. and A.P.; software – A.O. and A.P.; validation – G.D., J.O, A.O. and A.P.; formal analysis – G.D. and A.P.; investigtion – G.D., A.O., J.O. and A.P.; data curation – G.D., A.O., J.O. and A.P.; writing – original draft preparation, G.D., A.O., J.O., and A.P.; writing – review and editing, G.D., A.P., A.O.; visualization – A.O. and A.P.; supervision – A.P. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Albuquerque, T.M., Ortez, O., Carmona, G.I. and Ciampitti, I.A. (2017) 'Soybean: Evaluation of Inoculation', Kansas Agricultural Experiment Station Research Reports, 3, available: https://doi.org/10.4148/2378-5977.7437

Bi, Y., Fan, C., Liang, W., Liao, Y., Han, D., Li, W., Chen, X., Liu, M., Liu, J., Di, S. et al. (2024) 'Combination effects of sulfur fertilizer and *Rhizobium* inoculant on photosynthesis dynamics and yield components of soybean', *Agronomy***,** 14, 794, available: https://doi.org/10.3390/agronomy14040794

Capatana, N., Bolohan, C. and Marin, D.I. (2017) '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', *Scientific Papers-Series Agronomy*, 60, 207-214.

Cober, E.R. and Morrison, M.J. (2010) 'Regulation of seed yield and agronomic characters by photoperiod sensitivity and growth habit genes in soybean', *Theoretical and Applied Genetics*, 120, 1005-1012, available: https://doi.org/10.1007/s00122-009-1228-6

Cordeiro, C.F.d. and Echer, F.R. (2019) 'Interactive Effects of nitrogen-fixing bacteria inoculation and nitrogen fertilization on soybean yield in unfavorable edaphoclimatic environments', *Scientific Reports*, 9, 15606, available: https://doi.org/10.1038/s41598-019-52131-7

Fordoński, G., Okorski, A., Olszewski, J., Dąbrowska, J. and Pszczółkowska, A. (2023) 'The effect of sowing date on the growth and yield of soybeans cultivated in north-eastern Poland', *Agriculture*, 13, 2199, available: https://doi.org/10.3390/agriculture13122199

Gai, Z., Zhang J. and Li, C. (2017). 'Effects of starter nitrogen fertilizer on soybean root activity, leaf photosynthesis and grain yield', *PloS ONE*, 12(4), e0174841, available: https://doi.org/10.1371/journal.pone.0174841

Głowacka, A., Jariene, E., Flis-Olszewska, E. and Kiełtyka- Dadasiewicz, A. (2023) 'The effect of nitrogen and sulphur application on soybean productivity traits in temperate climates conditions', *Agronomy*, 13, 780, available: https:// doi.org/10.3390/agronomy13030780

Griebsch A., Matschiavelli N, Lewandowska S. and Schmidtke K. (2020) 'Presence of *Bradyrhizobium* sp. under continental conditions in Central Europe', *Agriculture*, 10, 446, available: https://doi.org/10.3390/agriculture10100446

Igiehon, O.N. and Babalola, O.O. (2021) 'Rhizobium and mycorrhizal fungal species improved soybean yield under drought stress conditions', *Current Microbiology*, 78, 1615-1627, available: https://doi.org/10.1007/s00284-021-02432-w

Israilov I., Ssheralievk., Abdalovag. , Iminova., Allanov K. , Aziz Karimov A. , Khaitov B., and Kim, Y.C. (2023) 'Interactive effects of n fertilization and *Bradirhizobia japanicum* on agronomical traits of soybean in salt affected soils', *Turkish Journal of Field Crops*, 28(1), 15-25, available: https://doi.org/ 10.17557/tjfc.1189103

Janas, KM., Cvikrova, M., Pałagiewicz, A., Szafrańska, K. and Posmyk, MM. (2002) 'Constitutive elevated accumulation of phenylpropanoids in soybean roots at low temperature', *Plant Sciences*, 163, 369-373, available: https://doi.org/10.1016/S0168-9452(02)00136-X

Jarecki, W. and Bobrecka-Jamro, D. (2019) 'Influence of seed inoculation with commercial bacterial inoculants (*Bradyrhizobium japonicum*) on growth and yield of soybean', *Legume Research – An International Journal*, 42(5), 688-693, available: https://doi.org/10.18805/LR-485

