Absorption and Assimilation of Top Dressed Nitrate in Rice Plants Cultivated in Paddy Soil

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ABSTRACT The fate of ^{15}N in rice plants was investigated after [15]NO$_3$ was applied in surface water as a top dressing fertilizer. From ^{15}N analysis, it was suggested that NO$_3$ was absorbed mainly by leaf sheaths for the first one or two days, then roots began to absorb NO$_3$ thereafter. The absorbed ^{15}N was more rapidly assimilated into the amino acids and protein of leaf blades and leaf sheaths than those of the roots. The labelling pattern of glutamine, glutamic acid, aspartic acid and alanine was rapid and very similar in each organ. Asparagine, a major temporary storage amino compound in rice plants gradually incorporated ^{15}N, but the initial incorporation rate was relatively slow.

Before NO$_3$ addition, no NO$_3$ was detected in all plant parts. Immediately, NO$_3$ became detectable in the leaf sheaths within 4 hours after NO$_3$ addition. However, NO$_3$ could not be detected in the roots for one day after NO$_3$ application.

From the results obtained it was concluded that top-dressed NO$_3$ could be absorbed by leaf sheaths of rice plants, and the N was rapidly transported to the leaf blades and assimilated there.

key words: rice, nitrate, ^{15}N, top-dressing fertilizer

Introduction

It is generally accepted that rice plants cultured in paddy field usually utilize NH$_4^+$ as a major N source. However, it was shown that rice plants can absorb and utilize NO$_3$ if it is added in the medium.[8,9,11,21] In N fertilization for rice in paddy field, the denitrification process is one major problem to loose N from rice field. The NH$_4^+$ is converted to NO$_3$ in surface thin oxidized layer, then NO$_3$ is converted to N$_2$ in the reduced layer below. From this point, the practical application of NO$_3$ fertilyzer into rice paddy field seemed to be scarecely employed, although rice plants can utilize NO$_3$.

Concerning to NH$_4^+$ and NO$_3$ assimilation and transport in rice plant, several ^{15}N tracer experiments have been conducted[1,6~12,20~24]. When [15]NH$_4^+$ was top-dressed to the rice plants cultivated with solution culture, the applied [15]NH$_4^+$ was first assimilated into the amide group of glutamine and other amino acids and amides in the roots.[1,20] The assimilated N was transported to the shoots probably in the forms of glutamine and asparagine.

On the other hand, [15]NO$_3$ was shown to be assimilated in the same manner as added NH$_4^+$ in the roots. However, the assimilation rate of NO$_3$ in the roots was very slow compared with NH$_4^+$.[20, 21] It was suggested that the leaf blades are major site of NO$_3$ reduction and assimilation in the case of rice plants[11].

As for N fertilization for rice, many pot and field experiments have been carried out[7~12, 17~19]. These results suggested that most of all basally dressed N fertilizer is depleted until the end of June around young panicle formation stage[17]. And the top dressing treatment of this stage was very efficient compared with fertilization at later stage[18]. The top dressing of N at about young panicle formation stage was not only efficiently used but also this treatment promoted the absorption of soil N. The top dressed ammonium fertilizer at this stage was almost completely depleted within 10 days, and the fertilizer efficiency was about 50%. The percentage of top

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dressed ammonium N remained in soil accounted for 3 to 10% of applied amount of N. On the other hand, about 40-50% was estimated to be lost by denitrification. When $^{15}NO_3^-$ was employed for the top dressing in this stage, the fertilyzer efficiency was about 50-60% and not so different from NH_4^+ top dressing[8].

In this paper, a pot experiment was conducted for elucidating the fate of ^{15}N labeled NO_3^- in rice plants grown in paddy soil.

Materials and Methods

Rice (*Oryza sativa* L. var. Koshihikari) seedlings were transplanted to the a/5000 pots containing 3 Kg of the surface soil of Muramatsu Experimental Farm belong to Faculty of Agriculture, Niigata University. Ammonium sulfate (0.5g-N), superphosphate (0.8g-P_2O_5) and potassium sulfate (0.5$_g$-K_2O) had been applied to each pot as a basal dressing. The pots had been flooded prior to transplanting. One month after transplanting, one g of ^{15}N labeled calcium nitrate (171mg-N, 10.3 atom% ^{15}N) was applied to the surface water at 10 : 00 AM on June 22nd. Then the plants were successively harvested at 4 and 8 hrs, 1 day, 2, 3, 4 and 6 days after the onset of ^{15}N feedings. The plants were washed with tap water and separated into roots, leaf sheaths (including stem) and leaf blades, then they were throughly washed with deionized water.

