Absorption and Assimilation of Nitrogen by Rice Plants

— A Review on ^{15}N Study in Japan—

By TADAKATSU YONEYAMA

Department of Soils and Fertilizers, National Agriculture Research Center
(Yatabe, Ibaraki, 305 Japan)

The first report appeared in Japan on the use of ^{15}N as a tracer was the report of Ozaki and Mitsui (1950),[16] in which ^{15}N was chased after the application of ^{15}N-labelled ammonium sulfate at the late-vegetative stage of rice plants. Following this report, they published 4 additional reports,[17-20] on the utilization of ammonium-^{15}N in rice plants, and established that the ammonium absorbed by rice plants can be assimilated very quickly into amino acids and further into proteins in the root and shoot organs. In these works the amount of N required for the analysis of ^{15}N concentration was so large (5-10 mg), and the amount of ^{15}N-labelled substrates available for experiments was so small to conduct more detailed examination on nitrogen metabolism. The introduction and development of the emission spectroscopic method for the analysis of minute amounts (1 - 10 μg) of N enabled to conduct the detailed study on the absorption, transformation and translocation of N in rice plants. Since early 1970s, the understanding of metabolism and translocation of N in rice plants as well as in other plants has been greatly advanced. In this report a review on the absorption and assimilation of N by rice plants with emphasis on the works in Japan will be given.

Absorption of N

1) *Sources and forms of N utilizable by rice plants*

Rice plants can utilize ammonium and nitrate, but the former form is the major N source because in most fields rice plants are grown under paddy soil conditions. When the culture medium contains both ammonium and nitrate, rice plants prefer ammonium, but the absorption rate of ammonium is decreased by the coexsistence of nitrate compared with single absorption. There is an evidence that the rice plant can absorb amino acids under aseptic conditions before the decomposition to ammonia,[14] and paddy soils contain amino acids. However, there is no estimate of the extent to which soil amino acids are decomposed to ammonia before the absorption by rice plants in the field.

Rice plants themselves do not fix atmospheric N_2, but N_2-fixers, such as blue-green algae and azolla grown on paddy fields and heterotrophic bacteria living near or on rice roots can supply fixed-N to rice plants during their growth. When kept in the atmosphere containing ^{15}N-labelled N_2 gas for several days, the soil and rice plants became labelled with ^{15}N,[34] showing that the rice-soil system fixed atmospheric N_2 and that the rice plants absorbed some of the fixed N. The proportion of the contribution of fixed N druing growth of rice plants may be small, but the contribution of fixed-N accumulated in soils for a long time could be considerable. Other N sources coming from outside the paddy field are N compounds supplied through the irrigated water and from polluted air. The input of N through these routes is significant in the polluted areas of industrialized countries in these days. The confirmation and estimation of the contribution of NO_2, an important air pollutant, to plant N increment was conducted by the ^{15}N dilution method in which plant-available soil nitrogen was labelled by applying ^{15}N-labelled ammonium sulfate to the soil, and the decrease in ^{15}N concentration of NO_2-fumigated plants was used as the index of the contribution of NO_2.[9,10] The result showed that NO_2 was absorbed mainly through leaf stomata, and the estimated contribution of NO_2 in 0.03 ppm NO_2 atmosphere, a probable concentration near the urban area, was a few percent of plant N.

The important source of N for the metabolism during growth is the stored or translocatable N in plants. A considerable amount of N required for germination is supplied from the seed endosperm,[28] and a considerable fraction of N used for leaf[27] and root[13,26] growth is translocated from mature organs. At the reproductive stage most of the N in vegetative organs is translocated to the grains. These types of N are in the form of amino acids, and transported in the phloem, while the N absorbed by roots is transported in the xylem to the shoot organs in the forms of inorganic N (nitrate) and amino acids.

2) *Environmental, morphological and genetical effects on N absorption*

Decrease of temperature from 30 to 9°C showed a pronounced depressive effect on the absorption of ammonium and nitrate.[24] At the temperature lower than 15°C, nitrate absorption was very much depressed, but ammonium was absorbed significantly.[22] Fig. 1 shows the ^{15}N incorporation into rice seedling roots at 28 and 2-3°C. At 2-3°C, rice roots still had the ability of assimilating $^{15}NH_4^+$ into amino acids (Table 1).

