Response of the leaf photosynthetic rate to available nitrogen in erect panicle-type rice (Oryza sativa L.) cultivar, Shennong265

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ABSTRACT

Increasing the yield of rice per unit area is important because of the demand from the growing human population in Asia. A group of varieties called erect panicle-type rice (EP) achieves very high yields under conditions of high nitrogen availability. Little is known, however, regarding the leaf photosynthetic capacity of EP, which may be one of the physiological causes of high yield. We analyzed the factors contributing to leaf photosynthetic rate (Pn) and leaf mesophyll anatomy of Nipponbare, Takanari, and Shennong265 (a EP type rice cultivar) varieties subjected to different nitrogen treatments. In the field experiment, Pn of Shennong265 was 33.8 μmol m−2 s−1 in the high-N treatment, and was higher than that of the other two cultivars because of its high leaf nitrogen content (LNC) and a large number of mesophyll cells between the small vascular bundles per unit length. In Takanari, the relatively high value of Pn (31.5 μmol m−2 s−1) was caused by the high stomatal conductance (gs; 72 mol m−2 s−1) in the high-N treatment. In the pot experiment, the ratio of Pn/Ci to LNC, which may reflect mesophyll conductance (gm), was 20–30% higher in Nipponbare than in Takanari or Shennong265 in the high N availability treatment. The photosynthetic performance of Shennong265 might be improved by introducing the greater ratio of Pn/Ci to LNC found in Nipponbare and greater stomatal conductance found in Takanari.

Introduction

Food shortage is a growing threat in light of continued population growth and increasing competition between food and energy for arable land. One of the most important cereal crops, rice (Oryza sativa L.), is widely cultivated, especially in Asian countries. It constitutes a major source of carbohydrates for more than half of the world’s population (http://www.fao.org/docrep/018/i3107e/i3107e03.pdf). A group of high-yielding japonica rice cultivars, characterized by an erect panicle (EP), and leaves are expected to feed the growing population and were released as commercial varieties in China. Recently, EP varieties were cultivated on 1.3 million ha throughout China (Song et al., 2013). According to the previous studies, EP varieties achieve very high yields under conditions of high nitrogen availability (Chen et al., 2007; Li, 2003; Zhang et al., 2002), and some genes related to the high yield of EP varieties have been detected (Huang et al., 2009; Wang et al., 2009; Zhu et al., 2009). Little is known, however, regarding the leaf photosynthetic capacity of EP, in spite of its importance in determining the yield of rice.

In general, leaf nitrogen content (LNC) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content are factors that can potentially limit crop production and leaf photosynthesis (Makino et al., 1983; Sinclair & Horie, 1989). Besides, short and dark colored leaves have been observed in EP varieties (Lv & Cheng, 2010; Zhu et al., 2009), and these leaves may be related either to high LNC or to unique leaf anatomy. We hypothesized that leaves of EP cultivars have a greater leaf photosynthetic capacity under nitrogen-rich treatments and the capacity is sustained by unique mechanisms, which may include leaf morphology and mesophyll structure. To test this hypothesis, Shennong265 (a typical EP rice) was grown under different nitrogen fertilizer treatments, along with Nipponbare (a representative japonica rice) and Takanari (a high-yielding japonica–indica cross-variety). The response of Pn to the nitrogen treatments and its physiological or anatomical basis was examined for Nipponbare, Takanari, and Shennong265.

Materials and methods

Plant materials and growing conditions

For the field experiment, Nipponbare, Takanari, and Shennong265 were grown in a paddy field at the experimental...
farm of the Graduate School of Agriculture, Kyoto University (35° 2′ N, 135° 47′ E; altitude 65 m above sea level). Seeds of each cultivar were sown on 7 May and transplanted on 6 June 2013 to the paddy field in alluvial loam soil classified as Haplaquept. The size of each plot was > 12 m², and hill spacing was 0.15 × 0.30 m (density: 22.2 hills m⁻¹) with one plant per hill. The randomized block design was established with two replications. For the low-nitrogen treatment, Eco-long (JCAM AGRI), a slow release fertilizer, was applied at rates of 3.00, 2.36, and 2.79 g m⁻¹ for N, P₂O₅, and K₂O, respectively. The same fertilizer was applied at rates of 12.00, 9.43, and 11.14 g m⁻¹ for N, P₂O₅, and K₂O, respectively, for the high-nitrogen treatment. Additionally, 5 g m⁻¹ of LP cote (JCAM AGRI), a coated nitrogen fertilizer, was applied to the high-nitrogen treatment group as a basal fertilizer (Table 1).

