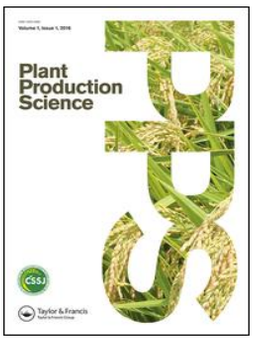

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The QTL Analysis of RuBisCO in Flag Leaves and Non-Structural Carbohydrates in Leaf Sheaths of Rice Using Chromosome Segment Substitution Lines and Backcross Progeny F_2 Populations

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Abstract : In rice (*Oryza sativa* L.), the maintenance of high photosynthetic rate of flag leaves and the carbon remobilization from leaf sheaths after heading is a critical physiological component affecting the yield. To clarify the genetic basis of RuBisCO content of the flag leaf, a major determinant of photosynthetic rate, and non-structural carbohydrate (NSC) concentration in the third leaf sheath at heading, we carried out quantitative trait loci (QTL) analysis with 39 Koshihikari/Kasalath chromosome segment substitution lines (CSSLs) and backcross progeny F_2 population derived from target CSSL holding the QTL/Koshihikari in the field. QTLs for RuBisCO content and NSC concentration at heading were detected between R2447-C1286 and R2447-R716 on chromosome 10, respectively, by comparing Koshihikari with four CSSLs for chromosome 10 (SL-229, -230, -231 and -232). The progeny QTL for RuBisCO content and for NSC concentration at heading *qRCH-10* and *qNSCLSH-10-1*, respectively, were detected at similar marker intervals between RM8201 and RM5708. In addition, QTLs for RuBisCO content at 14 d after heading, *qRCAH-10-1* and *qRCAH-10-2*, were detected in regions different from that of *qRCH-10*. No QTL for NSC concentration at 14 d after heading was detected between RM8201 and R716, the region analyzed in this study. The QTLs *qRCH-10* and *qRCAH-10-1* for RuBisCO content would have additive effects. These QTLs for RuBisCO content and NSC concentration newly found using CSSLs and their backcross progeny F_2 population should be useful for better understanding the genetic basis of source and temporary-sink functions in rice and for genetic improvement of Koshihikari in terms of their functions.

Key words : Backcross progeny, Chromosome segment substitution lines (CSSLs), Leaf sheath, Non-structural carbohydrate (NSC), QTL, Rice (*Oryza sativa* L.), RuBisCO.

Sink and source functions, and their relationships are fundamental physiological basis of biomass production and yield in cereal crops. The source function in rice (*Oryza sativa* L.) leaves includes photosynthetic carbon fixation, sucrose biosynthesis and sucrose loading into sieve elements. On the other hand, the sink function in rice panicles includes sucrose unloading from the phloem to the endosperm and starch biosynthesis in the endosperm. In addition, culms and leaf sheaths play a role in temporal carbon storage before and after heading; these organs function as a carbon sink before heading and as a carbon source after heading (Ohsugi, 2005).

Interspecific differences or cultivar differences in the apparent photosynthetic rate per unit leaf area have been studied because the apparent photosynthetic rate per unit leaf area is considered to be closely related to the growth rate and yield of rice; a high positive correlation is observed between the apparent photosynthetic rate per unit leaf area of flag leaf after anthesis and the grain yield (Cook and Evans, 1983; Sasaki et al., 1986; Ishii, 1995). The rice apparent photosynthetic rate per unit leaf area tends to be higher in modern high-yielding cultivars (Hayami, 1982; Jiang et al., 1988; Nakazawa et al., 1990; Ishii, 1995; Xu et al., 1997). These cultivar differences in the apparent photosynthetic rate per unit leaf area are closely related to the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO, EC 4.1.1.39) protein, which is a criterion of source function in mature leaves (Makino et al., 1984; Ishii, 1995).

