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THE EFFECT OF SELECTED STRAINS OF *TRICHODERMA* SPP. ON SEEDS SOWING VALUE AND VIGOUR OF BLUE LUPIN

Summary

Laboratory analyses were performed at the Department of Agronomy, the Poznań University of Life Sciences. Experiments were conducted on two strains of Trichoderma ssp. to investigate the aftereffect of sprinkling irrigation of blue lupin plants and seed soaking time (20, 40, 60 min). Results indicate a significant effect of Trichoderma ssp. strain and sprinkling irrigation on basic parameters of the value and vigour of blue lupin seeds. The greatest values of germinating energy and germination capacity were recorded after the application of the strain T. atroviride. Sprinkling irrigation deteriorated seeds sowing value and seed vigour in blue lupin. Seed soaking time did not modify germination energy or germination capacity, while significant differences were found in the seedling growth test, seedling growth rate test and the vigour index.
***Key words**: Trichoderma ssp., blue lupin, germination capacity, vigour*

WPŁYW WYBRANYCH SZCZEPÓW *TRICHODERMA* SPP. NA WARTOŚĆ SIEWNĄ I WIGOR NASION ŁUBINU WĄSKOLISTNEGO

Streszczenie

Badania laboratoryjne wykonano w Katedrze Agronomii Uniwersytetu Przyrodniczego w Poznaniu. Doświadczenia uwzględniały dwa szczepy Trichoderma ssp., następczy wpływ deszczowania roślin łubinu wąskolistnego oraz czas moczenia nasion (20, 40, 60 min). Uzyskane wyniki wskazują na istotny wpływ szczepu Trichoderma ssp. oraz deszczowania na podstawowe parametry wartości siewnej oraz wigor nasion łubinu wąskolistnego. Najwyższą energię i zdolność kiełkowania odnotowano po zastosowaniu szczepu T. atroviride. Deszczowanie pogarszało jakość siewną i wigor nasion łubinu wąskolistnego. Czas moczenia nasion nie wpływał na energię i zdolność kiełkowania, ale istotne różnice stwierdzono w przypadku testu wzrostu siewki, testu szybkości wzrostu siewki oraz indeksu wigoru.
***Słowa kluczowe**: Trichoderma ssp., łubin wąskolistny, zdolność kiełkowania, wigor*

1. Introduction

For several years now we have been observing increased interest in legume growing both in Poland and in the other EU countries. Nevertheless, in view of the increasing demand for protein the share of this crop group in the cropping structure is still low. In Poland the area cropped to legumes in 2014 was approx. 190 thousand ha, and it was primarily faba bean, peas and lupins. Among the 250 species in the genus *Lupinus* only three, i.e. *L. albus, L. luteus* and *L. angustifolius*, are crop plants well adapted to the soil and climatic conditions of the temperate zone [5]. Blue lupin exhibits a high yielding potential, high thermal tolerance and a shorter vegetation period [15; 1]. This species is a valuable source of protein and may be used as feed in the form of seeds, green forage and silage [3]. Yielding of blue lupin is to a considerable extent affected by weather conditions, particularly precipitation deficit in the flowering and pod setting periods [12]. Under precipitation deficit yielding may be improved by sprinkling irrigation [14]; however, as it is indicated by literature sources, this procedure frequently has a negative effect on germination capacity of produced seeds [2].

Withdrawal of an increasing number of pesticides, but also increased public awareness concerning the requirements of environmental protection, result in farming practice with the reduced application of chemical pesticides as well as increased interest in the use of natural-origin preparations. Commonly known microorganisms, fungi *Trichoderma* spp., are applied increasingly commonly in plant production thanks to their effect enhancing plant growth and development [6; 10]. Improvement of seed quality and vigour under the influence of *Trichoderma* spp. was reported in rice by Febri Doni et al. [4], in maize and beans by Okoth et al. [11], in melon by Kaveh et al. [8] and in tomato by Mastouri et al. [9]. However, literature source report practically no information on the effect of *Trichoderma* ssp. strains on germination and vigour of lupin seeds.

The aim of this study was to determine the effect of selected strains of *Trichoderma* ssp. on seed value and vigour of blue lupin.

