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Influence of Micromycetes on Lectin and Antioxidant Activity in Wheat Germs.

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ABSTRACT

We have performed a research of infection contamination of spring wheat plants of Omskaya 33 variety with fungal pathogen on content of malondialdehyde (MDA), activity of lectin proteins and antioxidant enzymes: catalase, ascorbate-peroxidase and soluble peroxidase. The seeds were inoculated in suspension with spores of phytopathogens ($1 \bullet 10^4$ CFU/cm^3) within one day. Infectious agents were represented by Fusarium root rot *Fusarium (F.) oxysporum Schlectend.:Fr.* And saprophyte mold fungus *Aspergillus niger.* Fungi were sustracted from of spring wheat's seeds, area-specific for varieties of the Republic of Tatarstan and selected lines. It has been shown that both pathogenic microorganisms inhibited activity of soluble lectins, but only phytopathogen *F. oxysporum* caused increased activity of lectins, located in cell walls. At the same time *F. oxysporum* increased content of malondialdehyde, which is evidenced by oxidative burst in cells of wheat's roots. Activity of antioxidant enzymes was differently changed under the influence of pathogenic agents: *A. niger* increased activity of catalase and ascorbate-peroxidase, while *F. oxysporum* increased activity of soluble peroxidase. It appears that depending in phytopathogen's specialization, plants activate various signal systems, necessary for formation of defense reactions.

Keywords: Spring wheat, phytopathogens, lectins, lipid peroxidation, antioxidant enzymes.

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INTRODUCTION

Infectious diseases, caused by fungous phytopathogens, cause reduction in yields of cereal crops and limit increase of agricultural goods' production. In this connection study of molecular mechanisms of formation of resistance to plants' diseases becomes essential, in particular, with regards to fusarial rot.

Since wheat's lectin shows high specificity to N-acetylglucosamin and chitin's oligomers, an assumption was put forward about its defensive role in contamination of plants with chitin-iferous phytopathogens [1]. Intercellular recognition is evidently the first stage of interrelation of host plant and microorganism, and the role of receptors in recognition of foreign infectious structures may be performed by lectins that are localized in cellular wall [2].

It is known that pathogenesis of pants is accompanied by intensification of oxidative processes, which play an important role for realization of defensive reactions [3]. Newly formed active forms of oxygen may influence metabolic processes of both plant and phytopathogen. However, stable plants may compensate oxidizing processes at the account of higher content of antioxidants.

Since mechanisms of plants' stability to specific and non-specific phytopathogens are presented by complex and understudies processes, the aim of our work was to find out the influence of contamination with specific and non-specific phytopathogens on pro-antioxidant status of spring wheat.

MATERIALS AND METHODS

The object of our research was represented by germs of spring wheat *Triticum aestivum* L. of Omskaya 33 variety. The seeds were sterilized with 70% ethyl alcohol, washed with sterile distilled water and inoculated in suspension of phytopatogenic spores ($1 \bullet 10^4$ CFU/cm^3) within one day. Infectious agents were represented by Fusarium root rot *Fusarium (F.) oxysporum Schlectend.:Fr.* And saprophyte mold fungus *Aspergillus niger.* Fungi were subtracted from spring wheat's seeds, area-specific for varieties of the Republic of Tatarstan and selected lines. After the seeds were contaminated, they were grown in cuvettes in piped water at artificial light with 12-hour photoperiod within 7 days. Control plants were grown on piped water. Assessment of contamination of plant material was performed according to method [4]. Roots of 7-day germs were used for determination of content of malondialdehyde, activity of lectin proteins and antioxidant enzymes.

Soluble lectins were extracted with 0,05% HCl and lectins of cellular walls were extracted with 0,05% solution of Triton X-100 according to method described in the work [5]. For determination of quantity of proteins Bradford method [6] has been used. Lectin activity was calculated according to minimal quantity of proteins that causes agglutination of erythrocytes (mcg of protein/ml)$^{-1}$ [7]. The level of lipid peroxidation (LP) was defined based on accumulation of its product malondialdehyde (MDA) [8]. Activity of soluble peroxidase was defined per method [9], activity of ascorbate oxidase was defined according to method [10], and activity of catalase was defined according to method [11]. Experiments were performed in three biological replications. Results of experiments are presented on pictures and in tables as arithmetic average and their standard errors.

RESULTS AND THEIR DISCUSSION

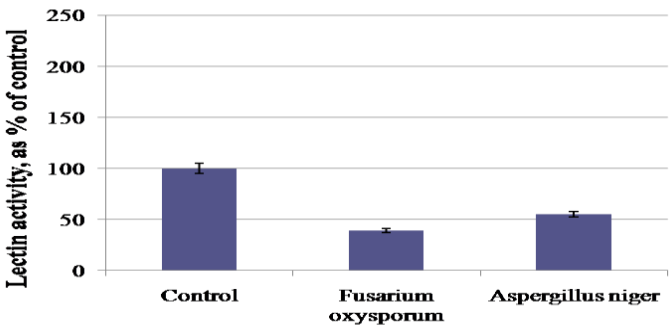

Figure 1: Activity of soluble lectins of spring wheat of Omskaya 33 variety while infected with pathogenic fungi.

