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THE EFFECT OF INOCULATION OF *BRADYRHIZOBIUM SP. LUPINUS* ON PLANT DEVELOPMENT AND YIELDING OF NARROW-LEAVED LUPIN

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ABSTRACT

A field experiment was conducted in the Wielkopolska region at the Gorzyń Research Station, Poland (52.34°N, 15.54°E) in Central Europe. The study was conducted over a 3-year period (2017, 2018, 2019) as a two-factorial design with four replications in the RCBD. The aim of the research was to determine the effect of the cultivar ('Bolero', 'Tytan') and the inoculation (Nitragina–seeds inoculation, Nitroflora I–seeds inoculation, Nitroflora II–soil inoculation, HiStick® Lupin–seeds inoculation) on plant development, seeds chemical composition and yielding of narrow-leaved lupin. The weather conditions and experimental factors significantly influenced on productivity of narrow-leaved lupin 'Tytan'. Drought during the growing season reduced seeds and protein yields. After inoculation of HiStck the seeds yield was significantly greater by 12.4% ($p < 0.01$) and the protein yield after application of Nitroflora I or HiStick by 13.9% ($p < 0.01$) and 19.2% ($p < 0.01$), respectively. Correlation coefficients showed strong relations between number of pods and seeds per plant in both cultivars regardless of the inoculation variant, however the strongest relations in both cultivar were proved on HiStick treatment.

Keywords: Biological nitrogen fixation, chemical composition, legumes, protein efficiency, yielding

INTRODUCTION

In modern agriculture the application of environmentally friendly practices is dictated by European Union law (EU Directive 2009/128) and recommendations for integrated protection/ cultivation have been applicable in Poland since 2014 (Sulewska et al., 2019). Under sustainable farming systems can be cultivated also legume crops (Księżak et al., 2009). Legume plant cultivation in crop rotation is very advantageous due to direct harvesting of plant protein for the production of highly valuable feeds (Etemadi et al., 2016), and contributes indirectly to the biodiversity of systems and crop rotation (Singh et al., 2013; Preissel et al., 2015; Lybæk and Hauggaard-Nielsen, 2019). These plants' capacity for biological reduction of molecular nitrogen contributes to an increase in the amount of nitrogen in the soil-plant system, which can significantly reduce the dose of nitrogen applied in the form of mineral fertilizers (Kalembasa et al., 2021). Sustainable, innovative and cost-efficient processing methods to produce high-protein ingredients should be devised to guarantee the socio-economic value of the crops (Lucas et al., 2015). The agricultural usability of bio-fertilizers, particularly including microbiological seed inoculation meet the recommendations for integrated protection/cultivation applicable in Poland and thanks to seed inoculating, it is possible to reduce fertilization costs and reduce environmental impacts (Ozturk and Yildirim, 2013; Sulewska et al., 2019). The highly diversified results of research on the effectiveness of inoculating soybean seeds or a simultaneous inoculation and application of mineral nitrogen fertilization in various countries, and, above all, the effectiveness of soybean inoculation have triggered similar studies in Poland (Prusiński et al., 2020). Soybean is an important legume cultivated worldwide and due to the high biological value of protein it is considered the most important protein plant in the world (Stein et al., 2008; de Visser et al., 2014; Thrane et al., 2017; Tsukamoto et al., 2018; Księżak and Bojarszczuk, 2022). However, in our country more important legumes are lupines and especially narrow-leaved lupin. Pulse crops, such as white lupin (*Lupinus albus* L.), yellow lupin (*Lupinus luteus* L.), and narrow-leaved lupin (*Lupinus angustifolius* L.) are native European plants, and could provide an excellent source of plant protein (Sujak et al., 2006; Annicchiarico et al., 2010). In compare in Poland, the average area of soybean stand is only about 7 170 ha but lupines 170 540 ha (FAOSTAT, 2022). According to Prusiński et al. (2020) and Księżak and Bojarszczuk (2022), the inoculation seeds with *Bradyrhizobium japonicum* bacteria stimulated the development and yielding of soybean. Using the optimal bacterial strain is critical to obtain the expected results of nitrogen fixation in agricultural practice (Pudełko and Żarnicka, 2010). The hypothesis of our research stand is that the same effect we will achieve in narrow-leaved lupin after inoculation of *Bradyrhizobium sp. Lupinus*.

