INFLUENCE OF SOME ORGANIC ACIDS ON THE ACTIVITY OF THE MALATE DEHYDROGENASE OF COTTON SEEDS

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The investigation of the laws of the action of various growth regulators on the activity of enzyme systems may assist the directed search for physiologically active substances. Papers have appeared in which the influence of gibberellic acid and β-(indol-3-yl)acetic acid (IAA) on the induced synthesis of enzymes and also their action as effectors causing a change in the physicochemical state of enzymes have been reported [1]. Some communications give information on the activating effect of various enzyme systems of organic acids (dicarboxylic acids, some acids of the Krebs cycle), raising the energy level of the whole plant organism [2]. Very little is known about the action of growth regulators on plant malate dehydrogenase (MDH) [3, 4], and therefore we were interested to study the influence of some organic acids on the activity of MDH of cotton seeds, both in dormant seeds and in the process of their sprouting.

The investigation was performed with an extract obtained from defatted cottonseed flour by a method described previously [5]. The MDH activity was determined in the presence of IAA and succinic, ascorbic, butyric, α-aminobutyric, γ-aminobutyric, and phenylbutyric acids and also 2,4-dichlorophenoxyacetic acid

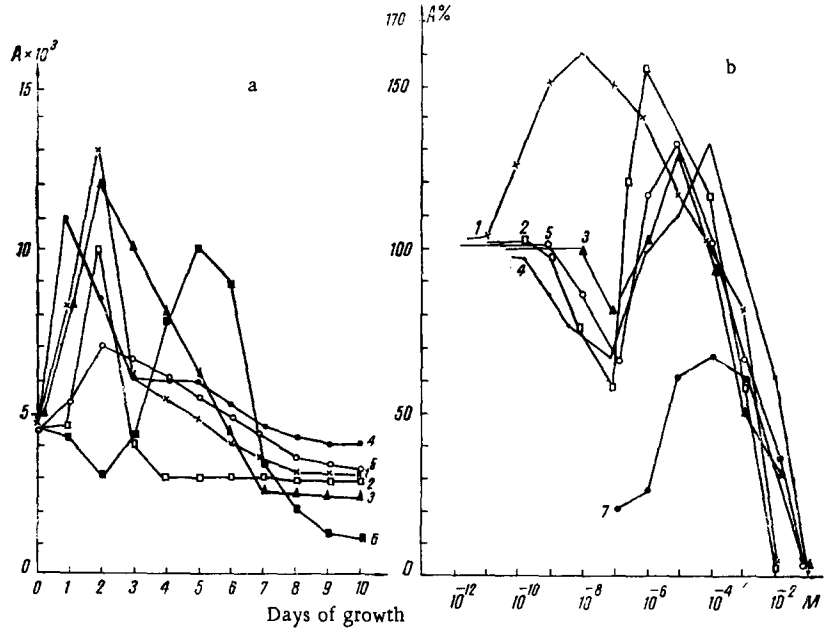

Fig. 1. Influence of some organic acids on the activity of the MDH of dormant (a) and sprouting (b) cotton seeds: 1) β-(indol-3-yl)acetic acid; 2) phenylbutyric acid; 3) butyric acid; 4) α-aminobutyric acid; 5) γ-aminobutyric acid; 6) distilled water; 7) 2,4-D.

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(2,4-D) in concentrations of 10^{-1}–10^{-15} M. The greatest activating effect on MDH was shown by IAA in a concentration of $1 \cdot 10^{-8}$ M and phenylbutyric acid in a concentration of $1 \cdot 10^{-6}$ M. At all concentrations, 2,4-D suppressed the activity, while succinic and ascorbic acids had no effect (Fig. 1b).

The change in the activity of MDH in a 10-day period of the sprouting of the seeds was also studied (see Fig. 1a). It is not constant over the period mentioned but on the fifth day it reaches a maximum which exceeds the activity of the dormant seeds by a factor of 2.5. The preliminary steeping of the seeds in solutions of the acids mentioned sharply changes the pattern, and the maximum activity appears on the first and second days. β-(Indol-3-yl)acetic, phenylbutyric, butyric, and α-aminobutyric acids increase the activity, while γ-aminobutyric acid has an inhibiting effect.

Thus, the facts given show the considerable influence of these acids on the MDH activity. A determination of the nature of the changes caused is the subject of further study.

EXPERIMENTAL

The protein in the solutions was determined by the biuret reaction and by the Warburg-Christian method [6].

The activity of the malate dehydrogenase was determined spectrophotometrically from the oxidation of NAD · H_2 and the decrease in the optical density at 340 nm [5].

The experimental mixture for determining the influence of the acids mentioned consisted of 0.51 μmole of oxaloacetic acid neutralized with 2% KOH solution and 0.17 μmole of NAD · H_2. The acid under investigation was added in an amount of $1 \cdot 10^{-1}$ to $1 \cdot 10^{-15}$ M after previous neutralization with a 2% solution of alkali and 2-5 μg of enzyme. The total volume of the reaction mixture was brought up to 3 ml with 0.1 M phosphate buffer, pH 7.4. The phosphate buffer was used as control. The activity was measured every 30 sec for 3 min.

The enzyme was incubated in a solution of the substance being studied for 10 min. The oxaloacetic acid and the NAD · H_2 were added at the beginning of the reaction.

Sprouting. The seeds were steeped in the substance under investigation in the following concentrations: $1 \cdot 10^{-8}$ M indolylacetic acid; $1 \cdot 10^{-6}$ M phenylbutyric acid; $1 \cdot 10^{-5}$ M butyric and γ-aminobutyric acids; $1 \cdot 10^{-4}$ M α-aminobutyric acid neutralized with 2% KOH solution; and, in the case of the control, in distilled water, in a ratio of 30 ml of solution to 100 g of seeds. The mixture was vigorously shaken for 6 h and was then placed in sand that had been carefully washed with hot water. The seeds were sprouted in the light at room temperature (22-25°C) and were watered with distilled water every day.

SUMMARY

1. β-(Indol-3-yl)acetic acid (IAA) and butyric, phenylbutyric, α-aminobutyric, and γ-aminobutyric acids have an activating effect on the malate dehydrogenase (MDH) of dormant and sprouting cotton seeds, while γ-aminobutyric acid inhibits the MDH of the sprouting seeds.

2. The maximum MDH activity is found on the fifth day of sprouting of cotton seeds. The preliminary steeping of the seeds in solutions of organic acids changes the time of maximum activity to the first and second days.

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