Effect of Septoria on the specific activity of Glycines max (L.) Merr hydrolases

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Abstract. Soybean (Glycines max (L.) Merr) is culture-sensitive to diseases and infections. The article states that a detailed study of the physiological processes at the biochemical level is required. It induces oxidative stress to create a cultivar resistant to S. glycines. Enzymes play a leading role in adaptation to stressors. The hydrolase enzymes we have selected for the study have selective neutral behaviour (concerning S. glycines on the studied soybean varieties), which allows them to be used as protein markers for the selection of the most resistant cultivar to S. glycines at the molecular level.

1. Introduction
One of the priority directions of modern biology is to decipher the ways of activating defence reactions in plants infected with pathogens. In-plant tissues, several signalling molecules have been identified that trigger a cascade of biochemical responses in the plant organism upon contact with a pathogen [1]. It is assumed that signalling molecules both participate in the formation of primary responses and determine the nature of subsequent biochemical and physiological processes, which determine a degree of adaptation and resistance of plants to the action of pathogens [2]. Fungal pathogenesis is the main cause of yield decline in many parts of the world. When a plant interacts with a pathogen, cross-linking of biochemical reactions occurs. A reaction is initiated when a pathogen comes into contact with the surface of the plant and it begins to secrete some non-protein and protein effectors called pathogen-associated molecular forms. Invasion of pathogens is successful when translocated hosts suppress the plant's immune system, thereby facilitating pathogen penetration [3,4].

Septoria (S. Nodorum, S. Soybean (Glycines max (L.) Merr) is culture-sensitive to diseases and infections that affect the plant at all stages of growth, i.e. from the beginning of seed germination to the formation of beans. Septoria, which is caused by fungi of the genus Septoria (S. Nodorum, S. Graminum, S. Tritici, S. Hordei, S. Secalis, etc.), is a harmful pathogen of G. max all over the world. Scientists estimate the annual loss of soybean yield from this disease on average from 10 to 30%, and in some cases from 60 to 70% in seriously damaged areas [5].

Measures to control the pathogen include the development of resistant varieties and a detailed study of physiological processes at the biochemical level, assessment of plant adaptation to the effects of factors inducing oxidative stress [6].

A critical condition for protection from pathogens by any living organism is the ability to perceive signals specific to an infection or a virus and depends on the existence of receptors, proteins or protein complexes that interact with signals of pathogenic origin and transfer them into cellular responses [7].
Enzymes play a leading role in maintaining intracellular homeostasis and adaptation to stressors [7]. For soybeans, the research on protective enzymes that neutralize abiotic and anthropogenic factors is of paramount importance. Thus, resistance testing can be based on the determination of specific enzyme activity.

The enzymes of the hydrolytic complex selected for research have wide substrate specificity and play an important role in the metabolism of lipids, carbohydrates, and nucleic acids. According to the information taken from scientific literature, ribonuclease (RNase) (EC 3.1) and acid phosphatase (EC 3.1.3.2) [8] are enzymes with wide substrate specificity. Ribonucleases are ubiquitous components of living cells and are involved in a wide variety of processes [9]. The genetic material of the majority of plant viruses is represented by RNA; therefore, it can be assumed that wound-induced extracellular RNases are one of the components of antiviral defence at the initial stages of infection [10]. Phosphatases are enzymes involved in the hydrolysis of phosphoric acid esters. They are extremely widespread in all living cells. The main amylolytic enzymes that make up the amylase complex include α-amylase, β-amylase (EC 3.2.1.1 and 3.2.1.2), glucoamylase, oligo-1,6-glucosidase, which are involved in starch hydrolysis, as a result, which produces products such as glucose, maltose and maltotriose [11, 12]. Enzymes of the esterase complex (EC 3.1.1) are involved in the hydrolysis of ester bonds, including lipids. In our laboratory, we are interested in studying the role of soybean oxidoreductases and hydrolases in mobilization situations under stress conditions caused by exposure to Septoria Glycines Hemmi. In this article, the research aims to study the specific activity of hydrolases (for example, amylase, esterase, ribonuclease, and acid phosphatase) of cultivated soybeans of various maturity groups and their resistance to S. glycines.

2. Materials and methods

The objects of the research were soybean seeds infected and not infected with septoria: Kruzhevnitsa, Sentyabrinka (early maturing, resistant to pathogenic fungus); Umka, Vereteika, Lydia (early maturing, moderately resistant to pathogenic fungus); Dauria, Zolushka (mid-season, resistant to pathogenic fungus); Lazurnaya (mid-season, medium-resistant to fungal diseases); Topaz (ultra-early maturing, resistance to pathogenic fungus is not yet known).

