Introgression of two chromosome regions for leaf photosynthesis from an *indica* rice into the genetic background of a *japonica* rice

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Abstract

Increases in rates of individual leaf photosynthesis (Pₙ) are critical for future increases of rice yields. A previous study, using introgression lines derived from a cross between *indica* cultivar Habataki, with one of the highest recorded values of Pₙ, and the Japanese elite cultivar Koshihikari, identified four QTLs (qCAR4, qCAR5, qCAR8, and qCAR11) that affect Pₙ. The present study examined the combined effect of qCAR4 and qCAR8 on Pₙ in the genetic background of Koshihikari. The pyramided near-isogenic line NIL(qCAR4+qCAR8) showed higher Pₙ than both NIL(qCAR4) and NIL(qCAR8), equivalent to that of Habataki despite being due to only two out of the four QTLs. The high Pₙ of NIL(qCAR4+qCAR8) may be attributable to the high leaf nitrogen content, which may have been inherited from NIL(qCAR4), to the large hydraulic conductance due to the large root surface area from NIL(qCAR4), and to the high hydraulic conductivity from NIL(qCAR8). It might be also attributable to high mesophyll conductance, which may have been inherited from NIL(qCAR4). The induction of mesophyll conductance and the high leaf nitrogen content and high hydraulic conductivity could not be explained in isolation from the Koshihikari background. These results suggest that QTL pyramiding is a useful approach in rice breeding aimed at increasing Pₙ.

Key words: Hydraulic conductance, leaf nitrogen content, Oryza sativa, photosynthesis, quantitative trait locus, stomatal conductance.

Introduction

Increasing the rates of leaf photosynthesis (Pₙ) should increase the yield potential of rice (*Oryza sativa* L.), since Pₙ affects dry matter production via photosynthesis within the canopy (Long et al., 2006; Murchie et al., 2009). The use of natural genetic variation in photosynthesis within species can be an effective strategy for crop improvement (Flood et al., 2011). Wide variations in Pₙ among rice cultivars have been shown in a number of studies (Murata, 1961; Takano and Tsunoda, 1971; Cook and Evans, 1983; Yeo et al., 1994; Osada, 1995; Xu et al., 1997; Masumoto et al., 2004; Kanemura et al., 2007; Jahn et al., 2011). However, most of the natural genetic resources have yet to be tapped.

Quantitative genetics is useful in assessing the genetic factors underlying the variation in photosynthesis and in designing breeding programmes (Flood et al., 2011). The complete genome sequence of rice and many DNA markers are already available (IRGSP, 2005). Several advanced populations, including backcrossed inbred lines and chromosome segment
substitution lines, have been developed to facilitate the genetic analysis of rice (Yamamoto et al., 2009; Fukuoka et al., 2010). Recent improvements in the quantification of photosynthesis have reduced measurement times while maintaining accuracy in the field (Long and Bernacchi, 2003). These advances facilitate the identification of quantitative trait loci (QTLs) and isolation of the underlying genes. In recent studies, several QTLs for \( P_n \) have been identified in rice (Teng et al., 2004; Hu et al., 2009; Takai et al., 2010; Gu et al., 2012), and one gene has been isolated (Takai et al., 2013).

The combination of multiple QTLs—QTL pyramiding—offers a straightforward and useful way for improving target traits in rice (Wang et al., 2012). To evaluate the precise effects of the combination of QTLs, it is necessary to develop near-isogenic lines (NILs), which carry a single target QTL in a unique genetic background to eliminate background noise, and to cross these NILs (Ashikari and Matsuoka, 2006; Hospital, 2009). However, there has been limited effort so far to develop NILs for rice \( P_n \) (Gu et al., 2012) and no attempt to evaluate the effect of pyramiding QTLs on \( P_n \).