Some part of fresh tissues were macerated and extracted thrice with hot 80% ethanol, then separated into the soluble and insoluble fractions. N content was determined by Kjeldahl digestion method. An aliquot of 80% ethanol extract was evaporated in vacuum, and redissolved in water. The NO_3^- concentration in this solution was determined by ion chromatography. Each amino acids and amides was separated by two dimensional thin layer chromatography with Silica gel G-60 developed successively with phenol-H_2O (4 : 1) and butanol-acetic acid-H_2O (4 : 1 : 1). Amino compounds on a silica gel plate were detected by spraying ninhydrin-butanol solution, then each spot was collected separately in a pyrex glass tube. The ^{15}N abundance wan determined by emission spectrometry[13].

Results and Discussion

At the time of ^{15}N treatment, the growth stage was shortly before young panicle formation stage. Most of the basal dressed N fertilizer was expected to be depleted or lost until this stage[17]. At the onset of $^{15}NO_3^-$ treatment on June 22nd, the shoot length of plants were about 40$_{cm}$, the number of tillers was approximately 50, and the fifth leaf was observed to be developing.

Table 1. Fresh weight, dry weight and N content of each
part of rice plants treated with $^{15}NO_3^-$ top-dressing.

	Roots	Leaf sheaths	Leaf blades	Total
Fw (g/pot)	37.5	28.8	13.6	79.9
DW (g/pot)	2.2	4.5	4.1	10.8
Total N (mg-N/pot)	53	106	183	342
Soluble N (mg-N/pot)	4.2	17.7	9.5	31.4
Insoluble N (mg-N/pot)	49	88	173	310

The fresh weight (FW), dry weight (DW), and N content of rice plants was shown in **Table 1.** Although the FW of roots was nearly the half of total FW of the plants, but the DW of roots accounted for only 27% of total DW. The DW of leaf sheaths and leaf blades were almost the same. Total N content in leaf blades was much higher than that of leaf sheaths and roots. Nearly 95% of total N in leaf blade was insoluble N (protein N). On the other hand, the leaf sheaths contained relatively high concentration of soluble N.

Fig. 1 shows the changes in ^{15}N abundance in total N, soluble N and insoluble N in each part of plants. From the figure of total N (left), it was obvious that ^{15}N was most rapidly incorporated into leaf sheaths among organs. During early period just after ^{15}N addition, only leaf sheaths showed significant enrichment of ^{15}N. The initial ^{15}N incorporation into soluble fraction of leaf sheaths was quite rapid and showing hyperbolic curve without a lag-phase (middle). The increase in ^{15}N abundance in total N of roots and leaf blades was initially low, but promptly increased after 4th day of treatment. This result may suggest that some part of the top dressed NO_3 in surface water was initially absorbed by leaf sheaths, rather than the roots.

Fig. 2 shows the N content (mg-N/pot) from top-dressed $^{15}NO_3$ in each part. In leaf sheaths (middle) ^{15}N was mainly distributed in soluble fraction for the first 4 hrs, and ^{15}N was evenly accumulated in soluble and insoluble fractions until 3rd day of ^{15}N treatment, then more ^{15}N was distributed in the insoluble fraction thereafter. The ^{15}N was readily incorporated into the insoluble fraction of leaf blades, especially after the 4th day of treatment (right). On the other hand, N content from $^{15}NO_3$ in roots was negligible during the first day of treatment (left).

