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The effect of ammonium ions on the activity of glutamate dehydrogenase, alanine aminotransferase and aspartate aminotransferase in *Cucumis sativus* L. seedlings

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Abstract

Changes in the activity of glutamate dehydrogenase (GDH), alanine aminotransferase (GPT) and aspartate aminotransferase (GOT) were studied in various organs of *Cucumis sativus* L. seedlings in relation to the uptake of mineral nitrogen (in form of NO_3^- or NH_4^+) from the medium. Activity of GDH, GPT, and GOT was higher in young leaves and roots of cucumber seedlings if the plants developed in an ammonium medium. No similar changes of aminotransferases activity were noted in the cotyledons. Factors affecting varying effect of ammonium ions upon GPT and GOT activity are discussed for particular organs of cucumber seedlings.

INTRODUCTION

Studies by Joy (1969), Kretovich et al. (1974), Caldas and Caldas (1976) presented the effect of NH_4^+ ions upon the activity of glutamate dehydrogenase (GDH). In plants uptaking ammonium ions, activity of this enzyme was usually higher than in plants uptaking nitrate ions (Weissman 1972, Ehmke and Hartmann 1976). On the other hand, there are some disagreements as to the effect of nitrate ions upon the GDH activity. Works by Kretovich et al. (1974), Sarkissian and Fowler (1974) showed that NO_3^- ions affect the enzyme in some plant fragments, whereas studies by other authors suggest that nitrate ions do not induce any changes in the GDH activity (Ingle et al. 1966, Sahulka et al. 1975).

Studies related to the regulation of GDH activity by ammonium ions do not take into account the role of this ion in subsequent stage of

the primary amino acid biosynthesis. This role consists of transferring the amino groups from glutamic acid to proper keto acids through the transamination process. In this process a significant role is played by alanine aminotransferase (GPT) and aspartate aminotransferase (GOT), which provide the source of amino acids for the biosynthesis of proteins and which are the primary substrate in the biosynthesis of other amino acids. In our laboratory the effect of ammonium ions upon the assimilation and reduction of nitrates in cucumber seedlings was studied (Buczek 1979, Buczek and Burzyński 1979). The present paper constitutes a continuation of these studies. It presents an analysis of the effect of NH_4^+ ions on the activity of GDH, GPT, and GOT in cotyledons, leaves, and roots of cucumber seedlings.

MATERIAL AND METHODS

Experiments were carried out on cucumber (*Cucumis sativus* L. of the "Monastyrski" variety) seedlings. Conditions during the germination and composition of ammonium and nitrate media have been described in the previous paper (Kubik-Dobosz and Soroka 1979). The media contained nitrogen in the amount of 200 mg/dm³. All plants grew in conditions of 17 h light period (5000 lux) and 7 h dark period. Cotyledons and roots seedlings growing for 4 days in the media were used for studies, as well as leaves and roots of seedlings growing for 13 days.

Analytical methods. Glutamate dehydrogenase (GDH) was extracted from plant material with the method of Harper and Paulsen (1969), whereas alanine aminotransferase (GPT) and aspartate aminotransferase (GOT) — with the method of Hedley and Stoddart (1971). After homogenization the material was centrifuged for 15 min. at 20 000 × g. Activity of the enzymes was determined in the supernatant.

Glutamate dehydrogenase was determined after incubation with 2-ketoglutarate, $(NH_4)_2SO_4$ and NADH in a tris-HCl buffer (pH 8.0) (Pahlich and Joy 1971). GDH activity was expressed in μmoles of oxidized NADH per min per g of fresh mass. Alanine aminotransferase was measured in the presence of a substrate containing L-alanine and 2-ketoglutarate in a tris-HCl buffer (pH 7.4), while aspartate aminotransferase was determined after incubation with L-aspartate and 2-ketoglutarate in a tris-HCl buffer (pH 7.4) (Hedley and Stoddart 1971). Activity of GPT and GOT is presented in μmoles of the product per min per g of fresh mass. All the results constitute a mean from 3 measurements.

