Nitrogen Content of Leaves Affects the Nodal Position of the Last Visible Primary Tiller on Main Stems of Rice Plants Grown at Various Plant Densities

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Abstract: The nitrogen content of leaves in rice plants at various planting densities in the field and under high and low levels of nitrogen in pots were comparatively examined, and thereby the effect of the nitrogen content of leaves on the nodal positions of the last visible primary tiller on the main stem was clarified. The nodal positions of the last visible primary tiller, which determine the potential number of cumulative tillers, were negatively correlated with the planting density. However, critical nitrogen contents of leaves for tillering on a leaf-area basis ($N_A$) and those on a dry-weight basis ($N_W$) were estimated as $1.4 \sim 1.6 \text{ g m}^{-2}$ and $3.8 \sim 4.5\%$, respectively, at the time when the last visible primary tiller emerged, even when the planting densities varied from 24 to 197 plants m$^{-2}$. The critical $N_A$ for tillering of rice plants grown under high and low levels of nitrogen fertilization was also nearly the same at the time when the primary tiller ceased to emerge. Therefore, the higher nodal position of the last visible primary tiller caused by lower plant density was attributable to the delayed canopy development and delayed competition for soil nitrogen resources. Suppression of the emergence of the primary tiller when the $N_A$ was less than $1.6 \text{ g m}^{-2}$ by an insufficient supply of nitrogen was explained satisfactorily by assuming an insufficient supply of assimilates from leaves to a primary tiller bud.

Key words: Direct sowing, Last visible primary tiller, Leaf nitrogen content, Nodal position, Plant density, Rice.

Cereal grasses emerge and develop tillers in response to the environmental conditions in which they are planted and grown. The number of fertile tillers is an important yield component in grain production. Tillering is regulated by the interaction between the genetic and environmental factors. Weather, management, and plant competition affect patterns of tiller production in cereal grasses in the field. Plant density that alters plant competition is a key factor determining tiller development. Reduced tillering caused by high planting densities has been observed in cereal grasses.

The potential number of cumulative tillers per plant is determined by the nodal position of the last visible primary tiller on the main stem in rice plant (Matsuba, 1988). For that reason, studies on the correlation between the plant density and the nodal position of the last visible primary tiller are necessary to predict rice-plant growth and development. Our previous study showed that the nodal position of the last visible primary tiller was negatively correlated with the plant density in two rice cultivars, irrespective of year (Sasaki et al., 2004). The water depth of a paddy field influenced the percentages of tillers emerged from the lower node on the main stem (Sasaki et al., 2002). Consequently, the decrease of the nodal position of the last visible primary tiller by increasing seedling density was not influenced under deep water management, but the nodal position was increased by deep water management by 0.6–1.3 nodes compared with those in normal water management (Sasaki et al., 2004). This phenomenon was attributed to the relative delay of canopy development and competition for nitrogen and light resources.

There are numerous studies on the effects of nitrogen supply on tillering in rice plant. Ishizuka and Tanaka (1963) suggested that nitrogen content of leaf blades in rice plants was at least more than 3.5% for good production of tillers; tiller emergence did not occur when the nitrogen content was less than 2.5%. However, a comprehensive understanding of the contribution of nitrogen content of leaf to emergence of tillers is lacking. Predictive modeling of tillers and leaf area development in response to various plant populations requires information about developmental regulation according to nitrogen content. The knowledge of the nitrogen content at the time when the last visible primary tiller on the main stem

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Abbreviations: DAS, days after sowing; LAI, leaf area index; $N_W$, nitrogen content of leaf blade on a dry-weight basis; $N_A$, nitrogen content of leaf blade on a leaf-area basis.
emerges is valuable for elucidating the physiological mechanisms responsible for plant competition.

The present work was intended to evaluate the nitrogen content of leaves at the emergence of the last visible primary tiller on the main stem. We examined the nitrogen content of leaves of rice plants grown under field conditions at various plant densities and in pots with high and low levels of nitrogen fertilizer.

