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Nitrate reduction and assimilation in rice plants (*Oryza sativa* L.)

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Abstract. Nitrogen assimilation was studied in two rice varieties, Taichung Native I, a high nitrogen tolerant strain and Ponni, a moderate nitrogen feeder. Nitrate and ammonia were supplied to the seedlings of the two strains at 3 levels 21, 42 and 84 μg N/ml of culture medium. Assimilation of nitrate and ammonia was followed by estimating levels of nitrate and nitrite reductases, glutamine synthetase and glutamate dehydrogenase activities. Growth, total nitrogen content, nitrate content and amino acid pool pattern were also determined at different age levels. It turned out that the high nitrogen tolerant variety T(N)1, most probably gets its nitrate reduction and ammonia assimilation done in the leaves with the photosynthetically generated reductant and energy, whereas in Ponni, much of these processes seem to occur in the roots at the expense of translocated photosynthates. Ammonia assimilation is primarily *via* GS/GOGAT pathway rather than *via* GDH and T(N)1 was higher in its assimilatory efficiency than Ponni.

Keywords. Nitrate reduction; GS/GOGAT pathway; Taichung Native 1; Ponni; nitrate reductase inactivating factor.

1. Introduction

Crop productivity during the last 50 years is highly correlated with fertilizer-N input. Maximised efficiency of use of soil and fertilizer-N is one of the main concerns of the fertilizer input technology and research imperatives directed towards this goal have often been emphasized.

Nitrogen utilization efficiency at the plant level involves a number of steps, such as uptake of nitrogen from the soil, reduction of nitrate and/or biosynthesis of amino acids in either root or shoot, transport of either reduced or unreduced forms of nitrogen, biosynthesis of enzymes involved in the processes and their regulation, compartmentation and remobilization of storage nitrogen. It is thus obvious that the problem is a complex one and involves the integration of all these aspects.

Nitrate reductase (NR) (EC 1.6.6.1) is the first enzyme in the assimilation path of nitrate—the predominant form of nitrogen available for crop plants in the field. Significant positive correlation exists between this enzyme and the nitrogen status of some higher plant systems, and that growth, yield or protein content are sometimes correlated with this enzyme level in seeds or leaves (Hageman 1979; Srivastava 1980).

Recently, Arima and Kumazawa (1975) have shown by the [^{15}N]-tracer method that glutamine is a primary product of ammonium assimilation and is synthesized from glutamic acid and newly absorbed ammonium by the catalytic activity of the enzyme glutamine synthetase (GS) (EC 6.3.1.2). The synthesis of [^{14}C]-glutamine from [^{14}C]-glutamate was differentially affected by the source of the nitrogen (Iyer *et al* 1981) and GS activity is reversibly repressed with increase in the external ammonium concentration (Rhodes *et al* 1975; Arima *et al* 1976). On the other hand, the universal existence of

glutamate dehydrogenase (GDH) (EC 1.4.1.4) in plants is well known, but the physiological significance of this enzyme is still not clear, especially as regards its high K_m to ammonia and inhibition by ATP.

A comparison of the nitrogen assimilation efficiencies of two strains of paddy namely Taichung Native I (T(N)1), a high nitrogen tolerant variety and Ponni (Mashuri) which is only a moderate feeder, has been studied by growing them on three levels of nitrogen equivalents of nitrate and ammonium sources, 42 μg N/ml being the optimum level and two other levels, one suboptimal and the other twice the normal level, representing high nitrogen level. Ammonium grown plants were included to see whether its assimilation proceeds *via* glutamine synthetase/glutamate synthase (GS/GOGAT) pathway or *via* glutamate dehydrogenase and also to indicate the tissue where it occurs primarily. Furthermore, ammonium fertilizers are usually favoured for rice culture.