It is widely acknowledged that \( P_n \) is closely related to leaf nitrogen content (LNC) in rice, because large amounts of N are invested in ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the primary CO\(_2\)-fixation enzyme (Cook and Evans, 1983; Makino et al., 1992). Wide varietal differences in LNC have been observed even at the same rate of N application (Kanemura et al., 2007; Hirasawa et al., 2010). \( P_n \) is also affected by the diffusion of CO\(_2\) from the atmosphere to the chloroplasts. Varietal differences in stomatal conductance \( (g_s) \) have been observed even at a small vapour pressure deficit (Ohsumi et al., 2008; Hirasawa et al., 2010). Since \( g_s \) decreases as leaf water potential decreases, the hydraulic conductance from roots to leaves \( (C_h) \), which controls the water balance in plants, would affect the value of \( g_s \) (Hirasawa et al., 1989; Hirasawa and Ishihara, 1992; Brodribb et al., 2007). Mesophyll conductance \( (g_m) \), with respect to the diffusion of CO\(_2\) from the intercellular airspace to the chloroplasts, might also be important to improving rice \( P_n \) (Makino, 2011). Recent studies report genetic differences in \( g_m \) among Oryza species (Scafaro et al., 2011) and rice lines (Adachi et al., 2013). In addition to Rubisco content, LNC is associated with also \( g_s \) and \( g_m \) (Makino et al., 1988; Ishihara, 1995; Li et al., 2009). To clarify factors underlying the differences in \( P_n \) among rice cultivars and lines, the influences of differences in LNC should be considered carefully.

The \( P_n \) of young, newly expanded leaves ranges between \( \sim 20 \) and \( \sim 30 \, \mu\text{mol} \, \text{CO}_2 \, \text{m}^{-2} \, \text{s}^{-1} \) among rice cultivars at an ambient CO\(_2\) concentration of 370–400 \( \mu\text{mol} \, \text{mol}^{-1} \) under light-saturating and unstressed conditions (Kanemura et al., 2007; Hirasawa et al., 2010; Jahn et al., 2011). The high-yielding indica cultivar Habataki has one of the highest recorded rates of \( P_n \) among rice cultivars, at 30–33 \( \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \) (Asanuma et al., 2008). In contrast, Koshihikari, the most popular cultivar in Japan, has a relatively low \( P_n \) of 25–28 \( \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \).

In previous studies using introgression lines derived from a cross between Koshihikari and Habataki, this study group identified four QTLs \( (q\text{CAR}4, q\text{CAR}5, q\text{CAR}8, q\text{CAR}11) \) on chromosome 5, \( q\text{CAR}8 \) on chromosome 8, and \( q\text{CAR}11 \) on chromosome 11, Habataki alleles of which increased \( P_n \) (Sueyoshi et al., 2009; Adachi et al., 2011). The present work developed a pyramided line, NIL\((q\text{CAR}4+q\text{CAR}8)\), by crossbreeding NIL\((q\text{CAR}4)\) and NIL\((q\text{CAR}8)\), each of which has a single chromosome segment from Habataki substituted in the genetic background of Koshihikari, and quantified the effects on the rate of leaf photosynthesis and on processes related to photosynthesis.

### Materials and methods

#### Plant materials

Four QTL-NILs carrying a single chromosome segment from Habataki in the genetic background of Koshihikari were developed (Fig. 1; Sueyoshi et al., 2009; Adachi et al., 2011). NIL\((q\text{CAR}4)\), which carries a chromosome segment from Habataki on the long arm of chromosome 4, was crossed with NIL\((q\text{CAR}8)\), with a segment from the short arm of chromosome 8, and plants homozygous for Habataki in both regions were selected, to create the line NIL\((q\text{CAR}4+q\text{CAR}8)\). \( F_2 \) progeny were used in field experiments and \( F_3 \) progeny were used in pot experiments.

#### Cultivation of rice plants

Plants were grown at the university farm (35° 40’ N 139° 28’ E). In the field experiment, seedlings at the fourth-leaf stage were transplanted into the paddy field (alluvial clay loam) at a density of 22.2 hills m\(^{-2}\) (30 × 15 cm), with one plant per hill. As a basal dressing, manure was applied at 15 t ha\(^{-1}\) and inorganic fertilizer was applied at 30 kg N, 60 kg P\(_2\)O\(_5\), and 60 kg K\(_2\)O ha\(^{-1}\). One-third of the total N was applied as nitrogen sulphate, one-third as LP-50 elution-controlled urea (Chisso Asahi Fertilizer, Tokyo, Japan), and one-third as LPS-100 elution-controlled urea. No topdressing was applied.