Grain formation, grain filling and grain quality, such as percentage of chalky or milky-white kernel, are affected by the amount of nonstructural carbohydrates (NSC) supplied from culms and leaf sheaths after heading, which is considered to contribute to 10–40 percent of the total carbohydrate filled in the grain

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(Weng et al., 1982; Sumi et al., 1996; Kodani and Kuroda, 2006; Nakagawa et al., 2006; Yamaguchi et al., 2006). The third leaf sheath (the leaf sheath of the second leaf below the flag leaf) accumulates much more starch than the others (Hirose et al., 1999), and there were considerable variations of the NSC in the third leaf sheath among cultivars with different yield productivity (He et al., 2005).

It is important to investigate sink and source functions on a molecular and genetic basis to understand their complex network and find a new target for breeding (Ohsugi, 2005). In rice, the usefulness of quantitative trait locus (QTL) analysis using chromosome segment substitution lines (CSSLs) that substituted a small segment of the chromosome has already been demonstrated (Kubo et al., 2002; Ebitani et al., 2005; Ishikawa et al., 2005). For example, 39 Koshihikari/Kasalath CSSLs have a high detection power but low epistatic effect because basically only a partial segment of one chromosome of Koshihikari was substituted with a Kasalath segment. Furthermore, the combination of QTL analysis using CSSLs and backcross progeny population derived from target CSSL holding the QTL of Koshihikari was also recommended in order to obtain the QTLs with high resolution and to estimate the accurate QTL effects (Ebitani et al., 2005; Kanbe et al., 2008).

Previously, QTLs for RuBisCO content (Ishimaru et al., 2001) and NSC concentration (Nagata et al., 2002) have been studied using segregated populations which include epistasis under the complex gene interactions. However, those reports were still far from practical use of QTLs directly toward the development of valuable near isogenic lines (NIL). QTLs detected in CSSLs can be useful in the transition to breeding of NIL(Ando, 2005). Furthermore, the CSSL itself including valuable allelic effects from Kasalath on a certain QTL on the genetic background of Koshihikari might be useful for breeding new cultivars.

The present work aims to find some QTLs for RuBisCO content on the flag leaf and NSC concentration on the third leaf sheath at heading by the combination of QTL analyses using 39 Koshihikari/Kasalath CSSLs in multiyear tests and backcross progeny derived from target CSSL which had the QTL of Koshihikari under the comparatively small effects of complex gene interactions. In addition, we tried to find some QTLs for RuBisCO content of the flag leaf and NSC concentration in the third leaf sheath at 14 d after heading using backcross progeny population because of the importance of maintaining source activity and remobilization of NSC to panicle after heading (Matsushima and Wada, 1959; Kusutani, 1988; Ohsugi, 2003).

Materials and Methods

1. Phenotyping and QTL detection in CSSLs

The 39 (SL-201-SL-239) Koshihikari/Kasalath CSSLs (Rice Genome Resource Center, 2003; Ebitani et al., 2005) and Koshihikari were grown on the experimental paddy field (35°44' N, 139°32' E, ASL 58 m) of Field Production Science Center at the University of Tokyo by the conventional cultivation method in 2002, 2003 and 2004. Seeds were sown in a greenhouse on 19 April 2002, 25 April 2003 and 27 April 2004. Four leaf-age seedlings were transplanted into paddy field (one plant per hill), at intervals of 30×30 cm (11.1 hills m^{-2}) on 21 May in 2002, 27 May in 2003 and 25 May in 2004, respectively. The 50 g m^{-2} compound fertilizer for paddy field (N: P_2O_5: K_2O=12: 16:18%) was applied to the paddy field as a basal dressing. The 39 CSSLs and parents were planted in single row (65.8 m^2) with two replications. At the heading of each CSSL or Koshihikari, the flag leaf and the third leaf sheath on the main stem were harvested, frozen with liquid N and stored at –80°C until measurement (n=3).

One-way ANOVA post hoc test (Dunnett's pairwise multiple comparison test using Koshihikari as the control) with SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan) was used to detect differences between the mean values of the trait in Koshihikari and each CSSL. When we observed a significant difference (<0.05 probability level) between Koshihikari and particular CSSL in multiple years, the putative QTL region was assumed to be located in the substituted chromosomal segment in that CSSL (Kanbe et al., 2008).