2. Materials and methods

Rice plants were raised in culture solutions as per the procedure of Yoshida *et al* (1976). Seeds of T(N)1 and Ponni were obtained from the Paddy Experimental Station, Aduthurai, Tamil Nadu. The composition of the culture medium was as stated by Marwaha and Juliano (1976) and the source of nitrogen was from KNO_3 or $(NH_4)_2SO_4$, supplied in 3 different concentrations, namely 21, 42 and 84 μg N/ml. Rice plants were grown for the required duration in the green house under conditions of broad day light (8 K lux) and the pH of the culture medium was adjusted daily to 5·0.

Cell-free enzyme extracts were prepared from leaves and roots of 3, 5, 7, 10 and 12 days-old seedlings by grinding them separately at 4°C in a pre-chilled corning glass mortar and pestle using 0·01 M K-phosphate-KOH buffer (pH 7·5), containing 5 mM cysteine with 1:4 (W/V) ratio of the tissue to the extraction medium. The homogenate was squeezed through 8 layers of muslin cloth and centrifuged at 18,000 g for 30 min. The resulting clear supernatant was employed as crude enzyme source.

Nitrate reductase was assayed by the method of Hageman and Flesher (1960) as used by Marwaha and Juliano (1976). The reaction mixture (2 ml) contained in μ mol: K-phosphate-KOH buffer, 100 (pH 7·5); KNO_3, 20; NADH, 0·68, and shoot extract, 0·2 ml or root extract, 0·3 ml.

Nitrite reductase (NiR) (EC 1.6.6.4) was assayed by the method of Joy and Hageman (1966) with a slight modification as employed by Marwaha and Juliano (1976). The assay mixture (2 ml) contained in μ mol: K-phosphate-KOH buffer, 100 (pH 7·5); $NaNO_2$, 1·5; methyl viologen, 0·6; $Na_2S_2O_4$, 7·5 and shoot extract, 0·2 ml or root extract, 0·3 ml.

In suitable aliquots, the quantity of nitrite was determined with sulphanilamide-N-1-naphthylethylene diamine reagent at 540 nm. Enzyme activity was expressed as n mols of NO_2^- formed or consumed/15 min/mg protein.

Glutamine synthetase activity was measured by a modification of the procedure of Elliott (1953) in which the production of γ-glutamyl hydroxamate was measured colorimetrically (Shapiro and Stadtman 1970). The incubation mixture (3 ml) contained 0·2 M Tris-HCl buffer (0·5 ml, pH 7·5); 50 mM·ATP (0·2 ml, pH 7·0); 0·5 M sodium glutamate (0·5 ml; pH 7·5); 0·1 M NH_2OHCl (0·3 ml, pH 7·5); 0·1 M cysteine (0·1 ml, pH 7·5); 0·1 M $MgSO_4.7H_2O$ (0·1 ml) and shoot extract (0·3 ml) or root extract

(0·5 ml). After 15 min of incubation at 30°C, the reaction was stopped by the addition of ferric chloride reagent (Shapiro and Stadtman 1970; Ferguson and Sims 1971) and absorption was read at 540 nm against a reagent blank devoid of ATP. Specific activity was expressed as n mols of γ-glutamyl hydroxamate formed/15 min/mg protein.

Glutamate dehydrogenase (GDH) (EC 1.4.1.4) was assayed by the method of Bulen (1956) as followed by Perez *et al* (1973). The enzyme activity was measured by following the initial rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD at 25°C in a UNICAM sp 700 B recording spectrophotometer.

The reaction mixture (3 ml) contained 0·2 M K-phosphate-KOH buffer (2·0 ml, pH 8.0); $1{\cdot}67 \times 10^{-4}$ M NADH (0·5 ml, pH 8·0); $1{\cdot}33 \times 10^{-2}$ M α-Ketoglutarate (0·2 ml, pH 8·0); 0·067 M $(NH_4)_2SO_4$ (0·2 ml, pH 8·0) and enzyme extract from shoot or root (0·1 ml). Specific activity was expressed as n mols of NADH oxidised/15 min/mg protein.

