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PROTEINS RESPONSIBLE FOR SEED PUBESCENCE OF COTTON GENETIC LINES

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The activities of fiber-forming enzymes (glucansynthetase, peroxidase, cellulase) from smooth- and pubescent-seeded cotton were compared. Protein inhibitor from the smooth-seeded line was isolated and studied. One of the aspects of the mechanism regulating growth and differentiating epidermal cotton ovule cells into fiber was investigated.

Key words: cotton, proteins, glucansynthetase, peroxidase, cellulase, gene-inhibitor.

Questions about the biochemical characteristics of cotton genetic lines that differ in degree of seed pubescence, fiber-forming enzyme activity, and protein composition of sprouts, fiber, and integument have not yet been sufficiently answered.

The activities of glucansynthetase, peroxidase, and cellulase, which are involved in cotton fiber formation, have been studied in 7-day sprouts and developing fiber 10 and 20 days after flowering. A study of these enzymes in initial parental forms revealed high activities for glucansynthetase (1.5×10^{-6} units/mg-protein) and peroxidase (0.821 U/mL) in a pubescent line and low activities (4.2×10^{-7} and 0.288 U/mL, respectively) in a smooth-seed line [1]. The activities of the enzymes in pubescent lines is apparently increased due to changes in the expression level of genes that code for the proteins necessary for cellulose synthesis [2, 3].

An inverse dependence of enzyme activity on seed pubescence was observed during the study of cellulase activity of cotton lines with different amounts of seed pubescence. High activity, 13.0 units/mg-protein, was noted in the smooth-seed line L-70 whereas it was less, 7.9 units/mg-protein, in pubescent line L-489.

Enzyme activity in fiber (pubescent) and integument (pubescent and smooth-seed) of the examined lines was investigated as the cotton developed. Bolls were collected 10 and 20 days after flowering.

The activity of glucansynthetase in developing fiber of L-489 was 3.3×10^{-6} units/mg-protein; of cellulase, 43.1 units/mg-protein. The activivty of glucansynthetase in seed integument was less, 2.8×10^{-6} (L-489); cellulase, 6.25 units/mg-protein. For smooth-seed line L-70, the values were 11.8×10^{-7} units/mg-protein (glucansynthetase) and 10 units/mg-protein (cellulase). The analytical results for the enzymes revealed low activities in integument of smooth-seed cotton.

Peroxidase activity in fiber of line L-489 was 0.280 U/mL; in integument, 0.200 in L-489 and 0.100 U/mL in L-70. The high enzyme activity in the pubescent line is probably related to the presence of a comparatively rich and wide assortment of phenolic compounds, especially in 10-day fiber. Then, their numbers decreased as the fiber developed [4]. The drop of peroxidase activity in fiber after formation of the secondary cell wall (20-day fiber) is explained by the decrease of total content of the phenolic compounds that are the substrate for this enzyme. The reduction in the total content of phenolic compounds in 10-day fiber coincided with intensified cellulose synthesis and accumulation.

The low enzyme activity in sprouts of smooth-seed L-70 was mentioned above. The low activity in integument of developing ovules of this line is explained by the dominance of the gene-inhibitor of seed pubescence. The results agree with published theoretical prerequisites [3].

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TABLE 1. Activities of Enzymes Involved in Fiber-Formation of First-Generation Cotton Genetic Lines

Line	Protein content, μg/mg			Enzyme activity, units								
	S	F	I	glucansynthetase × 10^{-6}			peroxidase			cellulase		
				S	F	I	S	F	I	S	F	I
L-489	240	323	350	4.6	6.1	0.4	0.324	0.133	0.013	79.3	50	20.5
L-70	240	-	261	0.6	-	0.05	0.236	-	0.009	87.2	-	32.9

S, sprouts; F, fiber; I, integument.

