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PROTEINS OF BARE- AND DOWNY-SEEDED COTTON VARIETIES

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An investigation of the composition of fiber-forming proteins and enzymes established a correlation between the degree of seed downiness in genetic cotton varieties and enzyme activity.

Key words: fiber-formation, cotton, proteins, peroxidase, glucansynthetase, cellulase.

Proteins from various plant species and varieties differ in composition. The emergence in hybrid varieties of new protein components that are absent in the parental varieties could be the result of activating genes that were previously regressive in the parental varieties. In certain instances the reverse phenomenon is observed, i.e., some protein components decrease in the hybrids compared with the parents as a result of regression or elimination in the hybrid of the gene responsible for the protein [1, 2]. It has been proven that the genetic material in all cells of an organism is identical whereas the differentiation mechanism that activates separate parts of the genome in various organs is as yet unknown. Valuable information about the plant specimen can be obtained by using electrophoresis or isoelectric focusing to determine the relative content of proteins. Genetic cotton varieties with improved fiber properties, high productivity, etc., can be selected using the fiber-forming activity of parental varieties in early growth stages (sprouts).

We investigated activities of glucansynthetase, peroxidase, and cellulase, which are involved in cotton cellulose synthesis, and found that the glucansynthetase activity in 5-day sprouts of bare-seeded genetic variety L-70 is an order of magnitude less than that in downy specimens: L-70, 4.2×10^{-7}; L-37, 4.2×10^{-6}; Golib-1, 1.5×10^{-6} units/mg protein (Fig. 1). The bare-seeded varieties contain an inhibitor gene that suppresses the activity of down structural genes in cotton [3]. Apparently the presence of the inhibitor gene lowers the activity of glucansynthetase, which participates in the final stage of cotton cellulose biosynthesis.

An inverse dependence on enzyme activity was observed in the study of cellulase in the studied cotton varieties with various degree of seed downiness (Fig. 1). Bare-seeded variety L-70 has high activity (13.0 arb. units/mg protein) whereas the downy varieties Golib-1 and L-37 have lower activities (7.9 and 6.3 arb. units/mg protein, respectively). Apparently the cellulase activity increases because genes that code for proteins that suppress cotton cellulose formation are expressed.

An investigation of peroxidase activity for the specific substrate ABTS [ammonium 2,2-azinobis(3-ethylbenzothiazolin-6-sulfonate) found that varieties with downy seeds have enzyme activities significantly greater than bare-seeded ones: Golib-1, 0.821 units/mL; L-70, 0.228 units/mL.

The spectrum of peroxidase showed differences in the relative enzyme mobility (REM) of isozymes: isozymes with REM 0.43 were characteristic of Golib-1; 0.45, L-37; and 0.16 and 0.19, L-70 (Fig. 2a).

We detected different total protein content in sprouts of the studied cotton varieties: 313 μg/mg dry weight for L-37; 473, Golib-1; 416, L-70.

The composition of soluble proteins isolated from sprouts differed: Golib-1 contained a polypeptide with molecular weight 47 kDa; L-37, 27 and 72 kDa; L-70, 54 kDa (Fig. 2b).

The enzyme activity in fiber (downy) and integument (downy and bare-seeded) varieties was investigated as a function of development. We collected 10- and 20-day (from the day of flowering) bolls for the analysis.

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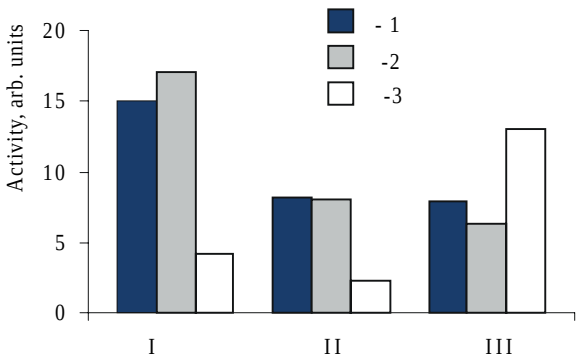

Fig. 1. Enzyme activity of 6-day sprouts of cotton genetic varieties differing in seed downiness: glucansynthetase (I), peroxidase (II), cellulase (III). Golib-1 (1), L-37 (2), L-70 (3).

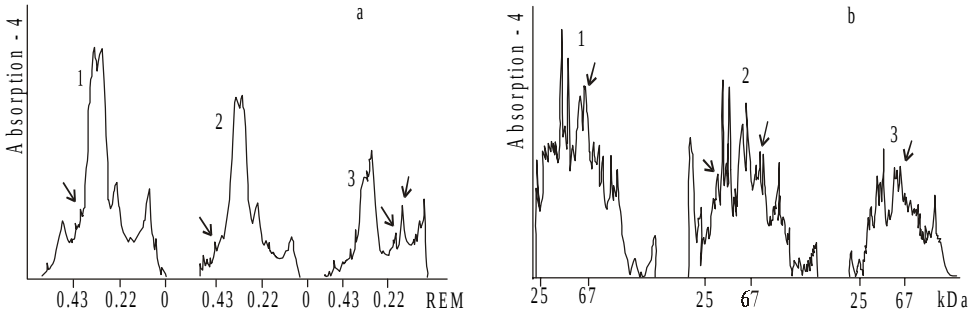

Fig. 2. PAAG densitogram of peroxidase isoforms (a) and sprout protein composition (b). Golib-1 (1), L-37 (2), L-70 (3).