Hundred milligrams of dried plant material or 1 g of fresh tissue (shoots and roots of 5 day-old rice seedlings) was boiled for 5 min in 5 ml of distilled water and then ground in a mortar. The aqueous extract was made up to 20 ml with distilled water and nitrate was estimated as outlined by Woolley *et al* (1960).

Total nitrogen content of shoots and roots was estimated by the modified micro-kjeldahl method (nesslerisation) (Umbreit *et al* 1972).

Amino acids from shoots and roots of 5 day-old rice seedlings were resolved by 2-dimensional thin layer chromatography on silica gel-G as described by Brenner and Nieder-wieser (1967). The extraction of amino acids was done in 80% (v/v) ethanol and after drying, the residue was dissolved in 1 ml of 10% (v/v) isopropanol. The supernatant was used for the amino acid analysis.

Protein was determined following the procedure of Lowry *et al* (1951). Bovine serum albumin (BSA) was used as the standard.

Assays were always run in triplicate and average of two concurrent values expressed. Experiments were repeated at least once and values were found to be reasonably close. Controls and blanks were run with the experiments as usual.

3. Results

The roots and shoots of T(N)1 registered an increase in nitrate content with increase in nitrate concentration of the culture medium (table 1). But, in the shoots of Ponni it showed an opposite trend. Maximum nitrate content was observed when it was raised in 42 μg/ml nitrate-N. Further increase brought about decline in the nitrate content.

Table 1. Nitrate content of shoot and root of T(N)1 and Ponni grown in nitrate for a duration of 5 days.

N-concentration in μg/ml	Nitrate in μg/100 mg dry weight of material*			
	T(N)1		Ponni	
	Shoot	Root	Shoot	Root
21	3·73	183·3	172·0	212·2
42	276·8	402·7	167·7	487·2
84	526·2	483·2	128·2	259·3

* Average of six determinations.

Nitrate content was higher in the shoots of T(N)1 than those of Ponni, except at 21 μg/ml nitrate-N. Conversely, roots of Ponni, except at 84 μg/ml nitrate-N, registered higher amount of nitrate than T(N)1. The shoots of T(N)1 cultured in ammonia had higher N content than those grown in nitrate. Similarly, shoots of Ponni reared in ammonia showed higher N content except the 3 and 10 day old plants grown in 42 μg/ml. Barring those of 5 day old plants grown in 21 μg/ml nitrate-N and 7 day in 42 μg/ml nitrate and ammonia-N, shoots of T(N)1 recorded higher N content than those of Ponni. At the highest level of nitrate supplied, T(N)1 roots accumulated higher quantity of N than those of Ponni, while at the lower levels, omitting 3 day old plants grown in 21 μg/ml ammonia-N and 5 and 10 day in 42 μg/ml nitrate and ammonia-N, Ponni had an edge over that of T(N)1 (tables 2 and 3).

Table 2. Total nitrogen content and specific activity of nitrate reductase, nitrite reductase, glutamine synthetase and glutamate dehydrogenase in shoots (S) and roots (R) of T (N)1 grown in NO_3^- and NH_4^+ at 21, 42 and 84 μg N/ml of culture medium.

