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Activity of aminotransferase in tomato seedlings depending on Ca : Mg ion ratio in the nutrient medium

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ABSTRACT

Change from the normal Ca : Mg ion ratio (3 : 1) to 3 : 30 in a medium in the presence of nitrates causes reduction of dry weight content in the tomato seedlings. This reduction is not observable if ammonium salts are the source of nitrogen. It was found that the GOT and GPT activity depends on the form of mineral nitrogen and on the Ca : Mg ion ratio in the medium. An excess of magnesium causes a distinct decrease of the activity of both enzymes in roots of plants grown in nitrate media. In the remaining organs of the plants the GOT activity tends to grow at increased magnesium dose in the medium in the presence of nitrates. Excess of Mg^{2+} ions in the medium containing ammonium salts increases the activity of GPT in the roots of the tomato seedlings and the GOT activity in the roots, shoots and leaves of tomatoes.

The possibility of indirect influence of the Ca : Mg ratio in the medium upon the activity of both enzymes has been discussed.

INTRODUCTION

Experiments with tomatoes conducted by Buczek and Leonowicz-Babiak (1971) showed that a change of Ca : Mg ion ratio either in the medium or in soil from the optimal (3 : 1) to an abnormal value (3 : 30) causes among other things a decrease of protein level in the tomato seedlings. It is supposed that the abnormal ratio of calcium to magnesium leads to disturbances in protein synthesis. Further experiments of Buczek et al. (1974) proved the excess of Mg^{2+} ions in relation to Ca^{2+} ones in a medium with nitrates as nitrogen source, to cause reduction of the dry weight and protein content by weight and to inhibit the activity of nitrate reductase after a three-week culture. A similar dependance was

not found when ammonium ions were the source of nitrogen in the medium. Since in the experiments of the authors cited above, inhibition of protein synthesis occurred earlier than inhibition of nitrate reductase activity in the presence of magnesium excess, the reason of decrease of the protein level in the tomato seedlings grown at the abnormal Ca : Mg ratio is still unclear.

Alanine aminotransferase (GPT), (EC 2. 6. 1. 2) and aspartate aminotransferase (GOT), (EC 2. 6. 1. 1) play an important role in the production of amino acids in plant tissues. Both enzymes together with glutamate dehydrogenase fulfill a basic function in plant tissues in the primary synthesis of amino acids (Kirk and Leech, 1972). Recently, Lea and Miflin (1974) reported that in green tissues of plants specific systems of primary synthesis of amino acids with participation of glutamate synthetase and of glutamine synthetase are present.

The purpose of this work was to study the influence of the changed Ca : Mg ion ratio in the media, where nitrates or ammonium salts constituted a source of mineral nitrogen, upon the activity of alanine aminotransferase and aspartate aminotransferase in tomato seedlings.

MATERIAL AND METHODS

Plant cultures. Tomato seeds (*Lycopersicum esculentum* variety Stonora) were sown on cheesecloth in one-quart dishes filled with tap water. After germination the seedlings were placed in one-litre glass jars with nutrient solutions. The jars were kept in a greenhouse for three weeks. The experiments were conducted in July. At 11-, 16- and 21-day intervals the length of roots and shoots was measured and the particular parts of the plants (roots, leafless shoots and leaves) were separately analysed for increment of dry weight and GOT and GPT activity.

The plants were grown on nutrient solution containing either nitrates or ammonium salts as a source of mineral nitrogen. In control medium the ratio Ca : Mg was 3 : 1. The tested solution differed from the control one only by a higher magnesium content, the other compounds remaining the same. The Ca : Mg ratio in the tested medium was 3 : 30. The composition of the medium with nitrate was the same as in the experiments of Buczek (1971). In ammonium nutrient solution nitrates were replaced with equivalent amounts of $(NH_4)_2SO_4$ and Ca^{2+} ions were supplemented with $CaCl_2$. The pH of solution was permanently verified and for tomato plants it was 6.8.

Preparation of enzyme extracts. Samples of roots, leafless shoots and leaves were excised, washed three times with ice- cold redistilled water and ground in a cold mortar and pestle with extraction medium. Six milliliters of medium were used per gram of fresh weight

of tissues. The extraction medium consisted of 0.04 M tris-HCl, pH 7.5, 0.25 mM EDTA and 2.0 mM of reduced glutathione (GSH). The brei was strained through four layers of cheesecloth moistened with extraction medium and centrifuged at 20 000 g for 20 min. The supernatant was analysed for enzyme activity. All described above manipulations were carried out at 0° to 2° C.

