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PLANT GROWING

Activity and Electrophoretic Spectra of Enzymes in Soy Leaves Affected by the Pathogens of Various Trophic Groups

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Abstract—The resistance of soybean plants to the damaging effect of pathogens with different types of trophicity is determined not only by their genetic nature but also by various physiological and biochemical mechanisms. Therefore, the study was aimed at investigating the activity of enzymes (peroxidase, catalase, and acid phosphatase) in the leaves of the soybean Garmoniya cultivar with moderate resistance, Sonata cultivar susceptible to septoriosis, and Dauriya cultivar strongly affected by peronosporosis. It was shown that the infection of soybean leaves with hemibiotroph (*Septoria glycines*) or biotroph (*Perenospora manshurica*) depends on the plant genotype and leads to an increase in peroxidase activity in moderately resistant varieties and a decrease in the activity of catalase and acid phosphatase in all cultivars studied. The causative agent of septoriosis leads to an increase in the heterogeneity of peroxidase and a decrease in the heterogeneity of catalase and acid phosphatase, while the causative agent of peronosporosis does not induce any changes in the electrophoretic spectra of catalase and acid phosphatase in soybean leaves. The data obtained indicate that the rate and direction of enzyme activity is determined by the genotype of soybean and by the type of trophicity of the pathogen.

Keywords: soybean, *Septoria glycines* T. Hemmi, *Perenospora manshurica* (Naum.) Syd. Syn, peroxidase, catalase, acid phosphatase, electrophoretic spectra

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INTRODUCTION

The pathogenic microflora of the Far East, where soybean has been cultivated for a long time and occupies large areas, is very diverse. Septoriosis is one of the most harmful soybean diseases in the region; the causative agent is the imperfect fungus *Septoria glycines* T. Hemmi, which is a hemibiotroph (optional saprophyte). The pathogen affects living tissues but survives on plant remains even after the host dies [1]. It is characterized by a broad organotrophicity and easily passes from one organ to another, affecting almost all of the plant's aboveground parts: cotyledons, leaves, stems, and beans [2]. Peronosporosis is also a common and harmful disease. The causative agent of peronosporosis, or downy mildew, is *Perenospora manshurica* (Naum.) Syd. Syn., which is an obligate parasite (biotroph). The life cycle of biotrophs is closely related to the host plant; they can grow and develop under natural conditions only on living plants [1].

The most typical reaction of plants to the disease manifests in changes in the enzymatic activity that controls the metabolism of the host. Among the enzymes involved in the regulation of the metabolism of an infected plant, an important role is played by peroxidase [3–6]. Literary data on the role of catalase in the formation of the host-parasite relationship are contradictory [5]. Acid phosphatase is a protective plant enzyme. It is able to neutralize the effect of a wide range of infections caused by various microorganisms [7, 8].

To study the biochemical mechanisms of resistance to the pathogens with different types of trophicity, we conducted a study that was aimed at determining the enzymatic activity of peroxidase, catalase, and acid phosphatase in soybean leaves affected by *S. glycines* and *P. manshurica*.

MATERIALS AND METHODS

The leaves of three soybean cultivars (Sonata, Garmoniya, and Dauriya), affected by the causative agents of septoriosis and peronosporosis were the objects of the study. Healthy leaves were used as a control. Earlier, we found that the Garmoniya cultivar has a moderate resistance to the whole complex of diseases and the Sonata and the Dauriya cultivars were most