2. QTL mapping using progeny F_2 population

The CSSL having the QTL for RuBisCO content and NSC concentration on chromosome 10 (SL-231) was backcrossed with Koshihikari as the pollen parent. The 440 F_2 individual seeds derived from F_1 plants by self-pollination were grown in a greenhouse. Seeds were sown on 28 April in 2005, transplanted into a paddy field in Field Production Science Center at the University of Tokyo on 23 May (30×30 cm, 11.1 hills m^{-2}), and the flag leaf and the third leaf sheath on the main stem were harvested in a similar manner as in the QTL analysis using the CSSL population described above.

To investigate the change of traits during grain filling stage, we sampled 220 out of 440 F_2 individuals at heading, and the remained half at 14 d after heading. To analyze leaf traits in detail, we measured flag leaf length, flag leaf width, and flag leaf area with a leaf area meter (AAM-6, Hayashi Denko Co. Ltd., Tokyo, Japan), and specific leaf weight was calculated from the leaf area and the leaf dry weight.

QTL F_2 progeny were detected by using a QTL Cartographer 2.5 (Wang et al., 2006) with above LOD

Table 1. Mean values of the investigated traits in Koshihikari and range of CSSLs in each year and *F* test of the values in CSSLs in three years.

Trait	Year	Koshihikari mean ± SD	Range of CSSLs
RuBisCO content on the flag leaf at heading ($g\ m^{-2}$)	2002	1.4 ± 0.7	1.2–3.2
	2003	1.4 ± 0.3	1.3–4.3
	2004	1.9 ± 0.2	1.4–3.2
		Year *F*	19.0***
NSC concentration on the third leaf sheath at heading (%)	2002	18.1 ± 4.4	10.7–50.2
	2003	11.4 ± 4.4	11.0–48.1
	2004	21.5 ± 1.8	20.5–34.5
		Year *F*	8.3***

*** indicates significant at 0.1% level.

value 2.5. Correlation analysis were carried out with SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan) for the traits among the F_2 progeny. The QTLs detected here were named according to McCouch et al. (1997).

3. Determination of RuBisCO content

Six leaf-disks 6 mm in diameter (1.7 cm^2) without midribs were sampled from each flag leaf blade sample on the main stem, and then homogenized with quartz sand in a buffer solution (pH 7.9) containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 0.5 mM EDTA-2Na, 5 M DTT, 0.2% (w/v) PVPP and 0.1% (w/v) Triton X-100. After the homogenate was centrifuged at 19,000 ×g for 20 min, the supernatant was treated with 0.625 M Tris-HCl (pH 6.8), 2.0% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) BPB and 5.0% (v/v) 2-mercaptoethanol at 100ºC for 3 min. The RuBisCO content was determined by densitograph gel documentation system (Densitograph Series No.2 Ver.1.0, ATTO, Tokyo, Japan) after CBB R250-staining and 15.0% (w/v) SDS-PAGE (mini slab gel electrophoresis apparatus AE-6500W, ATTO, Tokyo, Japan) according to Makino et al. (1986). A calibration curve was made with bovine serum albumin (BSA) and reference RuBisCO solution extracted from the leaf of rice cultivar Nipponbare.

4. Determination of NSC concentration in the third leaf sheath

The third leaf sheaths were dried at 80ºC for 3 d. The NSC concentration was determined gravimetrically using 1,4-α-D-glucan glucohydrolase (Amyloglucosidase, EC 3.2.1.3) according to the method of Sasaki et al. (2005). Dry weight of the third leaf sheath and the total amount of NSC of the third leaf sheath (NSC content) were also measured.

