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Partitioning of ^{15}N Applied at Reproductive Stages among Grains and in Grain Tissues of Rice Plants*

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Abstract : Hydroponically-grown rice (*Oryza sativa* L.) plants were pulse-labelled with ^{15}N-ammonium sulphate at various stages of reproductive growth, and partitioning of ^{15}N among grains or grain tissues were examined at full maturity for a better understanding of the ripening process of rice panicle, which consisted of a large number of grains.

Labelled nitrogen applied before heading was equally distributed to all grains. ^{15}N applied at heading or the milk-ripe stage was mainly distributed to the early developing grains located on the upper part of the panicle, while that administered at a later period of ripening was principally transported to the later developing grains on the basal part. Thus, the position of grains actively accumulating nitrogen moved down from the upper to the basal part of the panicle with ripening.

Active incorporation of ^{15}N into husk continued up to the milk-ripe stage. At the later stages of grain filling, ^{15}N uptake in the embryo became lower than that in the endosperm, indicating the formation of embryo preceded the endosperm. ^{15}N levels incorporated into the inner parts of the endosperm decreased earlier than the outer parts ; this suggested that the deposition of nitrogen in the endosperm was first completed at the central part then extended to the peripheral layer.

Based on these results, it could be concluded that the increase in the source-sink ratio is not sufficient to improve grain filling in the rice plants. It is also very important to maintain the physiological activities of leaves and roots until the later stages of ripening when the inferior spikelets continue to accumulate materials.
Key words : Competition, Grain, ^{15}N, Nitrogen, Partitioning, Position on panicle, Rice, Ripening.

水稲の生殖生長期に与えた ^{15}N の穂上分布と粒内分布 : 岡野邦夫**・河野恭広（名古屋大学農学部・**野菜・茶業試験場）

要　旨 : 多数の穎果から構成される水稲の穂の登熟過程を解明するために，水耕法を用いて生殖生長各時期に ^{15}N 標識の硫安を与え，完熟期における ^{15}N の穂上分布と粒内分布を調べた．

出穂期前に与えた ^{15}N は全ての穎果に均等に分布したが，出穂期から乳熟期に与えた ^{15}N は穂の上部に位置する発育の早い穎果に，一方登熟後期に与えた ^{15}N は穂の下部に位置する発育の遅い穎果に，主として移行した．すなわち活発に窒素集積を行っている穎果の位置は，登熟の進行につれて穂の上部から下部へと順次移動した．

籾殻への ^{15}N の活発な取り込みは乳熟期まで続いた．胚は胚乳に比べると登熟後期における ^{15}N 取り込み活性の低下が早く，胚の形成は胚乳に先行することを示した．胚乳の内層部は外層部より ^{15}N の取り込み活性が早く低下することから，胚乳でのタンパク質の蓄積は中心部から始まり，順次周辺部へ及ぶものと考えられた．

以上の結果に基づく考察から，水稲の登熟向上にはソースシンク比の増大だけでは不十分であり，弱勢果が物質集積を続けている登熟後期まで，葉や根の生理活性を高く維持することが非常に重要であると結論された．
キーワード : イネ，穎果，^{15}N，競合，穂上位置，窒素，登熟，分配．

Panicle of rice plant consists of a large number of spikelets differing in the degree of ripening. Spikelets located on the upper part of a panicle bloom earlier and usually attain good ripening, while those on the basal part flower later and often exhibit poor ripening[2,14]. The former is referred to as "superior spikelet (grain)" and the latter, as "inferior spikelet (grain)"[9,10,15]. Occurrence of the inferior spikelets is one of the limiting factors preventing the improvement of grain

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yield in rice.

Experimental manipulations[2,15,16,24,26,28] such as defoliation, shading, removal of spikelets, or nitrogen top-dressing which may alter the source-sink balance, can largely affect the ripening of the inferior spikelets and the occurrence of various types of ill-ripened grains. These results led many investigators[9,10,16] to the consideration that main cause for poor ripening of inferior spikelets and the occurrence of the ill-ripened grains must be the competition of insufficient photosynthates among the spikelets. This idea is, however, based on indirect and circumstantial evidence. It is important to clarify the actual state of assimilate partitioning among the grains for better understanding of the processes of grain filling. Although some workers[5,18,27] conducted ^{14}C or ^{13}C tracer experiments on the distribution of photosynthates among the grains in rice, more investigations are needed to criticize the existence of competition.