Chemicals. Substrates for enzymatic reactions were purchased from Sigma Chemical Co., the other chemical (analytical grade) were obtained from P.O.Ch. Gliwice.

Abbreviations used. GDH — glutamate dehydrogenase, GPT — alanine aminotransferase, GOT — aspartate aminotransferase.

RESULTS

Data presented in Fig. 1 show that, independently of the lenght of seedlings growth period (4 and 13 days), activity of GDH in the roots of cucumbers developing in the ammonium medium was 4 times higher than in the roots of seedlings from the nitrate medium. In the cotyledons and leaves of seedlings uptaking ammonium nitrogen the GDH activity increased 2-3 fold.

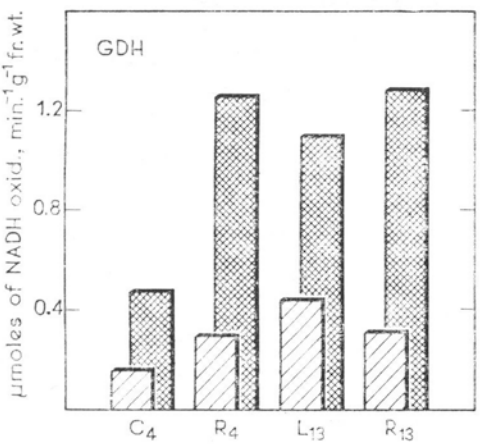

Fig. 1. Activity of GDH after 4 and 13 days of seedlings growth in a nitrate (light blocks) or ammonium (dark blocks) medium

C_4 — cotyledons after 4 days, R_4 — roots after 4 days, L_{13} — leaves after 13 days, R_{13} — roots after 13 days

As it results from data given in Fig. 2, ammonium ions affected also the level of GPT activity in the leaves and roots of 13-day old cucumber seedlings. GPT activity in these organs increased by 51 and 21% respectively compared to GPT activity in the some organs of plants uptaking NO_3^- ions. Similar dependence on the NH_4^+ as a source of nitrogen was observed in the roots of 4-day old cucumbers. In the cotyledons, activity of this enzyme was similar whether the plants grew in the nitrate or ammonium medium.

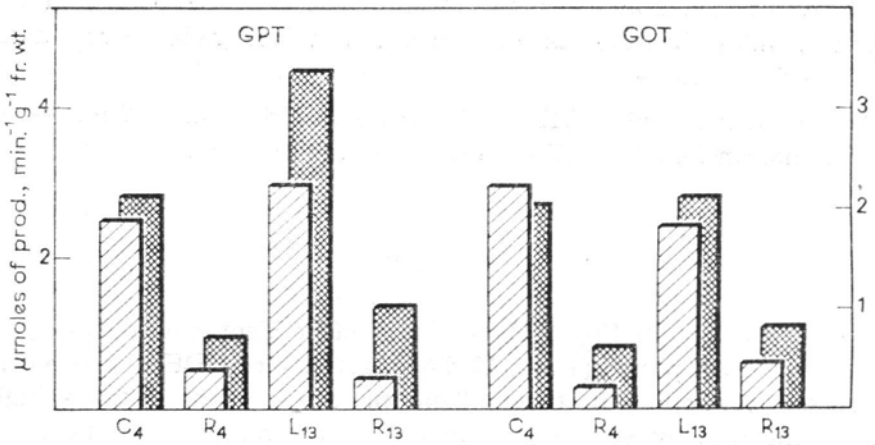

Fig. 2. Activity of GPT and GOT after 4 and 13 days of seedlings growth in a nitrate (light blocks) and ammonium (dark blocks) medium. Explanations as in Fig. 1

It was also found that GOT activity in the roots was higher if the seedlings grew in the ammonium medium (Fig. 2). Increase of GOT activity in the leaves of plants growing in the presence of ammonium ions was insignificant. No effect was noted of NH_4^+ ions on the activity of GOT in cucumber cotyledons.

