

The Remobilization of Nitrogen Related to Leaf Growth and Senescence in Rice Plants (*Oryza sativa* L.)

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Rice plants (*Oryza sativa* L.) grown in a nutrient solution were fed with $(^{15}\text{NH}_4)_2\text{SO}_4$ during the 5 days of their young panicle formation.

At the end of that time in the youngest leaf blade, which had started to emerge during the labelling, absorbed-nitrogen accounted for 37% of the increased nitrogen of the tissue; in the next developing leaf blade it accounted for 55%. Thus, remobilized-nitrogen originating from older parts of the plant made up 63 and 45%, respectively, of their total nitrogen. The important contribution of the remobilized-nitrogen to the development of a leaf is evident.

The remobilization of nitrogen in the 12th leaf blade on the main stem was examined in detail after labelling during its developing stage. The ^{15}N level started to decrease soon after the end of the labelling period and continued to decrease until full senescence, although the total nitrogen in the same leaf increased until just after its complete expansion, suggesting that even a young leaf plays a role as a supplier of remobilized-nitrogen.

During the rapid decrease in the total nitrogen after its peak at full expansion of the leaf, the actual proportion of labelled absorbed nitrogen remained nearly the same, indicating that influx of new nitrogen into a senescing leaf is very limited.

Key words: Growth (leaf) — Nitrogen — *Oryza sativa* — Remobilization (nitrogen) — Rice leaf — Senescence.

Leaf senescence in cereal crops is usually pronounced during their reproductive stage. There are two remarkable events accompanying the leaf senescence: one is a decline in photosynthetic activity and the other is a remobilization of nutrients. Therefore, leaf senescence during the reproductive stage in cereal crops is directly related to crop productivity. Nitrogen is one of the most remobilizable elements during the reproductive stage in rice plants (Tanaka 1956), and its content in leaves is closely related to photosynthetic activity (Murata and Osada 1959).

By the use of ^{15}N , the distribution and redistribution of nitrogen in rice plants during their reproductive stage have been studied by many workers (Muhammad and Kumazawa 1972, 1974, Muhammad et al. 1974, Ozaki and Mitsui 1950, 1952, Wada et al. 1973). The influx and efflux of nitrogen in leaves have been studied in tobacco (Oertli 1966), soybean (Kato and Kitada 1979) and rice seedlings (Yoneyama and Sano 1978). But few studies have related the remobilization of nitrogen to leaf growth and senescence.

This paper focuses upon the nitrogen dynamics of a single leaf of a rice plant at its reproductive stage, following the leaf's development and senescence by means of

a ^{15}N tracer. $(^{15}\text{NH}_4)_2\text{SO}_4$ was fed to the plants at the stage of young panicle formation in order to examine the distribution and redistribution of nitrogen.

Materials and Methods

Plant culture—Rice seeds (*Oryza sativa* L. cv. Sasanishiki) pre-treated with a fungicide were germinated on a saran net floating in tap water, the pH of which was adjusted to 5.0. Eight seedlings were transplanted to each pot in a greenhouse 24 days after sowing. Care was taken to select uniform seedlings, because a preliminary experiment showed that growth differences between plants after transplanting was mainly due to their initial size. The plants were grown in 3.5 liters of culture solution per pot, changing the concentration of nutrients over the course of their growth. The nutrient solution was renewed every 5–7 days.

The basal nutrient solution contained the following nutrients in tap water (mM): NH_4NO_3 , 1.0; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.6; K_2SO_4 , 0.3; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.6; EDTA-Fe, 4.5×10^{-2} ; H_3BO_3 , 5×10^{-2} ; $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 9×10^{-3} ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 3×10^{-4} ; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 7×10^{-4} ; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 1×10^{-4} . The strength of the nutrient solution was changed as follows: 0, May 28–Jun. 7 and Sept. 17–Oct. 11; one eighth, Sept. 3–Sept. 17; one fourth, Jun. 7–Jun. 14 and Aug. 27–Sept. 3; one half, Jun. 14–Jun. 27; nine tenths, Jul. 27–Aug. 1 (the period of labelling with ^{15}N); full strength, Jun. 27–Jul. 27. The age of the leaves on the main stems of all the plants was checked by marking them with ink. At about 32 days before heading time, when most of the 12th leaf blades on the main stems had emerged from the 11th leaf sheaths, the nutrient solution of all pots was changed to that containing ^{15}N -labelled ammonium sulfate (6.25 atom %) instead of ammonium nitrate. After 5 days, the plants were again cultured in non-labelled nutrient solution until harvest.

