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Absorption, Translocation and Assimilation of Ammonium- and Nitrate-Nitrogen in Rice Plants as Affected by the Supply of Potassium, Calcium and Magnesium

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Rice plants (*Oryza sativa* L., var. Asominori) were treated with complete, minus K (-K), Ca-limited (-Ca) and minus Mg (-Mg) nutrient solutions for 10 days. Absorption, translocation and assimilation of $^{15}\text{NH}_4\text{-N}$ and $^{15}\text{NO}_3\text{-N}$ fed for 2 days to the treated plants were investigated, and *in vivo* nitrate reductase activity (NRA) was intermittently determined after the initiation of the treatment. 1) Absorption of $^{15}\text{NH}_4\text{-N}$ was not significantly different among treatment, while lower absorption of $^{15}\text{NO}_3\text{-N}$ was observed in -K and -Ca treatment. 2) Translocation of $^{15}\text{NH}_4\text{-N}$ from root to upper parts was not affected by treatment, but translocation of $^{15}\text{NO}_3\text{-N}$ was inhibited by -K and -Ca treatment. 3) NRA of rice plants was decreased by -K and -Ca treatment, and repressed when $\text{NO}_3\text{-N}$ was replaced with $\text{NH}_4\text{-N}$ alone and in combination with $\text{NO}_3\text{-N}$. 4) Assimilation of $^{15}\text{NH}_4\text{-N}$ into amino acids and protein was increased by -K treatment, but assimilation of $^{15}\text{NO}_3\text{-N}$ was inhibited by -K and -Ca treatment. These results indicate that nitrogen metabolism was disturbed by -K and -Ca treatment when rice plant was fed with $\text{NO}_3\text{-N}$ but not when plant fed with $\text{NH}_4\text{-N}$.

INTRODUCTION

Most plant species are different in their ability to absorb $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ as nitrogen sources. The high ability of rice plant to absorb and utilize $\text{NH}_4\text{-N}$ has been reported by many investigators (Fried *et al.*, 1965; Oji and Izawa, 1970; Dijkshoorn and Ismunadji, 1972). Absorption of ions from a culture medium may be influenced by other ions through effects of competition at the site of absorption. Ions more similar to NH_4^+ can compete with it and may decrease its absorption. Bange *et al.* (1965) found that K^+ inhibited the NH_4^+ absorption by barley root. Recently, Rosen and Carison (1984) found that increasing K^+ concentration had no significant effect on the NH_4^+ uptake by plants. Thus it seems that the influence of K^+ on NH_4^+ absorption may be not yet fully understood. Nitrate absorption is usually associated with increased uptake and transport of cations (Minotti *et al.*, 1968; Ben-Zioni *et al.*, 1970; Ben-Zioni *et al.*, 1971; Cox and Reisenauer, 1973; and Blevins *et al.*, 1974). Matsumoto *et al.* (1980) observed that NO_3^- content of cucumber leaves

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was decreased by Ca deficiency. Burstrom (1954) and Shrader and Hageman (1967), however, claimed that Ca did not appear to be essential for absorption of $\text{NO}_3\text{-N}$. The relationship between Mg content and nitrogen metabolism has been reported by Webster (1961) and Haeder and Mengel (1969). The investigators frequently suggested that K deficiency inhibited protein synthesis in plants because amino acids accumulated (Koch and Mengel, 1974). On the other hand, Hsiao *et al.* (1970) and Besford (1975) did not find any direct effect of K on the protein content of plant. This finding, however, was not always accepted. As nitrate reduction is the first step in nitrate assimilation (Hageman *et al.*, 1961), a lower activity of nitrate reductase leads to declined assimilation of $\text{NO}_3\text{-N}$.

In this study we have compared metabolism of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in K-, Ca- and Mg-deficit rice plants to make clear roles of K, Ca and Mg in nitrogen metabolism.