For the pot experiment, seeds of the same varieties were sown on 7 May and transplanted on 6 June 2013 into 3.8-L pots filled with soil from the field (alluvial loam soil) at a density of one plant per pot with five replicates. Ammonium sulfate (Sumitomo Chemical), monocalcium phosphate (Taki Chemical), and potassium chloride (Mitsubishi) were applied at rates of 0.5–0.5–0.5 g per pot (N–P₂O₅–K₂O) as the basal fertilizer, respectively. For standard N and high N treatments, additional N fertilizer (ammonium sulfate) for the standard N and high N treatments was applied every two weeks to total nitrogen supplies to 1.2, and 2.1 g, respectively (Table 1).

### Table 1. The total amount of N fertilizer applied for the pot and field experiments.

|                  | Pot experiment (g pot⁻¹) | Field experiment (g m⁻²) |
|------------------|--------------------------|--------------------------|
| Low-N            | 5                        | 3                        |
| Standard-N       | 1.2                      | Not applicable           |
| High-N           | 2.1                      | 17                       |

**Measurement of leaf area index**

In the field experiment, the aboveground parts of four plants per plot were harvested at the panicle initiation and heading stages. Shennong265, Takanari, and Nipponbare reached the panicle initiation stage on 9, 20, and 25 July 2013, respectively, and reached the heading stage on 1, 13, and 16 August 2013, respectively. Based on the number of tillers per plant, 3 samples were taken in total from 12 plants in each plot to balance the rice canopy. Green leaf blades were separated from the plant, and leaf area was measured with a portable leaf area meter (Li-3,080; Li-COR). The leaf area index (LAI) was calculated by dividing the measured leaf area by the planted area.

**Quantitation of LNC**

In both field and pot experiments, the leaves were collected after the measurement of \( P_n \), frozen immediately with liquid nitrogen, and stored at −80 °C until use. The area of each leaf was determined with the portable leaf area meter. After the measurement of area, leaves were oven-dried at 80 °C for a minimum of 72 h and weighed to determine dry weight. Leaf mass per area (LMA) was calculated from single-leaf area and dry weight. Nitrogen concentration was quantified by the indophenol method after Kjeldahl digestion (Kjeldahl, 1883). LNC was calculated by multiplying LMA by nitrogen concentration. For LNC and LMA, four samples per plot were collected and then the values were averaged.

**Analysis of leaf mesophyll anatomy**

Samples for microscopic observation were taken from the uppermost, fully expanded leaves of plants grown in the pots. Cross sections of leaf mesophyll with 5-μm and 10-μm thicknesses were cut on a sliding microtome (REM-710; Yamato Kohki) and stained with 1% toluidine blue. The samples were observed with a light microscope (BHS-323; Olympus) at 200 × magnification. The microscopic images were recorded using a digital camera. The number of mesophyll cells between the two small vascular bundles (CN) was counted. The length between the small vascular bundles (L) was measured, and the cell number per unit area was calculated by dividing CN by L. The leaf thickness at the small vascular bundles was measured. Mean mesophyll cell area was determined by dividing the area occupied...
by the mesophyll cells (the dotted line in Figure 1) by CN. Mesophyll cell occupancy was calculated as the ratio of the area enclosed by the dotted line to the area framed by the thick line (%; Figure 1). These anatomical characteristics were determined with Image J software (NIH).

