CHANGE IN THE ACTIVITY OF THE PROTEOLYTIC ENZYMES OF COTTON SEEDS WHEN THE PLANTS ARE AFFECTED BY WILT

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The change in the proteolytic activity of the seeds of the cotton plant varieties Tashkent-1- Tashkent-6, S-4727, and 108-F when the plants are affected by wilt has been studied. A relationship has been established between the decrease in activity for seeds of the Tashkent-1 and Tashkent-6 varieties and the degree of attack by wilt. A hypothesis has been put forward on the existence of variety differences in the response reaction of the cotton plant to the introduction of the pathogen. The appearance of distinctive electrophoretic protein components in the seeds of wilt-affected cotton plants of the Tashkent-1 and Tashkent-6 varieties has been shown. A hypothesis has been made of a possible increase in the amount of proteinase inhibitors in response to the infection of the plants by wilt, a consequence of which is a sharp decrease in proteolytic activity and the appearance of distinctive electrophoretic components.

At the present time, even wider interest is being aroused by the question of the specific role of proteinase inhibitors in the pathogen-plant interrelationship. There are certain facts concerning the action of proteinase inhibitors on pathogenic microorganisms. A positive correlation is observed between the activity of the inhibitors and the resistance of the plant to fungal diseases [1-3]. A. M. Yamaleev, comparing the proteolytic activities of two varieties of wheat — resistant, and susceptible to infection by loose smut — found that the activity of the susceptible variety was twice as high as that of the resistant variety. In the latter, all the proteinases were inactivated by inhibitors, while in the susceptible variety only 26.4% of the activity of the enzyme was bound with inhibitors. These results indicate differences in the response reaction of resistant and susceptible varieties of wheat to the introduction of a pathogen. The resistant variety is characterized by an increase in the amount of inhibitors and by a sharp decrease in the activity of the proteinases in the seeds of a plant infected by a pathogen, while in the susceptible variety there are no inhibitors and by a sharp decrease in the activity of the proteinases in the seeds of a plant infected by a pathogen, while in the susceptible variety there are no inhibitors and the proteolytic activity is high [4].

We have made a comparative study of the change in the activity of the proteinases of seeds of the Tashkent-1, Tashkent-6, and S-9063 varieties of healthy cotton plants relatively resistant to infection by wilt which were collected in the experimental field of the G. S. Zaitsev Institute of Selection and Seed Production. The results of the investigation are given in Table 1.

TABLE 1

Variety	Amount of protein, mg in 1 ml of seed extract		Proteinase activity OD units per 1 g of seed protein	
	healthy	infected	healthy	infected
Tashkent-1	8,1	7 2	0,5	0,05
Tashkent-6	8,1	7,1	0.48	0,23
S-9063	6 7	5,27	0,9	0,8

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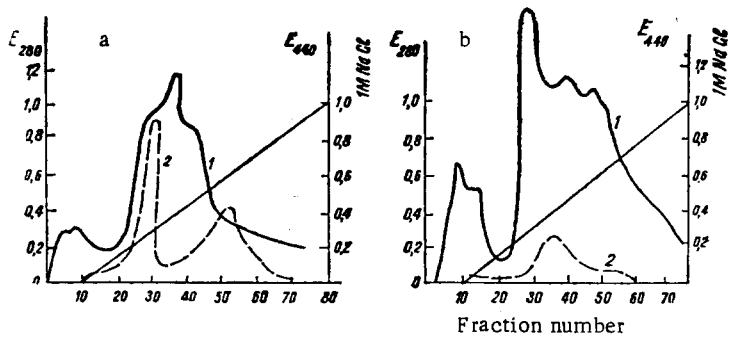

Fig. 1. Separation of the total protein fractions from healthy (a) and wilt-infected (b) seeds of cotton plants of the Tashkent-1 variety: 1) E_{280}; 2) E_{440}.

When a plant was 50% infected by wilt, in the seeds of the Tashkent-1 variety only 10-20% of the activity of the proteolytic enzymes was retained, the corresponding figure for the Tashkent-6 variety being 54% and for the S-9063 variety 80-90%. We studied the dependence of the change in the proteolytic activity of the seeds of cotton plants of the Tashkent-1 and Tashkent-6 varieties on the degree of infection by wilt, which was evaluated on a four-point system (see the Experimental part). Table 2 gives the average values of three samples of each variety.

