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Nitrogen Assimilating Enzyme Activity of Tomato Plant in Response to the Supply of Ammonium or Nitrate or Both

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The changes in the *in vitro* activity of nitrate reductase, glutamine synthetase and glutamate dehydrogenase of leaves and roots of tomato plant (*Lycopersicon esculentum* Mill.) with the supply of different nitrogen sources (ammonium, nitrate, or both) were examined. Nitrate reductase activity of leaves and roots became very low when nitrate was omitted from nutrient media. Plants supplied with ammonium nitrate had a detectable level of nitrate reductase activity. Glutamine synthetase activity increased similarly in roots of plants supplied with ammonium alone and of plants with ammonium nitrate, but it was not affected in leaves by the supply of different nitrogen sources. On the contrary, glutamate dehydrogenase activity was not clearly affected in roots by the supply of different nitrogen sources whereas it became much higher in leaves of plants supplied with ammonium alone and slightly higher in those of plants with ammonium nitrate than in those of plants with nitrate alone. It is considered that lower accumulation of ammonium in leaves of plants supplied with ammonium nitrate may be due to stimulation of metabolic steps other than the primary ammonia assimilation.

INTRODUCTION

Most of crop plants are able to utilize ammonium and nitrate as the sole source of nitrogen. There are differences among plant species in which form of nitrogen (ammoium or nitrate) is a better source (Moritsugu and Kawasaki 1980). Tomato plants grew better on nitrate media than when those received ammonium alone (Woolhouse and Hardwick 1966). However, the simultaneous supply of nitrate and high concentrations of ammonium prevented ammonium injury of tomato plants and resulted in identical growth to that of plants grown on nitrate media (Ikeda and Yamada 1984). It is supposed that the presence of nitrate in media containing ammonium and nitrate may affect the absorption and assimilation of ammonium by tomato plant. In this study we determined the activity of nitrogen assimilating enzymes of tomato plants supplied with different nitrogen sources (ammonium, nitrate or both).

MATERIALS AND METHODS

Plant material

Tomato (*Lycopersicon esculentum* Mill. cv Fukuju nigo) seedlings were grown in dilute Hoagland solution containing 3.75 mM $NaNO_3$ in a greenhouse at controlled temperature, 25°C/20°C day/night and relative humidity varied between 60 and 80%.

Forty-day-old plants were treated with three one-half strength Hoagland solutions containing 7.5 mM $NaNO_3$, 7.5 mM NH_4Cl or 7.5 mM NH_4NO_3 (Downs and Hellmers 1975). The growth solution was aerated and changed every three days. The solution pH was adjusted at 6.0 everyday.

Enzyme extraction and assay

For nitrate reductase, leaves and roots were extracted in 0.1 M Tris-HCl (pH 7.5) containing 1 mM cystein with a small amount of polyvinylpolypyrrolidone. The homogenate was filtered through Miracloth and centrifuged at 15,000 g for 20 min. Nitrate reductase activity of the supernatant was determined at 30°C by the method of Hageman and Hucklesby (1971). The reaction mixture contained in a final volume of 2 ml, 50 μmol potassium phosphate (pH 7.5), 20 μmol KNO_3, 0.4 μmol NADH and 0.3 ml of enzyme extract. Nitrite formed was determined by the method of Scholl et ***al.*** (1974).

An extract was prepared for glutamine synthetase by grinding leaves and roots in 50 mM Tris-HCl (pH 7.5) containing 20 mM $MgSO_4$, 1 mM EDTA and 10 mM mercaptoethanol. The homogenate was filtered through Miracloth and centrifuged at 15,000 *g* for 20 min. Glutamine synthetase activity was determined according to the method of Oaks et al. (1980). The reaction mixture (pH 7.8) contained in μmol, Tricine 400 ; glutamate 160 ; hydroxylamine 12 ; ATP 16 ; $MgSO_4$ 8 ; EDTA 0.2, in a final volume of 2 ml. The reaction was initiated at 30°C for 20 min. After incubation, the reaction was stopped by the addition of 2 ml of a solution containing equal volumes of 30% TCA, 5.5 N HCl and 8% $FeCl_3$ in 0.1 N HCl. After centrifugation the absorbance of the supernatant was measured at 540 nm. Glutamine synthetase activity was calculated using y-glutamylhydroxamate as a standard.