Mature rice grain contains approximately 70% starch, 10% protein and other components. Although the growth of grain is usually expressed as dry weight or carbon content, nitrogen content has been also demonstrated to be an useful indicator for grain growth[2]. In the present study, ^{15}N-labelled ammonium sulphate was applied at different stages of reproductive growth in rice plant, and the distribution of ^{15}N among the grains or grain tissues was examined at full maturity. The present experiments were designed to clarify the changes in the pattern of nitrogen partitioning among grains with the progress of ripening and the processes of nitrogen deposition in the grain.

Materials and Methods

Plants

Seeds of rice (cv. Chukyo-asahi) were sown on a nursery bed containing alluvial soil. At the emergence of 7th leaf, each of the four seedlings were transplanted to a water culture container (made of polypropylene, 12 litres in volume) filled with Kasugai's rice culture solution. The seedling was inserted into a rift of urethane resin floating on the solution. The concentration of nitrogen in the solution was set at 15 ppm. The solution was renewed once a week and adjusted to pH 5.5 with 1N HCl and 1N KOH. The plants were grown in a glasshouse. At heading (September 3), panicles with the same heading date were tagged. Heading was designated by the flowering of terminal spikelet on the uppermost primary rachis-branch.

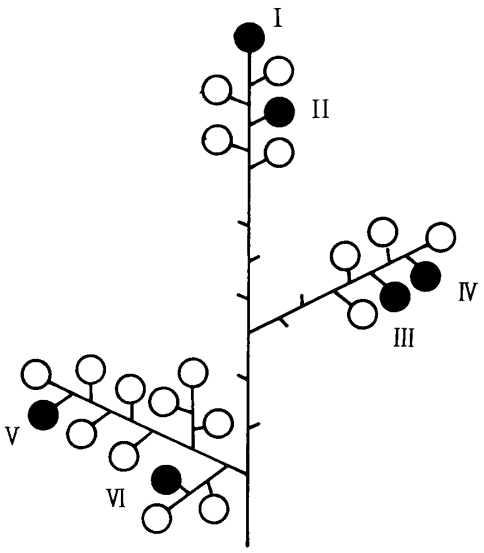

Fig. 1. A schematic diagram of rice panicle representing the position of spikelets used in the present experiment. I : grain I, II : grain II, III : grain III, IV : grain IV, V : grain V and VI : grain VI (for details, see text).

Partitioning of ^{15}N among grains in a panicle

At various stages of reproductive growth from 20 days before to 28 days after heading (see legend in Fig. 2), each of 16 plants in four containers were transferred to a nutrient solution containing ^{15}N-labelled ammonium sulphate (10 atom% ^{15}N, 40 ppm N). After three -days culture in the labelled solution, the plants were returned to the unlabelled solution of 15 ppm N, and were raised under the same environmental conditions.

At full maturity (45 days after heading), 10 panicles with the same heading date and equal size were harvested from each plot. According to the classification of ear type by Sasahara et al[21]., cv. Chukyo-asahi may belong to type I or II, where the number of spikelets on the secondary rachis-branch was numerous in basal position of the panicle and became less towards the top of the panicle. Among a lot of spikelets in a panicle, representative six positions were selected (Fig. 1). Flowering progresses in order from spikelet I to VI within a

period of five to six days[1]. Spikelets I and II on the uppermost primary rachis-branch correspond to the superior spikelets, while spikelet VI on the secondary rachis-branch of lowermost primary rachis-branch is a typical inferior one. The harvested spikelets were dried in an oven at 80°C for three day, then husked grain was used for ^{15}N analysis. Ten grains from each position on panicle were analysed in one lot. In this experiment, chemical analysis of the vegetative organs was not conducted.

Distribution of ^{15}N in a grain

In another series of experiments, distribution of ^{15}N among endosperm, embryo, seed coat and husk in a spikelet was examined. At various growth stages from 20 days before to 25 days after heading (see Table 2), 16 plants in four containers were pulse-labelled with ^{15}N -ammonium sulphate (10 atom% ^{15}N, 40 ppm N) for three days as described above.

At full maturity, 10 panicles with the same heading date and equal size were harvested from each plot. Superior spikelets, that is, the lst, 4th, 5th and 6th spikelets on the uppermost primary rachis-branch were collected and used for ^{15}N determination. These spikelets usually reach anthesis within one or two days. Approximately 40 spikelets were divided into their component parts. Husk and embryo were first detached from the spikelet. After removing both dorsal and ventral portions of endosperm by a razor, the remaining endosperm was dissected into inner and outer parts. Seed coat was stripped off by tweezers. Although most of the aleurone layer was included in the outer part of the endosperm, a part of it got mixed in the seed coat fraction.

