THE EFFECT OF PHYTOHORMONES TO THE DYNAMICS OF PROTEIN BIOSYNTHESIS AND ENZYME ACTIVITY IN LINTED AND NAKED SEED COTTONS

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Abstract

We determined the effect of exogenous indolil-3-acetic acid, α-naphthyl-3-acetic acid and gibberellic acid-3 on enzymatic activity of glucansynthase, peroxidase and cellulase in ovule development of naked and linted cotton (*Gossypium hirsutum* L.) seeds. We isolated a protein–inhibitor of 37 kDa with pI 4.2 from integument tissue of naked cotton seeds. In addition, we studied its inhibitory activity to the biosynthesis of cellulose after gibberellic acid-3 treatment of ovules of linted cottonseed. The results should be useful for understanding of lint development in cotton.

Introduction

The study of the regulatory pathways of cotton fiber development is the important theoretical and practical interest. Understanding cotton fiber development mechanisms is not possible without detail knowledge of expansion of the fiber cell walls structure, mechanisms of synthesis of constitutive components, and regulation of these processes (Polevoy, 1982; Polevoy, 1986). In this aspect phytohormones are considered to strongly influence. The mechanism of phytohormone effect on the plant cell includes activation of DNA transcription, biosynthesis of mRNAs, followed by synthesis of enzymes, and leading to the complete activation of cell metabolisms. Hence, phytohormone-induced permeability of cell membranes and the increase of ATP-biosynthesis are important. Phytohormones regulate a level of growth and developmental processes in the plants as constitutive natural products of metabolism. Activation of cell polysaccharide synthetases induces rapid polysaccharide synthesis.

Phytohormones are organic, low-molecular compounds which facilitate the interaction of cell, tissues and organs. These compounds trigger physiologic programs in plants such as seed germination, and growth maturation, morphogenesis, and flowering at very low concentrations. Wide-spectrum of physiological activity of phytohormones provides the exogenous phytohormones as a primary factor in the in vitro control of morphogenesis. The physiological action of phytohormones occurs by means of hormone-receptor complex formation, which determines the phytohormone effect (Tarchevchkiy, 2001; Polevoy et al., 2001). In the case of cotton fiber initiation is influenced by physiological changes in the plant. The number of lint fibers per ovule is affected by environmental and agronomic conditions such as planting date (Bowman, 2000; Lewis, 2000), and mean minimum daily temperature (Lewis, 2000).

Studies of the effects of phytohormones on fiber development have been conducted (Basra et al., 1999; Gialvalis et al, 2001; Momtaz, 1998, Beasly, 1971; Beasly et al., 1973; 1974). Analysis of hormone content in young ovules and fibers grown in plant indicates that auxin levels are high initially (0 d post-anthesis) and then drop significantly 8 d post-anthesis (Chen et al., 1996; John, 1994; Nayyar et al., 1989). This research supports previous observations of Jasdanwala et al. (1980) indicating an increase in indolil-3-acetic acid (IAA) levels is important for epidermal cells to differentiate them into fibers. There are only a few studies concerning hormone regulation of cotton cellulose synthesis in recent publications. These studies deal with investigation of the role of endogenous hormones in cell metabolism, but not effects of exogenous action of phytohormones (Tarchevchkiy, 2001). IAA and gibberellic acid-3 (GA3) phytohormones have a large influence on intensification of fiber cellulose formation (Lozovaya et al., 1987).

It is significant that each phytohormone group influences specific processes in plant morphogenesis. For example, auxines are essential participants in morphogenesis coordination. They have an impact on fission, straining and differentiation of cells. These hormones initiate DNA replication. High concentrations of auxin

can cause mitosis in somatic plant cells. Auxin activates synthesis of specific proteins including proteins secreted to cell walls which are not inhibited by actinomycin D. By data of Musaev (1974) for allopolyploid cotton cultivars G. hirsutum L. four genes exist for lint and naked seed traits. These genes have been separated into different groups: 1) basic genes; 2) additional gene; 3) gene-inhibitor. Dominant alleles of the basic genes control the development of linter of seed. The amount of lint depends on the expressing of genes in the dominant alleles. The naked seed of G. hirsutum L. depends on two factors: first – the presence of a dominant gene-inhibitor in the genotype and second – the presence of recessive genes of linter of seed. However, investigations of phytohormone action on the ovule development of naked seed cotton have not been performed.