Analytical methods. Alanine aminotransferase (GPT) was determined by incubation (30 min) at 37° C with 1.0 ml of substrate and appropriate amounts of enzyme extracts (0.1 to 0.5 ml). 0.1 M this buffer at pH 7.4 was added to the final volume 1.5 ml of incubation medium. The substrate solution consisted of 0.2 M DL-alanine, 2.0 mM of α-oxoglutarate in 0.1 M tris-HCl at pH 7.4.

Aspartate aminotransferase (GOT) was assayed in similar fashion as GPT with the exception of times of incubation (60 min) and substrate solution (0.1 M L-aspartate, 2.0 mM α-oxoglutarate in 0.1 M tris-HCl of pH 7.4). The product of enzyme reaction was assayed by measurement of optical density at 546 nm by the method of Reitman and Frankel (1957). The specific activity was calculated as keto-acids released per hour by 1 mg protein. All figures given are the means of duplicate samples. Soluble protein was determined according to the standard methods of Lowry et al. (1951).

Chemicals. Tris-(hydroxymethyl)-aminomethan was purchased from Koch-Light Laboratories LTD, DL-A-alanine, L-aspartic acid and α-ketoglutaric acid were obtained from Sigma Chemical Company.

RESULTS

The tomato seedlings grown in nitrate medium exhibited a high sensibility to change of the Ca : Mg ratio in the medium. As seen from Table 1 a change of Ca : Mg ratio in the nitrate medium caused shortening of the shoot and root length. Decrease of length is observable already after 11 days of seedling growth, the roots being more sensitive than the shoots. On the following days differences in size between control and studied plants became wider. After 21 days of seedling growth in nitrate medium with Ca : Mg ratio 3 : 30, the length of the roots and shoots reached only 48 and 67 per cent of these lengths in the control, respectively. It is worth mentioning that only the length of internodes decreased and their number did not. Similar dependences were not found in media where ammonium salts served as nitrogen sources (Table 1).

Change of Ca : Mg ion ratio by an increase of magnesium amount, the calcium level being constant, caused a distinct reduction of dry weight content in the seedlings growing in nitrate medium (Table 2). On the other hand, in the presence of ammonium ions, independently of the Ca :

: Mg ratio in the medium, the dry weight of the seedlings remained at the same level all the time, the dry weight of leaves excepted. In these organs in seedlings grown in medium with Ca : Mg ratio 3 : 30, a significant increment of dry weight after 16 and 21 days of seedling growth was observed.

Table 1

Average length of roots and shoots

Nutrient solution	Days of growth	Shoots			Roots		
		3:1	3:30		3:1	3:30	
		mm	mm	%	mm	mm	%
NO_3^-	11	86	66	77	159	97	61
	16	129	83	64	206	97	47
	21	165	111	67	220	107	49
NH_4^+	11	71	76	107	190	184	97
	16	104	107	103	209	202	97
	21	140	143	102	209	204	98

Table 2

Average content of dry weight in leaves, leafless shoots and roots per plant

Nutrient solution	Days of growth	Leaves			Leaftless shoots			Roots		
		3:1	3:30		3:1	3:30		3:1	3:30	
		mg	mg	%	mg	mg	%	mg	mg	%
NO_3^-	11	25.3	15.7	62	12.5	10.5	84	5.4	3.2	59
	16	75.5	34.6	46	41.2	21.7	53	14.3	8.6	60
	21	128.9	60.4	47	85.2	41.6	49	30.7	11.9	39
NH_4^+	11	30.4	31.8	105	11.7	12.2	104	7.6	8.3	109
	16	61.8	74.5	120	25.5	26.9	105	11.4	13.2	116
	21	88.4	112.1	127	55.8	63.7	114	18.7	19.6	105

Experiments concerning the activity of aminotransferase showed that the form of nitrogen utilized by the plants grown in the media with Ca : : Mg ratio changed, has a great influence upon the GPT and GOT activity level in the particular organs of tomato seedlings. Data shown in Fig. 1 indicate that an excess of magnesium in relation to calcium in ammonium medium causes an increase of the GOT activity in tomato roots, shoots and leaves. The widest differences in the activity of aspartate aminotransferase accured in the roots on the particular days of measurement, however a significant increase of GOT activity with magnesium excess in other organs may also be observed. On the other hand, the GOT activity in seedlings grown in nitrate medium develops differently. An excess of magnesium in relation to calcium inhibited significantly the GOT activity in the roots on all days of measurements and also in shoots in the

initial growth phase (after 11 days of growth). At the same time in the leaves the differences were not significant. On the following days of measurement, the GOT activity in the shoots and leaves of the seedlings grown in medium with an excess of magnesium, was always higher than in plants grown at normal Ca : Mg ratio (3 : 1).

