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CELLULOSE BIOSYNTHESIS OF COTTON TREATED WITH RETARDANTS

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The effect of retardants on cellulose biosynthesis and protein composition in seedlings and fiber of two cotton species is investigated. The activity of glucansynthetase and the synthesis of "de novo" polypeptides increase under the influence of morphonol and Pix.

Many synthetic compounds that are foreign to a living system agressively interfere with metabolism and disrupt its normal functioning. They themselves also undergo various chemical transformations. Retardants, growth regulators, occupy a special place among compounds that actively affect vegetative processes in plants. Retardants cause growth to cease and nutritive substances to accumulate in fruiting bodies by exerting an inhibitory effect on gibberellins.

Studies of the effect of retardants on cotton concern mainly the study of their influence on the growth of plants, the chlorophyll content in leaves, the preservation of fruiting bodies, increased productivity, etc. [1, 2]. The action of these physiologically active compounds on the biochemical processes occurring during the development of cotton fiber has been little studied [3]. Therefore, we examined the effect of retardants on the synthesis of cotton-fiber cellulose, in particular, on one of the principal enzymes involved in this process, glucansynthetase.

An extract isolated from seedlings and fiber of two species of cotton, Andizhan-9 and C-6524, was used in the study of the effect of the retardants Pix and morphonol on cotton cellulose formation.

One of the rapid methods for studying the action of physiologically active compounds on enzyme activity is to add them directly to the incubation medium in which the studied compound is being biosynthesized. It was found previously [1] that retardants at a concentration of 0.001% positively affect the enzyme activity of cotton proteins. Therefore, the action of Pix and morphonol *in vitro* was studied at this concentration.

Increased enzyme activity was observed during a determination of the glucansynthetase activity of proteins in seedlings and fiber of both cotton species. The stimulating effect of morphonol is greater than that of Pix (Table 1). Thus, the activity in seedlings increased by an average of 90%; in Andizhan-9 fiber, by 50%; in C-6524, by 88%. The positive effect of Pix was slightly less. In Andizhan-9 seedlings, the activity increased by 70%; in fiber, by 30%, in C-6524, by 40 and 28%, respectively.

Then the effect of industrial concentrations of retardants, 0.016% Pix and 0.25% morphonol, on cellulose synthesis in fiber of these cotton species was studied after spraying the plants with solutions of the compounds. At first the activity of glucansynthetase in fiber treated with Pix exceeded the activity of the control for Andizhan-9 by 44%; in C-6524, by 52%. Activation by morphonol was noticeably less (Fig. 1).

The increased enzyme activity is explained by the stimulating effect of Pix and morphonol on metabolic processes occurring in the cell. Chlorophyll formation is known to increase in leaves after treatment of cotton with retardants [3-5]. In all probability this is indicative of increased photosynthesis and, correspondingly, increased formation of carbohydrates such as glucose, saccharose, etc.

The enzyme activity of glucansynthetase decreases during formation of the secondary cell wall (17-20 days after spraying). The retardant effect on cellulose synthesis diminishes as the cotton fiber develops. The glucansynthetase activity begins to increase by the 30th day. For Andizhan-9, it is two times the control value.

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TABLE 1. *In vitro* Effect of 0.001% Pix and Morphonol Solutions on Andizhan-9 and C-6524 Cotton Seedling and Fiber Glucansynthetase Activity

Variety	GS-activity			
	AU/mg protein·10^{-7}	%	AU/mg protein·10^{-7}	%
	Seedlings		Fiber	
Andizhan-9, control	2.0	100	1.4	100
Andizhan-9 + Pix	3.4	170	1.8	130
Andizhan-9 + morphonol	3.9	195	2.1	150
C-6524, control	7.6	100	6.9	100
C-6524 + Pix	10.5	140	8.8	128
C-6524 + morphonol	14.7	193	13.0	188

