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CHARACTERIZATION OF LECTINS FROM WHEAT SEEDLINGS INFECTED WITH *FUSARIUM GRAMINEARUM* AND TREATED BY JASMONIC ACID

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Fusarium head blight is one of the most serious diseases of wheat caused by a range of Fusarium fungi, which infects the heads of the crop, reducing grain yield. Lectins that specifically bind carbohydrate ligands of various chemical nature and Jasmonic acid (JA) as a key regulator of plant development play an important role in plant protective responses to biotic factors. The goal of the study was to determine the activity and biochemical characteristics of soluble lectins in wheat seedlings of varieties 'Lastivka odeska' with a high resiliency to Fusarium graminearum and 'Nikonia odeska' susceptible to Fusarium graminearum. Wheat seedlings were grown on the media containing pathogenic infection or JA solution. Lectins were purified by affinity chromatography and separated by electrophoresis in 15% PAGE. Lectin activity was determined by the method of trypsinized blood erythrocytes hemagglutination. Molecular mass of the main components of lectins from 'Lastivka odeska' seedlings was determined to be 67, 60, 45 kDa, and of the main component of lectins from 'Nikonia odeska' seedlings – 45 kDa. Lectins isolated from the control untreated seedlings had preferential affinity for N-acetylglucosamine, D-galactosamine and D-fructose-6-phosphate. It was shown that both at pathogen action or JA treatment, lectin activity in the seedlings of resistant variety 'Lastivka odeska' was increased while in the seedlings of susceptible variety 'Nikonia odeska' it was decreased as compared to control. At the joint action of pathogen and JA, lectin activity in the seedlings of susceptible variety increased compared with the infected seedlings. The results obtained can be used for the development of biochemical methods for assessing the degree of wheat varieties resiliency to fusariose.

K e y w o r d s: wheat, Fusarium graminearum, resilience to fusariose, soluble lectins, affinity to carbohydrates, jasmonic acid.

Fusarium head blight (FHB), predominately caused by *Fusarium graminearum*, is one of the most serious wheat diseases in the world. Yield and quality losses can be devastating and mycotoxins produced by Fusarium pathogens compromise food and feed safety [1]. Therefore, FHB-resistant varieties would help to increase yield, to lower production cost (fungicide use) and to improve food and feed security [2]. In various countries of the world, wheat breeding programs successfully select the source material and promising genotypes using biochemical and molecular resiliency markers. Their implementation will allow shortening the time of creation of resistant varieties of agricultural crops and, as a result, to obtain high yields. Protective proteins play an important role in biochemical processes associated with disease resiliency [3]. One of the groups of protective proteins are lectins. Lectins are proteins that can reversibly and specifically bind carbohydrate ligands of various chemical nature. Plant lectins have been classified into seven families of proteins which are related structurally and evolutionarily. These are lectins of phloem from *Cucurbitaceae*, chitin-binding lectins, legume lectins, ribosome-

inactivating protein type 2, mannose-binding lectins of monocots, jacalin-related lectins and the family of amarantins. Also, plant lectins can be classified according to the specificity of their interactions with mannose/glucose, mannose/maltose, galactose/N-acetylgalactosamine, N-acetylglucosamine/N-acetylglucosamine, galactose, mannose, fucose and sialic acid among others [4]. Lectins are the most likely candidates for performing a protective function in plants against fungal pathogens due to their affinity for N-acetyl-D-glucosamine and chitin oligomers. Such proteins include, for example, the water-soluble, plasmalemma-bound potato lectin, GlcNAc-specific lectin II from *Griffonia simplicifolia*, as well as wheat germ agglutinin (WGA) etc. De novo WGA synthesis in meristematic tissues of roots and above-ground part of seedlings of wheat has been established [5-7]. It is known that plant lectins and proteins with one or more lectin domains represent a major part of some membrane-bound and soluble pattern recognition receptors (PRRs). The important role of lectins in plant defense has been demonstrated using transgenic plants with genes for different plant lectins [8, 9]. Some purified plant lectins have antifungal properties against fungus of genus *Fusarium* [4]. It is known that lectins localized in different parts of the cell take part in the formation of plant defense reactions under the action of biotic and abiotic factors. It has been shown that lectins can be in the soluble fraction or be associated with subcellular structures [9]. Most of the constitutively expressed lectins are synthesized with a signal peptide, and are sequestered in the vacuole or secreted to the extracellular space, most of the inducible plant lectins reside in the nucleus and the cytoplasm of a plant cell [10]. The presumed involvement of plant lectins in defense mechanisms is inferred from an analysis of the biochemical, physiological, cellular biological and molecular biological properties of plant lectins.