N Source	Concentration (μg N/ml)	Days germinated	Total N (mg/g dry wt.)		Enzyme activity in n moles of product formed or disappeared or oxidised/15 min/mg protein							
					NR		NiR		GS		GDH	
			S	R	S	R	S	R	S	R	S	R
NO_3^-	21	3	39	26	2	0	195	510	300	280	7·50	94·50
		5	42	17	5	0	225	255	600	610		
		7	49	20	8	0	300	225	520	450	7·35	127·50
		10	41	15	3	0	630	495	770	300		
		12	42	14	2	0	495	585	580	350	25·50	175·50
	42	3	48	24	69	0	195	315	540	240		
		5	70	30	91	0	150	225	580	290		
		7	32	16	44	0	105	285	540	180		
		10	63	21	32	0	195	585	700	180		
		12	46	16	2	0	255	270	630	210		
	84	3	51	30	158	63	120	585	820	430	8·40	96·00
		5	76	30	114	8	135	240	510	300		
		7	83	38	133	4	345	900	980	360	11·40	150·00
		10	58	27	4	0	210	210	760	290		
		12	74	32	9	0	270	330	670	1100	13·50	183·00
NH_4^+	21	3	49	32	0	0	75	30	260	570	6·60	75·00
		5	49	19	0	0	90	120	480	410		
		7	56	18	0	0	120	15	480	530	10·50	106·50
		10	55	17	0	0	195	750	750	540		
		12	44	12	0	0	150	555	600	440	24·00	156·00
	42	3	60	28	0	0	45	75	420	210		
		5	74	28	0	0	30	285	530	360		
		7	44	18	0	0	60	240	530	300		
		10	70	25	0	0	150	735	630	110		
		12	48	20	0	0	30	105	570	150		
	84	3	58	28	0	0	90	300	590	380	9·60	9·00
		5	79	28	0	0	75	330	510	520		
		7	85	61	0	0	90	675	740	500	14·25	69·00
		10	71	29	0	0	30	345	760	450		
		12	78	37	0	0	15	360	640	1200	15·00	106·50

Table 3. Total nitrogen content and specific activity of nitrate reductase, nitrite reductase, glutamine synthetase and glutamate dehydrogenase in shoots (S) and roots (R) of Ponni grown in NO_3^- and NH_4^+ at 21, 42 and 84 μg N/ml of culture medium.

N Source	Concentration (μg N/ml)	Days germinated	Total N (mg/g dry wt.)		Enzyme activity in n moles of product formed or disappeared or oxidised/15 min/mg protein							
					NR		NiR		GS		GDH	
			S	R	S	R	S	R	S	R	S	R
NO_3^-	21	3	32	26	3	0	375	675	200	190	14·10	72·00
		5	46	28	13	0	240	330	380	290		
		7	32	22	9	0	255	420	360	290	16·35	174·00
		10	35	19	2	0	270	105	560	500		
		12	38	18	1	0	285	360	530	210	31·20	175·50
	42	3	39	35	10	0	250	315	150	140		
		5	49	16	21	0	255	540	480	140		
		7	42	25	22	0	240	360	690	250		
		10	46	16	3	0	300	345	620	280		
		12	41	18	2	0	315	675	860	400		
	84	3	49	30	19	5	15	165	270	230	12·00	100·50
		5	51	28	73	0	195	270	520	150		
		7	53	29	46	0	225	405	450	130	16·65	63·00
		10	27	19	15	0	150	450	660	80		
		12	39	21	7	0	480	735	530	60	16·80	178·50
NH_4^+	21	3	39	26	0	0	165	195	190	360	11·55	90·00
		5	46	32	0	0	30	45	340	400		
		7	41	26	0	0	15	45	440	660	15·30	160·50
		10	44	20	0	0	15	60	525	580		
		12	39	19	0	0	45	285	580	600	30·15	160·50
	42	3	35	31	0	0	15	45	170	480		
		5	49	20	0	0	45	75	380	550		
		7	46	27	0	0	15	15	560	790		
		10	44	18	0	0	45	585	620	330		
		12	44	20	0	0	45	450	1000	380		
	84	3	55	36	0	0	0	60	270	380	22·65	100·50
		5	66	30	0	0	15	210	420	500		
		7	56	30	0	0	120	390	540	380	28·35	49·50
		10	39	23	0	0	60	315	600	130		
		12	51	23	0	0	225	390	640	210	25·95	158·50