5. DNA marker analysis

DNA was extracted from leaves at the early tillering stage by alkali extraction method (Wang et al., 1993). On the basis of the database (Sakata et al., 2000; Jaiswal et al., 2006; Cold Spring Harbor Laboratory and Cornell University, 2007; Rice Genome Research Program, 2007), a linkage map used for QTL detection consists of 6 alternative SSR markers (McCouch et al., 2002) on the target QTL region. PCR was conducted with a thermocycler (GeneAmp 9700, Applied Biosystems Japan Ltd., Tokyo, Japan) using Smart Taq DNA Polymerase (SP-1000, Nippon genetics, Tokyo, Japan).

Results

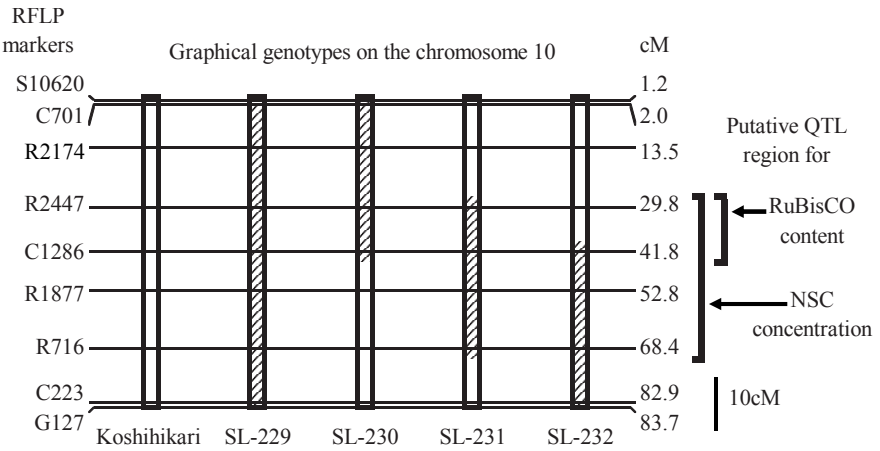
1. Phenotypic range of CSSLs in three years

The Koshihikari/Kasalath CSSL showed a wide variation for RuBisCO content and NSC concentration in each year of 2002, 2003 and 2004 (Table 1). The fluctuations of RuBisCO content and NSC concentration were smaller in 2004 than in the other years. Both *F* test of RuBisCO content and NSC concentration between the years was significant at the 0.1% level, showing that there was a high inter-annual variation. The *F* value of RuBisCO content was much higher than that of NSC concentration in the three years.

2. Mapping of the QTL region for RuBisCO content and NSC concentration on chromosome 10 using CSSL

Fig. 1 shows putative QTL region for RuBisCO content and NSC concentration on chromosome 10 estimated by comparing the phenotypic difference between Koshihikari and 4 CSSLs in each year. Significant CSSLs genotype×year interactions were detected by two-way ANOVA; we used one-way ANOVA post hoc test to detect QTL. The other CSSLs did not show significant differences in RuBisCO content or NSC concentration from Koshihikari in multiple years (data not shown).

SL-229 which has the Kasalath homozygous region on chromosome 10, showed a significant RuBisCO content increase, compared to Koshihikari in 2003 and a significant NSC concentration increase in 2 years. On the other hand, SL-232, which has the Kasalath



Year					
	RuBisCO content (g m^{-2})				
2002	1.4	1.6 ns	1.5 ns	1.7 ns	1.8 ns
2003	1.4	3.0 **	3.1 **	3.3 ***	1.9 ns
2004	1.9	1.9 ns	1.8 ns	2.5 *	2.3 ns
	NSC concentration (%)				
2002	18.1	26.5 ns	18.2 ns	37.3 ***	19.7 ns
2003	11.4	20.8 **	11.7 ns	34.9 ***	16.0 ns
2004	21.5	26.2 **	23.1 ns	31.7 ***	24.8 ns

Fig. 1. Mapping putative QTL for RuBisCO content (g m^{-2}) and NSC concentration (%) by comparing Koshihikari with 4 CSSLs on chromosome 10 in each year. Open and hatched regions in graphical genotype indicate Koshihikari homozygous and Kasalath homozygous, respectively. Mean values are shown. ns, *, ** and *** show no significant difference and significant difference from Koshihikari at 5, 1 and 0.1% levels (Dunnett's pairwise multiple *t*-test), respectively.

