Diurnal and Phenological Changes in the Rate of Nitrogen Transportation Monitored by Bleeding in Field-Grown Rice Plants (*Oryza sativa* L.)

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Abstract: Nitrogen uptake is essential for rice growth and yield. Thus, the development of a simple and rapid method for monitoring nitrogen absorption is strongly required. We examined the fundamental properties of nitrogen transportation monitored by bleeding, including diurnal and phenological changes, to discuss whether the analysis of bleeding sap could be used for monitoring nitrogen uptake by rice. The rate of nitrogen transportation monitored by bleeding was estimated from a combination of the nitrogen concentration in bleeding sap and bleeding rate. We found a clear diurnal change in the rate of nitrogen transportation monitored by bleeding; it was higher in the daytime than at night. In this study, the diurnal change in nitrogen uptake was not influenced by soil temperature but by the light condition. The rate of nitrogen transportation monitored by bleeding showed a phenological change with a peak around the panicle formation stage, while the bleeding rate peaked at around heading and was correlated with root length. The nitrogen concentration in bleeding sap continued to decrease gradually from the early growth stage. The cumulative amount of nitrogen uptake estimated by the bleeding sap analysis was less than half of that estimated by the plant analysis before the maximum tiller number stage, but the difference between these values decreased with plant growth. There was a significant positive correlation between the cumulative amount of nitrogen uptake estimated by these analyses throughout the growing period.

Key words: Bleeding sap, Diurnal change, Nitrogen uptake, Phenological change, Rice, Root length.

Nitrogen is an important nutritional element for photosynthesis (Ishihara et al., 1979), growth and yield (Wada et al., 1989; Wada and Sta Cruz, 1990) of rice (*Oryza sativa* L.), but nutritional diagnosis based on the conventional analysis of rice plants requires tedious procedures such as grinding and extraction. For this reason, a simple and rapid method for monitoring nitrogen uptake is strongly required to improve rice cultivation.

Bleeding sap is the xylem sap exuded from detopped plant stumps, and is probably caused by root pressure (Schurr, 1998). The bleeding rate, the weight of sap bled per unit time after detopping, is known to relate with physiological traits of the root system such as the root respiratory rate (Yamaguchi et al., 1995) and the specific absorption rate of nitrogen (Samejima et al., 2004). In addition, concentration of various nutrients in the xylem sap is affected by both nutrient concentration in culture medium and root function of active absorption (Yoneyama et al., 1995). So the chemical analysis of bleeding sap could be effective for plant nutritional diagnosis. In fact, Sakaigaichi et al. (2005) showed that the analysis of bleeding sap is quite effective for obtaining information on the uptake of top-dressed nitrogen. However, fundamental information on nitrogen dynamics in bleeding sap, especially through whole growing period, is still quite limited.

In this paper, diurnal and phenological changes in the rate of nitrogen transportation monitored by bleeding in relation to root growth were examined in rice plants grown in a farmer’s paddy field. At the same time, phenological changes in nitrogen uptake were estimated by bleeding sap analysis to compare with results from conventional chemical analysis of the plant to examine whether analysis of bleeding sap could be a rapid and reliable method to monitor nitrogen uptake by rice plants.

Materials and Methods

1. Plant materials

The leading cultivar of lowland rice in Japan, Koshihikari, was grown in a farmer’s paddy field in Sakura, Chiba Prefecture, Japan (latitude 35°43’N, longitude 140°13’E), in 2002. Seedlings at the 3.0 through 3.5-leaf stage were mechanically transplanted...
on April 27 where the planting density was about 18 hills m$^{-2}$ (29 cm × 19 cm spacing). Chemical fertilizer was applied at the rate of 1.35 g N, 6.75 g P$_2$O$_5$, and 3.24 g K$_2$O m$^{-2}$ as basal dressings before transplanting, and at the rate of 1.05 g N, 0.28 g P$_2$O$_5$, and 1.05 g K$_2$O m$^{-2}$ as topdressing at the panicle formation stage on July 17. The paddy field was continuously flooded except for the periods of midseason drainage (June 21-30) and ponding water release (August 16-28). The maximum tiller number stage was on June 25, and the heading date was August 1. Average ear weight of rice plants was about 844 g m$^{-2}$ at the time of harvesting (August 28).

2. Collection and nitrogen analysis of bleeding sap

The diurnal change in the rate of nitrogen transportation monitored by bleeding was examined 21 and 22 d before heading (i.e., July 3-4). Bleeding sap was collected every 2 hr (0900-1000 on July 3 to 0900-1000 on July 4) from different groups of six hills with an average number of stems to clarify diurnal changes in nitrogen uptake. Additionally, bleeding sap was analyzed every week from 50 d before heading (June 12) to 27 d after heading (August 28) to clarify phenological changes in nitrogen uptake. For each measurement, bleeding sap was collected from eight hills with an average number of stems in the morning (0900-1000).

