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Dynamics and Polypeptide Composition of Lectins in Wheat Germs Infected With Plant Pathogenic Fungi.

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ABSTRACT

The activity of the soluble and associated with the cell wall lectins and the polypeptide composition of proteins of the cell wall of the winter wheat Kazanskaya 560 infected with the specific phytopathogene *Fusarium oxysporum Schlectend.:Fr.* and non-specific mold fungus *Aspergillus niger* were investigated. The activity of lectin proteins under influence of *A. niger* was increased 24 hours after contamination and under influence of *F. oxysporum* - after 72 hours. In the elution profile of the cell wall proteins of the control plans Kazanskaya 560 the lectin activity was detected in the fractions of proteins with the molecular weight 89, 77, 61, 54, 43, 37 kDa. In the case with *A.niger* the cell wall lectins with the molecular weight 72, 61, 47, 37 kDa were found. At the same time the content of the low molecular polypeptides that apparently participate in the reaction and neutralization of nonspecific phytopathogenes was increased. By infecting with *F.oxysporum* all lectin proteins peculiar to the control plants were preserved and new ones with the molecular weight 72, 69, 63 and 47 kDa appeared. It is likely that both the high- and low molecular proteins participate in the response reaction to infecting with specific fungous pathogenes.

Keywords: *Triticum aestivum* L., fungous pathogenes, lectin activity, resistance.

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INTRODUCTION

Diseases caused by fungous phytopathogenes result in great loss in the grain harvest and limit the increase in the output of agricultural products. In this regard the study of the molecular mechanisms of formation of resistance to plant diseases, in particular such as gain in considerable importance particular such as fusarium disease gain in particular importance.

The mechanism of the plant resistance to fungous pathogens represent complex and under investigated processes. An important role in establishment of relations between the host plant and pathogen is assigned to lectins. The studies of different researchers confirm the participation of lectins in the plant immunity mechanisms [1-4].

Lectin is a specific class of proteins featuring the ability to be reversibly and specifically associated with the carbohydrate residues of different chemical origin. Since the cell walls of phytopathogenes contain carbohydrate components this property of lectins may ensure the plant ability to recognize a wide range of phytopathogenes even by the slight differences in the carbohydrate structure of the microorganism surfaces. Lectins contained in the cell walls may be considered as receptors in the cell-cell interaction with phytopathogenes.

There is quite much information about involvement in formation of protective plant reactions of the so-called classical lectins located in cytoplasm and vacuole. These lectins may be associated with spores of pathogenic fungi and other infectious structures of pathogens and inhibit the growth and development thereof. However, the physiological-biochemical interactions between the pathogen and the host plant during the process of infecting feature complex temporal and spatial dynamics which complicates the decoding of mechanisms of interactions between two organisms.

In this regard the objective of our research is determination of effect of phytopathogenic fungi on the activity and polypeptide composition of the cell wall lectins in the winter wheat germs.

MATERIALS AND METHODS

The object of the research was the roots of the winter wheat germs *Triticum aestivum* L. of the Kazanskaya 560 grade. Prior to sowing the germs were sterilized with the use of 70% ethyl alcohol, rinsed with sterile distilled water and grown in cuvettes with the use of tap water under artificial lighting with the 12-hours photoperiod. As the infectious agents *Fusarium oxysporum Schlectend.:Fr.* and the saprophytic mold fungus *Aspergillus niger* were used. The choice of phytopathogenes was determined by their specialization, i. e., confinedness to a specific nutritious substrate – *Fusarium* fungi (*F. oxysporum, F. culmorum, F. avenaceum, F. moniliforme*) being agents of the root blight of wheat [5] while *A.niger* is the excitant of the black mold rot of onion and garlic. The fungi were extracted from the wheat germs of the grades and selective series released for the Republic of Tatarstan.

The seven-days old wheat germs during one hour were inoculated in the suspension of the pathogenic spores ($1 \bullet 10^4$ CFU/cm^3) after which the plants continued to grow in the laboratory conditions. The initial level of activity of the soluble and associated with the cell wall lectins was determined 1 hour after completion of infecting. Further samples were taken each 24 h during 4 days.

The assessment of the infection rate of the plant material was performed according to the method [6]. Soluble lectins were extracted with the use of 0,05н HCl, lectins of the cell wall – by 0,05% solution of triton-X-100 according to the procedure [7]. For counting of the protein content the Bradford method was used [8]. The lectin activity was estimated by the minimum amount of the protein causing hemagglutination $(\mu g \text{ of protein/ml})^{-1}$[9]. Analysis of the cell wall proteins was performed with the use of a chromatographic system with a column filled with Sephadex G-150 [10]. The tests were performed in three biological replications. The test resukts are presented in the Figures as the mean arithmetical and standard mean square errors.