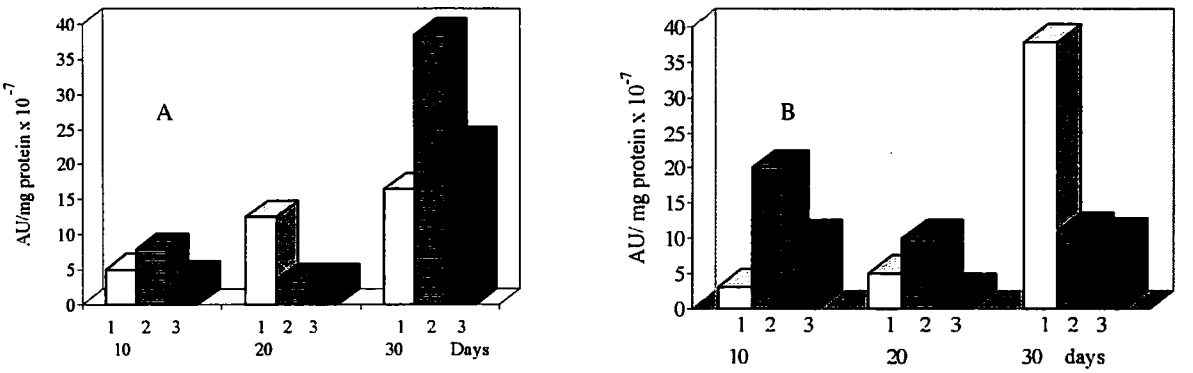

Fig. 1. Effect of Pix and morphonol on cotton fiber glucansynthetase activity: type Andizhan-9 (A), type C-6524 (B), control (1), Pix (2), morphonol (3).

Pix and morphonol exhibit different effects on glucansynthetase of the different cotton species during maximal manifestation of enzyme activity (30th day after flowering). Whereas the activity in Andizhan-9 is 1.5-2 times higher that of the control, it is 3-4 times less than the control in C-6524.

Electrophoresis of fiber proteins in cotton treated with retardants showed that Pix and morphonol change the protein composition. The composition differs from the controls in the appearance of specific polypeptides. For Andizhan-9, the appearance of polypeptides of molecular mass 32, 37, 44, and 47 kDa is characteristic of treatment with Pix; of 7, 12.1, and 43 kDa, with morphonol (Fig. 2A). For C-6524, Pix causes the appearance of polypeptides of molecular mass 31, 36, 41, and 78 kDa; morphonol, 17 and 64 kDa (Fig. 2B).

The quantitative protein content increases during fiber development under the influence of morphonol and Pix compared with the control.

Thus, it can be concluded that Pix and morphonol stimulate the activity of cotton glucansynthetase, which sharply increases in the first days of cotton fiber development. Polypeptides appear in fiber proteins after treatment with Pix and morpholon. These may act as regulators of the gene responsible for the synthesis of these proteins. The difference in the action of retardants on the different cotton species must be considered. The use of seedlings for accelerated testing of the effect of newly synthesized growth regulators on plant enzyme systems can be recommended. In particular, this concerns enzymes involved in cotton fiber cellulose synthesis.