Table 2. QTL analysis of the RuBisCO content at heading, NSC concentration in the third leaf sheath at heading and RuBisCO content at 14 d after heading in progeny F_2 population derived from SL-231/Koshihikari cross on chromosome 10.

Trait	*QTL*	Marker interval	Map location (cM)	LOD	Effects on the phenotype			
					a	*d*	*d/a*	R^2 %
RuBisCO content on the flag leaf at heading (g m^{-2})	*qRCH-10*	RM8201 – RM5708	30.1	6.0	0.60	–0.03	–0.04	33.0
NSC concentration on third leaf sheath at heading (%)	*qNSCLSH-10-1*	RM8201 – RM5708	27.1	2.8	0.94	0.18	0.19	6.1
	qNSCLSH-10-2	RM5708 – RM5304	35.2	2.7	0.87	0.61	0.70	7.0
RuBisCO content at 14 DAH (g m^{-2})	*qRCAH-10-1*	RM258 – RM7020	50.3	2.9	0.24	–0.11	–0.46	9.6
	qRCAH-10-2	RM7020 – RM8202	60.5	3.2	0.20	–0.27	–1.37	8.3

a; additive effect of Kasalath allele on each traits, *d*; dominant effect of the Kasalath allele, *d/a*; degree of dominance, R^2 %; percentage of phenotypic variance explained by each QTL

homozygous region on the side of the long arm, showed no significant difference from Koshihikari for RuBisCO content and NSC concentration in 3 years. SL-230 had a significantly higher RuBisCO content than Koshihikari in 2003, but showed no significant difference from Koshihikari in NSC concentration in 3 years. SL-231 showed a significantly higher RuBisCO content in two years and NSC concentration in three years, compared to Koshihikari. The putative QTL region for RuBisCO content was estimated to be between R2447 and C1286 (29.8–41.8 cM) from the results of SL-229, -230 and -231. The putative QTL region for NSC concentration was also estimated to be between R2447 and R716 (29.8–68.4 cM) from the increase of NSC concentration in SL-229 and -231. Based on these estimates, SL-231, which has both QTLs for RuBisCO content and NSC concentration, was chosen as the material for analysis of the backcross progeny F_2 populations.

3. QTL detection using backcross progeny F_2

In order to validate the QTL detected on

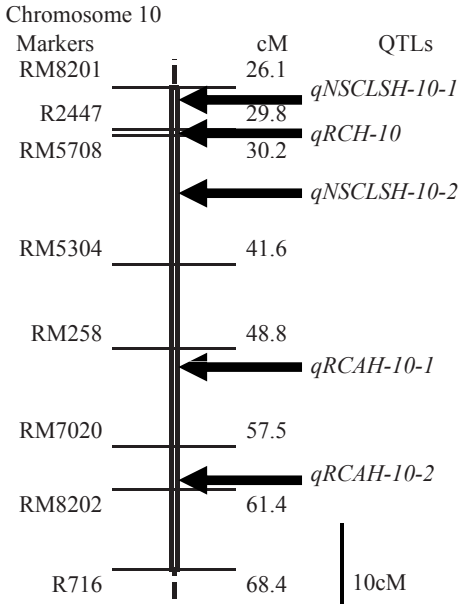


Fig. 2. Mapping progeny QTLs for RuBisCO content at heading, NSC concentration in the third leaf sheath at heading and RuBisCO content at 14 d after heading in progeny F_2 population derived from SL-231/Koshihikari cross in the chromosome region containing QTL in SL-231. Arrows indicate the position of QTL.