MATERIALS AND METHODS

Material

Three preparations available on the market were used in the experiment containing live strains of rhizobial bacteria (*Rhizobium*) under the name nitragina (produced by the Institute of Soil Science and Fertilization - National Research Institute, IUNG-PIB, PL), nitroflora (produced by MYKOFLOR company, PL) and HiStick® Lupin (produced by BASF Agricultural Specialities Limited company, GB). Immediately prior to sowing, the seeds were inoculated according to the label with a properly prepared suspension of rhizobia bacteria. The study used narrow-leaved lupin seeds (*Lupinus angustifolius* L.) of the 'Bolero' and 'Tytan' variety registered in 2016, originating from HR Smolice Spółka z o.o. The IHAR Group. The seeds were sown on a 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 chemical properties of the field soil are presented in Table 1. The soil type, according to the World Reference Base, is an Albic Luvisol that overlies a gray–brown podzolic.

Table 1. Selected chemical properties of soil at the study site

Chemical soil properties	
pH in 1M KCl	6.4
C (g kg^{-1})	10.5
N (g kg^{-1})	527
P (available mg kg^{-1})	13.9
K (available mg kg^{-1})	10.9
Mg (available mg kg^{-1})	61

The weather course in the years of the study (2017–2019) was presented in Table 2.

During the study period, mean air temperatures were ranged from 14.3 in 2017 to 17.3 in 2018 (Table 2). Annual precipitation differed considerably between the years of the study: it was the highest in 2017 and the lowest in 2019. In compare to long term mean values higher mean temperatures were noticed in 2018 and 2019. In these two years sum of rainfall was lower than mean long term value by 35.4 mm and 49.7 mm, respectively.

Table 2. Air temperature (°C) and rainfall sum (mm) during the vegetation period in 2017–2019.

Day of Month	Mean Air Temperature (°C)					Rainfall Sum (mm)				
	Month/Year									
	Apr	May	Jun	Jul	x̅	Apr	May	Jun	Jul	∑
	2017									
1st–10th	10.2	8.2	16.3	17.2	13.0	3.3	28.2	32.9	38.5	102.9
11th–20th	5.8	15.4	18.3	17.9	14.4	25.5	6.9	11.1	27.2	70.7
21st–30th/31st	6.4	16.2	18.3	21.6	15.6	3.7	44.5	95.4	96.6	240.2
x̅ */∑ **	7.5*	13.3	17.5	18.9	14.3	32.5**	79.6	139.4	162.3	413.8
	2018									
1st–10th	9.8	15.0	20.4	19.4	16.2	2.3	14.8	15.3	14.3	46.7
11th–20th	15.0	16.2	19.8	19.5	17.6	29.9	3.5	0.1	69.0	102.5
21st–30th/31st	13.5	18.7	17.9	22.7	18.2	23.3	4.0	8.4	0.9	36.6
x̅ */∑ **	12.8*	16.6	19.4	20.5	17.3	55.5**	22.3	23.8	84.2	185.8
	2019									
1st–10th	8.3	9.3	21.3	16.8	13.9	0.0	9.4	2.3	13.4	25.1
11th–20th	6.9	11.9	23.2	17.9	15.0	1.6	37.4	4.6	25.4	69.0
21st–30th/31st	13.6	15.2	21.6	22.5	18.2	4.9	39.5	0.0	33.0	77.4
x̅ */∑ **	9.6*	12.1	22.0	19.1	15.7	6.5**	86.3	6.9	71.8	171.5
Long term mean value	8.2	14.4	17.1	18.8	14.6	47.8	33.8	61.5	78.0	221.2