A number of signaling compounds have been found in plant tissues, which trigger a cycle of biochemical reactions in response to stressors of various natures. For example, this role can be performed by jasmonic acid (JA). JA is an oxidized lipid-derived derivative of cyclopentanone and it functions as a plant hormone to regulate diverse developmental processes and defense responses. Many jasmonate responses are mediated via changing levels of synthesis of the jasmonate-regulated proteins (JRP). Some of these JRPs with diverse physiological functions have been characterized in detail, such as proteinase-inhibitors, thionins, phytoalexin-synthesizing enzymes, cell wall proteins, pathogenesis-related proteins and osmotin, lipoxygenase. There is also data to suggest that some lectins may be also involved in cellular regulation and signaling, plant defense. For example, Ma et al cloned and characterized a novel jacalin-like lectin gene (Ta-JA1) from wheat (*Triticum aestivum* L.), which codes a modular JRP with disease response and jacalin-related lectin (JRL) domains which present only in the Gramineae family. Their results suggest that Ta-JA1 may confer a basal but broad-spectrum resistance to plant pathogens [11]. It would be interesting to know about influence JA on wheat soluble lectins and their role in plant resiliency to *Fusarium graminearum*. Thus, the isolation and investigation of soluble lectins from the wheat plants distinguished by the level of resiliency to Fusarium graminearum and grown on the media containing pathogenic infection and solution of JA are urgent and important for finding out the biochemical properties of these lectins and their role in defense reactions of the wheat plants. In this connection the goal of our study is to identify the changes in the activity and biochemical characteristics of soluble lectins in wheat seedlings with different resiliency to *Fusarium graminearum* at the infection by fusariosis agents and the action of JA in order to research the role of these proteins in the wheat protective reactions and development the new biochemical methods for assessing variety resilience to this phytopathogen.

Materials and Methods

Plants of bread winter wheat (*Triticum aestivum* L.) varieties, created in Plant Breeding& Genetics Institute-National Center of Seed and Cultivar Investigation, Odessa, Ukraine, were used for an investigation. Variety ‘Lastivka odeska’ is characterized as having a high-level resiliency to fungal phytopathogens, including *Fusarium graminearum*. Variety ‘Nikonia odeska’ is characterized as susceptible to *Fusarium graminearum* [12].

The seeds were disinfected for 30 min with a 1% $KMnO_4$ solution and washed with distilled water. Then they were germinated for 4 days in the dark at 24°C in Petri dishes on filter paper moistened with distilled water or suspension which contained 1×10^5 conidia/ml of the highly pathogenic strain K-90 *Fusarium graminearum*. Colonies of the fungus K-90 *Fusarium graminearum* from the collection of

strains of the Department of Phytopathology and Entomology of the PBGI-NCSCI were grown on potato agar. To obtain a filtrate, the spore suspension was added to a liquid potato medium and cultivated for 10 days at a temperature of 24°C. Treatment of seedlings with JA was conducted by spraying with 1 μM solution of JA on the 3rd day of germination (duration of exposure was 24 h). Further seedlings were treated with cycloheximide, an inhibitor of protein synthesis, at a concentration of 10 mg/l for 2 h. The concentration and exposure time were selected based on preliminary experiments [13].

The exposure being over, the prepared overground part of sprouts was frozen at –70 °C, freeze-dried and ground.

The lectin activity was determined by their ability to agglutinate trypsinized white rat erythrocytes at room temperature. The activity was taken as the reciprocal of the minimum protein concentration when erythrocyte agglutination occurs (μg protein/ml)$^{-1}$ [14]. The total protein content in the extract was determined by the Lowry method [15]. Receiving erythrocytes and trypsinization was carried out according to the method [14].

Soluble lectins were extracted from the seedlings tissues by 0.05 N HCl at 4°C for 1 hour at the ratio mass:volume 1 : 10. Homogenate was centrifuged for 20 min at 20 000 g (4°C). The precipitate was washed by 0.05 N HCl. The supernatant was collected, neutralized by 10% KOH solution in a ratio of KOH : extract 1 : 2 to pH 7.4, re-centrifuged and used to determine lectin activity and further lectin purification. Our purification plan includes 3 stages.

Stage 1. Salting out. The supernatant was salted out by ammonium sulphate at saturation 60% (162.5 g/l). Sediment was separated by centrifugation during 20 min at 6000 g (4 °C).