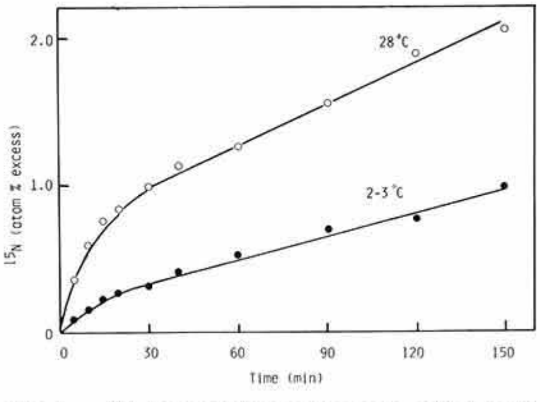

Fig. 1. ^{15}N incorporation in the roots of 21-day-old plants

Plants were kept at 28 or 2-3°C during the treatment with ^{15}N-labelled ammonium sulfate (30 atom % excess) at 30 ppm N (Yoneyama's unpublished data).

Table 1. ^{15}N incorporation into ammonia, free amino acids and protein of rice seedling roots kept at 28 or 2-3°C during $^{15}NH_4^+$ feeding (see Fig. 1.)

	Temperature (°C)	
	28	2-3
Ammonia	17.7	13.9
Free amino acid		
Glutamic acid	12.1	2.68
Aspartic acid	4.18	0.34
Serine	0.80	0.11
Alanine	3.94	1.75
Protein	0.12	0.00

The roots were fed with ^{15}N-labelled ammonium sulfate (30 atom % ^{15}N) at 30 ppm N for 1 hr in the light (Yoneyama's unpublished data).

Shading of rice plants also affected the absorption of $^{15}NH_4^+$ and $^{15}NO_3^-$.[24] Complete darkness reduced the uptake and translocation of $^{15}NH_4^+$ and $^{15}NO_3^-$, especially the latter.[30] The mechanism of the reduction of N uptake by the decrease of light has not been further investigated. However, the reduction of transpiration and carbohydrate supply from the shoot could be the important factors. The pH of the culture medium also influences uptake of ammonium and nitrate: low pH reduced the uptake rate.[5]

As roots emerge from successive nodes of a rice plant, in harmony with the emergence of leaves from the nodes, roots emerged from lower nodes are more aged than those produced on upper nodes: usually the *N*th leaf grows together with the roots from the *N*-3th node. The roots of rice plants at the vegetative stage were separated into 3 parts depending on their age, and then, an uptake rate of $^{15}NH_4^+$ and translocation of ^{15}N to leaves were compared.[25] The lower mature roots had higher activity in absorption and translocation of ^{15}N than the young upper roots. The lower roots transported ^{15}N more to the lower leaves, and the upper roots to the upper leaves. The young upper roots imported ^{15}N from the mature lower roots. These differences of ^{15}N distribution to leaves depending on the root age were not observed at the ear-formation stage.[3] At this stage the upper and lower roots exported ^{15}N to the leaves in a similar distribution pattern. The tips of young roots are active in ammonium assimilation, so that the removal of root tips decreased the incorporation of $^{15}NH_4^+$ into the free amino acid fraction and protein fraction.[33] However, application of cytokinin to the tip-removed root segments enhanced $^{15}NH_4^+$ absorption to a level similar to or higher than that of intact segments. This work suggests the importance of the root tip as a supplier of plant hormones, which may play some roles in nitrogen absorption and metabolism.