The selection of seeds infected with S. glycines was carried out in laboratory conditions by visual inspection of the seed material according to morphological characteristics, i.e., the presence on the cotyledons of rounded red-brown spots with a diameter of 6 ... 10 mm with numerous pycnidia, as well as uninfected seeds in terms of purity, germination, uniformity and phytosanitary condition of seeds (health, weediness).

The specific activity (ribonuclease, acid phosphatase, esterase and amylase complexes) and the concentration of malondialdehyde were determined in soybean seeds in two biological and three analytical replicates to analyze of plant response to stress caused by the fungal infection of Septoria glycines Hemmi.

The experiment scheme is as follows: for control, seeds were taken without infection with Septoria glycines Hemmi (1 - Kruzhevnitsa, 2 - Sentyabrinka, 3 - Vereteika, 4 - Lydia, 5 - Umka, 6 - Dauria, 7 - Zolushka, 8 - Lazurnaya, 9 - Topaz). For the experiment, seeds were infected with Septoria glycines Hemmi (1 - Kruzhevnitsa, 2 - Sentyabrinka, 3 - Vereteika, 4 - Lydia, 5 - Umka, 6 - Dauria, 7 - Zolushka, 8 - Lazurnaya, 9 - Topaz).

Soybean protein extract was obtained by homogenizing seeds (500 mg) in 0.15 M NaCl at 4° C for 15 minutes. The resulting extract was centrifuged at 3000 rpm for 15 min. Protein was determined by the Lowry method (1951).

MDA (malonic dialdehyde) content was carried out based on the property of this substance at high temperatures in an acidic medium to react with thiobarbituric acid (TBA), generating a coloured trimethylene complex by the guidelines specified in the workshop on plant physiology and biochemistry.

The activities of ribonuclease, acid phosphatase, esterases, and α- and β-amylase were determined spectrophotometrically on a spectrophotometer (Cary50 13620-08, Australia). The specific activity was
expressed in units per mg of protein and was measured according to methods for studying polymorphism of soybean enzymes.

The obtained experimental data were processed using Statistica 10 software, graphical data presentation was processed using Excel (2010). The results were expressed as a mean (n=6) ± standard deviation, differences were considered statistically significant at p <0.05.

3. Discussions

The earliest response of a plant organism to the introduction of a pathogen is the local generation of reactive oxygen species (ROS), i.e., an oxidative explosion that triggers a chain of subsequent defence reactions [13]. Thus, in plant tissues during pathogenesis, the concentration of free radicals (superoxide O₂-, hydroxyl OH-) and hydrogen peroxide (H₂O₂) sharply increases. Despite the presence of numerous experimental data on the participation of ROS in the induction of a protective response in plants, the sources and methods of generation of these compounds during pathogenesis remain poorly understood [14]. A degree of membrane damage under the action of ROS and H₂O₂ is usually judged by the level of lipid peroxidation (LPO). The rate of formation of MDA, a final product of LPO, serves as a marker of the level of oxidative stress in a plant cell [15].

In the course of our research, it was found that, in general, when the cultivated soybean is infected with Septoria, the MDA content increases relative to the control (figure 1). It should be noted that the greatest increase in the concentration of MDA was noted in short-season variety, resistant and moderately resistant to fungal diseases: Kruzhevnitsa, Umka, Vereteyka, and in the mid-season and moderately resistant to fungal diseases cultivar Lazurnaya soybean. Earlier, when studying the effect of heavy metals on soybean seedlings [10], we also noted an increase in the MDA content. The plant sensitivity to phytopathogens may result in the expression of genes coding for the synthesis of new proteins ("pathogenesis-related proteins" (PR)) by the host plant in response to the pathogen. The name "PR-proteins" was formed due to the proteins found in virus-infected plant tissues [16]. However, for each group of season duration, seeds of soybean varieties (Lydia (short-season variety)), Dauria (mid-season variety) and Topaz (ultra-short season varieties) were identified, in which the concentration of MDA remained at the control level, which indicates the stability of these varieties under conditions of infection with a fungal disease. Note that the soybean varieties Lydia is not only the most popular among local producers (25.5% of the total area of soybeans in the Amur Region), but it is also applied as a standard in research.
Figure 2. Specific activity of RNases of seeds of soybean varieties under conditions of septoria infection: 1 - Kruzhevnitsa, 2 - Sentyabrinka, 3 - Vereteyka, 4 - Lydia, 5 - Umka, 6 - Dauria, 7 - Zolushka, 8 - Lazurnaya, 9 - Topaz.

