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Uptake, Assimilation and Translocation of Nitrogen by Crops

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Abstract
Nitrogen metabolism in some selected upland crops was investigated using a stable isotope, ^{15}N, as a tracer. An optical emission spectrometer as well as a mass spectrometer employed for the investigations made great advance in the detailed analyses. Characteristics of nitrate and ammonium uptake by roots and foliar uptake of nitrogen dioxide (an air pollutant) were clearly identified. The kinetic studies using ^{15}N suggested that all the inorganic nitrogen be mainly assimilated into glutamine (particularly into amide), followed by transference to other amino acids and proteins in both the roots and shoots. Nitrate was partly reduced in roots and shoots, but largely kept in storage nitrate pools before reduction. Significant transfer of nitrogen from mature leaves to growing leaves and tops as well as to roots was demonstrated directly by ^{15}N and by a compartment analysis.

Discipline: Solis, fertilizers and plant nutrition
Additional key words: glutamine synthetase, ^{15}N, nitrate reductase, nitrogen cycling, tracer experiment

Introduction

A principal source of nitrogen absorbed by upland crops is nitrate, whereas that for paddy crops like rice is ammonia[25]. In upland fields, ammonium and amino acids are also present, and probably absorbed to some extent. But a large fraction of these reduced nitrogen is transformed into nitrate in the soil and move to the root rhizosphere and then absorbed by crops.

A series of experiments have been undertaken in Japan during the last two decades to examine uptake and assimilation of nitrate and translocation of nitrogen in crops by using ^{15}N as a tracer. To analyze ^{15}N abundances in a small amount of nitrogen from a small section of plant tissues and individual amino acids containing about 1 to 10 μg of nitrogen, an emission spectroscopic method was introduced and developed[4,28,31]. An advancement in the method based on a gas chromatography-mass spectrometer (GC-MS) was also achieved for the analysis of ^{15}N abundance of amino acids in plant samples[19]. This report attempts to review the results of the recent studies conducted in Japan, pertaining to uptake, assimilation and translocation of nitrogen by upland crops. Subjects on nitrogen metabolism in relation with nitrogen fixation in soybean plants are discussed by Dr. Akao separately in this volume.

Absorption of N

The uptake of ^{15}N-nitrate and distribution of absorbed-N in komatsuna (*Brassica campestris* L. var. *rapa*) plants are shown in Fig. 1. Nitrate uptake is linear with time. An increase of nitrate concentration in the medium results generally in increased uptake of nitrate, though in the presence of excessively high nitrate concentration, growth of plants and nitrate uptake are retarded. Distribution of the absorbed-N varies depending on the concentration in the medium. At high concentrations, more nitrogen absorbed is distributed to the leaves and less to the roots.

The patterns of ^{15}N-nitrate and ^{15}N-ammonium uptake in the roots of maize seedlings were compared[26]. The data obtained indicate that the

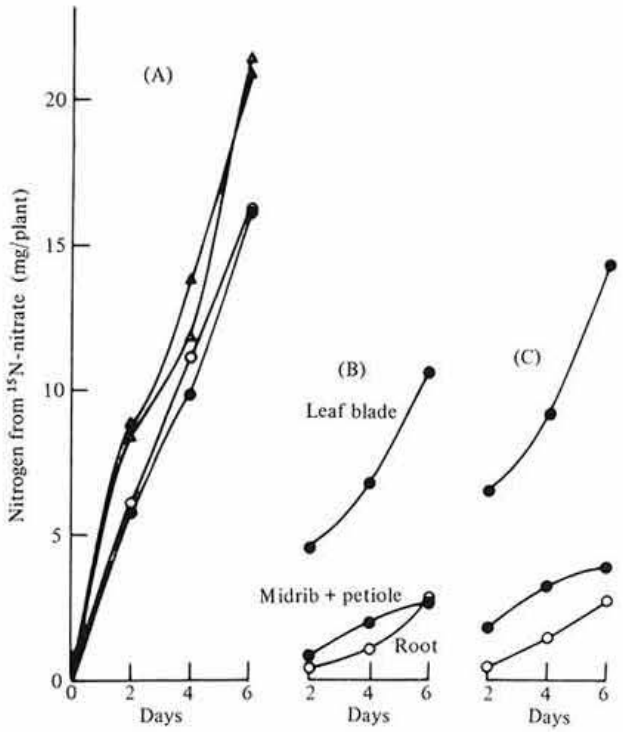

Fig. 1. Nitrate absorption by komatsuna plants from the medium containing ^{15}N-labelled nitrate

