Identification of Chromosomal Regions Controlling the Leaf Photosynthetic Rate in Rice by Using a Progeny from *Japonica* and High-yielding *Indica* Varieties

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Abstract: The whole-leaf photosynthetic rate in rice plants is controlled by various physiological processes. In a high-yielding *indica* rice variety, Habataki, the leaf photosynthetic rate (LPR) of the uppermost fully expanded leaves was approximately 130 to 140% of that in a *japonica* variety, Sasanishiki, from booting to the early ripening stage. We characterized the difference in the LPR between Habataki and Sasanishiki. Leaves of Habataki contained higher levels of nitrogen and, as a consequence, of Rubisco, and had higher stomatal conductance that was associated with higher hydraulic conductance from roots to leaves than those of Sasanishiki. These features were responsible for the higher LPR of Habataki. An analysis of chromosome segment substitution lines (CSSLs) in which chromosome segments from Habataki were substituted into the genetic background of Sasanishiki showed that three genetic regions on chromosomes 4, 5 and 11 were responsible for the increase in the LPR. Each of these regions was estimated to increase the LPR by 15 to 30%, and we showed that they were associated with higher activity of mesophyll photosynthesis due to higher leaf nitrogen content and greater stomatal conductance. Leaf nitrogen content and stomatal conductance may be useful parameters for further quantitative trait locus analysis of efficient photosynthesis in leaves.

Key words: Chromosome segment substitution line, Hydraulic conductance, Nitrogen content, *Oryza sativa*, Photosynthesis, Quantitative trait locus, Stomatal conductance.

The increase in leaf photosynthetic rate (LPR) is important to increase the yield potential of rice (Long et al., 2006) because the LPR affect dry matter production via photosynthesis within the canopy. The light use efficiency for leaf photosynthesis in the canopy was increased significantly by improving the architecture of the canopy (e.g. the increase in the inclination angle of leaves in the canopy), thus contributing to the yield increase in the last century (e.g., San-oh et al., 2008; Taylaran et al., 2009). However, the differences in LPR among the bred cultivars and the genetics of inheritance of traits that influence LPR are not yet fully understood (e.g., Hubbart et al., 2007; Zhao et al., 2008).

The LPR is normally measured at full leaf expansion under conditions of saturating light, the common ambient atmospheric concentration of CO₂, optimum temperature and a low vapor pressure deficit. We refer to this rate here as the maximum photosynthetic rate (Murata, 1961). However, it is reduced by the midday and afternoon depression that results from abiotic stresses, such as water stress (Ishihara and Saito, 1987), and also by senescence (Makino et al., 1984; Jiang et al., 1987, 1999).

There have been several reports on the differences of LPRs among individual leaves (Ishii, 1995; Agarie, 2003), among *japonica* varieties (e.g., Murata, 1961; Kuroda and Kumura, 1990; Sasaki and Ishii, 1992), and among *indica* and *japonica* varieties and other species of *Oryza* (e.g., Cho and Murata, 1980; Cook and Evans, 1983; Masumoto et al., 2004). Some clear differences have been observed among varieties, among species of *Oryza*, and among progeny...
derived from crosses between species at a given rate of nitrogen application. However, the LPR is strongly influenced by the level of leaf nitrogen (Ishihara et al., 1979; Makino et al., 1988). The rate is also affected significantly by stomatal conductance (Ishihara and Saito, 1987; Hirasaawa et al., 1988) and the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content of each leaf (Makino et al., 1987). We refer here to these effects respectively as stomatal and non-stomatal factors of photosynthesis. Stomatal conductance (Ishihara et al., 1979) and Rubisco content (Makino et al., 1988) are, in turn, strongly affected by leaf nitrogen content. Although there are several reports on the varietal differences in LPR at an ambient concentration of CO2, little attention has been paid, to our knowledge, to the effects of nitrogen on varietal differences in LPR (Ishii, 1995) and to the effects of stomatal and non-stomatal factors on these varietal differences.