Thus, our study is connected with the investigation of biochemical characteristics of cottons differing by the lint of seeds, fiber maturating enzyme activity, protein composition of cotton fiber and integument tissue of naked and linted seed cotton. Thus, we investigated specific proteins and modulation of glucansynthase, peroxidase and cellulase activity by α-naphthyl-3-acetic acid (NAA), IAA, and GA3 phytohormones in the integument of naked seed cotton, and the fiber and the integument tissue of linted cotton.

Materials and Methods

General

The subject of research is proteins from 20 dpa ovule integument of naked seed cotton (line L-70) and fiber and integument of linted seed cotton (AN-Bayaut-2 cultivar) *Gossypium hirsutum* L. (Institute of Cotton Breeding, Republic of Uzbekistan), which were treated with IAA, NAA and GA3 (10-6M) by hand using a syringe on the day of flowering.

Protein isolation from cotton integument and fiber (line L-70 and AN-Bayaut-2 cultivar)

Plant material was ground with liquid nitrogen. Total protein was extracted with 0.05 M Tris-HCI (pH 7.8), containing 1M NaCl (1:4) for 30 min at 4oC, and centrifuged for 5 min at 1500 g. The supernatant was collected. The precipitate was suspended in a small volume of the same buffer and centrifuged for 5 min at 1500 g. The supernatants were combined and centrifuged for 30 min at 5000 g. The total protein was precipitated by addition of 5 volume cold acetone, centrifuged (15 min at 6000 g), lyophilized, dissolved in small volume of the same buffer, and desalted on a column with Sephadex G-10 (2.5x80 cm) in distilled water and lyophilized.

Electrophoresis

Electrophoretic analysis of proteins was performed in gradient (10 to 15%) polyacrylamide gel (PAG) by the method of Laemmly (1971) using vertical apparatus (Himifil, Estonia) with Sigma reagents. Protein content was determined by the method of Lowry O.N. (1951). Isoelectrofocusing was performed using ampholins with pI 3.5 to 9.6 (Sigma).

Protein chromatography on TSK-gel column

Lyophilized total protein was dissolved in 1 mL of 0.05 M sodium phosphate (pH 7.0) and put on TSK HW-55F column (0.7x30 cm) in the same buffer. Flow rate was 2 mL/h.

Ion-exchange chromatography of proteins

Fractions collected from the TSK gel were dissolved in 0.7 ml of 0.001 M sodium acetate (pH 4.6) and put on a DEAE-TSK column (1.2x4 cm) using the same buffer. Elution was performed using a linear gradient of sodium chloride in the same buffer. Flow rate was 20 mL/h. Obtained fractions were dialyzed against distilled water and lyophilized.

Determination of enzyme activity

The glucansynthase, peroxidase and cellulase activity was determined by the method described in (Akhunov et al., 2001).

Microscopic assay

The cotton ovule's morphology was assayed using an optical microscope MBI-6 and universal optical microscope Neophot-2 (Carl Zeiss, Germany).

Statistical analysis

All experiments were repeated at least three times. Data were subjected to analysis of variance (ANOVA), and differences between treatments assessed by Student's two-sample t-test at $P<0.05$.

Results and Discussion

We analyzed the effect of IAA, NAA and GA3 phytohormones on protein content and enzyme activity of glucansynthase, peroxidase and cellulase of 20 dpa integument tissue of naked seed (line L-70) cotton, and fiber and integument of linted (AN-Bayaut-2) cotton. The phytohormone NAA showed no effect on total protein content in AN-Bayaut-2, whereas in L-70 it caused an increase of 13% (Table 1). GA3 increased the protein by 35% in AN-Bayaut-2 and by 6% in line L-70. We suggested that NAA and GA3 activate genes causing a synthesis of RNA, and lead to synthesis of new kinds of enzymes or their activation (Privalov, 1983). This fact demonstrated increase of protein concentration in all 5-, 10- and 20 dpa integument tissues of seed-bud examples.

