J. Plant Biol. (2010) 53:173–179
DOI 10.1007/s12374-010-9096-9

ORIGINAL RESEARCH

Response of Ammonia Assimilation in Cucumber Seedlings to Nitrate Stress

Xiaoyu Yang · Xiufeng Wang · Min Wei · Shoko Hikosaka · Eiji Goto

Received: 30 October 2009 / Revised: 14 December 2009 / Accepted: 30 December 2009 / Published online: 10 April 2010

Abstract The influence of increased nitrate concentration—14 (control) and 140 mmol L^{-1} (T)—in hydroponic culture on ammonia assimilation in cucumber (*Cucumis sativus* L. cv. Xintaimici) seedlings was investigated. The results showed that NH_3 accumulation in the roots and leaves of T seedlings increased significantly, indicating that NH_3 toxicity might be involved in nitrate stress. Under control conditions, GS and GOGAT activity were much higher in the leaves than in the roots, whereas GDH activity was much higher in the roots than in the leaves. Correlation analysis showed that NH_3 concentration had a strong negative linear relationship with GDH activity in the roots but had a strong negative linear relationship with GS and GOGAT activity in the leaves. These results indicate that NH_3 might be assimilated primarily via GDH reaction in the roots and via GS/GOGAT cycle in the leaves. Short-term nitrate stress resulted in the increase of GS and GOGAT activity in the roots and GDH activity in the leaves of T seedlings, indicating possible shifts in ammonia assimilation from the normal GDH pathway to GS/GOGAT pathway in the roots and from the normal GS/GOGAT pathway to the GDH pathway in the leaves under nitrate stress, but with the increase of treatment time, GS, GOGAT, and GDH activity in the roots and leaves of T seedlings decreased possibly due to low water potential and NH_3 toxicity.

Keywords Ammonia · Cucumber · Glutamate dehydrogenase · Glutamate synthase · Glutamine synthetase · Nitrate stress

Introduction

Nitrogen is needed in large amounts by plants because it is a constituent of macromolecules such as protein. However, only some plants living in association with nitrogen-fixing bacteria can use dinitrogen contained in the air. The majority of plants rely on nitrate and ammonium that originate from decomposition of organic materials and are taken up from soil. Thus, the availability of nitrogen often limits plant growth and development. Complicating this situation for agriculture is the fact that often less than 50% of nitrogen fertilizer applied to crops ultimately may be utilized by crops because nitrate is highly mobile and is not absorbed by soil colloid (Allison 1966). To satisfy the nitrogen demand, agriculturers often add nitrogen in large quantities to maintain adequate level in the rhizosphere (Zhu et al. 2005). This excessive use of nitrogen fertilizer has resulted in undesirable conditions such as the accumulation of nitrate in plant and soil. The large accumulation of nitrogen in the soil, on one hand, has contaminated the ground water (Barker and Mills 1980), on the other hand, has resulted in soil secondary salinization in the protected farmland because of a lack of leaching by rainfall and strong evaporation of soil water (Kitamura et al. 2006).

X. Yang · X. Wang (✉) · M. Wei
College of Horticulture Science and Engineering,
Shandong Agricultural University,
Tai'an, Shandong 271018, People's Republic of China
e-mail: xfwang@sdau.edu.cn

X. Wang · M. Wei
State Key Laboratory of Crop Biology,
Tai'an, Shandong 271018, People's Republic of China

X. Yang · S. Hikosaka · E. Goto
Graduate School of Horticulture, Chiba University,
Matsudo, Chiba 271-8510, Japan

China has the largest area of protected crops and is now the leading country in the world for protected agriculture, including multispan greenhouse, solar lean-to greenhouse, and plastic tunnels (Jiang and Du 2000). However, secondary salinization has seriously limited sustainable development of protected agricultural production in China (Yu et al. 2005). According to the previous studies, accumulation of ions in the protected farmland is greatly different from in the seaside or inland. In the protected farmland, the main cation and anion are Ca^{2+}, K^{+}, and NO_3^{-}, respectively, whereas Na^{+} and Cl^{-} are the main forms of ions in the seaside or inland (Ju et al. 2007).

In the past several years, lots of research on salt stress to plants has been done, but most of it has been focused on NaCl (Stepien and Johnson 2009; Zhu 2002). So far, there have been few investigations about nitrate stress to horticultural crops. Cucumber is one of the most important horticultural crops. It has been reported that the large accumulation of nitrate seriously inhibited the growth and development of cucumber in the protected farmland of China (Lü et al. 2007). Yang (2008) reported that excessive nitrate supply greatly influenced nitrate reduction and resulted in large accumulation of NO_3^{-} and NO_2^{-} and serious repression of in vivo nitrate reductase activity in cucumber seedlings. However, it is still unclear how ammonia assimilation, which is catalyzed mainly by glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH), responds to nitrate stress in cucumber seedlings (Fig. 1).