MATERIALS AND METHODS

Plant culture and feeding of ^{15}N labeled nitrogen sources

Rice (*Oryza sativa* L., var. Asominori) seeds were sterilized by 70 % ethanol and then 2 % (w/v) calcium hypochlorite, washed with tap water, and germinated on moist filter paper in an environment controlled room (natural light, 25°C , 70 % RH). Then seedlings were transferred to moist sand in plastic pots and supplied with half strength of the nutrient solution (Table 1) for a few days. The seedlings were then transplanted to a plastic plate with holes which was held over a plastic pot containing tap water for a few days in the greenhouse, and then tap water was replaced with half strength of the nutrient solution for several days. The complete nutrient solution of full strength was supplied at 27 days after germination. The nutrient solution was renewed once a week in the first three weeks, then twice a week, and

Table 1. Composition of the complete nutrient solution used.

Element	Concentration (mg/l)	Compound
N*	28	NH_4NO_3
P	31	$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
K	78	KCl
Ca	80	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
Mg	18	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
S**	24	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
Si	50	Na_2SiO_3
Fe	3	$\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_8\text{NaFe} \cdot 3\text{H}_2\text{O}$
Mn	0.2	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$
B	0.2	H_3BO_3
Zn	0.05	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
Cu	0.05	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
Mo	0.05	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$

* N sources were changed to 4mM $^{15}\text{NH}_4\text{Cl}$ and $\text{Na}^{15}\text{NO}_3$ at 52-day age of plant for 2 days (during ^{15}N -feeding).

** In Mg-omitted solution Na_2SO_4 was used as S source.

every 2 days during treatment. The aeration system was used during solution culture. The pH of nutrient solution was adjusted at 5.6 twice a day with 1N H_2SO_4 or NaOH using a pH meter. The nitrogen source was NH_4NO_3 before the supply of ^{15}N . In the treatment of minus K (-K) and minus Mg (-Mg), K and Mg were omitted from the complete (control) nutrient solution, respectively, and 4 ppm of Ca was added in Ca-limited (-Ca) treatment. Treatment was continued for 10 days. The experiment was done to determine the absorption, translocation and assimilation of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ under different K-, Ca- and Mg-supply conditions. The duplicated treatment was begun at 42 days after sowing, and then 4mM $^{15}\text{NH}_4\text{Cl}$ (31.2 atom % ^{15}N) and $\text{Na}^{15}\text{NO}_3$ (30.8 atom % ^{15}N) were fed to 52-day-old plants for 2 days. During ^{15}N feeding 3 plants for each treatment were held in one plastic bottle containing 1 liter of nutrient solution. The plants were harvested at 54 days after sowing, separated to three parts as blade, stem+sheath, and root. Then they were freeze-dried for chemical analysis.

Determination of mineral content and total nitrogen

Potassium, Ca and Mg content were determined by combination of a dry combustion method and atomic absorption spectrophotometry. Total nitrogen was determined by the modified Kjeldahl method with salicylic acid and the steam distillation method.

Determination of ^{15}N abundance

The samples were prepared for ^{15}N analysis by the Kjeldahl digestion and distillation method. ^{15}N abundance of NH_3 in concentrated distillates was measured by Dumas method and optical spectrometry with a NIA-I ^{15}N analyzer (Nihonbunko Co. Ltd, Tokyo, Japan) (Fiedler and Proksch, 1975).

Determination of free amino acids and protein-N and its ^{15}N abundance

Free amino acids were extracted by addition of 60 ml of 80 % ethanol to 100 mg of powdered materials and boiling for 60 minutes on a water bath with a reflux condenser. After cooling the extract was filtered through Miracloth in a Guchi crucible. The filtrate was transferred into an evaporation flask to concentrate the sample until dryness under reduced pressure at 40°C. The remaining was dissolved by addition of 10 ml of H_2O and about 10 ml of CH_2Cl_2 with shaking, and kept in a separation funnel for several hours. The upper H_2O layer was centrifuged (1000 rpm for 12 minutes), and an aliquot of the supernatant was used for determination of free amino acids by an automatic amino acid analyzer (JLC 6 AH), according to the method described by Kedenburg (1971). Nitrogen in the residue on Miracloth was regarded as protein-N. Protein-N and its ^{15}N abundance were determined as described above.