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RESULTS AND DISCUSSION

As can be seen from the Figures 1 and 2 in response to inoculation of the germs by the pathogen spores the change in the activity of the soluble and associated with the cell wall lectins was observed.

In the plants infected with *F. oxysporum* the activity of the soluble lectins was changed by phases, i. e., we observed two peaks of the increase in activity: 24 and 72 hours after infecting (Figure 1) alternating with substantial drops in activity of soluble lectins after 48 h and 96 h. Such increase of the lectin activity may indicate the involvement of the soluble lectins in the response reaction of plants to infecting. In case with *A.niger* the activity of soluble lections were decreased already 1 h after plant treatment with the pathogen spores, and 24 h after infecting – was increased by 37% as compares to the control samples and then decreased again throughout the experiment (Figure 1).

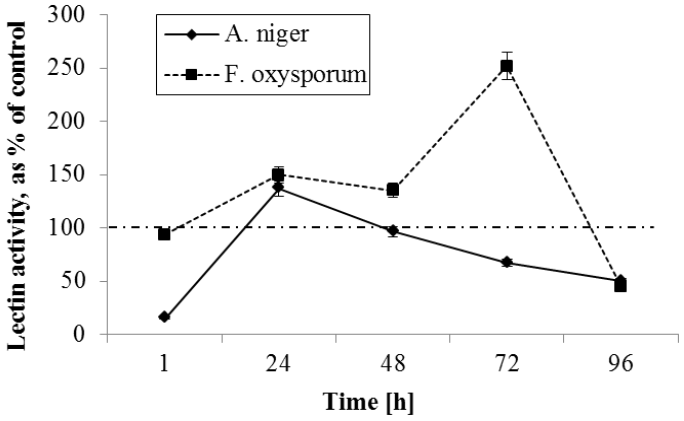

Figure 1: Dynamics of activity of lectins of the winter wheat Kazanskaya 560 infected with phytopathogenic fungi.

The activity of the cell wall lectins varied under influence of phytopathogenes as follows. Infecting with *F. oxysporum* caused inhibition of the cell wall lectin activity during the first two days after incubation of plants in the fungi spore suspension and then on the third day the significant increase in the activity of this group of proteins took place. After 96 h the level of the cell wall lectin activity gain became lower as compared to the control plants (Figure 2).

A. niger increased the activity of lectins associated with the cell wall only 24 h after the commencement of the experiment; during further measurements no increase in the activity of these proteins was observed (Figure 2).

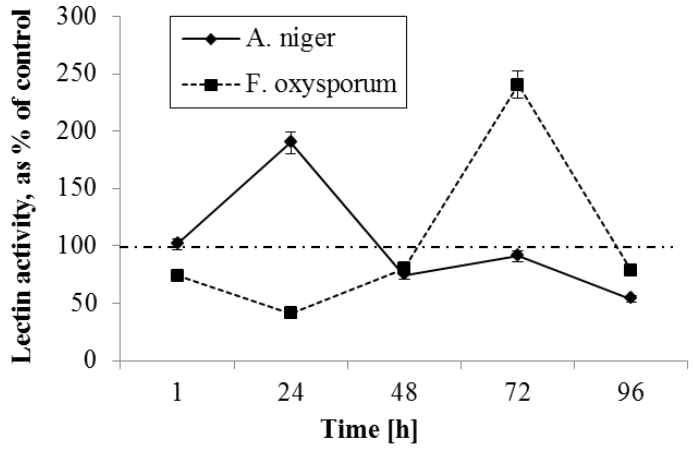

Figure 2: Dynamics of activity of the cell wall lectins of the winter wheat Kazanskaya 560 infected with phytopathogenic fungi.

Thus, by treatment of the germs of the winter wheat Kazanskaya 560 with the suspension of the pathogenic fungi spores the change in the activity of both the soluble and associated with the cell wall lectins was observed. The non-specific pathogenic fungus *A.niger* caused the peak of activity of the soluble and associated with the cell wall lectins after 24 h and the specific *F. oxysporum* - 72 h after infecting the germ roots. The data obtained speak of the participation of these protein groups in the reactions of the plant susceptibility/resistance as the qualitative change of the level or activity of lectins is one of teh indicators of involvement thereof in the pathogenesis processes [11].