Samples were taken 8 times between the start of ^{15}N -labelling and harvest; 32 plants were collected each time, and their roots were washed thoroughly, first with tap water, then with distilled water. The main stems were separated from the plants, then divided into individual parts: leaf blades, leaf sheath, stems and ears. The roots and top parts remaining were then separated from each other. After measuring their fresh weight, the plant parts were dried in an oven at 70°C for several days, then powdered with a mill for nitrogen analysis.

Nitrogen determination and ^{15}N analysis—The total nitrogen of each sample was determined by the distillation method after Kjeldahl digestion, and their ^{15}N contents measured by emission spectrography (Yoneyama et al. 1975) with a JASCO ^{15}N -Analyzer (NIA-1). The amount of nitrogen in each part absorbed by the plant during the labelling period was calculated as follows (Oertli 1966):

$$N = \frac{{}^{15}\text{N atom \% excess of each sample}}{{}^{15}\text{N atom \% excess of } ({}^{15}\text{NH}_4)_2\text{SO}_4 \text{ fed to the plants}} \times \text{total nitrogen per sample}$$

Results

Changes in the dry weight of the leaf blades, leaf sheaths, stem and ear on the

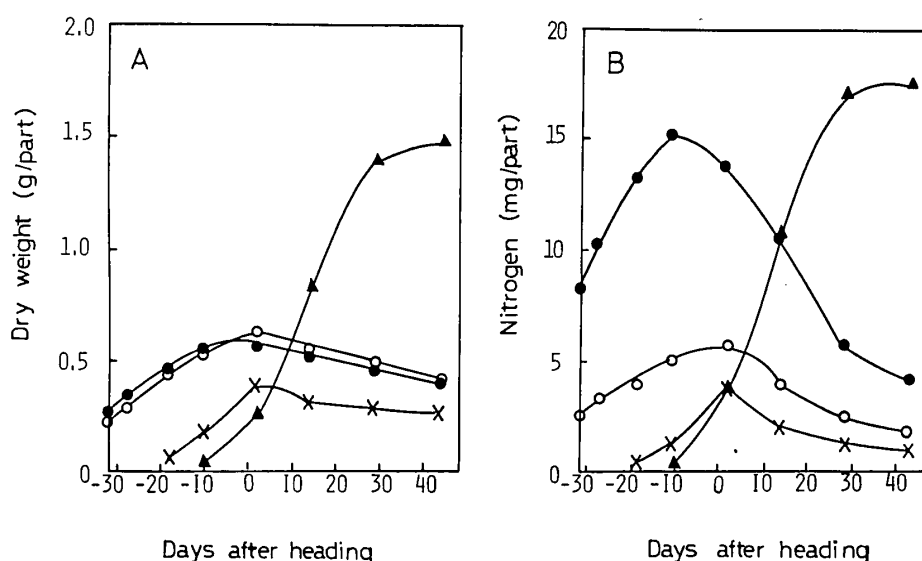


Fig. 1 Changes in the dry weight (A) and the nitrogen content (B) of the following parts of the main stem of the rice plant during its reproductive growth: total leaf blades (●), total leaf sheaths (○), stem (×) and ear (▲).

main stem of rice plants during their reproductive growth are shown in Fig. 1A. The dry weights of the leaf blades, leaf sheaths and stem reached their peak around heading time, then decreased gradually, while that of the ear increased progressively after heading. The loss of dry weight in the leaf blades, leaf sheaths and stem by harvesting time was 27, 33 and 22%, respectively, of peak values. As the dry weight of the leaf blades and leaf sheaths at harvesting time did not include lower leaves, which had already dropped off by them, the actual net loss in the dry weight was less than that indicated by Fig. 1A. The sum of the net dry weight loss from these three parts during the reproductive stage was only 21% of the increased amount of the dry weight in the ear during its ripening period.

