A Comparison of the Accumulation and Partitioning of Nitrogen in Plants between Two Rice Cultivars, Akenohoshi and Nipponbare, at the Ripening Stage

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Abstract: To clarify the factors responsible for the maintenance of a high rate of photosynthesis at the ripening stage in the high-yield rice cultivar Akenohoshi, as compared with that in a Japanese standard cultivar, Nipponbare, we investigated the nitrogen content of leaves, focusing on the accumulation and the partitioning of nitrogen in rice plants. The nitrogen content of leaves of plants that were grown in the field or in pots remained higher in Akenohoshi than in Nipponbare during the ripening stage, and there was a close correlation between the rate of photosynthesis and the nitrogen content irrespective of cultivar and treatment. The accumulation of nitrogen in the whole plant was greater in Akenohoshi than in Nipponbare before heading and during the ripening stage. The extent of partitioning of nitrogen to leaves was higher and that to ears was lower in Akenohoshi than in Nipponbare during the ripening stage. By application of additional nitrogen fertilizer to Nipponbare, the nitrogen content of leaves was increased as a result of the increased accumulation of nitrogen in the whole plant and the enhanced partitioning of nitrogen to leaves. Our results indicate that the higher nitrogen content of Akenohoshi leaves was due to the greater accumulation of nitrogen in the plant before heading and during the ripening stage and the more effective partitioning of nitrogen to leaves during the ripening stage, which resulted in the maintenance of a high rate of photosynthesis during ripening.

Key words: Leaf nitrogen content, Nitrogen accumulation, Nitrogen partitioning, Photosynthesis, Rice, Senescence.

The rate of photosynthesis in the leaves of rice plants decreases with time after leaves have fully expanded. No new leaves are formed after heading and, thus, the extent of the decrease in the rate of photosynthesis during the ripening stage has a considerable effect on the production of dry matter and grain yield. Akenohoshi is a cultivar of panicle-weight type and produces heavy dry matter and grain compared with Nipponbare, a Japanese standard cultivar of panicle-number type. The leaves of Akenohoshi maintain a higher rate of photosynthesis throughout the ripening stage than the leaves of Nipponbare (Jiang et al., 1988a; Jiang et al., 1988b). It is extremely important for future breeding of high-yielding cultivars to clarify the physiological characteristics that are related to the maintenance of a high rate of photosynthesis during senescence at the ripening stage.

The decrease in the rate of photosynthesis during senescence is closely related to the decrease in the amount of ribulose-1,5-bisphosphate carboxylase and oxygenase (Rubisco) in leaves (Makino et al., 1985). The leaf nitrogen content is not only a factor that limits the maximum rate of photosynthesis when leaves are fully expanded but it is also a factor that limits photosynthesis during senescence (Mae et al., 1983). There is a close correlation between leaf nitrogen content and Rubisco content because Rubisco accounts for between 25 and 30% of total leaf nitrogen (Makino et al., 1985; Evans, 1989). The Rubisco content of Akenohoshi remains higher than that of Nipponbare and contributes to the higher rate of photosynthesis at the ripening stage (Jiang et al., 1999). It is assumed that the maintenance of the high rate of photosynthesis in Akenohoshi is a result of maintenance of a high nitrogen content. The nitrogen content of leaves is affected by the nitrogen absorption of the plant, the distribution of the absorbed nitrogen to leaves and the translocation of accumulated nitrogen from leaves (Mae and Ohira, 1981). In the present study, we compared the nitrogen content of the leaves of two cultivars of rice (Oryza sativa L.), Akenohoshi and Nipponbare, and examined the possible causes of the difference that we found, focusing on the accumulation and the partitioning of nitrogen in the plants.

Materials and Methods

1. Plant materials and growth of plants

(1) Plants grown in the field

For field studies, intensive experiments were conducted in 1997 and the results were confirmed in 2000. We will describe only the experiment of 1997 here.

Seeds were sown in nursery boxes on May 3, 1997. Seedlings at the fourth-leaf stage were transplanted to the paddy field of the University Farm on alluvial soil of Tama River at a rate of three plants per hill on May 28. The planting density was 22.2 hills m⁻², with a spacing
of 15 cm × 30 cm. Manure was applied at the rate of about 10 t per ha before puddling, and compound fertilizer, containing 14% each of N, P₂O₅, and K₂O, was applied as basal dressing at a rate of 50, 50 and 50 kg each per ha, respectively. Top dressing was applied at a rate of 10 kg each per ha for N and K₂O on July 16 and 20 kg each per ha on August 1. The field was kept under submerged conditions throughout the course of experiments. Heading occurred on August 25 in both cultivars. Both cultivars were harvested on October 14. The experiment was laid out in a randomized complete block with three replicates (17 m² for each replicate).