In the pot experiment, plants were grown outdoors in 12-l pots filled with a 1:1 (v/v) mixture of paddy soil and upland soil (diluvial volcanic ash) at a density of three hills per pot, three plants per hill. Basal fertilizer was applied at 1.0 g N, 1.0 g P\(_2\)O\(_5\), and 1.0 g K\(_2\)O per pot, and additional N was applied at 0.5 g per pot at booting stage when the flag leaves had fully expanded. To examine the relationship between \( P_n \) and LNC, different amounts of N were applied to Koshihikari and Habataki at booting stage: at 0.25, 0.5, 1.0 or 2.0 g to Koshihikari and 0, 0.25 or 0.5 g to Habataki.

Plants were also grown in 3-l pots in a growth chamber (Koitotron, Koito Manufacturing, Tokyo, Japan) under a 12/12 light/dark cycle (28/23 °C, relative humidity 60/80%, and a photosynthetic photon flux density (PPFD) at the top of the canopy of 1000 \( \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \)). Basal fertilizer was applied at 0.5 g N, 0.5 g P\(_2\)O\(_5\), and 0.5 g K\(_2\)O per pot. No topdressing was applied.

#### Gas exchange measurements

Leaf gas exchange was measured with a portable gas-exchange system (LI-6400, LI-COR, Lincoln, NE, USA); flag leaves were measured at the full heading stage. The CO\(_2\) assimilation rate \( (A_{\text{ra}}) \) and stomatal conductance \( (g_s) \) were measured at an ambient CO\(_2\) concentration of 370 \( \mu\text{mol} \, \text{mol}^{-1} \), a PPFD of 2000 \( \mu\text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \), a leaf-to-air vapour pressure difference of 1.3–1.6 kPa, and an air temperature of 30 °C. The CO\(_2\) assimilation rate at an intercellular CO\(_2\) concentration \( (C) \) of 280 \( \mu\text{mol} \, \text{mol}^{-1} \) \( (A_{\text{ra}}) \) was also measured by changing the ambient CO\(_2\) concentration. Plants were examined from 08:30 to 11:00, when the photosynthetic rate was close to the daily maximum (Hirasawa and Ishihara, 1992; Ishihara, 1995). Measurements were made at the full heading stage. The measurement for each leaf was conducted once a day and was repeated on
day 2 or 3 and the mean of measurements calculated. Between five and seven leaves were used for each replicate.

**Determination of nitrogen content and dry matter weight**

The leaves were collected immediately after completion of the gas exchange measurements and were stored at –80 °C. The area of an 80-mm-long segment cut from the centre of the leaf was measured with a leaf area meter (AAM-9; Hayashi Denko, Tokyo, Japan). The segments were dried at 80 °C for 24 h to determine the nitrogen content with a CN analyser (MT700 Mark II, Yanako, Kyoto, Japan) and the dry matter weight.

**Determination of hydraulic conductance and conductivity**

The hydraulic conductance of the plants grown in 3-l pots, from the soil through the roots to the leaves ($C_p$, 10$^{-8}$ m$^3$ s$^{-1}$ MPa$^{-1}$) was calculated as $U_w$ \((\Psi_s - \Psi_l)\) (Hirasawa and Ishihara, 1991), where $U_w$ ($10^{-8}$ m$^3$ s$^{-1}$) is the water uptake rate of the whole plant, $\Psi_s$ (MPa) is the water potential of the soil immediately outside the root, and $\Psi_l$ (MPa) is the average water potential of the uppermost three leaves. Since plants were grown under submerged conditions and the water potential of the soil solution was high compared with $\Psi_l$ and was kept constant, $\Psi_s$ could be regarded as 0. Measurements were made in a controlled-environment chamber (air temperature 28 °C, air vapour pressure deficit 1.5 kPa, PPFD at the top leaves $1000 \mu$mol m$^{-2}$ s$^{-1}$). $U_w$ was determined from the rate of weight loss of the pot over 20 min after a steady state had been reached. To prevent evaporation from the surface of the pot, the top was covered with polystyrene foam and the gap between the foam and the stem was sealed with oil clay. After measurement of $U_w$, $\Psi_l$ of the uppermost three leaves was measured in a pressure chamber (model 3005, Soil Moisture Equipment, Santa Barbara, CA, USA). Measurements were conducted under the steady-state condition where the transpiration rate is equal to $U_w$. It is reported that the transpiration rate and $g_s$ do not influence hydraulic conductance when the transpiration rate is high (Fiscus, 1975; Hirasawa and Ishihara, 1991; Stiller et al., 2003). The $U_w$ per leaf area was sufficiently high (>2.0 mmol m$^{-2}$ s$^{-1}$) to eliminate the effect of the difference in water uptake rate on $C_p$. After roots had been washed gently in water, the root surface area of the total root system ($S_r$) was measured with an image analyser (Win-Rhizo REG V 2004 b, Regent, PQ, Canada). For comparing root hydraulic conductivity, the hydraulic conductivity of a plant ($L_p$, 10$^{-8}$ m s$^{-1}$ MPa$^{-1}$), defined as hydraulic conductance per root surface area (Steudle and Peterson, 1998).