TABLE 2. Activities of Fiber-Forming Enzymes in Ovules of Hybrids Produced by Crossing Smooth-Seed and Pubescent-Seed Lines

Hybrid	Protein, μg/mg	Enzyme activity, units/mg		
		glucansynthetase × 10^{-6}	peroxidase	cellulase
L-489 × L-70 (fiber)	220	1.4	0.050	57.7
L-489 × L-70 (integument)	180	0.28	0.016	12.5

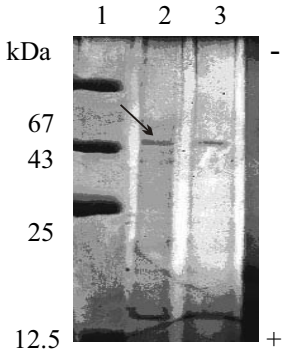


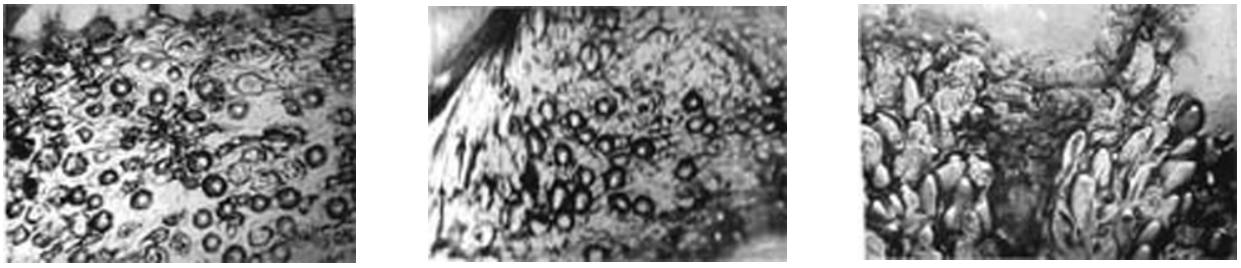
Fig. 1. Electrophoregram in PAAG (10%) of fractions obtained after ion-exchange chromatography over DEAE-cellulose: markers BSA, ovalbumin 43 kDa, chymotrypsin, cytochrome C (1); first fraction after ion-exchange chromatography (IEC) (2); second fraction after IEC exhibiting cellulose-formation inhibiting activity (3); arrows show polypeptides with MW 13 and 44 kDa.

Then we investigated first-generation genetic lines wth morphological and biochemical indicators of the parental forms. The inverse dependence between seed pubescence and enzyme activity was retained. Sprouts and integument of smooth-seed line L-70 had low enzyme activity whereas the pubescent lines had high glucansynthetase and peroxidase activities (Table 1).

It seemed interesting to track the inheritance of the smooth-seed trait upon crossing genetic lines differing in seed pubescence. Crossing in all versions produced seeds with down and fiber. According to the literature [3], the cotton structural genes responsible for seed pubescence are dominant in these instances whereas the gene-inhibitor is recessive. Proteins and enzymes involved in fiber formation were investigated in the produced hybrids. The activities of glucansynthetase, peroxidase, and cellulase were less in fiber and integument of hybrids than in parents (Table 2).

We investigated proteins that inhibit fiber formation.

Gel filtration over TSK-HW-65 gel separated proteins isolated from sprouts of the smooth-seed cotton genetic line (L-70). A study of the glucansynthetase activity in the resulting protein fractions showed that the first fraction had inhibitory activity. Purification of this fraction by ion-exchange chromatography over DEAE-cellulose produced fraction 2, which contained polypeptides of MW 13 and 44 kDa (Fig. 1). These were highly active inhibitors of cellulose synthesis. Isoelectric focusing of the fraction obtained after ion-exchange chromatography established that the proteins were predominantly acidic.



A B C

Fig. 2. Photographs of the epidermal surface of ovules of pubescent line L-489 in MS culture medium: control (A), in the presence of fraction 2 obtained after ion-excchange chromatography of smooth-seed cotton L-70 (B, C); inhibitor concentration 500 μg/mL. Magnification 350× (A, B); 500× (C).

Thus, proteins exhibiting inhibitory activity for glucansynthetase, which is involved in fiber formation, were produced from sprouts of smooth-seed cotton line L-70.