The activity of glucansynthetase in developing fiber of Golib-1 and L-37 was 3.3×10^{-6} and 6.0×10^{-6} (units/mg protein); of cellulase, 43.1 and 25.0, respectively (Fig. 3a). The glucansynthetase activity in seed integument was lower: 2.8×10^{-6} (Golib-1) and 7.9×10^{-7} (L-37); cellulase, 6.25 and 5.8; for bare-seeded variety L-70, 3.3×10^{-7} (glucansynthetase) and 10 (cellulase) (Fig. 3b). The results for enzyme activity revealed lower values in the integument of bare-seeded cotton.

Peroxidase activity in fiber of the studied varieties was more or less equal: 0.280 units/mL (Golib-1) and 0.200 (L-37) (Fig. 3a); in integument, 0.200 (Golib-1), 0.180 (L-37), and 0.100 (L-70) (Fig. 3b). The enzyme activity in downy varieties may be due to the presence of a comparatively rich and qualitatively varied set of phenolic compounds, especially in 10-day fiber. After that, their quantity decreased. The decline in peroxidase activity in fiber after formation of the secondary cell wall (20-day fiber) is explained by the decrease of total phenolic compounds, which are a substrate for this enzyme [4]. This is consistent with the accelerated synthesis and accumulation of cellulose. Apparently the phenolic compounds are involved in redox processess that accompany cellulose biosynthesis.

We found differences in the number and REM of isozymes in an investigation of the isoenzyme spectrum of peroxidase in 10- and 20-day fiber of the studied varieties. Comparison of the isoform composition of peroxidase of downy- and bare-seeded varieties showed an identical number of isoforms and different coloration of bands in the electrophoregram.

The low activity of enzymes in L-70 sprouts was mentioned above. An inhibitor gene for seed downiness probably appears also in late stages of plant development.

Next we isolated proteins from 20-day fiber of downy-seeded Golib-1 and L-37 and from seed integument of bare-seeded L-70. Proteins were obtained only from integument of germinating seeds owing to the absence of fiber.

The composition of soluble proteins isolated from various samples varied. The integument of L-37 contained a polypeptide of molecular weight (MW) 43 kDa; fiber, 26 and 58 kDa; integument of Golib-1, 37 and 56 kDa; fiber, 52 and 92 kDa (Fig. 4). Integument of bare-seeded variety L-70 contained polypeptides of MW 27 and 130 kDa.

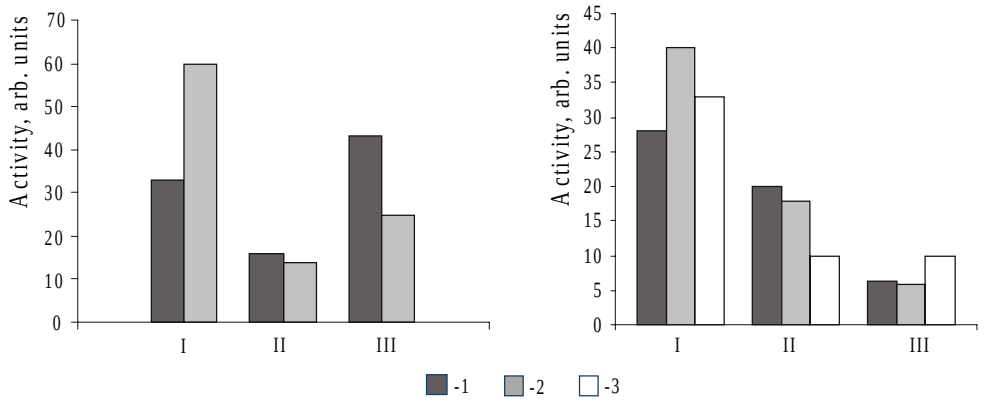

Fig. 3. Enzyme activity of 20-day fiber of downy cotton varieties (a) and integument of genetic varieties differing in degree of seed downiness (b): glucansynthetase (I), peroxidase (II), cellulase (III). Golib-1 (1), L-37 (2), L-70 (3).

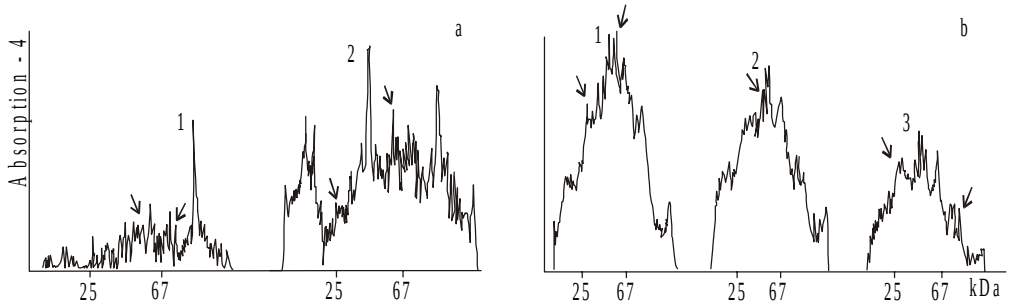

Fig. 4. PAAG densitogram of protein composition in cotton fiber (a) and integument (b). Golib-1 (1), L-37 (2), L-70 (3).