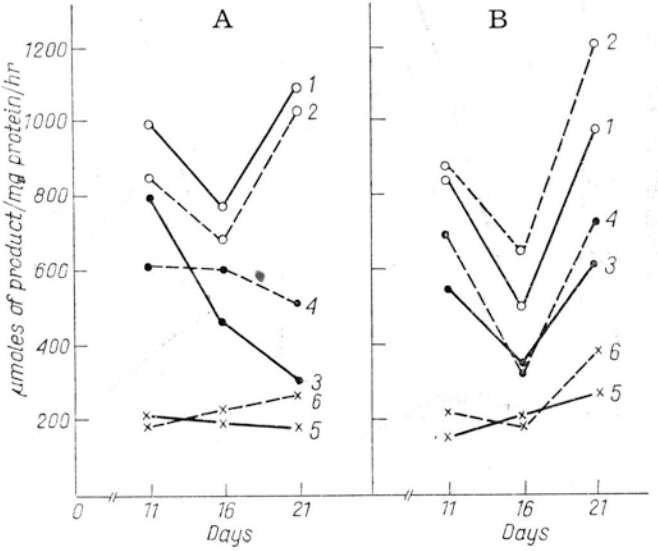

Fig. 1. Aspartate aminotransferase (GOT) activity in µmoles of product released per mg of soluble protein and hour.

A — nitrate nutrient solution, *B* — ammonium nutrient solution
1 — roots (Ca:Mg=3:1), *2* — roots (Ca:Mg=3:30), *3* — leafless shoots (Ca:Mg=3:30), *4* — leafless shoots (Ca:Mg=3:30), *5* — leaves (Ca:Mg=3:1), *6* — leaves (Ca:Mg=3:30)

As seen from Fig. 2, the GPT activity in the roots of the control plants (Ca : Mg ratio in medium 3 : 1), except the initial growth phase of the plants, was higher in the presence of nitrates than in the presence of ammonium ions. An excess of Mg^{2+} ions in relation to Ca^{2+} ions in the medium caused a drastic decrease of the GPT activity in the roots of tomatoes growing on nitrates. In the presence of ammonium ions an inverse phenomenon occurred, namely, the GPT activity in the roots of the seedlings grown with an excess of magnesium exceeded considerably the activity of this enzyme in the roots of the control plants. In the shoots the GPT activity, independently of the form of mineral nitrogen and of the Ca : Mg ratio, was relatively low and similar both in the control and the studied plants. The differences occurred not earlier than at the end of seedling growth (after 21 days).

The GPT activity in leaves is shown in Fig. 3. In the leaves of the seedlings grown in nitrate medium an influence of the exogenous Ca : Mg ratio upon the activity level of this enzyme on particular days of measu-

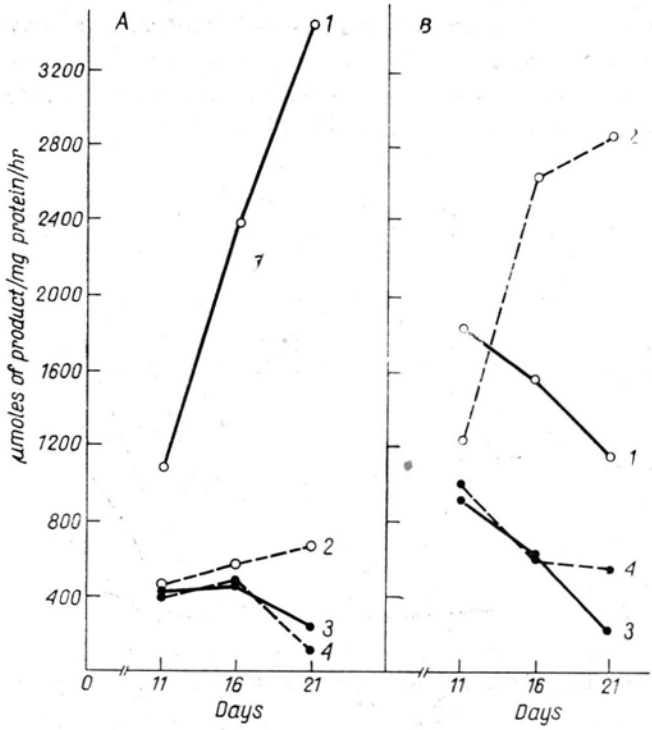

Fig. 2. Alanine aminotransferase (GPT) activity in µmoles of product released per mg of soluble protein and hour