Plants were treated with non-labelled 2 mM nitrate before ^{15}N feeding.

(A) Nitrate uptake from 0.4 mM nitrate added every day (●), 2.4 mM nitrate at the initial (○), 2 mM nitrate added every day (▲), and 12 mM at the initial (△).

(B) Distribution of labelled-N in roots, midribs plus petioles, and leaf blades when fed with 0.4 mM nitrate every day.

(C) Distribution of labelled-N in roots, midribs plus petioles, and leaf blades when fed with 2.0 mM nitrate every day.

Source: Kaneko & Yoneyama (unpublished data).

sites of active uptake of ammonium are root tips, while those of nitrate are an elongating zone and a root hair zone as well. Adsorption of ammonium was clearly observed in the roots but not in the case of nitrate uptake. Nitrate was linearly absorbed with time. The ^{15}N-nitrate transport from the cortex to the stele took a similar period of time (about 4 min) in both the apical and basal regions. In contrast, the translocation of ^{15}N-ammonium from the cortex to the stele was very fast in the apical region, but slow in the basal region.

Tea plants prefer ammonium than nitrate. When either $^{15}NH_4$–NO_3 or NH_4–$^{15}NO_3$ is added to the culture medium, the uptake and translocation of $^{15}NH_4$ is more intensive than that of $^{15}NO_3$ in tea plants[7]. It is also observed that the uptake and assimilation of NH_4 is activated in the presence of potassium ion[7].

In addition to the uptake of nitrogen from soil solution by the underground plant parts and from atmospheric N_2 through symbiotic fixation by nodules, plants absorb nitrogen through their aerial parts. Plant leaves treated with solution containing ^{15}N-labelled nitrogen, or with the ^{15}N-labelled at-

mospheric nitrogen, oxides or ammonia show the incorporation of ^{15}N into amino acids and proteins.

Ito and Kumazawa[2] treated sunflower leaves with ^{15}N-ammonium, while Karasuyama et al.[6] treated tea leaves with ^{15}N-urea. The results of these studies indicate that in those plants, ^{15}N is incorporated in the amino acide of the treated leaves as well as in the non-treated plant parts.

Yoneyama et al.[34] and Yoneyama and Sasakawa[32] conducted experiments, exposing spinach leaves to ^{15}N-labelled NO_2, an important air pollutant in Japan, and found that ^{15}N-nitrogen dioxide was absorbed into the leaves mainly through the stomata and incorporated into free and bound amino acids. Subsequent studies[27] using ^{15}N-nitrogen dioxide revealed that the ^{15}N absorbed by the mature leaves of sunflower and maize was transported to the non-treated young leaves and roots, but not to the other mature leaves.

As far as the quantity of nitrogen absorbed by plants from atmospheric nitrogen dioxide is concerned, estimations were made by using a ^{15}N dilution method[8-10,17]. The plant materials under those studies were all fed continuously with ^{15}N-labelled nitrate through the culture medium, and exposed to non-labelled nitrogen oxide. The extent of decrease in ^{15}N concentration in the NO_2-treated plants as compared to that in the non-treated plants is attributed to the contribution of nitrogen derived from the atmospheric NO_2. Sunflower and tomato plants were both active absorbers of the atmospheric NO_2, while sorghum and maize plants were poorer in this respect. In Japan, the proportion of nitrogen derived from air pollutant NO_2 in total plant nitrogen is estimated to be below a few percent.