Changes in the amount of nitrogen are shown in Fig. 1B. Obviously, the nitrogen loss from the leaf blades was greater than that from the leaf sheath and stem, amounting to 60% of the total nitrogen loss from the three parts during the reproductive growth period. The loss from other two parts was roughly equal. The total amount of nitrogen lost from these three parts was almost equal to the nitrogen gained by the ear during its grain-filling period.

The total nitrogen content of individual leaf blades changed as they aged (Fig. 2B), increasing continuously during their developing stage; just after their full expansion, the amount stayed constant for a while, then decreased rapidly during their senescing stage. The dry weight of each leaf blade also increased progressively during development, but the decline in dry weight during senescence was much smaller than the drop in nitrogen (Fig. 2A).

The distribution of ^{15}N in the plants was examined after 5 days of ^{15}N -labelling at the young panicle formation stage. At this time, the tops had taken up 90% of the total ^{15}N incorporated by the whole plant. Distribution of this ^{15}N into the leaf blades and leaf sheaths on the main stem is shown in Fig. 3. During the labelling period, the 13th leaf blade started to emerge, the 12th leaf blade was

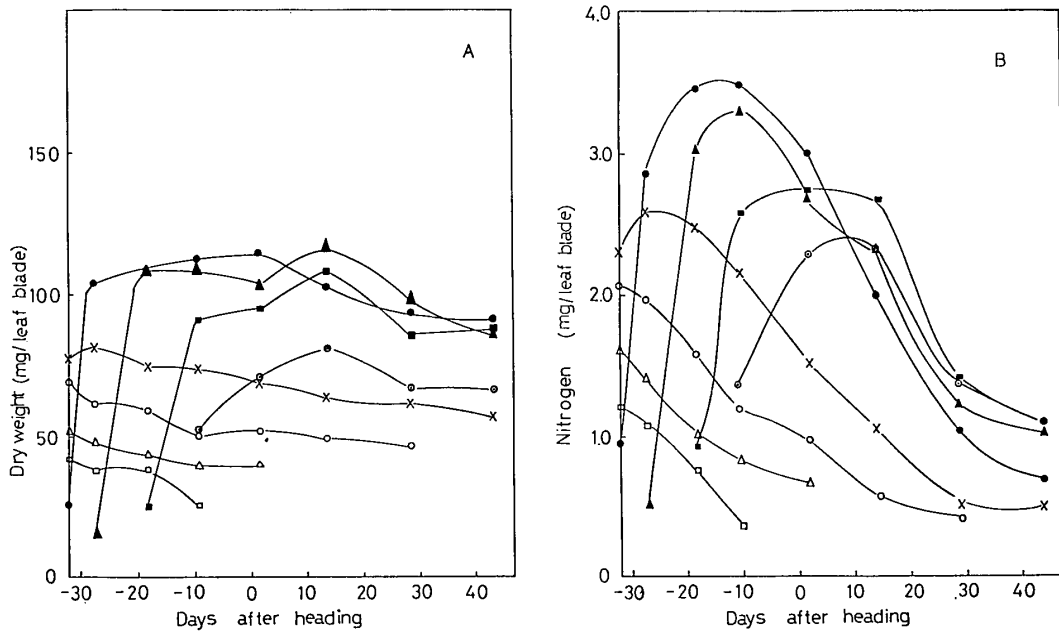


Fig. 2 Changes in the dry weight (A) and the nitrogen content (B) of the individual leaf blades on the main stem of the rice plant during its reproductive growth. Leaves were numbered from the first leaf after germination. Leaf number: 15th (○), 14th (■), 13th (▲), 12th (●), 11th (×), 10th (○), 9th (△), 8th (□).

developing and the 11th leaf blade finished expanding. The ^{15}N -labelled nitrogen was incorporated mostly into the parts actively growing during the labelling period.