**Results**

**Leaf photosynthesis**

The CO$_2$ assimilation rate at an ambient CO$_2$ concentration of 370 μmol mol$^{-1}$ ($A_{370}$) in the four NILs were significantly higher than that in Koshihikari—by 23% in NIL(qCAR4), 11% in NIL(qCAR5), 17% in NIL(qCAR8), and 9% in NIL(qCAR11) and significantly smaller than that in Habataki—7, 21, 12, and 13%, respectively (Fig. 2), indicating that the combination of the two QTLs additively increased leaf photosynthesis. Interestingly, $A_{370}$ in NIL(qCAR4+qCAR8) was comparable to that in Habataki despite being due to only two out of the four QTLs. Similar results were obtained in the pot experiments.

**Leaf nitrogen content**

In the field experiment, LNC was significantly higher in NIL(qCAR4) (by 27%) than in Koshihikari, but LNC in NIL(qCAR8) was similar to that in Koshihikari (Table 1). LNC in NIL(qCAR4+qCAR8) was higher than that in Koshihikari (by 24%) and similar to those in NIL(qCAR4) and Habataki. In the pot experiment, not only NIL(qCAR4)
but also NIL(qCAR8) showed higher LNC than Koshihikari. LNC in NIL(qCAR4+qCAR8) was even higher than that in NIL(qCAR4) (Table 1).

**Dry matter weight per leaf area**

In the field experiment, dry matter weight per leaf area (LMA) in Habataki was similar to that in Koshihikari (Table 1). LMA was significantly higher in NIL(qCAR4) (by 14%) than in Koshihikari, but LMA in NIL(qCAR8) was similar to that in Koshihikari. LMA in NIL(qCAR4+qCAR8) was higher than that in Koshihikari (by 12%) and similar to that in NIL(qCAR4). Similar results were obtained in the pot experiment.

**Stomatal conductance and hydraulic conductance**

In the field experiment, \( g_s \) was 24% higher in NIL(qCAR4) and 29% higher in NIL(qCAR8) than in Koshihikari (Table 1). It was 52% higher in NIL(qCAR4+qCAR8) than in Koshihikari and was higher than those in both NIL(qCAR4) and NIL(qCAR8), but still lower than that in Habataki. Similar results were obtained in the pot experiment.

\( \bar{C}_p \) was significantly higher in Habataki than in Koshihikari (Fig. 3A). It was significantly higher in NIL(qCAR4) (by 43%) and NIL(qCAR8) (by 40%) than in Koshihikari. It was 84% higher in NIL(qCAR4+qCAR8) than in Koshihikari, and was higher in NIL(qCAR4+qCAR8) than in NIL(qCAR4) (P=0.07) and NIL(qCAR8) (P=0.05), but still lower than in Habataki.

\( \bar{C}_p \) is related to \( S_t \) and \( L_p \) (Steudle and Peterson, 1998). The high value of \( S_t \) in Habataki contributed to the high \( \bar{C}_p \) in Habataki (Fig. 3A, B). \( S_t \) in NIL(qCAR8) was similar to that in Koshihikari, while that in NIL(qCAR4) was higher (although not significantly) than that in Koshihikari (Fig. 3B). \( S_t \) in NIL(qCAR4+qCAR8) was higher than that in Koshihikari and similar to that in NIL(qCAR4). \( L_p \) in Habataki was lower than that in Koshihikari (Fig. 3C). \( L_p \) in NIL(qCAR4) was similar to that in Koshihikari, while \( L_p \) in NIL(qCAR8) was higher (although not significantly) than that in Koshihikari. \( L_p \) in NIL(qCAR4+qCAR8) was higher than that in Koshihikari and similar to that in NIL(qCAR8). These results indicate that the elevated \( \bar{C}_p \) in NIL(qCAR4+qCAR8) resulted from