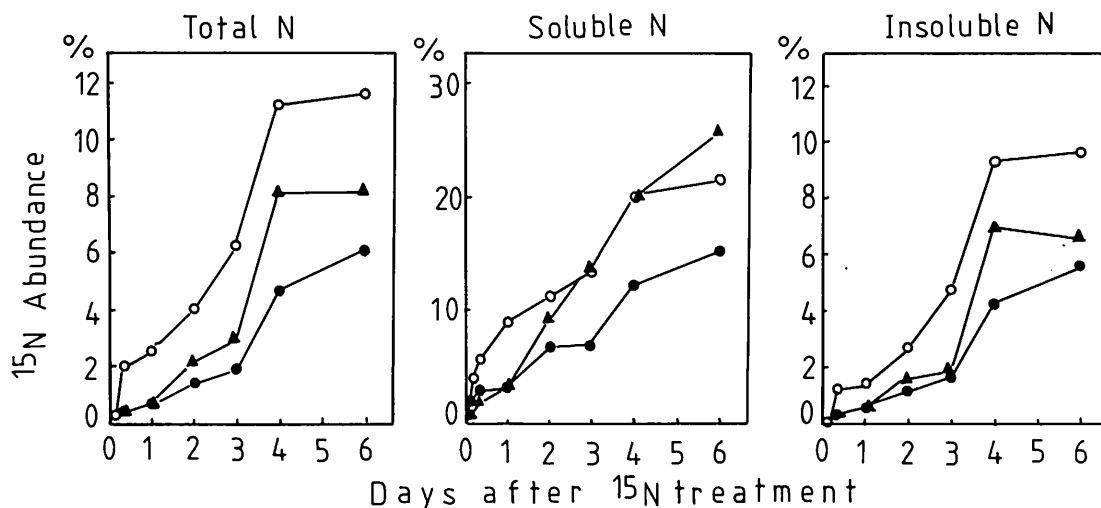**Fig. 3** shows the changes in soluble ^{15}N concentration and NO_3-N concentration in each part

Fig. 1
Changes in ^{15}N abundance of total N(left), 80% ethanol soluble N(middle)and insoluble N(right)in each part of rice plants after$^{15}NO_3$ addition.
▲ Roots, ○ Leaf sheaths, ● Leaf blades
*^{15}N abundance was expressed as percentage of N originating from ^{15}N labeled NO_3 calculated by following equation:
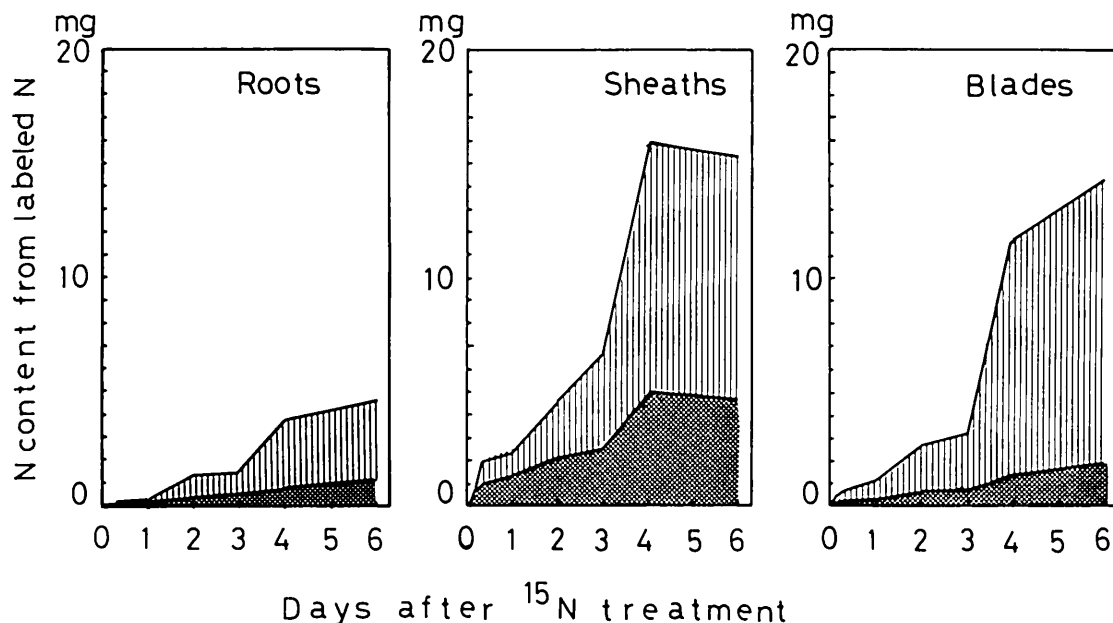atom% excess of sample ÷ atom% excess of $^{15}NO_3 \times 100$

Fig. 2

Changes in N content originating from $^{15}NO_3^-$ in soluble and insoluble fraction of roots (left), leaf sheaths (middle) and leaf blades (right).