Materials and Methods

1. Field experiment (Exp. I)

A field experiment was conducted in an experimental paddy field at the Hokuriku Research Center of the National Agricultural Research Center (37°06’45”N, 138°16’31”E) in 2002. Pre-germinated seeds of rice (Oryza sativa L.) cultivar Dontokoi (Uehara et al., 1995) were sown directly on the soil on 15 May. The soil in the field was clayey soil (LiC): it was puddled and leveled two days before sowing and the standing water was drained on the day of sowing. The respective sowing rates per square meter were 1.25, 2.25, 3.5, 5.0, 6.0, and 8.75 g. Plot sizes were 9.9 – 29.0 m$^2$, and larger plots were used for a lower sowing rate. A randomized complete design with three replications was used in the experiment. Chemical fertilizer was applied as a basal dressing at a rate of 4 g m$^{-2}$ of N, 8 g m$^{-2}$ of P$_2$O$_5$, and 7.2 g m$^{-2}$ of K$_2$O; nitrogen top dressing was applied at a rate of 2 g m$^{-2}$ of N on 23 July and 2 August. No water was applied for 12 days after sowing (DAS). The water level was subsequently maintained at about 3 cm above the soil surface. Water management, including midseason drainage and intermittent irrigation, were conducted ordinarily.

Six 0.25 m$^2$ quadrats (Sasaki et al., 2002) were set in each plot at the seedling establishment stage by counting the number of rice plants per quadrat. The standard numbers of rice plants were 6, 12, 20, 30, 35, and 50 per quadrat, which were 24, 48, 80, 120, 140 and 200 per m$^2$, designated respectively as PD$_{24}$, PD$_{48}$, PD$_{80}$, PD$_{120}$, PD$_{140}$, and PD$_{200}$, but thinning and additional transplanting of rice seedlings were done inside and outside the quadrats in some quadrats. Three quadrats were selected for each plant density. Then six rice plants in the selected quadrat were measured for plant age in leaf number and the nodal position of the last visible primary tiller on the main stem. Rice plants in two quadrats for each plant density were sampled 26, 30, 34, 41, 47, 55 DAS and those in three quadrats for each plant density were sampled 69 and 82 DAS. Numbers of rice plants and tillers were recorded; then roots of the rice plants were cut off. The rice plants that were sampled 26 – 55 DAS were then separated into two parts: leaf blades, and culms with leaf sheaths and dead parts. Leaf area, dry weight, and nitrogen content of each plant part were determined. The leaf area index (LAI) was measured for its green leaf area with a leaf area-meter (AAM-9; Hayashi Denko Co. Ltd., Tokyo, Japan). The dry weights of respective plant parts were determined after desiccation in an oven at 70°C for more than 48 h. Each dried sample was powdered with a mill and its nitrogen content was measured using Kjeldahl analysis. The dry weight and nitrogen content of shoots without roots of the rice plants sampled at 69 and 82 DAS were also determined.

Table 1. Linear regression coefficients for days after sowing vs. plant age in leaf number on the main stem during 34–58 days after sowing (Exp. I).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of plants per m$^2$</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD$_{24}$</td>
<td>24.0 ± 0.0</td>
<td>0.187</td>
<td>1.398</td>
<td>0.999</td>
</tr>
<tr>
<td>PD$_{48}$</td>
<td>49.3 ± 1.3</td>
<td>0.163</td>
<td>2.284</td>
<td>0.989</td>
</tr>
<tr>
<td>PD$_{80}$</td>
<td>80.0 ± 0.0</td>
<td>0.145</td>
<td>2.928</td>
<td>0.999</td>
</tr>
<tr>
<td>PD$_{120}$</td>
<td>121.3 ± 1.3</td>
<td>0.133</td>
<td>3.209</td>
<td>0.999</td>
</tr>
<tr>
<td>PD$_{140}$</td>
<td>138.7 ± 4.8</td>
<td>0.146</td>
<td>2.799</td>
<td>0.999</td>
</tr>
<tr>
<td>PD$_{200}$</td>
<td>197.3 ± 3.5</td>
<td>0.120</td>
<td>3.597</td>
<td>1.000</td>
</tr>
</tbody>
</table>

The numbers of plants are means and standard errors of three replications. DAS: days after sowing.

2. Pot experiment (Exp. II)

The rice cultivar Dontokoi was also used in this experiment. Pre-germinated seeds were sown in 1/5000 a Wagner pots containing an air-dried paddy soil, clayey soil (LiC), on 4 June 2003. Nitrogen fertilizer was applied at two levels of 0.2 g N (0.2 N) and 0.8 g N (0.8 N) per pot; phosphate fertilizer and potassium fertilizer were applied respectively at 1.0 g P$_2$O$_5$ and 0.9 g K$_2$O per pot. Eighteen pots were used for each nitrogen treatment. Before potting, the above fertilizers were mixed well with the soil. In each pot, 12
pre-germinated seeds were sown. The seedlings were thinned to four per pot at the second leaf stage. The water level was maintained at 2–3 cm above the soil surface throughout the experiment. Rice plants were grown under open-air conditions. Three pots for each nitrogen treatment were sampled at 34, 40, 43, 49, 55, and 62 DAS. The plant age in leaf number and the nodal position of youngest visible primary tiller on the main stem were measured for each rice plant sampled at 62 DAS. Roots of sampled rice plants were removed. Then rice plants were separated into three organs: leaf blades, culms with leaf sheaths, and dead leaf blades. The leaf area, dry weight, and nitrogen content of each plant part were determined as shown in Exp. I.