Stage 2. Dialysis. The sediment was dissolved by 0.05 M phosphate buffer at pH 7.2, and put to dialysis against 20-fold volume of distilled water during night at 4 °C, using dialysis membranes (Regenerated cellulose tubular membrane, Fisher Scientific, USA) with a pore diameter of 12-14 kDa. After dialysis, the lectin solution was centrifuged for 20 min at 6 000 g (4 °C), the lectin activity and protein content were determined in the supernatant. The latter was used for further purification.

Stage 3. Affinity chromatography. The lectin solution was lyophilically dried, dissolved in 0.05 M phosphate buffer at pH 7.4, containing 0.1 M NaCl. The lectin solution was applied to the column (1.6×20 cm) with bromine-activated ovomucoid-Sepharose 4B, balanced with the above buffer solution. Protein was eluted stepwise by 0.05 M phosphate buffer, pH 7.4, containing 0.1 M NaCl; 0.1 M acetic acid and 0.05 M phosphate buffer, pH 7.4, containing 0.1 M NaCl and 1% N-acetylglucosamine.

Carbohydrate specificity of lectins was determined by their affinity for carbohydrates [14]. The following carbohydrates were used: D-glucose, D-fructose, sucrose, D-xylose, D-galactose, D-raffinose, N-acetylglucosamine, D-galactosamine, D-fructose-6-phosphate in an initial concentration of 500 mM. The level of affinity of lectins to carbohydrates was judged by the lowest concentration of carbohydrates at which no agglutination was observed.

Protein electrophoresis was carried out in 15% polyacrylamide gel containing 0.1% SDS at pH 8.3 according to the Laemmli method [16] using equipment for vertical electrophoresis of Hem-Hoff (USA). Proteins-markers produced by Serva (Germany) were used to plot the calibration graph (phosphorylase B (97 kDa), bovine serum albumin (67 kDa), albumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), L-lactalbumin (14.4 kDa).

Statistical processing of the investigation results was made using the pack of programs “Analysis of the Data of Electron Tables Microsoft Excel”, image analysis program “Imagel”. The experiments were performed in triple biological and analytical repetitions. Mean values and their standard errors are presented in Fig. 1, differences between the experiment variants, distinguished by variety resiliency to pathogen, were considered reliable at the significance level $P < 0.05$ by the Student criterion.

Results and Discussion

One of the possible functions of lectins is their participation in formation of plant protection reactions against phytopathogens. To confirm this supposition, we have studied lectin activity in the control plants, those infected by *Fusarium graminearum* and treated by JA in wheat varieties distinguished by the level of resiliency to fusariosis agents. The conducted studies have shown that the 1.3- and 1.5-fold increase of lectin activity compared with control, respectively, was observed in the resistant wheat variety under the influence of pathogen and JA. The lectin activity of a susceptible genotype significantly decreased relative to control in the seedlings infected by *Fusarium graminearum*, treated by JA. Lectin ac-

tivity in the seedlings of susceptible variety at the joint action of pathogen and JA increased reliably at $P \leq 0.05$ compared with the infected seedlings (Fig. 1).

This is consistent with the literature data and our previous research about involvement of lectins in the formation of defense reactions in wheat against *Fusarium graminearum* [3, 13, 17]. Changes in lectin activity under the influence of JA suggest an important role of this signal molecule in the processes of regulating the activity of the soluble fraction of wheat lectins. It can be assumed that the level of lectin accumulation in the tissues of wheat seedlings under the influence of studied factors may be associated with a rate of their synthesis. Inhibitory analysis is widely used to study the mechanisms of controlling the activity and content of proteins, in particular the influence of protein synthesis inhibitors such as cycloheximide. Treatment of seedlings in different growing conditions (*Fusarium graminearum*, JA, JA+*Fusarium graminearum*) by cycloheximide significantly reduced the lectin activity in both resistant and susceptible wheat varieties.

The obtained results evidence for soluble lectins involvement in the regulation of cell metabolism of the wheat plants at the infection by *Fusarium graminearum* and at the action of JA. Also these results indicate the possibility of regulation the level of lectins in wheat seedlings under the influence of the studied factors at the level of protein biosynthesis processes.