Root glutamate dehydrogenase was prepared by grinding roots in a mortar with a pestle. The extraction medium contained 50 mM imidazol (pH 7.5), 1 mM dithiothreitol, 0.5 mM EDTA and small amounts of quartz sand and polyvinylpolypyrrolidone. The homogenate was centrifuged at 15,000 g for 20 min. The supernatant was used for root enzyme assay. For preparation of leaf glutamate dehydrogenase, leaves were ground in a mortar with a pestle. The grinding medium contained 0.87% potassium phosphate (pH 7.8), 10 mM mercaptoethanol and small amounts of quartz sand and polyvinylpolypyrrolidone (Damadaram and Nair 1938). The homogenate was centrifuged at 15,000 g for 20 min. After adjusting the pH of the supernatant at 5.2, solid $(NH_4)_2SO_4$ was added to it to 50% saturation. The precipitate was collected by centrifugation at 15,000 g for 30 min and then dissolved in 1/15 M potassium phosphate (pH 7.8) and dialyzed for 4 hr. After centrifugation the clear supematant was used as leaf enzyme. Glutamate dehydrogenase activity was determined by the method of Yamasaki and Suzuki (1969). The reaction mixture contained, in a final volume of 3 ml, 150 μmol Tris (pH 8.0), 750 μmol NH_4Cl, 120 μmol a-ketoglutarate, 0.75 μmol NADH, 1 μmol $CaCl_2$ and 0.5 ml of enzyme solution. The reaction was initiated by adding the enzyme solution and the oxidation of pyridine nucleotide was followed at 340 nm at 30°C for 10 min using a dual-beam spectrophotometer (Hitachi model 124, Japan). The blank consisted of the complete reaction mixture without NH_4Cl.

Analytical method

Protein was determined by the method of Lowry et al. (1951) with bovine serum albumine as a standard. Protein was precipitated with 30% TCA and resuspended in

0.1 N NaOH containing 0.2 M Na_2CO_3 prior to measurement. Ammonium and nitrate of dry matter were extracted in deionized water at 45°C for 60 min and quantified by the method of McCullough (1967) and Cataldo et *al.* (1975), respectively.

RESULTS

When tomato plants were supplied with ammonium alone and ammonium nitrate, ammonium content of roots greatly increased and gradually decreased after day 4. Ammonium content of roots and leaves remained at very low levels in plants which received nitrate alone. Although the supply of ammonium alone or with nitrate increased leaf ammonium content, that was much higher in plants which received ammonium alone than in those which received ammonium nitrate (Fig. 1).

Both leaf and root nitrate content rapidly decreased during treatments when nitrogen source was ammonium alone. The values of nitrate content of roots and leaves were highest in plants which received nitrate alone. Nitrate content, however, was lower when nitrogen source was ammonium nitrate than when that was nitrate alone though the same concentration of nitrate was added to the media (Fig. 2).

Tomato leaves had 8-fold higher nitrate reductase activity than did tomato roots. When nitrogen source was ammonium alone, nitrate reductase activity declined to very low levels in both roots and leaves (Fig. 3). In roots, nitrate reductase activity slowly increased in plants which received nitrate alone while it increased once and thereafter gradually declined in plants which received ammonium nitrate. Leaf nitrate

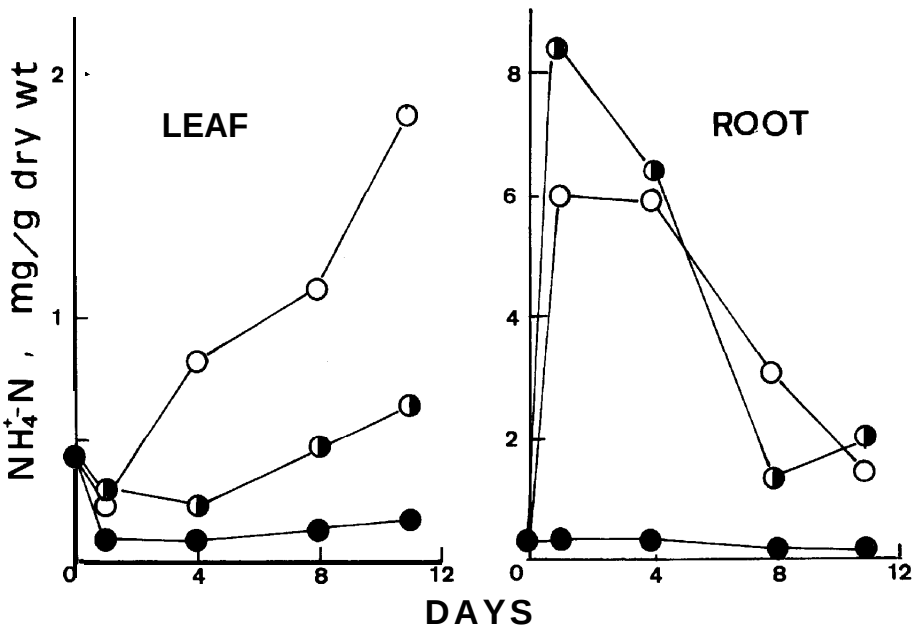

Fig. 1. Effect of nitrogen sources on ammonium content of leaves and roots of tomato plants. Symbols represent plants supplied with ammonium alone (O), ammonium nitrate (◑) and nitrate alone (●).