Determination of ^{15}N

Total nitrogen content of the samples was determined by the distillation method after semi-micro Kjeldahl digestion. The ^{15}N concentration was measured by emission spectrography[8,30] with a NIA-1 ^{15}N analyzer (Japan Spectroscopic Co. Ltd. Tokyo). Chemical analyses of the samples were repeated three times.

Results

Grain weight and nitrogen content

Dry weight and total nitrogen content of the grains located at various positions on the panicle are shown in Table 1. As the results were practically the same among the seven experimental plots, only the data from the plants fed with labelled nitrogen at heading is shown. The grain I, II and III showed the highest and equal values of both dry weight and nitrogen content, followed by the grain IV, V and VI. The grain VI, a typical inferior grain, had the lowest values of about 75% of the superior grains.

Partitioning of ^{15}N among grains

The changing pattern of ^{15}N partitioning among the grains with the progress of ripening is illustrated in Fig. 2. Generally, concentration of ^{15}N in a tissue means the sink activity of the tissue for the applied nitrogen. In the present experiment, results expressed by both the amount and the concentration of ^{15}N had the same tendency, since the differences in total nitrogen contents were small among the examined grains (Table 1). In plants fed with

Table 1. Dry weight and total nitrogen content of the grains located at various positions on panicle of rice plant fed with labelled nitrogen at heading.

Position on panicle[1]	Dry weight (mg grain^{-1})	Total nitrogen (μg grain^{-1})	Total nitrogen (% on D. W. basis)
Grain I	22.1 (100)	294 (100)	1.33
Grain II	22.8 (103)	306 (105)	1.34
Grain III	21.9 (97)	287 (98)	1.31
Grain IV	20.6 (93)	272 (93)	1.32
Grain V	20.1 (91)	257 (87)	1.28
Grain VI	17.3 (78)	213 (72)	1.23

1) See Fig. 1.

Values are the mean of triplicate analyses.

Numerals in parentheses are the relative values to Grain I (100).

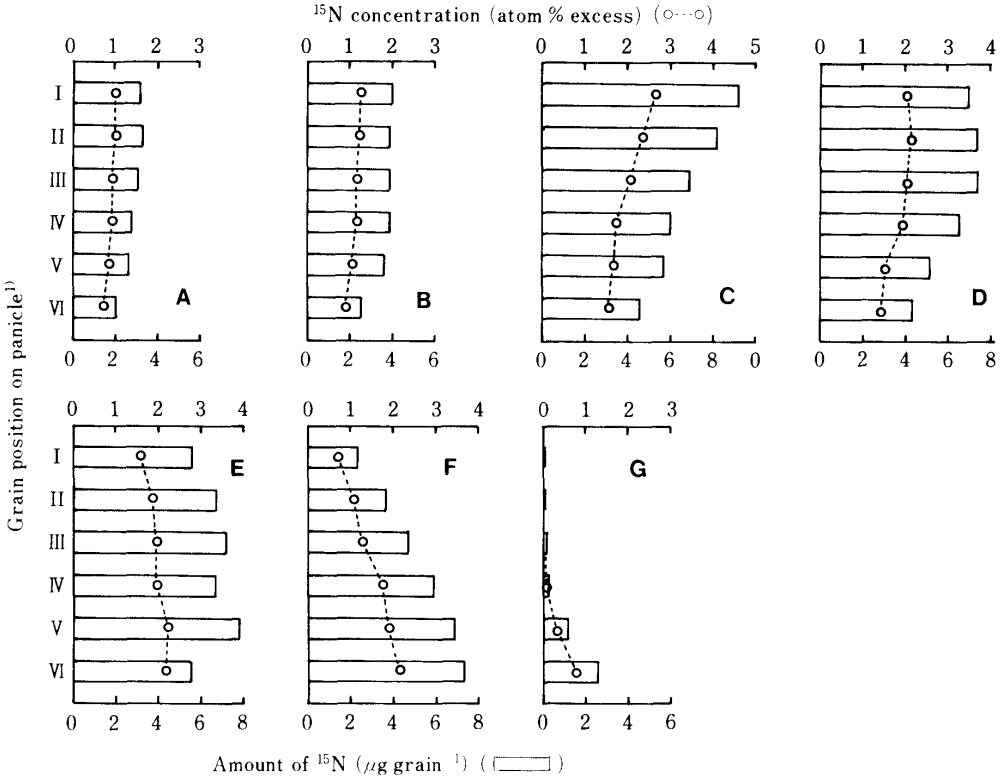

Fig. 2. Partitioning of ^{15}N among the grains located at various positions on rice panicle.
^{15}N-labelled ammonium sulphate was applied at (A) 20 days before heading, (B) 10 days before heading, (C) heading, (D) 7 days after heading, (E) 14 days after heading, (F) 21 days after heading and (G) 28 days after heading