One of the fundamental problems of modern biology involves the molecular mechanisms of growth or inhibition of plant cells. Therefore, the effect of the protein-inhibitors isolated from line L-70 on 1-day ovule of pubescent line L-489 was investigated by electron microscopy.

Direct experiments on living cotton ovules were set up in order to check the stated hypotheses about inhibition of cellulose and fiber formation by polypeptides from line L-70. We tested 1-day L-489 cotton ovules (*G. hirsutum*). Proteins with cellulose-forming inhibitory activity from the smooth-seed line at a concentration of 500 μg/mL decreased markedly the surface density of emerging fiber cells. Fiber cells on the ovule epidermis appeared only in the parts near the chalaza, which was especially evident at a polypeptide concentration of 500 μg/mL in MS medium. The emergence and growth of fiber cells was suppressed greatly on the epidermis surface of ovules in the very early stages of differentiation into fiber (Fig. 2).

It can be seen at high magnification that the ovule epidermal surface consists of bulging and slightly protruding epidermal cells that are ready to lengthen but are restrained from this genetically given tendency.

Direct optical microscopic observations of the epidermis of living ovules placed in cluture medium showed that fraction 2 that was isolated by ion-exchange chromatography from the smooth-seed cotton line and contained polypeptides of 13 and 44 kDa clearly suppressed growth of epidermal ovule cells into fiber. Therefore, one of the aspects of the mechanism regulating growth and differentiation of epidermal cells of cotton ovules into fiber was studied for the first time.

Thus, we analyzed and compared the activities of fiber-forming enzymes of smooth-seed and pubescent-seed cotton, isolated and studied protein-inhibitors from the smooth-seed line, and investigated one of the aspects of the mechanism regulating growth and differentiation of epidermal cells of cotton ovules into fiber.

EXPERIMENTAL

We studied cotton genetic lines obtained from the Division of Genetics and Cytoembryology of the National University of the Repubic of Uzbekistan, L-70, which is smooth-seed, and line L-489, which is pubescent-seed.

We used 7-day sprouts, 20-day fiber, and integument from the studied lines. Ovules of the pubescent line were collected on the day of flowering for the electron microscopy study.

Glucansynthetase and cellulase activities were determined as before [1].

Identification of Synthesis Product. The nature of newly synthesized polymer from cellulose precursor UDP-^{14}C-G and the cotton enzyme complex was established by TLC on Silufol plates (15 × 7.5 cm) using *n*-propanol:ethylacetate:water (7:1:2). Standards were glucose and cellobiose. Bands with radioactive material were cut into transverse strips 0.5-cm wide, transferred with silica gel into tubes, and counted in a β-counter.

Peroxidase and polyphenoloxidase activities were determined spectrophotometrically [5, 6].

Isolation and purification of protein that codes for inhibitor gene were performed over TSK-HW-65 gel followed by ion-exchange chromatography over DEAE cellulose. Protein was eluted by Tris-HCl buffer (0.01 M, pH 7.8) from LKB columns (Sweden) and detected on a UVcord (LKB) at 280 nm.

Effect of inhibitor protein from smooth-seed line L-70 on the development of ovule fiber of the pubescent line was investigated using Murashige and Skoog (MS) medium [7]. The experiment was performed under sterile conditions. One-day bolls of L-489 were sterilized initially by freeing them of flower petals and bracts, wetting them with alcohol, quickly passing them through a flame, and immersing them in sterile distilled water with ampicillin (0.5 g per 50 mL water) for 30 min. Then, the prepared ovules were washed three times with sterile distilled water and transferred into Petri dishes. Ovules were separated from bolls and placed (10-15 each) into tubes with MS medium so that the medium just covered the ovules. Doses of 500 and 200 μg/mL were tested. The control tube contained no protein fraction. Tubes with ovules were kept in a controlled chamber at 28°C on a day, night, and humidity schedule for cotton. Samples were collected after 3, 4, 5, and 7 days and investigated by microscopy using an MBI-6 optical microscope and a Neophot-2 universal optical microscope (Carl Zeiss Jena).

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