Metabolism of N in roots

Several results on ^{15}N-labelling of amino acids in root tissues treated with ^{15}N-labelled substrates are shown in Table 1. When maize roots were fed with ^{15}N-labelled ammonium or nitrate, glutamine (particularly the amido group) was the most highly labelled after a short period of time, markedly in 5 min of ^{15}N-ammonium feeding, followed by glutamic acid and aspartic acid[26]. These results suggest that the pathway of glutamine synthetase–glutamate synthase (GS–GOGAT)[11] operate in maize roots as the case in roots of rice plants[25]. In this

Table 1. Labelling of amino acids with ^{15}N in the root tissues treated with ^{15}N-labelled substrates

Amino acid	Corn roots[a] $^{15}NO_3^-$	Barley roots[b] $^{15}NO_3^-$	Corn roots[c] $^{15}NO_2^-$	Corn roots[d] $^{15}NH_4^+$
Glutamine	–	2.73	–	25.4
Amide	7.81	–	3.42	–
Amino	1.29	–	0.24	–
Glutamic acid	4.52	1.78	2.07	12.1
Asparagine	–	–	0.13	3.06
Amide	1.06	–	–	–
Amino	0.10	–	–	–
Aspartic acid	2.56	0.59	–	5.76
Alanine	1.12	0.28	0.50	1.80
Serine	0.29	0.65	0.08	0.84
Proline	0.00	–	–	0.14
(Reference no.)	(26)	(13)	(26)	(26)

a): 1.4 mM $K^{15}NO_3$ (translated to 100 atom % excess) for 30 min.
b): 0.7 mM $K^{15}NO_3$ (95.6 atom % excess) for 30 min.
c): 0.7 mM $Na^{15}NO_2$ (99.5 atom % excess) for 30 min.
d): 0.7 mM $(^{15}NH_4)_2SO_4$ (translated to 100 atom % excess) for 30 min.

context, nitrite metabolism in the roots of kidney bean, maize, and sunflower was investigated by using ^{15}N[29]. Nitrite reduction was active in the apical root zones with the reduced products incorporated into glutamine (amido), glutamic acid, and alanine in this order. All the nitrite absorbed was assimilated in the roots. No nitrite was detected in the shoots, but the ^{15}N from nitrite was incorporated into amino acids in the shoots of the above-noted three plants. The transfer of ^{15}N to the shoots was more active in the daytime, although the same rate of nitrite reduction in the roots was maintained during the day and night.

Oji and Wakiuchi[15] investigated the utilization of ^{15}N-labelled nitrate, nitrite, and ammonium in barley plants. Their conclusions are: nitrate is partly reduced in the roots and transported to the shoots as amino acids and nitrate, while almost all the ammonium and nitrite are assimilated in the roots and transported to the shoots in the form of amino acids. In tobacco plants[36], the assimilation of ^{15}N-ammonium into amino acids in the roots is more intensive than that of ^{15}N-nitrate. However, the transport of ^{15}N-nitrate to the leaves is more active than that of ^{15}N-ammonium, while both are assimilated at the same rate in the shoots.

Mori et al.[12] observed better growth of barley

plants when arginine rather than nitrate was used in the culture medium. The uptake and assimilation of ^{15}N-nitrate was more active in the NO_3^--grown barley than in the arginine-grown plants, but the uptake and metabolism of [ureido-^{15}N] arginine occurred at the similar rates in both plants. Barley plants absorbed more efficiently glutamine and arginine than nitrate under both high (20°C) and low (4–5°C) temperatures, and preferentially absorbed organic nitrogen (arginine and glutamine) in case where both the organic nitrogen and nitrate were present in the culture medium[13].

The nitrogen in seeds is an important N source besides the nitrogen added to the medium during the early stage of seedling growth. The utilization of seed and medium (ammonium or nitrate) nitrogen during the growth of roots and shoots of seedlings was compared by feeding ^{15}N-labelled ammonium or nitrate in rice, maize, and soybean[22]. The ^{15}N-ammonium was incorporated actively into glutamine, asparagine, glutamic acid and aspartic acid, but poorly into proline, arginine, and lysine, while the reverse was true in the utilization of seed nitrogen. In maize seedlings, ^{15}N-nitrate was incorporated actively into glutamine, glutamic acid, and alanine, but poorly into proline, arginine, and lysine. The similar results were observed by Samukawa and Yamaguchi[20]. Utilization of medium nitrate by soybean seedlings was small and the growth of roots and leaves in 4 days after germination was greatly dependent on the seed nitrogen.