The objective of this work was to examine the effect of nitrate stress on ammonia assimilation in cucumber seedlings. Ammonia (NH_3) concentration, GS, GOGAT, and GDH activity in cucumber seedlings were investigated under nitrate stress with hydroponic culture.

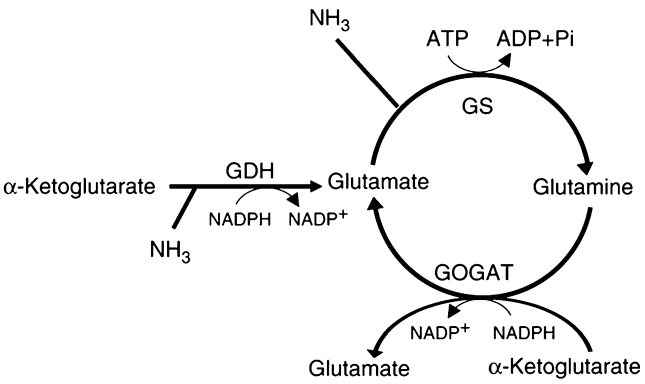

Fig. 1 Simple scheme of ammonia assimilation pathways (Yuan et al. 2009). The three enzyme circuit assimilates NH_3 and produces two central intermediates, glutamine and glutamate. GS catalyzes glutamine synthesis. Glutamate can be synthesized by the action of either GS/GOGAT or GDH, respectively, with high or low affinity for NH_3 (Yan 2007)

Materials and Methods

Plants, Growth Conditions, and Experimental Design

Cucumber (*Cucumis sativus* L. cv. Xintaimici, midtolerant to salinity stress; kindly provided by Xintai Research Institute of Cucumber, China), was used in all experiments. The seeds were sterilized with sodium hypochlorite containing 5% active HOCl for 5 min, soaked for 12 h in deionized water after being washed five times. The soaked seeds were raised in well-washed quartz sand and irrigated with tap water. The experiments were carried out in the greenhouse of Shandong Agricultural University from March to June in 2007. When there was one completely expanded leaf, the plants were washed with tap water to remove all substrate from the roots and then transplanted to hydroponic boxes (40×30×12 cm, eight plants per box) containing a complete cucumber nutrient solution (Guo 2004) with continuous aeration by an electric pump. The nutrient solutions in all the hydroponic boxes were renewed every 4 days. When the seedlings had developed three completely expanded leaves, nitrate was dissolved in nutrient solution directly. The stress by excess of nitrate was carried out in a split-plot design with three replications of completely randomized design, providing eight plants per replication. Two treatments were applied (Table 1):

(CK)complete nutrient solution (control);

(T) complete nutrient solution + $Ca(NO_3)_2$ 31.5 mmol·L^{-1} + KNO_3 63 mmol · L^{-1}

At 0, 1, 2, 4, 6, 8, and 12 days after treatment, the second and third completely expanded leaves counted from the top and lateral roots of cucumber seedlings were sampled and measured for NH_3 concentration, GS, GOGAT, and GDH activity.

Determination of NH_3 Concentration

Ammonia concentration was determined according to the method of Tang (1999). Sample (0.5 g) of fresh tissue was put in a mortar with 5 ml 10% (*V*/*V*) acetic acid and ground to a fine powder. Then the powder was diluted to 100 ml with deionized water and filtered into a 100-ml beaker. The reaction solution included 2 ml ammonia extraction, 3 ml ninhydrin reagent solution, and 0.1 ml 1% (*W*/*V*) ascorbic acid. The mixture was well stirred and boiled for 15 min. Reagent blank was incubation mixture in which the ammonia extraction was replaced by deionized water. After cooling to room temperature in a cold water bath, the reaction solution was made to 5 ml with alcohol and well

Table 1 Nitrate concentration and osmotic potential of solution

Treatment	$Ca(NO_3)_2$ ($mmol \cdot L^{-1}$)	KNO_3 ($mmol \cdot L^{-1}$)	NO_3^- ($mmol \cdot L^{-1}$)	Osmotic potential before treatment (MPa)	Osmotic potential after treatment for 3 days (MPa)
CK	3.5	7	14	−0.256	−0.218
T	35	70	140	−0.570	−0.567

stirred. The absorbance was recorded with spectrometer (160A, Shimadzu, Japan) at 580 nm (Tang 1999).

Determination of GS Activity

Sample (1 g) of fresh tissue was extracted at 4°C with a pestle and mortar. The extraction medium consisted of 0.05 mol L^{-1} sodium phosphate buffer (pH 7.4), 0.4 mol L^{-1} sucrose, and 4 mmol L^{-1} cysteine. The ratio of tissue to medium was 1:4 (*W/V*). The homogenate was centrifuged for 10 min at 10,000×g at 4°C. The clear supernatant was used to determine GS activity. GS activity, expressed as OD per gram FW·per hour, was determined according to the method of Wang et al. (2002).