The bleeding rate, defined as the weight of xylem sap bled in the first hour after detopping, was measured using cotton traps (Morita and Abe, 2002). After the removal of shoots at around 15 cm above the ground, preweighed absorbent cotton (about 1-2 g, depending on the growth stage of rice) was placed on the cut surface of the stump and wrapped in plastic film with a rubber band. The cotton was weighed again after 1 hr and the increase was used to determine the bleeding rate. After weighed, the bleeding sap was collected in a plastic tube by squeezing the cotton, and it was preserved in a freezer at −20°C. In case of diurnal change, we collected the bleeding sap every 2 hr from six different rice plants to minimize the effect of shoot removal.

The nitrogen concentration of bleeding sap was measured by the alkaline potassium peroxodisulfate-ultraviolet spectrophotometric method (Tsuzuki and Uchino, 1994; Sakaigaichi et al., 2005). Namely, 250-times diluted potassium peroxodisulfate/sodium hydride solution was added to the sap to change all organic and inorganic nitrogen in the sap into the nitrate form (NO$_3^-$) by autoclave heating. Then, the nitrate concentration was measured with a spectrometer (DU640, Beckman Coulter K. K., Fullerton, CA USA). The rate of nitrogen transportation monitored by bleeding was calculated according to the following formula:

Fig. 1. Diurnal change in bleeding rate and the rate of nitrogen transportation monitored by bleeding. A, Bleeding rate; B, Nitrogen concentration in bleeding sap; C, Rate of nitrogen transportation monitored by bleeding. The vertical bars indicate standard error of the means of six hills.
Rate of nitrogen transportation monitored by bleeding \((\mu g \text{ hill}^{-1} \text{ hr}^{-1})\)
\[= \text{Bleeding rate (g hill}^{-1} \text{ hr}^{-1}) \times \text{Nitrogen concentration in bleeding sap (g g}^{-1}) \text{)} \quad (1)\]

Cumulative amount of nitrogen uptake based on bleeding sap analysis was estimated as follows:

Cumulative amount of nitrogen uptake based on bleeding sap analysis (g hill\(^{-1}\))
\[= \sum_{t}^{12} \left\{ \frac{(\text{Rate of nitrogen transportation monitored by bleeding at } (t_{i-1}) + \text{Rate of nitrogen transportation monitored by bleeding at } (t_{i}))}{2 \times (t_{i} - t_{i-1})} \right\} \quad (2)\]

where \(t_{i-1}\) and \(t_{i}\) are the time of adjoining measurements.

3. Nitrogen analysis of the plant

Three plants with an average number of stems were measured for nitrogen concentration in the plant at each growth stage. The rice plants were separated into leaf blades and culms with leaf sheaths and ears. Samples were then dried at 80°C for 48 hr and weighed. After weighed, dried samples were ground and digested using the Kjeldahl method, and the nitrogen concentration was then measured with an autoanalyzer (TRAACS-2000, Bran+Luebbe K. K., Norderstedt, Germany). The nitrogen content of the plant (g hill\(^{-1}\)) was calculated as the sum of nitrogen content of each plant organ; namely, the multiplied product of dry weight (g hill\(^{-1}\)) \(\times\) nitrogen concentration (%). Net nitrogen uptake was estimated from the increase in nitrogen content of the plant.

4. Root length

The phenological change of root length was investigated from 37 d before heading (June 25) to 27 d after heading (August 28). To measure root length,
we chose three typical hills from the eight hills used for bleeding sap collection based on stem number. Soil monoliths (15 cm in diameter and 15 cm in depth) with roots were taken using a metallic cylinder. After washing out the roots, root length was measured using a root length scanner (Comair Root Length Scanner, Commonwealth Aircraft Corp. Ltd, Melbourne, Australia).

**Results**

1. **Diurnal changes in the rate of nitrogen transportation monitored by bleeding**

   The bleeding rate began to increase before dawn (0300-0400) and reached the maximum value (4.0 g hill$^{-1}$ hr$^{-1}$) early in the morning (0700-0800), although the bleeding rate was rather stable in other periods (Fig. 1A). The nitrogen concentration in the bleeding sap began to increase rapidly just after sunrise and reached a maximum value (81 $\mu$g g$^{-1}$) in the morning (0900-1000). It decreased gradually during the day, and rapidly just after sunset (Fig. 1B). The rate of nitrogen transportation monitored by bleeding showed almost the same diurnal change as that of the nitrogen concentration in the bleeding sap (Fig. 1C). This was because the variation in bleeding rate was rather small (Fig. 1A). Soil temperature at 10 cm below the soil surface reached a maximum value (24.5$^\circ$C) at 1500-1600 and decreased to a minimum value (21.5$^\circ$C) at 0700-0800 in the morning (Fig. 2). Soil temperature had no significant positive correlation with bleeding rate and the rate of nitrogen transportation monitored by bleeding (data not shown).