labelled nitrogen 20 days or 10 days before heading, a relatively small but equal amount of ^{15}N was distributed to all grains, with lesser amount distributed to grain VI (Fig. 2 A, B). A large part of labelled nitrogen applied at heading was partitioned to the grains I or II, which flowered earlier and were located on the upper part of the panicle (Fig. 2 C). On the contrary, nitrogen applied at 21 days after heading was mainly transported to grains V or VI, which flowered later and were situated on the basal part of the panicle (Fig. 2 F). When labelled nitrogen was applied at 7 days or 14 days after heading, a relatively equal and large amount of ^{15}N was partitioned to all grains (Fig. 2 D, E). In plants fed with labelled nitrogen 28 days after heading, only a small amount of ^{15}N was transported to grains V and VI, and no labels were detected in grains I and II (Fig. 2 G).

Fig. 3 shows the total amount of ^{15}N accumulated in the six grains examined (grain I -VI), which is calculated from the data in Fig. 2. The result would indicate the efficiency of panicle recovery of nitrogen applied at various growth stages. Nitrogen applied from heading to 14 days after heading was most efficiently transported to the panicle. Nitrogen transport to the panicle slightly decreased at 21 days after heading. Thereafter, only a small amount was recovered by the panicle. Panicle recovery of nitrogen applied before heading was about half of that applied after heading, and the value tended to decrease as the time of nitrogen application was earlier before heading.

Partitioning of ^{15}N among grain tissues

Table 2 shows the ^{15}N concentration in different tissues of the grains which were collected from the uppermost primary rachis -branches of rice plants fed with labelled nitrogen at various growth stages. This kind of data indicates the changes in sink activity of different tissues for the applied nitrogen. When labelled nitrogen was applied before heading, ^{15}N concentration in the husk was higher compared to that in the other parts. The value was kept at a high level until application at 5 days after heading, and thereafter,

declined abruptly. From the application of labelled nitrogen before heading, ^{15}N concentration in the embryo was higher than in the endosperm. While from the application after heading, the situation was quite the reverse where it was consistently higher in the endosperm than in the embryo. Both the embryo and the endosperm showed the highest value from the application just after heading, whereas decline of the value was faster in the embryo than in the endosperm. During the early period of grain filling, the inner and the outer parts of the endosperm showed the equal values of ^{15}N concentration, but a rapid decline was observed in the inner part at the later period of grain filling. ^{15}N concentration in the seed coat was lower than the other tissues during the early and middle period of grain filling, but it was kept at a relatively higher level until the later period.

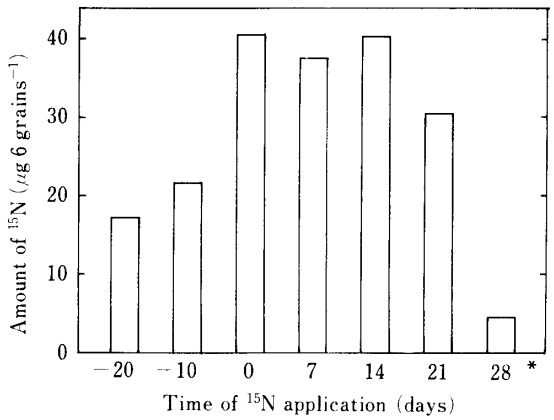

Fig. 3. Total amount of ^{15}N in the six grains (grain I-VI) which was calculated from the data in Fig. 2.
* Days before or after heading.

Fig. 4 indicates the absolute amount of ^{15}N accumulated in each of the spikelet tissues. In all of the ^{15}N application plots, 80 to 90% of labelled nitrogen transported to the grains was partitioned to the endosperm. Accumulation of ^{15}N in the husk virtually ceased by the application at 5 days after heading, whereas the endosperm and the embryo continued to incorporate the applied nitrogen over a relatively long period. Nitrogen applied at heading or 5 days after heading was most efficiently transported to the grains, while that applied at 25 days after heading was scarcely recovered. These results were similar to those in Fig. 3.