* mean monthly air temperature (°C) ** monthly rainfall sum (mm).
Data source: Meteorological Station, Gorzyń, Poland

Field experiment

Field experiments were conducted in 2017-2019 at the Department of Agronomy of the University of Life Sciences in Poznań, in the field of the Research and Education Center Gorzyń, branch in Gorzyń (52.34°N, 15.54°E). The experiments were run as two-factors in four replicates, in the RCBD. The first experimental factor was narrow-leaved lupin cultivar: 'Bolero' and 'Tytan'. The second factor was the inoculant: Nitragina –seed inoculant, Nitroflora I–seed inoculation, Nitroflora II–soil inoculation, HiStick® Lupin – seed inoculant. According to the manufacturer's 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 prior to sowing, the lupin seeds were inoculated with a prepared suspension of Rhizobia bacteria and in 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 covering.

The agrotechnical and cultivation treatments were carried out in accordance with the principles of good agricultural and experimental practice for this species. Seeds were sown in row spacing of 18 cm, sowing rate of 100 germinating seeds per m^2, and the sowing depth of 4 cm. Both cultivars were sown in the last week of March, depending on weather conditions. The plots for sowing were 24 m^2 in size (plot area: 4 m width × 6 m length) and for harvesting. In all the tillage systems plots were drilled with a double disk drill (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, the soil had been ploughed after fore-crop (winter wheat) in Autumn and had been harrowed in spring before lupin was sown. At the beginning of the trial, in early spring in each year phosphorus (P) and potassium (K) fertilization was applied in doses of 82.5 P_2O_5 and 80 kg K_2O $_5$·ha^{-1}, respectively. There was not nitrogen fertilization use. During the growing season, recommended pesticides were used for particular target species as *Colletotrichum lupine* (Gwarant 500 SC) and *Charagmus gressorius* (Mospilan 20 SP). Plants were harvested at the full maturity stage of narrow lupin in each year in July, at one stage with a 1.5-metre wide Wintersteiger plot combine. Lupin seeds were harvested from the whole plot area.

Plant Biometric Assesment

According to the experimental practice in the phase of full plant maturity, 15 plants were randomly taken from each plot and the number of pods and seeds on plant, along with the number of seeds per plant pod and the height of plants were determined. To evaluate the influence of inoculants on nodulation, during flowering phase, 10 whole plants were randomly collected for the nodulation measurements involving the nodules dry mass (laboratory drier, 80 ºC/48 h). Plant density was assessed before harvest using frame method on 1 m^2 area. After harvesting, the seed yield per 1 ha, seed humidity (15%), and 1000 seed weight (2 × 500 seeds were counted and weighed) were determined (PN-68/R-74017).

Chemical analysis of narrow lupin seeds

Analyses of the chemical composition of narrow-leaved lupin seeds (milled into fine powder) were performed using the commonly accepted methods at the laboratory of the Department of Agronomy, the Poznań University of Life Sciences. Crude protein (AOAC, 2011) and fibre (Van Soest, 1963) was determined according to Kjeldahl, oil contents according to Soxhlet method. Samples were dried at 105 °C to a constant weight, as well as ash content by ashing the sample at 925 °C to constant weight (Erbas et al., 2005). Nitrogen-free extractives were determined by subtraction from 100% contents of the other components. All the determinations were expressed on a dry weight basis. Protein content in g kg^{-1} was recalculated as protein yield (kg ha^{-1}).

Statistical Analysis

The results were statistically analyzed with the use of the two-way analysis of variance (ANOVA) 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 for two varieties, while confidence intervals for the means of LSD (α = 0.05) were used. Correlation coefficient (Pearson's method) was determined to show the relationship between the selected parameters (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Cultivar was found to modify biometrical features and yield components (Table 3). A significantly greater plant height (by 2.4 cm), dry mass of nodules per plant (by 0.1 g), number of pods per plant (by 22.4%) and number of seeds per plant (by 11.4%) were observed in the Tytan cultivar, and a significantly lower number of seeds per plant pod (by 7.5%) and 1000 seed mass (by 10.8 g) compared to Bolero. Inoculation intensity substantially affected only the numbers of seeds per plant. However, this factor did not have an impact on plant high, dry mass of nodules per plant, plant density, number of pods per plant, number of seeds per plant pod or the 1000 seed mass. A significantly greater number of seeds per plant (by 13.2%) was observed after the application of HiStick than in control.