2. Material and methods

The laboratory analyses were conducted at the Department of Agronomy, the Poznań University of Life Sciences, on seeds of blue lupin cv. Baron harvested in the years 2011–2012 in the fields of the Experimental Teaching Station in Gorzyń, branch in Złotniki. In each year the trials were performed in the completely randomized, three-factorial design, with 4 replications for each combination. The experimental factors included: 1st order – strains of *Trichoderma* ssp.- S1, S2 and the control (seeds soaked in deionised water), 2nd order – the after-effect of the water variant with seeds coming from the field trial from non-irrigated treatments (the control) and from irrigated treatments, while seed soaking time in strains of *Trichoderma* ssp. was the 3rd order factor (20, 40 and 60 min).

Isolates of *Trichoderma* ssp. came from the collection of strains at the Department of Microbiology, the Institute of Horticulture in Skierniewice. Strain S1 belongs to *T. atroviride*, which was isolated from the soil, while strain S2 of *T. harzianum* was isolated from a mushroom farm. Strains were proliferated at the Department of Environmental Microbiology, where these strains were cultured on a potato starch glucose medium PDA (Sigma) on Petri dishes (90 mm). Incubation was run for 7 days at 25°C, initially in the dark and next exposing the culture to visible light in order to increase sporulation efficiency of these fungi. After the incubation period the Petri dishes were flooded with 10 ml of sterile 0.85% NaCl solution and mycelia were removed from the substrate surface using a sterile scalpel and transferred to sterile flasks. In order to remove conidial spores from mycelial hyphae a hand blender (Braun) was used, while spore density was determined using a hemacytometer. The final spore density of used isolates obtained in 100 ml NaCl solution was 107 ml^{-1}. Strain isolates were applied as spore suspensions.

Seeds of blue lupin collected in each year with moisture contents in the years of harvest of 10.6% and 10.0% were stored under controlled conditions, i.e. 4 °C with no access to light. Each year after 4-month storage respective to the combination selected clean seeds were soaked and placed on filter paper of medium filtration rate for analyses of seeds sowing value. Germination quality was expressed based on the first count (germination energy) and the final count (germination capacity) as well as seed vigour in accordance with ISTA [7]. Vigour was determined in the vigour tests, i.e. the seedling growth test and the seedling growth rate test. In the seedling (shoot) growth test 25 kernels were placed in rolled filter paper in 4 replications. Filter paper sheets were moistened with water and placed in a thermostat at 20°C. After germination was completed, the length of normally germinated seedlings was measured (cm). The seedling growth rate test was performed after the completion of the seedling growth test. Normal seedlings from each roll (without seed fragments) were dried for 24 h at 80°C and next the mass of a single seedling was determined. Additionally, the seed vigour index was calculated as a product of the mean shoot length (cm) and mean germination capacity (%).

Obtained results were analyzed statistically and the smallest significant difference was estimated using the Tukey test at the significance level $\alpha = 0.05$.

3. Results and discussion

The germination phase is considered to be one of the most critical moments in plant development, as seedlings are exposed to numerous stress factors such as damage, disease or environmental stresses [13]. The germination capacity recorded in this study meets the requirements imposed on certified seeds, which for blue lupin is 75%. Statistical analysis of recorded results showed improvement seeds sowing value of blue lupin as a result of the application of fungi *Trichoderma* ssp. The greatest germination energy and germination capacity levels were observed after the application of strain *T. atroviride* (tab. 1). In comparison to the control the increase in seed germination energy after inoculation with *T. atroviride* was 13 percentage points, while for *T. harzianum* it was 3 percentage points. In turn, for germination capacity this increase amounted to 6 and 4 percentage points, respectively. Seed inoculation with *Trichoderma* ssp. had no significant effect on the share of abnormally germinating and dead seeds, we could only observe a trend towards a lower share of these seeds in relation to the control following the application of both tested strains. Improved germination capacity of soy depending on seed inoculation with strains of *Trichoderma* ssp. was also reported by Mukhtar et al. [10], who showed the greatest germination capacity (96%) after the application of *T. harzianum* and *T. hamatum*. In turn, Okoth et al. [11] under the influence of seed inoculation with this fungus showed improved germination capacity of maize seeds, whereas it had no significant effect on seed germination in beans.

Evaluation of seed material quality depending on the water variant showed a significant deterioration both in germination energy and germination capacity under the influence of sprinkling irrigation, with the differences amounting to 5 and 2 percentage points, respectively. Moreover, seeds coming from irrigated treatments were characterized by a greater share of dead seeds. This is confirmed the studies by Faligowska et al. [2], in which sprinkling irrigation reduced seed germination energy and capacity as well as increased the share of healthy non-germinating seeds in blue lupin.

Seed soaking time in strains of *Trichoderma* ssp did not significantly modify seeds sowing value of blue lupin.