With a slight change in the amount of protein, the proteolytic activity of the seeds decreased as the degree of infection, increased, reaching 80% in the Tashkent-1 variety and 44% in the Tashkent-6 variety. To determine the qualitative change in the composition of the proteinases of dormant seeds of a cotton plant of the Tashkent-1 variety collected from plants 50% infected by wilt, in comparison with the seeds of healthy plants, total protein extracts were separated on a column of DEAE cellulose and the proteolytic activities of the fractions eluted was determined. A graph of the separation is shown in Fig. 1.

Graphs of the separation of the total protein obtained from the healthy seeds (a) and the wilt-infected seed (b) were fairly similar, while graphs of the manifestation of proteolytic activity differed sharply. From the total extract of the healthy seeds, on its separation by means of an ion-exchanger, two fractions with proteolytic activity were obtained, A_1 and A_2, which were eluted from the column with an increase in the ionic strength of the buffer solution: A_1 at 0.2 M NaCl, and A_2 at 0.45 M NaCl. In the extracts obtained from the seeds of the infected plant, the A_2 fraction was completely absent while A_1 amounted to only 20% in comparison with the results obtained from the healthy seeds. Consequently, the greatest decrease in proteolytic activity, of about 90%, on the infection of a plant by wilt is observed in the seeds of the Tashkent-1 variety, as compared with other varieties. By analogy with the literature information given above, it may be assumed that the response to the introduction of the pathogen into the plant there is possibly an increase in the amount of proteinase inhibitors which primarily inhibit their own proteinases, which explains the sharp decrease in proteolytic activity. The higher the resistance of the plant, the greater the amount of inhibitors that are synthesized in it and the lower is the activity of the proteolytic enzymes. The different degrees of decrease in proteolytic activity in the seeds of the Tashkent-1, Tashkent-6, and S-9063 varieties can obviously be explained by differences in the degree of resistance of these varieties to infection by wilt.

TABLE 2

Degree of infection	Amounts of protein, mg, in 1 ml of extract		Activity, OD units for azocasein		Activity, OD units per 1 mg of protein	
	Tashkent-1	Tashkent-6	Tashkent-1	Tashkent-6	Tashkent-1	Tashkent-6
Control	8,1	8 1	2	1,8	0,5	0,48
1 point	8,5	6,7	2	1,6	0,47	0,47
2 points	7,7	7,6	1,5	0,9	0,37	0,27
3 points	7,4	6,7	1,35	0,61	0,36	0,21
4 points	7,3	7,1	0,4	0,84	0,1	0,23
Loss of activity, %			80	44		

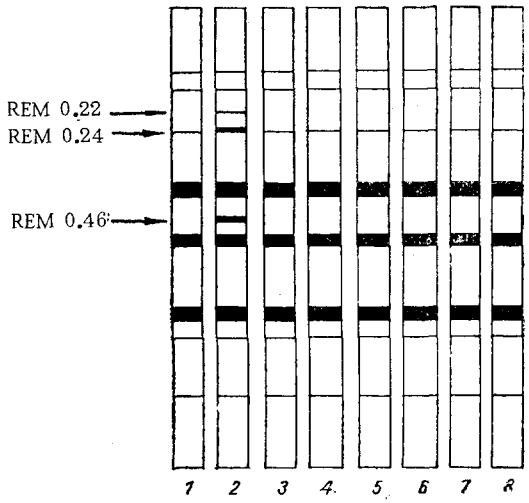

Fig. 2. Sketches of electrophoretograms of the total protein fractions of healthy (1, 3, 5, 7) and infected (2, 4, 6, 8) seeds of cotton plants of the varieties Tashkent-1, Tashkent-6, 108-F, and S-4727.

