Aspects of Nitrogen Metabolism in the Rice Seedling[1,2]

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ABSTRACT

The effects of nitrogen source NO_3^- or NH_4^+ on nitrogen metabolism during the first 2 weeks of germination of the rice seedling (*Oryza sativa* L., var. IR22) grown in nutrient solution containing 40 $\mu g/ml$ N were studied. Total, soluble protein, and free amino N levels were higher in the NH_4^+-grown seedling, particularly during the 1st week of germination. Asparagine accounted for most of the difference in free amino acid level, in both the root and the shoot. Nitrate and nitrite reductase activities were present mainly in the shoot and were higher in the NO_3^--grown seedling, whereas the activity of glutamate dehydrogenase and glutamine synthetase in the root tended to be lower than that of the NH_4^+-grown seedling during the 1st week of germination. Glycolate oxidase and catalase activities were present mainly in the shoot. Maximum activity of the above five enzymes occurred 7 to 10 days after germination. Differences in the zymograms of nitrate reductase, glutamate dehydrogenase, and catalase were mainly between shoot and root and not from N source. Nitrite reductase bands were observed only in plants grown in NO_3^-.

Ten-day-old seedlings of three rices differing in level of grain protein did not differ in the level of N fractions and of enzyme activities, which were consistent with their differences in grain protein content.

Rice (*Oryza sativa* L.) is capable of growing in both flooded and upland culture and can grow well in nutrient culture containing either NH_4^+ or NO_3^- N, even without aeration (29). Although several papers have been published on the subject of growth and N metabolism of rice plants grown in different sources of N, they only consider particular aspects of interest to the investigators. In plants grown in NH_4^+, absorbed inorganic N is converted in the roots by reductive amination of α-keto acids (21). By contrast, in NO_3^--grown plants, NO_3^- is absorbed by the roots and is largely translocated into the leaves, where it undergoes reduction to NH_4^+, and, subsequently, reductive amination of α-keto acids (22). Nitrate reductase, which limits the rate of conversion of NO_3^- to NO_2^- in plants, is present in the leaf and requires light for activity (1, 10). In addition, Mitsui *et al.* (18, 19) reported that rice roots contain an active glycolate oxidase enzyme which is absent in other cereals, but Chiba *et al.* (4) reported that it is absent also in rice roots. In view of these considerations, particularly of the difference in the tissue in which reductive amination mainly occurs, levels were assayed in rice seedlings grown in nutrient medium with NH_4^+ or NO_3^- as N.

source of the following: various nitrogenous compounds and sugars; enzymes involved in nitrogen metabolism; and glycolate oxidase.

A positive relationship has been observed in some upland cereal crops between leaf nitrate reductase activity and grain protein production (1, 5). No such relationship was noted among four rices that differed in grain protein content for 1-month-old seedlings transplanted and grown under flooded conditions to maturity (24). Seedlings of three rices differing in grain protein content were tested for possible differences in levels of nitrogenous compounds and of various enzymes to check if younger plants show an index of grain protein content.

MATERIALS AND METHODS

Seeds of IR22, IR8, and IR480-5-9 rice (*Oryza sativa* L.) were obtained from the plant breeding department of the Institute. Seeds were sterilized by soaking in 0.64% HCHO for 15 min, rinsed with distilled H_2O, and germinated on a framed aluminum screen above a modified Hoagland nutrient solution (38) in a glasshouse under natural sunlight. The basal medium contained, per liter, 50 mg $NaH_2PO_4 \cdot 2H_2O$, 90 mg K_2SO_4, 111 mg $CaCl_2$, 405 mg $MgSO_4 \cdot 7H_2O$, and traces of Mn, Mo, B, Zn, Cu, and Fe. Nitrogen source was 40 $\mu g/ml$ N from KNO_3, $(NH_4)_2SO_4$, or NH_4NO_3. The culture solution was adjusted twice daily to pH 4.5 to 5 and was changed twice per week. Supplementary lighting was applied on cloudy days to provide about 8 klux light intensity at the level of the leaf blades.