The standard deviations calculated for plant high, plant density, number of pods and seeds per plant, number of seeds per plant pod, the 1000 seed mass were lower for inoculation than in cultivar, which may indicate more stable values.

Mut et al. (2012) assessed the effect of cultivar, inoculation, and sowing date in white and narrow-leaved lupin. In these experiments, seed yield, thousand seed weight, plant height, first pod height, number of pods per plant, pod length, number of seeds per pod were investigated. In terms of these investigated features it was determined that there were differences among varieties. Besides, inoculation with rhizobium increased seed yield and yield components of lupin. In experiment of Tounsi-Hammami et al. (2020), who studied potential of native inoculum to improve the nodulation and growth of white lupin in Tunisia, reported that the rhizobial inoculation led to growth parameters similar to those obtained by applying 90 units of nitrogen ha^{-1}. Several studies have reported an increase in biometrical features others species of legumes by seed inoculation with rhizobia. Ouma et al. (2016) were found that the inoculation with efficient rhizobial strains significantly increased plant height, and shoot and root dry weights compared to uninoculated common bean and soybean plants. In contrast, Samudin and Kuswantoro

(2018) reported that rhizobial inoculation significantly increased the root dry weight of soybean, but did not affect plant height and shoot dry weight. Księżak and Bojarszczuk (2022) were found that soybean plants in cultivation with seed inoculation and different nitrogen fertilization rates were characterized by larger seeds, and set more pods and seeds on the plant than plants without fertilization and inoculation. In this study, the number of nodules as well as their weight were small, although more were recorded on the root system of plants that received the HiStick Soy. In addition, the cv. Annushka set slightly more nodules than the cv. Aldana.

Table 3. Means of agronomical characteristics and yield components measured in the experiment

Yield component	PH	DMN	PD	NP	NS	NSP	MTS
				Cultivar			
SD	8.91	0.07	2.30	3.03	10.10	0.34	14.45
Bolero	54.2	1.0	74.0	8.5	34.2	4.0	179.0
Tytan	56.6	1.1	74.0	10.4	38.1	3.7	168.2
LSD value	1.60**	0.03*	ns	0.44**	1.03**	0.26**	2.01**
				Inoculant			
SD	1.03	0.09	1.60	0.54	1.75	0.19	1.30
Control	56.4	1.2	73.1	9.5	34.2	3.6	174.5
Nitragina	56.2	1.0	75.6	9.4	36.7	3.9	174.9
Nitroflora I	55.8	1.0	73.8	8.8	34.8	4.0	171.5
Nitroflora II	54.1	1.0	75.6	9.3	36.4	3.9	173.7
HiStick	54.5	1.1	71.9	10.3	38.7	3.8	173.4
LSD value	ns	ns	ns	ns	2.65*	ns	ns

ns: not significant; * $p < 0.05$ and ** $p < 0.01$. SD: standard deviation. 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 4 shows the chemical composition of the narrow-leaved lupin isolates reported on the dry matter basis. In the narrow-leaved lupin seeds, the organic components and ash content were not affected by the cultivar, or by the interaction between the cultivar and inoculant. The aim of Panasiewicz's (2022) research was to determine the effect of the cultivar and tillage system on the chemical composition of three lupin species seeds. The results of her study were similar to our and the organic components and ash in narrow-leaved lupin seeds did not depend on the variety of this species. The lowest crude protein content was observed in narrow-leaved lupin seeds, indeterminate 'Dalbor' (323 g kg^{-1} DM) and determinate 'Regent' (327 g kg^{-1} DM).