Nicotine in tobacco plants is synthesized in roots and transported to leaves. As sources of nitrogen in nicotine, the nitrogen absorbed by the roots and the amino acids transported from the leaves are important[38]. A greater amount of ^{15}N-ammonium was incorporated into the alkaloid fraction in roots in the daytime than in the night, and the labelling of alkaloids with ^{15}N in leaves gradually increased with time[37]. The ^{15}N-ammonium was incorporated into both the pyridine and pyrrolidine rings of nicotine in the roots and shoots. The labelling of both rings with ^{15}N was continuous for at least 187 hr after 5 hr's feeding of ^{15}N-ammonium[39].

Theanine (γ-glutamylethylamide) which is an important compound for green tea quality is mainly synthesized in roots, and transported to young leaves to be harvested. The incorporation of ^{15}N into theanine as well as into other amino acids was very intensive when $^{15}NH_4$ was fed as compared to $^{15}NO_3$ feeding[7].

Table 2. Labelling of amino acids with ^{15}N in the leaf tissues treated with ^{15}N-labelled substrates in the light and darkness

Amino acid	Isolated spinach leaf cells[a], $^{15}NH_4^+$		Sunflower leaf discs[b] [^{15}N-amide] Glutamine		Sunflower leaf discs[c] ^{15}N-Glutamic acid		Tea leaves[d] ^{15}N-urea	Spinach leaves[e] $^{15}NO_2$ gas
	Light	Dark	Light	Dark	Light	Dark	Light–Dark	Light
Glutamine	–	–	–	–	–	–	30.5	14.5
Amide	19.8	20.3	25.4	23.0	1.87	5.68	–	23.2
Amino	1.41	0.62	0.64	1.54	1.59	1.44	–	5.8
Glutamic acid	2.63	1.82	2.22	1.07	10.6	9.04	27.07	17.4
Asparagine	0.90	0.64	–	–	–	–	–	8.01
Aspartic acid	1.93	1.20	1.75	1.07	2.95	1.40	15.71	13.5
Alanine	1.51	0.83	1.89	0.83	2.77	1.75	–	13.4
Serine	0.96	0.65	1.25	0.15	1.65	0.25	–	12.6
γ-amino butyric acid	1.73	1.34	1.54	0.69	4.08	2.11	–	14.3
Arginine	–	–	–	–	–	–	21.04	1.90
Theanine	–	–	–	–	–	–	0.60	–
(Reference no.)	(4)		(3)		(3)		(6)	(32)

a): 0.2 mM $(^{15}NH_4)_2SO_4$ (97.3 atom % excess) for 5 min.
b): 1 mM [^{15}N-amide] glutamine (49.0 atom % excess) for 30 min.
c): 1 mM ^{15}N-Glutamic acid (50.0 atom % excess) for 30 min.
d): 0.83 % (w/v) ^{15}N-urea (translated to 100 atom % excess) was sprayed 3 times every 2 days.
e): 4 ppm $^{15}NO_2$ (95.1 atom % excess) gas for 2.5 hr.

Metabolism of N in shoots

Several results on ^{15}N labelling of amino acids in leaf tissues treated with ^{15}N-labelled substrates are shown in Table 2. The application of ^{15}N-ammonium to sunflower leaves[3] and to cells isolated from spinach leaves[4] resulted in the highest ^{15}N labelling of glutamine, followed by glutamic acid and aspartic acid. The incorporation of ^{15}N into the amide of glutamine was more active than that into the amino of glutamine or glutamic acid[3], and the ^{15}N transfer from the [^{15}N] amide of glutamine to glutamic acid was greatly enhanced by light illumination[4]. These results suggest that the GS–GOGAT pathway[11] operates during ammonia assimilation in the leaves and that the glutamate synthase reaction be highly dependent on light probably due to the involvement of ferredoxin as electron donor to this reaction.