Table 1. Enzyme activity of GS, PO, and Ce isolated from 20 dpa integument tissues.

Samples	Protein content mg/ml (%)		Enzyme activity					
	Integument	Fiber	GS cpm/min/mg of protein (%)		PO unit/mg of protein (%)		C unit/mg of protein (%)	
			Integument	Fiber	Integument	Fiber	Integument	Fiber
Line L-70								
Control	5.217±0.2 (100)	-	1710±80 (100)	-	7.30±0.4 (100)	-	7.67±0.4 (100)	-
Treatment with IAA	5.999±0.3 (115)	-	6120±300 (358)	-	8.60±0.4 (118)	-	8.25±0.4 (108)	-
Treatment with NAA	5.913±0.3 (113)	-	5870±290 (343)	-	9.20±0.5 (126)	-	8.46±0.4 (110)	-
Treatment with GA_3	5.530±0.2 (106)	-	620 ± 30 (36)	-	9.20±0.5 (126)	-	5.94 ±0.3 (77)	-
Sort AN-Bayaut-2								
Control	4.696±0.2 (100)	1.438± 0.07 (100)	1000±45 (100)	3460± 150 (100)	13.40±0.7 (100)	0.256 ±0.01 (100)	6.39±0.3 (100)	0
Treatment with IAA	5.166±0.2 (110)	1.610± 0.08 (112)	1350±59 (138)	6890± 330 (199)	13.1± 0.6 (98)	0.287 ±0.01 (112)	5.98± 0.3 (94)	0
Treatment with NAA	4.696±0.2 (100)	1.508± 0.07 (105)	1200± 60 (120)	6440± 300 (186)	12.30±0.6 (92)	0.304 ±0.02 (119)	5.75± 0.25 (90)	0
Treatment with GA_3	6.348±0.3 (135)	1.780± 0.09 (124)	10,588± 500 (1060)	13625 ±600 (394)	11.10±0.5 (83)	0.342 ±0.02 (134)	5.51±0.25 (86)	0

The electrophoretic investigation of the total proteins isolated from the control and those treated with GA3 and NAA plant integument showed wide range of minor components and expressed proteins having different molecular masses (Fig.1).

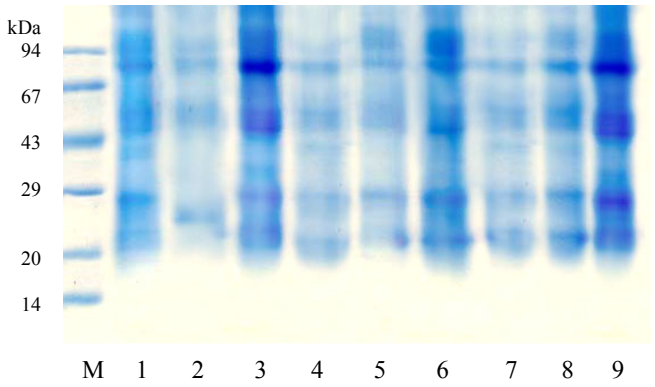

Figure 1. Electrophoretogram of total protein from integument of AN-Bayaut-2 in gradient PAG (10 to 15%) after 5, 10 and 20 dpa: M –markers; 1 – control; 2 – treatment with NAA; 3 – treatment with GA3 (5dpa); 4 – control; 5 – treatment with NAA; 6 – treatment with GA3 (10 dpa); 7 – control; 8 – treatment with NAA; 9 – treatment with GA3 (20 dpa).

