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HEALTHINESS OF PEA SEEDS VARIETY TARCHALSKA AFTER BACTERIAL INOCULATION

Summary

The aim of the study was to determine the health status of seeds and the quality of seedlings of pea of Tarchalska variety after the use of Nitragina vaccine and a non-commercial bacterial vaccine IUNG. It was observed that in 2010 treatment with vaccines reduced the amount of seeds with pathogenic changes. Fungi belonging to 11 species were isolated from the pea seeds. Alternaria alternata species and Penicillium genus were the most abundant.
***Key words**: Rhizobium, seeds, pea, fungi*

ZDROWOTNOŚĆ NASION GROCHU SIEWNEGO ODMIANY TARCHALSKA PO ZASTOSOWANIU SZCZEPIONEK BAKTERYJNYCH

Streszczenie

Celem pracy było określenie zdrowotności nasion oraz jakości siewek grochu siewnego odmiany Tarchalska po zastosowaniu szczepionki Nitragina oraz niekomercyjnej szczepionki bakteryjnej IUNG. Obserwowano, że w 2010 r. zaprawianie szczepionkami wpłynęło na zmniejszenie ilości nasion ze zmianami patogennymi. Z nasion grochu wyizolowano grzyby należące do 11 gatunków. Najliczniej był reprezentowany gatunek Alternaria alternata oraz rodzaj Penicillium.
***Słowa kluczowe**: Rhizobium, nasiona, groch, grzyby*

1. Introduction

In 2017 the area of leguminous plants sowing for grain in Poland was approx. 272 thousand hectares [1]. The economics of these crops and interest in them increase in relation to the implementation of agri-environmental programs, the development of an integrated and organic production system within the framework of the functioning CAP. Since last year, the new rules for granting the payments for high-protein plants are an additional incentive. A payment was introduced to the area of leguminous plant cultivation for grain, including pea, if the grain was harvested. Pea in moderate climate conditions is a valuable species for sustainable or organic farming due to its high production potential, combined with the versatility of seed use, for food or feed [2]. A significant advantage of peas consists also in the ability of this plant to use atmospheric nitrogen by way of symbiosis with root-nodule bacteria [3].

An important issue in pea cultivation is related to seeds treatment with *Rhizobium* spp. bacteria. These bacteria provide plants with adequate nitrogen supply, increasing the effectiveness of its biological binding [4, 5]. Thus, they affect the growth and development of plants, and thus their yields. It is also observed that vaccines containing symbiotic bacteria may have a positive effect on plant health. This is related to the inhibition of phytopathogenic microorganisms activity and the induction of plant resistance [6-9].

The aim of the study was to determine the health status of seeds and the quality of seedlings of pea of Tarchalska variety after the use of Nitragina vaccine and a non-commercial bacterial vaccine IUNG.

2. Materials and methods

The Tarchalska variety pea seeds used in the study were collected from plants grown in field experiments in the 2010 and 2011 seasons. A close field experiment was carried out at the Bayer Polska® Experimental Station, located in Modzurów (50°09'N 18°07'E), at an altitude of 274 m above sea level, in the province of Silesia (Raciborski poviat) on the soil classified as the second bonitation class and the very good wheat complex. Agro-climatic conditions in the pea vegetation period were variable due to the differentiated amount of rainfall and average air temperature in particular months (Table 1). The year 2010 turned out to be very humid, mainly due to heavy rainfall in May and July, when precipitation totals were 193 and 208 mm, respectively. Lower sums of rainfall were recorded in the following year, although July was relatively wet, because the monthly sum of rainfall was 167.5 mm. Analysis of the course of average monthly temperatures indicates that 2011 was warmer.

Seeds before sowing on the plots were treated with bacterial vaccines: commercial product Nitragina™ by Biofood® and IUNG preparation produced by the Department of Microbiology of the Institute of Cultivation and Fertilization - National Research Institute in Puławy.

In the laboratory experiment, the external appearance, quality and fungal colonization of seeds collected from plants subject to vaccination before sowing were determined. The assessment of the external appearance of seeds was conducted by taking 50 seeds in 4 replicates for each object. Next, with the use of the magnifying glass, symptoms such as insect and mechanical damage as well as pathogenic changes in the form of discoloration and necrosis were observed [10, 11].