chromosome 10 and to analyze it on a narrower chromosomal area, we developed F_2 populations derived from backcross between SL-231 and Koshihikari. The progeny QTL for RuBisCO content of flag leaf blade at heading *qRCH-10* was detected adjacent to RM5708, having a high LOD value of 6.0 and R^2% value 33.0 (Table 2, Fig. 2). Two QTLs for NSC concentration of the third leaf sheath at heading were detected: *qNSCLSH-10-1* between RM8201 and RM5708 with moderate genetic effects, which had the same marker interval as *qRCH-10*, and *qNSCLSH-10-2* between RM5708 and RM5304. In addition, *qRCAH-10-1* and *qRCAH-10-2* for RuBisCO content at 14 d after heading were detected in the regions different from that of *qRCH-10* for RuBisCO content at heading. These QTLs showed an LOD value of 2.9 and 3.2, and R^2 % value 9.6 and 8.3, respectively. There was no QTL for NSC concentration at 14 d after heading. Based on the degree of dominance, the effects of *qRCH-10* (–0.04), *qNSCLSH-10-1* (0.19) and *qRCAH-10-1* (–0.46) were referred to as additive. On the other hand, *qNSCLSH-10-2* (0.70) and *qRCAH-10-2* (–1.37) showed a dominant and recessive effect, respectively.

4. Correlation analysis of related traits

Table 3 shows Pearson's product moment correlation coefficient of RuBisCO content , NSC concentration and related traits (flag leaf length, flag leaf width, flag leaf area, specific leaf weight on the flag leaf, dry weight of third leaf sheath and NSC content of third leaf sheath) investigated at heading and 14 d after heading. The LOD values of these related traits in QTL analyses were below threshold value of 2.5 (data not shown). The coefficient of the correlation of RuBisCO content at heading with that of the other 7 traits showed a low absolute value in the analysis. The coefficient of correlation between RuBisCO content and the other 3 traits (dry weight of third leaf sheaths, NSC concentration and NSC content) at 14 d after heading also showed very low values. In this progeny F_2 population, SLW did not influence the RuBisCO content and NSC concentration at all. On the other hand, NSC concentration was closely related to NSC content (r=0.73 and 0.88, P<0.001) in both growth stages.

Table 3. Pearson's product moment correlation coefficient of traits investigated at heading and 14 d after heading among progeny F_2 population derived from SL–231/Koshihikari cross on chromosome 10.

Growth stage	Trait	RC	FLL	FLW	FLA	SLW	DWLS	NSC%
Heading	FLL	0.25 ***						
	FLW	0.10 ns	0.50 ***					
	FLA	0.21 *	0.96 ***	0.62 ***				
	SLW	0.12 ns	0.12 ns	0.09 ns	0.12 ns			
	DWLS	0.02 ns	0.34 ***	0.43 ***	0.39 ***	0.22 ***		
	NSC %	0.07 ns	0.33 ***	0.13 ns	0.31 ***	–0.17 *	0.35 ***	
	NSCC	0.05 ns	0.41 ***	0.37 ***	0.43 ***	0.08 ns	0.89 ***	0.73 ***
14 d after heading	DWLS	–0.14 *	–	–	–	–		
	NSC %	0.05 ns	–	–	–	–	–0.00 ns	
	NSCC	–0.01 ns	–	–	–	–	0.47 ***	0.88 ***

RC; RuBisCO content, FLL; flag leaf length; FLW; flag leaf width, FLA; flag leaf area, SLW; specific leaf weight of the flag leaf, DWLS; dry weight of third leaf sheath, NSC%; NSC concentration of third leaf sheath, NSCC; NSC content of third leaf sheath. ns, * and *** indicate no significance, significant correlation (two-tailed test) at 5 and 0.1% levels, respectively.