The amount of dry matter and nitrogen accumulated within the plant and the partitioning of nitrogen to leaves all affect the levels of leaf nitrogen. On the other hand, the response of stomata to environmental conditions is sensitive. For example, stomatal conductance is affected by air humidity which affects transpiration rate significantly (Hirasaawa et al., 1988). Stomata of rice start to close even at a small reduction in leaf water potential (Hirasaawa et al., 1988). The reduction in leaf water potential with the increase in the rate of transpiration is suppressed in the plants with a larger hydraulic conductance from roots to leaves. There was a close relationship between stomatal aperture and hydraulic conductance in rice (Hirasaawa et al., 1992).

Various DNA markers have been developed and used for the genetic analysis of rice (Sasaki, 2003; International Rice Genome Sequencing Project, 2005). Various quantitative trait loci (QTLs) that are responsible for a variety of traits have been identified (Yamamoto et al., 2009). Chromosome segment substitution lines (CSSLs) have recently been developed for the identification of QTLs (Yano, 2001; Yamamoto et al., 2009). The population of CSSLs is much smaller than other primary mapping populations, such as an F2 population or a population of recombinant inbred lines (RILs), and, thus, phenotypic analysis is simplified considerably (Ebitani et al., 2005).

The high-yielding indica variety Habataki produces particularly heavy grains. It has a higher LPR in the morning, that is, a higher maximum photosynthetic rate than a japonica commercial variety, Sasanishiki, from the booting to the early ripening stage (Asanuma et al., 2008). This is a cause of the high yield in Habataki (Asanuma et al., 2008). Recently, a new set of CSSLs derived from a cross between Sasanishiki and Habataki for the genetic analysis of particular traits were developed (Ando et al., 2008). In the present study, we analyzed the causes of the higher LPR in Habataki than in Sasanishiki, focusing on stomatal and non-stomatal factors. Then we identified the chromosome regions responsible for the elevated photosynthetic rate using the CSSLs generated from Sasanishiki and Habataki and investigated the functions of these regions in leaf photosynthesis in association with leaf nitrogen content and stomatal conductance.

### Materials and Methods

1. **Plant materials**

Rice plants (O. sativa) of the japonica variety Sasanishiki, the indica variety Habataki, and 38 CSSLs with chromosome segments of Habataki on the genetic background of Sasanishiki were used in this study. Details of the development of the CSSLs were reported previously by Ando et al. (2008).

2. **Cultivation**

Rice plants were grown in the paddy field of the University Farm (35° 40' N, 139° 28' E) in alluvial soil (clay loam) from the Tama River in 2005 and 2006. Details of cultivation were basically the same in the two years. Seedlings at the fourth-leaf stage were transplanted at a rate of 22.2 hills m$^{-2}$ (spacing, 30 cm x 15 cm) with one plant hill$^{-1}$. As basal dressing, manure was applied at a rate of approximately 20 t ha$^{-1}$ and chemical fertilizer at 50, 136 and 72 kg ha$^{-1}$ of N, P$_2$O$_5$ and K$_2$O, respectively. One-third of the total N was applied as nitrogen sulfate; one-third as elution-controlled urea (LP-50; Chisso Asahi Fertilizer, Tokyo); and one-third as elution-controlled urea (LPS-100; Chisso Asahi Fertilizer). No topdressing was applied. The experiments were designed with three randomly arranged replicates in each case.

Rice plants were also grown in 12-L, and 3-L pots filled with a mixture of paddy soil and Kanto diluvial soil (1:1, v/v) outdoors and in a growth chamber (Koito Manufacturing Co. Ltd., Tokyo), respectively. In the growth chamber, day/night temperature was 28°C/25°C, day/night relative humidity approximately 60%/80%, photoperiod 12 h, and photosynthetic photon flux density (PPFD) at the top of the canopy approximately 1,000 µmol m$^{-2}$ s$^{-1}$. Basal fertilizer was applied to 12-L pots at a rate of 1.0, 1.0 and 1.0 g pot$^{-1}$ for N, P$_2$O$_5$ and K$_2$O, respectively, and additional fertilizer (N) was applied at a rate of 0.5 g pot$^{-1}$ at the booting stage. We also applied nitrogen to the pots at 0.75, 1.5 or 2.0 g pot$^{-1}$ to change the levels of leaf nitrogen. In the case of the 3-L pots, basal fertilizer was applied at a rate of 0.5, 0.5 and 0.5 g pot$^{-1}$ for N, P$_2$O$_5$ and K$_2$O, respectively, and no additional fertilizer was applied.