The quality of the seeds was tested on Petri dishes with wet filtering paper, laying 100 seeds (20 plates of 5 seeds) in 4 replicates for each object. Incubation was carried out for 8 days at 20°C ± 0.5°C. Then the length of the stems and roots was measured and the seedlings health status was evaluated on a scale of 0-4, in which 0 meant seedlings

without disease changes, and 4 seedlings with more than 50% of the area with lesions. The obtained results were converted into an infestation index [12].

In order to determine fungal colonization, surface disinfected seeds were placed on 9 cm Petri dishes on agar-glucose-potato medium (Biocorp PDA), acidified with lactic acid to pH 4.5-5.0. 25 seeds (5 plates of 5 seeds) were tested for each object in 4 replications. Seeds were surface-sterilized by soaking in 0.6% NaOCl for 3 min with constant agitation, rinsing with sterile distilled water for 2 min, and drying on a sterile distilled paper towel before plating on PDA plates. After 7 days of incubation at 21°C ± 0.5°C, emerged fungal colonies were counted, and transplanted in order to obtain pure cultures. The identification of fungi was carried out on the basis of macro- and microscopic observations using the keys for fungi determination [13-15].

The results were subject to analysis of variance, and the significance was verified by Duncan's test (p=0.05).

3. Results and discussion

An evaluation of the external appearance of the seeds showed that the healthy seeds were significantly the most numerous in the seed samples from both years of the study (Table 2). It was observed that in 2010, treatment with vaccines reduced the amount of seeds with pathogenic changes. It was also noted that the use of bacterial vaccines increased the seed yield. This increase was higher in 2011, when the highest yield was obtained in combination with the use of IUNG treatment. However, yielding due to the applied seed vaccine was not significantly differentiated. In addition to vaccines, the yield of pea seeds was determined by the course of meteorological conditions during plants vegetation. In 2010, with more rainfall seed yields decreased. However, the opposite situation occurred in 2011, which was warmer and less humid, and the yield increased.

Symbiotic bacteria from the genus *Rhizobium* are one of the most important factors affecting the growth and development of legumes. The yielding role of these bacteria results from their ability to supply plants with nitrogen. Symbiotic pea bacteria quite commonly inhabit the soil in Poland. However, if the pea cultivation break in the field was longer than 4-5 years, the seeds should be treated with a bacterial vaccine. The use of vaccine preparations with *Rhizobium* gives positive results in the form of significant increases in pea yields [5, 16].

In the experiment conducted in this study, the vaccines affected an increase in the length of pea stems and roots (Table 3). However, these changes were not significant. The stems of the pea seedlings grown from the examined seeds were healthy. However, in 2010, treatment with Nitragina influenced the increase in the seedlings root infestation index.

Rhizobia species directly affect the growth of plants, producing phytohormones, such as indolylacetic acid [9]. Patten and Glick [17] observed that the inoculation of bean seeds with *Bradyrhizobium* sp. had a positive effect on elongation of internodes and an increase in gibberellins level. They also demonstrated that *Rhizobium leguminosarum* var. *trifolii*, besides gibberellins, also produces indolylacetic acid (IAA), which belongs to the natural hormones from the auxin group.

Table 1. Weather conditions in vegetation seasons
Tab. 1. Warunki pogodowe w sezonach wegetacyjnych

Meteorological components/	Years	III	IV	V	VI	VII	VIII
Monthly average temperatures [°C] in years	2010	4.0	7.5	11.7	16.7	20.4	18.5
	2011	2.0	9.7	13.2	17.4	17.3	18.9
Monthly average precipitation [mm] in years	2010	17.0	66.5	193.2	103.5	208.5	95.1
	2011	31.1	29.2	71.5	99.5	167.5	73.2

Source: own work / Źródło: opracowanie własne

Table 2. External appearance and yield of pea seeds of the Tarchalska cultivars after treatment with bacterial vaccines
Tab. 2. Ocena zewnętrznego wyglądu i plon nasion grochu odmiany Tarchalska po zastosowaniu szczepionek bakteryjnych