Determination of nitrate reductase activity

Rice plants were grown in the nutrient solution including 2 mM NaNO_3 before treatment was initiated as described above. At 42-day age of rice the plants were treated with 5 kinds of nutrient solution such as 2 mM

NaNO_3 (NO_3), 2 mM NH_4Cl (NH_4), 2 mM NH_4NO_3 (NH_4NO_3), NH_4NO_3 minus K ($\text{NH}_4\text{NO}_3\text{-K}$) and NH_4NO_3 limited Ca ($\text{NH}_4\text{NO}_3\text{-Ca}$) for 10 days. Thereafter, in a series of NH_4NO_3 treatment, the nitrogen source was replaced with 4mM NaNO_3 (so abbreviated NO_3 , $\text{NO}_3\text{-K}$, and $\text{NO}_3\text{-Ca}$). The latter treatment was continued for 6 days. The experiments were carried out in 2 replications. Nitrate reductase activity was determined by the *in vivo* assay method (Hageman and Hucklesby, 1971).

Determination of nitrate

Nitrate content in dry matter was determined according to the method of Cataldo *et al.* (1975).

Statistical analysis

The data of experiments were subjected to statistical analysis according to Duncan's multiple range test, and new LSD test (Duncan, 1955; Waller and Duncan, 1969).

RESULTS AND DISCUSSION

Effect of K, Ca and Mg deficit on plant growth and mineral content

Treatment of -K, -Ca and -Mg for 12 days had no statistically significant effect on the growth of rice plants (data not shown). It can be seen from Table 2 that K, Ca and Mg concentrations of plant tissues were decreased by -K, -Ca and -Mg treatment, respectively, but the concentration remained at the levels which were required for the maintenance of normal plant growth (Loneragan and Snowball, 1969; Draycott and Durrant, 1971; and Fageria, 1976).

Effect of K, Ca and Mg deficit on absorption of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in rice plants

In order to decrease the effect of pH on the absorption of NH_4^+ and NO_3^- ,

Table 2. Effect of mineral supply on mineral content of rice plants.

Treatment	K(%)			Ca(%)			Mg(%)		
	Root	Stem+ sheath	Blade	Root	Stem+ sheath	Blade	Root	Stem+ sheath	Blade
Ammonium-N									
Complete	4.85 a	3.25 a	2.93 a	0.10 a	0.13 a	0.36 a	0.12 a	0.13 a	0.15 a
-K	3.80 b	1.68 b	1.60 b	0.08 a	0.10 a	0.32 a	0.13 a	0.17 b	0.26 b
-Ca	4.85 a	2.30 a	2.35 a	0.09 a	0.08 b	0.21 b	0.12 a	0.11 a	0.16 a
-Mg	5.10 a	3.60 a	2.53 a	0.08 a	0.11 a	0.33 a	0.09 b	0.07 c	0.07 c
Nitrate-N									
Complete	5.00 a	3.10 a	2.53 a	0.16 ac	0.14 a	0.36 a	0.13 a	0.14 a	0.14 a
-K	4.25 a	1.55 b	1.30 b	0.14 a	0.12 a	0.32 a	0.14 a	0.18 b	0.28 b
-Ca	5.00 a	2.25 a	2.55 a	0.10 b	0.09 b	0.21 b	0.13 a	0.11 a	0.17 a
-Mg	4.85 a	3.75 c	2.45 a	0.18 c	0.15 a	0.36 a	0.09 a	0.07 c	0.08 c

Within a column, means followed by the same letter are not significantly different according to Waller and Duncan, using new LSD test for $k=100$.

the pH of nutrient solution was adjusted at 5.6 twice a day and recorded before the pH was corrected. The deviations from the desired pH value (5.6) were not so great among treatment (data not shown). The data in Table 3 indicate that absorption of $\text{NH}_4\text{-N}$ was not affected by $-\text{Ca}$ and $-\text{Mg}$ treatment. The absorption of $\text{NH}_4\text{-N}$ in $-\text{K}$ treatment was slightly larger than the other treatment, although the difference was statistically insignificant. This difference is probably due to lack of the effect of a monovalent cation (K^+) competing with NH_4^+ for selective binding sites in the absorption process. There was a paper that a relative high K^+ concentration in nutrient solution resulted in somewhat reduced uptake of NH_4^+ by rice roots (Fried et al., 1965). The absorption of $\text{NO}_3\text{-N}$ was decreased by 38 % and 30 % by $-\text{K}$ and $-\text{Ca}$ treatment, respectively, as compared to control treatment (Table 3).