Seedlings were cut into shoots, roots, and residual grain and were thoroughly washed with H_2O. A portion of the samples was freeze-dried and weighed. Plant material (1 g) was homogenized in 1 g acid-washed sea sand in a mortar and pestle with 5 ml 10 mM K-phosphate buffer (pH 7.5) containing 5 mM cysteine. The homogenate was centrifuged at 15,000g for 20 min and the supernatant liquid used as the crude enzyme preparation. All of the operations were done at 0 to 4 C.

Enzyme Assays. All enzyme assays were done on the crude extract except for *in vivo* nitrate reductase assay, which was performed on fresh segments of shoot and root according to Perez *et al.* (24). Activities were expressed in $\mu mol/min \cdot g$ fresh tissue. Nitrate reductase activity was assayed by the method of Hageman and Flesher (10). The reaction mixture (2 ml) contained 105 μmol K-phosphate buffer (pH 7.5), 20 μmol KNO_3, 0.68 μmol NADH, and 0.3 ml enzyme. After 20 min incubation at 30 C, the reaction was stopped by adding 0.1 ml 1 M zinc acetate and 1.9 ml 70% (v/v) ethanol. Nitrate was determined with sulfanilamide-N-1-naphthylethylenediamine reagent at 540 nm.

Nitrite reductase was determined by a modification of the method of Joy and Hageman (12). The assay was done in tubes (1 × 7.5 cm) at 30 C with a thin layer of mineral oil over the reaction mixture to prevent rapid oxidation of reduced methyl viologen. The reaction mixture (2 ml) contained 75 μmol K-phosphate buffer (pH 7.5), 1.5 μmol $NaNO_2$, 0.6 μmol methyl viologen, and 0.2 ml enzyme. The reaction was started by pipetting 7.5 μmol $Na_2S_2O_4$ below the oil layer and gently

stirring the contents with a thin glass stirrer. The reaction was terminated after 20 min by vigorously shaking the contents until the methyl viologen was completely oxidized. Tubes containing boiled enzyme extract served as controls. Residual nitrite was determined in 0.1-ml aliquots (12).

Glutamate dehydrogenase was assayed by the method of Bulen (2) as used by Perez et al. (24). Activity was expressed in μmol NADH oxidized and corrected for the NADH loss in the blank without α-ketoglutarate.

Glutamine synthetase activity was measured by a modification of the procedure of Elliott (7). The incubation mixture contained 0.5 ml 0.2 M Tris-HCl buffer (pH 7.5), 0.2 ml 50 mM ATP (pH 7), 0.5 ml 0.5 M sodium glutamate, 0.1 ml 1 M MgSO₄, 0.3 ml freshly prepared 0.1 M NH₂OH, 0.1 ml 0.1 M cysteine, 0.5 ml enzyme solution, and water to make up to 3 ml. Reaction was started by adding glutamate. After 15 min at 30 C, the γ-glutamyl hydroxamate formed was reacted with ferric chloride reagent and the color was read at 540 nm.

Glycolate oxidase was assayed by a modification of the procedure of Soda et al. (28). The incubation mixture contained 0.9 ml 1 M glycine-HCl (pH 8), 0.2 ml 0.1 M sodium glycolate, 0.1 ml 1 mM flavin mononucleotide, 0.6 ml freshly prepared 25 mM o-aminobenzaldehyde (Sigma), and 0.2 ml enzyme extract. The reaction was started by adding glycolate, which was omitted in the blank. After incubating for 15 min at 37 C, the reaction was stopped by adding 0.5 ml 10% trichloroacetic acid. The samples were centrifuged and the absorbance of the reaction product between glyoxylate and o-aminobenzaldehyde was read at 440 nm.

Catalase activity was determined by the method of Chance and Maehly (3) as employed by Palmiano and Juliano (23). Incubation time was 1.5 min.

Zymograms. Crude extracts were precipitated with 80% saturated (NH₄)₂SO₄ at 4 C and the protein was subjected to disc electrophoresis in 7% polyacrylamide according to the method of Davis (6). A sample of 200 μg protein was used for soluble protein electrophoresis and for shoot catalase, 250 μg for nitrate and nitrite reductase, 400 μg for root catalase, and 50 μg for glutamate dehydrogenase. Cysteine (2 mM) was added to the Tris-glycine buffer for the zymogram of nitrate and nitrite reductases. Nitrate and nitrite reductase bands were detected according to UpCroft and Done (32). Glutamate dehydrogenase bands were detected by the method of Shaw and Prasad (26) and catalase isozymes, by the method of Woodbury et al. (34). Protein bands were also stained with 1% Amido black B in 7.5% acetic acid.

