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EFFECT OF α-NAPHTHYLACETIC AND GIBBERELLIC ACIDS ON INHIBITOR PROTEIN CONTENT AND FIBER-FORMING ENZYME ACTIVITY OF GYMNOSPERM AND FUZZY COTTON

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The effect of the phytohormones α-naphthylacetic acid and gibberellic acid on the protein content; glucansynthetase, peroxidase, and cellulase enzyme activity; and development of gymnosperm and fuzzy cotton ovules was studied. The effect on cellulose synthesis of an inhibitor protein isolated from integument of gymnosperm cotton treated with gibberellic acid was investigated.

Keywords: gymnosperm, fuzzy cotton, peroxidase, phytohormones, proteins, glucansynthetase, peroxidase, and cellulase enzymes.

Phytohormones have developed through evolution and are a genetically stable system of compounds that regulate the activity of the genetic apparatus during growth processes and senescence and protect the whole plant or its separate organs from the effects of extreme environmental factors and microflora [1]. The phytohormonal system is a product of the genotype and has a large inductor or repressor influence on the activity of certain genes or their blocks. All this eventually affects the quantity and quality of the harvest.

The most important plant hormones are auxins and gibberellins. Only a few reports on the hormonal regulation of cotton cellulose synthesis have appeared. These mainly analyze the levels of endogenous hormones in cells of cotton seed fibers and not features of the exogenous action of phytohormones [2]. The phytohormones α-naphthylacetic acid (α-NAA) and gibberellic acid (GA) have great significance for increasing cellulose fiber formation [3].

We investigated the action of phytohormones α-NAA and GA on protein content, ovule development, and glucansynthetase (GS), peroxidase (PO), and cellulase (C) enzyme activity in 20-day integument of gymnosperm (line L-70) and fuzzy (variety AN-Bayaut-2) cotton.

According to the results, phytohormone α-NAA had no effect on the total protein content in AN-Bayaut-2 whereas it increased it in line L-70 by 13% (Table 1). GA increased the amount of protein by 35% in AN-Bayaut-2 and by 6% in line L-70. It has been hypothesized that α-NAA and GA activate repressor genes by interacting with low-molecular-weight repressor proteins, thereby inducing the synthesis of RNA molecules. This in turn leads to the synthesis of new types of enzymes or enhances the synthesis of already existing enzymes [1].

An electrophoretic study of total proteins from integuments of control plants and those treated with gibberellin and α-NAA showed that distinctive proteins with various molecular weights were present in addition to a large number of minor protein components (Fig. 1). The color intensity of proteins from samples treated with GA increased in fuzzy variety AN-Bayaut-2.

This indicated that the protein concentration increased in all ovule samples. The increased protein concentration caused by gibberellin may have been due to functional specifics of this phytohormone.

It has been reported [4] that the growth period increased upon treatment of plants with GA before flowering. In other words, it slowed the flowering process itself. However, treatment of the plant with this same phytohormone during flowering accelerated the fruit ripening process, because of which it can be concluded that the biosynthesis of proteins was accelerated by spraying flowers with gibberellin and also that their concentration increased.

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TABLE 1. Activity of Glucansynthetase (GS), Peroxidase (PO), and Cellulase (C) Enzymes Isolated from 20-Day Integument of Gymnosperm and Fuzzy Cotton Treated with Phytohormones α-NAA and GA

Sample	Protein content, mg/mL (%)	Enzyme activity		
		GS, counts/min/mg protein (%)	PO, units/0.1 mL (%)	C, units/mg protein (%)
		Line L-70		
Control	5.217 (100)	1710 (100)	7 (100)	7.67 (100)
Treatment with α-NAA	5.913 (113)	5870 (343)	9 (129)	8.46 (110)
Treatment with GA	5.530 (106)	620 (36)	9 (129)	5.94 (77)
		Variety AN-Bayaut-2		
Control	4.696 (100)	1000 (100)	13 (100)	6.39 (100)
Treatment with α-NAA	4.696 (100)	1200 (120)	12 (92)	5.75 (90)
Treatment with GA	6.348 (135)	10588 (1060)	11 (85)	5.51 (86)