Determination of GOGAT and GDH Activity

Enzymes were extracted according to the method described before by Srivastava and Ormrod (1984). Sample (5 g) of fresh tissue was extracted at 4°C with a pestle and mortar. The extraction medium consisted of 0.2 mol L^{-1} sodium phosphate buffer (pH 7.5), 2 mmol L^{-1} EDTA, 1 mmol L^{-1} cysteine, and 0.5% (*W/V*) casein. The ratio of tissue to medium was 1:4 (*W/V*). The extraction was centrifuged for 10 min at 20,000×g at 4°C. GDH activity was assayed in a part of the supernatant. The rest of the supernatant was centrifuged further for 20 min at 30,000×g at the same temperature. The supernatant thus obtained was assayed for GOGAT activity.

GOGAT activity was assayed for NADH specific activity according to the method described before (Srivastava and Ormrod 1984). The assay mixture consisted of 2 ml 0.2 mol L^{-1} sodium phosphate buffer (pH 7.5) containing 10 mmol L^{-1} glutamine, 0.4 ml 50 mmol L^{-1} α-ketoglutarate, 0.4 ml 0.1 mmol L^{-1} NADH, and 0.2 ml of enzyme preparation. The reaction was started by the addition of NADH followed immediately by the enzyme preparation. Reagent blank was the assay mixture in which α-ketoglutarate was replaced by deionized water. The oxidation of NADH was recorded at 340 nm.

GDH activity was determined for NAD^+ specificity by the method described before (Singh and Srivastava 1983; Tang 1999) with some modification. Reaction mixture contained 0.2 ml enzyme extraction and 2.8 ml 0.2 mol L^{-1} sodium phosphate buffer (containing 60 mmol L^{-1} glutamate, 0.1 mmol L^{-1} NAD^+, pH 7.5). The reaction was initiated by the addition of 0.2 ml enzyme extraction. Reagent blank was the reaction mixture in which glutamate was replaced by deionized water. The rate of the reduction of NAD^+ was determined by monitoring A at 340 nm.

Protein Determination

Protein content was determined by the dye-binding method of Bradford (1976) with bovine serum albumin as a standard.

Statistical Analysis

Data were analyzed with OriginPro8 (Version8E, OriginLab Corporation, Massachusetts, USA) and presented as means of three replicates ± standard errors.

Results

NH_3 Concentration

Figure 2 showed changes of NH_3 concentration in the roots and leaves of cucumber seedlings under nitrate stress. NH_3 concentration in the roots and leaves of CK seedlings had few changes over treatment course. NH_3 concentration in the roots of T seedlings increased rapidly during the first 8 days compared with CK. After 8 days, this increase of NH_3 concentration in the roots of T seedlings became slow and reached 1.61 times of CK at the end of treatment course. In the leaves of T seedlings, NH_3 concentration increased slowly during the first 8 days, and a dramatic increase occurred after 8 day. At 12 days, NH_3 concentrations in the leaves of T seedlings were 46% higher than CK.

GS Activity

Figure 3 showed changes of GS activity in the roots and leaves of cucumber seedlings under nitrate stress. GS activity was much higher in the leaves (from 9.50 to 10.54 OD g^{-1} FW h^{-1}) than in the roots (from 1.01 to 1.71 OD g^{-1} FW h^{-1}) under control conditions. With the increase of treatment time, GS activity in the roots of CK

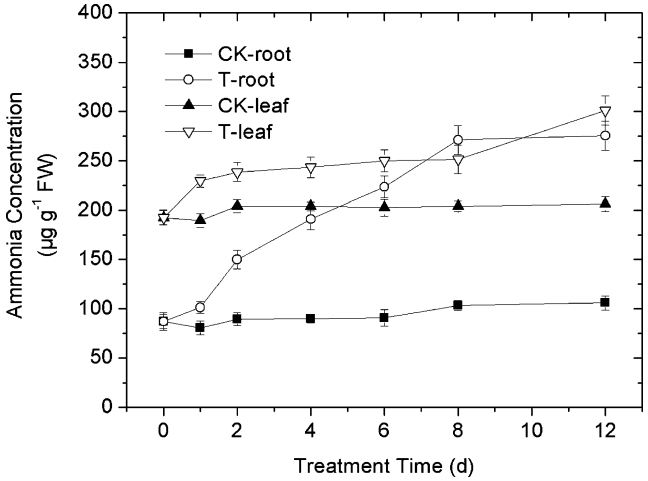

Fig. 2 Changes of ammonia concentration in the roots and leaves of cucumber seedlings under nitrate stress. Plants were grown in nutrient solution containing 14 (CK) and 140 mmol L^{-1} (T) nitrate during 12 days, respectively. *Vertical bars* represent the standard errors (n=3)

seedlings increased gradually. During the first 2 days, GS activity in the roots of T seedlings dramatically increased by 62.1% with respect to CK. Thereafter, the activity decreased. At 12 days, GS activity in the roots of T seedlings was 32.1% lower than CK. There were few changes in GS activity in the leaves of CK over treatment course. GS activity in the leaves of T seedlings had no significant difference from CK during the first 2 days. After 2 days, the activity decreased substantially. At 12 days, GS activity in the leaves of T seedlings was 42.7% lower than CK. Correlation analysis showed that NH_3 concentration had a strong negative linear relationship with GS activity in the leaves (r=−0.8549).