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**Table 1.** CO\(_2\) assimilation rate at a PPFD of 2000 \( \text{mol m}^{-2} \text{s}^{-1} \) and an ambient CO\(_2\) concentration of 370 \( \text{mol mol}^{-1} \), leaf nitrogen content, dry matter weight per leaf area, and stomatal conductance of plants grown in the field and in 12-l pots

|          | \( A_{370} \) (\( \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \)) | LNC (g m\(^{-2}\)) | LMA (g m\(^{-2}\)) | \( g_s \) (mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)) |
|----------|---------------------------------|-------------------|-------------------|------------------|
| Field    |                                 |                   |                   |                  |
| Koshihikari | 23.5 ± 1.1\(^c\)               | 1.46 ± 0.15\(^b\) | 58.4 ± 4.1\(^b\) | 0.66 ± 0.06\(^c\) |
| NIL(qCAR4) | 29.0 ± 0.1\(^{a,b}\) (123)   | 1.85 ± 0.06\(^c\) (127) | 66.7 ± 1.8\(^b\) (114) | 0.82 ± 0.07\(^b\) (124) |
| NIL(qCAR8) | 27.4 ± 1.4\(^b\) (117)       | 1.59 ± 0.15\(^{a,b}\) (109) | 59.1 ± 1.2\(^b\) (101) | 0.85 ± 0.06\(^c\) (129) |
| NIL(qCAR4+qCAR8) | 31.0 ± 1.2\(^c\) (132)    | 1.81 ± 0.17\(^{a}\) (124) | 65.2 ± 0.4\(^b\) (112) | 1.01 ± 0.05\(^b\) (152) |
| Habataki  | 31.5 ± 0.9\(^c\) (133)        | 1.85 ± 0.07\(^d\) (126) | 57.6 ± 0.5\(^d\) (99) | 1.82 ± 0.12\(^d\) (274) |
| Pots     |                                 |                   |                   |                  |
| Koshihikari | 22.5 ± 1.5\(^c\)           | 1.35 ± 0.06\(^b\) | 55.8 ± 1.1\(^c\)  | 0.55 ± 0.05\(^c\) |
| NIL(qCAR4) | 28.5 ± 0.8\(^c\) (129)    | 1.67 ± 0.11\(^b\) (124) | 63.6 ± 2.8\(^b\) (115) | 0.82 ± 0.03\(^c\) (151) |
| NIL(qCAR8) | 26.1 ± 0.8\(^c\) (117)     | 1.54 ± 0.06\(^c\) (115) | 57.4 ± 1.1\(^d\) (104) | 0.70 ± 0.02\(^d\) (128) |
| NIL(qCAR4+qCAR8) | 31.0 ± 0.5\(^c\) (139)  | 1.91 ± 0.12\(^c\) (142) | 64.2 ± 3.1\(^c\) (116) | 0.87 ± 0.04\(^c\) (159) |
| Habataki  | 31.5 ± 1.8\(^c\) (141)       | 1.80 ± 0.06\(^d\) (133) | 53.8 ± 1.1\(^d\) (97)  | 1.81 ± 0.15\(^d\) (331) |

Values are means±SD (\(n=3–6\)). Different superscript letters indicate significant differences between rice lines (\(P<0.05\), Tukey’s test). Values in parentheses are percentages relative to Koshihikari. \( A_{370} \): CO\(_2\) assimilation rate at an ambient CO\(_2\) concentration of 370 \( \text{mol mol}^{-1} \); \( g_s \): stomatal conductance; LMA, dry matter weight per leaf area; LNC, leaf nitrogen content.
simultaneous increases of $S_n$, which may have been inherited from NIL(qCAR4), and $L_p$, from NIL(qCAR8).

Relationship between $P_n$ and LNC

$A_{370}$ was always higher in Habataki than in Koshihikari at a given LNC and it increased with increases in LNC in both cultivars (Fig. 4A). $A_{370}$ was also higher in NIL(qCAR4), NIL(qCAR8), and NIL(qCAR4+qCAR8) than in Koshihikari at the same LNC. It is possible to estimate photosynthetic activity without considering the effect of $g_s$ by measuring $P_n$ at identical $C_i$ (von Caemmerer and Farquhar, 1981). There was no difference in $A_{370}$ between Koshihikari and Habataki at all levels of LNC examined (Fig. 4B). This indicates that the difference in $A_{370}$ was entirely due to the differences in LNC and $g_s$ between Koshihikari and Habataki (Supplementary Fig. S1 available at JXB online). $A_{370}$ in NIL(qCAR8) was similar to that in Koshihikari at the same LNC, while $A_{370}$ in NIL(qCAR4) and NIL(qCAR4+qCAR8) was higher than that in Koshihikari at given values of LNC. This indicates that factors other than LNC and $g_s$ are related to the high $A_{370}$ in both NIL(qCAR4) and NIL(qCAR4+qCAR8).