3. **Measurements of LPR and stomatal conductance**

The LPR and the stomatal conductance of the flag leaf on the main stem were measured at the full heading stage under submerged soil conditions. The measurements in
the paddy field were made in a closed gas-exchange system with a gas-exchange apparatus (LI-6200; LI-COR, Lincoln, NE). Measurements were started at a concentration of CO₂ in air of approximately 370 μmol mol⁻¹ in the assimilation chamber. Measurements, for eight seconds each, were repeated three times and mean values were taken as the measured values. The leaves were exposed to natural light at a PPFD of higher than 1,200 μmol m⁻² s⁻¹. The leaves were irradiated supplementary with white light from an electric lamp (LA-180Me; Hayashi Tokei, Tokyo) when the PPFD of the sunlight was below 1,200 μmol m⁻² s⁻¹.

For measurements of photosynthetic rate and the stomatal conductance of potted plants, we used an open gas-exchange system with a gas-exchange apparatus (LI-6400; LI-COR) under the following conditions: leaf temperature, 30ºC; PPFD, 2,000 μmol m⁻² s⁻¹; and a leaf-air vapor pressure difference of approximately 1.5 kPa. The intercellular CO₂ concentration was calculated as described by von Caemmerer and Farquhar (1981).

### 4. Determination of the levels of nitrogen and Rubisco in flag leaves

Flag leaves were collected immediately after completion of measurements of CO₂ assimilation rate and stomatal conductance, and then they were stored at -80ºC prior to analysis. The area and fresh weight of each leaf were determined, and each leaf was separated into two equal parts for separate quantification of Rubisco and nitrogen. The halves of leaves were homogenized separately with a mortar and pestle in a solution that contained 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10 mM MgCl₂, 10 mM 2-mercaptoethanol, and 5% (w/w) insoluble polyvinylpyrrolidone (Polyclar VT; Wako Chem., Tokyo). Each homogenate was centrifuged at 10,000×g for 10 min at 4ºC. The supernatant was used for quantification of Rubisco by the single radial immunodiffusion method (Sugiyama and Hirayama, 1983) with rabbit polyclonal antibodies raised against purified Rubisco from rice. Nitrogen was quantified with a CN analyzer (MT600; Yanako, Kyoto).

### 5. Determination of hydraulic conductance and leaf water potential of plants grown in pots

Hydraulic conductance (Cₚ) from roots to leaves was calculated for plants grown in 3-L pots as follows:

\[ C_p = \frac{1}{S} \cdot \frac{U_w}{(\Psi_s - \Psi_l)} \]  

where S is the number of stems, U_w is the water-uptake rate of the whole plant, Ψ_s is the soil water potential, and Ψ_l is the leaf water potential. Since rice plants were grown under submerged conditions, Ψ_s could be regarded as negligible (zero) when compared with Ψ_l. Measurements were made in an environment-controlled chamber (air temperature, 28ºC; relative humidity, 58% to 62%; and PPFD at the top leaves, approximately 1,000 μmol m⁻² s⁻¹). The water-uptake rate was determined from the rate of weight loss of the pot after a steady state had been reached. The top of the pot was covered with polystyrene foam and oily clay was used to seal the gap between the foam and the stem to prevent evaporation from the surface of the pot. After measurements of the water-uptake rate, the leaf water potential of the uppermost three leaves was measured with a pressure chamber (model 3005; Soil Moisture Equipment Inc., Santa Barbara, CA) as described by Hirasawa and Ishihara (1991). The mean value of the water potential of the three leaves was used for calculation of Cₚ.

### 6. Detection of QTLs

The existence of a QTL was detected when the average value of a trait was significantly different between a CSSL and the recurrent parent, Sasanishiki, according to Dunnet’s multiple comparisons test. From the identity of the CSSLs, QTLs could be assigned to the substituted chromosomal segment. When QTLs were detected on overlapping chromosome segments in multiple CSSLs, their location could be narrowed down (Ando et al., 2008).