Discussion

Panicle recovery of absorbed nitrogen

It has been believed that nitrogen taken up by the rice plants after heading was once incorporated into leaf blades or leaf sheaths, then gradually retranslocated to the panicle[19,29]. Our previous study[3], however, demonstrated the existence of a direct route of nitrogen transport from roots to panicle. Oritani and Yoshida[17] also suggested that the nitrogen top-dressed at heading would be directly translocated to the panicle rather than the leaf blades or leaf sheaths. Although chemical analysis of the vegetative organs was not conducted in the present experiments, a major

Table 2. Concentration of ^{15}N (atom % excess) in different parts of the grains[1] of rice plants fed with labelled nitrogen at various stages of reproductive growth.

Parts of grain	Time of ^{15}N application (days before or after heading)							
	−20	−10	0[2]	5	10	15	20	25
Husk	1.02[3]	1.66	1.11	0.89	0.32	0.42	0.27	0.18
Embryo	0.95	1.36	3.00	3.71	2.07	1.61	1.03	0.20
Endosperm	0.83	1.21	3.26	3.85	2.68	2.23	2.02	0.33
Inner part	0.88	1.30	3.33	4.19	2.55	1.95	1.18	0.16
Outer part	0.81	1.19	3.36	3.83	2.73	2.26	2.00	0.35
Seed coat	—	—	2.72	3.24	1.84	1.59	1.71	0.54

1) Analysed grains were collected from the 1st, 4th, 5th and 6th spikelets on the uppermost primary rachis-branch.
2) Heading time.
3) Values are the mean of triplicate analyses.

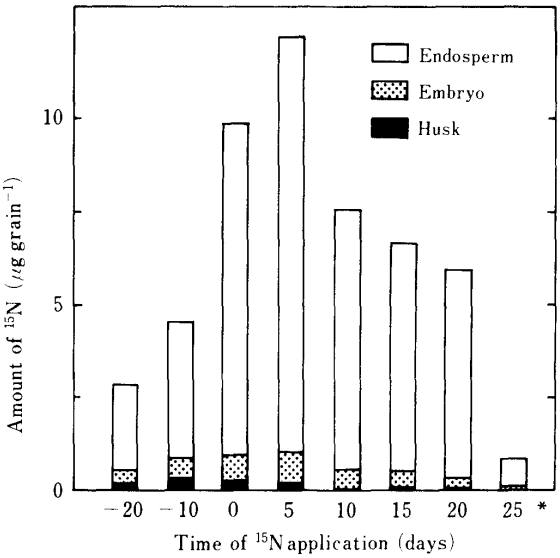

Fig. 4. Absolute amount of ^{15}N accumulated in husk, embryo and endosperm of rice spikelet.
^{15}N-labelled ammonium sulphate was applied at various growth stages of before or after heading.
* Days before or after heading.

part of labelled nitrogen absorbed after heading would be directly transported to the panicle within a short period, and the rest might be translocated to the vegetative organs then retranslocated to the panicle over a relatively long period.

Nitrogen applied at heading or 14 days after heading was most efficiently transported to the panicle, while panicle recovery of nitrogen administered before heading was about half of this (Fig. 3). Muhammad et al.[13] reported that nitrogen taken up during the reproductive stages of the rice plant was largely distributed to the grains at the harvesting stage and this trend was more remarkable by top-dressing carried out at the milk-ripe stage as compared to the early growth stages. Wade et al.[29] also observed the same results on the rice plants grown in a paddy field. These results suggest that nitrogen top-dressing at heading or the milk-ripe stage would be most effective for the purpose of increasing the protein contents of rice grains[6,20,25].

Nitrogen partitioning among grains

At full maturity, labelled nitrogen applied before heading was equally distributed to all grains on the panicle (Fig. 2 A, B). This implies that nitrogen absorbed before heading and incorporated into protein fractions of vegetative organs would begin to degrade after heading, and was gradually retranslocated to the panicle throughout the ripening period. Similar results were also observed on the ^{14}C-labelled carbohydrates assimilated by the rice plants before heading[18]. The grain VI received a smaller amount of ^{15}N absorbed before heading as compared to the other grains (Fig. 2 A, B). This may be due to the deficiency of reserved materials for the completion of the growth of the inferior grains, or the restriction of nitrogen deposition by the small size of the husk of the inferior spikelets[1].