Assimilation of N

1) Early works from 1950s to 1960s

A short-term experiment of $^{15}NH_4^+$ assimilation was conducted by Ozaki and Sasaki (1956).[20] They fed water-cultured rice plants at a late-vegetative stage with ^{15}N-labelled ammonium sulfate for 1 and 6 hr in the daytime, and analyzed the ^{15}N concentrations of non-protein and protein fractions (Table 2). The ^{15}N concentrations of the 2 fractions of the roots and leaves increased with the time of ^{15}N feeding. By 1-hr feeding, ^{15}N was detected in the root protein fraction, and by 6-hr feeding it was detected even in the leaf protein fraction. The transformation of absorbed $^{15}NH_4^+$ to the non-protein fraction was examined by separating it into NH_4-N, amido-N, and other soluble-N and by determining their ^{15}N concentrations.[18] By feeding rice plants with $^{15}NH_4^+$ for 24 hr, the ^{15}N concentration of amido-N became the highest, followed by NH_4-N and other soluble-N in the leaves.

Table 2. Incorporation of ^{15}N supplied as ammonium sulfate into non-protein and protein fractions[20]

^{15}N feeding	Tissue	Non-protein	Protein
		atom % excess	
1 hr	Roots	0.41	0.02
	Leaves	0.22	0.00
6 hr	Roots	0.68	0.25
	Leaves	0.71	0.08

Water-cultured rice plants (at the late-vegetative stage) were fed with $(^{15}NH_4)_2SO_4$ (11.89 atom % excess) for 1 and 6 hr in the daytime (10 am – 4 pm) in a greenhouse.

In the roots, as the amount of NH_4-N and amido-N was too small to analyze with a mass spectrometer available at that time, they were analyzed together as a mixture. From these works it was concluded that ammonium absorbed by rice plants could be actively assimilated as amides and amino acids. Examination by paper chromatography showed that glutamine and asparagine were major forms of amides in the rice plants.[18]

The enzymes associated with the assimilation of nitrite and ammonium were investigated in rice plants as in other higher plants. The *in vitro* activities of nitrate reductase,[6] nitrite reductase,[15] glutamate dehydrogenase,[21] and glutamine synthetase[7] were detected from rice plant tissues. The presence of these enzymes suggests that the rice plant can reduce nitrate to ammonium through nitrite and that the ammonium produced may be assimilated into glutamic acid and/or glutamine. A large amount of nitrate was found in the xylem sap when rice plants were fed with nitrate in the culture medium, but only trace of ammonium was detected in the xylem sap of ammonium-fed plants.[12] Nitrate may be reduced in the root and also in the leaf. Ammonium absorbed from the medium is assimilated into amino acids mostly in the root, and ammonium produced in the leaf by nitrate reduction and by other reactions may be transformed into amino acids in the leaf.

2) Recent works since 1970s

Introduction of the emission spectroscopic method[8] and the device of the method to determine ^{15}N concentration in amino acis developed on thin-layers[4,29] have enabled to trace the ^{15}N incorporation into small segments of tissues and individual amino acids when ^{15}N-labelled ammonium or nitrate was administered. The work by Yoneyama and Kumazawa[31] (1974) showed that the amino acid to which ^{15}N was incorporated to the highest extent was glutamine, and it was followed by glutamic acid, when ^{15}N-labelled ammonium sulfate was applied to rice seedlings at the concentration of 40 ppm N. This report also showed the rapid turnover of glutamine-N when the ^{15}N-fed plants were transferred to the non-labelled culture medium. These data indicate that ammonium may be assimilated first of all into

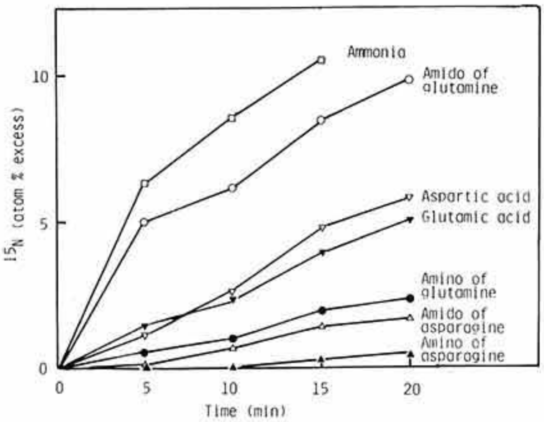

Fig. 2. ^{15}N incorporation into amino acids in rice seedling roots[1]