In both T(N)1 and Ponni, an increase in shoot NR activity was observed with increase in nitrate concentration of the culture medium. On the contrary, ammonia grown rice seedlings showed no NR activity. Also, with an increase in the concentration of nitrate-N from 21 to 42 μg/ml, the shoot NR activity in T(N)1 registered a 8 fold increase whereas in Ponni, it increased only 1 fold (tables 2 and 3). Maximum shoot NR activity was observed on the 5 day for T(N)1 and on 7 day for Ponni. At 84 μg/ml nitrate-N, shoot NR activity increased 4 fold in Ponni and several fold in T(N)1. For T(N)1, the peak of shoot NR activity was recorded on the 3 day which, declined on the 5 day only to rise again on the 7 day. In the case of Ponni there was a single peak occurring on the 5 day, declining gradually thereafter.

Nitrate reductase activity was not deducted in the roots of Ponni grown either in nitrate or in ammonia. But, some amount of NR activity was recorded on the 3 day in the roots of T(N)1 when reared in 84 μg/ml nitrate-N (table 2). Presence of NR-inactivating factor in the roots of rice plants was already documented (Kadam *et al* 1974; Yamaya and Ohira 1978) and we have also recorded the occurrence of a proteinaceous NR-inactivating factor in the roots of these rice varieties (unpublished data).

During extraction, the addition of chemicals that increases NR activity in other plants—PVP, at 50% of the sample, which can bind phenols (Wallace 1973) or 1 or 3% BSA (Schrader *et al* 1974) did not enhance NR activity of either the shoot or root of these rice varieties.

Nitrite reductase activity was higher in rice seedlings grown in nitrate than in ammonia. Strangely, NiR activity was higher in roots than in shoots (tables 2 and 3). Taichung Native 1 recorded higher shoot NiR activity when grown in 21 μg/ml nitrate-N, while Ponni showed higher shoot NiR activity at 42 μg/ml nitrate-N. However, when cultured in 84 μg/ml nitrate-N, T(N)1 edged over that of Ponni, barring the 5 and 12 days. In general, the development of shoot NiR activity kept pace with the greening process.

At 21 μg/ml nitrate-N and at the early stages of growth, roots of Ponni showed higher NiR activity, while T(N)1 registered higher activity in the later stages (tables 2 and 3). At 42 μg/ml nitrate-N also root NiR activity was higher in Ponni, except on the 10 day. There were fluctuations in the level of root NiR activity of T(N)1 raised in 84 μg/ml nitrate-N, but in the roots of Ponni the NiR activity, though less than T(N)1, increased with increase in age. Regardless of the levels, ammonia reared T(N)1 exhibited higher root NiR activity than Ponni, the peak of activity occurring on the later stages of growth. The level of NiR was several fold higher than NR and did not seem to be limiting. Nitrite reductase activity was not inhibited, rather promoted by ammonia.

Glutamine synthetase activity in the shoots of T(N)1 was higher than that of Ponni except at 42 μg N/ml. At this level, on the 7 and 12 days, Ponni recorded higher GS activity than T(N)1. Nitrate at all levels, favoured higher GS activity than ammonia. However, GS activity in the roots was higher in ammonia grown seedlings. At 21 and 42 μg/ml ammonia-N, GS activity in the roots of Ponni was higher whereas at 84 μg/ml, T(N)1 roots registered higher activity. Though not linear, increase in N concentration brought about increase in GS activity in the shoots of both the rice varieties, while, in the case of roots of Ponni, increase in nitrate concentration of the culture medium brought about a decrease in the GS activity (tables 2 and 3). Glutamine synthetase activity in roots of T(N)1 decreased when the concentration of the culture medium increased from 21 to 42 μg/ml nitrate-N. Further raise to 84 μg/ml, resulted in an increase of activity to the extent of 5 fold. With regard to increase in ammonia-N concentration, GS activity in roots of T(N)1 followed a similar trend of decrease and increase, while in Ponni roots activity increased with increase in concentration from 21 to 42 μg/ml. Further increase brought about precipitous decline in the activity (table 3).