Table 3. Effect of mineral supply on the absorption of $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ by rice plants.

Treatment	^{15}N (mg/g dry weight)			
	Root	Stem-sheath	Blade	Whole plant
Ammonium-N				
Complete	4.72 a	5.37 a	4.22 a	4.79 a
$-\text{K}$	6.01 4.59 aa	5.67 3.60 aa	2.49 2.5 aa	5.47 3.50 aa
$-\text{Ca}$				
$-\text{Mg}$	4.99 a	4.77 a	3.23 a	4.19 a
Nitrate-N				
Complete				
$-\text{K}$	5.95 4.95 aa	2.55 4.25 ba	3.07 1.52 ab	4.14 2.57 b
$-\text{Ca}$	5.19 a	2.56 b	1.92 b c	2.89 b c
$-\text{Mg}$	4.94 a	3.72 a	2.40 a c	3.43 a c

Within a column, means followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

These results are in good agreement with those obtained by Koch and Mengel (1972, 1974) and by Matsumoto *et al.* (1980). The influence of K and Ca was stronger on the absorption of $\text{NO}_3\text{-N}$ than on that of $\text{NH}_4\text{-N}$. Mg deficit did not affect absorption of NO_3 .

Effect of K, Ca and Mg deficit on translocation of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ from root to upper parts of plants

It was supposed that inhibition of absorption of $\text{NO}_3\text{-N}$ in the plant may be caused through preventing the translocation of $\text{NO}_3\text{-N}$ from root to upper parts which may occur by the function of K^+ and Ca^{++} . In the data of Table 4 the translocation of ^{15}N supplied as $\text{NH}_4\text{-N}$ was not significantly different among treatment, but the translocation of $\text{NO}_3\text{-N}$ was inhibited by $-\text{K}$ and $-\text{Ca}$ treatment. These results strongly suggest that translocation of $\text{NO}_3\text{-N}$ was stimulated by the supply of K and Ca. Similar results have been reported by Frost *et al.* (1978).

Table 4. Effect of mineral supply on ^{15}N distribution in rice plant tissues.

Treatment	^{15}N distribution* (%)			
	Root	Stem+sheath	Blade	Shoot
Ammonium-N				
Complete	20.4 a	45.8 a	33.8 a	79.6 a
-K	22.1 a	44.6 a	33.3 a	77.9 a
-Ca	25.7 a	45.1 a	29.2 a	74.3 a
-Mg	23.2 a	45.6 a	31.2 a	76.8 a
Nitrate-N				
Complete	28.8 a	42.9 a	28.3 a	71.2 a
-K	35.4 b	40.0 a b	24.6 b	64.6 b
-Ca	38.6 b	36.5 b	24.9 b	61.4 b
-Mg	27.6 a	44.2 a	28.2 a	72.4 a

* ^{15}N distribution in each part was expressed as % of the total amount of ^{15}N absorbed.

Within a column, means followed by the same letter are not significantly different according to Waller and Duncan, using new LSD test for $k=100$.

Effect of nitrogen source and mineral supply on nitrate reductase activity of rice plants

Higher activity of nitrate reductase was found in NO_3 treatment while lower activity was found in NH_4 treatment. In NH_4NO_3 treatment the activity was slightly decreased as compared to NO_3 treatment as shown in Fig. 1. The decreases of levels of blade nitrate reductase activity in NH_4 and NH_4NO_3 treatment were 86% and 32 % at 10 days of treatment as compared to NO_3 treatment, respectively. This effect may be caused by ammonium inactivation of nitrate reductase (Orebamjo and Stewart, 1975 a, b) or by decreases in NO_3 content of rice plant (Fig. 2). A positive relationship was frequently observed between NO_3 content and nitrate reductase activity of plant tissues (Woodson *et al.*, 1984). Nitrate reductase is dependent upon the presence of nitrate in the tissue (Beevers *et al.*, 1965), which may be related to nitrate flux from roots to shoots (Heimer and Filner, 1971) where the reduction takes place (Muhammad and Kumazawa, 1974).