glutamine and that the produced glutamine is rapidly releasing its nitrogen to other amino acids. A further study by Arima and Kumazawa[1] has established that the amido-N of glutamine is more labelled with ^{15}N than its amino-N (Fig. 2). Treatment with L-methionine DL-sulfoximine, an inhibitor of glutamine synthetase, decreases the ^{15}N incorporation into amino acids from $^{15}NH_4^+$.[2] The label administered as amido of glutamine was transferred to glutamic acid at the highest rate and to other amino acids at lower rates.[2] These data clearly indicate the operation of glutamine synthetase/glutamate synthase pathway at the primary step of ammonium assimilation, which was found in some bacteria and in other higher plants.[11] This pathway is very effective in the assimilation of NH_4^+ of low concentrations due to the high affinity of glutamine synthetase to ammonium. Table 3 shows the ^{15}N incorporation to amino acids when 0.4 ppm N of ^{15}N-labelled ammonium sulfate was administered to rice seedlings. Glutamine was rapidly labelled and reached the maximum level at 65 min due to the disappearance of medium ammonium, although the increase in ^{15}N labelling in other amino acids continued upto 120 min.

By the feeding of ^{15}N-labelled nitrate, a similar pattern of ^{15}N labelling in amino acids was observed: the highest labelling was in glutamine, and it was followed by glutamic acid and other amino acids.[32] This indicates that nitrate is assimilated into amino acids through glutamine synthetase/glutamate synthase pathway after reduction of nitrate to ammonium. In this experiment the ^{15}N abundance of ammonium was higher than that of glutamine during the early period of $^{15}NO_3^-$ feeding but soon levelled off and overwhelmed by glutamine.[32] This suggests that the ammonium pool produced by nitrate reduction is small and probably localized near the nitrate reduction sites.

Table 4 shows the time-course changes in ^{15}N concentration of amino acids of xylem sap obtained from the stem base of rice seedlings to which ^{15}N-labelled ammonium sulfate was given. The amount of glutamine was largest and its ^{15}N concentration was most quickly increased. These data indicate that the transport of assimilated N from the root to the shoot, immediately after the assimilation, is made mainly in the form of glutamine.

Table 3. ^{15}N abundance of amino acids in rice seedling roots fed with 0.4 ppm N of ^{15}N-labelled ammonium sulfate (99.5 atom %)

Amino acid	^{15}N feeding (min) 25	65	120
	atom % excess ^{15}N		
Glutamic acid	3.65	18.9	20.5
Aspartic acid	4.20	14.9	17.5
Serine	0.37	3.53	6.35
Glutamine	11.7	23.8	23.7
Asparagine	1.30	2.17	3.13
γ-amino butyrate	0.90	3.99	5.32
Alanine	1.40	5.97	—
Valine	0.10	1.82	—
Leucine+isoleucine	0.16	0.71	1.17
Tyrosine	0.12	0.82	1.60
Phenylalanine	0.00	0.01	0.02
Proline	0.17	0.80	1.46
Arginine	0.11	0.63	1.80

(Yoneyama's unpublished data)

Table 4. ^{15}N **abundance of amino acids in xylem sap obtained at the stem base when rice seedlings were fed with** ^{15}N**-labelled ammonium sulfate (98.6 atom %) at 40 ppm N**

Amino acid	Color intensity (ninhydrin reaction)	Sampling time (min of ^{15}N treatment)					
		0-10	10-20	20-30	30-40	40-50	50-60
		atom % excess ^{15}N					
Glutamic acid	+	0.00	0.00	0.00	0.02	—	0.02
Aspartic acid	+	0.00	0.00	0.00	0.09	0.00	—
Serine	++	0.00	0.00	0.01	0.00	—	0.04
Alanine	+	0.00	0.00	0.00	—	0.14	0.05
Glutamine	++++	0.01	0.29	0.53	1.71	2.92	2.59
Asparagine	+++	0.00	0.00	0.03	0.06	0.19	0.24
Valine + methionine	+	0.00	0.00	0.00	0.01	0.03	—
Arginine	+	0.00	0.00	0.00	0.00	—	—

(Yoneyama's unpublished data)

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