Partitioning of nitrogen applied after heading was not equal among the grains (Fig. 2 C to G). Nitrogen applied at the early period of ripening was mainly distributed to the grains located at the upper part of the panicle, while that applied at the later period of ripening was principally transported to the grains on the basal part. Oshima[18] found that ^{14}C photoassimilated at heading or the milk-ripe stage was principally distributed to the grains located at the upper part of the panicle, while that incorporated at the later ripening stages was mainly partitioned to the grains at the basal part. Similar results were also reported with autoradiography[5] or ^{13}C tracer experiment[27]. These results indicate that the pattern of assimilate partitioning depends on the developmental stage of each grain, and that the position of grains actively accumulating materials moves down from the upper to the basal part of the panicle with ripening. The time lag in active accumulation observed among the grains must originate from the differences in flowering order[1,14] and further in differentiating order of the spikelet on the apical meristem[12]. These results imply that even the inferior spikelets could complete the grain filling if enough assimilates might be supplied at the later stages of ripening.

It is well known that the superior grains accumulate materials rapidly and in a short duration at the early period of ripening, while the inferior ones accumulate materials very slowly and over a long duration at the later period of ripening[10,14]. This fundamental feature of the inferior grains was hardly affected by the changes in source-sink balance. In the previous paper[2], we reported that the nitrogen top-dressing at heading could increase the

rate of nitrogen accumulation in the inferior grains, but not affect the duration or the time of active accumulation. Thus, the differences in the growth characteristics among the grains seems to be the result of inherently determined programs. The competition of assimilates[9,10,16], even if it should take place, would merely facilitate the predetermined differences in the growth characteristics among the grains. The occurrence of various types of poorly-ripened grains which were observed both in the superior and the inferior spikelets, might be related to the temporary shortage of translocating substances as pointed out by several workers[4,10,16,26]. From these considerations, it could be concluded that the increase in source-sink ratio would not be enough for the improvement of grain filling in rice plant. In addition, it seems to be very important to maintain the physiological activities of leaves and roots until the later stages of ripening period when the inferior spikelets continue to accumulate the materials.

Processes of nitrogen deposition in grain tissues

Concentration of ^{15}N in the husk was kept at a higher level when labelled nitrogen was applied by 5 days after heading (Table 2). This indicates that active incorporation of nitrogenous compounds into the husk continued to occur till this stage. Seo and Ota[23] also reported that the amount of nitrogen in the husk increased till the milk-ripe stage, and thereafter began to decrease. They considered that the husk plays an important role in the process of grain development as a temporary pool for the translocating substances.

^{15}N concentration in the seed coat was lower than the other tissues up to the middle period of ripening, while it continued to keep a higher level at the final stages of grain filling. This might be related to the fact that the seed coat fraction included the dorsiventral vascular bundles or the nucellar epidermis which were considered to be a pathway of translocating substances into the endosperm[7,11,22].

Ozaki et al.[20] reported that ^{15}N concentration was highest in embryo, followed by endosperm and bran as a result of the application of ^{15}N-ammonium sulphate at the beginning of shooting. Muhammad et al.[13] stated that the highest ^{15}N abundance was found in the inner part of the endosperm from dressings at the milk-ripe stage. These results are not consistent with those obtained in the present experiment. The reason for the discrepancy is not clear, but it might be partly explained by the differences in the method of grain dissection or the position of grains examined. In the present study, when labelled nitrogen was applied before heading, ^{15}N concentration in the embryo was higher than that in the endosperm. Further, from the application of labelled nitrogen at the later stages of ripening, the decline of the ^{15}N concentration was faster in the embryo than in the endosperm. These results imply that the formation of the rice embryo precedes that of the endosperm.

A rapid decline in ^{15}N incorporation was observed in the inner part of the endosperm as compared to the outer part at the later period of ripening. Oshima[18] and He et al.[5] observed with autoradiography that ^{14}C photoassimilated after the middle stages of ripening distributed only to the outer part of the endosperm and not to the inner part. In rice grains, the translucent area of the endosperm spreads from the central to the outer part as the grain ripens[4]. All of these results indicate that the deposition of storage materials in the rice endosperm is first completed at the central part, then extends to the peripheral layer. During this process, if there is a shortage of assimilates for some reason, the endosperm tissues would not be able to accumulate them, and the result would be various types of ill-ripening[4,10,16,26].

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