In successive experiment seedlings grew in a medium devoid of nitrogen, at proper level of other macro- and microelements. After 4 days the seedlings were transferred to the media containing NH_4^+ or NO_3^- ions. Activity of GDH, GPT, and GOT was measured after 3, 6, 9, and 24 hours. Rapid increase of activity of all three enzymes was observed in the roots of cucumbers transferred to the ammonium medium, whereas no changes were noted in the cotyledons (Fig. 3).

In case of seedlings transferred to the nitrate medium only the GPT activity increased in the roots after 9 and 24 h (Fig. 4). Activity of GDH and GOT in the roots remained unchanged. After 24 h the GDH activity in the cotyledons decreased to 57%.

DISCUSSION

Blackwood and Miflin (1976) stated that leaves of maize seedlings uptaking ammonium ions were able to synthetize much more alanine than leaves of seedlings uptaking nitrate ions. Data presented in this paper point to the fact that stimulating effect of NH_4^+ ions on GPT and GOT depends upon the plant organ. Increase of the activity of the aminotransferases under study was noted in cucumber leaves

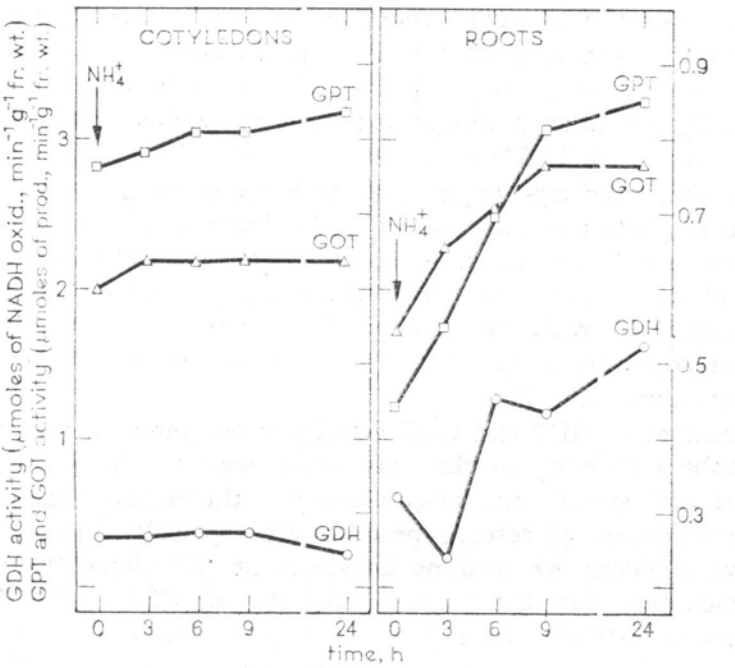

Fig. 3. Activity of GDH, GPT, and GOT in cotyledons and roots of cucumbers exposed to NH_4^+ ions