Results

1. Shoot development (Exp. I)

No difference was observed in the leaf appearance rate on the main stem among the six populations with various densities during 34 days after sowing (DAS) (Fig. 1). The plant age in leaf number on the main stem was 7.7–7.9 at 34 DAS. Thereafter, however, leaf appearance on the main stem was delayed by increasing planting density (Fig. 1 and Table 1).

Planting density did not significantly affect tiller production before 34 DAS, as indicated by the tiller number per plant in Fig. 2. The planting density, however, markedly altered the increase rate of the tiller number after 34 DAS: the maximal tiller numbers increased with decreasing planting density. In addition, the tiller numbers per plant in PD200 increased until 41 DAS, but those in PD48 increased continuously until 55–69 DAS. A tendency was revealed: the maximum tiller number stage was delayed by reducing the planting density.

2. Nodal positions of the last visible primary tiller on the main stem and the time of emergence of the last visible primary tiller (Exp. I)

The nodal position of the last visible primary tiller on the main stem declined linearly with increasing planting density, from 7.9 in PD24 to 5.1 in PD200 (Table 2). The plant age in leaf number on the main stem at the emergence of the last visible primary tiller was not examined in this experiment. For that reason, the plant age in leaf number was estimated assuming the synchronous relation between developed leaves and tillers (Katayama, 1951). That is, when a certain leaf emerges on the main culm, the first leaf of tiller develops from the leaf axil of the third leaf downward from it. The date of the emergence of the last visible primary tiller was calculated using regression lines between days after sowing and plant age in leaf number, which is shown in Table 1. Table 2 shows the estimated plant age and the date of the emergence of the last visible primary tiller. The estimated plant age in leaf number ranged from 8.1 in PD240 to 10.9 in PD24. The estimated date ranged from 37 DAS in PD200

![Fig. 2. Changes in the number of tillers per plant at various plant densities (Exp. I). Tiller numbers per plant include the main stem. Data are means of two replications during 55 days after sowing and three replications at 69 and 82 days after sowing. Vertical bars indicate standard errors.](image-url)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodal position on main stem</th>
<th>Plant age in leaf number</th>
<th>Days after sowing</th>
<th>NW (%)</th>
<th>NA (g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD24</td>
<td>7.9 ± 0.2</td>
<td>10.9</td>
<td>51.1</td>
<td>4.4</td>
<td>1.6</td>
</tr>
<tr>
<td>PD48</td>
<td>7.2 ± 0.2</td>
<td>10.2</td>
<td>48.8</td>
<td>4.2</td>
<td>1.5</td>
</tr>
<tr>
<td>PD80</td>
<td>6.4 ± 0.2</td>
<td>9.4</td>
<td>44.9</td>
<td>4.4</td>
<td>1.5</td>
</tr>
<tr>
<td>PD120</td>
<td>5.9 ± 0.1</td>
<td>8.9</td>
<td>43.0</td>
<td>4.2</td>
<td>1.5</td>
</tr>
<tr>
<td>PD140</td>
<td>6.3 ± 0.0</td>
<td>9.3</td>
<td>44.6</td>
<td>3.8</td>
<td>1.4</td>
</tr>
<tr>
<td>PD200</td>
<td>5.1 ± 0.4</td>
<td>8.1</td>
<td>37.0</td>
<td>4.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Values of the nodal position of the last visible primary tiller on the main stem are means and standard errors of three replications. NW – nitrogen content of leaf on a dry-weight basis; NA – nitrogen content of leaf on a leaf-area basis.
to 51 DAS in PD₃⁴. The difference in the date of the emergence of the last visible primary tiller between PD₃⁰ and PD₄ was similar to the difference in the date of the maximum tiller number stage between the two densities (14 days). Apparently, the emergence of the last visible primary tiller was a few days earlier than the maximum tiller number stage.