In the shoots, nitrate favoured higher GDH activity than ammonia, when the concentration was lowest. At the highest concentration it was *vice versa*. Ponni shoots reared in 21 μg/ml nitrate-N showed higher GDH activity than T(N)1. At the highest level of ammonia-N, it was again Ponni that surpassed T(N)1 with regard to its GDH activity. Regardless of the source of N, it was shoots of Ponni that exhibited higher GDH activity than that of T(N)1 (tables 2 and 3).

Table 4. Intracellular amino acid pool patterns in shoots of T(N)1 and Ponni.

Amino acids in µg/g fresh weight	N-concentration in µg/ml											
	T(N)1						Ponni					
	21		42		84		21		42		84	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
Alanine	2·2	1·9	15·1	8·9	2·1	3·3	11·1	15·5	1·9	46·6	1·9	4·4
Arginine	4·0	8·1	37·5	6·1	5·1	8·1	4·0	12·2	1·0	–	–	–
Aspartic acid	18·0	54·0	64·8	63·0	45·0	67·5	72·0	32·0	18·0	54·0	28·0	97·0
Asparagine	91·0	404·0	264·0	313·0	127·0	448·0	454·0	505·0	50·5	76·0	50·5	404·0
Glutamic acid	22·2	2·2	36·0	24·4	13·2	6·6	31·1	17·1	7·9	6·6	5·3	23·5
Glutamine	56·1	10·3	5·1	11·25	15·4	13·0	61·8	56·6	3·8	30·9	9·3	20·8
Glycine	14·1	19·2	60·8	4·0	9·6	12·0	38·4	29·2	7·1	14·1	5·7	10·4
Histidine	–	33·0	–	–	–	–	–	–	–	–	–	13·1
α-Aminobutyric acid	1·7	2·1	12·3	–	3·7	1·1	8·6	10·8	–	–	–	–
Leucine	1·0	3·0	4·2	2·6	6·5	3·9	7·9	–	3·9	–	1·0	2·0
Lysine	6·8	17·0	10·9	6·8	5·0	3·3	6·8	37·3	1·6	–	1·3	7·5
Valine	1·7	6·1	5·9	1·7	2·6	0·8	3·4	8·7	3·9	–	–	–
Total	218·8	560·9	516·6	441·75	235·2	567·6	699·1	723·4	99·6	228·2	103·0	582·7

– Not detectable

Glutamate dehydrogenase activity was higher in roots than in shoots of both the rice varieties. Roots of nitrate grown seedlings recorded higher GDH activity than those grown on ammonia. Except at the highest level of nitrate-N, where T(N)1 roots exhibited higher GDH activity (table 2), at all other levels of nitrate-N and ammonia-N, Ponni roots registered higher GDH activity (table 3). Amino acid analysis (tables 4 and 5) indicated that major differences in the composition was in the extremely high level of asparagine in the ammonia grown seedlings of both the strains. Yoneyama and Kumazawa (1974, 1975) also reported high asparagine content in rice seedlings raised in ammonia than in nitrate, but its turn over rate was very slow indicating that it was mainly a storage form of N. Shoots of T(N)1 showed greater amounts of asparagine than the roots, but in Ponni it was *vice-versa*. Of the 3 levels of N employed, maximum quantity of asparagine was registered when Ponni seedlings were raised in 21 μg/ml ammonia-N. On the contrary, in shoots and roots of T(N)1, higher level of asparagine was found when seedlings were grown in 84 and 42 μg/ml ammonia-N, respectively.