(2) Plants grown in pots

Experiments were carried out in 1998 and 2001, and similar results were obtained. Here, we will describe the experiment of 2001 when intensive studies were made taking into consideration of the results obtained in 1998.

Seeds were sown in the nursery boxes on May 27, 2001. Seedlings at the fourth leaf stage were transplanted to Wagner pots (1/2000 a) filled with a mixture of alluvial soil from the Tama River and Kanto diluvial soil (1:1, v/v) and grown under submerged conditions. The planting density was three plants per hill and four hills per pot. Fertilizer was applied as basal dressing at the rate of 1 g each per pot of N, P₂O₅, and K₂O. Top dressing was applied at the rate of 1 g each per pot of N and K₂O on July 20 and on August 5. Ammonium sulfate was applied to the plants of some pots with Nipponbare at a rate of 2 g per pot of N as additional nitrogen fertilizer (NF) on September 1. Plants were grown under submerged soil conditions throughout the course of experiments. Heading occurred on August 31 in both cultivars. Pots for each plot were placed randomly in the upland field of the University farm with a spacing of 80 cm × 50 cm.

2. Determination of photosynthetic rate

The photosynthetic rate was measured, with the flag leaf attached to the main culm, in the laboratory with an open gas-analysis system equipped with an assimilation chamber. The concentration of CO₂ and the dew point of the air pumped into and out the chamber was measured with an infrared gas analyzer (ZALDD252-10, Fuji Electric. Inc., Tokyo) and dew point hygrometer (TS-1E, Heinz Walz Inc., Effeltrich), respectively. Light was provided by metal halide lamps (M1000B-U, Mitsubishi Electric. Inc., Tokyo). Leaf and air temperatures were measured with a copper-constantan thermocouple of 0.1 mm in diameter. The air temperature in the chamber was controlled via the circulation of temperature-regulated water. The rate of air flow through the chamber was regulated with a mass flow controller (SEC-521, Stec Inc., Kyoto). The intensity of light at the leaf surface, the rate of air flow, the concentration of CO₂, the air temperature in the chamber and the difference in water vapor pressure difference between the leaf and the air were 1.400 μmol m⁻² s⁻¹, 1.126 μmol s⁻¹, 350 ± 0.1 μL L⁻¹, 30 ± 1°C and 9 ± 1 mmHg, respectively.

3. Determination of the nitrogen content of plants

The nitrogen content of plants was determined as follows. Eight hills with an average number of stems were selected from each plot and plants were separated into leaves, culms plus leaf sheaths, and ears for the experiment in the field, and into the three above-ground organs and roots for the experiment in pots. Then samples were dried in an oven at 90°C for 72 h. After weighing, dried samples were powdered with a ball mill (MB-1, Choukako Inc., Aichi). The total nitrogen concentration was determined with a CN analyzer (MT-600, Yanako Inc., Kyoto) and nitrogen content was calculated as the product of dry weight and the nitrogen concentration. Leaves that have been subjected to measurements of photosynthetic rates were digested by the Kjeldahl-Gunning method and nitrogen concentrations were determined by the indophenol method (Weatherburn, 1967).

Results

1. Comparisons of rates of photosynthesis and leaf nitrogen contents at the ripening stage

We compared the time course of changes in the rate of photosynthesis in flag leaves during the ripening stage between two cultivars when they were grown in pots (Fig. 1A). The rate of photosynthesis was maximal at the heading stage and it was slightly higher in Akenohoshi than in Nipponbare. The rates of photosynthesis decreased with time in both cultivars after heading, but rates of decrease were smaller and remained higher in Akenohoshi than in Nipponbare throughout the ripening stage. Thus, we confirmed that rates of photosynthesis in leaves during senescence remained higher in Akenohoshi than in Nipponbare.

We next compared the time courses of changes in nitrogen content of the flag leaves used for the measurements of photosynthetic rates. There were no differences between the two cultivars in terms of leaf nitrogen content at the heading stage. After heading, the nitrogen content decreased more slowly in Akenohoshi than in Nipponbare, and Akenohoshi retained larger amounts of nitrogen in its leaves (Fig. 1B). The leaf nitrogen content was closely correlated with the photosynthetic rate irrespective of the cultivar, and the relationship between the nitrogen content and the photosynthetic rate did not differ between the two cultivars (Fig. 2).