Ito and Kumazawa[3] investigated the metabolism of ^{15}N-labelled nitrate, nitrite, hydroxylamine, ammonium, glutamine (amide), glutamic acid, alanine, glycine and leucine administered to sunflower leaf discs. ^{15}N from all the inorganic N compounds was most actively incorporated into amide of glutamine and less into the other amino acids in a similar manner, suggesting that the nitrogen in all the inorganic forms be incorporated into amino acids after being reduced to ammonia. In the transfer of amino N, transamination played an important role as glutamic acid, alanine, and aspartic acid were always actively labelled with ^{15}N, when the ^{15}N-labelled amino acids were administered. This result implies that these amino acids lie in the center of cellular amino acid metabolism.

The occurrence of nitrate and nitrite reduction under a dark condition was examined by feeding leaf discs with ^{15}N-labelled substrates[23].

Under the aerobic (in the presence of air) and anaerobic (in N_2 gas) conditions, both the nitrate and nitrite were reduced and partly assimilated into amino acids, although the nitrite formation from nitrate was more intensive under the anaerobic condition, while the nitrite assimilation into amino acids was more active under the aerobic condition. Application of *n*-propanol inhibited nitrite assimilation. These results imply that the *in vivo* assay of nitrate reductase activities may be underestimated due to nitrite assimilation during the incubation. The incubation in N_2 gas or the addition of *n*-propanol may be desirable as far as the quantity of nitrite formed is concerned.

Yamaya et al.[21] investigated the ammonium assimilation in the mitochondria isolated from pea and maize shoots by feeding with ^{15}N-labelled ammonium and glycine. These mitochondria were tolerant to 2–5 mM ammonium and could synthesize ^{15}N-glutamate from both ^{15}N substrates, indicating that the photorespiratory produced NH_4^+ is, at least in part, reassimilated by the mitochondrial glutamate dehydrogenase.

The ^{15}N-urea foliarly applied to tea leaves was incorporated into glutamine at the highest ^{15}N abundance, and to glutamate and arginine at the lower abundance[6]. Although large quantities of theanine existed in the leaves, the incorporation of the ^{15}N-urea into this compound was small, probably due to the fact that theanine might have been synthesized in the roots (using mainly soil and fertilizer nitrogen) and transported to the shoots[7].

Ohta et al.[14] examined the nitrate uptake and reduction in *Amaranthus tricolor* L. plants, wherein their growth was enhanced with a sodium application. The sodium application increased the activity of nitrate reductase as well as the rate of uptake of ^{15}N-labelled nitrate and its subsequent incorporation into the insoluble fraction in the plants.

The greenness of leaf vegetables is an important quality and it is recognized that ammonium fertilizers are more efficient than nitrate fertilizers in increasing the greenness of vegatable leaves. When water-cultured komatsuna plants were treated with ^{15}N-labelled nitrate or ammonium, the labelling of chlorophyll with ^{15}N-ammonium was about double that with ^{15}N-nitrate (Table 3).

Table 3. Incorporation of ^{15}N-ammonium and nitrate into chlorophylls of komatsuna leaves

Nitrogen source	Proportion of fed N (%)		
	Chl. a	Chl. b	Total N
$^{15}NO_3^-$	11.5	10.0	12.6
$^{15}NH_4^+$	19.2	18.1	18.3

Hydroponically cultured komatsuna plants were fed with 1 mM $Na^{15}NO_3$ or 0.5 mM $(^{15}NH_4)_2SO_4$ for 5 days. The chlorophylls separated on a thin layer chromatography were extracted, and analyzed for ^{15}N concentrations. Source: Yamada & Yoneyama (unpublished data).