Nitrogen in young growing tissue can be divided into two groups on the basis of its origin: that which is freshly assimilated and incorporated into the tissue during its growing period (absorbed-N); and that which pre-existed in the plant before the particular tissue start to grow and is mobilized from older parts of the plant into the

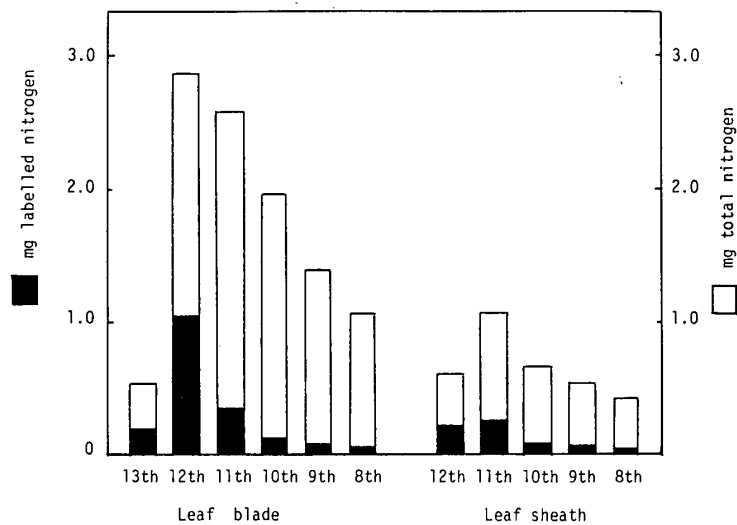


Fig. 3 The incorporation of ^{15}N into the leaf blades and leaf sheaths on the main stem of the rice during the 5 days of labelling with ^{15}N -ammonium sulfate at the young panicle formation stage.

new tissue during its growing period (remobilized-N). These two type of nitrogen could be separated experimentally by the use of ^{15}N . The proportion of absorbed-N and remobilized-N in the youngest (13th) leaf blade and the next youngest (12th) leaf blade and leaf sheath were determined by the use of ^{15}N as a tracer of absorbed-N. The results are shown in Table 1. In the youngest leaf blade, absorbed-N accounted for 37% of the increase in nitrogen during the labelling period; thus 63% of the increase in nitrogen was due to remobilized-N. In the next (12th) leaf blade, absorbed-N was 55% and remobilized-N was 45%. In the 12th leaf sheath, which started vigorous growth during labelling, absorbed-N was 36% and remobilized-N was 64%.

Remobilization of the original absorbed-N was then followed (Fig. 4 and 5). Just after labelling, 74% of the ^{15}N in the main stem was in the leaf blades and 24% in the leaf sheaths. At harvest, the amount of ^{15}N decreased to 20% in the leaf blades and to 8% in the leaf sheaths, while the ear contained 67% of the total ^{15}N . Thus remobilized-N was preferentially incorporated into growing parts successively.