Jarecki, W. (2023) 'Soybean response to seed inoculation or coating with *Bradyrhizobium japonicum* and foliar fertilization with molybdenum', *Plants*, 12, 2431, available: https://doi.org/10.3390/plants12132431

Jarecki,W. and Bobrecka-Jamro, D. (2021) 'Effect of sowing date on the yield and seed quality of Soybean [*Glycine max* (L.) Merr.]', *Journal of Elementology*, 26, 7-18, available: https://doi.org/10.5601/jelem.2020.25.4.2054

Kozak, M., Malarz, W., Kotecki, A., Cerný, I. and Serafin-Andrzejewska, M. (2008) 'The effect of different sowing rate and Asahi SL biostimulator', *Oilseed Crops*, 29, 217-230.

Księżak, J. and Bojarszczuk, J. (2022) 'The effect of mineral n fertilization and *Bradyrhizobium japonicum* seed inoculation on productivity of soybean (*Glycine max* (L.) Merrill)', *Agriculture*, 12, 11, available: https://doi.org/10.3390/agriculture12010110

Mandić, V., Đorđević, S., Bijelić, Z., Krnjaja, V., Pantelić, V., Simić, A. and Dragičević V. (2020) 'Agronomic responses of soybean genotypes to starter nitrogen fertilizer rate', *Agronomy*, 10, no. 4: 535, available: https://doi.org/10.3390/agronomy10040535

Mayhood,P. and Mirza, B.S. (2021) 'Soybean root nodule and rhizosphere microbiome: Distribution of rhizobial and nonrhizobial endophytes', *Applied Environmental Microbiol*ogy, 87, e02884-20, available: https://doi.org/10.1128/AEM.02884-20

Medić, J., Atkinson, C. and Hurburgh, C.R. (2014) 'Current knowledge in soybean composition', *Journal of the Amerocan Oil Chemists' Society*, 91, 363-384, available: https://doi.org/10.1007/s11746-013-2407-9

Niwińska, B., Witaszek, K., Niedbała, G. and Pilarski, K. (2020) 'Seeds of n-GM soybean varieties cultivated in Poland and their processing products as high-protein feeds in cattle nutrition', *Agriculture*, 10, 174, available: https://doi.org/10.3390/agriculture10050174

Panasiewicz, K., Faligowska, A., Szymańska, G., Ratajczak, K. and Sulewska, H. (2023) 'Optimizing the amount of nitrogen and seed inoculation to improve the quality and yield of soybean grown in the southeastern Baltic Region', *Agriculture*, 13, 798, available: https://doi.org/10.3390/agriculture13040798

Patil, G., Vuong, T.D., Kale, S., Valliyodan, B., Deshmukh, R., Zhu, C., Wu, X., Bai, Y., Yungbluth, D., Lu, F. et al. (2018) 'Dissecting genomic hotspots underlying seed protein oil and sucrose content in an interspecific mapping population of soybean using high-density linkage mapping', *Plant Biotechnology* Journal, 16, 1939-1953, available: https://doi.org/10.1111/pbi.12929

Prusiński, J., Baturo-Cieśniewska, A. and Borowska, M. (2020) 'Response of soybean (*Glycine max* (L.) Merrill) to mineral nitrogen fertilization and *Bradyrhizobium japonicum* seed inoculation', *Agronomy*, 10, 1300, available: https://doi.org/10.3390/agronomy10091300

Ray, D.K., Mueller, N.D., West, P.C. and Foley, J.A. (2013) 'Yield trends are insufficient to double global crop production by 2050', *PloS ONE*, *8*(6), e66428, available: https://doi.org/10.1371/journal.pone.0066428

Salvagiotti, F., Cassman, K., Specht, J., Walters, D., Weiss, A. and Dobermann, A. (2008) 'Nitrogen uptake, fixation and response to fertilizer N in soybeans, A review', *Field Crops Research*, 108, 1-13, available: https://doi.org/10.1016/j.fcr.2008.03.001