Bacterial vaccine for pea seeds of the Tarchalska cultivars	Number of damaged seeds [%] and pea yielding [dt/ha]									
	2010					2011				
	mechanical damage	insect damage	pathogenic changes	healthy	yield of seeds	mechanical damage	insect damage	pathogenic changes	healthy	yield of seeds
Control without vaccine	3,5 abc	1,5 abc	5,0 bcd	89,5 h	31,3	16,5 f	0,0 a	1,3 abc	81,5 g	49,8
Nitragina	8,5 de	5,5 cd	1,0 abc	85,0 gh	32,4	12,5 ef	0,0 a	2,0 abc	85,5 gh	51,8
Vaccine IUNG	4,5 a-d	8,5 de	0,5 ab	87,0 h	31,9	13,5 f	0,0 a	0,0 a	86,5 h	54,7
NIR					NS					NS

Source: own work / Źródło: opracowanie własne

Table 3. Quality of the pea seedlings after treatment with bacterial vaccines
Tab. 3. Ocena jakości siewek grochu odmiany Tarchalska po zastosowaniu szczepionek bakteryjnych

Bacterial vaccine for pea seeds of the Tarchalska cultivars	Root and stem average length [cm] and infestation index [%]							
	2010				2011			
	root length [cm]	root infestation index [%]	stem length [cm]	stem infestation index [%]/	root length [cm]	root infestation index	stem length [cm]	stem infestation index [%]
Control without vaccine	3,7 ab	0,3 a	1,9 ab	0,0 a	5,0 bc	0,0 a	2,4 abc	0,0 a
Nitragina	4,9 abc	1,7 a	2,0 ab	0,0 a	5,9 c	0,0 a	2,8 abc	0,0 a
Vaccine IUNG	4,6 abc	0,1 a	5,5 c	0,0 a	4,9 abc	0,0 a	3,3 abc	0,0 a

Source: own work / Źródło: opracowanie własne

Table 4. Number of colony forming units (CFU) of fungi isolated from pea seeds of the Tarchalska cultivars in the years 2010-2011
Tab. 4. Ilość jednostek tworzących kolonie (jtk) grzybów wyizolowanych z nasion grochu odmiany Tarchalska w latach 2010-2011

Fungus / *Grzyby*	2010				2011			
	Control without vaccine	Nitragina	Vaccine IUNG	Total CFU	Control without vaccine	Nitragina	Vaccine IUNG	Total CFU
Alternaria alternata (Fr.) Keissler	210	100	170	480	176	120	76	372
Ascochyta pisi Libert	7	1	5	13	5	4	2	11
Aspergillus flavus Link	17	8	7	32	2	1	1	4
Botrytis cinerea Pers. Fr.	36	23	26	85	25	10	12	47
Fusarium equiseti (Corda) Sacc.	6	3	3	12	10	5	5	20
Mucor spp.	16	10	10	36	14	0	12	26
Penicillium spp.	120	48	30	198	69	46	20	135
Phoma exigua Desm.	4	2	2	8	2	0	1	3
Rhizoctonia solani Kühn	6	2	3	11	4	0	0	4
Sclerotinia sclerotiorum (Lib.) de Bary	2	0	0	2	0	1	0	1
Trichoderma viride Pers.	1	1	0	2	2	1	1	4
Total CFU	425	198	256	879	309	188	130	627

Source: own work / Źródło: opracowanie własne

IAA secreted by root-nodule bacteria influences the action of plant auxins during root growth, stimulation of division and elongation of plant cells [18]. Seed treatment and soil inoculation with *Rhizobium* bacteria stimulate seed germination and plant growth [19]. The positive effect of vaccines containing in their composition bacteria binding atmospheric nitrogen also involves the inhibition of the effects of phytopathogenic microorganisms and the creation of systemic immunity of plants [6, 8, 20-22]. Biocontrol mechanisms of *Rhizobia* may involve antibiotics, HCN and siderofores. *Rhizobia* also appear to influence the plant defence mechanism by stimulating the production of phytoalexins by plants [6, 9]. The beneficial effects of root-nodule bacteria may be disrupted, since their survival on leguminous plants seeds decreases over time from the use of the vaccine. *Rhizobium* bacteria are very sensitive to drying [5].