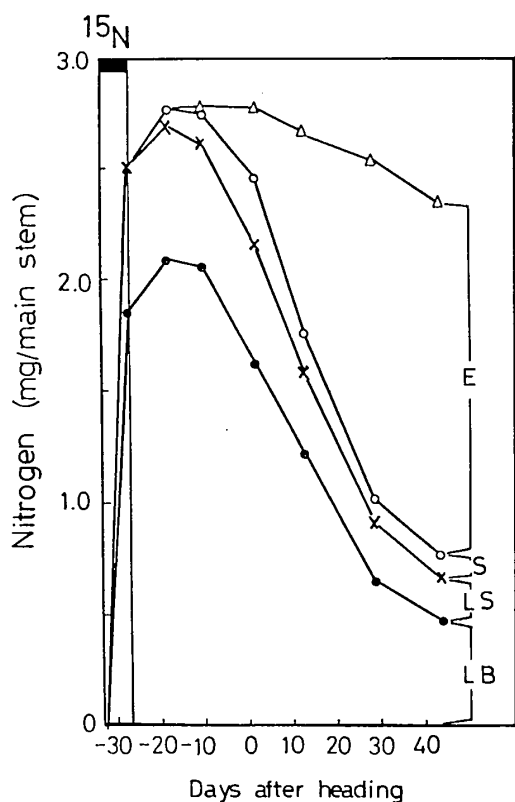


Fig. 4

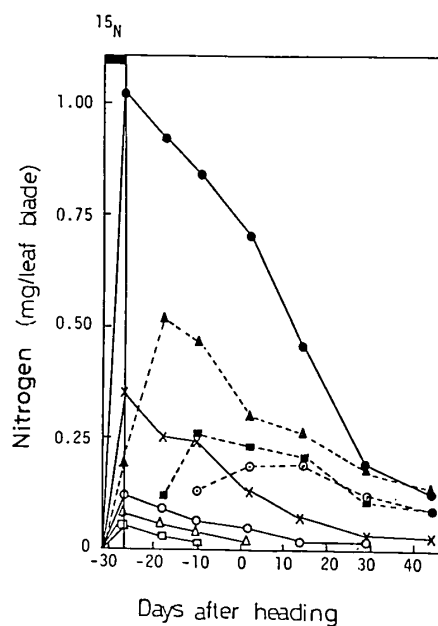


Fig. 5

Fig. 4 Remobilization of nitrogen: ^{15}N in each part of the main stem of the rice plant during its reproductive growth, derived from that absorbed by the plant during young panicle formation. The bar in the top left-hand corner indicates the period of ^{15}N uptake. LB, leaf blades; LS, leaf sheaths; S, stem; E, ear.

Fig. 5 Remobilization of nitrogen: ^{15}N in each leaf blade of the main stem of the rice plant during its reproductive growth, derived from that absorbed by the rice plant during young panicle formation. The bar in the top left-hand corner indicates the period of ^{15}N uptake. Leaf number: 15th (\circ), 14th (\blacksquare), 13th (\blacktriangle), 12th (\bullet), 11th (\times), 10th (\circ), 9th (\triangle), 8th (\square).

Table 1 Proportion of absorbed-N and remobilized-N in the growing leaves on the main stem of the rice plant after the 5-day ^{15}N -absorption period

Sample	Nitrogen amounts (mg)			Origin of new nitrogen (mg)	
	Just prior to absorption (A)	At the end of absorption (B)	N-increase during absorption (B) - (A)	Absorbed-N ^a (C)	Remobilized-N (B) - (A) - (C)
13th leaf blade (youngest)	negligible	0.52	0.52	0.19 (37%) ^b	0.33 (63%) ^b
12th leaf blade	0.95	2.86	1.91	1.05 (55%)	0.86 (45%)
12th leaf sheath	negligible	0.59	0.59	0.21 (36%)	0.38 (64%)

^a Absorbed-N was calculated from the ^{15}N atom % excess of each tissue as shown in **Materials and Methods**.

^b Values in parentheses show the percentage of the total N which is absorbed-N or remobilized-N.

To examine the influx and efflux of nitrogen in a leaf as it aged, the total nitrogen and the ^{15}N content of the 12th leaf blade were followed in detail from the beginning of the ^{15}N labelling (32 days before the heading time), when the leaf blade had just emerged from the 11th leaf sheath, through senescence (Fig. 6). At the end of the 5 days' labelling period, the leaf had reached 83% of its full length, becoming fully expanded about 24 days before heading time; 14 days after heading, the tip started to dry up; by 29 days after heading, a quarter of it had dried up; and at harvest, the whole leaf was dead. Total nitrogen increased until just after full expansion, and stayed constant before declining sharply, to 30% of its peak value. The ^{15}N level started to decrease soon after the end of the labelling period and continued to decrease until harvest. The proportion of labelled nitrogen in the leaf blade decreased

