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REACTION OF WHITE LUPINE (*Lupinus albus* L.) TO SEED INOCULATION WITH NITRAGINA

Wacław Jarecki, Dorota Bobrecka-Jamro
University of Rzeszów

Abstract. In the years 2009-2011 field experiment was carried out, whose aim was determination of reaction of two white lupine cultivars to bacterial inoculation, Nitragina. It was found that the applied inoculation prolonged stages of budding and flowering by two days and the stage of maturity (technical and full) by three days compared with the control (without Nitragina). Under the effect of Nitragina, the number of pods per plant increased significantly as well as the number of seeds per pod, however the weight of 1000 seeds did not change. The result of the use of bacterial inoculation was a significant increase in the seed yield by 0.29 $Mg \cdot ha^{-1}$, i.e. 9% compared with the control. Yields of both cultivars were high, on average 3.38 $Mg \cdot ha^{-1}$ in the years of research. Protein content in the dry weight of seeds increased by 2.7% after inoculation of seeds with bacteria, compared with seeds from the control. In the seeds of both cultivars total protein concentration reached a statistically similar level.

Key words: Nitragina, *Rhizobium*, white lupine cultivars, yield, yield components

INTRODUCTION

Leguminous plants cover approximately 1% of cropping system in Poland, however this is diversified in particular voivodeships. Decrease in production observed in recent years concerned mainly edible seeds [Jarecki and Bobrecka-Jamro 2010, Księżak *et al*. 2009]. Many national species of leguminous plants now have little economical significance, though they are characterized by a great yield-forming potential. One of them is white lupine. Under favourable habitat conditions, its yield is over 3 $Mg \cdot ha^{-1}$ [Prusiński 2002, Borowska and Prusiński 2005, Księżak and Kawalec 2006], with a satisfactory concentration of protein [Prusiński 2001, Wiatr *et al.* 2007] and mineral components in seeds [Krejpcio *et al*. 2007]. Lampart-Szczapa and Łoza [2007] classify lupine seeds as ‘functional food’, while in some people its protein may cause allergic reactions. The reasons of little interest in the discussed species can be found in late maturation, competitiveness of field bean [Prusiński *et al.* 2000, Podleśny 2006, 2007,

Corresponding author – Adres do korespondencji: dr inż. Wacław Jarecki, Department of Plant Production of the University of Rzeszów, Zelwerowicza 4, 35-601 Rzeszów, e-mail: waclaw.jarecki@wp.pl

Prusiński 2007] as well as little national requirement for the seeds. On the list of the Research Center for Cultivar Testing (COBORU), there are two registered cultivars of white lupine, Boros and Butan, which differ in the morphological structure. Borowska and Prusiński [2005] state that the use of the biological potential by white lupine depends on the complex effect of many factors. Among the most important they included: the course of weather conditions, hormonal balance and plant nutrition. In lupine, similarly to other leguminous plants, major role is also played by Rhizobium bacteria fixing nitrogen [Martynik and Oroń 2008, Carranca *et al*. 2009].

The aim of the research was determination of the reaction of two white lupine cultivars to bacterial inoculation, Nitragina. In the research hypothesis it was assumed that inoculation of white lupine seeds, will favourably affect the height and quality of the seed yield, and cultivar reaction to this treatment may be diverse with regard to the fact that they represent a different morphological type: cultivar Butan is indeterminate, while Boros determinate.

MATERIAL AND METHODS

Field experiments with white lupine were carried out in the years 2009-2011 at the Experimental Station of the Faculty of Biology and Agriculture of the University of Rzeszów in Krasne (50°03’ N; 22°06’ E) near Rzeszów. The experiment was realized according to the split-plot design in four replications. The studied factors included:

I – seed inoculation (without Nitragina, with Nitragina),

II – cultivars (Boros and Butan).

Weather conditions are given according to Weather Reports for Rzeszów from the Institute of Meteorology and Water Management (IMiGW) in Warsaw. In the research years, the rainfall total was unstable. In the period April-August it was: 372.5 mm in 2009, 651.8 mm in 2010 and 450.0 in 2011. Mean temperatures in the same period were less diversified (Table 1).