▓▓ Soluble fraction,　|||||| Insoluble fraction

based on g FW. No NO_3^- was detected in the plants harvested just before $^{15}NO_3^-$ addition, Therefore, all the NO_3^- in the plants was exclusively derived from top dressed $^{15}NO_3^-$.　In leaf sheaths (middle), NO_3^- concentration increased immediately after $^{15}NO_3^-$ addition.　Moreover, NO_3^--N concentration was almost equal to the ethanol soluble N concentration during initial 8 hrs, Then NO_3^- concentration kept constant or rather decreased until at the 3rd day of $^{15}NO_3^-$ treatment.　Then NO_3^- concentration began to increase again thereafter.　Also, after 3rd day, NO_3^- concentration increased in the roots (left).　These results may imply that the roots began to absorb appreciable amount of NO_3^- only after 3 days of ^{15}N treatment.　Before this period, most of NO_3^- was suggested to be absorbed from leaf sheaths.　The second increase in NO_3^- concentration in leaf sheaths at 3rd day may be due to the NO_3^- absorption form the roots. The NO_3^- concentration in leaf blades increased during initial two days but kept constant value at around 20 $\mu gN/gFW$.

　　　Time course of ^{15}N incorporation into amino compounds was measured.　In roots, alanine, glutamine, glutamic acid, and asparagine were the major free amino compounds.　In leaf sheaths, asparagine was a predominant amide and alanine, aspartic acid were relatively abundant. Glutamic acid, aspartic acid, alanine and asparagine were major amino compounds in leaf blades. **Fig.** 4 shows the changes in ^{15}N abundance of free amino acids in roots (left), leaf sheaths (middle) and leaf blades (right).　The initial ^{15}N incorporation rate in every amino acid was highest in leaf blades compared with roots and leaf sheaths.　Concerning to glutamine, glutamic acid, alanine, aspartic acid in leaf blades, the steep increase in ^{15}N was observed within initial 8 h after ^{15}N application, followed by steady incorporation thereafter.　On the other hand, this steep increase
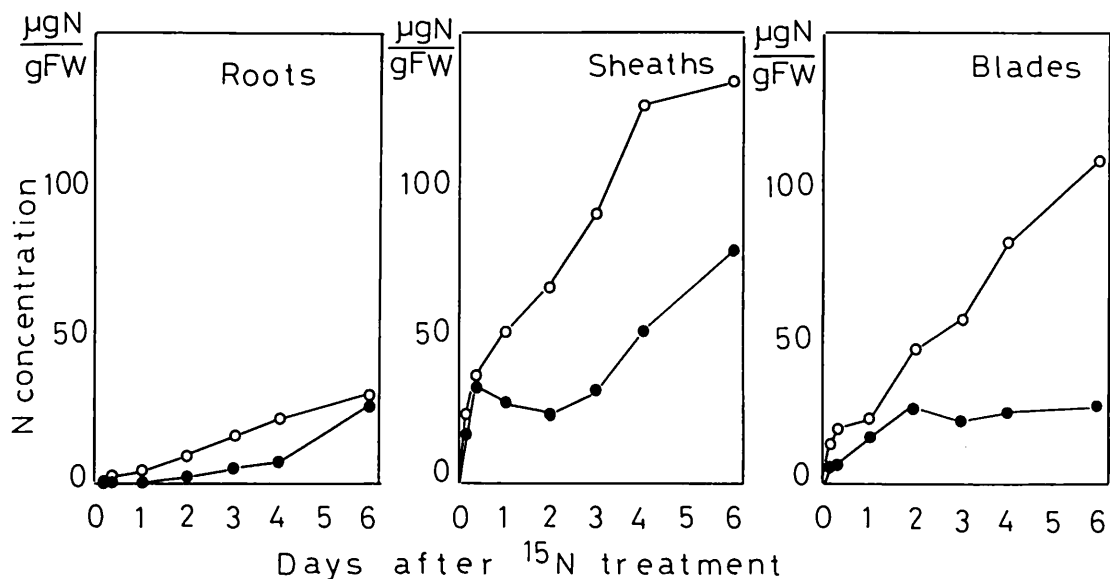
Fig. 3

Changes in NO_3^- concentration and N concentration originating from $^{15}NO_3^-$ in soluble fraction of roots(left), leaf sheaths (middle) and leaf blades(right).