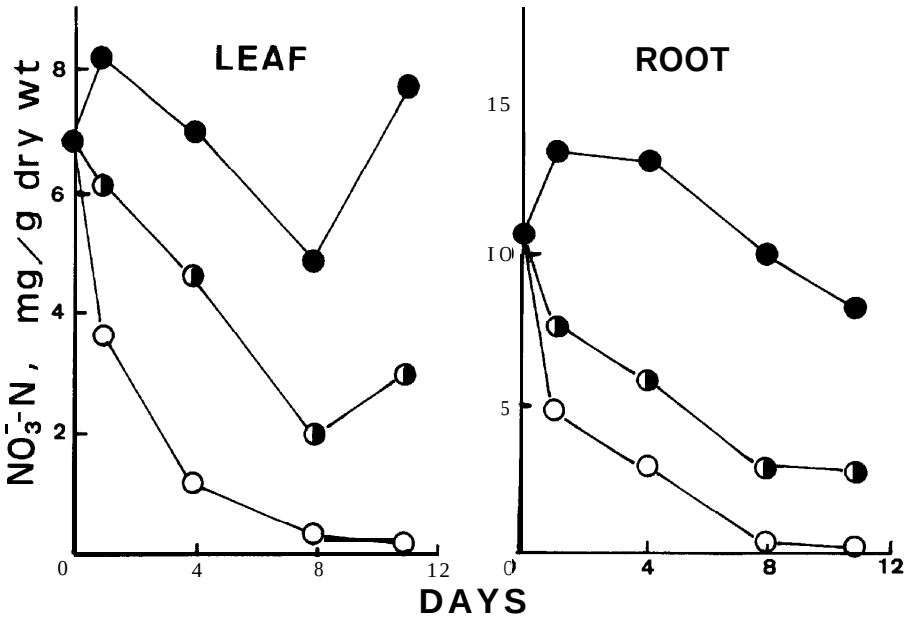

Fig. 2. Effect of nitrogen sources on nitrate content of leaves and roots of tomato plants. See legend to Fig. 1 for symbols.

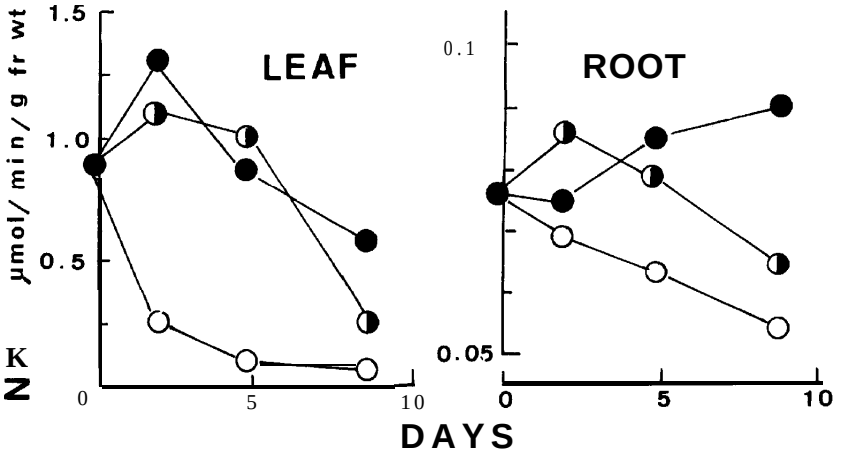

Fig. 3. Effect of nitrogen sources on nitrate reductase (NR) activity of leaves and roots of tomato plants. See legend to Fig. 1 for symbols.

reductase activity was similar both for plants which received nitrate alone and those which received ammonium nitrate.