GOGAT Activity

Figure 4 showed changes of GOGAT activity in the roots and leaves of cucumber seedlings under nitrate stress. GOGAT activity was much higher in the leaves (from 41.85 to 45.92 NADH micromoles per milligram protein per minute) than in the roots (from 22.74 to 27.08 NADH micromoles per milligram protein per minute) under control conditions. With respect to CK, GOGAT activity dramatically increased by 38.2% in the roots of T seedlings during the first 2 days. From 2 to 4 days, a rapid decrease in the activity occurred and after 4 days the decrease became slow. At 12 days, GOGAT activity in the roots of T seedlings was 51.5% lower than CK. In the leaves of T seedlings, significant decrease in GOGAT activity occurred over treatment course. At 12 days, GOGAT activity in the leaves of T seedlings was 74.4% lower than CK. Correlation analysis showed that NH_3 concentration had a strong negative linear relationship with GOGAT activity in the leaves (r=−0.9720).

GDH Activity

Figure 5 showed changes of GDH activity in the roots and leaves of cucumber seedlings under nitrate stress. GDH activity was much higher in the roots (from 58.36 to 64.51 NADH micromoles per milligram protein per minute) than in the leaves (from 31.58 to 39.29 NADH micromoles per milligram protein per minute) under control conditions. Over treatment course, GDH activity had few changes in the roots of CK but increased slightly in the leaves. GDH activity in the roots of T seedlings decreased with respect to CK. At 12 days, GDH activity in the roots of T seedlings was 41.3% lower than CK. In the leaves of T seedlings,

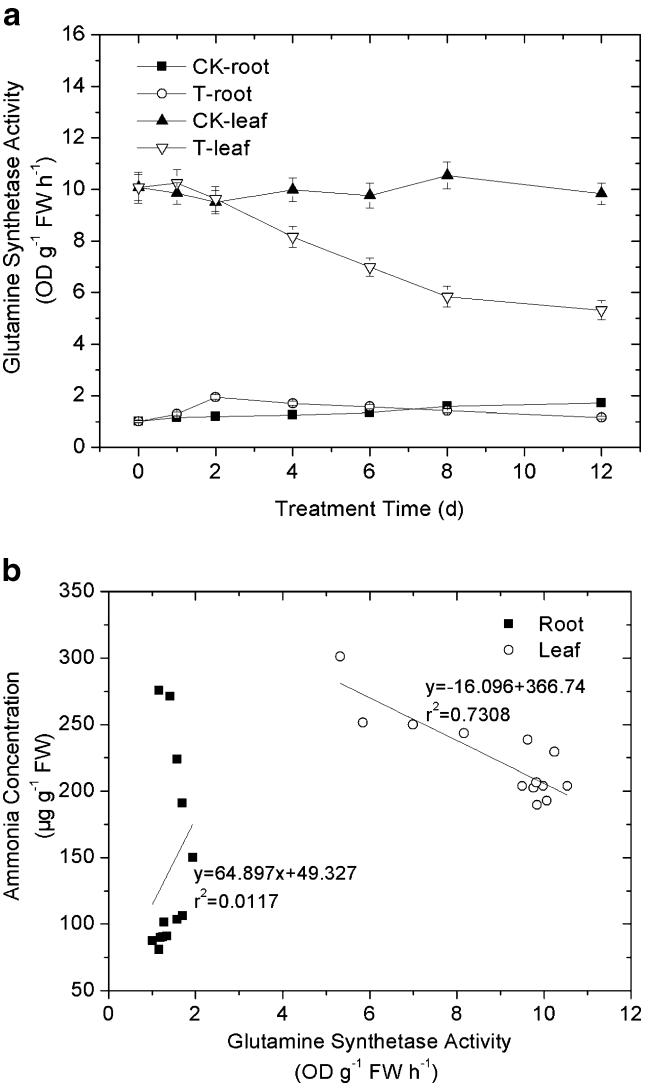

Fig. 3 Changes of glutamine synthetase activity (**a**) and correlation analysis between glutamine synthetase activity and ammonia concentration (**b**) in the roots and leaves of cucumber seedlings under nitrate stress. Plants were grown in nutrient solution containing 14 (CK) and 140 mmol L^{-1} (T) nitrate during 12 days, respectively. *Vertical bars* represent the standard errors (n=3)