Evaluation of seed vigour in blue lupin depending on the strain of *Trichoderma* ssp. also showed a significant ef-

Table 1. Seeds sowing value of blue lupine depending on strain of *Trichderma* ssp., sprinkling irrigation and soaking time
Tabela 1. Wartość siewna nasion łubinu wąskolistnego w zależności od szczepu Trichderma ssp., następczego wpływu deszczowania oraz czasu moczenia nasion

Factor	Level	Energy capacity [%]	Germination capacity [%]	Abnormally germinated seeds [%]	Rotted seeds [%]
Strain of *Trichderma* ssp.	*T. atroviride*	86	92	3	4
	T. harzianum	76	90	3	4
	control object	73	86	4	5
$LSD_{0.05}$		1.96	1.39	n.s.*	n.s.
Water variant	non irigation	80	90	3	4
	irigation	75	88	3	5
$LSD_{0.05}$		1.30	1.32	n.s.	0.3
Soaking time	20	78	90	3	4
	40	77	88	3	3
	60	77	89	3	4
$LSD_{0.05}$		n.s.	n.s.	n.s.	n.s.*

*n.s. – not significant differences

Source: own study / *Źródło*: opracowanie własne

Table 2. Seed vigour of blue lupin depending on strain of *Trichderma* ssp., sprinkling irrigation and soaking time
Tabela 2. Wigor nasion łubinu wąskolistnego w zależności od szczepu Trichderma ssp., następczego wpływu deszczowania oraz czasu moczenia nasion

Factor	Level	Seedling growth test [cm]	Seedling growth rate test [mg/seedling]	Vigour index	Fresh mass of seedling [mg/seedling]
Strain of *Trichderma* ssp.	*T. atroviride*	10.1	27.6	908	581
	T. harzianum	9.85	27.9	905	532
	control object	9.59	28.8	826	563
LSD $_{0.05}$		0.23	0.84	21.9	39.7
Water variant	non irigation	9.97	29.5	894	585
	irigation	9.74	26.7	865	532
LSD $_{0.05}$		0.11	0.39	15.4	45.9
Soaking time	20	9.60	28.6	865	562
	40	9.89	27.8	875	552
	60	10.1	27.9	899	562
LSD $_{0.05}$		0.18	0.65	27.5	n.s.*

n.s.* – not significant differences

Source: own study / *Źródło*: opracowanie własne

fect of this factor on values of the seedling growth test, the seedling growth rate test, seed vigour and seedling fresh weight.

Both tested strains of *Trichoderma ssp.* in relation to non-inoculated seeds increased in seedling length and the vigour index, while the greatest effects for these traits were recorded after the application of the strain of *T. atroviride* (tab.2). Analysis of seedling dry weight (the seedling growth rate test) in relation to the control treatment showed a lower seedling weight in treatments following the application of fungal isolates, whereas no significant differentiation was found between dry weights of seedlings grown from inoculated seeds. Febri Doni et al. [4] when comparing seven strains of *Trichoderma* ssp. showed seedlings with the greatest mass, length and the vigour index after the application of *Trichoderma* ssp. strain SL2. According to Zheng and Shetty [16], improvement of seed vigour after the application of *Trichoderma* ssp. results from the induction of phenolic compounds during seed germination.

Sprinkling irrigation caused deterioration of seed vigour in blue lupin, as indicated by all parameters investigated in this study. Seedlings grown from seeds from irrigated treatments were by 0.23 cm lower, i.e. by 2.3% than those from non-irrigated treatments. Also dry and fresh seedling weight and the vigour index confirm the negative effect of sprinkling irrigation on seed vigour. Similarly, Faligowska et al. [2] showed a negative effect of sprinkling irrigation on seed vigour in blue lupin.

Seed soaking time had a diverse effect on the results of vigour parameters, since the longest seedlings and the greatest vigour index were recorded after the longest soaking time (60 min), whereas seedlings of greatest weight were obtained after 20-minute seed soaking time.

4. Concluding remarks

Tested strains of *Trichoderma* ssp. significantly modified germination energy and germination capacity of blue lupin seeds. Higher seed material quality was recorded after the application of *T. atroviride*. Seeds of blue lupin coming from irrigated treatments were characterised by inferior seeds sowing value in comparison to non-irrigated treatments. Sprinkling irrigation of plants deteriorated seed vigour of blue lupin.

5. References

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We would like to thank Dr. Magdalena Szczech from the Institute of Horticulture in Skierniewice for her kind permission to use Trichoderma strains.