The increase of protein concentration under GA3-treatment might possibly connect with functional properties of this phytohormone. Basra et al. (1999) showed that plants treated with GA3 before flowering increased the generative period, otherwise the flowering process was slowly inhibited, and the process of fruit ripening was activated after this phytohormone-treatment during the flowering period. The authors concluded that the process of protein biosynthesis is activated and their concentration increases after treatment of the flower with GA3. The treatment with NAA, before blooming, caused a large increase of fuzz amount on the epidermal surface of cells compared to GA3-treatment, according to optical microscopic of control and phytohormone-treated ovules. The treatment of unfertilized seed-buds revealed the reverse effect: GA3 increased a fuzz number compared to NAA. Therefore an exogenous phytohormone effect depends on period of influence on cotton. Studying of AN-Bayaut-2 cotton ovules structure on epidermis surface showed "blubber" cells especially near chalazal holes. The process of fuzz appearance and their elongation was timely increased under GA3-treatment.
The surface of L-70 cotton ovules remained largely unchanged after treatment with NAA and GA3. The increase in the number of ovule cells which lead to distortion of lank cell surface, to appearance of folds, and to longitudinal deepening. However appearance of fuzz was not observed.

The investigation of the influence of phytohormones to the activity of glucansynthase (GS), peroxidase (PO) and cellulase (Ce) which considered as a fiber development associated enzymes revealed that a treatment of naked seed ovules with NAA increased activity of GS by 243%, PO by 29%, and Ce by 10% (Table 1). The activation of the analyzed enzymes in 20 dpa integument tissue of L-70 under NAA was associated with an increase in protein quantity. Protein synthesis and the increase of enzyme activity are closely interconnected (Sakalo et al. 2004). According to our results, the increase of enzyme activity correlates with increase of protein content under NAA-treatment in L-70. NAA also increased the activity of GS in linted AN-Bayaut-2 by 20%, while PO and Ce activities were reduced by 8 and 10% respectively. Furthermore, treatment of ovules with GA3 reduced GS activity in integument tissue of L-70 by 64%; Ce by 23%, and PO acclivity increased by 29%. The activity of GS increased (by 960%) in integument of AN-Bayaut-2, while activities of PO and Ce were reduced by ~15%.

The specificity of plant reaction to different phytohormones is controlled by genetic background of the cultivar (Privalov, 1983). The cause of physiological activity has been explained by two hypotheses: 1) the reaction of plants to exogenous gibberellin is condition the plant to reconstruct gibberellin to the active form; and 2) plant cells have different specific receptors to gibberellins, and gibberellins activity depends on the degree of their affinity to molecular structure of specific receptor. In our case, the various effects of phytohormones may be caused by the presence of different receptors in line L-70 and AN-Bayaut-2 cultivar. Several means of phytohormone inactivation exist in plant cells such as binding of proteins, glycolysis, PO and polyphenoloxidase oxidation (Kefeli, 1970). In our previous study (Akhunov et al., 2001) we showed the presence of proteins which inhibited GA activity from integument tissue of naked seed of L-70 line. In the current investigation, we studied the effect of protein fractions of phytohormone-treated integument tissue on the GS activity. Proteins from integument of naked seed L-70 line suppressed GS activity both in control and in experimental variants (Table 2). The first fraction from GA3-treated integument tissue, obtained after gel filtration on TSK-gel

column, suppressed GS activity by 96%. In this case the gene-inhibitor is in the dominant state (Musaev, 1988) and, possibly, during GA3 treatment an activation of inhibitor gene occurred. The increase of GS to 170% was observed under the effect of proteins from integument. The activation of GS (or intensification of synthesis this enzyme) stimulated under the action of this phytohormone.

Table 2. Effect of proteins isolated from 20-days integument of naked seed on GS activity

Sample	Percent of inhibition, %
Line L-70. Control integument	
Initial material	84
1st fraction	77
2 fraction	14
Integument treated with IAA	
Initial material	Stimulation 47
1st fraction	Stimulation 168
2 fraction	39
Integument treated with NAA	
Initial material	Stimulation 40
1st fraction	Stimulation 170
2 fraction	40
Integument treated with GA_3	
Initial material	36
1st fraction	96
2 fraction	20

Electrophoretic determination of protein components from ovule integument tissue of naked seed L-70 line revealed that in the control and GA3-treated plants, there are two proteins detected with molecular masses 37 and 13 kDa, respectively, in addition to a large number minor protein components (Fig.2). Proteins bands of samples treated with GA3 are expressed more clearly than control.