The level of glutamine was much less than that of asparagine. Shoots of Ponni cultivated in ammonia contained greater amounts of glutamine than those grown in nitrate, except at the lowest level. But in T(N)1, barring those that were grown in 42 μg/ml, glutamine was higher in shoots of seedlings grown in nitrate than those raised in ammonia. In both the varieties of rice, roots recorded higher levels of glutamine than shoots. Ponni roots raised in ammonia contained higher amounts of glutamine than those grown in nitrate, whereas it was the otherway about in T(N)1. Shoots in general, contained greater amounts of glutamic acid than roots. It was present in higher concentration in nitrate-grown shoots of both the rice varieties, except in shoots of Ponni raised in 84 μg/ml ammonia-N. Roots of Ponni reared in ammonia recorded higher level of glutamic acid than those grown in nitrate except at 84 μg/ml nitrate-N. In T(N)1 shoots, the opposite trend was observed.

4. Discussion

Nitrate reductase is the rate limiting enzyme in the biochemical reduction of nitrate to ammonia (Beevers and Hageman 1969). Since it is an inducible enzyme (Hageman and Flesher 1960; Beevers *et al* 1965), its activity increased in the presence of nitrate and ammonia completely repressed the enzyme synthesis (Pate 1973; Oaks *et al* 1977). The response of the two rice varieties to nitrate supply with respect to the development of shoot NR activity was significantly different. The leaves of T(N)1 showed a 8 fold increase in NR activity when supplied with 42 μg/ml nitrate-N, while there was 1 fold increase only in the case of Ponni leaves (tables 2 and 3). Ponni performed better at lowest concentration of nitrate-N, while T(N)1 exhibited several fold increase at highest concentration. Not only the NR activity in T(N)1 was several fold higher, but the development of very high activity was evident even on the 3 day (table 2).

Roots of Ponni showed almost no NR activity that of T(N)1, when grown in 84 μg/ml nitrate-N registered some activity on the 3 day only (table 2). Interference in the isolation of NR is less in young plant materials and this probably may account for the recovery of activity in root tissue at 3 day but not later. Further, the NR-inactivating factor reported to be present in the roots of rice seedlings (Kadam *et al* 1974; Yamaya and Ohira 1978) may also prevent the appearance of NR activity. It may also suggest that the absorbed nitrate was transported to the leaves where it got reduced and

Table 5. Intracellular amino acid pool patterns in roots of T(N)1 and Ponni.

Amino acids in µg/g fresh weight	N-Concentration in µg/ml											
	T(N)1						Ponni					
	21		42		84		21		42		84	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NO_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
Alanine	3·1	8·9	8·9	7·7	15·0	10·5	6·2	12·4	1·0	10·3	5·3	19·3
Arginine	2.0	–	9·6	–	–	3·0	2·8	–	4·0	19·2	1·5	4·0
Aspartic acid	25·0	9·0	42·0	81·0	21·6	27·0	25·0	37·0	22·5	20·0	25·0	30·0
Asparagine	50·5	127·0	140·0	234·0	50·5	192·0	284·0	568·0	50·5	454·5	61·0	303·0
Glutamic acid	4·6	2·2	5·2	2·5	6·2	9·5	3·1	6·2	3·8	5·2	3·5	3·0
Glutamine	35·7	23·1	34·0	18·0	9·3	2·5	21·5	72·1	6·4	72·3	5·9	11·1
Glycine	18·6	7·1	18·0	22·2	10·2	16·8	6·7	33·1	7·1	38·4	4·7	28·2
Histidine	–	–	–	–	–	–	–	–	–	–	–	–
α-Aminobutyric acid	3·2	6·4	10·0	–	7·7	5·4	3·0	12·1	1·7	19·8	2·6	5·0
Leucine	1·9	2·6	12·0	–	3·1	1·3	1·8	3·6	–	6·0	1·0	1·6
Lysine	7·1	3·3	8·0	11·9	4·0	2·5	4·7	14·2	1·9	15·6	1·2	10·9
Valine	2·4	1·7	8·1	–	1·7	1·7	2·4	2·4	–	8·0	–	–
Total	154·1	191·3	305·8	377·3	129·3	272·2	361·2	761·1	98·9	669·3	111·7	413·1

– Not detectable.

incorporated. Nitrate reduction and assimilation if taking place in the leaves utilizing photosynthetically generated reductant and energy would be advantageous to the plant as it is a highly energy-demanding process (Raven 1976).