Discussion

In this study, we used CSSLs and backcross progeny F_2 population to detect valuable and reliable QTLs for RuBisCO content and NSC concentration. We detected putative QTLs for RuBisCO content and NSC concentration on chromosome 10 by comparing the 4 CSSLs for chromosome 10 with Koshihikari (Fig. 1). We also detected 5 QTLs for RuBisCO content and NSC concentration using backcross progeny F_2, using SL-231, the CSSL including both QTLs for RuBisCO content and NSC concentration, for the parental line of progeny (Fig. 2, Table 2). The QTL for RuBisCO content varied with the experimental year in CSSLs, but a distinctive QTL was detected with progeny F_2. On the other hand, the QTL for NSC concentration was clearly detected in CSSLs, but the genetic effects of two QTLs detected in progeny F_2 were low. To explain the difference of NSC concentration among SL-230, -231 and -232, we assumed the existence of negative allelic effects of Kasalath in S10620-R2447 and R716-G127 to counterbalance the QTL. However, it is difficult to explain by this assumption why SL-229 had a higher NSC concentration in 2003 and 2004, compared to Koshihikari. There might be another allele(s), which affects the NSC concentration on chromosome 10, or there might be complicated gene interactions among alleles having a positive or negative effect on NSC concentration when all these alleles were present on chromosome 10, although it is beyond the scope of the present study to dissect every genetic trait of the alleles in the candidate QTL region.

Considering the error of measurement, as Kenney-Hunt et al. (2006) have defined, *qRCH-10*, *qNSCLSH-10-1* and *qRCAH-10-1* showed additive allelic effects of Kasalath. These allelic effects of the QTLs were considered to be valuable for the improvement of RuBisCO content and NSC concentration in an actual breeding scheme.

The QTL *qRCH-10* we detected on chromosome 10 with the genetic effect of the Kasalath allele for increasing RuBisCO content at heading was not detected in a previous study using Nipponbare/Kasalath backcross inbred lines (BILs) population (Ishimaru et al., 2001). They detected QTLs for the amount of RuBisCO protein at 5 or 25 d after heading on the chromosome 1, 5, 8, 9 and 12. Since, the CSSL population shows a higher detection power of QTLs than other mapping populations (Ebitani et al., 2005), *qRCH-10* on the chromosome 10 detected in this study might be elicited from complex gene interactions.

The RuBisCO enzyme in higher plants is known to consist of two different subunits; the large subunit is encoded by the chloroplast genome (*rbcL*), and the small subunit by the nuclear genome (*rbcS*) (Spreitzer, 1999). We measured the content of large subunits of RuBisCO. Ishimaru et al. (2001) suggested that the QTLs for the content of RuBisCO large subunit, soluble protein and total leaf N is not directly related to structural genes, such as *rbcS*. The *qRCH-10* did not overlap with any *rbcS* gene (National Bioresource Project and National Institute of Genetics, 2000; Kurata and Yamazaki, 2006; Cold Spring Harbor Laboratory and Cornell University, 2007). Therefore, the QTL gene(s) on chromosome 10 might function as a link between the nuclear genome and plastid gene expression. Furthermore, in rice leaves, RuBisCO protein consists of approximately 50% total soluble protein and 25% total nitrogen (Makino, 2003). Regardless of irradiance, temperature or CO_2 concentration during growth, the ratio of RuBisCO to total N in a rice leaf appears to be determined only by the amount of N (Ishimaru et al., 2001). Senthilvel et al. (2008) quantified genotype×nitrogen level interaction and mapped QTLs associated with nitrogen use efficiency and other agronomic traits. They demonstrated that the QTLs for number of productive tillers under different nitrogen usage condition corresponded to that for RuBisCO content on the chromosome 9 in Ishimaru et al. (2001). The QTL for RuBisCO content in this study might also be related to various factors of nitrogen usage including its absorption, metabolism and translocation.

The photosynthetic rate in rice grown under low nitrogen supply may be limited only by the amount of RuBisCO protein (Makino et al., 1984; Ishii, 1995; Nurul Amin et al., 2002). A linear correlation was observed between maximum photosynthetic rate and RuBisCO content less than approximately 2.0 to 3.0 g m^{-2} in various rice cultivars at different nitrogen supply levels (Uchida et al., 1980; Makino et al., 1994; Fukayama et al., 1996; Nurul Amin et al., 2002). The slope of the relationship decreased significantly above 4 g m^{-2} of RuBisCO content (Makino et al., 1994). In this study, the RuBisCO content of Koshihikari was in the 1.4–2.2 g m^{-2} range (data not shown) under the 6 g N m^{-2} basal dressing condition. The QTLs for RuBisCO content detected here might be attractive for enhancing the photosynthetic rate under a low nitrogen condition, which may lead to environmentally-friendly agriculture, although it is necessary to examine the response of this QTL to different nitrogen conditions.