2. **Phenological changes in the rate of nitrogen transportation monitored by bleeding**

   The bleeding rate increased with shoot growth to a maximum value of 9.3 g hill$^{-1}$ hr$^{-1}$ around heading. It then decreased rapidly during the grain-filling period (Fig. 3A). The nitrogen concentration in bleeding sap decreased gradually from much earlier growth stages than the bleeding rate (Fig. 3B). The rate of nitrogen transportation monitored by bleeding showed phenological changes similar to the bleeding rate because variations in the nitrogen concentration were rather small. It reached maximum values at an earlier growth stage than bleeding rate, around 10-20 d before heading (Fig. 3C).

3. **Relationships between the rate of nitrogen transportation monitored by bleeding and root length**

   The root length increased to a maximum value at around heading and decreased rapidly thereafter

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Fig. 4. Phenological change in root length. The vertical bars indicate standard error of the means of three hills.

Fig. 5. Correlation of between root length with the bleeding rate, and the rate of nitrogen transportation monitored by bleeding. A, Relationship between root length and bleeding rate. ** indicates significant difference at 1% level of probability; B, Relationship between root length and the rate of nitrogen transportation monitored by bleeding. * indicates significant difference at 5% level of probability.
There was a significant positive correlation between the bleeding rate and root length throughout the growing period (Fig. 5A). The rate of nitrogen transportation monitored by bleeding showed linear relationships with root length, but the regression lines between them were different before and after heading (y = 1.83x + 156 before heading and y = 1.07x + 53.7 after heading). The rate of nitrogen transportation monitored by bleeding per root length was much lower after heading than before heading (Fig. 5B).

4. Comparison of the nitrogen uptake estimated by bleeding sap and plant analyses

The nitrogen content of the plant (shoot in Fig. 6) increased with the growth to reach the maximum value at 12 d after heading (Fig. 6). The cumulative nitrogen uptake estimated by the bleeding sap analysis was less than half of that estimated by the plant analysis before the maximum tiller number stage, and the differences between these values decreased with plant growth (Fig. 7A). The cumulative nitrogen uptake estimated by the two analyses did not fit completely, but there was a significant positive correlation between these values throughout the whole growing period (Fig. 7B).

Discussion

1. Diurnal changes in the rate of nitrogen transportation monitored by bleeding

There was a clear diurnal change in the rate of nitrogen transportation monitored by bleeding where it was greater in the daytime than at night (Fig. 1C). This is probably the common pattern of diurnal change in rice plants under a flooded condition, and we previously reported a similar diurnal change in pot-grown rice plants at the panicle formation stage (Sakaigaichi et al., 2005). Shoot removal for the collection of sap might affect the rate of nitrogen uptake, but Delhon et al. (1995) reported quite similar diurnal changes in the nitrate uptake by intact soybean plants that was determined by the depletion of nitrate in the culture medium. In addition, they found a decrease in the nitrate uptake rate during the dark period, although there was no such decrease under continuous light conditions. The light condition might be one of the most important factors affecting nitrogen uptake. Soil temperature may affect the rate of nitrogen transportation monitored by bleeding because both bleeding rate and root respiratory rate depend on temperature (Yamaguchi et al., 1995). However, there was no significant correlation...
between soil temperature and the rate of nitrogen transportation monitored by bleeding in this study (data not shown).

The rate of nitrogen transportation monitored by bleeding was calculated from the combination of the bleeding rate and nitrogen concentration in bleeding sap. Because variations in the diurnal change of nitrogen concentration in bleeding sap were much greater than that of bleeding rate (Fig. 1A, B), it is likely that diurnal change in the rate of nitrogen transportation monitored by bleeding is greatly influenced by the nitrogen concentration in bleeding sap.