Although glutamine synthetase activity was higher in leaves than in roots, leaf glutamine synthetase had no response to different nitrogen sources (Fig. 4). When ammonium was supplied in the absence or presence of nitrate in media, root glutamine

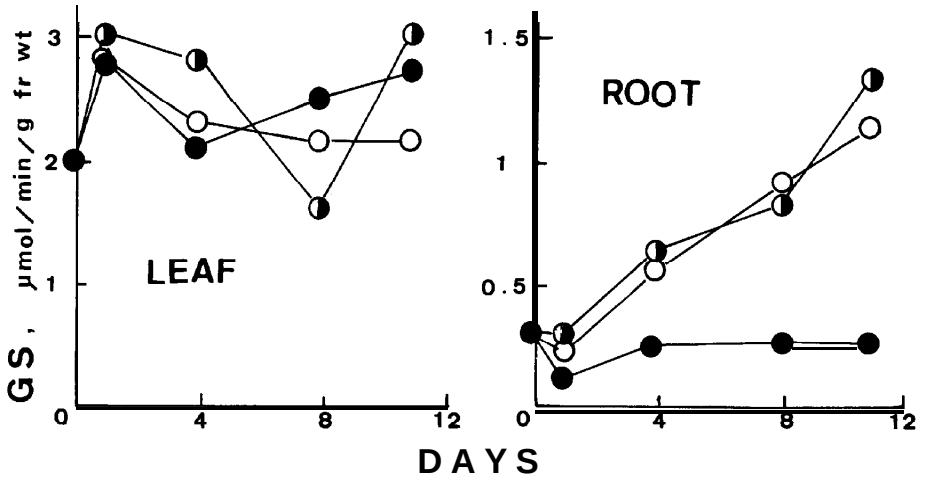

Fig. 4. Effect of nitrogen sources on glutamine synthetase (GS) activity of leaves and rocks of tomato plants. See legend to Fig. 1 for symbols.

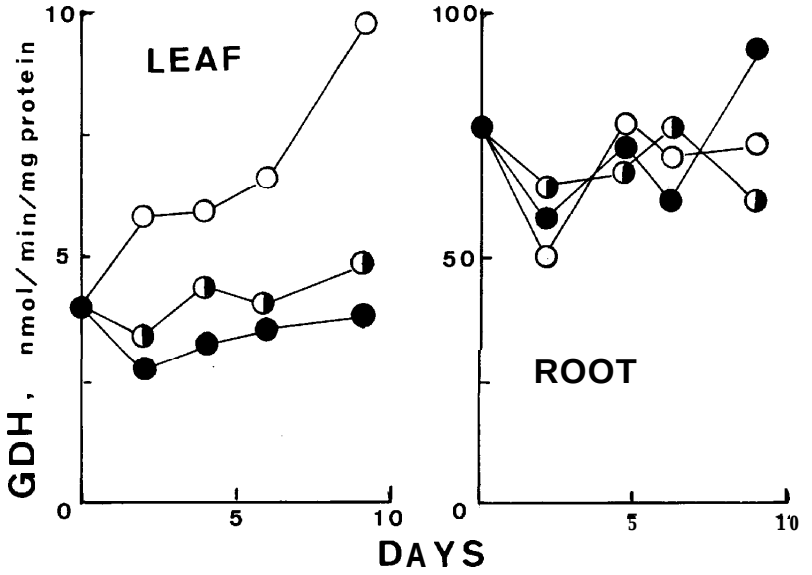

Fig. 5. Effect of nitrogen sources on glutamate dehydrogenase (GDH) activity of leaves and roots of tomato plants. See legend to Fig. 1 for symbols.

synthetase activity continued to increase with time. The activity remained at low and constant levels in plants which received nitrate alone.

When a crude extract prepared from tomato leaves was used in glutamate dehydrogenase assay, glutamate dehydrogenase activity could not be measured. Hence leaf soluble protein fraction was collected by salting-out with ammonium sulfate and the precipitated protein was used for glutamate dehydrogenase assay after dialysis to

remove ammonium from enzyme solution. The activity of glutamate dehydrogenase was 20-fold higher in roots than that in leaves when compared on a basis of protein. The activity of root glutamate dehydrogenase was not clearly affected by the addition of ammonium with or without nitrate to nutrient media (Fig. 5). When nitrogen source was ammonium alone, the activity of leaf glutamate dehydrogenase continued to increase greatly during a period of the treatment. The activity of leaf glutamate dehydrogenase, however, slightly increased in plants which received ammonium nitrate and remained at almost constant levels in plants which received nitrate alone.

DISCUSSION

Ammonium and nitrate content of leaves and roots are determined as an integrated conseguence of uptake, translocation, storage and assimilation of these ions. Except for ammonium content of roots, both ammonium and nitrate content in plants supplied with ammonium nitrate were between respective content in plants supplied with ammonium alone and that in plants supplied with nitrate alone (Fig. 1 and 2). Generally nitrate content is lower in plants supplied with ammonium nitrate than that in plants with nitrate alone due to the inhibition of nitrate uptake by the presence of ammonium in nutrient media and the accumulation of nitrate with no toxic reaction. It was reported that ammonium content of tomato leaves was reduced by the addition of nitrate to nutrient media which contained high concnetrations of ammonium (Ikeda and Yamada 1984). Similar results were obtained in radish plants (Goyal et *al. 1982,* Ota and Yamamoto 1989). Because there was little difference in ammonium content of roots of tomato plants supplied with ammonium alone and those with ammonium nitrate, it is suggested that the supply of nitrate in nutrient media containing ammonium may affect the accumulation of ammonium through the translocation and assimilation of ammonium rather than the uptake of ammonium.