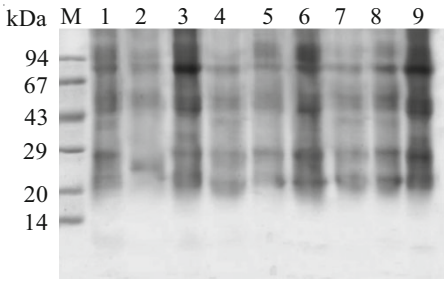

Fig. 1. Electrophoregram of proteins from integument of AN-Bayaut-2 ovules in PAAG (10-15%): M, marker proteins; 5-day: control (1), NAA treated (2), gibberellin treated (3); 10-day: control (4), NAA treated (5), gibberellin treated (6); 20-day: control (7), NAA treated (8), gibberellin treated (9).

Optical microscopy of control fruit and that treated with phytohormones suggested that treatment with α-NAA before flowering caused a greater increase in the number of fibers on the epidermal cell surface than treatment with GA. Treatment of fertilized ovules caused the opposite effect. GA increased the number of fibers compared with α-NAA. Therefore, the exogenic effect of the phytohormones depended on the time of application to the cotton ovule.

Bulging cells were observed, especially near the chalaza opening, on the epidermal surface of AN-Bayaut-2 cotton ovules during a study of their structure. The production of fibers and their length increased sharply under the influence of GA.

The L-70 cotton ovule surface remained almost the same for treatment with GA or α-NAA solutions regardless of the type of hormone and development time. The increase in the number of ovule cells distorted the relatively smooth cellular surface of the ovules and caused the appearance of wrinkles and longitudinal depressions. However fibers did not appear. This indicated that gymnospermicity is a genetically imparted signature.

The action of the studied phytohormones on the activity of GS, PO, and C enzymes, which are involved in cotton fiber formation, showed that treatment of ovules of gymnosperm cotton with α-NAA increased the activity of GS by 243%; PO, 29; and C, 10 (Table 1). The activation of these enzymes in 20-day integument of L-70 by the action of this phytohormone was due to the increased protein content. Slowing protein degradation is a necessary condition for a high level of cellular metabolism. Protein synthesis and increased enzyme activity are closely interrelated [5]. According to our data, the increased enzyme activity (Table 1) caused by α-NAA in L-70 correlated with increased protein content. In fuzzy variety AN-Bayaut-2, this phytohormone increased the activity of GS by 20% although those of PO and C decreased by 8 and 10%, respectively.

A different picture was observed upon treatment of ovules with GA. The GS activity in integument of L-70 decreased by 64%; C, by 23%. However, PO increased by 29%. The GS activity in integument of variety AN-Bayaut-2 increased dramatically (by 960%) whereas that of PO and C decreased by ~15%.

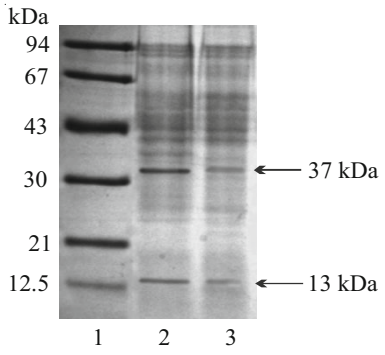

Fig. 2. Electrophoresis of proteins from 20-day integument of cotton variety L-70: marker proteins (1), from samples treated with gibberellin (2), extract of control samples (3).

Specific reactions of plants to various phytohormones were due to genetic features of the variety [1]. Two hypotheses clarify the reason for the specific physiological activity of gibberellins. 1) Plants react differently to exogenic gibberellin due to different abilities to adapt gibberellin into an active form and 2) plant receptors have different specificities for gibberellins and gibberellin activity depends on the degree of their affinity to the structure of the specific receptor molecule. In our instance, the different action of the phytohormones is probably explained by the presence in line L-70 and variety AN-Bayaut-2 of receptors that differ in degree of affinity for gibberellins and auxins. An excess of phytohormones can be inactivated by several methods in plant cells. These are binding with proteins, glycolization, PO oxidation, and polyphenoloxidases [6].