Translocation of N

The ^{15}N-nitrate was partly transported from roots to shoots in an unchanged form, while all the nitrite was reduced in roots and transported to shoots as amino acids[29]. The question whether or not ammonium is transported in the xylem fluids is still subject to debate, because the damage of ammonia to plants is closely related to the accumulation of ammonium in the leaves[1]. Ikeda and Yoneyama (unpublished) analyzed the ammonium concentration in the xylem sap of faba bean and tomato plants treated with nitrate or ammonium by a ^{15}N dilution method. Ammonium was present in the xylem sap by 3 to 9% of xylem sap N in the nitrate-treated plants and those percentages were higher in the ammonium-treated plants.

Little is known about the long-distance transport of nitrogen from mature leaves through the phloem. To trace the movement of nitrogen, the ^{15}N has to be administered to the mature leaves in large quantities enough to be analyzed at the sink organs. There have been several reports of the studies, in which the ^{15}N-labelled nitrate or urea was applied to leaves over several days[18]. However, in such a long period of time, it is most likely that the following movements of nitrogen take place in a mingled fashion: entering and leaving the plant organs, and circulating in the plant. As a consequence, there are great difficulties in the analysis of nitrogen transport in the phloem. An attempt was made by administering ^{15}N-labelled NO_2 to the mature leaves for about one hr just as $^{14}CO_2$ feeding. The NO_2 was absorbed through stomata and metabolized into amino acids[32] without any damage on leaves if the atmospheric NO_2 concentration was 2–4 ppm. The $^{15}NO_2$ fed to leaves of sunflower[27] and maize[33] was incorporated into amino acids, and subsequently partly utilized to synthesize proteins. The remaining ^{15}N moved from the leaves to the other parts of the plants. The ^{15}N-labelled proteins were mobilized to amino acids for translocation from the leaves. The main sinks of ^{15}N were the growing leaves and roots. Only trace amount of ^{15}N was detected in the other mature leaves. These results suggest that the amino acids in the leaves be translocated before utilization for protein synthesis and after remobilization of proteins[33], and that the movement of nitrogen from the leaves be slower but more persistent than that of the photosynthetically fixed carbon[27].

Nitrate reduction and partitioning of nitrogen in the whole komatsuna plant was analysed by using ^{15}N-nitrate[35]. Based on the distribution of ^{15}N in nitrate, amino acids, and proteins of the roots, stems plus petioles, and leaf blades, compartment analysis of nitrogen flux in different nitrogen pools in these plant parts was conducted. Nitrate was present in small metabolic and large storage pools. Nitrate reduction occurred in the three plant parts, but largely in the leaves during the daytime. Amino acids produced by nitrate reduction were partly incorporated into the storage pools, but largely transported in the xylem and phloem. It was confirmed that the amino acids used for protein synthesis were supplied in the following three routes: i.e. by direct nitrate reduction, through transport in the xylem and phloem, and from the storage pools of amino acids.

The importance of reduced N in cycling through the plant for N feeding of each organ was also investigated in barley by using ^{15}N-labelled nitrate and nitrite[16]. In this experiment, an assessment was made in regard to nitrate reduction in the shoots and roots and transport of nitrate and reduced N in the xylem and phloem. The transport and transformation of nitrogen were modulated by the magnitude of reduced-N demand in each organ and light–dark cycles.

Wheat plants were grown in culture solution by feeding them with ^{15}N-labelled nitrate at different stages of growth, and the distribution of ^{15}N in the different plant organs were investigated[24]. The sources of nitrogen in the growing organs were both of the newly absorbed and stored (retranslocated) nitrogen as in the case of rice plants[25]. Ears, i.e. the final sink, also got nitrogen from those two sources, with a larger quantity coming from the latter source. The contribution of N absorbed at different stages of plant growth to various proteins (separated by the Osborne method) did not vary, but that to the amino acids was different. The dependence of lysine, histidine plus arginine, and valine on the early absorbed N (probably this N was stored in the plants and mobilized for retranslocation) was relatively higher than that of glutamic acid, aspartic acid, and alanine, while the reverse status was observed for the N absorbed in the later stage of growth.

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