In order to reveal the protein components appearing in the seeds of a wilt-infected cotton plant, we carried out an electrophoretic investigation of the total protein extracts obtained from the seeds of healthy plants of varieties not less than 50% infected by wilt. Electrophoresis was performed on plants in a thin layer of polyacrylamide gel at pH 8.3 and 4.5 No differences were observed in the electrophoretogram of obtained at pH 4.5, but there were differences on the electrophoretograms obtained at pH 8.3 (Fig. 2). The extracts obtained from the seeds of healthy and wilt-infected plants of the 108-F and S-4727 varieties did not differ with respect to the spectra of their electrophoretic components, while in the spectra of extracts obtained from the seeds of the Tashkent-1 and Tashkent-6 varieties distinct components were found with relative electrophoretic mobilities, REPs, of 0.22, 0.46, and 0.24. The electrophoretic results agree well with those obtained on the change in proteolytic activities. The sharp decrease in proteolytic activity in the seeds of wilt-infected plants of the Tashkent-1 and Tashkent-6 varieties can be explained by an increase in the amount of proteinase inhibitors, which was detected in the electrophoretic spectra in the form of components with REPs of 0.22, 0.46, and 0.24.

EXPERIMENTAL

Determination of the Dependence of the Change in Proteolytic Activity of Cotton Seeds on the Degree of Infection by Wilt. Samples of healthy and wilt-infected cotton plants of the Tashkent-1, Tashkent-6, S-4727, and 108-F varieties were obtained in the experimental field of the G. S. Zaitsev Institute of the Selection and Seed Production of the Cotton Plant. The degree of infection was determined on a four-point system from the degree of colored corking up of the vessels in sections of the stems. Seeds from three plants were taken for each score and for the control. The seeds were cooled with liquid nitrogen, and crushed, and the husks were separated from the kernels. The cooled kernels were ground and were defatted by means of acetone with the addition of dry ice. The defatted flour (0.5 g) was extracted with 15 ml of acetate buffer having pH 5.5. The extract was centrifuged at 18,000 rpm for 30 min. The amount of protein in the extract obtained was determined by the Warburg–Christiani method [5], and the proteolytic activity with respect to azocasein, with measurement of the optical density at 440 nm followed by the recalculation of the proteolytic activity of LD units per 1 mg of protein [6].

Separation of the Total Protein Extract on a Column of DEAE-Cellulose. The defatted flour of the Tashkent-1 seeds (50 g) was extracted with 100 ml of 0.1 M phosphate buffer, pH 7.4, for 3 h, then the extract was centrifuged at 18,000 rpm for 30 min. The protein was precipitated from the supernatant liquids by 80% saturation with $(NH_4)_2SO_4$, with centrifugation at 18,000 rpm for 30 min. The precipitate was dissolved in the minimum amount of 0.005 M phosphate buffer, pH 7.4, and was dialyzed against the same buffer for a day, centrifuged, and deposited on a column (2.5 × 15 cm) of DEAE-cellulose equilibrated with 0.05 M phosphate buffer, pH 7.4. The protein was eluted from the column at the rate of 20 ml/h, 5-ml fractions being collected every 15 min. The conditions for the extractions of proteins obtained from the seeds of the healthy and wilt-infected cotton plants and of its separation on the DEAE-cellulose column were absolutely identical. The proteolytic activity in each fraction was determined by a method described above.

Electrophoretic Separation of the Total Proteins. The water-soluble proteins of cotton seeds were extracted with 0.05 M Tris-H [sic] buffer, pH 8.3 [7]. The isolated proteins were freeze-dried and were used for the electrophoretic separation. Electrophoresis was performed in 7.5% PAAG in basic buffer (pH 8.9) using vertical plants with dimensions of 115 × 115 × 1 mm.

An AVGE apparatus for vertical gel electrophoresis was used. Electrophoresis was performed in Tris-glycine buffer, pH 8.3 for 2.5 h at U = 250 V and I = 33 mA per plate. After separation, the plates were fixed in 7% TCA solution for 20-30 min and were stained with a 0.2% solution of Coomassie Bright Blue R-250 for 1 h. The excess of dye was washed out with a mixture of 7% acetic acid and 10% ethanol unitl the background of the plate was clear.

CONCLUSIONS

1. A comparative study has been made of the proteolytic activities of the seeds of healthy and wilt-affected cotton plants of the Tashkent-1, Tashkent-6, S-4727, and 108-F varieties. The decrease in proteolytic activity for the Tashkent-1 and Tashkent-6 varieties has been found to depend on the degree of wilt infection.

2. The appearance of distinctive electrophoretic protein components in the seeds of wilt-affected cotton plants of the Tashkent-1 and Tashkent-6 varieties has been shown.

3. The results obtained give grounds for assuming the existence of variety differences in the response reaction of the cotton plant in its infection by wilt.

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