A number of studies shows that infecting the wheat germs with phytopathogenes results in significant accumulation of the wheat germ agglutinin that increases as far as the diseases develops [12]. At the same time the dynamics of the lectin content features at transient peak and later and more lasting qualitative changes, and if the first one in most cases correlates to the plant resistance the latter changes reflect the disease symptoms, i.e., the plant susceptibility to the pathogen. The changes of activity of the soluble and associated with the cell wall lectins infected with *F. oxysporum* and *A.niger* that where detected during our experiments most probably speak of the wheat resistance to *A.niger* and susceptibility to *F. oxysporum*.

According to the established concepts it is the cell wall lectin that participates in the processes of phytopathogen recognition and establishment of relationships within the system the host plant – phytopathogen. During the recent years the lectin domains were found in the composition of receptor kinases. The extracellular domain of the lectin-like receptor kinases of plants is similar to the soluble lectins of legumes and Arabidopsis or chitinase. Expression of genes of some lectin-like kinases increases by infecting treatment with elicitors [13]. Normally, lectin of receptor complexes are present in a cell in small amounts, however, during the pathogenesis process their quantity may increase by times. These proteins participate in the process of specific recognition both inside and outside the cell.

Therefore, in the next series of experiments we performed chromatographic separation of the cell wall proteins of the Kazanskaya 560 wheat germ roots infected with phytopathogenes. In the elution profile of the cell wall proteins of control plants Kazanskaya 560 the lectin activity was detected in the protein fractions with the molecular weight 89, 77, 61, 54, 43, 37 kDa (Figure 3a).

Infecting the plants of the Kazanskaya 560 grade with *A.niger* and *F.oxysporum* resulted in the change of the entire elution profile of the cell wall proteins.

In the case with *A.niger* the composition of the cell wall lectins has changed as follows: the proteins with the molecular weight 89, 77, 54, 43 kDa disappeared and two new lectin proteins -72 kDa and 47 kDa appeared. At the same time the content of high molecular proteins reduced and the concentration of low molecular polypeptides 47 kDa and 37 kDa that were forming the two specific peaks increased (Figure 3b). We assume that induction of production of low molecular proteins –phyto-agglutinins is necessary for effective interaction and neutralization of nonspecific fungous pathogens.

The lectin 37 kDa may be the classical wheat lection – wheat germ agglutinin (WGA). As is known, along with cytoplasm WGA may be located within the space between the cell membrane and cell wall [14]. Apparently, the WGA the rapid accumulation of which takes place under different unfavorable conditions relates to the extractable proteins the extraction of which into the outer environment may be relevant in terms of prevention of the weakened plants from the possible soil infection [15].

By infecting with *F.oxysporum* the number of peaks was increased as compared to control samples: all the lectin proteins peculiar to the control plant were preserved and the new ones with the molecular weight 72, 69, 63 and 47 kDa appeared (Figure 3c). Just like by infecting with *A.niger,* the specific phytopathogen significantly increased that content of the lection 37 kDa. Apparently, in the response reaction to infecting with the fungous pathogenes both the high- and low molecular proteins. Previously we showed that the lectin proteins with the molecular weight 72 and 69 kDa appeared in the wheat cell wall through hypothermia [16]. The appearance of these proteins may be referred to the triggers of formation of nonspecific protective reactions of the plant cells.