We showed earlier that proteins that suppress biosynthesis of cotton cellulose fiber are present in integument of gymnosperm line L-70 [7]. Therefore, we studied the activity of protein fractions isolated from integument treated with phytohormones of line L-70 fruits on the activity of glucansynthetase, the principal enzyme involved in cellulose synthesis.

We found during a study of proteins from integument of gymnosperm line L-70 that they suppress GS activity in both the control and test varieties. The first protein fraction obtained after gel filtration over G-75 of integument treated with GA decreased GS activity by 96%. According to the theory of Musaev [8], the inhibitor gene in gymnosperm cotton occurs in a dominant state. Apparently synthesis of proteins coded by this gene is activated through the action of GA. The enzyme activity was increased by 170% through the action of proteins from integument treated with α-NAA. It is possible that GS was activated (or the synthesis of this hormone was enhanced) by this phytohormone.

The action of proteins isolated from 20-day integument of gymnosperm line L-70 and fuzzy variety AN-Bayaut-2 treated with phytohormones on GS activity is shown below.

Sample	*Percent GS activity inhibition, %*
Line L-70	
Control integument, not treated with PH	
Starting material	84
1st fraction	77
2nd fraction	14
Integument treated with α-NAA	
Starting material	Stimulation 40
1st fraction	Stimulation 170
2nd fraction	40
Integument treated with GA	
Starting material	36
1st fraction	96
2nd fraction	20

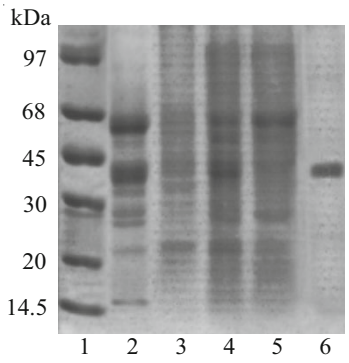

Fig. 3. Electrophoresis of fractions obtained after chromatography over DEAE-TSK: marker protein mixture (1), fraction 1 (2), fraction 2 (3), fraction 3 (4), fraction 4 (5), fraction 5 (6).

An electrophoretic study of total proteins from integument of gymnosperm line L-70 found that two proteins with molecular weights 37 and 13 kDa, respectively, were present in addition to a large number of minor protein components in integuments of control plants and those treated with gibberellin (Fig. 2). Bands corresponding to these proteins were more pronounced than for the control in electrophoregrams of samples treated with gibberellin.

The results led to the conclusion that proteins exhibiting pronounced inhibitor activity for GS were present in total protein extract of integuments of cotton line L-70 treated with GA.

Considering the presence of two dominant components (proteins with MW 37 and 13 kDa) and previous data [7], we assumed that namely these proteins exhibited the inhibitor activity for GS.

Highly purified forms of these proteins should be obtained for a more detailed characterization. For this, we used gel exclusion chromatography over TSK-HW-55F.

Separation of total proteins over TSK-HW-55F gave three fractions. Fraction 1 (62%) exhibited the highest inhibitor activity toward GS and was purified by ion-exchange chromatography over DEAE-TSK gel.

We found that GS activity decreased most (by 63%) for the first fraction after separation over a column of TSK-HW-50F whereas the second and third fractions suppressed the activity by 20 and 25%, respectively, compared with the control.

The activity of protein fractions obtained after separation over a column of TSK-HW-50F gel of line L-70 integument treated with GA on GS activity of fuzzy variety AN-Bayaut-2 sprouts is shown below:

Sample	*GS activity inhibition, %*
1st fraction	63.2
2nd fraction	19.6
3rd fraction	25.3
Control, distilled water	–

The component composition of the resulting fractions was studied by electrophoresis (Fig. 3), which showed that fraction 5 contained a homogeneous component, the molecular weight of which was of the order of 37 kDa.

Thus, the studies established that phytohormones α-NAA and GA have different effects on the degree of enhancement of protein biosynthesis and fiber-forming enzyme activity for GS, PO, and C. The study of ovule development in integument of fuzzy and gymnosperm cotton as influenced by phytohormones found that the number and length of fibers was increased in fuzzy cotton. The surface structure of ovules changed in gymnosperm cotton. However, fibers did not appear. This indicated that the gymnosperm nature is a genetically imparted signature. The content of a GS inhibitor protein with MW 37 kDa increased significantly in integument of gymnosperm cotton under the influence of GA.