● NO_3^- concentration, ○ Soluble ^{15}N concentration

in ^{15}N was not observed in asparagine in leaf blades. It was characteristic that the labelling pattern of each amino compound in leaf sheaths was rather lower than leaf blades, although leaf sheaths were the primary organ for NO_3^- absorption especially for first few days. This seems to imply that the most of the absorbed NO_3^- in leaf sheaths might be directly transported to the leaf blades, then the NO_3^- was reduced and assimilated into amino acids and protein in blades. This agrees with the assumption that leaf blades are major sites of nitrate reduction and assimilation.

The fact that the increase in NO_3^- was very rapid in leaf sheaths (**Fig. 3**) but the labelling pattern of amino acids was rather slower than those in leaf blades might be coinside with the previous hypothesis that most of all NO_3^- was reduced in leaf blades and the assimilated amino compounds was recycled to leaf sheaths and roots from blades. ^{15}N incorporation in amino acids in roots was found prior to NO_3^- absorption in the roots. So especially in early stage, most of all amino acids in roots seemed to be originating from leaves. FUKUMORITA and CHINO[2] found that major transport form of N in the phloem of rice was asparagine and other amino amino acids, although low concentration of NO_3^- was detected[4].

Rice roots have ability to reduce NO_3^-, so after NO_3^- penetrated into rhizosphere, the roots could reduce some part of NO_3^- adsorbed. Probably leaf sheaths may have some NO_3^- reducing activity. It is unclear how much NO_3^- was reduced and assimilated in leaf sheaths and roots. The percentage of NO_3^- reduced in roots and shoots is known to depend on plant species, NO_3^- concentration or the other environmental conditions. In the case of soybean about half of NO_3^- absorbed was transported in the form of NO_3^- and remained half was transported as asparagine[15,16]. The relative importance for NO_3^- reduction between roots and shoots of rice plants are not fully elucidated.
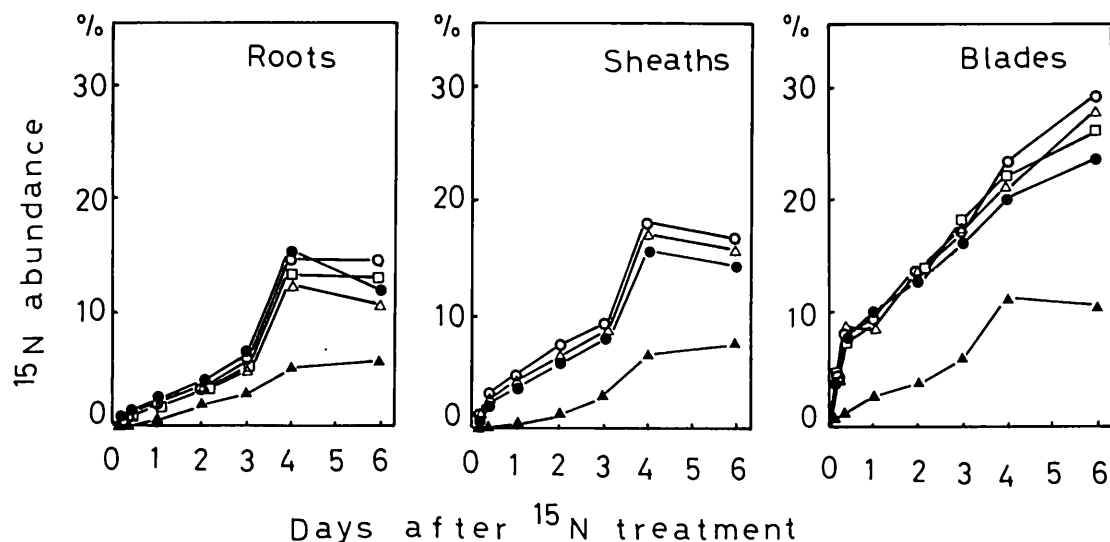
Fig. 4

Changes in ^{15}N abundance of free amides and amino acids in roots(left), leaf sheaths(middle)and leaf blades(right).

● Glutamine, ○ Glutamic acid, ▲ Asparagine,

△ Aspartic acid, □ Alanine

*[15]N abundance was expressed as percentage of N originating from ^{15}N labeled NO[3] by the same equation as Fig. 1.