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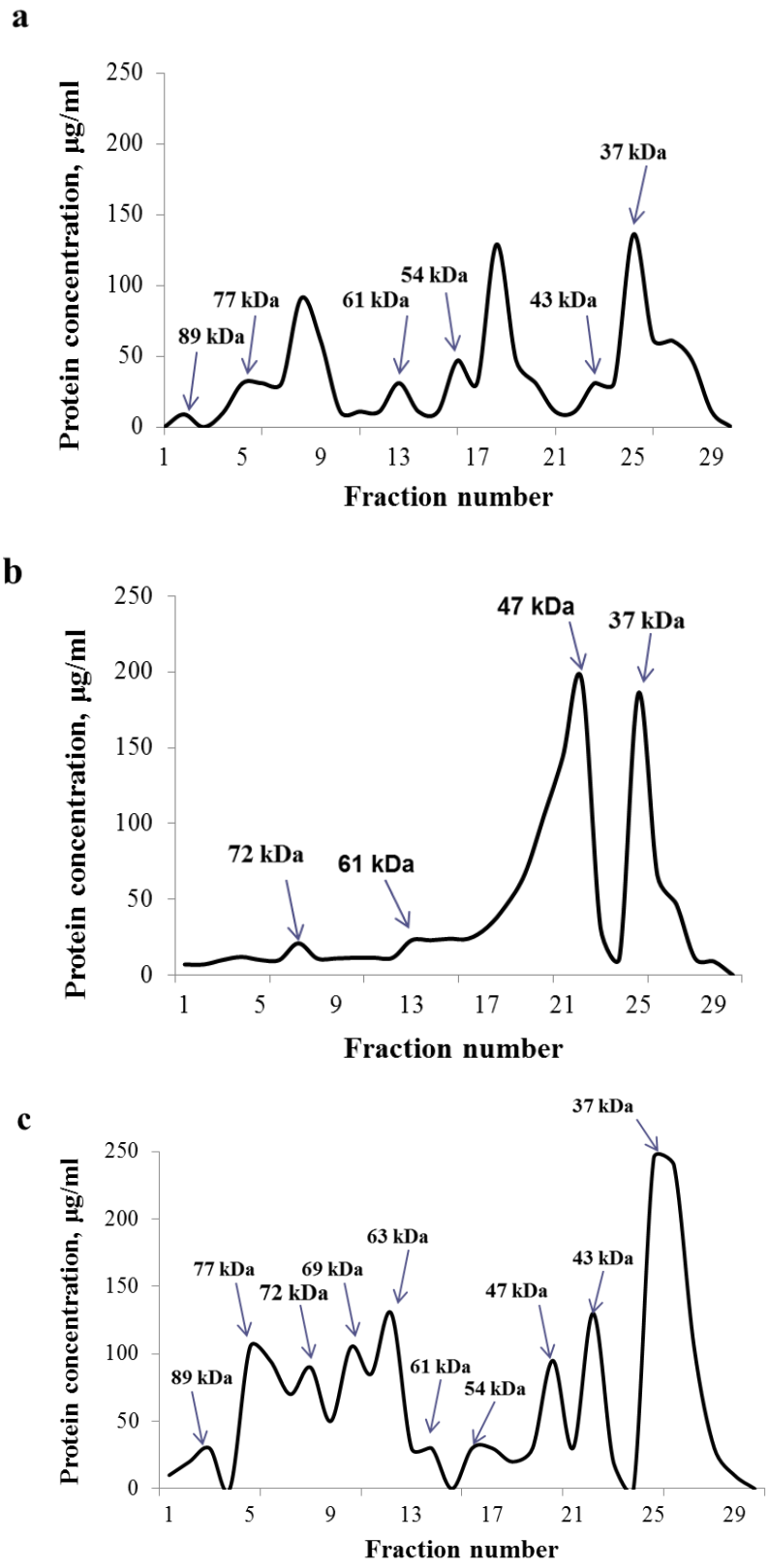

Figure 3: Elution profile by gel-penetrating chromatography of the cell wall proteins of the winter wheat germs Kazanskaya 560: a – control, b - *A.niger*, c - *F.oxysporum.*

CONCLUSIONS

- Infecting the winter wheat germs changed the activity of the soluble and associated with the cell wall lectins. At that under influence of *A.niger* the significant increase in the lectin activity was observed after 24 hours and by influence of *F.oxysporum* - 72 hours after infecting which speaks of the better plant resistance to *A.niger*.

- It was found that the mold micromycete *A.niger* caused in the plants of Kazanskaya 560 grade the increase in the content of low molecular lectin cell wall proteins and the specific phytopathogen *F.oxysporum* – that of the low- and high molecular lectins. Apparently, these two groups of proteins participate in recognition and inactivation of pathogens: the low molecular ones interact with non-specific agents and the high-molecular ones with the specific.

SUMMARY

Thus, the research performed showed that infecting plants both with specific and non-specific phytopathogenes resulted in qualitative and quantitative changes of the cell wall proteins. In the roots of the Kazanskaya 560 germs by infecting with non-specific phytopathogenes the content of low molecular proteins in the cell wall was increased and by infecting with specific ones – both of the high- and low molecular. These data speak of participation of these two groups of proteins in the interaction with different pathogenic microorganisms – the high molecular ones participate in recognition of pathogenic microorganisms and the low molecular ones – of non-pathogenic.

At the same time the quantitative and qualitative changes in the composition of the cell wall proteins may be the result of the damaging effect of phytopathogenes on a plant body. However, the high specificity of lectins, in particular of the wheat germ agglutinin to the chitin oligomers allows referring the changes in the polypeptide composition of the cell wall to the protective reactions of the wheat germs during pathogenesis that promotes to the increase in their resistance to biotic stresses.

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