A — nitrate nutrient solution, *B* — ammonium nutrient solution
1 — roots (Ca:Mg=3:1), *2* — roots (Ca::Mg=3:30), *3* — leafless shoots (Ca:Mg=3:30), *3* — leafless shoots (Ca:Mg=3:30)

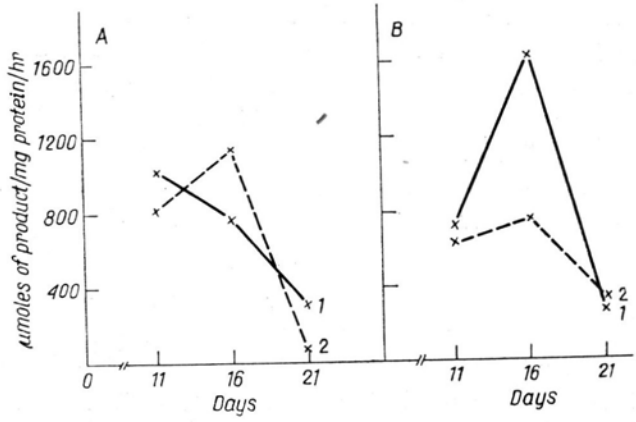

Fig. 3. Alanine aminotransferase (GPT) activity in µmoles of product released per mg of soluble protein and hour

A — nitrate nutrient solution, *B* — ammonium nutrient solution
1 — leaves (Ca:Mg=3:1), *2* — leaves (Ca:Mg=3:30)

rement was observed. In the combination with ammonium salts an excess of magnesium in the medium limited the GPT activity in leaves as compared to the control plants.

For interpretation of the above relations the authors carried out an analysis on samples *in vitro* on the influence of different concentrations of calcium and magnesium upon both enzymes.

To the incubated samples $CaCl_2$ and $MgCl_2$ of concentrations ranging from 0.1 to 10 mM were separately added. No greater changes of enzyme activity were observed. Only as the ion concentration of magnesium increased a slight increase of the GOT activity was noticed. The influence of different Ca : Mg ratios upon aminotransferases was also examined in samples *in vitro,* but no significant differences in their activities were obtained.

DISCUSSION

The above described experiments concerning the GOT and GPT activity in tomato seedlings grown in media with different sources of mineral nitrogen (nitrates or ammonium salts) and different Ca : Mg ratios showed that the activity of both enzymes is dependent both on the nitrogen form and on the ratio of bivalent cations. Futhermore the results showed differences in the activity of the studied enzymes in particular organs of the seedlings.

The presence of nitrates, serving as source of mineral nitrogen in the medium, increased the GOT and GPT activity in the roots as compared with the activity of those enzymes in the roots of seedlings grown in ammonium medium. On the other hand, in the leaves the activity of both studied enzymes remained more or less at a constant level. In the presence of nitrates an excess of magnesium in the medium in relation to calcium, caused a reduction of GOT activity and a drastic drop of GPT activity in the roots of tomatoes. However in green tissues (leaves, shoots) the magnesium excess did not influence the activity of both enzymes so distinctly, however, an increase of GOT and GPT activity as an effect of increased magnesium dose in the medium is observed. If ammonium salts were the source of mineral nitrogen for the plants, the magnesium excess distinctly increased the GOT activity in the roots, and a drastic increase of the GPT activity in those organs was noted. An excess of Mg^{2+} ions in the medium did not, however, cause an increase of GOT activity in the leaves of seedlings grown in ammonium media, similarly as it happened in the combination with nitrate salts, but it distinctly inhibited GPT activity as compared with the combination having a normal magnesium dose after 16 days of seedling growth.

The above mentioned facts point to: (1°) significant differences in the activity of both enzymes depending upon the organ of the plant (roots,

leaves) and (2°) the dependence of GOT and GPT activity upon the source of mineral nitrogen with which the plants are supplied (nitrates, ammonium salts).