Next, we compared the nitrogen contents of plants grown in the field with reference to leaf positions (Table 1). The respective nitrogen contents of the flag and fifth leaves were similar in the two cultivars at the heading stage, while the nitrogen content of the third leaf was higher in Akenohoshi than in Nipponbare. At the late-ripening stage, the leaves of Akenohoshi contained more nitrogen than those of Nipponbare irrespective of posi-
Fig. 1. Changes in rates of photosynthesis (A) and in nitrogen content (B) of flag leaves in plants grown in the field at the heading stage.

○, Nipponbare; ●, Akenohoshi.

* and **: Values are significantly different at the 5% and 1% level, respectively (Student's t-test). Values in parentheses are values relative to those at the heading stage.

Fig. 2. Relationships between nitrogen content and the rate of photosynthesis of flag leaves in plants grown in the pots after heading.

○, Nipponbare; ●, Akenohoshi.

Fig. 3. Comparison between the nitrogen contents of above-ground parts of plants grown in the field.

○, Nipponbare; ●, Akenohoshi.

Means followed by different letters are significantly different at the 5% level (Student's t-test). The increase in nitrogen content (mg hill⁻¹) is indicated in the inserted table. Nip and Ake refer to Nipponbare and Akenohoshi, respectively. Periods (1) and (2) refer to Aug. 5-Aug. 25 and Aug. 25-Oct. 4, respectively.

was significantly different in the fifth leaves between the two cultivars.

2. Comparisons of the accumulation and partitioning of nitrogen between cultivars

(1) Plants grown in the field

Figure 3 shows the nitrogen contents of the above-ground parts of plants grown in the field at the panicle-formation, heading and late-ripening stages. The nitrogen content increased markedly from the panicle-formation stage to the heading stage and increases were smaller after heading in both cultivars. There were no differences in terms of nitrogen content between the two cultivars at the panicle-formation stage. At heading, the nitrogen content was higher in Akenohoshi than in Nipponbare and the difference between the two cultivars had become much larger by the late-ripening stage. The increase in nitrogen content from the panicle-formation stage to the heading stage and from the heading stage to late-ripening stage was approximately 1.8 and 1.5 times greater in Akenohoshi, respectively, than in Nipponbare (Fig. 3).

Details of the partitioning of nitrogen in plants of two
whole plants of Nipponbare could be increased to that of Akenohoshi by the application of nitrogen (NF). The whole plants between the two cultivars prompted us to in nitrogen content from the heading stage to the late-ripening stage. Thus, the concentrations of nitrogen in leaves were slightly higher at the heading stage and 1.5 times higher at the late-ripening stage in Akenohoshi than in Nipponbare. This difference was the result of the more extensive accumulation of nitrogen in above-ground parts (Fig. 3) and the greater partitioning of nitrogen to leaves in Akenohoshi. Thus, the nitrogen concentrations of leaves were similar at the panicle-formation and heading stages but higher in Akenohoshi than in Nipponbare at the late-ripening stage (Table 2).

(2) Plants grown in pots
To compare the accumulation of nitrogen by whole plants, including roots, we determined the nitrogen contents of the plants grown in pots (Table 3). At the heading stage, the nitrogen content of whole plants tended to be higher in Akenohoshi than in Nipponbare, resembling the results obtained in the field. The increase in nitrogen content from the heading stage to the late-ripening stage was 2.3 times higher and, as a result, the nitrogen content of whole plants was 1.2 times higher in Akenohoshi than in Nipponbare at the late-ripening stage.

The large differences in nitrogen accumulation in whole plants between the two cultivars prompted us to investigate whether the accumulation of nitrogen by whole plants of Nipponbare could be increased to that of Akenohoshi by the application of nitrogen (NF). The increase in the nitrogen content from the heading stage to the late-ripening stages was approximately ten times higher in NF-treated Nipponbare than that in controls. As a result, the nitrogen content of whole plants was very much higher in NF-treated Nipponbare than in the controls at the late-ripening stage (Table 3).

The partitioning of nitrogen to the various organs is summarized in Table 4. There were no differences in the partitioning to leaves at the heading stage. At the late-ripening stage, the extent of partitioning was 29% in Akenohoshi, as compared with 23% in Nipponbare, while the partitioning to ears was 44% in Akenohoshi, as compared with 52% in Nipponbare. More residual nitrogen was partitioned to roots and less to culms plus leaf sheaths in Akenohoshi, as compared with Nipponbare. As a result of the greater accumulation of nitrogen in whole plants and the greater partitioning of nitrogen to leaves, the nitrogen content of leaves was slightly higher at the heading stage and 1.5 times higher at the late-ripening stage in Akenohoshi than in Nipponbare. Thus, the concentrations of nitrogen in leaves were higher in Akenohoshi than in Nipponbare at the late-ripening stage (Table 4).

