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CHANGES IN SOME ENZYME ACTIVITIES OF RICE AND CHINESE CABBAGE DUE TO NITROGEN STATUS

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It is generally accepted that a plant species has a preferred nitrogen form, i. e., nitrate-nitrogen (N) or ammonium-nitrogen (N). Although many plants can utilize either nitrate or ammonium salts, nitrate is the preferable nitrogen source for various crops[8]. Many workers have investigated the relative values of nitrate and ammonium salts as nitrogen sources[5,8,9]. Several environmental factors concerning uptake and utilization of these nitrogen sources for plant growth have been investigated in agricultural studies directed toward establishing fertilizer practice.

The external pH and oxygen supply in the culture solution are important factors that affect the uptake and utilization of nitrogen[3,8]. Besides these factors, metabolic capabilities of plants toward different nitrogen sources could be reflected in the different preferences to nitrogen forms[1,7,10,11].

In this study, some characteristic behaviors of nitrate reductase (NR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) under different nitrogen status were investigated using rice plants which tend to prefer ammonium-N and chinese cabbage which strictly prefer nitrate[8].

MATERIALS AND METHODS

Plant materials——Seeds of rice (*Oryza sativa* cv. Akebono) and chinese cabbage (*Brassica rapa* cv. Kashin-hakusai) were allowed to germinate approximately 10 days after sowing, the seedlings were cultured in a standard culture solution containing the following minerals: 1 mM NaH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 4 mM KNO_3, 1 mM $NaNO_3$, 0.5 ppm B, 0.5 ppm Mn, 0.05 ppm Zn, 0.02 ppm Cu, 0.01 ppm Mo and 1 ppm Fe. The solution was adjusted to pH 5.5. The culture solution diluted 4 fold was given during the first week. Rice was given an additional 2 mM H_2SiO_3. The plants in the greenhouse were aerated throughout the experiment and the culture solution was renewed weekly. After approximately 1 month of culture, the samples were used for the experiment.

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Enzyme preparation and assays——Enzyme preparation was carried out at 2–6°C. Samples were weighed, washed by distilled water, and then homogenized with three times their volume of 0.1 M Tris-HCl buffer (pH 7.5) containing 10 mM cysteine in a Waring blender for 2 min. Polyvinylpyrolidone corresponding to 1/5 the sample weight was added during homogenization. The homogenate was passed through 4 layeres of cheese cloth and centrifuged at 15,000 x *g* for 20 min. The resulting supernatant was used as the crude enzyme.

The reaction components and assay conditions for NADH-nitrate reductase (NR), glutamine synthetase (GS) and glutamate dehydroge-

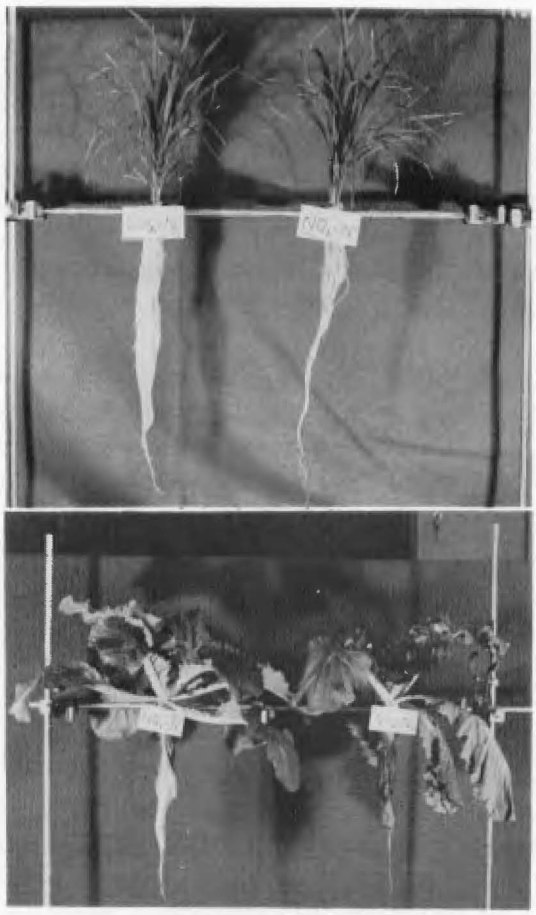

FIG. 1. Rice and chinese cabbage fed with either 5 mM ammonium-N or nitrate-N for 5 days. Note that chinese cabbage, especially roots, was injured by ammonium-N. Chinese cabbage (lower): treated with ammonium-N (right) and nitrate-N (left). Rice (upper): treated with ammonium-N(left) and nitrate-N (right).