In our research a significant effect of the inoculant ($p < 0.05$) was observed on the protein content and on the N-free extract content. These two components are usually in high correlation: an increase of the former causes a decrease of the latter and vice versa (Faligowska et al., 2017). In a study of Faluyi et al. (2000) an increase of the carbohydrate level was accounted of decrease in protein of white lupin seeds.

Our analysis of mean showed greater content of crude protein was found after the application of Nitroflora I (by 12.4 g kg^{-1}) and HiStick (by 19.1 g kg^{-1}). Moreover, for HiStick treatment lower N-free extract content was recorded by 21.2 g kg^{-1} in contrast to the control seeds. The average standard deviation calculated for crude protein and crude fiber were lower for the inoculant, which may indicate more stable values. Zetochová et al. (2020) studied the influence of the inoculant on the content of biogenic elements in ten varieties of white lupine (*Lupinus albus*) and three varieties of grass pea (*Lathyrus sativus* L.). In conclusion, inoculation did not significantly affect the chemical composition of selected legume species. In study of Księżak and Bojaszczuk (2022), Nitragina or HiStick Soy and fertilization with mineral nitrogen increased the content of protein and fiber in seeds of both soybean cultivars, as well as reduced the amount of ash and fat. The seeds of cv. Aldana had a higher amount of protein and ash than cv. Annushka, but a similar amount of fat and fiber. Differences in the protein content in seeds of both cultivars were found also in experimet of Prusiński et al. (2020). The highest protein content in seeds of cv. Annushka was recorded after the use of HiStick + 60 kg N ha^{-1}. The protein content in seeds of the cv. Aldana was significantly higher after the application of HiStick without N as well as with both rates of N (30 and 60 kg N ha^{-1}).

Table 4. Means of the chemical content of seeds (g kg^{-1} DM)

Yield component	Crude protein	Crude lipids	Crude fiber	Crude ash	N-free extract
			Cultivar		
SD	159.72	167.21	28.63	22.63	58.23
Bolero	340.5	59.3	158.1	41.7	400.4
Tytan	340.0	57.5	165.3	42.1	395.1
LSD value	ns	ns	ns	ns	ns
			Inoculant		
SD	156.60	183.87	25.20	47.60	83.06
Control	332.0	58.0	164.0	41.6	404.3
Nitragina	334.6	59.1	165.2	41.8	399.3
Nitroflora I	344.4	55.6	164.1	42.2	393.7
Nitroflora II	338.8	59.5	151.5	41.7	408.5
HiStick	351.1	60.0	163.5	42.0	383.1
LSD value	11.97*	ns	ns	ns	20.19*

ns: not significant; * $p < 0.05$ and ** $p < 0.01$. SD: standard deviation. DM: dry matter.

The seeds yield and protein yield were different between years (Table 5), the lowest were recorded in 2018 and the highest in 2017. However, the statistical evaluation showed a significant differentiation only for Tytan cultivar, namely in compare to 2017 the seeds yield was lower in 2018 by 2.47 t ha^{-1} and the protein yield by 715.3 kg ha^{-1}. The relationship between years in protein yield was similar to those in seed yield, because protein yield was calculated as the amount of protein in seed yield. The interaction between years of experiment and inoculation was not significant. Two of three years of experiment was dry (2018 and 2019) and drought stress significantly reduced nodulation (Lumactud et al., 2023). Legumes are very sensitive to weather conditions during vegetation period, especially to water availability. Rainfall deficit combined with high temperatures is particularly unfavourable during flowering and pod formation, resulting in shedding of flowers and pod setting, and as a consequence reducing the seed yield (Atkins and Smith, 2004). According to Mut et al. (2012) annual environmental conditions that changed according to the years may have affected yield and some of the yield components in the white and narrow-leaved lupin cultivars. The correlation analysis showed a significant relationship between the sum of rainfall in June–August and the seed yield of soybean: with the increase in rainfall by 0.36 mm, the yield increased by 0.012 t ha^{-1} (Prusiński et al., 2020). The favorable distribution of rainfall in 2018 significantly affected the growth and development of soybean, and the plants were characterized by a more favorable structure, which also resulted in a higher yield level (Księżak and Bojarszczuk, 2022). In our study the most favorable vegetation period was in 2017, when May and June were relatively wet (precipitation in shedding of flowers and pod setting 79.6 and 139.4 mm, respectively), which was enough to obtain the highest yields, especially in 'Tytan'.