After the application of additional nitrogen fertilizer to Nipponbare, the nitrogen contents of all organs increased, and nitrogen concentrations were much higher than in controls. In addition, large differences in nitrogen partitioning to respective organs were apparent. The partitioning of nitrogen to leaves increased and was 30%
Table 4. Comparison of nitrogen contents, nitrogen concentrations and nitrogen partitioning in plants grown in pots.

| Organ       | Nitrogen content (mg hill⁻¹) | Nitrogen concentration (%) | Nitrogen partitioning (%) |
|-------------|------------------------------|----------------------------|---------------------------|
|             | Heading (Aug.31) | Late ripening (Sep.25) | Heading (Aug.31) | Late ripening (Sep.25) | Heading (Aug.31) | Late ripening (Sep.25) |
| Leaves      |                 |                            |                           |                           |                 |                            |
| Nipponbare (control) | 292.4 a         | 142.9 a                    | 2.24 a                    | 1.21 a                    | 49.4 a           | 22.6 a                     |
| Akenohoshi (control) | 324.1 a         | 219.5 b                    | 2.19 a                    | 1.55 b                    | 50.1 a           | 29.3 b                     |
| Nipponbare (NF) | 307.2 c         |                            |                            |                            | 30.0 b           |                            |
| Culms + leaf sheaths | 202.0 a         | 114.1 a                    | 0.76 a                    | 0.58 a                    | 34.0 a           | 17.9 a                     |
| Akenohoshi (control) | 190.3 a         | 122.8 a                    | 0.70 a                    | 0.56 a                    | 29.3 b           | 16.3 b                     |
| Nipponbare (NF) | 202.3 b         |                            |                            |                            | 19.7 c           |                            |
| Ears        |                 |                            |                           |                           |                 |                            |
| Nipponbare (control) | 58.2 a          | 328.7 a                    | 1.34 a                    | 1.32 a                    | 9.8 a            | 51.5 a                     |
| Akenohoshi (control) | 68.3 b          | 331.5 a                    | 1.42 a                    | 1.31 a                    | 10.6 a           | 44.3 b                     |
| Nipponbare (NF) | 459.4 b         |                            | 1.64 b                    |                            | 44.8 b           |                            |
| Roots       |                 |                            |                           |                           |                 |                            |
| Nipponbare (control) | 39.8 a          | 50.1 a                     | 0.93 a                    | 0.84 a                    | 6.8 a            | 8.0 ab                      |
| Akenohoshi (control) | 64.3 b          | 74.6 b                     | 0.88 a                    | 0.85 ab                   | 10.0 b           | 10.1 a                      |
| Nipponbare (NF) | 55.7 ab          |                            |                            |                            | 5.5 b            |                            |

Nitrogen partitioning is expressed as the nitrogen content of the respective organ, calculated as a percentage of the total nitrogen content of the whole plant. Means followed by different letters are significantly different at the 5% level (LSD).

Our results demonstrated that, in Akenohoshi, the nitrogen content of leaves remained high because of the greater accumulation of nitrogen in plants before heading and during ripening and because of the greater partitioning of nitrogen to leaves at the ripening stage, which resulted in the maintenance of a higher rate of photosynthesis during ripening. We also found that a high level of nitrogen in leaves could be maintained by the application of additional nitrogen fertilizer, as a result of the elevated partitioning of nitrogen to leaves, in addition to the increase in nitrogen accumulation by the whole plant.

Discussion

The nitrogen content of leaves during senescence remained higher in Akenohoshi than in Nipponbare, as did, the rate of photosynthesis, and there was a close correlation between the rate of photosynthesis and the leaf nitrogen content in both cultivars. It was reported previously that the rate of photosynthesis during leaf senescence is closely correlated to the amount of Rubisco, which accounts for 25 to 30% of the leaf nitrogen and, also, that there is a close correlation between the Rubisco content and the nitrogen content in many plant species (Makino et al., 1984; Makino et al., 1985). Akenohoshi retains a higher level of Rubisco in leaves than Nipponbare and there is a close correlation between the Rubisco content and the photosynthetic rate at the ripening stage in both Akenohoshi and Nipponbare (Jiang et al., 1999). We confirmed that a high rate of photosynthesis and a high level of Rubisco were sustained by the retention of large amounts of nitrogen in leaves of Akenohoshi at the ripening stage.