nase (GDH) were the same as those reported previously[2,6,12]. Protein was determined by the method of Lowry *et al.*[4]

RESULTS AND DISCUSSION

Characteristic Growth of Rice and Chinese Cabbage Cultured by Either Nitrate or Ammonium Salts

As shown in Fig. 1, rice plants grew normally on either nitrate or ammonium-N, while chinese cabbage was severely injured by ammonium-N. This supports the previous finding that nitrate-N is the strictly preferred nitrogen source for the growth of chinese cabbage[8].

Nitrate Reductase Activity from Rice and Chinese Cabbage

NR activity from the leaves of rice and chinese cabbage which were grown with nitrate medium showed nearly the same activity on a protein basis (Fig. 2). The reaction progressed linearly up to 300 μg protein in both enzymes. It can be said that the level of NR activity might not be an important factor determining the preference of nitrogen form of rice and chinese cabbage. This implies that other factors, 1) NR regulating system[13], 2) the capacity of absorption/accumulation of nitrate and 3) the tolerance to ammonium-N, may be responsible for the different response of rice and chinese cabbage to each nitrogen form.

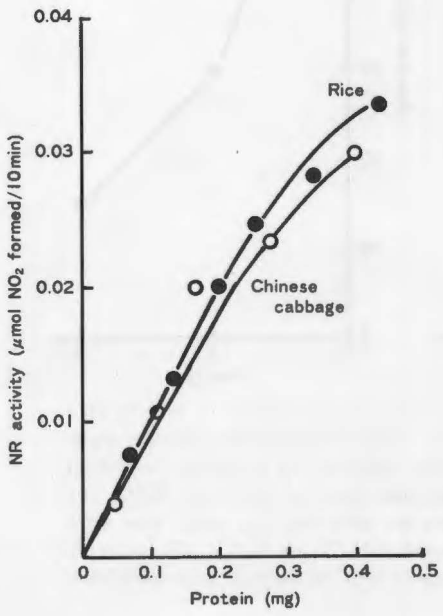

FIG. 2. NR activity in leaves of rice and chinese cabbage as a function of enzyme volume. Samples were cultured with nitrate-N added for approximately 1 month.

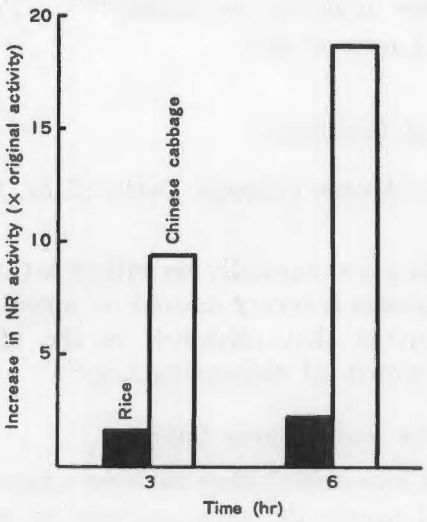

FIG. 3. The rate of induction of NR in excised leaves of rice and chinese cabbage. Rice and chinese cabbage were grown with a standard culture solution for 1 month followed by nitrogen starvation for 2 weeks. Nitrogen starved leaves were cut into small segments(approximately 5 mm squares) and floated on 0.1 M KNO_3 in 0.1 M potassium phosphate buffer (pH 7.5) under 30,000 lux light at 30°C for 0, 3 and 6 hr. The values are expressed by % increase in the activity of the non-induced sample.