When NO[3] was applied to the rice roots which had been cultivated in N-free solution culture, NO[3] absorption occurred linearly without lag-phase[3]. The initial pathway of NO[3] assimilation in rice roots depends on GS/GOGAT pathway[21] as same as in the case of NH[4][1, 20]. The ammonium pool intimately linked with NO[3] reduction seemed to be very small and about 1 % of total NH[4] pool in rice roots[21].

The results obtained here suggested that the major part of NO[3] absorbed by leaf sheaths was directly transported to the blades then reduced and assimilated there.　Generally the remobilized N from mature leaves are suggested to play an important role for supplying N for the growing parts in rice[22-24] as well as sunflower[5] and soybean plants[14].　The recycle N is considered to be transported via phloem either downward to the roots or upward to newly developing leaves or reproductive parts.

Fig. 5 shows a model for the initial events of NO[3] absorption, transport and assimilation after NO[3] was applied as a top dressing N fertilizer. Before NO[3] penetrated into rhizosphere, NO[3] is mainly absorbed from leaf sheaths contact with surface water, probably because NO[3] penetration might be slow in paddy soil. After NO[3] penetrates to the soil where roots had been distributed, roots become a major site of NO[3] absorption.

The absorbed NO[3] is rapidly transported to the leaf blades, and it is photochemically reduced and assimilated there. Then the N was recycled to leaf sheaths and roots probably as in the form of amino N via phloem.
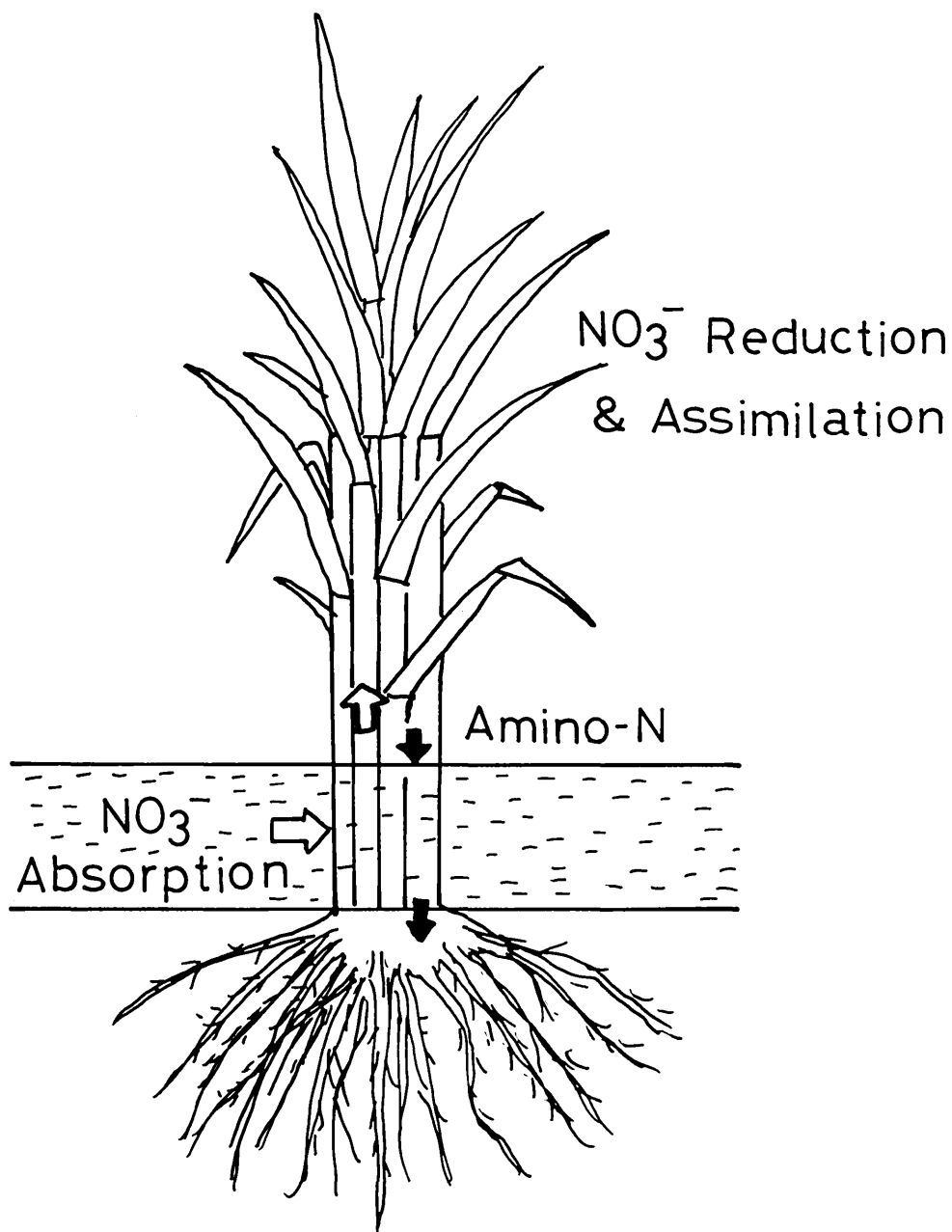
Fig. 5
A Model for initial absorption, translocation, and assimilation process of NO_3^- in rice plants cultivated with soil.