Since NiR activity was several fold higher than NR activity, particularly in roots, this enzyme was neither rate-limiting nor was it inhibited by ammonia. On the contrary, ammonia enhanced the enzyme activity as reported by Miflin (1974). Ponni roots in general, exhibited higher NiR activity than that of T(N)1 and such a high level of activity was suggestive of NR activity also, which perhaps was masked by the presence of NR-inactivating factor reported to be present in the roots. Higher level of NiR activity in the shoots of T(N)1 as against that of Ponni was in accordance with the observed higher level of shoot NR activity in T(N)1, and this as per Marwaha and Juliano (1976) reflects the relatively higher rate of oxidation of ammonia in the leaves.

Higher activity of GS in the shoots of both the rice varieties (tables 2 and 3) regardless of the form of nitrogen probably is an indication of GS/GOGAT pathway being a preferred mode of assimilation of ammonia-N. Taichung Native 1 appeared to be more efficient than Ponni. The higher tolerance for nitrogen in the former most probably was due to the fact that most of its inorganic nitrogen assimilation activities were performed in the shoots at the expense of photosynthetically generated reductant and energy.

Glutamate dehydrogenase activity was higher in roots than in shoots of both the rice varieties in both the sources of nitrogen. Ponni registered higher GDH activity than T(N)1. A relatively prominent role of GDH was ascribed when plants were grown in excessive concentration of ammonia (Barash *et al* 1973; Shepard and Thurman 1973). In the roots of Ponni, GDH activity was stimulated when the seedlings were exposed to higher levels of ammonia (table 3), and this brought about a steep decline in the GS activity. The repression of NR in the roots of Ponni and a parallel repression of GS with increased ammonia supply appeared to be associated with a switch over of ammonia assimilation from *via* glutamine to assimilation *via* glutamate. This was an indication of the poor efficiency of Ponni with regard to nitrogen utilization which appeared to be confined to the roots and hence energetically less economical from the point of view of the energy budget of the plant.

Analysis of the intracellular amino acid pool indicated a pattern consistent with the conclusions drawn. Glutamine level in roots of T(N)1, with increasing ammonia supply decreased, while a high level of glutamine was noticeable in the roots of Ponni. In the case of Ponni shoots, increase in nitrate supply caused a precipitous decline in glutamine level, increasing ammonia resulted only in a gradual reduction (table 4). This is in consistent with the pattern of nitrogen assimilation in the roots and transportation of reduced nitrogen in the form of glutamine to the above ground parts in Ponni. Similarly, greater amounts of asparagine was recorded in the shoots of T(N)1, whereas Ponni roots contained higher levels of this amide.

From the distribution of NR, NiR, GS and GDH activities and total nitrogen, nitrate and amino acid contents in the two rice varieties, it could be concluded that nitrogen assimilation in T(N)1 occurs predominently in the leaves while it seems to take place in the roots in Ponni. Nitrogen assimilation when confined to the roots deprives the plant of the option of the direct use of photo-produced co-factors rather than respiration-derived co-factors, which could be uneconomical from the point of view of the energy budget of the plant (Raven 1976). Nitrogen assimilation also involves availability of carbon skeleton for synthesis of nitrogen containing organic assimilates. These are

directly available in leaves but they are to be translocated to the roots in the first place if nitrogen assimilation were to take place in the roots.

From the comparison of these two rice varieties, it appears that compartmentation of nitrogen assimilation reactions in the leaves could confer greater efficiency of nitrogen utilization and thus might account for the higher nitrogen tolerance of T(N)1. This not only imposes a pH stress in the shoots (Raven and Smith 1976; Smith and Raven 1979; Pate 1980), but also competition for the photo-produced energy and reductant for carbon fixation and nitrogen assimilation and these have to be modulated without sacrificing the efficiency of one or the other.

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