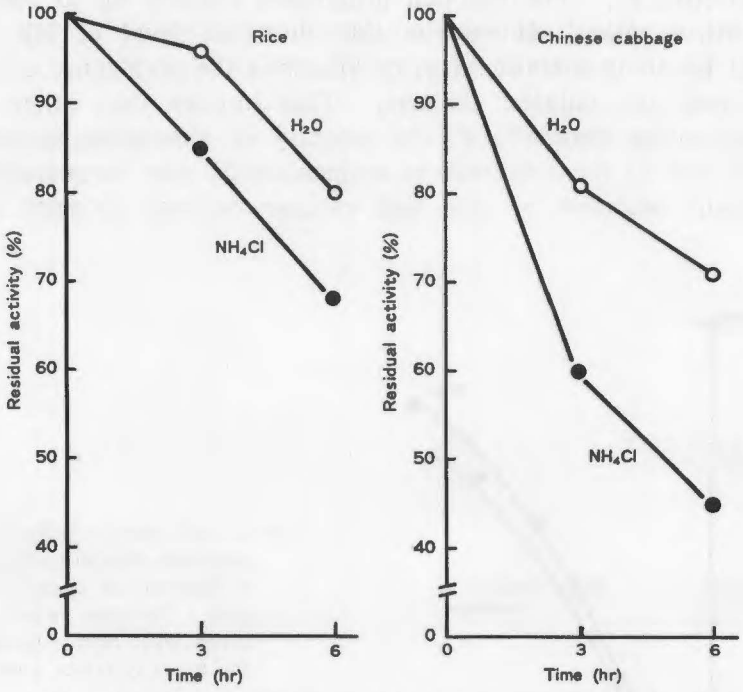

FIG. 4. Effect of ammonium-N on *in vivo* stability of NR of rice and chinese cabbage leaves. Rice and chinese cabbage were grown normally for 1 month followed by nitrogen starvation for 2 weeks. Then the samples were given 5 mM KNO_3 for 48 hr to induce NR. The leaves were cut into small segments and floated on either 100 mM NH_4Cl or H_2O. NR activity was measured and the value at 0 hr of floating was calculated as 100 %.

It is well known that NR is an inducible enzyme. The rate of induction within a short period was compared in the following experiment. Rice and chinese cabbage were grown normally for one month and then in a nitrogen deficient condition for another two weeks. Then the leaves of nitrogen starved plants were cut into small segments and floated on nitrate solution to induce NR. The rate of NR induction of rice after 3 and 6 hr was 1.8 and 2.5 fold, and that of chinese cabbage 9.4 and 18.7 fold, respectively (Fig. 3). However, the NR activity of nitrogen-starved rice and chinese cabbage was 0.62 and 0.04 μmol NO_2 formed per mg protein for 10 min, respectively. These results suggest that chinese cabbage effectively responds to the nitrate supplied concerning NR induction.

Effect of Ammonium-N on in vivo Stability of NR

As shown in Fig. 4, NR activity in both leaf segments decreased after they were floated on water, and the loss was slightly bigger in chinese cabbage than in rice plants. Furthermore, the loss was promoted by the floating of leaf segments on the solution containing ammonium-N. Nearly 67% of the activity of rice NR remained after 6 hr, but only 45% remained in chinese cabbage leaves. This suggests that the NR in chinese cabbage becomes less stable by coexisting ammonium-N than NR of rice does. This may be connected to the fact that ammonium-N represses the induction of NR in chinese cabbage as will be shown later. In the following experiments, the effects of ammonium-N on NR, GS and GDH were investigated further.

Effect of Ammonium-N on the Activities of NR, GS and GDH

GS and GDH play an important role in the assimilation of ammonia. NR is the key enzyme in the process of reducing nitrate to ammonia. Thus the effects of ammonium treatment on the activities of GS and

TABLE 1. NR activity of rice and chinese cabbage fed with different nitrogen forms

N-form	NR specific activity in leaf*	
	Rice	Chinese cabbage
NO_3-N 5 mM	13×10^{-2}(100%)	13×10^{-2}(100%)
NO_3-N 2.5 mM, NH_4-N 2.5 mM	10×10^{-2}(76.9)	9×10^{-2}(69.2)
NH_4-N 5 mM	3×10^{-2}(23.1)	0.6×10^{-2}(4.6)

Samples cultured with a standard culture solution for 1 month were treated with nitrogen starvation for 2 weeks. Then the samples were fed with different nitrogen forms for 72 hr.

* Values mean μmol NO_2 formed/mg protein/10 min.