Table 5. Means of cultivar of seeds yield (t ha^{-1}) and protein yield (kg ha^{-1}) in years of research

Cultivar	Year		
	2017	**2018**	**2019**
		Seeds Yield	
Bolero	2.60	1.57	2.42
Tytan	3.60	1.13	2.47
LSD value		1.815**	
		Protein Yield	
Bolero	751	455	700
Tytan	1041	326	713
LSD value		529.5**	

LSD value; ** $p < 0.01$.

In the seeds yield and protein yield differences both experimental factors and their interaction were observed (Table 6). The highest values of both yields were found for Tytan and significantly lower for Bolero by 0.20 t ha^{-1} in seeds yield and 58 kg ha^{-1} in protein yield. The noted interaction resulted from the fact that the inoculant differentiated the values of these features only in Tytan cultivar. In compare to control the significantly greater variation of seeds yield was found with HiStick (the increase by 0.28 t ha^{-1}) and protein yield with Nitroflora I and HiStick (the increase by 88 kg ha^{-1} and 122 kg ha^{-1}, respectively). The statistical evaluation showed that the inoculant significantly modified the protein yield, the greatest value on average was recorded after the application of HiStick.

The results the analysis of variance of experiments with white and narrow-leaved lupin showed that the effects of cultivars and its combinations on seeds yield can be significant (Mut et al., 2012). The average of all treatments, the highest seeds yield was obtained with the local genotype within each year and the lowest yield was obtained with the ‘Boruta’ cultivar of narrow-leaved lupin. In the cultivars tested, the inoculation of rhizobium showed higher seeds yield. Increased yield with inoculation was 0.86 t ha^{-1} in 2007 and 0.95 t ha^{-1} in 2008. Seed inoculation is an effective way for improving the productivity of legumes (Murtaza et al., 2014). The results showed that seed inoculation with *Rhizobium japonicum* and *Pseudomonas fluorescens* improved the seeds yield of soybean, as compared to un-inoculated control. The genotypes did not differ significantly in soybean seeds yield (Yousaf et al., 2018). Księżak and Bojaszczuk (2022) were found that the inoculation of soybean seeds with *Bradyrhizobium japonicum* increased the yield of soybeans. In addition, the protein yield compared to the control and inoculation of HiStick Soy favored a better yield than Nitragina. The results of Prusiński’s et al. (2020) showed that cultivar of soybean ‘Annushka’ demonstrated a higher yield of seeds and protein than ‘Aldana’. Both cultivars responded with an increase in the seed yield after seed inoculation with HiStick, also with an application of 30 and 60 kg N, as well as with Nitragina with 60 kg N. According to Zveushe et al. (2023), the efficiency of biological nitrogen fixationdepends on factors, such as bacterial species and strain, crop cultivar, symbiosis specificity, and prevailing environment (Campo et al., 2009; de Borja Reis et al., 2021). 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 (Hungria and Vargas, 2000).

Table 6. Means of cultivar and inoculation of yield of protein (kg ha^{-1}) and seeds (t ha^{-1})

Cultivar (C)	Inoculant (I)					Mean
	Control	Nitragina	Nitroflora I	Nitroflora II	HiStick	
	Seeds Yield					
Bolero	2.28	2.07	2.10	2.33	2.20	2.20
Tytan	2.25	2.46	2.47	2.28	2.53	2.40
Mean	2.27	2.26	2.28	2.31	2.37	-
LSD value	C – 0.138**; I – ns; C × I – 0.258*					
	Protein Yield					
Bolero	643	591	616	673	654	635
Tytan	635	697	723	656	757	693
Mean	639	644	669	664	706	-
LSD value	C – 37.47**; I – 55.33*; C × I – 78.25*					

ns: not significant; * $p < 0.05$ and ** $p < 0.01$.

