INFLUENCE OF GROWTH REGULATORS ON THE LIPASE ACTIVITY OF COTTON SEEDS. I.

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The action of growth regulators on the sprouting of seeds leads to a change in the activity of some enzymes in them [1], including lipases [2]. It is also known that in early ripening varieties of cotton a higher level of lipase activity is achieved on sprouting [3]. It appeared of interest to determine the correlation between the action of a growth regulator and the level of lipase activity. To study this question, we have investigated the action of sucrose and of indolylacetic, succinic, and ascorbic acids at those concentrations of their solutions at which they are stimulators. The indolylacetic and succinic acids were also used in the higher concentrations at which they exhibit a herbicidal effect.

Figure 1 shows curves of the changes in the lipase activity, and in the contents of lipids, proteins, fatty acids, and choline during the sprouting of cotton seeds in the absence of growth regulators. Under ordinary conditions, the lipase activity first fell (first day of sprouting) and then rose rapidly, reaching a maximum value on the fourth or fifth day (see Fig. 1, curve 1) [4]. With an intensification of lipolysis, the amount of lipids decreased (curve 2), and the amounts of fatty acids (curve 3) and protein (curve 4) fell

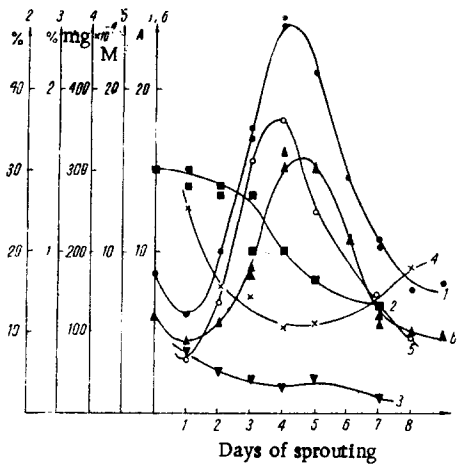

Fig. 1

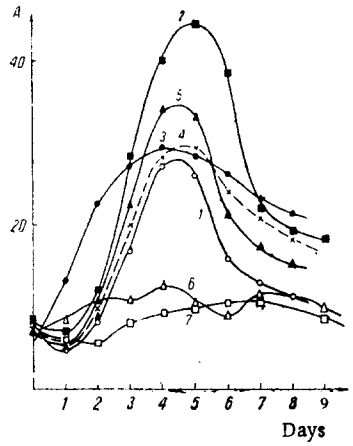

Fig. 2

Fig. 1. Change in the contents of the natural components of cotton seeds on sprouting: 1) lipase activity with tributyrin; 2) lipids; 3) fatty acids; 4) proteins; 5) choline; 6) lipase activity with cottonseed oil.

Fig. 2. Action of growth regulators on lipase activity: 1) control; 2) indolylacetic acid (10^{-5} M); 3) ascorbic acid; 4) sucrose; 5) succinic acid (10^{-4} M); 6) succinic acid (10^{-1} M); 7) indolylacetic acid (10^{-1} M).

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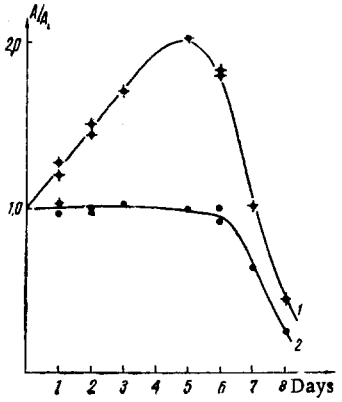

Fig. 3. Change in lipase activity in cell-free extracts of cotton seeds: 1) incubation temperature 20°C; 2) 4°C (medium: 0.1 M phosphate buffer; pH 7.2) (activity referred to the activity of a freshly isolated extract).

slightly, probably because of the greater rate of their consumption in other metabolic processes [5] and because of the synthesis of proteinase [6]. A parallelism is observed between the level of free choline and the magnitude of the lipase activity.