The top dressed NO_3^- was efficiently used for the protein synthesis of leaf blades and leaf sheaths.

The advantage of NO_3^- top dressing to rice plants may be speculated as follows. First, the

high concentration of NO_3^- is less toxic than that of NH_4^+, and plants can accumulate NO_3^- in vegetative parts. Second, the elongation of shoots and lodging may be less severe with NO_3^- top dressing than NH_4^+ application. It was suggested that ^{15}N originating from $^{15}NH_4^+$ was preferentially transported to the growing leaves, but ^{15}N from $^{15}NO_3^-$ was translocated to the mature leaves. Third, NO_3^- is hardly fixed in the soil and the movement of N to the rhizosphere may be easier than NH_4^+. The biggest disadvantage of NO_3^- top dressing to paddy rice field is N loss by denitrification. Further researches are required for NO_3^- top dressing to the rice cultivaiton in paddy field.

References

1. ARIMA Y. and KUMAZAWA K. 1975: A kinetic study of amide and amino acid synthesis in rice seedling roots fed with ^{15}N-labelled ammonium. J. Sci. Soil and Manure, Japan, **46**, 355 -361 (in Japanese)

2. FUKUMORITA, T. and CHINO, M. 1982: Sugar, amino acid and inorganic contents in rice phloem sap. Plant Cell Physiol., **23**, 273-283

3. HBSEGAWA H. 1990: Short period uptake of nitrate by rice seedlings. Japan J. Crop Sci. **59**, pp498-502

4. HAYASHI H. and CHINO M. 1985: Nitrate and other anions in the rice phloem sap. Plant Cell Physiol. **26**, 325-330

5. ITO O. and KUMAZAWA K., 1976: Nitrogen assimilation in sunflower leaves and upward and downward transport of nitrogen. Soil Sci. Plant Nutr., **22**, 181-189

6. KUMAZAWA K. and GOLEB J. A. 1969: Optical emission spectrographic studies on the distribution and accumulation of nanogram amounts of ^{15}N in rice seedlings. Plant Cell Physiol. **10**, 725-731

7. MUHAMMAD S. and KUMAZAWA K. 1972: Use of optical spectrographic ^{15}N analyses to trace nitrogen applied at the heading stage of rice. Soil Sci. Plant Nutr., **18**, 143-146

8. MUHAMMAD S. and KUMAZAWA K, 1974: The absorption, distribution, and redistribution of ^{15}N-labelled ammonium and nitrate nitrogen administered at different growth stages of rice. Soil Sci. Plant Nutr., **20**, 47-55

9. MUHAMMAD S.,KIM U. J. and KUMAZAWA K, 1974: The uptake, distribution, and accumulation of ^{15}N-labelled ammonium and nitrate nitrogen top-dressed at different grwth stages of rice. Soil Sci. Plant Nutr., **20**, 279-286

10. MUHAMMAD S. and KUMAZAWA K, 1974: Assimilation and transport of nitrogen in rice. I. ^{15}N-labelled ammonium nitrogen. Plant Cell Physiol., **15**, 747-758

11. MUHAMMAD S. and KUMAZAWA K, 1974: Assimilation and transport of nitrogen in rice. II. ^{15}N-labelled nitrate nitrogen. Plant Cell Physiol., **15**, 759-766