The next step in our research was isolation, purification of soluble lectins and investigation of some biochemical characteristics of these proteins. Isolation and purification of lectins were carried out using salting out by 60% ammonium sulfate, dialysis, column affinity chromatography. Initial lectin activity in extracts was 0.034-0.052 (μg protein/ml)$^{-1}$. After salting out by ammonium sulfate with following dialysis, the protein was concentrated and lectin activity increased 2.19-2.65 times. After dialysis, the concentrated lectin solution was applied to the column with bromine-activated ovomucoid-Sepharose 4B, balanced with the above buffer solution. Protein was eluted stepwise by 0.05 M phosphate buffer, pH 7.4, containing 0.1 M NaCl; 0.1 M acetic acid and 0.05 M phosphate buffer, pH 7.4, containing 0.1 M NaCl and 1% N-acetylglucosamine. The lectin was eluted from the column by 2 peaks: peak 1 with 0.1 M acetic acid and peak 2 with 1% N-acetylglucosamine. The fractions with maximum lectin activity were extracted. Maximum lectin activity was found in the second peak (Fig. 2).

After the affinity chromatography lectin activity increased 98.4-115.2 times. As a result of using salting out by ammonium sulfate with following dialysis, affinity chromatography, the yield of soluble lectin from the control samples was 81,9% with purification coefficient 115.2; from seedlings treated by JA – 90.4% with purification coefficient 108.9; from infected seedlings – 65.1% with purification coefficient 98.4 and from infected seedlings after

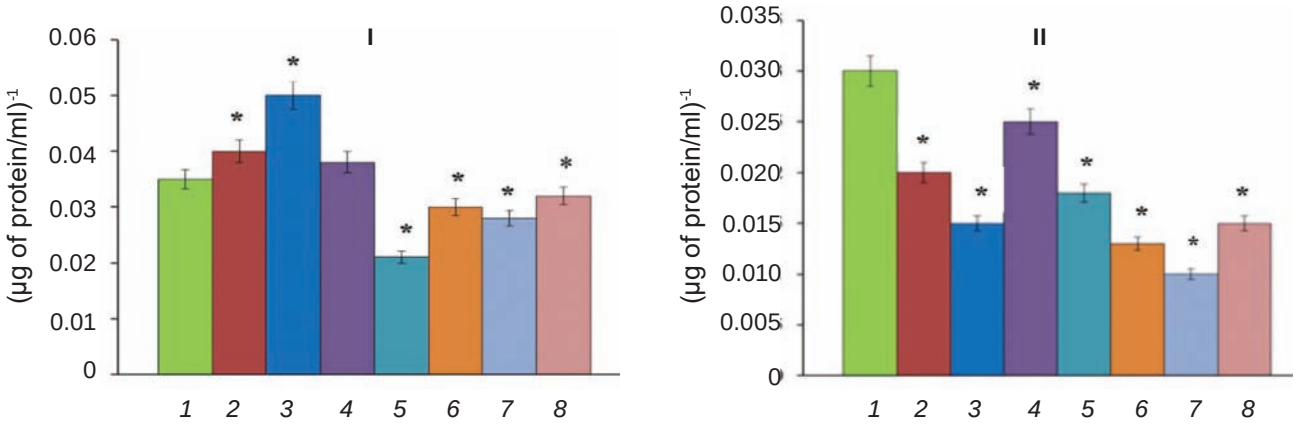

*Fig. 1. Lectin activity in wheat seedlings at the infection by Fusarium graminearum and at the action of JA, CG. **I** – Resistant variety 'Lastivka odeska'; **II** – susceptible variety 'Nikonia odeska'; JA – jasmonic acid, CG – cyclohexemide. 1 – Control; 2 – JA; 3 – Fusarium graminearum; 4 – JA+Fusarium graminearum; 5 – CG; 6 – JA+CG; 7 – Fusarium graminearum+CG; 8 – JA+Fusarium graminearum+CG. *Differences are significant a $P < 0.05$*