**Results**

**Photosynthetic capacity, LNC, and LAI in the field experiment**

The value of $P_n$ varied from 26.8 μmol m$^{-2}$ s$^{-1}$ for Nipponbare in the low-N treatment to 33.8 μmol m$^{-2}$ s$^{-1}$ for Shennong265 in the high-N treatment in the field experiment (Table 2). $P_n$ of Shennong265 was the highest among the three cultivars under both N conditions. The value of $g_s$ varied from .52 mol m$^{-2}$ s$^{-1}$ for Nipponbare in the low-N treatment to .72 mol m$^{-2}$ s$^{-1}$ for Takanari in the high-N treatment (Table 2). In both high and low-N treatments, the $g_s$ of Takanari and Shennong265 was higher than Nipponbare. The $P_n/C_i$ ratio varied from .088 μmol m$^{-2}$ s$^{-1}$/μmol mol$^{-1}$ for Nipponbare in the low-N treatment to .114 μmol m$^{-2}$ s$^{-1}$/μmol mol$^{-1}$ for Shennong265 in the high-N treatment (Table 2). In comparison with two other cultivars, Shennong265 exhibited high LNC values of 1.72 g m$^{-2}$ and 1.63 g m$^{-2}$ in the high-N and low-N treatments, respectively (Table 2). The effect of cultivars and N fertilizer levels on $P_n$, $g_s$, and $P_n/C_i$ was significant ($p < .01$), whereas the interaction of these variables was not (Table 3). For the variation of LNC, the effect of cultivars and the interaction of cultivars and N fertilizer levels were significant ($p < .01$ and $p < .05$, respectively), whereas that of N fertilizer levels was not (Table 3). At the panicle initiation stage, LAI varied from .89 m$^2$ m$^{-2}$ for Shennong265 in the low-N treatment to 6.00 m$^2$ m$^{-2}$ for Nipponbare in the high-N treatment, respectively. At the heading stage, it varied from 1.84 m$^2$ m$^{-2}$ for Shennong265 in the low-N treatment to 6.63 m$^2$ m$^{-2}$ for Takanari in the high-N treatment, respectively (Figure 2). The LAI of Shennong265 was the lowest among the three cultivars under both N fertilizer levels. At panicle initiation stage, LAI of Shennong265 was only 36% and 30% against that of Nipponbare in low-N and high-N treatments, respectively. At heading stage, these values were 52% and 80%, respectively (Figure 2).

![Figure 1](image-url) The conceptual figure of mesophyll cell occupancy. Mesophyll cell occupancy was calculated as the ratio of the area enclosed by the dotted line to the area framed by the thick line (%). Tissues are bulliform cells (BF), small vascular bundle (SVB), epidermis (e), and mesophyll cells (M). The scale bar corresponds to 50 μm.

|                | $P_n$ (μmol m$^{-2}$ s$^{-1}$) | $g_s$ (mol m$^{-2}$ s$^{-1}$) | $P_n/C_i$ (μmol m$^{-2}$ s$^{-1}$/μmol mol$^{-1}$) | LNC (g m$^{-2}$) |
|----------------|-------------------------------|-------------------------------|-----------------------------------------------|-----------------|
| **Nipponbare** |                               |                               |                                               |                 |
| low            | 26.8 ± 1.8                    | c .52 ± .06                   | .088 ± .007                                   | 1.26 ± .09      |
| high           | 29.8 ± 2.5                    | bc .58 ± .07                  | .099 ± .009                                   | .86 ± .37       |
| **Takanari**   |                               |                               |                                               |                 |
| low            | 28.2 ± 1.4                    | c .59 ± .03                   | .091 ± .005                                   | .64 ± .04       |
| high           | 31.5 ± 1.6                    | ab .72 ± .04                  | .102 ± .006                                   | .99 ± .13       |
| **Shennong265**|                               |                               |                                               |                 |
| low            | 33.0 ± 1.7                    | a .62 ± .06                   | .111 ± .007                                   | 1.63 ± .06      |
| high           | 33.8 ± 3.1                    | a .69 ± .07                   | .114 ± .011                                   | 1.72 ± .02      |