Some of the nitrogen taken up by rice plants evaporates into the atmosphere as ammonia from the leaves (Sekimoto et al., 1985) but the amount of such evaporated nitrogen is small enough to ignore. Therefore, we can regard the amount of nitrogen accumulated in a whole plant as the nitrogen absorbed during its growth. There have been many studies on nitrogen absorption by plants grown under different conditions and by different cultivars and these investigations included determinations of changes in nitrogen accumulation (Miyama and Okano, 1986; Higuchi and Yoshino, 1986). The absorption of nitrogen by rice plants increases rapidly from the tillering stage to the panicle-formation stage and then it decreases from the panicle-formation stage to the heading stage (Mae, 1982), until, after heading, it becomes to be very limited. There are large differences in nitrogen absorption after heading among the methods of application of fertilizer and among cultivars, which resulted in differences in the maintenance of a high rate of photosynthesis at the ripening stage (Wada et al., 1971; Miyama and Okabe, 1986; Higuchi and Yoshino, 1986). In some cases, no absorption of nitrogen is detectable after heading while, in other cases, it accounts for 30 to 40% of the total nitrogen absorbed (Mae 1982). In the present study, the accumulation of nitrogen by above-ground parts was very considerable before heading in rice plants grown in the field. While the accumulation of nitrogen became relatively limited after heading, a difference in accumulation between cultivars was clear. In other words, at the panicle-formation stage, the absorption of nitrogen accounted for more than 60% of the total accumulation in above-ground parts in both
The nitrogen content of ears (Wada et al., 1973). The proportion of nitrogen translocated to ears from other organs depends on whether a larger amount of nitrogen or a limited amount of nitrogen is taken up after heading (Wada et al., 1973), being higher in the latter case than in the former (Wada et al., 1973). The less extensive partitioning of nitrogen to ears and the greater partitioning of nitrogen to leaves in Akenohoshi (Table 2 and Table 4) might be caused by the high capacity of this cultivar for nitrogen absorption. The dry weight of ears per hill was significantly greater in Akenohoshi than in Nipponbare. However, the difference in terms of the nitrogen content between the ears of the two cultivars was very small compared with that between the leaves because of the lower concentration of nitrogen in the ears of Akenohoshi than in those of Nipponbare (Table 2). This observation indicates that nitrogen partitioning might be affected by other mechanisms in these plants. After application of additional nitrogen fertilizer to Nipponbare, the concentrations of nitrogen in all organs increased because of the enhanced absorption of nitrogen. There were differences in terms of the extent of the increase in nitrogen concentration between respective organs. The increase in nitrogen concentration was large in leaves, while it was small in ears and roots, and the extent of partitioning of nitrogen to leaves was large, and that to ears and roots was small. These results indicate that nitrogen absorption affects nitrogen partitioning to various organs in rice plants and that increases in absorption of nitrogen enhance the partitioning of nitrogen to leaves.

Plant hormones might participate in the partitioning of nitrogen among the organs of rice plants. Cytokinin affects not only leaf senescence but also the partitioning of nitrogen in whole plants (Beck, 1996; Wagner and Beck, 1993; Simpson et al., 1982; Jordi et al., 2000). It suppresses the senescence of lower leaves, in particular (Jordi et al., 2000). The amounts of cytokinin transported from the roots to the above-ground parts of plants grown in the field are larger in Akenohoshi than in Nipponbare (Soejima et al., 1992; Soejima et al., 1995). In the present study, the activities of cytokinin in the xylem sap of plants grown in pots were also higher in Akenohoshi than in Nipponbare, and the activities were increased by the application of additional nitrogen fertili-
lizer to Nipponbare (Table 5). These results suggest nitrogen partitioning might be affected by cytokinin and that cytokinin might be one of the factors responsible for the partitioning of larger amounts of nitrogen to leaves in Akenohoshi as compared with Nipponbare. It has also been reported that cytokinin enhances nitrogen absorption (Tweedy and Ries, 1967; Parkash, 1982; Trckova and Kaminek, 1999). The effects of cytokinin on levels of leaf nitrogen, nitrogen absorption and nitrogen partitioning to leaves remain to be investigated.

In this study, we showed that the maintenance of a high level of nitrogen in leaves during the ripening stage and, therefore, the high rate of photosynthesis in Akenohoshi, as compared with that in Nipponbare, was a result of the high capacity of Akenohoshi for nitrogen absorption at the panicle-formation and ripening stages and of the partitioning of large amount of nitrogen to leaves and limited amount of nitrogen to ears.

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*In Japanese with English abstract.
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