GDH together with NR were investigated. The samples treated for nitrogen deficiency were fed either with nitrate-N or with ammonium-N, or a mixture of both N sources for 72 hr. As shown in Table 1, ammonium-N strongly affected the level of NR in the leaves of rice and chinese cabbage, especially in the latter. The NR activity of chinese cabbage fed with ammonium-N was suppressed to only 4.6% of that of the nitrate-fed plants. The coexistence of equal concentrations of ammonium- and nitrate-N reduced the NR activity by approximately 70% of that of the nitrate-fed plants. On the other hand, rice plants were much less affected by ammonium-N. The NR activity was suppressed by ammonium-N to 77% of the NR activity of nitrate-fed plants. These results show that ammonium-N affects the level of NR more prominently in chinese cabbage than in rice as shown in Fig. 4.

The effects of ammonium-N on GS activity in rice and chinese cabbage were also clearly different (Table 2). GS activities in both leaves

TABLE 2. GS activity of rice and chinese cabbage fed with different nitrogen forms

N-form	GS specific activity*			
	Rice		Chinese cabbage	
	Root	Leaf	Root	Leaf
NO_3-N 5 mM	0.30 (100%)	1.01 (100%)	0.67 (100%)	2.10 (100%)
NO_3-N 2.5 mM + NH_4-N 2.5 mM	0.88 (293)	0.98 (97)	0.60 (90)	1.84 (88)
NH_4-N 5 mM	1.02 (340)	1.40 (139)	0.10 (15)	1.21 (58)

Samples cultured with a standard culture solution for 1 month were treated with nitrogen starvation for 2 weeks. Then the samples were treated with different nitrogen forms for 72 hr.

* Values mean μmol γ-glutamylhydroxamate formed/mg protein/10 min.

and roots of rice were increased by ammonium treatment, while those of chinese cabbage were strongly suppressed. The increase in GS activity was especially prominent in the roots of rice, GS activity being 340% of that of the sample without ammonium treatment. Also the presence of ammonium-N in rice roots caused an increase in GS activity, which was 2.9 fold that of the nitrate-fed plants.

These results coincide with those obtained on the induction of GS in rice roots by increasing amounts of ammonia[2]. Unlike in rice plants, GS activity of chinese cabbage was strongly suppressed as was the case for pea roots[10] and *Osmunda regalis*[11]; the rate of inhibition was 42% in leaves and 85% in roots of chinese cabbage.

The GDH activity of rice as affected by ammonium treatment was also somewhat different from that of chinese cabbage. In leaves of rice

TABLE 3. GDH activity of rice and chinese cabbage fed with different nitrogen forms

N-form	GDH specific activity*			
	Rice		Chinese cabbage	
	Root	Leaf	Root	Leaf
NO_3-N 5 mM	8.1×10^{-5}(100%)	1.7×10^{-5}(100%)	24.0×10^{-5}(100%)	0.8×10^{-5}(100%)
NO_3-N 2.5 mM, NH_4-N 2.5 mM	8.1×10^{-5}(100)	1.6×10^{-5}(94)	15.9×10^{-5}(66)	1.1×10^{-5}(138)
NH_4-N 5 mM	13.5×10^{-5}(167)	2.9×10^{-5}(171)	15.9×10^{-5}(66)	1.8×10^{-5}(225)

Samples cultured with a standard culture solution for 1 month were treated for nitrogen starvation for 2 weeks. Then the samples were given different nitrogen forms for 72 hr.

* Values mean nmol NADH oxidized/mg protein/min.

and chinese cabbage and in roots of rice, GDH activity increased due to ammonium treatment (Table 3). These results are supported by the findings that GDH activity is induced by ammonium-N in several plant species[7,12]. However, a clear suppression of GDH activity was observed in the roots of chinese cabbage treated with ammonium-N. This is apparently in contrast to the roots of rice which show an increase in GDH activity by 67% of the nitrate-fed plants.

The above results suggest that NR, GS and GDH activities in chinese cabbage which strictly prefers nitrate-N are inhibited more by the treatment with ammonium-N than rice plants are.