Discussion

Pyramiding of qCAR4 and qCAR8, which are QTLs for leaf photosynthesis, additively increased $P_n$ in rice. Similar approaches have yielded additive increases in number of grains (Ashikari et al., 2005; Ando et al., 2008), grain yield (Ohsumi et al., 2011; Wang et al., 2012), grain quality (Wang et al., 2012), plant height (Wang et al., 2012), and heading date (Shibaya et al., 2011; Wang et al., 2012). This is the first report to show that QTL pyramiding is effective at improving rice leaf photosynthesis.

Habataki has one of the highest recorded values of $P_n$ among rice cultivars, and at least four QTLs for leaf photosynthesis are associated with the difference in $P_n$ between Koshihikari and Habataki (Asanuma et al., 2008; Sueyoshi et al., 2009; Adachi et al., 2011). Because $A_{370}$ in NIL(qCAR4+qCAR8), with two of the four QTLs, was the same as in Habataki, with all four QTLs (Table 1), the possible reasons for the high $A_{370}$ need to be discussed from both genetic and physiological viewpoints.

Possible reasons for the high rate of leaf photosynthesis in the pyramided line

LNC strongly affects $P_n$ because it is closely related to the content of Rubisco (Makino et al., 1992). A difference in Rubisco content is a key factor in varietal differences in the capacity for leaf photosynthesis in rice (Hubbart et al., 2007; Hirasawa et al., 2010). In addition, the diffusion of CO$_2$ from the atmosphere to the chloroplasts, which is regulated by $g_s$ and $g_{m}$, is another important determinant (Ohsumi et al., 2008; Hirasawa et al., 2010; Makino, 2011). The high $P_n$ of Habataki might result from the high LNC, due to its elevated capacity for N accumulation, and from the high $g_s$, due to the large hydraulic conductance of plants, in turn due to the large root surface area compared with Koshihikari (Adachi et al., 2011).

In the field experiment, the higher LNC in NIL(qCAR4+qCAR8) would have contributed to the higher $A_{370}$ than that in Koshihikari (Table 1). The high LNC may have been inherited from NIL(qCAR4), because the values of LNC in these two NILs were similar and reached the level of Habataki. In a preliminary study, NIL(qCAR5) and NIL(qCAR11) also showed higher LNC.
than Koshihikari (data not shown). This result suggests that at least three QTLs are involved in the difference in LNC between Koshihikari and Habataki. The large increase in LNC in NIL(qCAR4+qCAR8) and NIL(qCAR4) cannot be explained in isolation from the Koshihikari background: that is, there might be other QTLs, the Koshihikari alleles of which increase LNC, or genetic interactions between qCAR4 and other unknown QTLs. The detailed genetic mechanisms should be examined in future research. In the pot experiment, LNC in NIL(qCAR8) was also higher than that in Koshihikari. This suggests that qCAR8 is also associated with LNC under some growing conditions. The interaction between genes related to photosynthesis and the environment would be another subject to study.

The high gs in NIL(qCAR4+qCAR8) may also have contributed to the higher A370 than in Koshihikari (Table 1). gs was additionally increased by the combination of qCAR4 and qCAR8. Although gs in Habataki was significantly higher than that in NIL(qCAR4+qCAR8), a greater gs might not increase photosynthesis in rice further (Hirasawa et al., 1988), as Habataki had a much greater gs but no greater A370. The critical water potential for stomatal closure is very much higher in rice than in other crop plants (Hirasawa, 1999). The value of gs in Koshihikari was already decreased by the reduction in leaf water potential because of its low Cp even when the vapour pressure deficit was as low as ~1.5 MPa, whereas the higher gs in Habataki was supported by the maintenance of higher leaf water potential through the higher hydraulic conductance (Adachi et al., 2011). This is clear because water-stress-relaxation treatments increased A370 and gs in Koshihikari leaves to the same level as in Habataki at similar LNC but had no effect in Habataki leaves (Adachi et al., 2011). This means that rice gs is affected markedly by Cp (Taylaran et al., 2011). Because the NILs in this research were derived from the cross between Koshihikari and Habataki and the measurement conditions of both A370 and Cp were exactly the same as in Adachi et al. (2011) and Taylaran et al. (2011), the connection between Cp and gs may be the case with the rice lines in this research. The combination of NIL(qCAR4) and NIL(qCAR8) may have resulted in the higher Cp in NIL(qCAR4+qCAR8) than in Koshihikari (Fig. 3A) and thus in the high gs. The even greater Cp in Habataki might thus explain the far larger gs than that of NIL(qCAR4+qCAR8). These assumptions should be tested by further water-stress-relaxation experiments.

