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Lectin activity of winter wheat seedlings affected by eyespot causal agent under the action of Bacillus subtilis 537/Б1 bacterial isolates

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The purpose. To study effect of bacterial isolates Bacillus subtilis 537/Б1 upon change in lectin activity and content of malonic dialdehyde (MDC) in plantlets of winter wheat of different on resistance varieties — Myronivska 808 and Renan — affected by causal organism of eyespot spot Pseudocercosporella herpotrichoides (Fron) Deighton. **Methods.** Preparation of lectin-containing extract was carried out by Lutsyk method. Lectin activity was determined by erythro-agglutination method. Protein content in lectin-containing extracts was determined by Bradford method. Level of generated MDC as a product of lipid peroxidation was estimated according to Dhindsa and Matowe. Statistical analysis of gained results was made by method of dispersion analysis in the program Microsoft Excel. Reliability of variance between alternatives was evaluated by Student t-test number at $P \leq 0,05$. **Results.** It is shown that treatment of plantlets of winter wheat of varieties Myronivska 808 and Renan wth bacterial isolates of B. subtilis 537/Б1 in pathogeny conditions promoted the maximum growth of lectin activity. And for variety Myronivska 808 such treatment was more efficient. It was established that oxidative stress developed both in sprouts and seeds infected with conidia suspension of pathogenic fungi and suspension of B.subtilis 537/Б1 bacterial isolates. That was confirmed by intensification of lipid peroxidation (LPO) processes. **Conclusions.** Treatment of plantlets and seeds of winter wheat of varieties Myronivska 808 and Renan with bacterial isolates B. subtilis 537/Б1 had protecting character under the action of eyespot causal agent. The obtained data may become a basis for the further researches and consideration of opportunities for creation of new efficient biofungicides on the basis of B. subtilis 537/Б1 for protection cereal crops against causal organisms of different diseases.

Key words: *PR-proteins, Bacillus subtilis 537/Б1, Pseudocercosporella herpotrichoides, pathogenesis, MDC content, lectin activity.*

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The global trend of reducing the doses of agrochemicals determines the need to increase the use in plant growth of new, additional sources of mineral nutrition and biological means of plant protection. Recently, scientists' attention has been focused on the development of biopreparations based on Plant Growth-Promoting Bacteria (PGPB) [1]. Endophytic bacteria have the particular interests among them. They can mutualistic live inside plant tissues which allow them, in comparison with other microorganisms, to be less dependent on external environmental factors and show a complex of economically useful properties.

Despite the wide assortment, there are no effective biofungicides to protect crops from diseases such as root rot, powdery mildew, septoria. One of the main reasons constraining creation of endophyte-based biopreparations is the absence of works about the systematic study of the molecular mechanisms of the relationship between plant systems-PGPB and the plant-PGPB-phytopathogen.

Analysis of recent research and publications. These and other facts force the use of nature biological compounds, including microorganisms and their metabolites, which do not have a harmful effect to humans and the environment instead of potent chemical plant protection product (CPPP). The principle of their action differs from classic CPPP. It is based on the regulation of the pathogens number, formation of competition with pathogens and induction of systemic resistance. Most of them are considered as triggers which initiates a cascade of protective reactions due to the production of various metabolites [2]. Plant own protection

mechanisms known as "induced systemic resistance" (ISR) and "systemic acquired resistance" (SAR) are initiated in plants under the influence of PGPB [3]. The pathogen-induced systemic acquired resistance is accompanied by a coordinated activation of pathogenesis-related genes (PRs, from pathogenesis-related). These genes encode proteins with antimicrobial activity [4] and controlled by the redox-regulated protein NPR1 (from non expressor of PR genes 1), which is activated by salicylic acid, is its receptor [5] and functions as a transcriptional co-activator of a large number of PR genes [6].

Chitin-binding proteins (PR-4 group) belong to a large group of proteins – the lectins that are the PR-proteins. Lectins are proteins that can selectively bind to polysaccharides, glycoproteins and glycolipids without causing their chemical transformation [7]. Currently, the system of carbohydrate-protein recognition is considered complementary to the genetic code. However, despite the intensive study of the functions of lectins, there is absolutely no data of the regulation mechanisms of their activity.