3. Nitrogen content of plants grown at various densities (Exp. I)

The nitrogen contents of shoots – leaf blades and culms with the leaf sheath – on a dry-weight basis, were highest around 30 DAS regardless of planting density (Fig. 3A). No difference in the nitrogen contents of shoot among plant densities was observed at 30 DAS. Thereafter, nitrogen contents decreased in all treatments until 82 DAS. In rice plants grown at higher densities, the nitrogen content decreased more rapidly than in the plants grown at lower densities. The pattern of change of nitrogen content on a dry-weight basis (NW) after sowing in leaf blades was similar to that in shoots (Fig. 3B). From 30 DAS, NW decreased, and the degree of decrease varied with the planting density. The NW at the time when the last visible primary tiller emerged was estimated by interpolating the measured data. The obtained NW was 3.8 – 4.5%, and the average was 4.3% (Table 2).

Fig. 3C shows the change in the nitrogen content of leaves on a leaf-area basis (NA) in rice plants grown at various densities. No difference was apparent among plant densities in NA at 24 DAS and 30 DAS; the NA was higher than 1.7 g m⁻². However, the differences among various planting densities in the NA became clear after 34 DAS: NA in the plants grown at a high density declined more rapidly than in the plants grown at a low density. The NA at the emergence of the last visible primary tiller on the main stem was also estimated similarly. The estimated Nₐ were within the narrow range of 1.4–1.6 g m⁻² (Table 2). These results suggested that the planting density did not cause marked differences in the nitrogen content of leaves at the time when the last visible primary tiller emerged.

4. Last visible primary tiller on the main stems of rice plants grown under different fertilizer nitrogen conditions (Exp. II)

In Exp. II, rice plants were grown under high and low levels of nitrogen fertilizer, and the NA was measured to elucidate the effects of nitrogen content of leaves on primary-tiller emergence. Nodal position of the last visible primary tiller was higher in the pots with 0.8 g N per pot (0.8 N) than in 0.2 N (Fig. 4A). The emergence of the primary tiller in the pots with 0.8 N depended mostly on the synchronous relation between developed leaves and tillers. However, the leaf appearance on the main stem was later in the pots with 0.2 N. The emergence of the primary tiller did not synchronize with the appearance of leaves (data not shown).

The primary tiller on the main stem emerged continuously when the NA was higher than 1.5 g m⁻² (Figs. 4A and 4B). However, the emergence of the primary tiller was unstable when the NA was reduced to around 1.5 g m⁻². Moreover, the primary tiller did not appear when the NA was decreased to less than 1.5 g m⁻². The overall pattern of changes of NW was similar to that of the NA (Fig. 4C). The primary tiller of rice plants did not emerge in the pots with 0.8 N when the NW was reduced to less than 4.4%. Fig. 4C shows, in contrast, that the NW in the pots with 0.2 N was 3.8% at 43 DAS. The primary tiller emergence tended to be suppressed at a lower NW.
Reductions of tillering caused by a high planting density have been observed in several cereal grasses. The present study revealed that a high planting density reduced the tiller production in rice (Fig. 2). The potential number of cumulative tillers per plant is determined by the nodal position of the last visible primary tiller on the main stem in rice plant (Matsuba, 1988). Therefore, lowering the planting density increased the maximal tiller number per plant, presumably because the nodal position of the last visible primary tiller was higher in the plants grown at a low planting density. The nodal position of the last visible primary tiller is therefore closely related to tiller production. Sasaki et al. (2004) grew two rice cultivars at various densities, and found that the nodal position of the last visible primary tiller was negatively correlated with the density, irrespective of cultivars. The high planting density presumably advanced the time of severe competition among the plants for space, light and nutrients. Subsequent discussion will address the relation between the nitrogen content of leaves and emergence of primary tiller on the main stem, a key parameter of tiller production.

Fig. 2 shows that a high planting density advanced the date of the maximum tiller number stage but reduced the leaf emergence rate (Fig. 1). This phenomenon can be explained adequately by the decline of nodal position of the last visible primary tiller at the high planting density (Table 2). The nodal position of the last visible primary tiller was 7.9 at PD24, and 5.1 at PD200. However, the Nw was 4.4% and 4.5%, respectively, and the Na was 1.6 and 1.4 g m⁻², respectively, at the stage of emergence of the last visible primary tillers (Table 2). An interesting finding is that the critical level in Na and Nw for tillering was little altered by planting density. According to Ishizuka and Tanaka (1963), the vigorous emergence of tillers requires nitrogen content of 3.5% or higher in the active leaves. Tillering was suppressed when the nitrogen content decreases to less than 2.5%. The critical nitrogen content of leaves observed in the present study differed from that reported by Ishizuka and Tanaka. They investigated nitrogen content on individual leaves on the main stem, whereas we investigated nitrogen content of whole leaves on the main stem and tillers. Furthermore, their investigation was not conducted at the stage of the emergence of the last visible primary tiller. Then, the difference in the critical nitrogen content between the two experiments may be attributed to the different method of investigation. Another reason for the discrepancy might be the different culture methods: rice plants were grown in water solution in the study of Ishizuka and Tanaka, whereas rice plants were grown by soil culture in the present study. Further study is needed to elucidate the discrepancy in the critical nitrogen content of leaves between the two experiments.