EXPERIMENTAL

We studied proteins of 20-day integument from gymnosperm (line L-70) and fuzzy (variety AN-Bayaut-2) cotton treated with phytohormones α-NAA and GA. Ovules were treated with phytohormones at 10^{-6} M concentrations by manual spraying on the day of flowering.

Isolation of Proteins from Integuments of Cotton Line L-70 and AN-Bayaut-2. Plant material was ground in a porcelain mortar with liquid nitrogen. Total proteins were extracted by Tris-HCl buffer (0.05 M, pH 7.8) containing NaCl (1 M) (1:4) for 30 min on a stirrer at 4°C. The extract was centrifuged at 1,500 rpm for 5 min to remove plant tissues. The supernatant liquid was collected and preserved in the cold. The precipitate was ground in a small volume of the same buffer and again centrifuged at 1,500 rpm for 5 min. The supernatant liquids were combined and centrifuged for 30 min at 5,000 rpm. Total proteins were precipitated by adding five volumes of cold acetone, separated by centrifugation (15 min at 6,000 rpm), dried, dissolved in a small volume of the same buffer, and again centrifuged. The supernatant was desalted over a column of Sephadex G-10 (2.5 × 80 cm) in distilled water and lyophilized.

Component composition of proteins was determined by electrophoresis in a PAAG gel gradient (from 10 to 15%) in the presence of sodium dodecylsufate (SDS) [9]. Protein content was determined by the Lowry method [10].

Chromatography of Integument Proteins over a Column of TSK Gel. Lyophilized total proteins were dissolved in sodium phosphate buffer (1 mL, 0.05 M, pH 7.0) and placed on a column of TSK gel (HW-55F, 0.7 × 30 cm) in the same buffer. The flow rate was 2 mL/h.

Ion-exchange Chromatography of Proteins over DEAE-TSK. Fractions obtained after separation over TSK gel were dissolved in NaOAc buffer (0.7 mL, 0.001 M, pH 4.6) and placed on a column of DEAE-TSK (1.2 × 4 cm) in the same buffer. Proteins were eluted by a linear NaCl gradient in the same buffer (0-1 M) at flow rate 20 mL/h. The resulting fractions were dialyzed against water and lyophilized.

Enzyme activity of GS, PO, and C was determined as before [6].

Microscopy studies used an MBI-6 optical microscope and Neofot-2 (Carl Zeiss Jena) universal optical microscope.

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REFERENCES

1. G. F. Privalov (ed.), *Role of Phytohormones in Manifestation of Certain Signatures in Plants*, A Collection of Scientific Works, Sib. Br., Acad. Sci. USSR, Novosibirsk, 1983.
2. H. T. Kim and B. A. Triplett, *Plant Physiol.*, **127**, 1361 (2001).
3. V. A. Lozovaya, M. N. Markova, and I. A. Tarchevskii, *Fiziol. Rast. (Moscow)*, **34**, 1, 97 (1987).
4. S. Givalis and R. W. Scagull, *J. Cotton Sci.*, **5**, 252 (2001).
5. V. D. Sakalo and V. M. Kurchii, *Fiziol. Rast. (Moscow)*, **51**, 2, 205 (2004).
6. V. I. Kefeli, *Usp. Sovrem. Biol.*, **69**, No. 3, 446 (1970).
7. A. A. Akhunov, Z. Golubenko, F. A. Ibragimov, N. A. Abdurashidova, E. Ch. Mustakimova, and G. O. Akbarova, *Khim. Prir. Soedin.*, 317 (2001).
8. D. A. Musaev, M. F. Abzalov, and Sh. Turabekov, in: *Progress in Modern Genetics* [in Russian], No. 15, Moscow, 1988, p. 241.
9. J. King and U. K. Laemmli, *J. Mol. Biol.*, **62**, 465 (1971).
10. O. H. Lowry, N. G. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).