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INDUCTION BY CYTODEF OF THE BIOSYNTHESIS OF ENDOGENOUS ETHYLENE AND CELLULASE AND THE CHANGE IN THE COMPOSITION OF THE PROTEINS IN THE LEAF-SHEDDING ZONE OF THE COTTON PLANT

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The influence of the defoliant Cytodef (Tsitodef) on the formation of ethylene and the activity of cellulase in the separating zone of cottonplant leaves has been investigated. It has been established that Cytodef is an inductor of a number of biochemical reactions taking place at the membrane and genetic levels and leading to leaf shedding.

An important direction in the investigation of biochemical aspects of the leaf-shedding process is a study of the influence of phytohormones and defoliants on the process of forming the abscission layer. According to modern ideas, the mechanism of defoliation is based on an enhancement of the production of ethylene, which activates the biosynthesis of enzymes hydrolyzing the polysaccharides of the cell walls in the abscission zone [1].

We have investigated the correlation between the formation of ethylene, cellulase activity, and leaf shedding that arises in the process of treating a cotton plant with the defoliant Cytodef (Tsitodef):

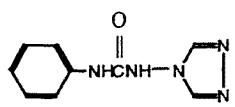

Table 1 shows a comparative analysis of the evolution of ethylene from leaves treated with Cytodef alone and with Cytodef in combination with activators (chloroethylphosphonic acid (CEPA), aminocyclopropanecarboxylic acid (ACPA), and an inhibitor of the biosynthesis of ethylene (cobalt chloride).

As can be seen from Table 1, a direct relationship exists between the defoliating activity of Cytodef and the synthesis of endogenous ethylene. Inhibition of the evolution of ethylene by cottonplant leaves after preliminary treatment with cobalt chloride was accompanied by the complete neutralization of the defoliating effect of Cytodef. It may also be concluded that some threshold concentration of ethylene is necessary for the manifestation of defoliating activity. A further increase in the level of ethylene does not lead to an appreciable enhancement of the defoliation effect.

Thus, for example, on the preliminary treatment of the plants with ACPA, an ethylene precursor, stimulation of the evolution of ethylene was observed after only 24 h, which indicates a rapid inclusion of ACPA in the biosynthesis of endogenous ethylene. However, this was not appreciably reflected in the defoliating activity of Cytodef. A similar pattern was observed under the action of CEPA, which is a source of exogenous ethylene [3]. In this case, in spite of a 6.5-fold increase in the level of ethylene, the defoliating activity exceeded that for Cytodef by 10% on the 9th day and by 5% on the 12th day. This effect can be ascribed partially to the contact action of CEPA [4].

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TABLE 1. Action of Cytodef, Cobalt Chloride, ACPA, and CEPA on the Synthesis of Ethylene and the Shedding of Leaves by the Cotton Plant

Variant of the experiment	Evolution of ethylene, nm/g		Leaf shedding, %			
	in 24 h	in 48 h	3 days	7 days	9 days	12 days
1. Control (water)	–	0.55	–	–	–	–
2. Cytodef 1%	2.17	4.55	–	–	75	80
3. Cobalt chloride 0.15% and, after a day, 1% Cytodef	–	0.92	–	–	–	–
4. ACPA 0.1% and, after a day, 1% Cytodef	17.74	5.05	–	65	70	85
5. CEPA 0.15% and, after a day, 1% Cytodef	8.35	29.5	–	80	85	95

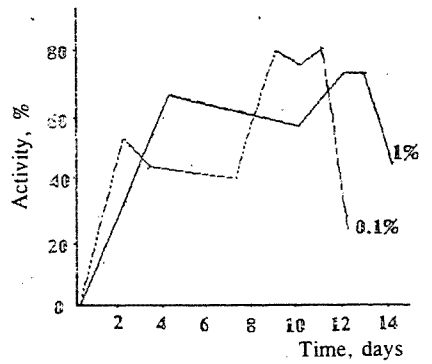

Fig. 1. Dynamics of the change in cellulase activity in the separating layer of the petiole of a cottonplant leaf after treatment with Cytodef.