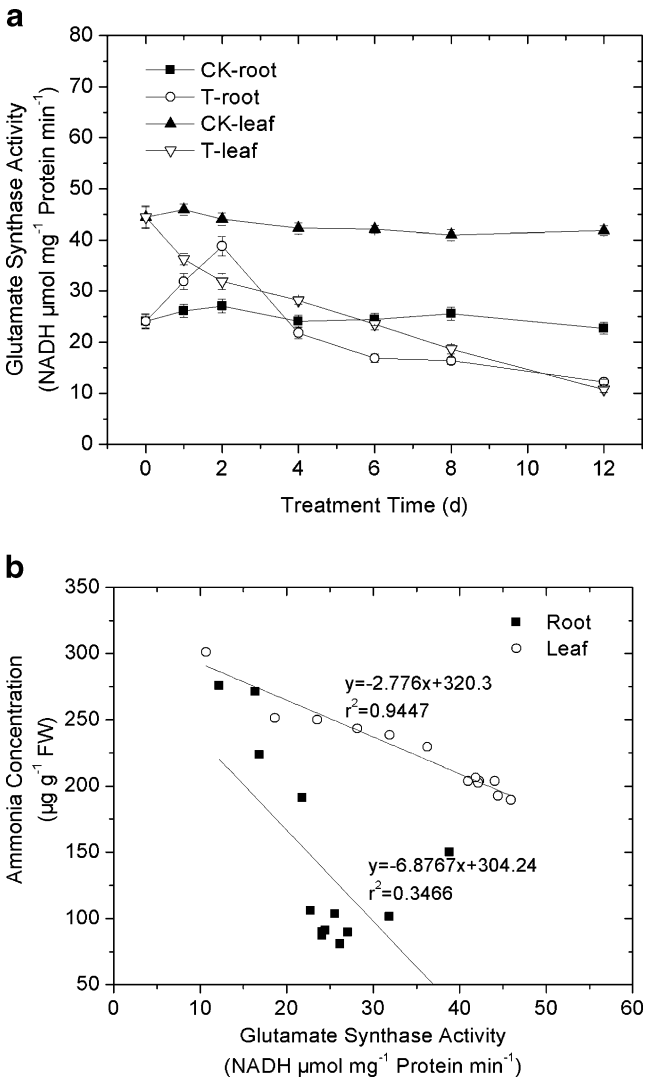

Fig. 4 Changes of glutamate synthase activity (**a**) and correlation analysis between glutamate synthase activity and ammonia concentration (**b**) in the roots and leaves of cucumber seedlings under nitrate stress. Plants were grown in nutrient solution containing 14 (CK) and 140 mmol L^{-1} (T) nitrate during 12 days, respectively. *Vertical bars* represent the standard errors (n=3)

GDH activity rapidly increased by 39.5% with respect to CK at 1 day and decreased thereafter. At 12 days, GDH activity in the leaves of T seedlings was 24.0% lower than CK. Correlation analysis showed that NH_3 concentration had a strong negative linear relationship with GDH activity in the roots (r=−0.9299).

Discussion

NH_3, which originates from a wide variety of metabolic processes such as nitrate reduction (Hirel and Lea 2001), is the final form of inorganic nitrogen prior to the synthesis of organic nitrogen compounds and plays a critical role in plant development (Masclaux-Daubresse et al. 2006), but excessive accumulation of NH_3 is toxic to plants (Cao et al. 2009). Unlike many other molecules or ions, NH_3 is difficult to compartmentalize because it is membrane mobile (Roubelakis-Angelakis and Kliever 1992). Consequently, plants are unable to use compartmentalization, which is often used with other harmful materials where the vacuole serves to isolate them from the cytoplasm with movement restricted by the tonoplast (Qiu et al. 2003), as a protection strategy against elevated NH_3. Therefore, NH_3 assimilation is considered as the only way by which plants can reduce elevated NH_3 level (Roubelakis-Angelakis and Kliever 1992). In the present study, nitrate stress resulted in large amounts of NH_3 accumulation in the roots and leaves