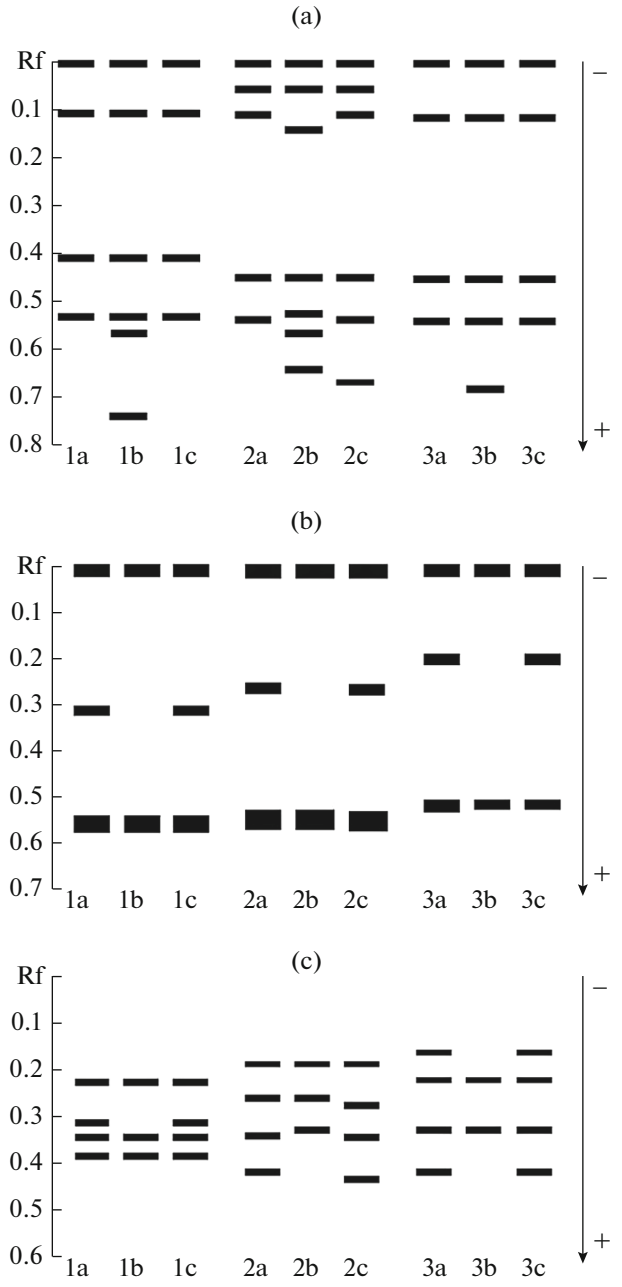

Fig. 1. Electrophoretic spectra of (a) peroxidase, (b), catalase, and (c) acid phosphatase in the leaves of soybean cultivars: 1—Garmoniya; 2—Dauriya; 3—Sonata; a—healthy; b—affected by septoriosis; c—affected by peronosporosis.

severely affected by septoriosis and peronosporous, respectively [9].

The activity of a peroxidase (EC 1.11.1.7) was determined by the rate of oxidation of benzidine under the action of the enzyme contained in plants prior to the formation of *n*,*n*'-diaminodiphenylquinone. The activity of a catalase (EC 1.11.1.6) was determined using the gasometric method by the amount of oxygen released after adding the peroxide hydrogen to the plant extract. The activity of an acid phosphatase (EC 3.1.3.2) was determined with a use of a colorimetric method based on the quantitative counting of inorganic phosphorus [10]. The amount of protein in the extracts was determined according to Lowry et al. [11]. The specific activity of the enzymes was expressed in the units of activity calculated for 1 mg of protein.

Electrophoretic spectra of healthy and affected soybean leaves were detected in the phase of bean formation at a high degree of development of diseases. Electrophoresis of soluble proteins was carried out in a 7.5% polyacrylamide gel, pH 8.3, according to Davis in the modification of V.I. Safonov and M.P. Safonova [12, 13]. The main criterion for characterizing the multiple molecular forms of enzymes was their relative electrophoretic mobility Rf. The statistical processing of the results was carried out according to standard methods [14].

RESULTS AND DISCUSSION

The activity of peroxidase in the leaves of the Garmoniya cultivar infected with septoriosis increased in all phases of soybean development; in the leaves of the Dauriya cultivar, it increased only in the phase of bean formation (by 1.6 times as compared with the control) (Table 1). In the affected and healthy leaves of the susceptible Sonata cultivar, no significant differences in peroxidase activity were detected.

The activity of the enzymes in leaves affected by *P. manshurica* was determined in the flowering and bean formation phases. In all studied varieties, leaves affected by peronosporosis did not have any necrosis, but only chlorotic spots were noted. The leaves of the Dauriya cultivar were most strongly affected (Table 1). Garmoniya and Sonata cultivars with a moderate resistance to peronosporosis were characterized by a high activity of peroxidase in the affected leaves: in the bean formation phase, it increased by 1.7 and 1.3 times, respectively. In a highly sensitive Dauriya cultivar, the activity of the enzyme in the affected leaves increased slightly (by 29% compared to the control).

The affection of soybean plants with *S. glycines* led to changes in the electrophoretic spectra of the enzyme. The phytopathogen induced the synthesis of additional forms of peroxidase with Rf 0.54 in the leaves of Garmoniya and Dauriya cultivars (Fig. 1a). In addition, the appearance of highly mobile forms with Rf ranging from 0.60 to 0.73 was observed in the leaves of all moderately resistant or susceptible studied plants affected by the causative agent of septoriosis. According to Chant and Bates [15], the appearance of additional peroxidase isoenzyme in the leaves of affected plants may be caused by their premature aging.

No qualitative changes in the spectrum of peroxidase were observed when the leaves of moderately

Table 1. Specific activity of peroxidase (U/mg of protein × 10^{-3}), catalase (U/mg of protein × 10^{-4}), and acid phosphatase (U/mg of protein × 10^{-6}) in soybean leaves, 2008–2011