Table 1. Weather conditions in the growing period of white lupine in the years 2009-2011
Tabela 1. Warunki pogodowe w okresie wegetacji łubinu białego w latach 2009-2011

Month – Miesiąc	Rainfall total – Suma opadów, mm				Mean temperature – Średnia temperatura, °C			
	2009	2010	2011	1986-2008	2009	2010	2011	1986-2008
April – kwiecień	3.7	49.9	50.0	50.6	11.1	8.9	10.3	8.7
May – maj	102.6	177.0	49.2	80.8	13.8	14.3	13.9	13.9
June – czerwiec	146.4	126.1	88.5	82.0	16.6	17.9	18.1	17.0
July – lipiec	98.0	200.2	233.7	88.2	20.7	20.8	18.6	19.0
August – sierpień	21.8	98.6	28.6	68.8	19.4	19.5	19.0	18.2

The experiment was set up on the soil of good wheat complex, bonitation class IIIa, with pH in the range from 5.10 (2009) to 5.94 (2010). The content of assimilable phosphorus, potassium and microelements was average, however magnesium content was very low (Table 2). Soil samples were collected to a depth of 20 cm. Soil analysis was carried out at the Regional Chemical-Agricultural Station in Rzeszow, according to the accepted methods.

Table 2. Reaction and chemical properties of soil
Tabela 2. Odczyn i właściwości chemiczne gleby

Year Rok	pH in – w KCl	Assimilable – Przyswajalne							
		macroelements – makroskładniki $mg \cdot kg^{-1}$ soil – gleby			microelements – mikroskładniki $mg \cdot kg^{-1}$ soil – gleby				
		P	K	Mg	Cu	Mn	Zn	Fe	B
2009	5.10	61.5	141.1	21	4.1	188.0	5.3	1210	1.55
2010	5.94	48.8	107.9	19	4.5	139.5	4.8	965	1.50
2011	5.67	64.5	120.4	21	3.2	143.2	5.0	1440	1.50

Seed material was dressed with Sarfun T 450 FS and was sown on the following dates: 16 April 2009, 13 April 2010 and 05 April 2011. Bacterial inoculation came from the Institute of Soil Science and Plant Cultivation – State Research Institute (IUNG-BIP), Puławy. It was applied wet, as a dose per area unit, just before sowing the seeds. The amount of sown seeds was 110 seeds$\cdot m^{-2}$ for cultivar Boros, and 100 seeds$\cdot m^{-2}$ for cultivar Butan. Winter wheat was the forecrop of white lupine in every year of research. The area of a plot was 15 m^2 (for harvest 12 m^2). Mineral phosphoric-potassic fertilization (triple superphosphate 46% and potassium salt 60%) was applied under autumn ploughing at the following rates: 35 P kg$\cdot ha^{-1}$, 100 K kg$\cdot ha^{-1}$. Nitrogen fertilization was not applied. Directly after sowing lupine, preparation Afalon Dyspersyjny 450 SC was used for weed control at a rate of 1.5 $dm^3 \cdot ha^{-1}$. Pesticides for the control of pests and diseases were not applied.

Every year, on the area of 1 m^2 plant density of white lupine was calculated in the stage of emergence and before its harvest. In the vegetation period, occurrence of more important development stages of plants was observed, i.e. emergence, budding (full), flowering (beginning and end) and maturity (technical and full). Lodging was evaluated in the scale from 1^o to 9^o. In the stage of full seed maturity, from each plot, 20 plants were collected at random, and their yield components were determined: the number of pods per plant, number of seeds per pod, and weight of one thousand seeds (with 15% moisture).

The harvest was conducted in a single stage on the following dates: 20 August 2009, 24 August 2010 and 19 August 2011. During harvest, from each plot, seed yield was determined calculating it per area of 1 ha, and leading it to a constant moisture of 15%. In the seeds the total nitrogen content was determined with the use of Kjeldahl method, and it was calculated into total protein with the use of a multiplier 6.25.

Obtained results for the yield, yield structure and protein content were elaborated statistically with the use of analysis of variance. Significance of difference between trait values were tested based on Tukey's honestly significant difference test, with significance level P = 0.05. Statistical program ANALWAR–5FR was used for calculations.

RESULTS AND DISCUSSION

It was found that plant losses during vegetation were smaller than on plots with Nitragina, compared with the control. Plant density after emergence was on average 103.5 plants$\cdot m^{-2}$ in cultivar Boros and 98 plants$\cdot m^{-2}$ in cultivar Butan (Table 3). Similar results concerning mean plant losses in white lupine were obtained by Podleśny [2006, 2007]. Prusiński [2002] adds that, with morphological diversity of white lupine cultivars, it is important to maintain a suitable plant density. It has a fundamental significance for the yield and cultivation costs.