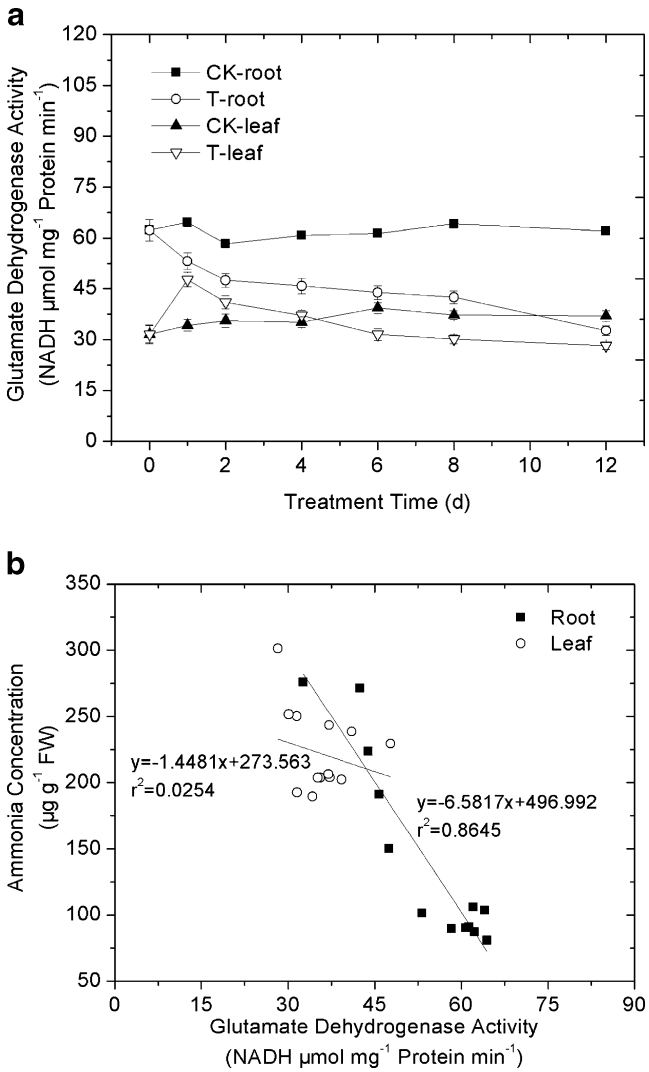

Fig. 5 Changes of glutamate dehydrogenase activity (**a**) and correlation analysis between glutamate dehydrogenase activity and ammonia concentration (**b**) in the roots and leaves of cucumber seedlings under nitrate stress. Plants were grown in nutrient solution containing 14 (CK) and 140 mmol L^{-1} (T) nitrate during 12 days, respectively. *Vertical bars* represent the standard errors (n=3)

of cucumber seedlings (Fig. 2) because of great inhibition of NH_3 assimilation enzymes activity (Figs. 3a, 4a, and 5a). Although in short-term treatment, GS and GOGAT activity in the roots and GDH activity in the leaves of T seedlings were stimulated to some extent, this stimulation seemed few effects on preventing from excessive accumulation of toxic NH_3 in cucumber seedlings. These results indicate that NH_3 toxicity might be involved in nitrate stress to plants.

In higher plants, there are three enzymes—GS, GOGAT, and GDH—involved in NH_3 assimilation reactions (Lam et al. 1996). The three enzymes constitute two NH_3 assimilation pathways—GS/GOGAT and GDH pathways (Suárez et al. 2002). Before 1974, GDH pathway was considered to be the key reaction in NH_3 assimilation (Miflin and Lea 1980). Since 1974, GS/GOGAT pathway has been considered to be the primary route for the initial assimilation of NH_3 (Masclaux-Daubresse et al. 2006) because GS has a much higher affinity of NH_3 than GDH (Stewart and Rhodes 1978). However, some evidence challenges this view. It has been reported that GS and GDH activity were detected in grape root and leaf extracts, whereas GOGAT activity was not detected in grape roots (Ghisi et al. 1984; Roubelakis-Angelakis and Kliever 1983). This result indicates that other NH_3 assimilation pathways (e.g., GDH reaction) could be more important in grape than in other plants (Roubelakis-Angelakis and Kliever 1992). Melo-Oliveira et al. (1996), Oaks (1995), and Yamaya and Matsumoto (1985) have also provided supporting evidence for an important role of GDH in NH_3 assimilation. These results lead to the hypothesis that alternative pathways might operate ammonia assimilation under particular physiological conditions when the GS/GOGAT pathway may not be able to fulfill its function (Harrison et al. 2003).

Interestingly, our research showed organ dependence in NH_3 assimilation pathways of cucumber seedlings, partially supporting the hypothesis above. Under control conditions, GS and GOGAT activity were much higher in the leaves than in the roots (Figs. 3a and 4a), indicating that GS and GOGAT possibly played a more important role in ammonia assimilation in the leaves (Miflin and Habash 2002). In contrast, GDH activity was much higher in the roots than in the leaves (Fig. 5a), indicating that GDH possibly played a more important role in ammonia assimilation in the roots (Turano et al. 1997). Furthermore, correlation analysis showed that NH_3 concentration had a strong negative linear relationship with GDH activity in the roots, whereas NH_3 had a strong negative linear relationship with GS and GOGAT activity in the leaves (Figs. 3b, 4b, and 5b). Based on this evidence, we presume that NH_3 assimilation is possibly controlled by different pathways in the roots and leaves of cucumber seedlings. In the roots, NH_3 may be assimilated primarily via GDH pathway, whereas in the leaves, NH_3 assimilation may be primarily controlled by GS/GOGAT pathway.