Aim. To investigate the effect of *Bacillus subtilis* 537/Б1 bacterial isolates to the lectin activity (LA) of winter wheat seedlings infected by eyespot causal agent *Pseudocercosporella herpotrichoides* (Fron) Deighton

Materials and methods. Two winter wheat (*Triticum aestivum* L.) varieties with different susceptibility to eyespot causal agent (*Pseudocercosporella herpotrichoides*): Myronivska 808 – a sensitive to pathogen and Renan – a resistant to pathogen were used. The seedlings were grown on the sand in chemically neutral containers under controlled laboratory conditions (16-h photoperiod, light intensity 15 000 lx, air temperature 25/20 °C (day/night), air humidity 60%); 25–35 ml of the Hoagland-Arnon nutrient solution was added to each container. The moisture level of the substrate was maintained at a constant (70%) level using an additional nutrient solution.

Conidial suspension of fungus *Pseudocercosporella herpotrichoides* (Fron) Deighton was used to the infection. The high virulent strain *P. herpotrichoides* was used, which was kindly provided by our colleagues from the V.M. Remeslo Myronivka Institute of wheat National Academy of Agrarian Sciences of Ukraine.

Bacteria *Bacillus subtilis* 537/Б1 were cultivated at 30°C for 16h (overnight). Cell culture medium was centrifuged at 10.000 g for 5 min and washed twice by 0.85% NaCl. The supernatant was discarded and bacterial cells were resuspended in sterile 0.85% NaCl adjusting to the final concentration 3x108 CFU/mL 10 (OD=0.3).

Live cell suspension of bacterial isolates was applied on seedlings immediately after preparing.

The used experimental variants: 7-day-old wheat seedlings: 1) – control; 2) –infected with a conidia suspension of pathogenic fungi; 3) – inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 4) – infected with a conidia suspension of pathogenic fungi and suspension of *B.subtilis* 537/Б1 bacterial isolates; 5) – from seeds inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 6) – from seeds inoculated with suspension of *B.subtilis* 537/Б1 bacterial isolates and infected with conidia suspension of pathogenic fungi. The control was sprayed with distilled water.

Selection of plant material for biochemical studies was carried out 4, 24, 48 hours after infection.

Lectin activity was determined by erythro-agglutination assay [8]. Lectin-like proteins of cell walls and cell organelles fractions were isolated according to the method described by Lutsyk [9]. It was calculated as reversed value to a minimum protein concentration, which caused the agglutination of rat erythrocytes $(\mu g/ml)^{-1}$.

LA = Titer/Protein concentration

The protein content in the obtained extracts was determined by the method of Bradford spectrophotometrically at 595 nm wavelength [10].

The level of generated malonic dialdehyde (MDA) as a product of lipid peroxidation was estimated according to Dhindsa and Matowe [11]

Each experiment was performed in triplicate. The data were subjected to analysis of variance (ANOVA) with subsequent Student's t-test or Duncan's multiple range test at $P<0.05$.

Results and Discussion. The analysis of the obtained results showed the oxidative stress development in wheat seedlings as well as in wheat seedlings from seeds infected with a conidia suspension of pathogenic

fungi and also with the suspension of *B. subtilis* 537/Б1 bacterial isolates. This is confirmed by the enhancement of the intensity of lipid peroxidation (LPO) processes characterized by the level of generated malonic dialdehyde (MDA). The genetic features of each variety and the duration of exposure greatly affect the intensity of lipid peroxidation.

The MDA content in photosynthetic tissues was no significant difference with the control in winter wheat seedling of Myronivska 808 variety in 4 hours after inoculated with the suspension of *B. subtilis* 537/Б1 bacterial isolates. But in winter wheat seedling from seeds inoculated with the suspension of *B. subtilis* 537/Б1 bacterial isolates, the MDA content slightly increased (by 6%), in wheat seedlings from seeds inoculated with the suspension of *B. subtilis* 537/Б1 bacterial isolates and infected with the conidia suspension of pathogenic fungi this parameter significantly decreased (by 23%). A tendency to decrease of MDA content in all experimental variants was observed after 24 hours of exposure (Fig. 1).