The amount of nitrogen taken up by a rice plant varies with the amount of applied fertilizer nitrogen and mineralized nitrogen in the soil. In general, ammonium nitrogen in soil and the soil solution become scarce around and after the maximum tiller number stage (Shoji and Mae, 1984). Mineral nitrogen sufficient to satisfy the demand of rice plants in the early developmental stage is supplied from fertilizer. Nevertheless, at the maximum tiller number stage, the amount of applied fertilizer nitrogen and mineralized nitrogen in the soil are apparently depleted. Consequently, nitrogen stress occurred early in rice plants grown at a high planting density. In Exp. II, the effect of increased application of nitrogen fertilizer on the nodal position of the last
visible primary tiller on the main stem was investigated. The increased nitrogen fertilizer, as basal dressing, markedly elevated the nodal position of the last primary tiller, as expected. However, the nitrogen content of rice plants when they emerged the last primary tiller was nearly equal in the two nitrogen treatments; the critical values of nitrogen content of leaves were identical to those obtained in Exp. I. The rice plants growing at a higher density had a smaller land area per plant. Therefore, it was suggested that the decline of the nodal position of the last visible primary tiller under the increased planting density might result from the limited supply of fertilizer nitrogen and mineralized nitrogen per plant.

The differentiation of tiller buds is unaffected by environmental conditions, such as the amount of fertilizer nitrogen (Yamazaki, 1960) and plant spacing (Yamazaki, 1960; Kakizaki, 1965), but the growth of tiller buds after differentiation is affected by various environmental conditions. As suggested by Sato (1961), and Ishizuka and Tanaka (1963), young tillers with less than three leaves are considered to grow mainly using energy resources from mother culms. Emergence of tillers depends on supply of carbohydrates and various constructing nutrients to the tiller buds from the mother culm.

Nitrogen is an essential component for the synthesis of photosynthetic enzymes and chlorophyll. A close correlation between $N_a$ and the level of Rubisco, a key enzyme in photosynthesis, has been reported by Makino et al. (1988). Furthermore, Sinclair and Horie (1989) reviewed the correlation between $N_a$ and leaf CO$_2$ assimilation. They showed a strong correlation between them suggesting a nearly straight line response of CO$_2$ assimilation rate per unit area to $N_a$ from 0.3 g m$^{-2}$ to about 1.6 g m$^{-2}$. The response was curvilinear when the $N_a$ was higher than about 1.6 g m$^{-2}$. Thus the suppression of the emergence of the primary tiller when the $N_a$ decreased to less than 1.6 g m$^{-2}$ was attributable to an insufficient supply of assimilates from leaves to primary tiller buds. The main stem supplied much more assimilates to the primary tiller buds than to the secondary tillers. In addition, the upper nodal primary tiller received more assimilates from the mother stem than did the lower nodal primary tiller (Wang and Hanada, 1982). However, the assimilates are primarily translocated to newly formed organs, and then to tiller buds. A sufficient supply of assimilates to tiller buds is indispensable for the regular emergence of tillers (Honda, 1977). These results suggest that the lower nodal position of the last visible primary tiller on the main stem caused by a higher planting density is attributable to lack of available assimilation for sustaining new tiller production.

In conclusion, the nodal position of the last visible primary tiller on the main stem, which determines the potential number of cumulative tillers, was negatively correlated with the plant density. The content of leaf nitrogen on a leaf-area basis ($N_a$) at the time when the last visible primary tiller emerged was estimated as 1.4 -1.6 g m$^{-2}$, even when the planting density changed from 24 to 197 plants m$^{-2}$. The $N_a$ values of rice plants grown under high and low levels of fertilizer nitrogen in the pot experiment were also nearly the same at the time when the primary tiller ceased to emergence. These results suggest that higher nodal position of the last visible primary tiller in the plants at a lower planting density is attributable to the delay of canopy development and competition for nitrogen resources in soil. Suppression of the emergence of the primary tiller by an insufficient supply of nitrogen when $N_a$ was less than 1.6 g m$^{-2}$ can be rationalized satisfactorily by assuming an insufficient supply of assimilates from leaves to a primary tiller bud.

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*** Translated from Japanese by the present authors.