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Fig. 1. Comparison of (A) photosynthetic rate at an ambient CO₂ concentration of 370 μmol mol⁻¹, (B) stomatal conductance at an ambient CO₂ concentration of 370 μmol mol⁻¹, (C) photosynthetic rate at an intercellular CO₂ concentration of 280 μmol mol⁻¹, and (D) the nitrogen content of flag leaves between Sasanishiki (Sasa) and Habataki (Haba) grown in 12-L pots and examined at the full heading stage. The photosynthetic rate was measured with a LI-6400. Bars represent standard deviations (n=5) and different alphabetical letters represent significant differences at the 5% level (t-test).
1. Difference in LPR between Habataki and Sasanishiki

LPR at an ambient CO\textsubscript{2} concentration of 370 µmol mol\textsuperscript{-1}, stomatal conductance, LPR at an intercellular CO\textsubscript{2} concentration of 280 µmol mol\textsuperscript{-1} and nitrogen content of the flag leaf are shown in Figure 1. LPR was 38% higher in Habataki than in Sasanishiki (Fig. 1A). Stomatal conductance was also significantly greater in Habataki than in Sasanishiki (Fig. 1B). The intercellular CO\textsubscript{2} concentration in the flag leaf at an ambient CO\textsubscript{2} concentration of 280 µmol mol\textsuperscript{-1} ranged from 280 µmol mol\textsuperscript{-1} (in Sasanishiki) to 300 µmol mol\textsuperscript{-1} (in Habataki). We were able to estimate the leaf photosynthetic activity without considering the effect of stomatal conductance by measuring LPR at identical intercellular concentrations of CO\textsubscript{2}. At an intercellular CO\textsubscript{2} concentration of 280 µmol mol\textsuperscript{-1}, LPR was 28% higher in Habataki than in Sasanishiki and the difference between the respective rates was lower than it was at the ambient CO\textsubscript{2} concentration of 370 µmol mol\textsuperscript{-1} (Fig. 1C). The nitrogen content of the flag leaf was higher in Habataki than in Sasanishiki (Fig. 1D). These observations indicated that although stomatal conductance is a cause of the higher LPR in Habataki, the mesophyll photosynthetic activity is predominantly responsible for the higher LPR in Habataki via the accumulation of more nitrogen in leaves.

We examined the relationships between leaf nitrogen content and LPR in Habataki and Sasanishiki. LPR at an ambient CO\textsubscript{2} concentration of 370 µmol mol\textsuperscript{-1} increased with increases in leaf nitrogen content in both varieties (Fig. 2). The LPR was higher in Habataki than in Sasanishiki at a leaf nitrogen content of approximately 1.6 g m\textsuperscript{-2}. Although the rate of increase in LPR with increasing leaf nitrogen content was lower in Habataki than in Sasanishiki, LPR was still much higher in Habataki than in Sasanishiki even at a leaf nitrogen content of approximately 2.0 g m\textsuperscript{-2}.

The stomatal conductance of leaves remained constant irrespective of the leaf nitrogen content in the two varieties, and it was far higher in Habataki than in Sasanishiki (Fig. 3A). By contrast, no differences were found in LPR at an intercellular CO\textsubscript{2} concentration of 280 µmol mol\textsuperscript{-1} between the two varieties at all levels of leaf nitrogen examined (Fig. 3B). Moreover, there was no difference in the relationship between the nitrogen concentration of 370 µmol mol\textsuperscript{-1} and nitrogen content of the flag leaf at the full heading stage in Sasanishiki (open circles) and Habataki (closed circles) grown in 12-L pots. The photosynthetic rate was measured with a LI-6400 at an ambient CO\textsubscript{2} concentration of 370 µmol mol\textsuperscript{-1}.