Nitrate reductase activity declined very rapidly in plants supplied with ammonium alone although it was still detectable 9 days after the discontinuation of nitrate supply, indicating that most of nitrate reductase is a substrate-inducible enzyme in tomato plants. In plants supplied with ammonium nitrate, nitrate reductase activity did not reduce during an early period of the treatment, but reduced 5 days after the treatment (Fig. 3). This could be related to a decrease in nitrate content of those plants. It is considered from the present results that nitrate reduction may be continued in plants supplied with ammonium nitrate. Nitrate reduction is accompanied by an alkaline shift of pH in the plant cells, resulting in malate accumulation through the stimulation of phosphoenolpyruvate carboxylase activity (Dijkshoorn 1962, Raven and Smith 1976). The malate may be utilized as a source of carbon skeleton necessary for the detoxification of ammonium (Ikeda and Yamada 1984, Dahlbender and Strack 1986). Hence the supply of nitrate together with ammonium in nutrient media promotes the growth of tomato plants without injury which otherwise takes place when tomato plants were supplied solely with ammonium (Ikeda and Yamada 1984).

It has been considered that the entry of ammonia into the organic form is catalyzed mainly by glutamate dehydrogenase or glutamine synthetase. Recent evidence for a new enzyme glutamate synthase coupled to glutamine synthetase now established glutamine synthetase is more important than glutamate dehydrogense,

especially when a level of available ammonium is low (Miflin and Lea 1980). Increasing availability of ammonium reduced the activity of glutamine synthetase and glutamate synthase in *Lemna minor* (Rhodes et *al. 1976).* On the other hand, in rice and barley plants the supply of ammonium greatly increased glutamine synthetase activity of roots whereas it had almost no effect on leaf glutamine synthetase activity (Matsumoto *et al.* 1983, Lewis ***et al.*** 1982). Both leaf and root glutamine synthetase activity of Chinese cabbage were unaffected by the supply of ammonium (Matsumoto *et al.* 1983). In tomato roots glutamine synthetase continued to increase by the supply of ammonium during the present experimental period (Fig. 4). The increased levels of glutamine synthetase activity stimulate the fixation of ammonium nitrogen entering from nutrient media into organic nitrogen in the plants. On the other hand, leaf glutamine synthetase activity did not respond to different nitrogen sources although ammonium content of leaves was clearely different among three treatments. It was reported that tomato leaves have only chloroplastic glutamine synthetase (McNally *et al.* 1983). This glutamine synthetase could play a role in the reassimilation of ammonia resulting from photorespiration in addition to the assimilation of ammonia from nitrate reduction, and was present in sufficient quantities, leading to relatively constant glutamine synthetase activity in tomato leaves despite of different nitrogen sources supplied (Wallsgrove *et al.* 1980).

Glutamate dehydrogenase activity of tomato roots which first contacted to a high concentration of ammonium in nutrient media did not seem to be responsive to different nitrogen sources, while the activity greatly increased in leaves of plants supplied with ammonium alone (Fig. 5). Unlike the present results, it was reported that glutamate dehydrogenase was induced by the supply of ammonium in roots of rice, maize and *Arabidopsis thaliana* (Kanamori *et al. 1972,* Loyola-Vargas and Sanchez de Jimenz 1984, Commaerts and Jacobs 1985). It is assumed that in tomato leaves glutamate dehydrogenase might play a role in the detoxification of ammonia in place of glutamine synthetase which was not responsive to the supply of ammonium in nutrient media.

Ota and Yamamoto (1989) considered that assimilation of ammonium was remarkably stimulated in radish plants in the presence of a small amount of nitrate and consequently accumulation of ammonium was reduced in plants supplied with ammonium plus nitrate. In tomato plants, however, neither glutamine synthetase nor glutamate dehydrogenase activity was higher when ammonium nitrate was supplied than those when ammonium alone was supplied. This suggests that metabolic steps other than the primary step of ammonia assimilation might be stimulated by the supply of nitrate together with ammonium as ammonium nitrate in nutrient media, resulting in better growth of tomato plants which were supplied with ammonium nitrate.

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