Ammonia assimilation is highly regulated by environmental factors such as light and water supply. The study of the expression of the nuclear genes encoding the chloroplastic form of GS in maize and Arabidopsis has revealed that the gene for this GS isoform is tightly regulated by light in a process at least in part mediated by phytochromes (Oliveira and Coruzzi 1999; Peterman and Goodman 1991; Sakakibara et al. 1992). Light can affect genes expression of ammonia assimilation by acting not only directly via phytochromes activation but indirectly via changes in levels of carbon metabolites. One well-described indirect effect of light is the activation of photosynthesis leading to a subsequent increase in the biosynthesis of carbon metabolites such as sucrose (Oliveira and Coruzzi 1999), which is an important regulator for NH_3 assimilation. Garg et al. (1998) reported that the activity of GS and GOGAT decreased and GDH activity increased under water stress in cluster bean, indicating a possible shift in the pathway of ammonia assimilation from the normal GS/GOGAT pathway to the GDH pathway under intense water stress. In the present study, GS and GOGAT activity in the roots and GDH activity in the leaves of T seedlings were stimulated during the former treatment period (Figs. 3a, 4a, and 5a), indicating possible shifts in the pathways of ammonia assimilation from the normal GDH pathway to GS/GOGAT pathway in the roots and from the normal GS/GOGAT pathway to the GDH pathway in the leaves under high nitrate stress. These shifts might be involved in NH_3 tolerance of cucumber seedlings. Thereafter, GS, GOGAT, and GDH activity in the roots and leaves all decreased (Figs. 3a, 4a, and 5a) possibly due to osmotic effects and NH_3 toxicity. High nitrate concentration in the root medium resulted in low water potential and eventually might lead to water stress (Table 1), which possibly resulted in the decrease of photosynthesis. Excessive accumulation of NH_3 under high nitrate might also result in the decrease of photosynthesis due to the severe inhibition of Hill reaction by NH_3 accumulation (Izawa 1977). The subsequent decrease in the biosynthesis of carbon metabolites might therefore lead to the significant inhibition of ammonia assimilation enzymes.

Acknowledgment We thank Dr. Weichang Yu (Faculty of Science, Chinese University of Hong Kong) for proofreading the manuscript. This work has been supported by National Natural Science Foundation of China (grant no.: 30471187).

References

Allison FE (1966) The fate of nitrogen applied to soils. Advan Agron 18:219–258

Barker AV, Mills HA (1980) Ammonium and nitrate nutrition of horticultural crops. Hort Rev 2:395–423

Bradford MM (1976) A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principles of protein-dye binding. Anal Biochem 72:248–254

Cao T, Xie P, Ni LY, Zhang M, Xu J (2009) Carbon and nitrogen metabolism of an eutrophication tolerative macrophyte, *Potamogeton crispus*, under NH_4^+ stress and low light availability. Environ Exp Bot 66:74–78

Garg BK, Vyas SP, Kathju S, Lahiri AN (1998) Influence of water deficit stress at various growth stages on some enzymes of nitrogen metabolism and yield in cluster bean genotypes. Indian J Plant Physiol 3:214–218

Ghisi R, Iannini B, Passera C (1984) Changes in the activities of enzymes involved in nitrogen and sulphur assimilation during leaf and berry development of *Vitis vinifera*. Vitis 23:257–267

Guo SR (2004) Soilless culture. China Agricultural Press, Beijing

Harrison J, Pou de Crecenzo MA, Sené O, Hirel B (2003) Does lowering glutamine synthetase activity in nodules modify nitrogen metabolism and growth of *Lotus japonicus* L.? Plant Physiol 133:253–262

Hirel B, Lea PJ (2001) Ammonium assimilation. In: Lea PJ, Morot-Gaudry JF (eds) Plant nitrogen. I. Springer, Heidelberg, pp 79–99

Izawa S (1977) Inhibition of electron transport. In: Trebst A, Avron M (eds) Encyclopedia of plant physiology, new series, Vol. 5, Photosynthesis I: photosynthetic electron transport and photophosphorylation, vol 5. Springer Verlag, Berlin, pp 266–279

Jiang WJ, Du DY (2000) Present situation and suggestion for sustainable development of protected horticulture in mainland China. Chin Agri Sci Bul 16(3):61–63

Ju XT, Kou CL, Christie P, Dou ZX, Zhang FS (2007) Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. Environ Pollut 145:497–506

Kitamura Y, Yano T, Honna T, Yamamoto S, Inosako K (2006) Causes of farmland salinization and remedial measures in the Aral Sea basin-research on water management to prevent secondary salinization in rice-based cropping system in arid land. Agric Water Manage 85:1–14

Lam HM, Coschigano KT, Oliveiva IC, Melo-Oliveira R, Coruzzi GM (1996) The molecular genetics of nitrogen assimilation into amino acids in higher plants. Ann Rev Plant Physiol Plant Mol Bio 47:569–593

Lü J, Wang XF, Wei M, Yang FJ, Gao QH, Du DL, Yang XY (2007) Effect of different salt treatments on growth and physiological characteristics of cucumber seedlings. Plant Nutr Fert Sci 13:1123–1128