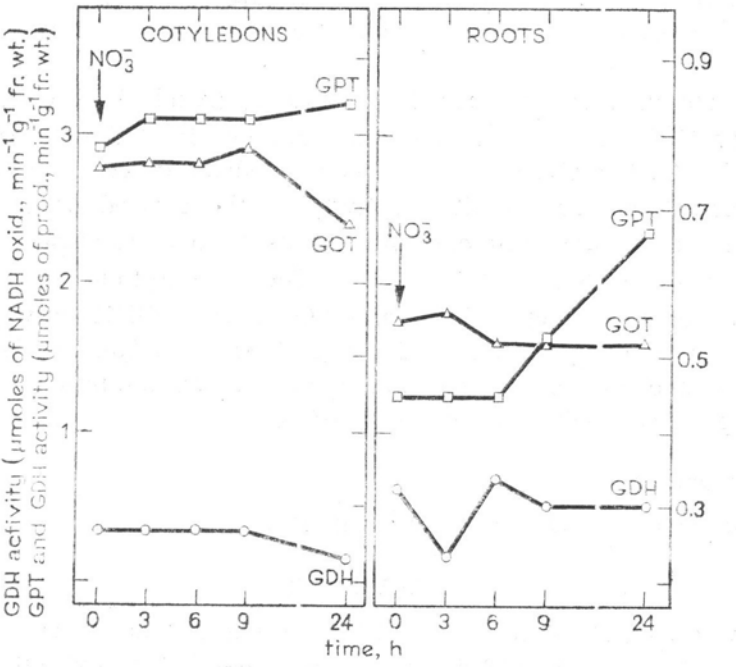

Fig. 4. Activity of GDH, GPT, and GOT in cotyledons and roots of cucumbers exposed to NO_3^- ions

and roots, i.e. in the organs characterized by intensive biosynthesis related to the formation of amino acids included into newly synthetized proteins. Increase of GPT and GOT activity in these organs is preceded by a similar (although less visible) increase of GDH activity. Wakiuchi et al. (1971) studied changes of GDH activity in 30-day old cucumber seedlings exposed to high concentrations of NH_4^+ ions. They did not note any increase of GDH activity in the leaves and roots during the first 2 days of the experiment. A significant increase of the activity of this enzyme was noted after 5 days. Our studies, however, have shown that rapid increase of GDH activity in cucumber roots occurs already after a few hours if 4-day old seedlings were treated with ammonium ions (Fig. 3).

No changes of GPT and GOT activity were found in the cotyledons of cucumbers growing in the ammonium medium. This fact may be connected with specific role of cotyledons as the reserve organs. Processes of degradation of reserve proteins are especially intensive in the first days of cucumber seedling development (Kłobus 1980). It may be assumed that during this period the role of GPT and GOT in the cotyledons is restricted mainly to katabolytic functions (decomposition of alanine and aspartate acid). Splittstoesser et al. (1976) state that high concentrations of alanine inhibit GPT activity in the cytoplasmatic fraction. Significant pool of amino acids liberated during decomposition of reserve proteins can constitute one of the factors limiting the increase of activity of aminotransferases in cucumber cotyledons.

Data obtained in the previous studies (Kubik-Dobosz and Soroka 1979) suggest that NO_3^- ions uptaken from the extermal solution inhibit GDH activity in cucumber cotyledons. It seems probable that the observed increase of GDH activity in the cotyledons, induced by NH_4^+ ions, is an apparent one and results from a decrease of enzyme activity in the presence of NO_3^- ions. These suggestions are supported by studies on the effect of NH_4^+ and NO_3^- ions on GDH activity (Fig. 3 and 4), as well as by studies of Pahlich et al. (1978). The latter studies were undertaken *in vitro* and pointed to an inhibition by nitrate ions of purified GDH from pea cotyledons.

Acknowledgments

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Wpływ jonów amonowych na aktywność dehydrogenazy glutaminianowej, aminotransferazy alaninowej i aminotransferazy asparaginianowej w siewkach Cucumis sativus L.

Streszczenie

Badano zmiany aktywności dehydrogenazy glutaminianowej (GDH), aminotransferazy alaninowej (GPT) i aminotransferazy asparaginianowej (GOT) w różnych organach siewek *Cucumis sativus* L. w zależności od pobieranej z pożywki formy azotu mineralnego (NO_3^- lub NH_4^+). W młodych liściach i korzeniach ogórków aktywność GDH, GPT i GOT była wyższa jeżeli rośliny rosły w pożywce amonowej. W liścieniach ogórków nie stwierdzono podobnych zmian aktywności badanych aminotransferaz. Dyskutowano przyczyny zróżnicowanego oddziaływania jonów amonowych na aktywność GPT i GOT występujących w różnych organach siewek ogórka.