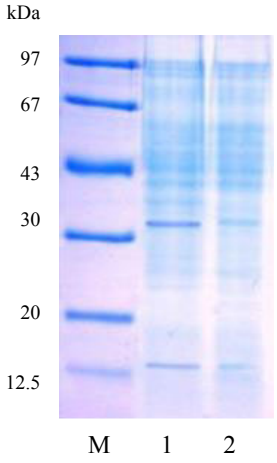

Figure 2. Electrophoresis of proteins from 20 dpa integument tissue of line L-70: M – markers; 1 – proteins from GA_3-treated samples; 2 – extract of control samples.

The obtained results suggest that proteins which possess inhibitory effect to GS activity are present in total protein extract of GA3-treated integument tissue of L-70 line. To purity this protein fraction we used size-exclusion chromatography on TSK-HW-55F. The separation of total protein on TSK-HW-55F showed the presence of three fractions. The first fraction demonstrated 63% of GS activity decrease, while the second and the third fractions had lower inhibitory effect (20 and 25%, respectively) as compared with control. The protein fraction composition was investigated by electrophoresis (Fig. 3, a). There is homogeneous component in fraction 5. The molecular mass of this component is ~ 37 kDa. The pI is 4.2 (Fig. 3, b).

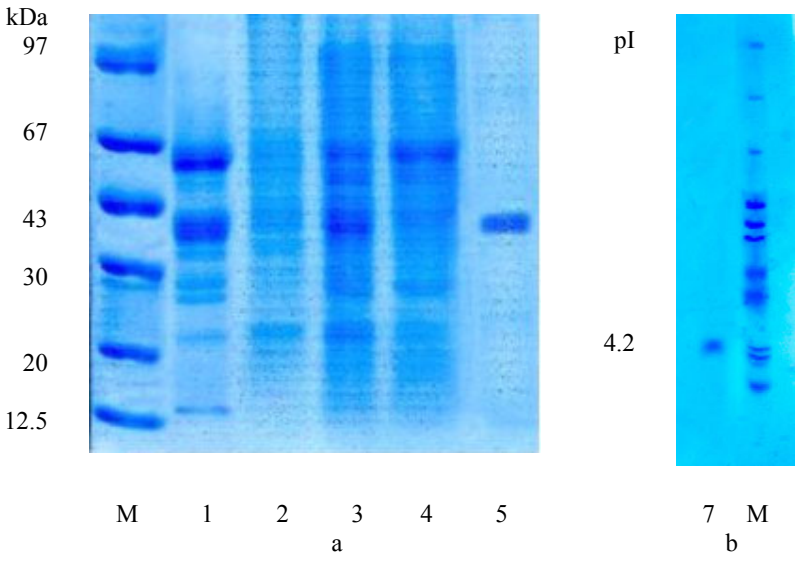

Figure 3. Electrophoresis of fractions obtained after chromatography on DEAE-TSK (a): M – mixture of markers; 1 – fraction 1; 2 – fraction 2; 3 – fraction 3; 4 – fraction 4; 5 – fraction 5. Isoelectrofocusing in 7% PAG at presence of ampholins in diapason pH 3.5-10 (b): M – mixture of pI markers; 1 – protein – inhibitor

Summary

Thus, phytohormones IAA, NAA and GA3 have different effects on protein biosynthesis and alter the activity of glucansynthase, peroxidase and cellulase enzymes active during fiber formation. The study showed an increase in the number and length of fiber in linted cotton under the action of phytohormones. The changes in structure of ovule surface of naked seed were marked, but appearance of fiber was not observed. This suggests that naked seeding of L-70 cotton line is genetically determined. Furthermore, the content of protein-inhibitor with Mr 37 kDa increased in integument tissue of naked seed cotton under the effect of GA3.

The research of modulators of cotton fiber development will allow understanding the typical mechanisms of development of fiber with different quality. The knowledge about these properties is discovered aspects for the choice of parental pairs during cotton selection, and control of growth processes.

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