Fig. 3. Correlation of nitrogen content with (A) stomatal conductance, (B) photosynthetic rate at an intercellular CO\textsubscript{2} concentration of 280 µmol mol\textsuperscript{-1} and (C) Rubisco content of flag leaves at the full heading stage in Sasanishiki (open circles) and Habataki (closed circles). Each point represents the average value of measurements for each rate of nitrogen top-dressing (0.5, 0.75, 1.5 and 2.0 g pot\textsuperscript{-1}) with standard deviations (n=5). Values of r represent correlation coefficients in Sasanishiki (n=15) and Habataki (n=14), respectively, and asterisks * and ** represent significance at the 5% and 1% levels, respectively.
content and the Rubisco content of leaves between the two varieties (Fig. 3C). From the results shown in Figures 2 and 3, we concluded that when leaf nitrogen contents were identical, stomatal conductance was responsible for the varietal differences in LPR and there was no difference in the mesophyll photosynthetic activity between Sasanishiki and Habataki.

The LPR was also higher in Habataki than in Sasanishiki at approximately 1.5 kPa of leaf-air vapor pressure difference in the laboratory even when the difference in leaf nitrogen content was small (Fig. 4). When a leaf was excised at the base of the leaf blade after leaf gas exchange had reached a steady state, the LPR of the excised leaf clearly increased for a few minutes in Sasanishiki. This phenomenon is well known and is referred to as the Ivanov effect (Slavik, 1974). By contrast, the corresponding increase in the rate was very small in Habataki. We found no difference in the maximum LPR attained after the leaf excision between Sasanishiki and Habataki. These results indicated that the leaves in which we had quantified the photosynthetic rate might have been under greater water stress in Sasanishiki than in Habataki even when the vapor pressure difference was small. Actually average leaf water potentials of Sasanishiki and Habataki were -0.41 and -0.29 MPa, respectively (Fig. 5). The maintenance of the high water potential in Habataki, in which stomata are fully open and transpiration rate is high, suggests that hydraulic conductance in Habataki is high. In fact, hydraulic conductance was far higher in Habataki than in Sasanishiki (Fig. 6).

2. Comparison of LPR between Sasanishiki and CSSLs

To identify chromosomal region that control the LPR, we compared the LPR in CSSLs with that in Sasanishiki at the full heading stage in the paddy fields (2005 and 2006) and in pots (2006 and 2007). There were considerable variations in LPR among 38 CSSLs, with average values ranging from 19.3 to 27.9 μmol m⁻² s⁻¹ in the paddy field in 2005 (data not shown). We selected several lines with relatively high LPR and we grew them in the paddy field and in pots to verify the difference among them in LPR intensively in 2006 and 2007. Among the selected CSSLs, three lines (SL414, SL416 and SL434) consistently showed significantly higher LPR than Sasanishiki (Table 1). The LPR of each line was 115% to 130% of that of Sasanishiki across 3 years and different growth conditions. Figure 7 shows the LPR of the three lines as an example. Other CSSLs sometimes showed a higher LPR than in Sasanishiki, but not always.

We analyzed causes of the higher LPR in the three lines by using plants grown in pots. Stomatal conductance in SL414 and SL416 was significantly greater than that in Sasanishiki (Fig. 8A). In addition, their leaf nitrogen contents tended to be higher (significant at 10% level; Fig. 8B) and LPR at an intercellular CO₂ concentration of

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**Fig. 4.** Changes in photosynthetic rate of flag leaf after excision at the base of the leaf blade after leaf gas exchange had reached a steady state in Sasanishiki (open circles) and Habataki (closed circles). The plants were grown in 3-L pots, and the photosynthetic rate was measured with a LI-6400. The leaf-air vapor pressure difference was kept approximately 1.5kPa before the leaf excision, but it was not controlled after the excision. Leaf nitrogen contents were 2.1 and 1.8 g m⁻² in Sasanishiki and Habataki, respectively.

**Fig. 5.** Water potential of flag leaf at the full heading stage in Sasanishiki (Sasa) and Habataki (Haba) that had been grown in 3-L pots. Bars represent standard deviations (n=5) and different alphabetical letters represent significant differences at the 5% level (t-test).

**Fig. 6.** Hydraulic conductance from roots to leaves at the full heading stage in Sasanishiki (Sasa) and Habataki (Haba) that had been grown in 3-L pots. Bars represent standard deviations (n=5) and different alphabetical letters represent significant differences at the 0.1% level (t-test).
280 μmol mol⁻¹ were significantly higher in SL414 and SL416 than in Sasanishiki (Fig. 8C). The stomatal conductance, leaf nitrogen content and the LPR at an intercellular CO₂ concentration of 280 μmol mol⁻¹ in SL434 all tended to be greater than the corresponding values in Sasanishiki although statistically significant differences were not observed.