The indicating effect and indirect effect via other traits on seed yield by using path analysis is prerequisite (Ton et al., 2021). Relations between yield and number of seeds and pods per plant in ‘Tytan’ cultivar (r>0.6) in each of inoculation treatments were stronger than in ‘Bolero’, where in control without any inoculation treatment were found poor relations (r=0.35, 0.47, respectively). The strongest (practically functional) relations between yield and number of seeds and pods per plant were found in Nitragina inoculation treatment both in ‘Tytan’ (r=0.97, 0.95, respectively) and ‘Bolero’ (r=0.76, 0.87, respectively). Strong relations were also found between number of pods and seeds per plant in both cultivars regardless of the inoculation variant, however the strongest relations (r>0.92) in both cultivar were proved on HiStick treatment (Figure 1). In exeperiment with narrow-leaved lupin (the indeterminate cv. Kalif and the determinate cv. Regent) functional relationships were observed between the number of pods/plant and the number of seeds/plant, and seeds yield and protein yield (Faligowska et al., 2017). Significant relattionships were also found in study with soybean, where ‘Aldana’, due to a significant decrease in plant density, produced a higher number of pods, seeds per pod and the 1000 seed weight per plant (Prusiński et al., 2020). The others results have shown that the inoculation can also modified the chemical composition of soybean seeds (Szpunar-Krok et al., 2021; Szpunar-Krok and Wondołowska-Grabowska, 2022).