Phenological phase	Leaves	Cultivar		
		Garmoniya	Dauriya	Sonata
		Peroxidase activity		
First ternate leaf	Healthy	227 ± 36	201 ± 19	243 ± 52
	Affected by:			
	septoriosis	933 ± 79	126 ± 7	200 ± 39
	penosporosis	–	–	–
Flowering	Healthy	331 ± 42	379 ± 27	361 ± 20
	Affected by:			
	septoriosis	599 ± 94	363 ± 7	409 ± 59
	penosporosis	587 ± 12	437 ± 13	468 ± 13
Bean formation	Healthy	1216 ± 26	1007 ± 14	986 ± 18
	Affected by:			
	septoriosis	3943 ± 22	1575 ± 39	1002 ± 19
	penosporosis	2059 ± 22	1112 ± 45	2378 ± 30
		Catalase activity		
First ternate leaf	Healthy	36 ± 2	32 ± 6	69 ± 4
	Affected by:			
	septoriosis	17 ± 2	17 ± 3	50 ± 5
	penosporosis	–	–	–
Flowering	Healthy	79 ± 5	74 ± 10	82 ± 4
	Affected by:			
	septoriosis	42 ± 5	47 ± 8	56 ± 12
	penosporosis	44 ± 5	73 ± 2	69 ± 4
Bean formation	Healthy	163 ± 17	138 ± 16	121 ± 8
	Affected by:			
	septoriosis	62 ± 7	44 ± 2	54 ± 4
	penosporosis	120 ± 6	25 ± 2	46 ± 8
		Acid phosphatase activity		
First ternate leaf	Healthy	73 ± 16	62 ± 12	70 ± 4
	Affected by:			
	septoriosis	54 ± 11	41 ± 14	35 ± 5
	penosporosis	–	–	–
Flowering	Healthy	178 ± 19	131 ± 18	219 ± 20
	Affected by:			
	septoriosis	107 ± 16	106 ± 20	94 ± 10
	penosporosis	144 ± 2	99 ± 2	141 ± 3
Bean formation	Healthy	384 ± 26	313 ± 20	334 ± 39
	Affected by:			
	septoriosis	261 ± 18	178 ± 24	141 ± 10
	penosporosis	319 ± 3	175 ± 6	200 ± 2

resistant Garmoniya and Sonata cultivars were affected by *P. manshurica* biotroph (Fig. 1a). The susceptible Dauriya cultivar had an additional highly mobile form (Rf 0.67). The synthesis of this component, perhaps, is also related to the aging of the leaves.

The specific activity of catalase in the leaves of soybean affected by septoriosis in the phases of the third ternate leaf and flowering was lower than in the control. Its significant decrease (by 2.2–3 times) was noted in the phase of the formation of beans in plants of all cultivars (Table 1), which may be caused by the destruction of chlorophyll. It is known that the amount of chlorophyll in the leaves decreases under the influence of most phytopathogenic fungi, because the microorganisms destroy chloroplasts. According to the literary data [16], partial or complete destruction of chlorophyll always leads to a drop in catalase activity.

The affection of plants with peronosporosis or septoriosis also reduces the activity of the catalase. In the flowering phase, it is 1.2–1.8 times lower than in healthy plants. The catalase activity continues to decrease until the phase of bean formation: by 1.4 times in the Garmoniya cultivar, by 2.6 times in the Sonata cultivar, and by five times in the Dauriya cultivar (Table 1).

Necrosis observed during septoriosis leads not only to a significant decrease in catalase activity but also to a decrease in the number of multiple molecular forms due to the disappearance of components with an average electrophoretic mobility. Affection of soybean plants with peronosporosis did not affect the qualitative and quantitative composition of the electrophoretic spectra of catalase (Fig. 1b). Probably, *S. glycines* causes more stress to affected plants than the biotrophic pathogen.

Studies of acid phosphatase in plants affected by pathogens are few. It is known that the viral damage of plants leads to its increase, which indicates the association between the infectious process and the violation of metabolic processes [18]. The specific activity of acid phosphatase in the leaves of all studied cultivars affected by septoriosis and peronosporosis is lower than in healthy ones. Essentially, the activity of the enzyme in the leaves of the Sonata cultivar affected by septoriosis and the Dauriya and Sonata cultivars affected by peronosporosis decreases significantly (by 50–60, 24 and 44%, respectively) (Table 1). Quantitative changes are observed in the electrophoretic spectrum of acidic phosphatase during the septoriosis, and the components with low and medium electrophoretic mobility disappear (Fig. 1b). Peronosporosis did not affect the number of forms of acid phosphatase in the leaves of soybean plants with different resistance. The presence of an equal number of multiple molecular forms with the same mobility in both the affected leaves and control leaves indicates that the process of formation of the enzyme is not violated.

Thus, the change in the enzymatic activity depends on the genotype of a plant and the type of trophicity of the causative agent. The affection of soybean leaves with pathogens with both hemibiotrophic (*S. glycines*) and biotrophic (*P. manshurica*) type of nutrition leads to an increase in peroxidase activity in moderately resistant cultivars and a decrease in the activity of catalase and acid phosphatase in all the cultivars studied. The causative agent of septoriosis leads to an increase in the heterogeneity of peroxidase and a decrease in the heterogeneity of catalase and acid phosphatase. The causative agent of peronosporosis is less pathogenic and does not lead to any changes in the electrophoretic spectra of catalase and acid phosphatase in soybean leaves.

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