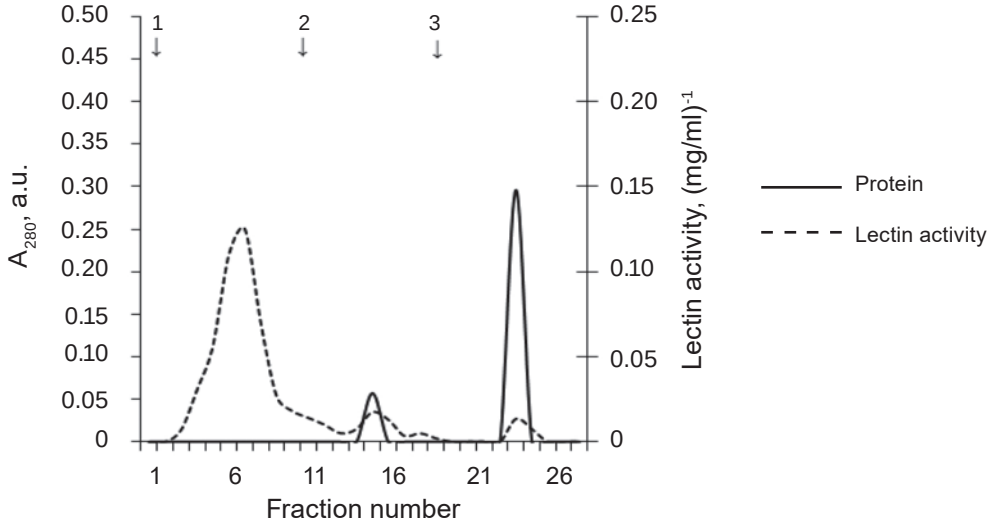

Fig. 2. Elution profile of soluble lectins from the control seedlings of wheat of variety 'Lastivka odeska' on bromine-activated ovomucoid-Sepharose 4B, column 1.6×20 cm, velocity 7.5 ml/cm²·h, fraction volume – 2.5 ml, elution by 0.05 M phosphate buffer pH 7,4. Protein is applied – 50.5 mg. 1 – 0.05 M phosphate buffer pH 7.4 +0.1 M NaCl; 2 – 0.1 M acetic acid, 3 – 0.05 M phosphate buffer, pH 7.4, containing 0.1 M NaCl and 1% N-acetylglucosamine

Table 1. Isolation of soluble lectins from wheat seedlings of variety 'Lastivka odeska'

Purification stages	Total protein, mg	Lectin activity, (μg of protein/ml)$^{-1}$	Output, %	Purification coefficient
Lectins from control samples				
Initial extract	50.5	0.034	100	1
Salting out + dialysis	70.3	0.082	100	1
Affinity chromatography	0.5	9.45	81.9	115.2
Lectins from seedlings, treated by JA				
Initial extract	52.5	0.042	100	1
Salting out + dialysis	72.3	0.092	100	1
Affinity chromatography	0.6	10.02	90.4	108.9
Lectins from infected seedlings				
Initial extract	53.1	0.046	100	1
Salting out + dialysis	75.5	0.122	100	1
Affinity chromatography	0.5	12.00	65.1	98.4
Lectins from infected seedlings after treatment by JA				
Initial extract	55.8	0.052	100	1
Salting out + dialysis	80.5	0.130	100	1
Affinity chromatography	0.5	12.9	61.6	99.2

treatment of seedlings by JA – 61.6% with purification coefficient 99.2 (Table 1).

The molecular mass of the main components of lectins, isolated from seedlings of resistant variety 'Lastivka odeska', determined by the method of electrophoresis, was 67, 60 and 45 kDa, the molecular mass of the main component of lectins, isolated from seedlings of susceptible variety 'Nikonia odeska' was 45 kDa (Fig. 3). It was shown that the intensity of bands of lectin components with molecu-