12. MUHAMMAD S. and KUMAZAWA K, 1976: Nitrogen distribution studies by emission spectrometry in free amino acids and amides of developing panicle of rice. Z. Pflanzenahr. Bodenkd. 592-536

13. OHYAYA T. 1982: Emission spectrometric ^{15}N analysis of amino acids. Radioisotopes, **31**, 212-221 (in Japanese)

14. OHYAMA T. and KAWAI S. 1983: Nitrogen assimilation and transport in soybean leaves: Investigation by petiole girdling treatment. Soil Sci Plant Nutr., **29**, 227-236

15. OHYAMA T., KATO N. and SAITO K. 1989: Nitrogen transport in xylem of soybean plants

supplied with $^{15}NO_3$.   Soil Sci Plant Nutr.,35, 131-137

16.   OHYAMA T., SAITO K. and KATO N. 1989:   Assimilation and transport of nitrate, nitrite and ammonia absorbed by nodulated soybean plants.   Soil Sci Plant Nutr., 35, 9-20

17.   SHOJI S., WADA G., SAITO K., and SHINBO I. 1971:   The fate of fertilizer nitrogen applied to the paddy field and its absorption by rice plant. II.   The fate of basal nitrogen in the paddy field.   Japan J. Crop Sci., 40, 281-286 (in Japanese)

18.   WADA G., SHOJI S.and TAKAHASHI J. 1971: The fate of fertilizer nitrogen applied to the paddy field and its absorption by rice plane. III. Fate of top-dressed nitrogen in the soil and its absorption by rice plant. Japan J. Crop Sci., 40, 287-293 (in Japanese)

19.   WADA G., SHOJI, S. and TAKAHASHI J. 1973:   The fate of fertilizer nitrogen applied to the paddy field and its absorption ay by rice planet. IV. Distribution of basal and top-dressed nitrogen in rice plant. Japan J. Crop Sci., 42, 84-90 (in Japanese)

20.   YONEYAMA T. and KUMAZAWA K., 1974:   A kinetic study of the assimilation of ^{15}N-labelled ammonium in rice seedling roots.   Plant Cell Physiol. 15, 655-661

21.   YONEYAMA T. and KUMAZAWA K., 1975:   A kinetic study of the assimilation of ^{15}N-labelled nitrate in rice seedling. Plant Cell physiol. 16, 21-26

22.   YONEYAMA T. and KUMAZAWA K., 1977:   Nitrogen nutrition and growth of the rice plant.   I.   Nitrogen circulation and protein turnover in rice seedlings.   Soil Sci Plant Nutr., 23, 237-245

23.   YONEYAMA T. and SANO C., 1978:   Nitrogen nutrition and growth of the rice plant.   II.   Consideration concerning the dynamics of nitrogen in rice seedling. Soil Sci Plant Nutr. 24, 191-198

24.   YONEYAMA T. 1978:   Nitrogen nutrition aod growth of the rice plant.   III. Origin of amino -acid nitrogen in the developing leaf. Soil Sci Plant Nutr. 24, 199-205

土耕栽培水稲に追肥した硝酸の吸収と同化

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摘　　要

　水稲を栽培した土耕ポットの田面水に¹⁵N標識硝酸カルシウムを追肥し，水稲植物体内での¹⁵Nの挙動を調べた。硝酸追肥直後，約1～2日目までは，¹⁵NO₃は田面水に接している葉鞘部分から主に吸収された。その後，根からも硝酸吸収が行われた。葉鞘で吸収された硝酸は，そのまま葉身へ移行し，葉身で還元を受け急速に遊離のアミノ酸やタンパク質等に同化された。その一部は，篩管を通って葉鞘や根へ再移動している事が示唆された。どの器官でもグルタミン，グルタミン酸，アスパラギン酸，アラニンの¹⁵Nの取り込みは急速で同じような継時変化を示した。稲の一時的な窒素の貯蔵形態と考えられているアスパラギンは徐々に¹⁵Nを取り込んだ。

　硝酸追肥直前に採取した植物では，どの部位でも硝酸は検出されなかった。硝酸を供与すると，葉鞘では4時間以内に硝酸の集積が検出されたが，根では一日後まで硝酸は検出できなかった。

　以上の結果から，水稲の葉鞘は田面水に追肥として施された硝酸を吸収することができ，その硝酸は主に葉身で還元同化されることが示された。

　キーワード：水稲，硝酸，¹⁵N，追肥

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