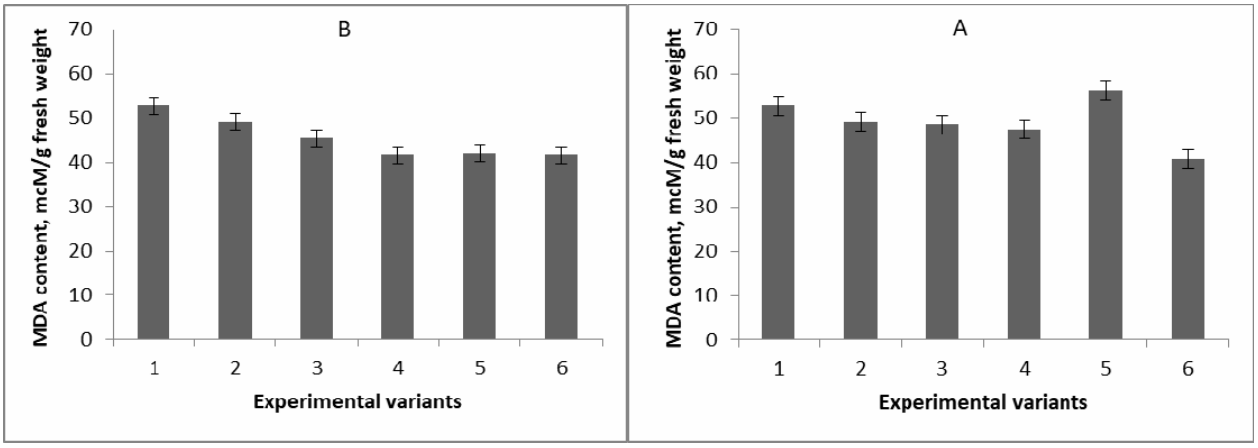

Figure 1. MDA content in winter wheat seedlings of Myronivska 808 variety: A – 4 hours, B – 24 hours of exposure. Experimental variants: 7-day-old wheat seedlings: 1) – control; 2) –infected with a conidia suspension of pathogenic fungi; 3) – inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 4) – infected with a conidia suspension of pathogenic fungi and suspension of *B.subtilis* 537/Б1 bacterial isolates; 5) – from seeds inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 6) – from seeds inoculated with suspension of *B.subtilis* 537/Б1 bacterial isolates and infected with conidia suspension of pathogenic fungi.

It should be admitted a significant reduction of MDA content in variants of seedlings and seed treatment with suspension of bacterial isolates (by 14% and 20% respectively). Exposure prolongation to 48 hours conducted the fluctuations in the MDA content (Fig. 2).

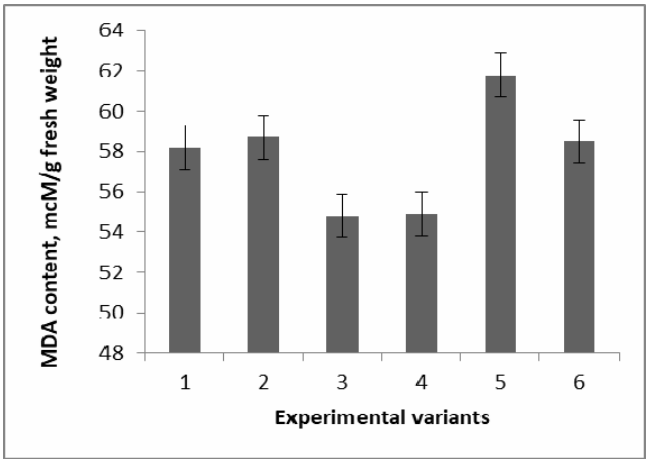

Figure 2. MDA content in winter wheat seedlings of Myronivska 808 variety after 48 hours of exposure. Experimental variants: 7-day-old wheat seedlings: 1) – control; 2) –infected with a conidia suspension of pathogenic fungi; 3) – inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 4) – infected with a

conidia suspension of pathogenic fungi and suspension of *B.subtilis* 537/Б1 bacterial isolates; 5) – from seeds inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 6) – from seeds inoculated with suspension of *B.subtilis* 537/Б1 bacterial isolates and infected with conidia suspension of pathogenic fungi.

Treatment with the suspension of *B. subtilis* 537/Б1 bacterial isolates and treatment with a conidia suspension of pathogenic fungi and suspension of bacterial isolates *B. subtilis* 537/Б1 caused the reduction of the LPO intensity in contrast of the control over 48 hours of exposure.