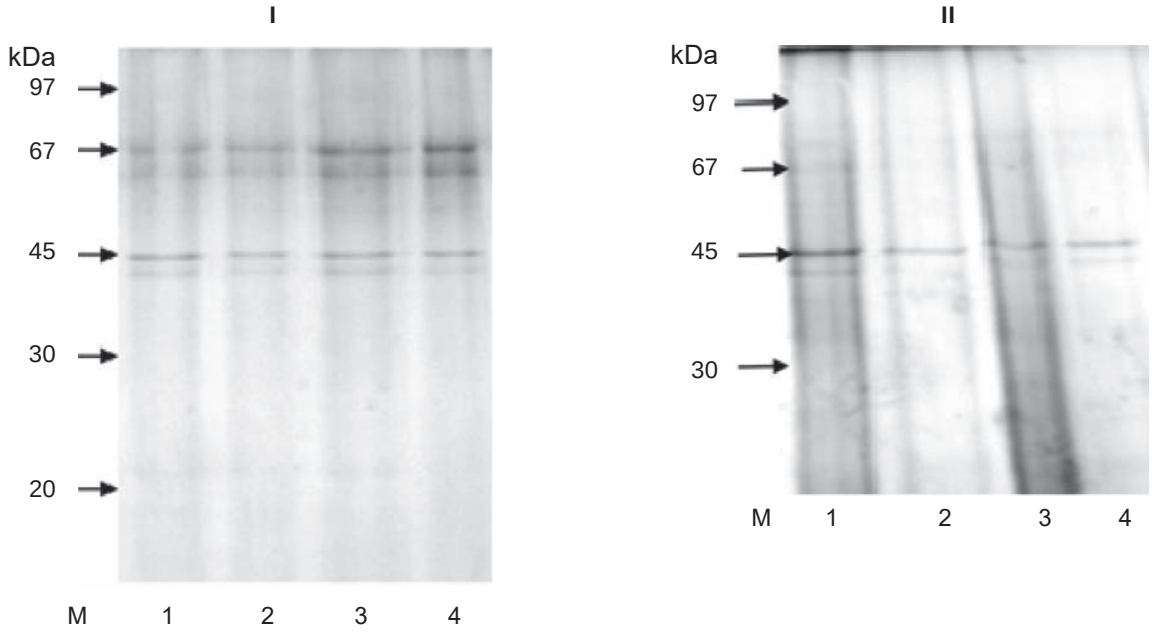

Fig. 3. Electrophoregrams of soluble lectin isolated from the overground part of wheat seedlings in the different germination conditions: 1 – control; 2 – JA; 3 – Fusarium graminearum; 4 – JA+Fusarium graminearum

lar mass of 67 and 60 kDa in the resistant variety increased at the infection by *Fusarium graminearum* and at the joint action of pathogen and JA. In the susceptible variety, the intensity of the bands of the component with a molecular weight of 45 kDa decreased relative to the control at the influence of pathogen and JA. It is evidence that lectin components with weight of 67 and 60 kDa could take part in protective reactions of wheat against *Fusarium graminearum.*

The basis of the biological activity of lectins is their participation in carbohydrate-protein interactions, so one of the tasks was to study the carbohydrate specificity of lectins for the qualitative characterization of these proteins.

Lectins isolated from control seedlings showed high affinity for N-acetylglucosamine, D-galactosamine and D-fructose-6-phosphate and negligible affinity for D-glucose, sucrose, D-xylose, D-fructose and D-raffinose. Lectins isolated from infected seedlings, from seedling, treated by JA, differed from control plants by a decrease of affinity for N-acetylglucosamine, D-galactosamine and D-fructose-6-phosphate but this affinity was higher in variety 'Nikonia odeska' compared to variety 'Lastivka odeska' (Table 2). These data indicate qualitative changes of lectin proteins at the infection by *Fusarium graminearum* and action of JA. Such changes in the carbohydrate specificity of soluble lectins of wheat seedlings at the influence of pathogen and JA may be caused conformational transformation of proteins as a result of which the accessibility of sugars to other carbohydrate-binding centers changes.

Thus, the obtained results showed that the activity of soluble lectins increased significantly in the seedlings of resistant variety and decreased in the susceptible variety at the infection by *Fusarium graminearum.* A similar picture was observed when we were studying the activity of cell wall lectins of wheat [13, 17, 18]. It indicates the participation of these proteins in formation of protective reactions of wheat against this pathogen. This may be related to the features of the component composition of soluble lectins, and the accumulation of lectin components with a molecular mass of 67 and 60 kDa in resistant wheat variety. Plant lectins can be composed of multiple lectin domains with different carbohydrate-binding properties [19]. The availability of carbohydrate-binding centers in the structure of lectins may change, and as a result, their ability to interact with the substrate (sugars) can increase or decrease at the infection by phytopathogen and at the action of JA.

The changes in the carbohydrate specificity of lectins at the action of *Fusarium graminearum* and JA that we discovered not only characterize the features of the "lectin-ligand" interaction in the studied plants but also allow us to assume a possible im-

Table 2. The concentration of carbohydrates (mM) that suppresses the activity of lectins isolated from the seedlings after infection by Fusarium graminearum and treatment by JA

Lectins	N-acetylglucosamine	D-galactozamin	D-fructose-6-phosphate	D-glucose	D-fructose	D-raffinose	D-xylose	Sucrose
			Variety 'Lastivka odeska'					
from control seedlings	8	15	31	1000	1000	1000	1000	1000
from seedlings, treated by JA	30.9	30.9	124.9	1000	1000	1000	1000	1000
from infected seedlings	30.9	30.9	124.9	1000	1000	1000	1000	1000
from infected seedlings after treatment by JA	30.9	30.9	124.9	1000	1000	1000	1000	1000
			Variety 'Nikonia odeska'					
from control seedlings	8	8	8	1000	1000	1000	1000	1000
from seedlings, treated by JA	16.5	31	123	1000	1000	1000	1000	1000
from infected seedlings	16.5	31	123	1000	1000	1000	1000	1000
from infected seedlings after treatment by JA	16.5	31	123	1000	1000	1000	1000	1000