In total 1506 CFU (colony forming units) of fungi belonging to 11 species were isolated from the pea seeds in the presented experiment, in both years of the study (Table 4). *Alternaria alternata* species was the most abundant: 852 CFU (56.7% of the total number of fungi), and the number of 333 CFU (22% of all fungi) was found for *Penicillium* genus. The pathogenic species: *A. pisi, B. cinerea, F. equiseti, P. exigua, R. solani* and *S. sclerotiorum* were observed on the seeds. These species were not numerous and accounted for 14.4% of total isolates. Higher number of CFU was obtained in 2010. Fewer fungi were isolated in the fields where seed bacterial vaccines were used.

Studies on fungi occurrence on pea seeds confirm that the most isolated species are: *A. alternata* and *Penicillium* spp. [23-26]. *Ascochyta* spp., *Botrytis cinerea*, *Cladosporium* spp., and *Rhizoctonia solani* are also often observed [25, 27, 28]. It is pointed that the frequency of fungi occurrence depends not only on their species, but to a large extent on the ambient conditions, which include weather conditions of the cultivated field. In the dry and hot season, Marcinkowska [23] did not find fungi from the genera *Mycospharella, Phoma* and *Ascochyta* causing ascochyta blight on the pea seeds. In our study, less fungi from the genus *Ascochyta* and *Phoma* were isolated from the seeds in 2011, that was more dry and warm.

Seed quality is one of the most important factors guaranteeing good storage, emergence and proper further growth and development of plants. Healthy, undamaged seeds with high germination ability should be used for pea sowing [16]. Seed sowing value depends, inter alia, on seed purity and health status. Fungi that often have a negative impact on the germination of seeds and plant emergence include species such as: *Ascochyta pisi, Botrytis cinerea, Sclerotinia sclerotiorum* and *Fusarium* spp. [29, 30]. Fungi of the genus *Ascochyta*, in addition to *B. cinerea* and *Fusarium* spp., are considered as the main pea pathogens [31]. Infected seeds are the main source of primary infection, and *Ascochyta* spp. pathogens can survive on seeds up to 12 years as long as the seeds are viable. Infection of pea seed is one of the major survival mechanisms of *Ascochyta* spp. and an important way of transmission into previously uninfected areas, but for some species can also represent a major source of inoculum for the developing crop [32]. Infection reduces seed germination, and seedlings that do develop from infected seeds may be diseased resulting in poor plant development and stands [33]. On the other hand the level of seed infection at planting had no impact on *A. pisi* disease severity on mature plants, on seed yield and size, or on the incidence of *A. pisi* infection of harvested seeds although *A. pisi* was the dominant species recovered from seeds [34]. Except *Ascochyta* spp., also *Phoma pinodella* is the cause of pea seedlings damping-off [27, 35].

The species composition of fungi may indicate the physiological state of the seeds. Shortly after harvesting, the occurrence of mainly the so-called field fungi from the genera *Alternaria, Botrytis, Cladosporium, Epicoccum* and *Fusarium* is observed. An increase in the number of microorganisms, mainly non-pathogenic ones, with a simultaneous decrease in infection by *Ascochyta* spp. and *Botrytis cinerea* pathogens is noted with seeds maturation [27]. In contrast, seeds with excessive humidity, stored at high air humidity, aging, damaged, are more often colonized by the so-called storage fungi from the genera *Aspergillus* and *Penicillium*. Among the fungi commonly found on the seeds, there are species that produce mycotoxins. These substances limit the use of seeds for direct consumption and/or processing. The presence of toxinogenic species was found on the examined seeds (Table 4). *A. flavus* produces mycotoxins with the highest toxicological importance. They include aflatoxins B1, B2, G1, G2 and M1. Also the fungi of the genus *Fusarium* are the producers of numerous mycotoxins. In turn, *A. alternata* produces tenuazonic acid. The presence of these species on seeds poses a threat. However, on the other hand, not all isolates, even of harmful species, can produce mycotoxins. Their number may also vary depending on various environmental factors, such as temperature or light [23, 26].

4. Summary

It was observed that, treatment with vaccines Nitragina i IUNG reduced the amount of seeds with pathogenic changes. It was also noted that the use of bacterial vaccines increased the seed yield. *Alternaria alternata* species and *Penicillium* genus were the most abundant. Fewer fungi were isolated in the fields where seed bacterial vaccines were used.

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