Table 2. The values of $P_n$, $g_s$, $P_n/C_i$ at 380 mol m$^{-1}$ of reference CO$_2$ concentration, and LNC of Nipponbare, Takanari, and Shennong265 at different nitrogen fertilizer levels in the field experiment. Values are means ± SD for $n = 2$ (LNC) and $n = 8$ ($P_n$, $g_s$, and $P_n/C_i$). Values followed by the same letters indicate no significant difference among cultivars or N treatments at $p < .05$ (Tukey’s test).
Photosynthetic capacity, LNC, and leaf mesophyll anatomy in the pot experiment

The value of $P_n$ ranged from 21.9 μmol m$^{-2}$ s$^{-1}$ for Nipponbare in the low-N treatment to 31.7 μmol m$^{-2}$ s$^{-1}$ for Shennong265 in the high-N treatment in the pot experiment (Figure 3). $P_n$ of Shennong265 was the highest among the three genotypes in two of the three N treatments. The effect of N fertilizer levels was significant ($p < .01$), whereas that of cultivars and the interaction of these variables was not (Table 3). The $g_s$ of Takanari was the highest across all nitrogen treatments (Figure 3). The ratio of $P_n/C_i$ ranged from .075 μmol m$^{-2}$ s$^{-1}$/μmol mol$^{-1}$ for Nipponbare in the low-N availability treatment to .112 μmol m$^{-2}$ s$^{-1}$/μmol mol$^{-1}$ for Shennong265 in the high-N treatment (Figure 3). The $P_n/C_i$ of Shennong265 in the high-N treatment was 11% higher than that of Takanari.

Figure 2. The values of LAI for Nipponbare, Takanari, and Shennong265 at different nitrogen fertilizer levels in the field experiment at panicle initiation stage and at heading stage. The error bars indicate the SD for $n = 2$. Columns with the same letters are not significantly different at $p < .05$ (Tukey’s test).

Figure 3. The values of $P_n$, $g_s$, and $P_n/C_i$ of Nipponbare, Takanari, and Shennong265 at different nitrogen fertilizer levels at 380 mol mol$^{-1}$ of reference $\mathrm{CO}_2$ concentration in the pot experiment. Vertical bars represent the SD for $n = 5$. Columns with the same letters are not significantly different at $p < .05$ (Tukey’s test).

Table 3. F values and significance of cultivar (C), nitrogen fertilizer level (N), and their interactions (C × N) to $P_n$, $g_s$, $P_n/C_i$, LNC, and $P_n/C_i$/LNC for the three cultivars in the field and pot experiments. A two-way analysis of variance (ANOVA) was conducted.

|                | $F$ value | Probability | $F$ value | Probability |
|----------------|-----------|-------------|-----------|-------------|
| $P_n$ Cultivar (C) | 28.6      | <.01        | 2.3       | NS          |
| Nitrogen fertilizer level (N) | 14.4      | <.01        | 21.7      | <.01        |
| C × N | 1.4 | NS | 1.5 | NS |
| $g_s$ Cultivar (C) | 18.6      | <.01        | 5.0       | <.05        |
| Nitrogen fertilizer level (N) | 28.0      | <.01        | 3.3       | <.05        |
| C × N | 1.5 | NS | .6 | NS |
| $P_n/C_i$ Cultivar (C) | 32.1      | <.01        | 3.1       | NS          |
| Nitrogen fertilizer level (N) | 12.2      | <.01        | 25.3      | <.01        |
| C × N | 1.4 | NS | 2.1 | NS |
| LNC Cultivar (C) | 28.0      | <.01        | -         | -           |
| Nitrogen fertilizer level (N) | .0        | NS         | -         | -           |
| C × N | 5.2 | <.05 | - | - |
| $P_n/C_i$/LNC Cultivar (C) | 110.8     | <.01        | 8.5       | <.01        |
| Nitrogen fertilizer level (N) | .8        | NS         | 58.3      | <.01        |
| C × N | 72.1 | <.01 | 2.8 | <.05 |