Nitrate reductase activity in response to supply of K and Ca is shown in Fig. 1. The results indicate that nitrate reductase activity in the leaves and stem of rice plants which were supplied with K and Ca was higher than in those of the plants which were deficient in K or Ca. Decreases of nitrate reductase activity in K-deficit leaves were 60, 58, 59 and 71% at 11, 12, 14 and 16 days of treatment, respectively, as compared to control plant. Nitrate reductase activity was also decreased by -Ca treatment. The decreases were 57, 50, 44 and 60 % as compared to control treatment (NO_3) at 11, 12, 14 and 16 days of treatment, respectively. These results are in good agreement with those obtained by Paulsen and Harper (1968), Sasakawa and Yamamoto (1977) and Matsumoto *et al.* (1980). As discussed above the results provided evidence for an important physiological role of K and Ca which influence the absorption and translocation of NO_3 -N. The results presented here also suggest

that K and Ca played a significant role in the nitrate reductase activity through their effect on $\text{NO}_3\text{-N}$ accumulation in the plant leaves, which arises from higher absorption and translocation by the presence of K and Ca. The data in Fig. 2 indicate that NO_3 content of rice plants was increased by increasing of K and Ca supply. A similar effect was obtained by Blevins et al. (1978).

Effect of K, Ca and Mg on assimilation of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ into amino acids and protein

The concentrations of asparagine, serine, glycine and total amino acids were higher in leaves of rice plants supplied with $\text{NH}_4\text{-N}$ than in those of plants supplied with $\text{NO}_3\text{-N}$ (Table 5). Likewise, protein content and rate of protein synthesis as shown in Tables 6 and 7 were higher in $\text{NH}_4\text{-N}$ fed plant

Fig. 1. Effect of nitrogen source and mineral supply on nitrate reductase activity of rice plants.

Fig. 2. Effect of nitrogen source and mineral supply on nitrate content of rice plants, (a) nitrate content in blade tissues was determined after 10 days of A, B, C, D and E treatment, (b) nitrate content in blade and stem+sheath tissues was determined after 12 days of complete, -K, -Ca and -Mg treatment (2 days of ¹⁵NO₃-feeding).

Table 5. Effect of nitrogen source and mineral supply on the concentration of free amino acids of rice leaves.

Free amino acid	Ammonium-N				Nitrate-N			
	Complete	-K	-Ca	-Mg	Complete	-K	-Ca	-Mg
	$\mu\text{mol/g dry weight}$							
Aspartic acid	15.0	10.0	10.0	13.0	14.0	9.9	8.9	12.0
Threonine	2.6	3.6	1.5	2.6	2.3	2.0	1.5	1.8
Serine	7.6	12.0	4.3	7.8	5.7	3.8	3.5	3.9
Asparagine	7.3	18.0	2.8	3.6	1.1	2.0	1.0	0.7
Glutamic acid	20.0	20.0	12.0	15.0	22.0	17.0	16.0	19.0
Glutamine	2.3							
Proline	0.9	1.3	0.8	0.9	0.7	0.6	1.0	0.7
Glycine	0.5	2.2	0.4	0.5	1.0	1.0	0.6	0.5
Alanine	7.6	10.0	7.3	7.1	8.1	8.3	8.7	8.9
Valine	0.8	1.1	0.9	1.0	0.9	0.9	0.6	0.7
Isoleucine	0.5	0.5	0.5	0.7	0.5	0.5	0.4	0.5
Leucine	0.4	0.4	0.4	0.5	0.5	0.4	0.3	0.4
Tyrosine	0.2	0.3	0.2	0.4	0.4	0.3	0.3	0.3
Phenylalanine	0.3	0.3	0.5	0.6	0.5	0.5	0.5	0.5
Lysine	0.4	0.8	0.3	0.4	0.4	0.5	0.3	0.3
Histidine	0.1	0.3	0.2	0.2	0.2	0.2	0.1	0.2
Arginine	0.3	2.8	0.3	0.6	0.3	0.4	0.2	0.3
Total	66.8	89.0	44.3	57.1	58.6	48.3	43.9	50.7
Ammonia	7.7	19.0	5.4	4.9	9.0	8.7	5.6	5.5

Table 6. Effect of mineral supply on protein- ^{15}N content of rice plants.