The high Cs in NIL(qCAR4) is attributable to the high S\textsubscript{i}d whereas the high Cp in NIL(qCAR8) is attributable to the high Lp (Fig. 3B, C). The higher Cs in NIL(qCAR4+qCAR8) seems to result from the combination of both. This result also suggests that it is possible to improve S\textsubscript{i} and Lp simultaneously in rice breeding. C\textsubscript{p} was lower in NIL(qCAR4) than in Habataki on account of its smaller S\textsubscript{i}. This suggests that other QTLs are associated with the difference in S\textsubscript{i} between Koshihikari and Habataki. Although L\textsubscript{p} in Habataki was somewhat lower than that in Koshihikari, L\textsubscript{p} in NIL(qCAR8) was even higher than that in Koshihikari, which seems to be a result of the combination of the Habataki chromosome segment with the Koshihikari background. It is known that LMA is also related to LNC (Ninemets et al., 1999). However, the correlation
coefficient between these two parameters in this study was not high (0.53 for paddy and 0.47 for pots), suggesting that the LNC was primarily controlled by factors other than LMA. This study cannot rule out the possibility that differences in the Michaelis–Menten constant, the maximum carboxylation rate, and the activation state of Rubisco are associated with the difference in $A_{370}$ (Ishikawa et al., 2011), although these traits seem to be similar among rice cultivars (Makino et al., 1987). Further studies should clarify which traits are related to the high $A_{370}$ in NIL(qCAR4) and NIL(qCAR4+qCAR8).

The flowering time of NIL(qCAR8) and NIL(qCAR4+qCAR8) was 7 days earlier than that of Koshihikari, while that of NIL(qCAR4) was similar to that of Koshihikari. This indicates that the genetic region of qCAR8 includes genes associated with flowering time. The causal link between flowering and photosynthesis is unknown at this time and should be examined in future research.

Potential for further enhancement of photosynthesis beyond Habataki

Despite having only two out of the four QTLs, NIL(qCAR4+qCAR8) had the same $A_{370}$ as Habataki. This phenomenon may be explained by the combined effect of the two Habataki chromosome segments in the Koshihikari genetic background. The process of photosynthesis is complex and is controlled by many genes (Shi et al., 2005). Its improvement might be a challenging task (Flood et al., 2011). However, the current results suggest that it is possible to increase $P_n$ to the highest level known by introducing a small number of genes from donor plants if the right combinations of cultivars are selected. It might be possible to develop a rice with a $P_n$ even higher than that of Habataki if the additional QTLs for $P_n$ on chromosomes 5 and 11 (Fig. 2) were stacked into NIL(qCAR4+qCAR8). To test this hypothesis, this study group is developing rice lines that carry multiple QTLs for $P_n$ in the genetic background of Koshihikari.

Conclusion

The $P_n$ of NIL(qCAR4+qCAR8) was as high as that of Habataki, even though the NIL had only two of the four known QTLs for $P_n$. The high values of LNC, $S_n$ and possibly $g_m$ found in NIL(qCAR4) and of $L_p$ in NIL(qCAR8) combined in NIL(qCAR4+qCAR8) to increase $P_n$. The increases in some of these traits cannot be explained in isolation from the Koshihikari genetic background. These results suggest that QTL pyramiding is a powerful approach in breeding of rice for increased $P_n$, and it should be possible to develop rice with even higher capacity for photosynthesis by stacking other QTLs into NIL(qCAR4+qCAR8).

Supplementary material

Supplementary data are available at JXB online. Supplementary Fig. S1. Relationships between leaf N content and stomatal conductance in flag leaves.

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