Winter wheat seedlings of Renan variety had a lower basal level of MDA. This result characterizes Renan as resistant variety. Response reactions developed from the 4th hour of exposure (Fig. 3a). MDA content decreased compare with the control variant in seedlings and seedlings from seeds treated with a suspension of *B. subtilis* 537/Б1 bacterial isolates. Inoculation of infected seedlings and seeds with the suspension of *B. subtilis* 537/Б1 bacterial isolates caused reduction of MDA content in photosynthetic tissues. Oxidation processes developed more intensively in the variants infected with the *P. herpotrichoides* after 24 hours of exposure (Fig. 3b).

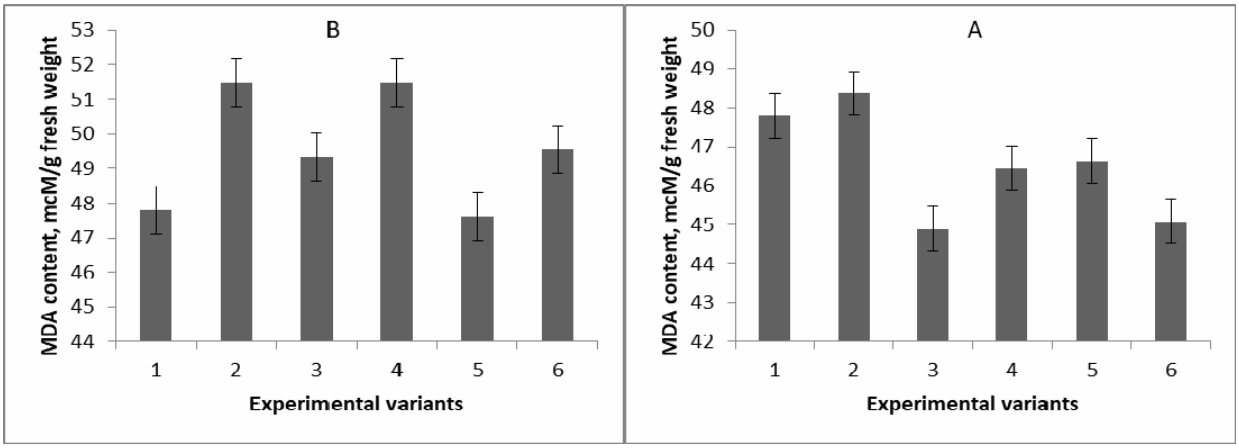

Figure 3. MDA content in winter wheat seedlings of Renan variety: A – 4 hours, B – 24 hours of exposure. Experimental variants: 7-day-old wheat seedlings: 1) – control; 2) –infected with a conidia suspension of pathogenic fungi; 3) – inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 4) – infected with a conidia suspension of pathogenic fungi and suspension of *B.subtilis* 537/Б1 bacterial isolates; 5) – from seeds inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 6) – from seeds inoculated with suspension of *B.subtilis* 537/Б1 bacterial isolates and infected with conidia suspension of pathogenic fungi.

The protective effect of *B. subtilis* 537/Б1 bacterial isolates under *P. herpotrichoides* infection in seedlings and seedlings from seeds inoculated with *B. subtilis* was more clearly at 48 hours of exposure. MDA content in these variants had been manifested in lower or at the level of control values (Fig. 4).

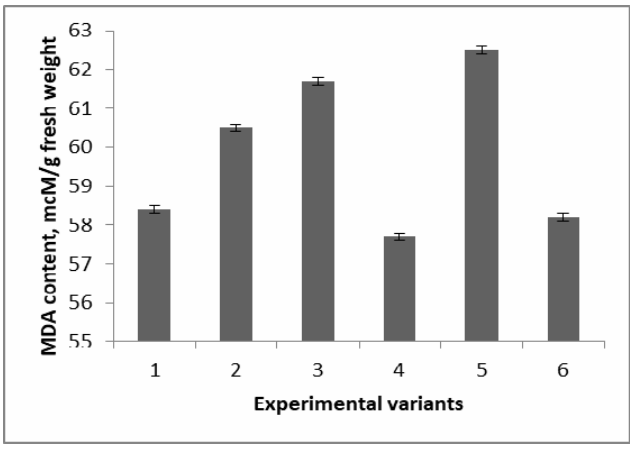

Figure 4. MDA content in winter wheat seedlings of Renan variety after 48 hours of exposure. Experimental variants: 7-day-old wheat seedlings: 1) – control; 2) –infected with a conidia suspension of pathogenic fungi; 3) – inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 4) – infected with a conidia suspension of pathogenic fungi and suspension of *B.subtilis* 537/Б1 bacterial isolates; 5) – from seeds

inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 6) – from seeds inoculated with suspension of *B.subtilis* 537/Б1 bacterial isolates and infected with conidia suspension of pathogenic fungi.

Consequently, the activation of LPO processes under treatment with bacterial preparations related to the fact that interaction between plant and fungi in early stages characterized by oxidative burst with ROS formation in increased quantities. Reduction of the oxidative processes intensity in variants with bacterial isolates *B. subtilis* 537/Б1 may be related with the fact that *B. subtilis* 537/Б1 bacterial isolates inhibit the development of various diseases in plants not only through the synthesis of various anti-fungal metabolites but also indirectly through the mechanism of the induced-resistance system [5]. It is regulated by produced hormones such as salicylic acid, abscisic acid, jasmonic acid, ethylene [6], and by cyclic lipopeptides [12].

There are enough data to show the lectin activity change due to the effects of various stress factors and the possible role of these proteins in the formation of nonspecific protective reactions of plants. The increase of LA compared to control was observed in all variants of the experiment for the Myronivska 808 variety (Fig. 5).

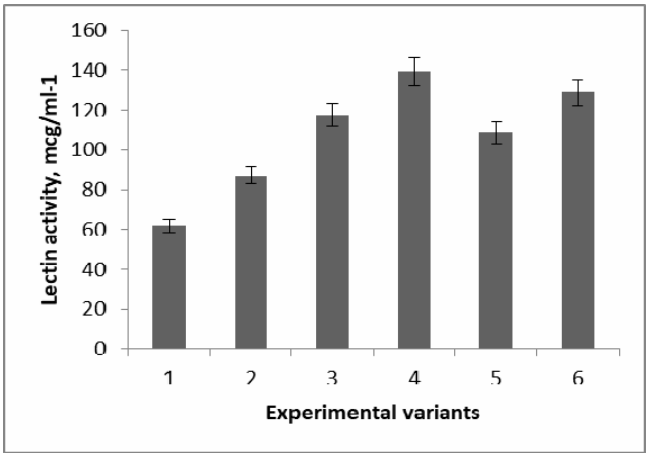

Figure 5. Lectin activity in winter wheat seedling of Myronivska 808 variety after 48 hours of exposure. Experimental variants: 7-day-old wheat seedlings: 1) – control; 2) –infected with a conidia suspension of pathogenic fungi; 3) – inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 4) – infected with a conidia suspension of pathogenic fungi and suspension of *B.subtilis* 537/Б1 bacterial isolates; 5) – from seeds inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 6) – from seeds inoculated with suspension of *B.subtilis* 537/Б1 bacterial isolates and infected with conidia suspension of pathogenic fungi.

Lectin activity of the Renan varieties was no significant difference with the control after 48 hours of suspension inoculation. But lectin activity increased significantly (by 2 times) in the seedlings infected with a conidia suspension of pathogenic fungi and suspension of *B. subtilis* 537/Б1 bacterial isolates (Fig.6).

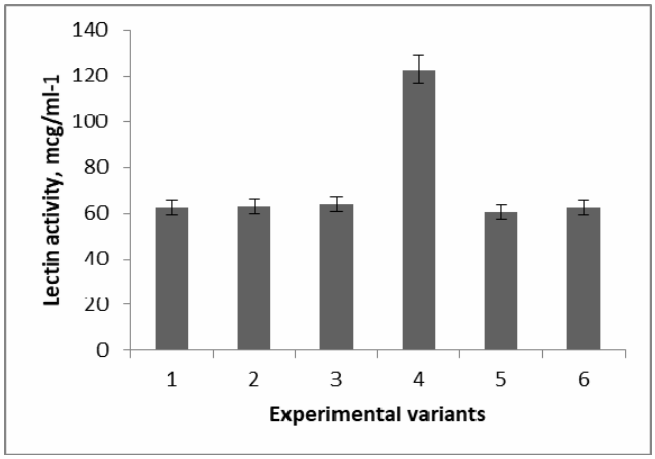

Figure 6. Lectin activity in the winter wheat seedling of Renan variety after 48 hours of exposure. Experimental variants: 7-day-old wheat seedlings: 1) – control; 2) –infected with a conidia suspension of pathogenic fungi; 3) – inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 4) – infected with a