Chemical Analysis. Total protein N was determined by micro-Kjeldahl method (13). Soluble protein was determined in the enzyme extract by the method of Lowry et al. (16). Free amino N and total sugars were determined on hot 80% (v/v) ethanol extract of the tissues. Free amino N was assayed by the ninhydrin reagent of Moore (20) with L-leucine as standard. Single column chromatographic analysis of free amino acids was done on a Beckman Spinco amino acid analyzer model 120B, using Beckman M72 resin according to Kedenburg (14). Nitrate was extracted from 200 to 300 mg fresh tissue by boiling for 5 min in 5 ml distilled H₂O. Nitrate content of the extract was determined by the method of Wooley et al. (35).

Total sugars were determined by the anthrone method (11). Reducing sugars were determined on the crude enzyme extract. The extract was treated with 5 volumes of ethanol at 4 C for 1 hr and centrifuged at 10,000g for 15 min. Reducing sugars were determined on the supernatant fluid by Nelson's copper reagent (11). Mono- and dicarboxylic α-keto acids were extracted from 300 to 500 mg freeze-dried shoot and root by grinding in 10% trichloroacetic acid at 0 to 4 C. The extract was centrifuged at 15,000g for 10 min and α-keto acids were determined in the supernatant solution by the procedure of Friedemann (9).

RESULTS AND DISCUSSION

Growth, Chemical Composition, and Nitrogen Metabolism. IR22 rice seedlings grown in NH₄⁺ tended to have a faster rate of shoot growth but a slower rate of root growth during the first week of germination than seedlings grown in NO₃⁻ (Table I). Chlorosis related to Fe deficiency was sometimes noted during the second week of growth of the seedling in NO₃⁻. Total N concentration of the shoot was consistently higher in the plant grown in NH₄⁺ than in that grown in NO₃⁻ and was also higher in the root during the first week of germination. A similar trend was noted for soluble protein N in both the shoot and root, and, in general, in the level of free amino acids. The results indicate that during the first 2 weeks of germination of IR22 rice, N absorption and assimilation were greater in the seedling grown in NH₄⁺ than in the seedling grown in NO₃⁻, particularly during the first week. The concentration of total N, soluble protein N, and free amino N decreased progressively with increasing age of the seedling regardless of N source. Depletion of seed N tended also to be faster in the NH₄⁺-grown seedling.

Nitrate content was higher in shoot and root of the seedling grown in NO₃⁻ but in the NH₄⁺-grown seedling, it was barely detected in the root and a small amount was present in the shoot (Table II). Nitrate reductase activity was higher in the shoot than in the root, and was higher in the NO₃⁻-grown seedling. These results agree with the substrate-inducible nature of nitrate reductase (1, 10, 27, 30). Activity of nitrate reductase by the in vivo assay using shoot segments was only a fraction of the activity by in vitro assay. In the in vivo assay, nitrate reductase activity in the root was higher than that of the shoot only in the 5-day-old seedling. Maximum activity of shoot in vitro nitrate reductase occurred 7 to 10 days after germination, whereas peak in vivo activity occurred 7 days after germination. During extraction, the addition of chemicals that increase nitrate reductase activity in other plants—PVP, at 10% of the sample, which can bind phenols, 0.25 mM phenylmethyl sulfonyl fluoride (33), or 1 or 3% BSA (25)—did not enhance the nitrate reductase activity of the root.

Nitrite reductase activity was higher in the seedling grown in NO₃⁻ than in that grown in NH₄⁺. It was higher in the shoot than in the root (Table II). Peak activity occurred 10 days after germination in the shoot and 7 to 10 days after germination in the root of the NO₃⁻-grown seedling, thus following closely the trend for nitrate reductase. Nitrite reductase activity was lower in the root than in the shoot of the NO₃⁻-grown seedling, except in the 5-day-old sample. The presence of higher levels of NO₃⁻, nitrate reductase, and nitrite reductase in the shoot than in the