Table 3. Plant density, plant losses during growth, lodging degree
Tabela 3. Obsada roślin, ubytki roślin podczas wegetacji, stopień wylegania

Seed inoculation Szczepienie nasion	Cultivar Odmiana	Number of plants after emergence, plants·m^{-2} Liczba roślin po wschodach, szt.·m^{-2}	Number of plants before harvest, plants·m^{-2} Liczba roślin przed zbiorem, szt.·m^{-2}	Plant losses during growth Ubytki roślin podczas wegetacji %	Lodging Wyleganie 1-9°
Control (without Nitragina) kontrola (bez Nitraginy)	Boros	102	85	16.7	9
	Butan	97	80	17.5	8
Nitragina	Boros	105	92	12.4	9
	Butan	99	85	14.1	7

Lodging of the canopy was observed on the plots with cultivar Butan, especially after the application of Nitragina. This phenomenon did not occur in cultivar Boros (Table 3). Podleśny [2006] did not observe lodging of white lupine plants, which was affected by a favourable distribution of weather conditions in the period of vegetation.

Plant emergence was observed on average after 14 days from the date of sowing, with oscillation from 12 days in 2011 to 16 days in 2009. According to Prusiński [2002], under unfavourable weather conditions this stage may extend even up to 30 days. The following development stages occurred earlier in cultivar Boros than in cultivar Butan (Table 4).

Table 4. Length of development stages of white lupine in days since the date of sowing
Tabela 4. Długość faz rozwojowych łubinu białego w dniach od daty siewu

Seed inoculation Szczepienie nasion	Cultivar Odmiana	Emergence Wschody	Full budding Pąkowanie Pełnia	Flowering – Kwitnienie		Maturity – Dojrzałość	
				beginning początek	end koniec	technical techniczna	full pełna
Control (without Nitragina) kontrola (bez Nitraginy)	Boros	14	51	59	79	121	129
	Butan	14	53	61	81	123	131
Nitragina	Boros	14	53	62	82	125	133
	Butan	14	55	64	84	127	135

The applied bacterial inoculation prolonged budding, flowering and maturation (the plants stayed green for a longer time). Vegetation period was on average 132 days, and was shorter than in the research of Prusiński [2002], in which it lasted from 145 to 157 days. Podleśny [2006] collected white lupine seeds after 115 days in the dry year and after 138 days in the wet year.

Inoculation of seeds with Nitragina resulted in the increase in the number of pods per plant and the number of seeds per pod, compared with the control (Table 5). Cultivar Butan developed on average 6.3 pods per plant, however cultivar Boros developed significantly less than that, on average by 2.1 pods. Number of seeds per pod was in cultivar Boros 3.8 seeds, and in cultivar Butan 3.4 seeds. These differences were in the range of statistical error. Podleśny [2007] observed a greater number of seeds per pod in determinate cultivar (Katon) compared with the indeterminate one (Bardo). Borowska and Prusiński [2005] obtained on average 8.77 pods in cultivar Butan; while Podleśny [2006] 6.9 pods per plant. In experiments on indeterminate white lupine, Prusiński [2005] considered it important to take into account yield components on the main shoot and lateral shoot, as the proportion of pods and seeds from lateral shoots in the total yield of this species is approximately 50%.

Table 5. Yield components of white lupine and total protein content in seeds
Tabela 5. Komponenty plonu łubinu białego i zawartość białka ogólnego w nasionach

Seed inoculation Szczepienie nasion (I)	Cultivar Odmiana (II)	Number of pods per plant Liczba strąków na roślinie	Number of seeds per pod Liczba nasion w strąku	Weight of one thousand seeds, g Masa tysiąca nasion, g	Total protein Białko ogólne %
Control (without Nitragina) kontrola (bez Nitraginy)	Boros	3.8	3.6	303.5	32.2
	Butan	5.6	3.2	255.4	33.5
Nitragina	Boros	4.6	4.0	308.5	34.8
	Butan	7.0	3.6	257.0	36.1
$LSD_{0.05}$ – $NIR_{0,05}$ for – dla:					
I x II		ns – ni	ns – ni	ns – ni	ns – ni
I x II x years – lata			ns – ni		
Control Nitragina	–	4.7	3.4	279.5	32.8
kontrola Nitragina		5.8	3.8	282.8	35.5
$LSD_{0.05}$ – $NIR_{0,05}$ for – dla:					
I		0,652	0.382	ns – ni	2.589
I x years – lata			ns – ni		
–	Boros	4.2	3.8	306.0	33.5
	Butan	6.3	3.4	256.2	34.8
$LSD_{0.05}$ – $NIR_{0,05}$ for – dla:					
II		0.532	ns – ni	27.15	ns – ni
II x years – lata			ns – ni		
Mean – Średnia		5.25	3.6	281.1	34.2

ns – ni – non-significant differences – różnica nieistotna

Nitragine did not have a significant effect on the seed plumpness. Weight of one thousand seeds was on average 281.1 g, and was significantly higher in cultivar Boros compared with cv. Butan (Table 5). In the research of Podleśny [2007], mean weight of 1000 seeds of particular cultivars was diverse and statistically greater in the indeterminate cultivar. Borowska and Prusiński [2005] obtained weight of 1000 seeds in cultivar Butan of 288 g.