So far many investigators have discussed why the nitrate-N is generally accepted to be the preferred from for optimal growth. Much attention has been paid to the conditions of the culture solution such as changes in pH and the amount of oxygen available[3,5,8]. The present work was designed to investigate the effect of ammonium- and nitrate-N on the growth of two plants whose preferred nitrogen forms are different. Particular emphasis was placed on the changes in enzyme activities concerned in the assimilation of nitrogen. It can be concluded that the strong preference for nitrate of chinese cabbage does not depend on the strong activity of NR (Fig. 2), but on its susceptibility to ammonium-N which suppressed the activities of NR, GS and GDH variously (Fig. 4, Table 1, 2 and 3). On the other hand, rice NR showing a similar activity to chinese cabbage NR is less suppressed by ammonium-N, and the level of GS and GDH in rice plants is even increased by ammonium treatment. Although such different behaviors of nitrogen assimilating enzymes upon the given treatments are reflected in different growth rate due to ammonium- and nitrate-N, there is as yet no evidence that these changes can be the direct cause of the different preference for nitrogen of rice and chinese cabbage.

SUMMARY

Comparison of the growth and nitrogen assimilating enzymes was made between rice and chinese cabbage which responded differently to nitrate- and ammonium-N. Rice grew normally on both nitrogen forms, while chinese cabbage was severly injured by ammonium-N. NR activities in leaves of rice and chinese cabbage were alike, when the plants were grown on nitrate-N. The rate of induction of NR in excised chinese cabbage leaves was strikingly higher than that of rice. The *in vivo* stability of NR in the presence of ammonium-N was smaller in chinese cabbage than in rice. The treatment with ammonium-N reduced the level of NR more strongly in chinese cabbage than in rice. GS activity in chinese cabbage and rice was inversely affected by ammonium treatment, and the activity in the chinese cabbage being suppressed. In addition, GDH activity in leaves and roots of rice was increased by ammonium treatment, while it was lowered in the roots of chinese cabbage. The results were discussed with respect to the difference in the preferred nitrogen form of chinese cabbage and rice.

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REFERENCES

1. Bayley, J. M., King, J. and Gamborg, O. L. 1972. The effect of the source of inorganic nitrogen on growth and enzymes of nitrogen assimilation in soybean and wheat cells in suspension cultures. Planta 105 : 15–24.
2. Kanamori, T. and Matsumoto, H. 1972. Glutamine synthetase from rice plant roots. Arch. Biochem. Biophys. 152 : 404–412.
3. Lee, R. B. 1978. Inorganic nitrogen metabolism in barley roots under poorly aerated conditions. J. Exp. Bot. 29 : 693–708.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 : 265–275.
5. Matsumoto, H. and Tamura, K. 1981. Respiratory stress in cucumber roots treated with ammonium or nitrate nitrogen. Plant Soil 60 : 195–204.
6. Matsumoto, H., Tanaka, T., Matoh, T., Hashizume, K. and Takahashi, E. 1979. Inhibition of NADH-nitrate reductase activity in cucumber leaves due to NADH oxidation. Plant & Cell Physiol. 20 : 573–582.
7. Mohanty, B. and Fletcher, J. S. 1980. Ammonium influence on nitrogen assimilating enzymes and protein accumulation in suspension cultures of Paul's Scarlet rose. Physiol. Plant. 48 : 453–459.
8. Moritsugu, M., Suzuki, T. and Kawasaki, T. 1980. Effect of nitrogen sources upon plant growth and mineral uptake. 1. Comparison between constant pH and conventional culture method. J. Sci. Soil Manure, Jpn. 51 : 447–456 (in Japanese).
9. Moritsugu, M. and Kawasaki, T. 1982. Effect of solution pH on growth and mineral uptake in plants under constant pH condition. Ber. Ohara Inst. landw. Biol., Okayama Univ. 18 : 77–92.

10. Sahulka, J. and Lisa, L. 1979. Regulation of glutamine synthetase level in isolated pea roots. 1. Differential effects of ammonium salts in sugar-supplied roots. Biochem. Physiol. Pflanzen 174 : 646–652.
11. Taylor, A. A. and Stewart, G. R. 1980. The effect of ammonia and light/dark transitions on the level of glutamine synthetase activity in *Osmunda regalis*. Plant Sci. Letters 20 : 125–131.
12. Wakiuchi, N., Matsumoto, H. and Takahashi, E. 1971. Changes of some enzyme activities of cucumber during ammonium toxicity. Physiol. Plant. 24 : 248–253.
13. Yamaya, T. and Ohira, K. 1976. Nitrate reductase inactivating factor from rice cells in suspension culture. Plant & Cell Physiol. 17 : 633–641.