Note. Inhibition of lectin activity is expressed in the minimum concentration of carbohydrates (mM), at which inhibition of hemagglutination was noted. Microtitration was performed in 3-fold repetition.

portant role of lectins, which they can play in the reception and transmission of signals of wheat plants upon infection of agents of fusariose and at the action of JA. It is known that lectins play an important role in the immune system of plants as PRRs. Carbohydrate structures on the surface of pathogens or released from host cells due to damage provoked by the pathogen are recognized by soluble or membrane-bound lectins that trigger a signaling cascade resulting in the induction of the defense mechanisms [20]. The response of wheat seedlings to the action of a protein synthesis inhibitor (cycloheximide) and the results of our previous studies on the character of lectin gene expression [18] indicate the presence of various ways of regulating the level of lectin. It is possible that changes in the lectin activity in vegetative wheat plants at the influence of various factors can be controlled both at the transcriptional, post-transcriptional and post-translational levels [4]. This situation is possible because wheat lectin is a protein characteristic of plants throughout ontogeny, and, most likely, a certain reserve of lectin mRNAs and lectin precursors is always present in cells, which once again confirms the vital importance of this protein for wheat plants. Changes in lectin activity at the action of JA suggest its participation in the processes of regulating the activity of the soluble fraction of wheat lectins.

*Conclusion*s. Therefore, it has been established that wheat varieties with different levels of resilience to *Fusarium graminearum* are characterized by contrasting activity and peculiarities of biochemical characteristics of soluble lectins at the infection by pathogen and action of JA. The changes in lectin activity at the action of JA suggest its participation in the processes of regulating the activity of the soluble fraction of wheat lectins. It is supposed that the content of soluble lectins in wheat seedlings under the influence of the studied factors could be regulated at the level of protein biosynthesis processes.

The obtained results may be of interest for the explanation and development of theoretical ideas of formation mechanisms of the protective responses of the wheat plants at the fungal infection, role of soluble lectins in these processes, structure and properties of these proteins and for development of biochemical methods for assessing and increasing the resiliency of wheat varieties to fungal pathogens, fusariosis in particular.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ХАРАКТЕРИСТИКА ЛЕКТИНІВ ІЗ ІНФІКОВАНИХ *FUSARIUM GRAMINEARUM* ТА ОБРОБЛЕНИХ ЖАСМОНОВОЮ КИСЛОТОЮ ПРОРОСТКІВ ПШЕНИЦІ

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Фузаріоз — одне з найсерйозніших захворювань пшениці, яке викликається рядом грибів *Fusarium*, які вражають колосся, знижуючи врожайність зерна. Важливу роль у захисних реакціях рослин на біотичні фактори відіграють лектини, які специфічно зв’язують вуглеводні ліганди різної хімічної природи та жасмонова кислота (ЖК) як ключовий регулятор розвитку рослин. Метою дослідження було визначення активності та біохімічних характеристик розчинних лектинів у проростках пшениці сортів “Ластівка одеська”, з високою стійкістю до *Fusarium graminearum* та “Ніконія одеська”, чутливої до *Fusarium graminearum*. Проростки пшениці вирощували на середовищах, що містять патогенну інфекцію, або розчин ЖК. Лектини очищали за допомогою афінної хроматографії та розділяли за допомогою електрофорезу в 15% PAGE. Активність лектину визначали методом гемаглютинації трипсинізованих еритроцитів крові. Визначе-

но молекулярну масу основних компонентів лектинів проростків сорту “Ластівка одеська” – 67, 60, 45 кДа та основного компонента лектинів проростків сорту “Ніконія одеська” – 45 кДа. Лектини, виділені з контрольних необроблених проростків, мали переважну спорідненість до N-ацетилглюкозаміну, D-галактозаміну та D-фруктозо-6-фосфату. Показано, що у разі інфікування збудником, так і при обробці ЖК активність лектину у проростків стійкого сорту “Ластівка одеська” була підвищена, а у проростків сприйнятливого сорту ‘Ніконія одеська’ – знижена порівняно з контролем. У разі спільної дії патогена та ЖК активність лектину у проростків сприйнятливого сорту підвищувалася порівняно з інфікованими проростками. Отримані результати можуть бути використані для розробки біохімічних методів оцінки ступеня стійкості сортів пшениці до фузаріозу.