Table I. *Fresh Weight, Total N, Soluble Protein N, Free Amino N and Residual Seed N of IR22 Rice Seedling Grown in NH₄⁺ and NO₃⁻ N*

N Source	Days Germinated	Fresh Wt Shoot	Fresh Wt Root	Total N Shoot	Total N Root	Soluble Protein N Shoot	Soluble Protein N Root	Free Amino N Shoot	Free Amino N Root	Residual Seed N
		mg/plant				mg × 10²/g fresh wt				µg
NH₄⁺	5	9.8	10.4	870	318	413	93	38	12	117
	7	30.9	15.9	827	264	422	80	24	10	64
	10	78.4	26.9	816	194	368	78	18	5	20
	14	113.5	39.4	755	146	360	69	13	5	11
NO₃⁻	5	6.8	9.7	752	282	290	82	37	6	125
	7	25.0	21.7	762	234	315	61	16	8	69
	10	72.0	32.7	686	186	328	53	14	4	27
	14	84.7	40.3	653	158	290	54	9	4	13
LSD (5%)	...	...		27	14	46	7	2	8	...

Table II. *Nitrate Content and Activities of Nitrate Reductase and Nitrite Reductase in IR22 Rice Seedling Grown in NH₄⁺ and NO₃⁻ N*
The data are given on a per g fresh weight.

N Source	Days Germinated	Nitrate Content		Nitrate Reductase				Nitrite Reductase	
		Shoot	Root	In vitro		In vivo		Shoot	Root
				Shoot	Root	Shoot	Root		
		μmol				*nmol NO₂⁻/min*			
NH₄⁺	5	2.1	trace	52	trace	2.0	2.9	41	34
	7	0.7	trace	62	trace	4.0	2.0	21	trace
	10	0.3	trace	34	trace	trace	trace	24	trace
	14	trace	trace	24	trace	trace	trace	trace	trace
NO₃⁻	5	48	81	104	19	4.8	8.6	109	202
	7	71	52	161	trace	7.8	4.9	377	325
	10	80	41	244	trace	4.6	4.9	764	311
	14	78	40	202	trace	1.4	2.0	639	158
LSD (5%)		6.2	11	25	8	0.8	1.6	48	44

Table III. *Activities of Glutamate Dehydrogenase, Glutamine Synthetase, Glycolate Oxidase and Catalase in IR22 Rice Seedling*
Enzyme activities are expressed per min per g fresh wt.

N Source	Days Germinated	Glutamate Dehydrogenase		Glutamine Synthetase		Glycolate Oxidase		Catalase	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
		μmoles NADH		μmoles γ-glutamyl hydroxamate		μmoles glyoxylate		mmoles O₂	
NH₄⁺	5	0.88	1.81	0.24	0.41	2.10	0.05	7.67	1.53
	7	0.57	2.70	0.30	0.49	2.05	0.05	7.83	1.07
	10	0.58	3.06	0.78	0.52	2.53	trace	8.13	1.05
	14	0.52	2.35	0.41	0.31	2.00	trace	7.77	1.13
NO₃⁻	5	0.66	0.76	0.29	0.24	1.60	trace	2.99	1.12
	7	0.61	2.09	0.33	0.34	1.76	0.02	3.83	1.59
	10	0.43	1.61	0.76	0.54	2.05	trace	3.39	0.99
	14	0.19	1.31	0.47	0.12	1.92	0.01	3.90	0.69
LSD(5%)		0.18	0.47	0.09	0.04	0.18	0.032	0.34	NS

root reflects the relatively higher rate of oxidation of NH_4^+ in the leaves.

The glutamate dehydrogenase activity was higher in the root than in the shoot. In the root, it was higher in the seedling grown in NH_4^+ than in that grown in NO_3^- (Table III). Peak activity in the root occurred 10 days after germination in the NH_4^+-grown seedling and 7 days after germination in the NO_3^--grown seedling. Glutamate dehydrogenase activity was higher 5 days than 7 days after germination in the shoot of the NH_4^+-grown seedling, but it decreased progressively in the seedling grown in NO_3^-.