Treatment	Protein ^{15}N (mg/g dry weight)			
	Root	Stem+sheath	Blade	Whole plant
Ammonium-N				
C-K complete	2.37 a	2.77 a b	2.80 a	2.70 a
-Ca	3.61 a	3.17 a b	3.74 a	3.47 a
-Mg	2.46 a	2.26 a b	2.66 a	2.32 a
Nitrate-N				
Complete				
-K	1.49 a	0.92 a b	2.18 a	1.95 a
-Ca	1.76 a	0.72 b	1.37 b c	1.09 b
-Mg	1.86 a	1.61 a	1.71 a c	1.19 b c

Within a column, means followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

than in $\text{NO}_3\text{-N}$ fed plant. Usually, the plants which have received $\text{NH}_4\text{-N}$ are clearly different in their nitrogenous composition from those which have received $\text{NO}_3\text{-N}$ (Clark, 1936; Takaki *et al.*, 1968) because NH_4^+ is directly incorporated into amino acids and then protein, but NO_3^- must be reduced to NH_3 before its incorporation to amino acids and protein. The present data show that the synthesis of amino acids from inorganic nitrogen may be more active in plants supplied with $\text{NH}_4\text{-N}$ than in those supplied with $\text{NO}_3\text{-N}$. There

Table 7. Effect of mineral supply on the rate of protein-¹⁵N to total ¹⁵N in each tissue of rice plants.

Treatment	Protein ¹⁵ N* (%)			
	Root	Stem+sheath	Blade	Whole plant
Ammonium-N				
Complete	53.3 a	51.5 a	75.4 b	56.5 a
-K	59.3 a	56.2 a	72.9 b	63.5 b
-Ca	88.6 b	49.8 a		66.9 b
-Mg	49.3 a	47.4 a	82.6 c	58.7 a
Nitrate-N				
Complete	36.2 a	39.8 a	70.8 a	47.0 a
-K				
-Ca	30.2 34.6 a a	36.2 b 27.9 c	70.0 71.1 a a	42.2 41.1 b b
-Mg	38.6 a	43.2 d	71.2 a	49.5 a

* Protein ¹⁵N was expressed as % of the total amount of ¹⁵N in each part.

Within a column, means followed by the same letter are not significantly different according to Waller and Duncan, using new LSD test for k=100.

is evidence that ammonium could accumulate only in very small amounts, and its change to amides or amino acids was very quick, but nitrate could accumulate in large amounts because ammonium ion (NH₄⁺) or its dissociation product (NH₃) is toxic but nitrate ion is not toxic. Ammonium ion is "detoxified" by union with amino acids. In contrast, nitrate can be absorbed and stored in various tissues without damage to the plant (Hageman, 1980).

Total free amino acid concentrations of rice plants which were supplied with NH₄-N were higher in -K treatment than those in the other treatment (Table 5). The increases in the amino acid concentration of -K treatment were 28 % as compared to control treatment. The stimulation of both amino acid and protein synthesis (Tables 5-7) in K-deficit plant supplied with NH₄-N may be attributed to higher absorption of NH₄-N, because excess ammonium must be in union with amino acid for detoxification of NH₄⁺. Treatment of -Mg had no effect on the synthesis of amino acids and protein from both the nitrogen sources. It thus seems that in Mg-deficit plant the Mg concentration required for enzyme activation may be still adequate in plant tissues (Table 2). In NO₃-N fed plant free amino acid and protein synthesis were decreased by -K and -Ca treatment (Tables 5-7). Total amino acids slightly decreased in K- and Ca-deficit plant supplied with NO₃-N (Table 5). The inhibition of protein synthesis in K- and Ca-deficit plant was 58.7 % and 47.1% as compared to control plant, respectively (Table 6). Lower absorption of NO₃ and nitrate reductase activity are considered to be responsible for limited assimilation of NO₃-N in K- and Ca-deficit plant. (Table 3 and Fig. 1).

In rice plant, conclusively, moderate K deficiency promotes the utilization of NH₄-N through the enhanced absorption of NH₄-N but moderate Ca and Mg deficiency had no effect on the utilization of NH₄-N. Both K and Ca have important roles in the absorption, translocation and reduction of NO₃-N in rice plant. Moderate Mg deficiency does not affect the utilization of NO₃-N.

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