Ключові слова: пшениця, *Fusarium graminearu*m, стійкість до фузаріозу, розчинні лектини, спорідненість до вуглеводів, жасмонова кислота.

References

1. El Chami J, El Chami E, Tarnawa Á, Kassai KM, Kende Z, Jolánkai M. Effect of Fusarium infection on wheat quality parameters. *Cereal Res Commun.* 2023; 51(1): 179-187.
2. Zhu Z, Hao Y, Mergoum M, Bai G, Humphreys G, Cloutier S, Xia X, He Z. Breeding wheat for resistance to Fusarium head blight in the Global North: China, USA, and Canada. *Crop J.* 2019; 7(6): 730-738.
3. Jain S, Kumar A. The pathogenesis related class 10 proteins in plant defense against biotic and abiotic stresses. *Adv Plants Agric Res.* 2015;2(7):305-314.
4. Santos AFS, da Silva MDC, Napoleão TH, Paiva PMG, Correia MTS, Coelho LCB. B. Lectins: Function, structure, biological properties and potential applications. *Curr Top Pept Protein Res.* 2014; 15: 41-62.
5. Oliveira MV, Osterne VJS, Lossio CF, Serna S, Reichardt NC, Nascimento KS, Van Damme EJM, Cavada BS. Purification and characterization of a highly thermostable GlcNAc-binding lectin from *Collaea speciosa* seeds. *Int J Biol Macromol.* 2021; 193(Pt B): 1562-1571.
6. Itakura Y, Nakamura-Tsuruta S, Kominami J, Tateno H, Hirabayashi J. Sugar-Binding Profiles of Chitin-Binding Lectins from the Hevein Family: A Comprehensive Study. *Int J Mol Sci.* 2017; 18(6): 1160.
7. Naithani S, Komath SS, Nonomura A, Govindjee G. Plant lectins and their many roles: Carbohydrate-binding and beyond. *J Plant Physiol.* 2021; 266: 153531.
8. Breitenbach Barroso Coelho LC, Marcelino Dos Santos Silva P, Felix de Oliveira W, de Moura MC, Viana Pontual E, Soares Gomes F, Guedes Paiva PM, Napoleão TH, Dos Santos Correia MT. Lectins as antimicrobial agents. *J Appl Microbiol.* 2018; 125(5): 1238-1252.
9. Van Damme EJM. 35 years in plant lectin research: a journey from basic science to applications in agriculture and medicine. *Glycoconj J.* 2022; 39(1): 83-97.
10. Lannoo N, Van Damme EJM. Lectin domains at the frontiers of plant defense. *Front Plant Sc*i. 2014; 5: 397.
11. Ma QH, Tian B, Li YL. Overexpression of a wheat jasmonate-regulated lectin increases pathogen resistance. *Biochimie*. 2010; 92(2): 187-193.
12. Babayants OV, Babayants LT. Bases of breeding and methodology of assessments of wheat resistance to pathogens. Odessa: VMV, 2014. 401 p. (In Russian).
13. Molodchenkova O.O., Adamovska V.G. Wheat defense reactions at the action of *Fusarium graminearum*, salicylic and jasmonic acids. *Plant Physiol Genet.* 2015; 47(5): 447-458. (In Ukrainian).
14. Lutsik MD, Panasyuk EN, Lutsik AD. Lectins. Lviv: Vishcha school, 1981. 155 p. (In Russian).
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951; 193(1): 265-275.
16. Osterman LA. Methods for the study of proteins and nucleic acids: Electrophoresis and ultracentrifugation. M.: Nauka, 1981. 288 p. (In Russian).
17. Molodchenkova OO, Adamovskaya VG, Tsiselskaya LY, Sagaydak TV. Isolation and properties of lectins from cell walls of wheat seedlings infected by *Fusarium graminearum*

and treated by salicylic acid. *Ukr Biokhim Zhurn.* 2010; 82(5): 111-117. (In Russian).
18. Molodchenkova OO, Ryshchakova OV. Participation of lectins in the formation of plant protective reaction of cereals. New stages of development of modern science in Ukraine and EU countries: Collective monograph. Riga: Baltija Publishing", 2019. P. 205-230.
19. Tsaneva M, Van Damme EJM. 130 years of Plant Lectin Research. *Glycoconj J.* 2020; 37(5): 533-551.
20. Schutter KD, Van Damme EJM. Protein-carbohydrate interactions as part of plant defense and animal immunity. *Molecules.* 2015; 20(5): 9029-9053.