Analysis of variance proved a significantly beneficial effect of Nitragina on the total protein content in the dry weight of seeds (Table 5). However, cultivar diversity was not observed in the content of the studied component in seeds. Wiatr *et al.* [2007] state that cultivar Butan is characterized by a high total protein content in the range 31.8-36.6% of dry weight of seeds.

Statistical analysis of the research results proved that Nitragina contributes to a significant increase in the seed yield. With reference to the control plots, the obtained increase was 0.29 $Mg \cdot ha^{-1}$, i.e. 9% (Table 6). Cultivars Boros and Butan yielded on a statistically similar level, 3.32 and 3.45 $Mg \cdot ha^{-1}$ respectively. However, in his research Prusiński [2002] proved a significant diversity in the yield of line R-525 (2.39 $Mg \cdot ha^{-1}$) and cultivar Bardo (3.17 $Mg \cdot ha^{-1}$).

Table 6. Yield of white lupine seeds, $Mg \cdot ha^{-1}$
Tabela 6. Plony nasion łubinu białego, $Mg \cdot ha^{-1}$

Seed inoculation Szczepienie nasion (I)	Cultivar Odmiana (II)	Year of research – Rok badań			Means from the years Średnie z lat 2009-2011
		2009	2010	2011	
Control (without Nitragina) Kontrola (bez Nitraginy)	Boros	3.34	2.90	3.12	3.12
	Butan	3.75	3.07	3.18	3.33
Nitragina	Boros	4.02	3.15	3.35	3.51
	Butan	4.09	3.20	3.38	3.56
$LSD_{0.05}$ – $NIR_{0,05}$ for – dla:					
I x II		0.324	ns – ni	ns – ni	ns – ni
I x II x years – lata				ns – ni	
Control Nitragina	–	3.54	2.99	3.15	3.23
Kontrola Nitragina		4.02	3.18	3.37	3.52
$LSD_{0.05}$ – $NIR_{0,05}$ for – dla:					
I		0.354	ns – ni	0.203	0.286
I x years – lata				ns – ni	
–	Boros	3.68	3.03	3.24	3.32
	Butan	3.92	3.14	3.28	3.45
$LSD_{0.05}$ – $NIR_{0,05}$ for – dla:					
II		0.218	ns – ni	ns – ni	ns – ni
II x years – lata				ns – ni	
Mean – Średnia		3.81	3.09	3.26	3.38

ns – ni – non-significant differences – różnica nieistotna

CONCLUSIONS

1. Bacterial inoculation: Nitragina significantly increased the number of pods per plant and number of seeds per pod compared with the control (without inoculation). Weight of 1000 seeds was not varied by this factor.
2. Inoculation of the seed material of white lupine with Nitragina significantly affected the increase of the seed yield and the total protein content in the seeds compared with the control.
3. Cultivar Butan was developing a significantly higher number of pods per plant, while it had a lower weight of one thousand seeds compared with cultivar Boros.

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REAKCJA ŁUBINU BIAŁEGO (*Lupinus albus* L.) NA SZCZEPIENIE MATERIAŁU SIEWNEGO NITRAGINĄ

Streszczenie. W latach 2009-2011 przeprowadzono ścisłe doświadczenie polowe, którego celem było określenie reakcji dwóch odmian łubinu białego na szczepionkę bakteryjną – Nitraginę. Stwierdzono, że zastosowana szczepionka wydłużyła fazę pąkowania i kwitnienia o dwa dni oraz fazę dojrzewania (techniczną i pełną) o trzy dni w porównaniu z obiektem kontrolnym. Pod wpływem Nitraginy istotnie zwiększała się liczba strąków na roślinie i liczba nasion w strąku, natomiast nie uległa zmianie masa tysiąca nasion. Efektem zastosowania szczepionki bakteryjnej był istotny wzrost plonu nasion o 0,29 $Mg \cdot ha^{-1}$, tj. 9% w porównaniu z obiektem kontrolnym. Plony obu odmian były wysokie; średnio w latach wynosiły 3,38 $Mg \cdot ha^{-1}$. Zawartość białka w suchej masie nasion wzrosła o 2,7% po inokulacji nasion siewnych bakteriami w stosunku do nasion z obiektu kontrolnego. W nasionach obu odmian koncentracja białka ogólnego kształtowała się na zbliżonym statystycznie poziomie.

Słowa kluczowe: bakterie brodawkowe, komponenty plonu, Nitragina, odmiany łubinu białego, plon

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