The reported presence of glutamate synthetase as an alternate pathway of N assimilation to glutamate dehydrogenase (15) makes glutamine synthetase an important enzyme in N metabolism, both in the shoot and root since the NH_4^+ that combines with α-ketoglutarate is derived from the amide group of glutamine. Glutamine synthetase activity was highest in the shoot of the 10-day-old seedling grown in NH_4^+ and NO_3^-. In the root, glutamine synthetase activity was higher in the seedling grown in NH_4^+ during the first week of germination. Root activity was maximum at 10 days after germination regardless of N source. The higher activity of this enzyme reflects the greater N assimilation in the seedlings grown in NH_4^+. Yoneyama and Kumazawa (37), using $^{15}NO_3^-$, showed that some ^{15}N incorporation into amino acids (principally glutamine and glutamate) also occurs in the root of rice seedlings in NO_3^-. Our data, thus, agree with nitrogen assimilation occurring mainly in the root in the rice seedling grown in NH_4^+ and in the shoot in the seedling grown in NO_3^-.

Glycolate oxidase was found to be present mainly in the shoot (Table III). The activity in the root was low and our results do not support those of Mitsui et al. (18, 19) of an "active" glycolate oxidase in rice roots. Tolbert (unpublished data) showed that this root α-hydroxy acid oxidase was a lactate oxidase. Peak activity occurred in the 10-day-old seedling regardless of N source. Glycolate oxidase activity was higher in 5- to 10-day-old seedlings grown in NH_4^+ than in those grown in NO_3^-. Catalase, the marker enzyme of peroxisomes (31), showed the same trend as glycolate oxidase, which is also a peroxisomal enzyme.

Chemical analysis of α-keto acids in the 10-day-old rice seedlings showed a lower level of α-keto dicarboxylate in the root of the NH_4^+-grown seedling than in the shoot of NO_3^--grown seedling (Table IV). No significant difference was noted in the root samples. Reducing sugar level tended to be higher in the root of the seedling grown in NH_4^+ than in the root of the NO_3^--grown seedling.

Amino acid analysis indicated that the major difference in composition was in the extremely high level of asparagine in the NH_4^+-grown seedling for both shoot and root (Table IV). Glutamine level was also higher in the NH_4^+-grown seedling together with ammonia, but the differences were much less than that for asparagine. By contrast, aspartate and glutamate were at similar levels in the shoot and the root, regardless of N source. Yoneyama and Kumazawa (36, 37) also found a higher asparagine content in the rice seedling grown in NH_4^+ than in the seedling grown in NO_3^-, but its turnover rate was very slow, indicating

Table IV. *Levels of α-Keto Acids, Reducing Sugars and Selected Amino Acids in 10-day-old IR22 Seedlings Grown in NH₄⁺ and NO₃⁻ N*

Constituent Per Gram Fresh Wt	NH₄⁺		NO₃⁻		LSD (5%)	
	Shoot	Root	Shoot	Root	Shoot	Root
α-Keto acids						
Monocarboxylate (nmoles pyruvate)	81	90	66	119	NS	NS
Dicarboxylate (nmoles α-ketoglutarate)	203	43	26⁰	40	20	NS
Total (nmoles)	284	133	335	159		
Reducing sugars (μmoles glucose)	11.5	3.77	11.7	3.16	NS	0.50
Ammonia (μmoles)	1.35	1.03	1.09	0.70	NS	NS
Aspartate (μmoles)	3.21	0.28	3.12	0.39	NS	0.08
Asparagine (μmoles)	8.57	2.48	0.28	0.10	0.30	0.51
Glutamate (μmoles)	2.74	0.38	2.64	0.54	NS	NS
Glutamine (μmoles)	2.82	0.33	0.56	0.61	NS	NS
Glycine (μmoles)	0.16	0.12	0.27	0.06	NS	NS
Serine (μmoles)	1.42	0.49	1.15	0.33	NS	0.11

that it is mainly a storage form of N. In their studies, turnover rate of ^{15}N in the root of rice seedling grown in both NH_4^+ and NO_3^- was fastest for glutamine, followed by glutamate, and then aspartate. The root of the NH_4^+-grown seedling had higher serine content, reflecting a lower rate of use of serine since root glycolate oxidase level was very low in the 10-day-old seedling (Table III).