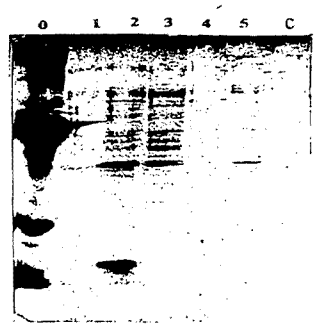

Fig. 2. Analysis of proteins by electrophoresis in PAAG. *0*) BSA; ch/t, p/s; *1*) 0.1% Cytodef (7 days) after treatment; *2*) 1% Cytodef (6 days); *3*) 1% Cytodef (10 days); *4*) 0.1% Cytodef (4 days); *5*) 0.1% Cytodef (3 days); C) control (the protein spectrum did not change over 7 days).

Judging from the literature, ethylene is a hormonal inductor of the leaf-shedding process connected with the induction of the activity of enzymes participating in a change in the cellulase–pectin complex of the cell walls of the abscission layer [5, 6]. In order to study the influence of Cytodef on the dynamics of the change in the cellulase activity, two-month-old plants were treated with Cytodef, taken in concentrations of 0.1 and 1% (Fig. 1).

As can be seen from Fig. 1, Cytodef stimulates the cellulase complex. It is characteristic here that the increase in enzymatic activity bears a two-phase nature. The maximum of the first phase came on the second day, and then a characteristic latent period was observed during which cellulase activity did not rise but even fell somewhat. The greatest cellulase activity was observed on the 8th-9th days after treatment. It was retained for three days and then fell sharply. The activation of the

cellulase complex by another defoliant — Butifos — takes place similarly [8], which makes it possible to assume a common nature of their defoliant activities.

At a concentration of Cytodef an order of magnitude lower, the nature of the curve of the dynamics of the change in cellulase activity was completely repeated. However, the maximum of the enzymatic activity was shifted in time. Such a dose-dependent effect is one more proof of the fact that the mechanism of the action of Cytodef is based on induction of the biosynthesis of a complex of cellulasic enzymes. It may be assumed that the change in the level of proteins in the leaf-shedding zone after treatment with the defoliant also follows a dose-dependent relationship. Analysis of the proteins isolated from cells of the separating layer by electrophoresis in PAAG basically confirmed this hypothesis. As can be seen from the electrophoregrams, the protein spectrum varies both qualitatively and quantitatively according to the concentration of defoliant and the time of exposure (Fig. 2).

Thus, the results obtained showed that Cytodef is a kind of trigger for a cascade of biochemical reactions taking place at the membrane and genetic levels the individual links of which require detailed consideration.

EXPERIMENTAL

Two-month cotton plants of the Yulduz variety grown in a climatic chamber on a mixture of sierozem and sand in a ratio of 3:1 were used.

The defoliating activity of Cytodef was determined 3-12 days after the treatment of the plants. A solution of Cytodef with a concentration of 0.05% was sprayed onto the surface of the plants. The result was expressed in terms of the number of shed leaves as a percentage of the total number of leaves. In the determination of defoliant activity 10 plants were used in each experiment.

Ethylene was determined in separate leaves on a Chrom-5 gas chromatograph with the support Poropak by a procedure described previously.

Some of the plants that had been treated with Cytodef were sprayed with solutions of cobalt chloride, ACPA, and CEPA. The treated plants were kept in a climatic chamber for 12 days at a mean air temperature of 25°C in illumination of 8000 lux, the time of a photoperiod being 16 h. In the determination of ethylene in a sample, 2-4 leaves were taken, depending on their size. Each variant was repeated five times.

Isolation of the Total Water-Soluble Proteins. Fifty to sixty 5-mm pieces of leaf-shedding zones, including the abscission layer, were washed first with distilled water and then with hypochlorite to prevent bacterial contamination of the tissues, and they were dried on filter paper and weighed. The material selected for analysis was triturated in the cold (0-4°C) in a mortar with the addition of 0.05 M phosphate buffer, pH 7.4. To adsorb phenolic substances, 50%, on the weight of the sample, of Kapron [polycaprolactam] powder was added to the homogenate, and the mixture was centrifuged in the cold at 10-12 thousand rpm for 10 min. To investigate the protein spectra the experimental and control samples were analyzed after predetermined intervals of time (3, 4, 6, 7, and 10 days) by electrophoresis in PAAG.

Electrophoresis in PAAG was conducted by Laemmli's method [9].

Cellulase activity was determined by the method of [7].

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