According to the results, the activity of enzymes involved in fiber formation, glucansynthetase and peroxidase, is greater in downy-seeded varieties than in bare-seeded ones whereas the cellulase activity is lower. It was also found that polypeptides characteristic of each variety are present in the proteins of fiber and integument.

EXPERIMENTAL

Genetic varieties of cotton obtained from the Genetics Department of the National University of Uzbekistan were investigated. These are L-70, bare-seeded, and L-37 and Golib-1, downy-seeded.

Enzyme activity was studied using 6-7-day sprouts and 10- and 20-day fiber.

Cotton seeds were bared by conc. H_2SO_4, quickly washed in a stream of cold water, and left for 24 h in tapwater. Swelled seeds were placed on paper sheets and grown for 5-6 days at 27°C in a thermostatted dark chamber.

Homogenate containing the enzyme preparation from sprouts and fiber was isolated by grinding material in liquid nitrogen and adding buffers to isolate separately each enzyme.

Determination of Glucansynthetase Activity. The crude membrane preparation obtained after destroying cells with liquid nitrogen was homogenized with tris-HCl buffer at pH 7.8. The homogenate was centrifuged at 2000 rpm for 5 min. The supernatant was centrifuged at 15,000 rpm for 45 min. The isolated enzyme preparation from sprouts was incubated in medium containing uridinediphosphate-^{14}C-glucose (UDP^{14}C-G) for 2 h at 27°C. The reaction was stopped by adding hot 96°C ethanol. Nonbonded label (UDP^{14}C-G) was removed by washing with ethanol (70%). The control was an enzyme preparation inactivated by heating on a boiling-water bath for 5 min before adding label. The radioactivity was counted in a β-counter.

Identification of the Synthesis Product. The nature of polymer newly synthesized 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 out in transverse sections of 0.5 cm width. Silica gel was transferred from them in vials and counted in a β-counter.

Isolation of Peroxidase Enzyme Preparation. Bolls for isolation were collected when the primary and secondary fiber cell wall formed. Cell walls were destroyed by liquid nitrogen. Then, the resulting powder was treated with 5-7 volumes of tris-glycine buffer at pH 8.3 containing NaCl (1 M). The extract was centrifuged for 30 min at 10,000 rpm. The supernatant was dialyzed against distilled water and lyophilized.

Peroxidase activity was determined by spectrophotometry [5]. Peroxidase was separated by electrophoresis in alkaline (pH 8.9) PAAG (7.9%) according to Davis [6]. Plates were visualized with benzidine.

Protein content was determined by electrophoresis in a PAAG gel gradient (from 10 to 15%) with SDS according to Laemmli [7]. Standards for determining MW were bovine serum albumin (BSA), chymotripsinogen, and cytochrome C. Protein content was found by the Lowry method [8]. PAAG plates were scanned on a 2202 ULTROSCAN Laser Densitometer.

Determination of Cellulase Activity for a Colored Insoluble Substrate. Substrate (150 mg) in acetate buffer (5 mL, 0.1 M, pH 4.5) in 50-mL Erlenmeyer flasks at 40°C on a rocker was treated with enzyme solution (2 mL, 0.1-1 units/mL of endoglucanase), incubated for 20 min, and filtered. The absorption at 490 nm was determined. The control was a suspension of substrate to which enzyme solution was added quickly and filtered. One arbitrary unit of enzyme activity equals 0.1 optical absorbance at 490 nm in 1 min.

REFERENCES

1. U. K. Nadjimov, M. S. Mirahmedov, B. U. Nosrullaev, G. N. Fathullaeva, and L. M. Scott, *J. Plant Growth Regul.*, **15**, 129 (1996).
2. M. F. Sanamyan and D. A. Musaev, *Genetika*, **28**, No. 11, 75 (1992).
3. D. A. Musaev and N. G. Gubanova, in: Abstracts of Papers of the Second All-Union Meeting "Growth Genetics," Tashkent (1990), Vol. 1, p. 108.
4. F. R. Nuritdinova and R. Zh. Allanazarova, *Theoretical and Applied Cartology* [in Russian], Kishinev (1989), p. 79.
5. A. I. Ermakov, ed., *Methods of Biochemical Research* [in Russian], Agropromizdat, Leningrad Div. (1987).
6. B. J. Davis, *Ann. N. Y. Acad. Sci.*, **121**, 404 (1964).
7. J. King and U. K. Laemmli, *J. Mol. Biol.*, **62**, 465 (1971).
8. O. H. Lowry, N. G. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).