conidia suspension of pathogenic fungi and suspension of *B.subtilis* 537/Б1 bacterial isolates; 5) – from seeds inoculated with a suspension of *B.subtilis* 537/Б1 bacterial isolates; 6) – from seeds inoculated with suspension of *B.subtilis* 537/Б1 bacterial isolates and infected with conidia suspension of pathogenic fungi.

Firstly, the using of *B. subtilis* bacterial isolates to plant protection usually associated with their competition with pathogenic microflora for nutrients and colonization niche [13]. Secondly, it is associated with the synthesis of various metabolites with antibiotic activity – antibiotics, biosurfactants, siderophores, hydrogen cyanide, etc. Thirdly, it is related to the synthesis of hydrolytic enzymes such as chitinase, glucanase, proteases, and lipase. They can destroy pathogenic fungi cells and a number of pathogen effector compounds [14]. Fourthly, it is associated with elicitor activity and the initiation of the induced and acquired systemic resistance [3]. These processes are carried out with the help of bacterial determinants (MAMPs - microbe-associated molecular pattern) such as flagellin, lipopolysaccharides (LPS), siderophores, antibiotics, biosurfactants and volatile organic compounds [15].

The results analysis suggests that the distinctive feature of induced systemic resistance, mediated with the *B. subtilis* bacterial isolates, is the resistance development under the mechanism of sensitization, which is called priming [16]. The phenomenon of priming with bacterial agents is the increase of plant cells sensitivity to the foreign substances influence and is characterized by faster and more powerful activation of cellular mechanisms of plant protection to pathogen attacks. It can last for a long time, which conducts to increase plants resistance. Induced systemic resistance develops by priming mechanism and is observed at different stages of plant-pathogen interaction. It begins with early responses, which are controlled by the hormonal system. It is activated by various signaling and protective proteins. The process ends with the long-term response reactions that involve the controlled chromatin changes and DNA methylation. Protective reactions that developed from induced systemic resistance under the action of bacterial isolates are characterized by rapid and early ROS accumulation (including H_2O_2) which activates the redox-sensitive transcription factors and PR-protein genes [17] and regulates the interaction of salicylic, jasmonic and ethylene signaling pathways. The induced systemic resistance mechanism can also be associated with the barrier formation to penetrate the pathogen by the callose deposition, strengthening of plants cell walls and the production of metabolites with antimicrobial activity (phytoalexins) [18].

ROS generation in plants that developed from induced systemic resistance under the action of bacterial isolates can play a critical role in the priming effect formation. Early ROS accumulation was observed in seedlings inoculated with the suspension of bacterial isolates after infection with a conidia suspension of pathogenic fungi. It is assumed this is due to their ability to produce the determinants [5]. ROS generation related to the expression of protective genes and could be associated with the protective effect of lipopolysaccharides [5]. It has been found a direct correlation between H2O2 generation and a surfactin concentration of different strains of *B. subtilis* which inoculate the tobacco cell suspensions [19]. The role of elicitors can also be performed by phytohormones at systemic resistance [5]. ROS also can activate transcription factors and regulate ROS-sensitive genes through them [20]. Investigation of plant transcriptome showed the transcription factors after inoculation of *B. subtilis* accumulate in plants and remain to be inactive until the pathogen infection. But they are supposed to give the plant ability to faster reaction to the pathogen attack. Hence, they provide a priming effect [5].

Conclusions

Inoculation of winter wheat seedlings and seeds of both varieties with *Bacillus subtilis* 537/Б1 bacterial isolates had a protective character under the action of eyespot causal agent. It has been shown that inoculation of winter wheat seedlings of Myronivska 808 and Renan varieties with suspension of *B. subtilis* 537/Б1 bacterial isolates under pathogenesis contributed maximum growth of lectin activity.

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