Masclaux-Daubresse C, Reisdorf-Cren M, Pageau K, Lelandais M, Grandjean O, Kronenberger J, Valadier MH, Feraud M, Jouglet T, Suzuki A (2006) Glutamine synthetase–glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. Plant Physiol 140:444–456

Melo-Oliveira R, Oliveira IC, Coruzzi GM (1996) Arabidopsis mutant analysis and gene regulation define a non-redundant role for glutamate dehydrogenase in nitrogen assimilation. Proc Natl Acad Sci USA 93:4718–4723

Miflin BJ, Habash DZ (2002) The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J Exp Bot 53:979–987

Miflin BJ, Lea PJ (1980) The biochemistry of plants. Academic, New York

Oaks A (1995) Evidence for deamination by glutamate dehydrogenase in higher plants: reply. Can J Bot 73:1116–1117

Oliveira IC, Coruzzi GM (1999) Carbon and amino acids reciprocally modulate the expression of glutamine synthetase in Arabidopsis. Plant Physiol 121:301–309

Peterman TK, Goodman HM (1991) The glutamine synthetase gene family of *Arabidopsis thaliana*: light-regulation and differential expression in leaves, roots and seeds. Mol Gen Genet 230:145–154

Qiu QS, Barkla BJ, Vera-Estrella R, Zhu JK, Schumaker KS (2003) Na^+/H^+ exchange activity in the plasma membrane of Arabidopsis. Plant Physiol 132:1041–1052

Roubelakis-Angelakis KA, Kliever WM (1983) Ammonia assimilation in *Vitis vinifera* L.: II Leaf and root glutamine synthetase. Vitis 22:299–305

Roubelakis-Angelakis KA, Kliever WM (1992) Nitrogen metabolism in grapevine. Hort Rev 14:407–452

Sakakibara H, Kawabata S, Takahashi H, Hase T, Sugiyama T (1992) Molecular cloning of the family of glutamine synthetase genes from maize: expression of genes for glutamine synthetase and ferrodoxin-dependent glutamate synthase in photosynthetic and non-photosynthetic tissues. Plant Cell Physiol 33:49–58

Singh RP, Srivastava HS (1983) Regulation of glutamate dehydrogenase activity by amino acids in maize seedlings. Physiol Plant 57:549–554

Srivastava HS, Ormrod DP (1984) Effects of nitrogen dioxide and nitrate nutrition on growth and nitrate assimilation in bean leaves. Plant Physiol 76:418–423

Stepien P, Johnson GN (2009) Contrasting responses of photosynthesis to salt stress in the glycophyte Arabidopsis and the halophyte Thellungiella: role of the plastid terminal oxidase as an alternative electron sink. Plant Physiol 149:1154–1165

Stewart GR, Rhodes D (1978) Nitrogen metabolism of halophytes.III. Enzymes of ammonia assimilation. New Phytol 80:307–316

Suárez MF, Avila C, Gallardo F, Cantón FR, García-Gutiérrez A, Claros MG, Cánovas FM (2002) Molecular and enzymatic analysis of ammonium assimilation in woody plants. J Exp Bot 53:891–904

Tang ZC (1999) Experimental guide of modern plant physiology. Science Press, Beijing

Turano FJ, Thakkar SS, Fang T, Weisemann JM (1997) Characterization and expression of NAD(H)-dependent glutamate dehydrogenase genes in *Arabidopsis*. Plant Physiol 113:1329–1341

Wang YF, Yu ZW, Li SX, Yu SL (2002) Effect of nitrogen nutrition on the change of key enzyme activity during the nitrogen metabolism and kernel protein content in winter wheat. Acta Agron Sinica 28:743–748

Yamaya T, Matsumoto H (1985) Influence of NH_4^+ on the oxygen uptake of mitochondria isolated from corn and pea shoots. Soil Sci Plant Nutr 31:513–520

Yan DL (2007) Protection of the glutamate pool concentration in enteric bacteria. Proc Natl Acad Sci USA 104:9475–9480

Yang XY (2008) Study on effects of nitrate stress on nitrogen assimilation of cucumber (*Cucumis sativus* L.) seedlings. Master Thesis, Shandong Agricultural University, Tai'an

Yu HY, Li TX, Zhou JM (2005) Secondary salinization of greenhouse soil and its effects on soil properties. Soils 37:581–586

Yuan J, Doucette CD, Fowler WU, Feng XJ, Piazza M, Rabitz HA, Wingreen NS, Rabinowitz JD (2009) Metabolomics-driven quantitative analysis of ammonia assimilation in *E. coli*. Mol Syst Biol 5:1–16

Zhu JH, Li XL, Christie P, Li JL (2005) Environmental implications of low nitrogen use efficiency in excessively fertilized hot pepper (*Capsicum frutescens* L.) cropping systems. Agric Ecosys Environ 111:70–80

Zhu JK (2002) Salt and drought stress signal transduction in plants. Ann Rev Plant Biol 53:247–273