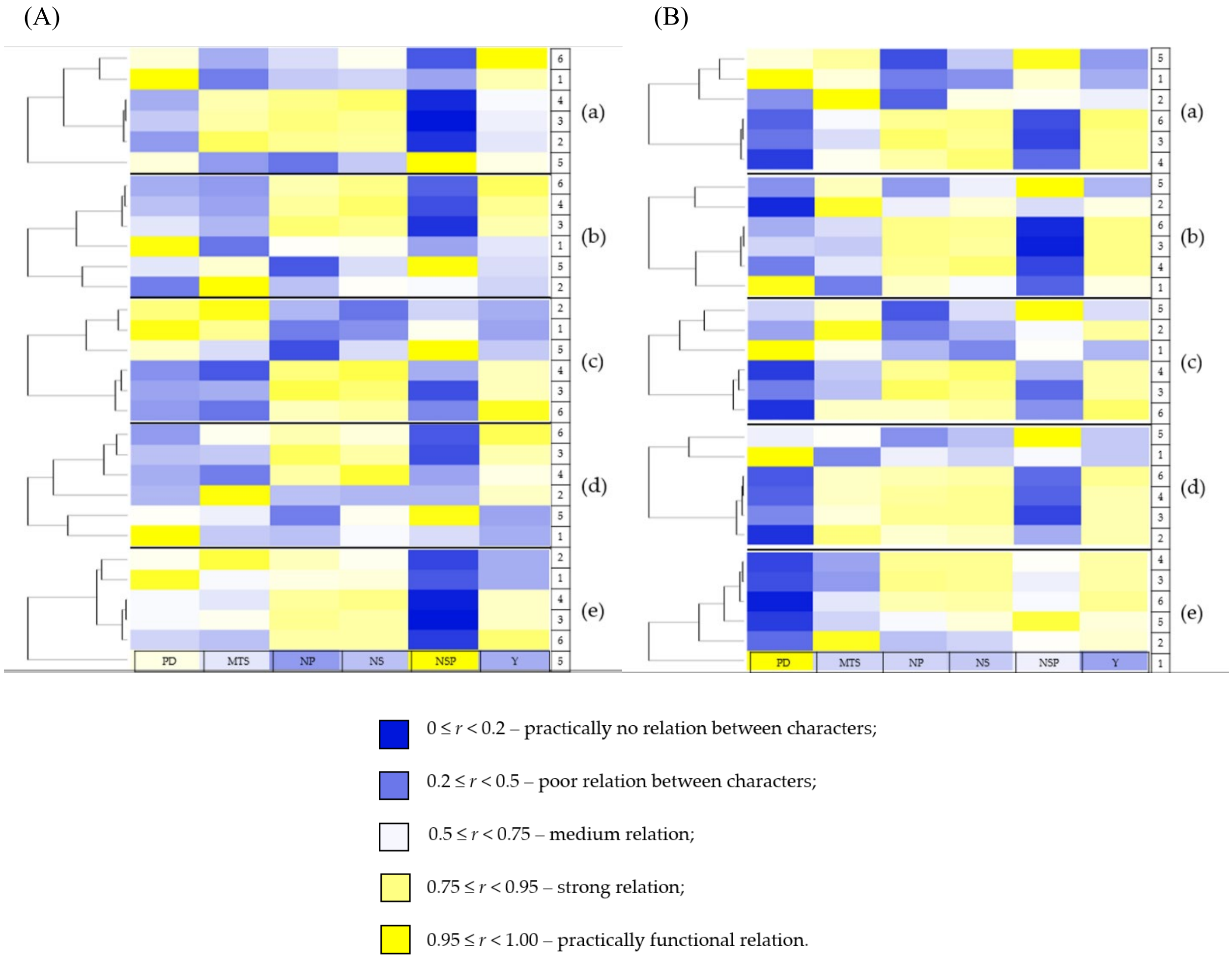

Figure 1. Correlation coefficients between features analyzed for cultivar 'Bolero' (A) and 'Tytan' (B) depending on inoculation treatment: control (a), Nitragina (b), Nitroflora I (c), Nitroflora II (d), HiStick (e). Features compared: PD, plant density (no. m^2); MTS, mass of 1000 seeds (g); NP, number of pods per plant; NS, number of seeds per plant; NSP, number of seeds per plant pod; Y, seed yield (t ha^{-1}). Interpretation of Pearson's linear correlation coefficient: $0 \leq p < 0.2$—practically no relation between characters; $0.2 \leq p < 0.5$—poor relation between characters; $0.5 \leq p < 0.75$—medium relation; $0.75 \leq p < 0.95$—strong relation; $0.95 \leq p < 1.00$—practically functional relation.

CONCLUSIONS

The thermal and humidity conditions had the direct impact on yielding of narrow-leaved lupin, especially for Tytan cultivar. A significant decrease in the yields over the year with rainfall deficit shows that soil moisture is an essential factor to obtain high protein yield and seeds yield. In our study, the relationships between experimenta factors in protein yield were similar to those in seed yield, because protein yield was calculated as the amount of protein in seed yield.

The inoculation with Nitroflora I or HiStick increased the content of crude protein in seeds. In addition, the effect of inoculation depended on cultivar. The protein yield was significantly greater after inoculation of Nitroflora I or HiStick and the seeds yield with HiStick but only in Tytan cultivar. In comapre to 'Bolero', 'Tytan' had also greater plant height, dry mass of nodules, number of pods and seeds per plant. Correlation coefficients were found that, the relations between yield and number of seeds and pods per plant in 'Tytan' cultivar in each of inoculation treatments were stronger than in 'Bolero'. In conclusion, seed inoculation with *Bradyrhizobium sp. Lupinus* improved yield (HiStick seed yield by 0.28 t ha^{-1} and protein yield 122 kg ha^{-1}). and quality of narrow-leaved lupin seed (Nitroflora I increased crude protein content by 12.4 g kg^{-1} and HiStick by 19.1 g kg^{-1}). over un-inoculated and could be used for improving the crop yield and quality of produce.

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