ACTIVITY OF TRYPsin INHIBITORS IN SEEDLINGS OF WHEAT AND RYE WHEN INFECTED WITH FUNGI – AGENT OF ROOT ROT


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The influence of infection, treatment with metabolites of pathogen Bipolaris sorokiniana on the level of activity of proteolytic enzymes and trypsin inhibitors in plant organs of wheat and rye was researched. These data demonstrate the successful development of protective responses in treated with metabolites plants that is an effect of induced resistance of the plant upon contact with the pathogen elicitors.

Keywords: wheat, rye, root rot, index of tolerance, trypsin inhibitors, proteolytic enzymes, elicitor, induced resistance.

Introduction

In modern conditions of agricultural production intensifies the negative impact of climatic and anthropogenic factors on plants. Harmfulness microbial phytopathogens is constantly increasing due to the emergence of new, more virulent, resistant to pesticides, forms [1]. For these reasons, there is a significant decline in real yields of agricultural crops. In this regard, relevant is the search for new methods and means to improve the adaptive abilities of plants. Alternative pesticides are chemical nature biological methods of plant protection, including methods for inducing and stimulating its own defense mechanisms of the plant organism [2, 3].

Protective mechanisms of modern cultivated plants formed during the long coevolution of the "plant-phytopathogenic" system. Substances of phytopathogens, which activate plant resistance responses, were called biogenic elicitors [4].

Elicitors activates different signaling systems of plant cell, which leads to the expression of protective genes, activation of the synthesis of specific proteins, the formation of phytoalexins, release of antipathogenic volatile compounds, etc. The result is the formation of the immunity to pathogens of plants. Numerous studies have shown that exogenous metabolites of pathogens have elicitor properties: stimulates the generation of active oxygen forms, the synthesis of protease inhibitors etc. [5–7].

Protein nature substances having antimicrobial activity, in particular inhibitors of proteolytic enzymes, are important constituents of plant defense mechanisms. Their role is to suppress the activity of exogenous enzymes of mammals, insects, pathogenic fungi and bacteria [8–10]. In the literature there is evidence about an increasing of amount (activity) of protease inhibitors in the tissues under the influence of various factors on plants [11, 12], which indicates the possibility of regulation of their activity. Gene activation of protease inhibitors enhances plant resistance to pathogens and pests. This is evidenced by the results of experiments on gene of these molecules transfer [13].

To identify in model experiment the eliciting activity of metabolites of the pathogen was chosen fungus – root rot pathogen of cereal crops – Bipolaris sorokiniana (Syn. Helminthosporium sativum).

In this article we study the effect of exogenous metabolites of pathogenic fungus Bipolaris sorokiniana on the activity of proteases and trypsin inhibitors in plants of wheat and rye.

Materials and methods

Experiments were performed on wheat (Triticum aestivum L.) seedlings varieties “Omsk 36” and rye (Secale cereale) varieties “Chulpan 7”.

Fungal material. Natural isolates of the fungus Bipolaris sorokiniana was cultured on Czapek agar medium. Spores (conidia ) was rinsed from 10-day colony, washed twice with distilled water, and their concentration was adjusted to 500 pcs/ml. For obtaining the liquid culture the conidia were washed off the solid medium to the liquid Czapek medium. The mycelia were grown in a liquid medium for 7 days at 23 °C, after this liquid was filtered, centrifuged and used for the treatment of seedlings. Inactivation of infectious material was produced by boiling of a suspension of conidia for 10 minutes.

Treatment of seedlings

Seeds were sterilized in a slightly pink solution of potassium permanganate and germinated with rolled method [14]. Each variant of the experiment used 30 seeds. Plants treated with conidia of B. sorokiniana and suspension of inactivated conidia at a concentration of 500 pcs/ml, with culture supernatant of 7-day culture of the fungus (CL – culture liquid), by adding to the root zone of 3-day-old seedlings of 50 µl of the appropriate material. Treatment was repeated three times with 24-hour intervals. At 7 days after the first treatment was measured the mass of roots and shoots and calculated the index of tolerance as the ratio of mass of the root and stem parts of seedlings to the mass of the same parts of the plants in the control variant.

To obtain a sample of tissue extracts the plant material was triturated in a porcelain mortar with quartz sand, filled with distilled water at a ratio of 1:4. It was...
incubated at 40 °C for 1.5 hours, filtered and centrifuged twice at 10,000 rpm in the centrifuge MRW-310 (Poland) for 10 min. In the supernatant was determined the activity of proteases using hydrolysis rate of the synthetic substrate Nα-benzoyl-D,L-arginine-4-nitroanilide (BAPNA) by the method of B. P. Erlanger [15]. Trypsin inhibitor activity was determined spectrophotometrically using the same substrate [16].

**Results discussion**

**Evaluation of the index of tolerance of seedlings when infected and processed with fungal metabolites**

It is known that the impact of the stressor on the plant can be measured by an index of tolerance [17]. In our experiments the index of tolerance was calculated by the ratio of the mass of the root and stem of the 10 control and experimental samples of seedlings (Table 1).