The nature of the change in the lipase activity during sprouting scarcely changed in the presence of growth regulators (Fig. 2), with the exception of ascorbic acid. However, the level of lipase activity and its maximum value at the fourth day of sprouting of the seeds depended on the type of growth regulator. Indolylacetic and succinic acids in low concentration increased the level of lipase activity.

High concentrations of indolylacetic and succinic acids led to a decrease in lipase activity. The sprouting of the seeds and the growth of the plants in these cases were also retarded or did not take place at all. The change in lipase activity in the presence of ascorbic acid and sucrose was proportional to their growth activity.

It follows from what has been said above that a correlation exists between the level of lipase activity in the sprouting plant and the stimulating action of a growth regulator. If this is the case, the level of lipase activity can be adopted as a measure of growth activity of a particular chemical compound, but it must be borne in mind that the activity of the lipase is not the only indicator of intracellular conditions; the action of growth regulators must be exerted on other enzymatic processes as well.

When extracts of cotton seeds in 0.1 M phosphate buffer with pH 7.2 were stored at 20°C, their lipase activity changed in the same way as in the sprouting seeds (Fig. 3). The only difference consists in the fact that no fall in lipase activity after the first day was found. This is apparently connected with the liberation of certain metabolism inhibitors. This fact, of course, increases the significance of the interrelationship between the level of lipase activity and the stimulating or herbicidal action of a chemical agent.

EXPERIMENTAL

The lipase activities were measured by a titrimetric method [7, 8], using as substrates tributyrin and cottonseed oil. The concentration of the substrates in the activity measurements was 2 mg/ml, the concentration of the enzyme 0.033 mg/ml, pH 8.8, temperature 25°C, and the medium was 0.1 M phosphate buffer. The concentration of Ca^{2+} ions and the conditions of their addition were the same as in the preceding investigation [8].

The samples were defatted by extraction with acetone and then with diethyl ether. The extracts from the defatted powders were prepared as described previously [4]. The concentration of protein was 10 mg/ml.

The seeds were sprouted in the light at 25°C on sand moistened with distilled water. The control seeds were moistened in distilled water. The concentrations of the solutions of the growth regulators for steeping the seeds were selected on the basis of literature information [2]. The concentrations of the indolylacetic acid were 10^{-5} and 10^{-1} M, of the succinic acid 10^{-4} and 10^{-1} M, of the sucrose 10^{-2} M, and of the ascorbic acid 10^{-4} M. The seeds were steeped in 30 ml of the solution with constant shaking for 5 h. Then they were sprouted on sterilized sand. Distilled water in measured amounts was added every day.

The protein content was determined by the biuret method, the fatty acids by titration [5], and the choline in the form of the complex with iodine [9]. All the titration operations were performed with an LPM-60M pH meter with glass and silver chloride electrodes.

SUMMARY

A method has been proposed for evaluating the action of some growth regulators by measuring the lipase activity of sprouting cotton seeds.

LITERATURE CITED

1. É. E. Khafkin, Induced Synthesis of Enzymes in the Processes of the Growth and Morphogenesis of Plants [in Russian], Moscow (1969).
2. D. N. Vyas and K. C. Patel, J. Amer. Oil Chemists' Soc., 46, 176 (1969).
3. A. V. Blagoveshchenskii and R. Kamilova, Dokl. Akad. Nauk SSSR, 95, 309 (1954).
4. M. M. Rakhimov and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 6, 782 (1970).
5. D. N. Vyas, K. C. Patel, and K. D. Patel, J. Amer. Oil Chemists' Soc., 46, 41 (1969).
6. I. N. Ihle and L. Dure, Bioch. Bioph. Res. Commun., 36, 705 (1969).
7. M. M. Rakhimov, Sh. R. Mad'yarov, N. R. Dzhanbaeva, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 6, 738 (1970).
8. M. M. Rakhimov, N. N. Dzhanbaeva, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 6, 642 (1970).
9. B. Shapiro, Biochem. J., 53, 653 (1953).