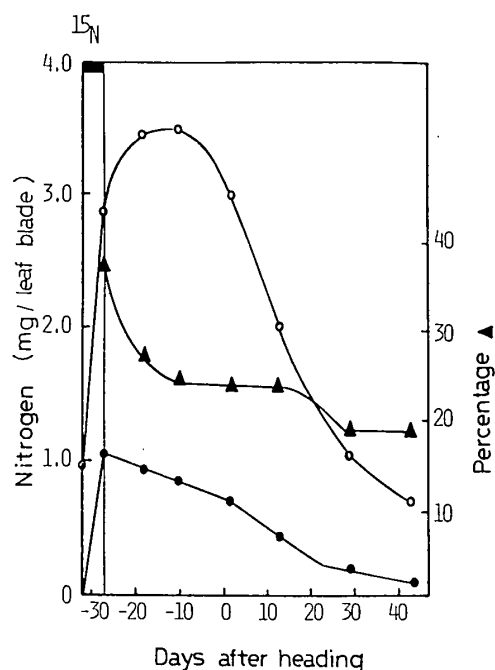


Fig. 6 Changes in the nitrogen levels in the 12th leaf blade on the main stem of the rice plant during its reproductive growth: total nitrogen (○); labelled nitrogen absorbed during its young panicle formation stage (●); percentage of total nitrogen which was labelled (▲). The bar in the top left-hand corner indicates the period of ^{15}N uptake.

sharply during its rapid growth period, then remained nearly the same until a quarter of the leaf blade had dried up, dropping a little at the end of senescence.

Discussion

As shown in Fig. 1, most of the nitrogen accumulated in the ear had been previously absorbed before heading time. A similar pattern has also been shown in the field-grown rice plant (Ishizuka and Tanaka 1953). Unlike nitrogen, a large part of the carbon accumulated in the ear was assimilated after the heading stage. For high yields, much more nitrogen is required than for reproductive development of the crop, and 30–40% of the total nitrogen is then absorbed during the grain-filling period (Murata and Matsuhima 1975). One possible explanation for this is as follows: the absorption of nitrogen after heading prevents a progressive leaf senescence either by reducing the rate of degradation of protein in the leaves or by supplying nitrogen to the leaves for protein synthesis. As a result, photosynthetic activity is maintained longer at a high level. When the leaves are the sole source of nitrogen for ear development, the progressive senescence of leaves may not be avoided.

The results shown in Fig. 4 and 5 clearly show the manner of remobilization during the reproductive period of the nitrogen which had been absorbed by the plant during young panicle formation. Remobilized nitrogen moves quite actively and preferentially into young and growing tissues, one after another. The amount of ^{15}N remobilized into the top part from the roots during the reproductive period was only 4% of the total absorbed nitrogen, suggesting that the roots are of little importance in this process. Instead, leaf blades are the major source of remobilized nitrogen, followed by the leaf sheaths and stems.

The large contribution of remobilized nitrogen from older parts to the growth of young leaves was shown in Table 1. Experiments using ^{15}N to distinguish remobilized and absorbed nitrogen have been done in tobacco (Oertli 1966), soybean (Kato 1973) and rice seedlings (Yoneyama and Sano 1978). All results showed that half or more of the total nitrogen in growing tissues were derived from remobilized-N. Thus it seems significant from the viewpoint of plant nutrition to classify the nitrogen of growing plant tissue on the basis of its origin.

Since the early work with ^{15}N by Hevesy et al. (1940), and Vickery et al. (1940), it has been generally considered that the level of nitrogen in a tissue is controlled by the rates of protein synthesis and breakdown (Beevers 1976), since protein is the most prevalent of the nitrogenous compounds in plants. Experimental data detailing the nitrogen dynamics throughout the life span of a single leaf is still limited, yet it seems likely that the extent of nitrogen influx and efflux is closely related to the process of leaf senescence. Our work demonstrated several interesting points. As shown in Fig. 6, efflux of nitrogen was observed even before the full expansion of the leaf; the substantial amount lost indicates that even a young leaf plays a role as a supplier of remobilized nitrogen. Efflux of the nitrogen from young leaves has also been observed in other plants (Kato and Kitada 1979, Oertli 1966, Yoneyama and Sano 1978).

During the rapid decrease in the total nitrogen after its peak at full expansion of the leaf, the actual proportion of labelled absorbed nitrogen remained nearly the

same, indicating that the influx of new nitrogen into the leaf was now very limited. At the end of senescence, the proportion of ^{15}N dropped a little. Since at this stage the influx of nitrogen into the leaf is negligible, this decrease may reflect that some unlabelled constituents were more resistant to degradation.

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(Received March 13, 1981; Accepted July 13, 1981)