Treatment of seedlings both wheat and rye with CL, infection with conidia decreased the weight of stems and roots, and the processing with Czapek medium – a slightly increased weight of wheat seedlings. As can be seen, when infected rye and wheat with *B. sorokiniana*, the root part of plants was inhibited greater then ground parts. In general, the mass of the roots of the treated plants was 25–40% less than that of the control group of the roots of plants. Mass of treated stems of plants decreased by only 15–20 %. Preferential inhibition of root growth in lesions of root rot pathogen also noted in the field [18].

The infection caused greatest growth inhibition of roots and leaves of plants. Reduced IT in processing of seedlings with viable conidia and CL of the fungi (without the development of infection) may be related both to the toxic effects of metabolites of the fungus and the change in intensity of the metabolic processes of the plant, which results in inhibition of growth and the synthesis of specific compounds. If this is a synthesis of the protective (antimicrobial) compounds, we can talk about the so-called eliciting effect of treatment of plants with the culture liquid and inactivated infectious material.

**The activity of proteolytic enzymes and trypsin inhibitors in sprouts**

Several studies have shown that treatment of plants with exogenous metabolites may cause synthesis of protective compounds, thereby preparing cell to penetration of the pathogen [6, 11]. Such protective connections include, in particular, active oxygen forms, a large group of PR-proteins, as protease, chitinase, glucanase, protease inhibitors etc. [5, 19, 20].

It is known that the activity of proteases in plant tissues varies with the defeat of plants by phytopathogenic fungi [21]. Most phytopathogenic microorganisms actively synthesizes hydrolytic enzymes (pectinases, cellulases, proteases etc.), causing the destruction of plant tissues and cell membranes that conducts pathogen penetration into cells, so an increase in proteolytic activity during infection may be due to the presence of enzymes of pathogen. However, in our experiments, the activity during infection not changes (Table 2).

In the stems of the seedlings in all experiments variant protease activity was reduced except the culture liquid treatment. In the roots of wheat an enzyme activity increases slightly in the case of the processing with inactivated conidia. In seedlings of rye in the stems there was no essential changes, in the roots an activity of proteolytic enzymes is reduced by 6–29%, depending on the processing variant.

In the dynamics of protease activity in the processing has not been possible to identify common patterns.

Further we investigated the change of the activity of trypsin inhibitors in the processing of plants. It is known that these molecules are not only inhibit the enzymatic activity of microorganisms, but also inhibit the growth of mycelium by adding them to the culture medium [22].

<table>
<thead>
<tr>
<th>Plant</th>
<th>Treatment variant</th>
<th>Ind. of Tolerance</th>
<th>Wheat</th>
<th>Rye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stem</td>
<td>root</td>
<td>stem</td>
<td>root</td>
</tr>
<tr>
<td>Intact conidia</td>
<td>0.81 ± 0.04</td>
<td>0.70 ± 0.04</td>
<td>0.88 ± 0.04</td>
<td>0.80 ± 0.05</td>
</tr>
<tr>
<td>Culture liquid</td>
<td>0.88 ± 0.04</td>
<td>0.80 ± 0.05</td>
<td>0.85 ± 0.04</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>Inactivated conidia</td>
<td>1.03 ± 0.5</td>
<td>1.06 ± 0.03</td>
<td>1.03 ± 0.5</td>
<td>1.06 ± 0.03</td>
</tr>
<tr>
<td>Czapek medium</td>
<td>1.03 ± 0.5</td>
<td>1.06 ± 0.03</td>
<td>1.03 ± 0.5</td>
<td>1.06 ± 0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment variant</th>
<th>Ind. of Tolerance</th>
<th>Wheat</th>
<th>Rye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1 (water)</td>
<td>161.6 ± 0.8</td>
<td>118.1 ± 0.3</td>
<td>121.4 ± 0.7</td>
</tr>
<tr>
<td>Control 2 (Czapek medium)</td>
<td>152.15 ± 0.8</td>
<td>136.55 ± 0.8</td>
<td>185.2 ± 0.2</td>
</tr>
<tr>
<td>Intact conidia</td>
<td>146.0 ± 0.5</td>
<td>124.45 ± 0.7</td>
<td>123.8 ± 1.1</td>
</tr>
<tr>
<td>Inactivated conidia</td>
<td>107.0 ± 0.3</td>
<td>144.7 ± 0.5</td>
<td>127.2 ± 0.7</td>
</tr>
<tr>
<td>Fungus culture liquid</td>
<td>204.6 ± 1.2</td>
<td>118.2 ± 0.4</td>
<td>121.25 ± 0.5</td>
</tr>
</tbody>
</table>
When processing with conidia and CL a level of activity of trypsin inhibitors in the seedlings (in stems and roots) increases. In this case, the processing of plant with control solution 2 (Czapek) does not increase the inhibitory activity. These facts are indicative of induction (activation) of protective mechanisms in the tissues when they are infected and processed with metabolites. Increased activity of inhibitors belonging to a group PR-proteins confirms assumptions concerning the inhibitor activity of these metabolites.

Thus, a substance contained in the culture liquid and allocated by root rot pathogen, including inactivated spores, induces (activates) defense responses of plants, which is appears by rising of level of activity of proteinase inhibitors. Since this effect is observed in various organs of plants (stems and roots), it is able to talk about the appearance of systemic stability.

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