A decrease in specific activity was observed relative to the control at the analysis of the specific activity of RNases of soybean seeds under conditions of infection with the fungal disease S. glycines. Earlier, we have obtained data on a decrease in the specific activity of RNases under conditions of oxidative stress [17]. In the short-season, medium resistant to pathogenic fungus variety of cultivated soybean Lydia, an increase in specific activity was noted by 2 times, relative to control (Figure 2). It can be assumed that wound-induced extracellular RNases are one of the components of antiviral defence taking into account the fact that in most plant viruses the genetic material is RNA. Sangaev and his research team showed that tobacco plants with increased activity of extracellular ribonuclease are characterized by increased resistance to the tobacco mosaic virus [18]. This confirms the hypothesis about the participation of plant extracellular ribonucleases in the formation of resistance to viruses. Thus, our results indicate a decrease in the virus resistance of the studied soybean varieties under the influence of septoria, except the reference soybean variety Lydia.

Figure 3. Specific activity of acid phosphatases in seeds of soybean varieties under conditions of septoria infection: 1 - Kruzhevnitsa, 2 - Sentyabrinka, 3 - Vereteyka, 4 - Lydia, 5 - Umka, 6 - Dauria, 7 - Zolushka, 8 - Lazurnaya, 9 - Topaz.
The analysis of the specific activity of acid phosphatases showed a decrease in the specific activity relative to the control, while the greatest decrease was observed in mid-season, resistant and medium-resistant soybean species such as Dauria, Zolushka and Lazurnaya by 5, 2 and 6 times, respectively (figure 3). The change in the specific activity of acid phosphatase is most likely associated with a violation of carbohydrate-phosphorus and lipid metabolism, which occurs under the influence of the pathogen [20]. It is also known that acid phosphatases are involved in signal transduction and metabolic regulation by protein dephosphorylation [21].

![Figure 4](image)

**Figure 4.** Specific activity of esterases of seeds of soybean varieties under conditions of septoria infection: 1 - Kruzhevnitsa, 2 - Sentyabrina, 3 - Vereteika, 4 - Lydia, 5 - Umka, 6 - Dauria, 7 - Zolushka, 8 - Lazurnaya, 9 - Topaz.

It is important to note that the specific activity of acid phosphatases in the seeds of the soybean varieties Lydia is practically at the control level. It is apparently since energy transfer does not slow down under metabolic regulation under the influence of *S. glycines*.

The analysis of the specific activity of esterases of soybean seeds of different ripeness groups showed a decrease in the specific activity in almost all varieties, relative to the control. The greatest decrease in the specific activity of the enzyme was found in the short-season variety Sentyabrina by 4 times. The mid-season varieties of Dauria and Zolushka soybeans showed a decrease by 2 times. The mid-season varieties Lazurnaya and ultra-short season variety Topaz showed it by 3 times, relative to the control. The short-season variety, medium resistant to pathogenic fungus soybean seeds of the species Lydia, revealed an increase in the specific activity of esterases by 1.5 times. It is probably due to an increase in metabolism caused by hydrolysis of choline esters, as well as the ability to react with organophosphorus compounds [22].

In short-season varieties, Kruzhevnitsa, Vereteika and Umka, infection with septoria led to an increase in the specific activity of amylases relative to the control. The specific activity of the amylase complex in the seeds of the soybean varieties Lydia was set at the control level. The rest of the studied varieties of cultivated soybeans, different in short-season variety, caused a slight decrease in the specific activity of the enzyme relative to the control. Earlier, studies of Yarulina and Akhatova and others showed that infection with strains of the causative agent of septoria of various aggressiveness was accompanied by an increase and decrease in the level of amylase and proteinase activity in plant tissues [23].
Figure 5. Specific activity of amylase of seeds of soybean varieties under conditions of septoria infection: 1 - Kruzhevitsa, 2 - Sentyabrinka, 3 - Vereteika, 4 - Lydia, 5 - Umka, 6 - Dauria, 7 - Zolushka, 8 - Lazurnaya, 9 - Topaz.

4. Conclusion
The increase in the concentration of MDA was observed as compared to the control, that indicates oxidative stress in the seeds of the studied soybean species, except for Lydia (early maturing), Dauria (mid-maturing), and Topaz (ultra-early maturing), affected by S. glycines.

The analysis of the specific activity indicators of hydrolases (ribonuclease, esterase complex, amylase complex and acid phosphatase) of seeds of soybean species affected by S. glycines showed its decrease relative to control samples. It indicates a decrease in the metabolism under the influence of pathogenic fungus. However, it was found that seeds of the species Lydia (early ripening, moderately resistant to pathogenic fungus), under conditions of infection with S. glycines, had increased specific activity of ribonucleases and an esterase complex. The specific activity of acid phosphatases and amylase complex remained at the control level, i.e., it indicates the stability of this species for pathogenic fungus at the biochemical level.

Thus, hydrolases exhibit selectively neutral behaviour towards S. glycines on the studied soybean species to be applied as protein markers for the selection of the most resistant cultivar to S. glycines at the molecular level.

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