2. Phenological changes in the rate of nitrogen transportation monitored by bleeding

The phenological changes in the rate of nitrogen transportation monitored by bleeding in field-grown rice plants remain unclear because of the lack of reports on this topic. Oritani and Yoshida (1970) showed that the rate of nitrogen transportation monitored by bleeding per day increased to a maximum value at the panicle formation stage and rapidly decreased thereafter in the pot-grown rice cultivar, Hounenwase. Our field experiment indicated a similar phenological change, although the peak was slightly later (10-20 d before heading), and the maximum rate of nitrogen transportation monitored by bleeding remained for about 2 wk (Fig. 3C). These results indicate that lowland rice cultivars grown under flooded conditions show a similar phenological change in nitrogen uptake having a peak around the panicle formation stage.

3. Factors affecting the phenological changes of the rate of nitrogen transportation monitored by bleeding

In this study, the rate of nitrogen transportation rate monitored by bleeding was calculated as the product of bleeding rate and nitrogen concentration in the bleeding sap.

Bleeding rate showed a large phenological change (Fig. 3A). We consider that root growth was a major factor determining the bleeding rate, because root length and bleeding rate showed quite similar phenological patterns, and there was a significant positive correlation between them throughout the growing period (Figs. 3A, 4, 5A).

The nitrogen concentration in bleeding sap decreased gradually from the early growth stage (Fig. 3B). The concentration of various nutrients in the xylem sap is affected by both nutrient concentration in culture medium and root function of active absorption (Yoneyama et al., 1995). The decrease in nitrogen concentration may be caused by (i) the soil nitrogen condition, and (ii) a decrease in the physiological activity of the whole root system because of the aging of individual roots.

In this study, chemical fertilizer was applied as a basal dressing (96 d before heading, 1.35 g N m\(^{-2}\)) and topdressing (15 d before heading, 1.05 g N m\(^{-2}\)). Although the nitrogen concentration in the soil is usually kept high enough until around the maximum tiller number stage, most of the soil ammonium nitrogen derived from the basal dressing disappeared before this growth stage (Ando et al., 1978). Such a decrease in nitrogen concentration in the soil may be a reason why the nitrogen concentration in bleeding sap decreased with growth. In later growth stages, mineralized organic nitrogen in soil may be the main source of nitrogen because most nitrogen top-dressed at the panicle formation stage is absorbed within 7-9 d (Ando et al., 1985). Sakaigaichi et al. (2005) also showed that ammonium sulfate top-dressed at the panicle formation stage increased the rate of nitrogen transportation monitored by bleeding drastically, but for only 7 d in rice. However, in this study, we measured the nitrogen concentration weekly, and detected only a slight fluctuation in nitrogen concentration caused by topdressing (15 d before heading in Fig. 3B). Therefore, the decrease in nitrogen concentration in the soil during the growing period may be responsible for the decrease in nitrogen concentration in bleeding sap with growth in this study.

The nitrogen concentration in bleeding sap gradually decreased during the growing period probably due to the aging and senescence of roots. Considering the life course of an individual root, its nitrogen uptake activity is low at early developmental stages and increases with the development of lateral roots. It then decreases gradually with root senescence (Tatsumi and Kono, 1980). The respiration rate of roots per unit root dry weight continues to decrease gradually with the growth of rice plants (Yamada et al., 1952). This decline in root respiration rate is probably due to an increase in the proportion of aged roots in the whole root system. In the present study, the relationship between the rate of nitrogen transportation monitored by bleeding and root length were different before and after heading (Fig. 5B). This indicates that the nitrogen uptake by the whole root system declined drastically after heading. The proportion of aged roots (i.e., roots with low activity) in the whole root system increases rapidly after heading because the formation of new crown roots usually terminates around heading in rice (Morita, 2000).

4. Validity of bleeding sap analysis as a monitoring method of nitrogen uptake

Nitrogen uptake estimated by analysis of the bleeding sap fit to nitrogen uptake by plant analysis very well after the maximum tiller number stage (Fig.
On the other hand, a rather large difference was found between the nitrogen uptake estimated by these two methods at the early stage of growth (Fig. 7A). Repeating the measurement for two or three days may be recommended to avoid the variation due to weather conditions.

A significant positive correlation existed between the cumulative amount of nitrogen uptake calculated based on the analyses of bleeding sap and whole plant throughout the growing period. Although the slope of the regression line of the correlation between the values determined by these two methods (ca. 0.85; Fig. 7B) showed a slight underestimate monitored by bleeding sap analysis, this difference would be solved by correction based on the known regression relationship.

In conclusion, the bleeding sap analysis could be an effective method for making a simple and rapid nitrogen diagnosis in rice plants. The reliability of bleeding sap analysis may be increased by applying an accurate calibration formula, which awaits elucidation of the detailed mechanism of rice response in nitrogen absorption to environmental factors.

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** In Japanese with English summary.
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**** In Japanese. The title was translated into English by the present authors.