Electrophoretic Characterization of Proteins. Disc electrophoresis indicated the close similarity in the protein bands of the shoot, regardless of N source (Fig. 1). The broad, intensely stained, slow migrating band that was absent in the root must be fraction I protein. Soluble protein of the root showed essentially the same electrophoretic pattern for seedlings grown in NH_4^+ and NO_3^- N, except for minor differences in mobility or in the presence or absence of minor bands. More minor bands were observed in the root of the NO_3^--grown seedling.

Nitrate reductase was shown to be detectable only in the shoot and only one isozyme band was shown that had an electrophoretic mobility similar to that of fraction I protein (Fig. 1). UpCroft and Done (32) also observed one nitrate reductase band in the shoot and root of wheat by starch gel electrophoresis. Nitrite reductase was only detected in the root and shoot of the NO_3^--grown seedling. Only one fast migrating nitrite reductase isozyme was detected in the shoot of the NO_3^--grown seedling, that was also present in the root together with a second slower migrating band. Two isozyme bands have also been reported for extracts of corn plants (32).

Differences in the zymogram patterns of the other isozymes were due to tissue specificity rather than to N source. Eight glutamate dehydrogenase bands were detected in the shoot regardless of N source. In the root, only seven isozymes were present since the broad, slow mobility band in the shoot was absent, also regardless of N source. Overloading the gel results in the fusion of all of the isozyme bands into one broad band. An identical effect of overloading has been reported by Yue (39). Three catalase isozymes were present in the shoot of which only the fast migrating isozyme was detected in the root, regardless of N source.

Seedlings Grown in Ammonium Nitrate. In general, the seedling grown in 40 $\mu g/ml$ NH_4NO_3 N showed properties intermediate between those grown in NH_4^+ and NO_3^- and the data are not

presented (17). Some properties, however, such as glutamine synthetase activity of shoot, approached those of the NH_4^+-grown seedling. Other properties approached those of the NO_3^--grown seedling, including nitrate and nitrite reductase activity of the shoot, and glutamate dehydrogenase activity of root and shoot. No inhibition of nitrate reductase by NH_4^+ was observed at a concentration of 20 $\mu g/ml$ N each coming from NH_4^+ and
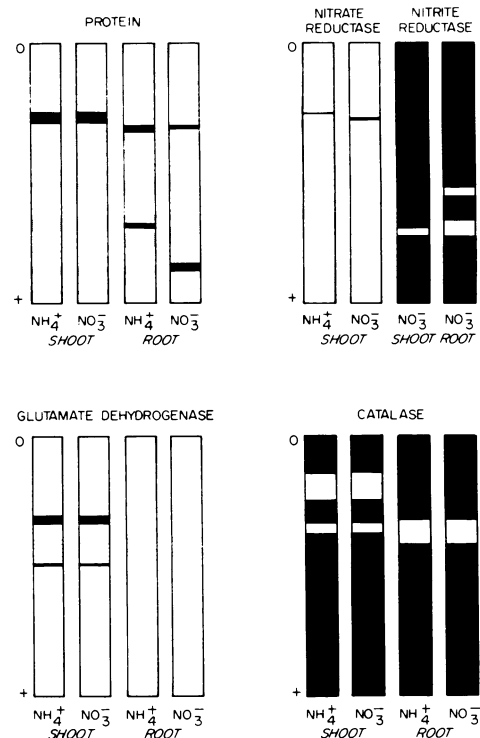

FIG. 1. Disc electrophoretic pattern of soluble protein and zymogram of nitrate reductase, nitrite reductase, glutamate dehydrogenase, and catalase in 10-day-old IR22 rice seedling. Enzyme bands were not detected for nitrate reductase in root and for nitrite reductase in the root and shoot of NH_4^+-grown seedling.

Table V. *Comparison of Properties of 10-day-old Seedlings of Three Rices Grown in NH_4^+ and NO_3^- N*

Enzyme activities are expressed per min per g fresh wt.