Notes. The total number of data points of field experiment except for LNC was 48 ($n = 48$), that of LNC was 12 ($n = 12$), and that of pot experiment was 45 ($n = 45$). NS indicates non-significance.
Although the effect of cultivars on CN/L was not significant, the effects of N fertilizer levels and their interactions on CN/L were significant \( (p < .05; \text{Table 4}). \)

### Discussion

In the present study, we found that Shennong265 had higher \( P_n \) than Takanari and Nipponbare under various N availabilities. The \( P_n \) value of \( C_3 \) plants is limited by either the capacity of RuBP carboxylation or that of RuBP regeneration, and the limitation varies with \( CO_2 \) concentration.
Sufficient varietal differences in LNC in rice cultivars even important parameters on photosynthesis (Evans, 1989; correlated with leaf Rubisco content, and one of the most V2006). Rubisco concentration is closely related to cmax, (Table 4). There are also apparent characteristics in the increase of the biomass production.

or greater planting densely, high photosynthetic capac- of dry matter production. By applying more N fertilizer LAI of Shennong265 should be a disadvantage in terms expansion. Since LAI is one of the most important factors in agricultural studies (Soltani & Galeshi, 2002), the low LAI of Shennong265 is much lower than in non-EP, regardless the nitrogen availability at both panicle initiation stage and heading stage (Figure 2). Thus, Shennong265 seems to have a tendency to distribute more N to leaf mesophyll structure rather than to LAI expansion. Since LAI is one of the most important factors in agricultural studies (Soltani & Galeshi, 2002), the low LAI of Shennong265 should be a disadvantage in terms of dry matter production. By applying more N fertilizer or greater planting densely, high photosynthetic capacity Shennong265 is expected to contribute to the further increase of the biomass production.

The value of Pn/Ci/LNC totally decreased as N fertilizer level increased (Figure 4), and it is suggested that the photosynthetic capacity was approaching to the saturation under the high N availability. There was varietal difference in the ratio of Pn/Ci to LNC among three cultivars (Table 3), and that of Shennong265 was likely to be lower compared with other cultivars (Figure 4). The value of Pn/Ci to LNC can be influenced by gm (mesophyll conductance), Rubisco activity, or the ratio of leaf Rubisco content to LNC (Makino et al., 1984a, 1984b). In this in vivo study, all of these are the possible interpretations to explain the variation of Pn/Ci/LNC. Especially, the observed difference in the mesophyll structure suggests the significance of gm (Adachi et al., 2013). The value of gm has recently been rec- ognized as one of the most important determinants of Pn, especially in C4 plants (Makino, 2011; Terashima et al., 2011; Warren, 2008). Furthermore, two backcrossed inbred lines derived from Takanari and Koshihikari (an elite japonica variety) have extremely high Pn, well-developed lobes of mesophyll cells, and a greater gm than Takanari (Adachi et al., 2013). Photosynthetic performance of Shennong265 might be further improved if it is combined with the better ratio of Pn/Ci to LNC seen in the other varieties examined in this study.

Greater LNC has been linked to high gs on several crop species (Yamori et al., 2011), and the value of gs of indica varieties was larger than that of japonica varieties (Maruyama & Tajima, 1990). The high Pn of Takanari, a high-yielding japonica–indica cross-variety, was due to high gs (Taylaran et al., 2011). The value of gs in Takanari was also much higher than that of Nipponbare or Shennong265, even under low-N treatment in the present study (Figure 3). Conversely, the value of gs was lower in Shennong265. These findings suggest that Pn of Shennong265 is also possible to be improved by combing with high gs derived from Takanari.

In conclusion, Shennong265 showed high leaf photosynthetic rate under various nitrogen availabilities. It was even higher than that of Takanari under some conditions. This phenotype was achieved by greater LNC and Pn/Ci, which may partly be explained by the mesophyll structure including the mesophyll cell number. Leaf photosynthetic capacity in Shennong265 could be further improved by introducing the greater stomatal conductance or higher Pn/Ci/LNC during future breeding.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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