These observations are confirmed by the results of studies conducted by Lea and Miflin (1974), who proved that in chloroplasts of pea leaves the greatest amounts of glutamate are produced in the presence of glutamine as donor and of α-ketoglutaric acid as acceptor. The smallest amount of glutamate was obtained when NH_4Cl was the donor of nitrogen. So the authors suppose that in the leaves of higher plants production of α-amino groups of amino acids takes place first of all with the participation of glutamine synthetase and glutamate synthetase while glutamate dehydrogenase is not involved. The authors do not exclude the role of GDH in this process, but they confine it to conditions in which a greater amount of available ammonia is present.

The pointed out differences in GOT and GPT activity depending upon the source of mineral nitrogen, as shown by the present studies, are closely connected with the ratio of bivalent cations in the medium. Namely the exogenous Ca : Mg ratio is not without influence on the endogenous ratio of calcium and magnesium in the particular tomato organs (Buczek and Leonowicz-Babiak, 1971). Thus, it is reasonable to anticipate the endogenous Ca : Mg ion ratios to have a decisive influence upon the activity of both enzymes. In the light of the experiments conducted by the auhors *in vitro* a direct influence of the Ca : Mg ratio upon the GOT and GPT activity was not found, this being in line with the results of Cooper and Hill-Cottingham (1974). The above cited authors found no direct influence of Ca^{2+} and Mg^{2+} ions upon the GOT activity.

In view of the foregoing facts it seems probable that the influence of bivalent cations upon the GOT and GPT activity is rather of indirect character what in turn seems to be confirmed by the results of Cooper and Hill-Cottingham (1974). These authors observed a distinct stimulation of glutamate dehydrogenase (GDH) providing substrates for GOT and GPT, by Ca^{2+} ions; but they found no influence of Mg^{2+} ions upon the GDH activity. The indirect influence of Ca^{2+} and Mg^{2+} ions upon GOT and GPT activity, observed by us in experiments with nitrates as nitrogen source, is also confirmed by the experiments of Buczek et al. (1974) concerning the activity of nitrate reductase in tomatoes. Buczek et al. found that an excess of magnesium in the medium in relation to calcium inhibits distinctly nitrate reductase activity already after 14 days of plant growing. It is known that nitrate reductase is the first link providing a substrate for GDH and indirectly for GOT and GPT.

The obtained results indicate that the abnormal Ca : Mg ratio in the medium, approximately 3 : 30, inhibits GPT activity in the roots of tomato seedlings grown in nitrate media. On the other hand, in ammonium me-

dia an excess of Mg^{2+} ions in relation to Ca^{2+} increases the GPT activity. The excess of magnesium also limits the GOT activity in the roots of plants grown on nitrates. In these roots the greatest drop of dry weight and protein content is also observed, as indicated by the experiments of Buczek et al. (1971, 1974). As reported by Hedley and Stoddart (1971), experiments, in which inhibitors of protein synthesis were used, showed that there is a correlation between the rate of amino acids binding and the level of GPT activity. More precisely: a more intensive synthesis is followed by a higher GPT activity. Limitation of synthesis may lead to an increase of alanine content, and consequently, to a decrease of GPT activity.

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Aktywność aminotransferazowa w siewkach pomidorów w zależności od stosunku jonów Ca : Mg w podłożu

Streszczenie

Nadmierne wprowadzenie jonów magnezu do pożywki azotowej przy zachowanym poziomie jonów wapnia ograniczyło wzrost oraz zawartość suchej masy w 3-tygodniowych siewkach pomidorów. Jeżeli sole azotanowe zastąpiono solami amonowymi nie zaobserwowano większych różnic pomiędzy wzrostem i suchą masą roślin rosnących przy prawidłowym (3 : 1) i zwiększonym (3 : 30) stosunku wapnia do magnezu.

Wykazano również zależność aktywności aminotransferazy alaninowej (GPT) i aminotransferazy asparaginianowej (GOT) od źródła azotu mineralnego i od stosunku jonów Ca : Mg w podłożu. W obecności azotanów wystąpiło znaczne zahamowanie aktywności GOT i GPT w korzeniach pomidorów. W liściach i łodygach z kolei, zaobserwowano pewne ograniczenie aktywności GPT i zwiększenie aktywności GOT po 3-tygodniowej uprawie. Zastosowanie soli amonowych w połączeniu z podwyższoną dawką magnezu spowodowało zwiększenie aktywności aminotransferazowych w korzeniach siewek pomidorów w 11, 16 i 21 dniu wzrostu oraz w 21 dniu zwiększanie aktywności tych enzymów w łodygach i liściach pomidorów.