In this study, QTLs for RuBisCO content were detected not only at heading but also at 14 d after heading. In addition, the regions of *qRCAH-10-1* and *qRCAH-10-2* for RuBisCO content at 14 d after heading were different from the region of *qRCH-10*, in particular, the *qRCAH-10-2* showed recessiveness by the Kasalath allele (Table 2, d/a=−1.37). These genetic effects were weaker than that of *qRCH-10*. This study suggested that the genetic factor(s) determining the RuBisCO content at heading are different from those that at 14 d after heading. These QTLs (RM258-

RM8020, 17.8–20.0 Mbps) completely overlapped with a digenic epistatic QTL *rrgf10* (RM304-RM147, 18.4–20.7 Mbps) for stay-green (index for the relative retention of greenness) of the flag leaf at 30 d after heading detected in doubled haploid population derived from Zhenshan 97/Wuyujing 2 cross (Jiang et al., 2004). Delayed leaf-senescence, or stay-green, is regarded as desirable characteristics for high yielding rice (Jiang et al., 2004). The maintenance of source activity (i.e. the apparent photosynthetic rate per unit leaf area or RuBisCO content) for 14 d after heading is also considered important since the most recent high-yielding cultivars possess high source activity for 14 d after heading and the assimilate produced during the ripening period heavily influenced the percentage of filled grains (Weng et al., 1982; Kusutani, 1988; Ohsugi, 2003).

The progeny QTLs for NSC concentration at heading (*qNSCLSH-10-1* and *qNSCLSH-10-2*) were newly detected in the vicinity of *qRCH-10*. Nagata et al. (2002) found QTLs for NSC concentration in leaf sheaths and culms on chromosome 5 and 11 but not on chromosome 10 using Sasanishiki/Habataki BILs population. NSC in the culms and leaf sheaths at heading might be controlled mainly by the balance of starch and sucrose metabolism including sucrose transport. The *qNSCLSH-10-1* detected between RM8201 and RM5708 on chromosome 10 (13.5-14.2 Mbps) were adjacent to the region in which some sucrose transporter genes were located (National Bioresource Project and National Institute of Genetics, 2000; Aoki et al., 2003; Kurata and Yamazaki, 2006; Cold Spring Harbor Laboratory and Cornell University, 2007). These sucrose transporter genes might be responsible for *qNSCLSH-10-1*, although fine mapping is necessary to clarify this relationship. No QTL was detected for NSC concentration at 14 d after heading between RM8201 and R716, the region analyzed in this study, suggesting that QTL(s) responsible for this trait might not be present or might be in other regions including different chromosomes.

Generally, the pleiotropy of QTL leads to the correlated response. Nagata (2006) suggested that QTLs for NSC have some pleiotropic effects. In this study, neither NSC concentration nor RuBisCO content showed a significant correlation coefficient, although *qNSCLSH-10-1*, *qNSCLSH-10-2* and *qRCH-10* were detected in similar chromosome region (Fig. 2, Table 2). These QTLs for source and temporary sink functions could be applied to breeding without any offset effect, although more detailed genetic analyses might be necessary for the actual breeding.

The results of this study showed the importance of the combination of CSSLs and their progeny F_2 population to detect QTLs under the comparatively small effects of complex gene interactions as demonstrated by Ebitani et al. (2005) and Kanbe et al. (2008). We also confirmed that it was possible to conduct genetic analysis of crop physiological traits using a smaller number of CSSLs (39 lines in this study) in comparison to F_2 or BILs, which could shorten the time and labor for analysis. Further work is under way to clarify the candidate genes in these QTL regions using some QTL-NILs.

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