Property	Tissue	NH₄⁺			NO₃⁻			LSD (5%)
		IR8	IR22	IR480-5-9	IR8	IR22	IR480-5-9	
Weight (mg/plant)	Shoot	100	74.6	122	114	72.0	117	
	Root	47.7	26.9	51.0	55.4	34.5	52.5	
Total N (mg/g fresh wt)	Shoot	7.41	7.27	6.43	6.23	6.09	5.52	0.19
	Root	2.10	2.04	1.89	2.17	2.04	1.83	0.089
Soluble protein N (mg/g fresh wt)	Shoot	4.60	4.72	4.49	3.93	3.56	3.42	0.35
	Root	0.73	0.78	0.78	0.67	0.66	0.62	N.S.[1]
Free amino N (mg/g fresh wt)	Shoot	0.20	0.16	0.14	0.13	0.11	0.10	0.01
	Root	0.08	0.07	0.05	0.03	0.03	0.03	0.01
In vitro nitrate reductase (nmol NO_2^- formed)	Shoot	31	38	60	226	251	236	27
	Root	trace	trace	trace	trace	trace	trace	N.S.
Nitrate reductase (nmol NO_2^- reduced)	Shoot	142	92	237	876	817	862	127
	Root	176	91	102	512	486	422	92
Glutamate dehydrogenase (μmol NADH used)	Shoot	0.52	0.58	0.39	0.23	0.43	0.33	0.18
	Root	0.43	0.85	0.72	0.60	0.53	0.53	N.S.
Glutamine synthetase (μmol γ-glutamyl hydroxamate formed)	Shoot	0.81	0.78	0.80	0.71	0.76	0.64	N.S.
	Root	0.37	0.52	0.56	0.52	0.54	0.46	0.08
Glycolate oxidase (μmol glyoxylate formed)	Shoot	1.86	2.11	1.86	1.82	1.97	1.95	N.S.
	Root	0.07	0.09	trace	0.05	0.03	trace	0.11
Catalase (nmol O_2 formed)	Shoot	16.12	18.72	15.34	3.25	3.58	3.90	1.32
	Root	1.17	1.08	1.56	0.96	0.81	1.20	N.S.

[1] N.S. = not significant.

NO_3^- in the medium. Fried *et al.* (8) previously reported that NH_4^+ is absorbed faster by rice plants than NO_3^- from 0.1 mM NH_4NO_3.

Comparison of Rices that Differ in Grain Protein. Properties were determined of the 10-day-old seedling of IR8, a low protein rice (7%), and of IR480-5-90, a high protein rice (11%) (13) together with IR22 (9% protein). The results indicated no consistent difference between these rices as affected by N source (Table V). Shoot weight was not always higher for the seedling grown in NH_4^+, nor was root weight higher for the seedling grown in NO_3^-. IR480-5-9 has a heavier leaf than the other rices. Growth in IR22 was slower than in the other two rices in both N sources and in both the shoot and root. No chlorosis was observed in the two other rices, although chlorosis was occasionally noted with the IR22 seedling grown in NO_3^-. The levels of total, soluble protein and free amino N, however, were consistently higher in the shoot of seedlings grown in NH_4^+, as previously noted in IR22 seedlings. No differences were noted in the activity of nitrate and nitrite reductases among the seedlings of the three rices grown in NO_3^-, although nitrate reductase activity was higher in the shoot of IR480-5-9 seedling than in that of IR8 in the NH_4^+ medium.

Essentially, no varietal differences were noted in the levels of glutamate dehydrogenase and glutamine synthetase, except that the activity of shoot glutamate dehydrogenase in IR8 seedling grown in NO_3^- was lower than that of the seedling grown in NH_4^+, and that the activity of glutamate synthetase in the root of IR480-5-9 seedling in NO_3^- was lower (Table V). Evidently, neither chemical analysis nor enzymic assays on seedlings may be used as an index of grain protein level in rice.

Glycolate oxidase activity was mainly in the shoot and was comparable to the level in seedlings grown in NH_4^+ and NO_3^-. This contrasts with the data on IR22 (Table III), in which the NH_4^+-grown seedling had higher activity. However, catalase activity was higher in the shoot than in the root and in the seedlings grown in NH_4^+, as earlier observed for IR22. The levels of these two enzymes in the shoot were not related to the protein content of the grain of the three rices.

The results on the three rices differing in grain protein content confirmed the absence of an early index of grain protein productivity in the young rice seedling grown both on NH_4^+ and NO_3^- N. The absence of such an index in the seedling stage is consistent with earlier findings that the major difference among rices differing in grain protein content is in the efficiency of translocation of foliar N to the developing rice grains rather than in the total N uptake by the plants (24).

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