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As it is known, an evidence of participation of lectin proteins in formation of mechanisms of resistance or vulnerability of plants to phytopathogens is the change of their content or activity. In our experiments activity of soluble lectins decreased under the action of both specific phytopathogen *F. oxysporum*, and non-specific phytopathogen *A. niger*, while inhibitive effect of *F. Oxysporum* on activity of soluble lectins was more expressed in comparison with *A. Niger* (Figure 1).

Similar effect was caused by studied pathogens on activity of cellular wall's lectins: *F. Oxysporum* inhibited their activity, while in the case with *A. niger* activity of lectins of cellular wall was similar to the one of control plants (Figure 2). The data received proves that lectins participate in the process of interaction of cells with specific and non-specific pathogens.

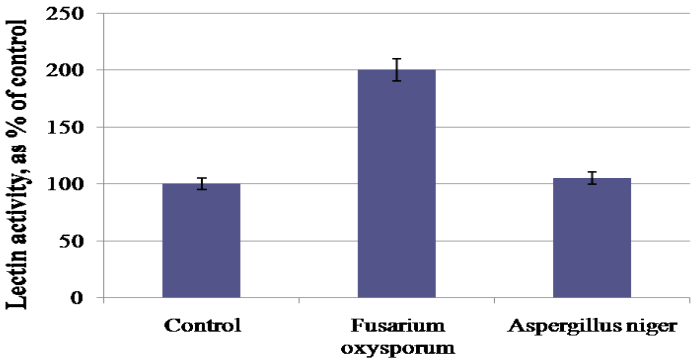

Figure 2: Activity of lectins of spring wheat's cellular wall (variety Omskaya 33) while infected with pathogenic fungi

As it is known, lectins, extracted from various plants, are able to specifically interact with various structures of fungous and bacterial cells, like: *Phytophtora infestans*, *P. megasperma var. sojae*, *P. megasperma f. sp. glycinea*, *Verticillium dahliae*, *Helminthosporium sativum*, *Ustilago tritici*, *Tilletia tritici*, *Ceratocystis fimriata*, *Trichoderma viride*, *Fusarium solani*, *Argobacterium tumefaciens*, *Pseudomonas solanacearum*. In the system "potato – *Phytophtora infestans"* interaction between lectins and pathogenic fungi is performed according to principle "receptor-ligand". At this, vegetable glycoprotein performs the functions of receptor, which interacts with ligand of phytopathogen while forming intercellular contact, which triggers reaction of hypersensitiveness of cells [12].

Basing on the data we obtained regarding counter reaction of lectins' activity on contamination, we may suppose that spring wheat plants of Omskaya 33 variety are more susceptible to *F. oxysporum* in comparison with fungus *A. niger*. This is proved by results of experiments dedicated to determination of influence of micromycetes on intensity of lipid peroxidation (LP) of membranes (Figure 3).

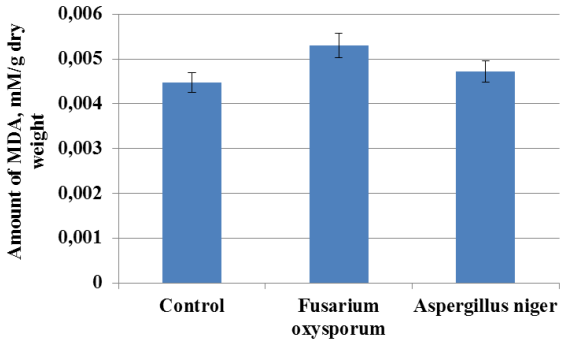

Figure 3: The level of lipid peroxidation (content of malondialdehyde) of spring wheat, variety Omskaya 33.

Activation of membranes' LP process, caused by intensified generation of active forms of oxygen (AFO), is one of the most recent effects, seen at plants when being exposed to various stress factors, including contamination with phytopathogens [13]. One of relatively stable products of lipid peroxidation of membrane lipids in cell is malondialdehyde (MDA), accumulation of which may help identifying the level of oxidizing stress

[14]. As it can be seen from Figure 3, phytopathogen *F. Oxysporum* increases intensiveness of formation of MDA in roots of spring wheat, while *A. niger* didn't influence this indicator.

As it is known, hydrogen peroxide H_2O_2 that forms at pathogenesis in plants' cells performs signal and defensive functions [3]. Various antioxidant enzymes participate in neutralization of H_2O_2, including catalase and peroxidase.

According to our researches, in the roots of wheat germs catalase's activity increased when being infected with *A. niger*, while *F. oxysporum* decreased activity of catalase (Figure 4).