Serafin-Andrzejewska, M., Helios, W., Jama-Rodzeńska, A., Kozak, M., Kotecki, A. and Kuchar, L. (2021) 'Effect of sowing date on soybean development in south-western Poland', *Agriculture*, 11, 413, available: https://doi.org/10.3390/agriculture11050413

Sogut, T. (2006) '*Rhizobium* inoculation improves yield and nitrogen accumulation in soybean (*Glycine max*) cultivars better than fertiliser', *New Zealand Journal Crop and Horticultural Science*, 34, 115-120, available: https://doi.org/10.1080/01140671.2006.9514395

Staniak, M., Czopek, K., Stępień-Warda, A., Kocira, A. and Przybyś, M. (2021) 'Cold stress during flowering alters plant structure, yield and seed quality of different soybean genotypes', *Agronomy*, 11, 2059, available: https://doi.org/10.3390/agronomy11102059

Stein, H.H., Berger, L.L., Drackley, J.K., Fahey, G.C., Jr., Hernot, D.C., Parsons, C.M. (2008) 'Nutritional properties and feeding values of soybeans and their co-products', In Soybeans, Chemistry, Production, Processing, and Utilization, Johnson, L.A., White, P.J., Galloway, R., Eds., AOCS Press, Urbana, IL, USA, pp. 613-660.

Sudarić, A., Kočar, M.M., Duvnjak, T., Zdunić, Z. and Kulundžić, A.M. (2019) 'Improving seed quality of soybean suitable for growing in Europe', In Soybean for Human Consumption and Animal Feed, available online: https://www.intechopen.com/online-first/improving-seed-quality-of-soybean-suitable-for-growing-in-europe. (accessed on 28 April 2024)

Szpunar-Krok, E., Bobrecka-Jamro, D., Pikuła, W. and Jańczak-Pieniążek, M. (2023) ‘Effect of nitrogen fertilization and inoculation with *Bradyrhizobium japonicum* on nodulation and yielding of soybean’, *Agronomy*, 13, 1341, available: https://doi.org/10.3390/agronomy 13051341

Szpunar-Krok, E., Wondołowska-Grabowska, A., Bobrecka-Jamro, D., Jańczak-Pieniążek, M,; Kotecki, A. and Kozak, M. (2021) ‘Effect of nitrogen fertilisation and inoculation with *Bradyrhizobium japonicum* on the fatty acid profile of soybean (*Glycine max* (L.) Merrill) seeds’, *Agronomy*, 11, 941, available: https://doi.org/10.3390/agronomy11050941

Toleikiene, M., Slepetys, J., Sarunaite, L., Lazauskas, S., Deveikyte, I. and Kadziuliene, Z. (2021) ‘Soybean development and productivity in response to organic management above the northern boundary of soybean distribution in Europe’, *Agronomy*, 11, 214, available: https://doi.org/10.3390/agronomy11020214

Wright, D. and Lenssen, A.W. (2013) ‘Inoculant use on soybean seed’, In *Agriculture and Environment Extension Publications.*, 192.

Wu, L., Misselbrook, T.H., Feng, L. and Wu, L. (2020) ‘Assessment of nitrogen uptake and biological nitrogen fixation responses of soybean to nitrogen fertiliser with SPACSYS’, *Sustainability*, 12, 5921, available: https://doi.org/10.3390/su12155921

Yang, S.H., Chen, W.H., Wang, E.T., Chen, W.F., Yan, J., Han, X.Z., Tian, C.F., Sui, X.H., Singh, R.P., Jiang, G.M. and Chen, W.X. (2018) ‘Rhizobial biogeography and inoculation application to soybean in four regions across China’, *Journal of Applied Microbiology*, 125, 853-866, available: https://doi.org/10.1111/jam.13897

Zhang, X., Huang, G., Bian, X. and Zhao, Q. (2013) ‘Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere’, *Plant Soil Environment*, 59(2), 80-88, available: https://doi.org/10.17221/613/2012-PSE

Zimmer, S., Messmer, M., Haase, T., Piepho, H.P., Mindermann, A., Schulz, H., Habekuß, A., Ordon, F., Wilbois, K.-P., Heß, J. (2016) ‘Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany’, *European Journal of Agronomy*, 72, 38-46, available: https://doi.org/10.1016/j.eja.2015.09.008