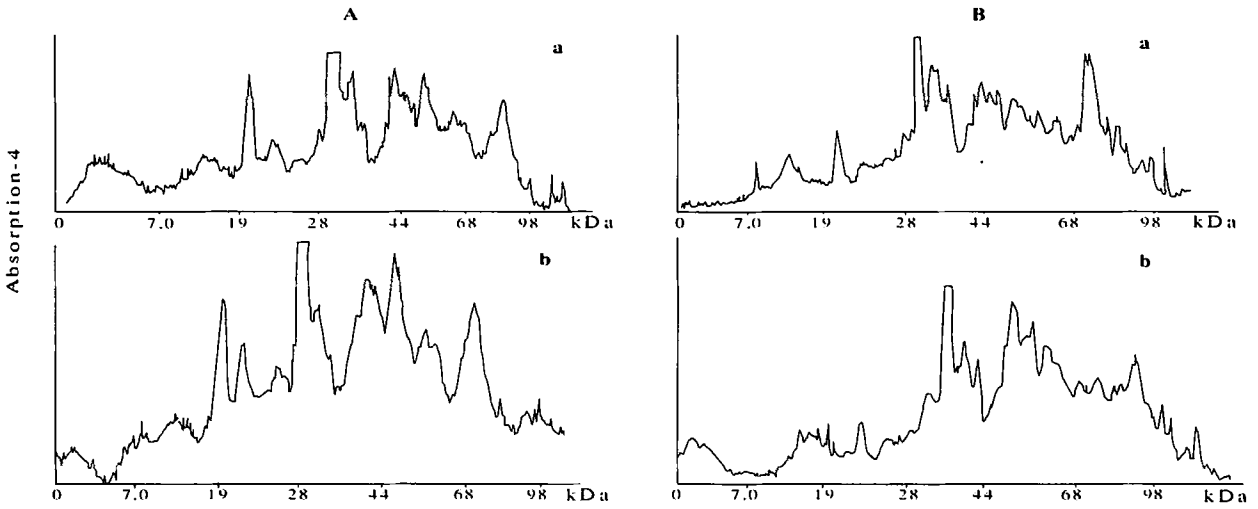

Fig. 2. Electrophoresis densitograms in 10-15% PAAG with sodium dodecylsulfate of soluble proteins of 20-day fiber of Andizhan-9 (A) and C-6524 (B) treated with the retardants Pix (a) and morphonol (b).

EXPERIMENTAL

Enzyme Preparation from Seedlings and Fiber. Stripped Andizhan-9 and C-6524 cotton seeds (*Gossypium hirsutum*) were soaked for one day in tapwater and sprouted in paper containers at 27°C for 4 days in a moist dark room. Blanched hypocotyls were ground in a porcelain mortar with added liquid nitrogen to destroy the cell walls, $NaHCO_3$ (1 g), and polyvinylpyrrolidone (PVP, 5 g) per 100 g of plant material. Tris-HCl buffer (0.001 M) at pH 7.8 was added at a 1:4 ratio as the grinding proceeded. The resulting mass was pressed through four layers of canvas and centrifuged at 2.000 rpm for 5 min. The supernatant was centrifuged at 15.000 rpm for 45 min. The precipitate was used in subsequent investigations. The enzyme preparation from fiber was prepared analogously.

Retardant Effect. The cotton species were grown on an experimental plot at the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan. Spraying with 0.016% Pix (mepiquat chloride) and 0.025% morphonol [N,N-di(β-hydroxyethyl)-morpholinium chloride] was conducted during full flowering. Plants were sprayed by hand with retardant preparations in the morning. Fiber samples were collected at 10 (primary cell-wall formation), 20 (secondary cell-wall formation), and 30 days of growth (appearance of maximal glucansynthetase activity).

The effect of Pix and morphonol *in vitro* was studied by adding retardant solutions (0.001%) directly to the incubation medium.

Glucansynthetase Activity. The enzyme preparation isolated from seedlings and fiber was incubated in a medium containing uridinediphosphato-^{14}C-glucose (UDP-^{14}C-G) for 2 h at 27°C [6]. The reaction was stopped by adding hot 96° ethanol. Nonbonded label was removed by washing four times with 70% C_2H_5OH. The control was an enzyme preparation inactivated by heating on a boiling water bath for 5 min before adding label. The radioactivity was measured on a β-analyzer. The protein was determined by the Lowry method [7].

Identification of Synthesis Product. The nature of the freshly synthesized polymer from the UDP-^{14}C-G cellulose precursor and enzyme complex from cotton seedlings and fiber was established by TLC on Silufol plates (15 × 7.5 cm) in solvents system of *n*-propanol—ethylacetate—water (7:1:2). The plates were developed with a solution of potassium permanganate (0.5%) in NaOH (1 H). The plates were heated to 100°C after spraying. Standards were glucose and cellobiose. Chromatograms with radioactive material were cut into 0.5-cm sections. The silica gel was transfered into vials. The activity was counted in a β-analyzer. The scintillant was ZhST.

Protein Composition of Cotton Fiber Treated with Retardants. Cotton fiber was ground in liquid nitrogen with addition of Tris-HCl (0.05 M) buffer at pH 7.8. The homogenate was centrifuged at 1500 rpm for 5 min. The supernatant was centrifuged at 6000 rpm for 30 min, dialyzed against distilled water, and lyophilized. The protein composition was determined

by gradient electrophoresis (10-15% in PAAG) by the method of Laemmly [8]; the quantitative protein content, by the method of Lowry [7].

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