LPR at an ambient CO₂ concentration of 370 μmol mol⁻¹ and stomatal conductance tended to be higher in SL414, SL416 and SL434 than in Sasanishiki irrespective of leaf nitrogen contents (Figs. 9A, B). There was no difference in the LPR at an intercellular CO₂ concentration of 280 μmol mol⁻¹ among Sasanishiki, SL414, SL416 and SL434 at any leaf nitrogen content (Fig. 9C), and there was no difference in terms of the relationships between Rubisco content and the LPR (Fig. 9D). The results shown in Figures 8 and 9 suggested that the three CSSLs have chromosomal regions associated with higher leaf nitrogen content and greater stomatal conductance.

There was no difference in LPR between Sasanishiki and SL413 (Fig. 10). It was concluded that the chromosome...
region defined by SSR markers RM3916 and RM2431 was involved in elevation of the LPR in chromosome 4. There was no difference in LPR between Sasanishiki and SL415 and the difference in LPR between Sasanishiki and SL417 was significant at 5% level in chromosome 5. SL416 contains a chromosome segment from Habataki on chromosome 7 (Ando et al., 2008). But any increased LPR was not found in SL422, which contained a Habataki chromosome segment on the same region of chromosome 7 (data not shown). The difference in LPR between Sasanishiki and SL434 was significant at 1% level in chromosome 11. These results suggest that the region RM6742 and RM5642 in chromosome 5 and the region RM7283 and RM1341 in chromosome 11 are involved in elevation of the LPR.

**Discussion**

1. Causes of varietal differences in LPR

In general, the LPR in rice plants is affected to a significant extent by the carboxylation rate and the rate of diffusion of CO₂ from the atmosphere into the leaf at common ambient concentration of CO₂ (Makino et al., 1984; Saito and Ishihara, 1987). We demonstrated previously that a high-yielding *indica* variety, Takanari, had a higher LPR, which was due to a higher leaf nitrogen content (Xu et al., 1997; Ohsumi et al., 2008; Hirasawa et al., 2010), and therefore, to a higher Rubisco content at a given level of nitrogen application, and due to the greater stomatal conductance of each leaf at a given level of leaf nitrogen, than in common *japonica* varieties (Hirasawa et al., 2010). In the present study, the LPR in the high-yielding *indica* variety, Habataki, was higher by approximately 30% to 40% than that in the *japonica* variety. The higher LPR in Habataki was also caused by the larger nitrogen content and the greater stomatal conductance. However, when leaf nitrogen contents were identical, no difference was observed in the mesophyll photosynthetic activity between Sasanishiki and Habataki (Figs. 3B, C). Our results confirmed the previous reports (Makino et al., 1987), although the rate of photosynthetic evolution of oxygen was higher in certain *japonica×indica* varieties than in common *japonica* varieties at the same
Leaf nitrogen content is determined by the amounts of dry matter and nitrogen accumulated and the partitioning of nitrogen to leaves. Takamari, which produced heavier dry matter than certain japonica varieties, accumulated a large amount of nitrogen but nitrogen partitioning to leaves was as great as in the japonica varieties examined (Taylaran et al., 2009). The greater accumulation of nitrogen was responsible for the higher nitrogen content of each leaf. Dry matter production was not lower in Habataki than in Sasanishiki (Asanuma et al., 2008), so the greater accumulation of nitrogen might also have been responsible for the higher nitrogen content of leaves in Habataki (Fig. 1). This possibility needs to be examined in future.