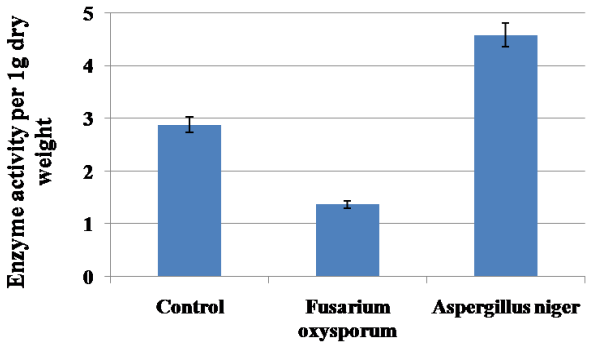

Figure 4: Activity of catalase of spring wheat, variety Omskaya 33.

It is known that pathogens or elicitors may not only activate, but also hamper expression of some genes, for example, of catalase and ascorbate peroxidase [15]. At the same time, activation of catalase intensifies pathogen's virulence at the account of decreasing concentration of H_2O_2 and suppression of oxidative burst [16]. Maintenance of certain level of H_2O_2 in wheat cells may be one of defensive mechanisms with reference to highly pathogenic agent *F. oxysporum.* On the other hand, according to some literature, in compensational mechanism of antioxidant defense a great role is played by ascorbate peroxidase [17], and effective defense from AOS is performed only at concomitant increase of catalase and ascorbate peroxidase's activity of [18]. In our experiments in response to infectious contamination with both *A. niger*, and *F. oxysporum*. It should be noted that lack of catalase's activity is often compensated with increase of activity of ascorbate peroxidase [19], which is apparently seen by us in case with *F. oxysporum.*

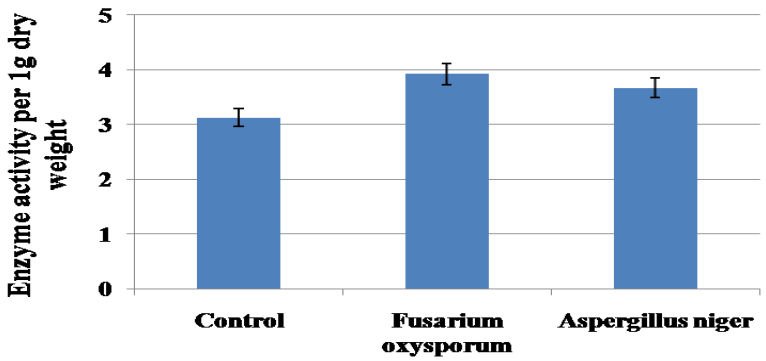

Figure 5: Activity of ascorbate peroxidase of spring wheat, variety Omskaya 33

In response to infectious contamination with phytopathogens activity of soluble peroxidase increased only under influence of *F. Oxysporum* (Figure 6). It should be noted that activation of peroxidase under the influence of infection is a typical biochemical response reaction of pants, which may be used to judge of plants' resistance. However, increased activity of peroxidase doesn't always correlate with stability and doesn't often stabilize it.

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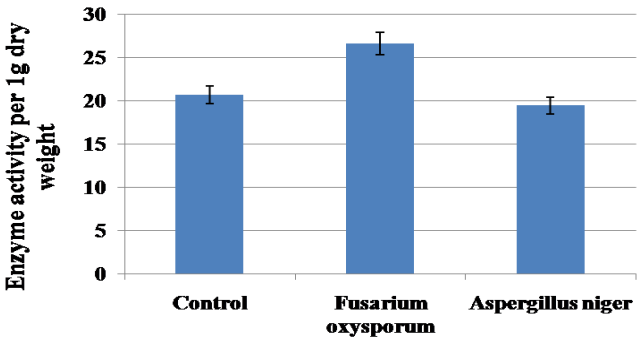

Figure 6: Activity of soluble peroxidase of spring wheat, variety Omskaya 33

SUMMARY

It has been stated that infectious contamination of wheat germs with phythopathogenic fungi *Fusarium spp.* and *Aspergillus niger* causes various reactions of antioxidant system of plants' defense. Oxidizing process, caused by *Aspergillus niger* fungi is mostly neutralized by enzymes ascorbate peroxidase and catalase, while in case of contamination with *Fusarium spp.* defense from AOS is performed by peroxidases.

CONCLUSION

As it can be seen from the above, following the experiments performed, we have stated that *F. oxysporum* causes oxidative burst in cells of wheat's roots, in extinction of which peroxidases take part. Peroxidases stimulate the processes of lignification and suberinization of cellular walls in the process of pathogenesis [20], which may represent one of mechanisms of defense from specific pathogens. In reponse to contamination with non-specific infectious micromycete *A. Niger* content of LP products didn't change, while catalase and ascorbate peroxidase were activated. Apparently, depending on specialization of phytopathogen, plants activate various signal systems that are necessary for formation of defensive reactions.

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