The stomatal conductance of rice leaf is very sensitive to air humidity. In an earlier study, stomatal conductance of a japonica variety decreased when the vapor pressure difference between the leaf and the atmosphere was above approximately 1 kPa (Hirasawa et al., 1988). The vapor pressure deficit of the atmosphere usually increases to approximately 1.5 kPa around at 9 a.m. on a clear day. Under such conditions, stomata of rice leaves start to close. Water balance in a plant is much affected by the plant hydraulic conductance. That is, with the increase in transpiration, leaf water potential decreases in the plant with small hydraulic conductance compared with the plants with large hydraulic conductance. The critical water potential for stomatal closure is very high in rice compared with other crop plants (Hirasawa et al., 1988). This might be a reason why the hydraulic conductance affected stomatal conductance. The increase in the LPR was large in excised Sasanishiki leaves (Fig. 4) since Sasanishiki has a low hydraulic conductance (Fig. 6), while it was very small in excised leaves of Habataki, which has higher hydraulic
conductance. It is well known that rice plants usually have a maximum value of the LPR in the morning under the mild condition of an atmospheric vapor pressure deficit and the rate decreases in the midday and afternoon under intense transpiration on a clear day (Ishihara and Saito, 1987). These observations indicate that stomata started to close in Sasanishiki as a result of water stress, even under the mild condition of a vapor pressure deficit of approximately 1.5 kPa, while those of Habataki remained open. This difference might explain the smaller stomatal conductance in Sasanishiki than in Habataki, even at the same level of leaf nitrogen. The significance of leaf hydraulic conductance in LPR has been indicated recently by Brodribb et al. (2007) in a wide range of C₃ species.

2. Chromosomal regions responsible for elevated LPR

Compared with intensive researches on QTLs associated with morphological and developmental traits such as plant height, days to heading and spikelet number (Yamamoto, 2009), QTL analysis related to photosynthesis has been very limited, perhaps because no simple method for evaluating LPR has been available and, also, because differences in LPR have not been well characterized. It has been reported that QTLs for the net assimilation rate are located on chromosomes 4 and 6 in a double haploid population derived from another culture of ZYQ8/JX17, a typical indica and japonica hybrid (Teng et al., 2004), and QTLs for Rubisco have been identified on chromosomes 1, 2, 3, 4, 6, 8, 9 and 12 in a population of backcross inbred lines of japonica Nipponbare and indica Kasalath by Ishimaru et al. (2001) and on chromosome 10 in Koshihikari/Kasalath CSSLs by Kanbe et al. (2009). A QTL for LPR under well-watered conditions has been identified on chromosome 10 in a population of recombinant inbred lines developed from Zhenshan 97B and IRAT 109 by Hu et al. (2009). However, these regions are not consistent with the regions identified in the present study. The sites of QTLs identified in previous studies might be related to other aspects of photosynthesis, or, alternatively, the regions including QTLs might differ with the parental lines examined.

We confirmed that stomatal and non-stomatal factors identified in the difference in LPR between parental varieties were also responsible for the elevated LPR in the CSSLs. The CSSLs with higher LPR had higher stomatal conductance probably due to the higher hydraulic conductance and the higher mesophyll photosynthetic activity due to higher nitrogen content. Hydraulic conductance as well as leaf nitrogen content and relating properties of the CSSLs with higher LPR remain to be investigated. The contribution of the stomatal factor to the increase in LPR was small in SL 434 compared with that in SL414 and SL416 (Fig. 9). The increase in the LPR was also small in the former plants compared with the latter plants. These results suggest that the locus for the high LPR in SL 434 might have a different function from that of SL414 and SL416. This possibility also remains to be examined in future research.

We have examined the LPR in all CSSLs only in 2005 in this study. There might be some other lines with the elevated LPR in the CSSLs. Because of convenience of measurements, leaf nitrogen content and stomatal conductance might be useful parameters for further QTL analysis of efficient photosynthesis in leaves and identifications of gene for the traits.

Conclusion

Variatel differences in LPR in rice were caused by the difference in nitrogen content of leaves and the difference in leaf stomatal conductance. By making a comparison of leaf nitrogen content and stomatal conductance as parameters of photosynthesis as well as CO₂ assimilation rate among CSSLs of Habataki with a Sasanishiki background, we found at least three regions on chromosome 4, 5 and 11 responsible for the increase in LPR. Leaf nitrogen content and stomatal conductance as well as whole-leaf photosynthetic rate might be useful parameters for further QTL analysis, including fine linkage mapping.

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* In Japanese with English abstract.
** In Japanese with English summary.
*** In Japanese.