

# Identification of QTLs for Improvement of Plant Type in Rice (*Oryza sativa* L.) Using Koshihikari / Kasalath Chromosome Segment Substitution Lines and Backcross Progeny F<sub>2</sub> Population

Takashi Kanbe<sup>1</sup>, Haruto Sasaki<sup>2</sup>, Naohiro Aoki<sup>1</sup>, Tohru Yamagishi<sup>1</sup>, Takeshi Ebitani<sup>3</sup>, Masahiro Yano<sup>4</sup> and Ryu Ohsugi<sup>1</sup>

<sup>1</sup>Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan;

<sup>2</sup>Field Production Science Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Midori-cho, Nishitokyo, Tokyo 188-0002, Japan;

<sup>3</sup>Toyama Prefectural Agricultural, Forestry and Fisheries Research Center, 1124-1 Yoshioka, Toyama, Toyama 939-8153, Japan;

<sup>4</sup>Department of Molecular Genetics, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan)

**Abstract** : Thirty-nine chromosome segment substitution lines (CSSLs) population derived from a Koshihikari / Kasalath cross was used for quantitative trait locus (QTL) analysis of plant type in rice (*Oryza sativa* L.). Putative rough QTLs (26.2~60.3cM of Kasalath chromosomal segments) for culm length, plant height, panicle number, chlorophyll content of flag leaf blade at heading and specific leaf weight, were mapped on the several chromosomal segments based on the comparison of CSSLs with Koshihikari in the field experiment for 3 years. In order to verify and narrow QTLs detected in CSSLs, we conducted QTL analyses using F<sub>2</sub> populations derived from a cross between Koshihikari and target CSSL holding a putative rough QTL. The *qPN-2*, QTL for panicle number was mapped on chromosome 2. In traits of flag leaf, the *qCHL-4-1* and *qCHL-4-2* for chlorophyll content was mapped on chromosome 4, and the *qSLW-7* for specific leaf weight on chromosome 7. All QTLs were detected in narrow marker intervals, compared with rough QTLs in CSSLs. The *qPN-2*, *qCHL-4-1* and *qCHL-4-2* had only additive effect. On the other hand, the *qSLW-7* showed over-dominance. It could be emphasized that QTL analysis in the present study with the combination of CSSLs and backcross progeny F<sub>2</sub> population can not only verify the rough QTLs detected in CSSLs but also estimate allelic effects on the QTL.

**Key words** : Backcross progeny, CSSLs, Leaf, Panicle, Plant type, QTL, Rice (*Oryza sativa* L.).

Recently, based on the improvement of the DNA marker and high-resolution genetic map, many novel mapping populations for quantitative trait locus (QTL) analysis were developed, resulting in identification of many valuable QTLs (Tanksley and Nelson, 1996; Paran and Zamir, 2003). Particularly, the analysis of QTL using a germplasm that substituted a small segment of a chromosome and the saturated genetic map with co-dominant DNA markers could reduce the effects of epistasis under the complex gene interactions, enabling to specify the QTL regions on the basis of phenotypic differences and genotypes (Tanksley and Nelson, 1996). For example, isogenic QTL mapping population such as near isogenic lines (NILs), chromosome segment substitution lines (CSSLs) and introgression lines (ILs) have already been demonstrated to be valid for the QTL detection of days to heading and cadmium concentration in rice (*Oryza sativa* L.) (Yamamoto et al., 2000; Kubo et al., 2002; Ebitani et al., 2005; Ishikawa et al., 2005), fruits qualities in tomato (Fridman et al., 2002; Liu

et al., 2003) and seed weight in sorghum (Tuinstra et al., 1997; Paran and Zamir, 2003; Serraj et al., 2005). Recently, 39 novel CSSL populations derived from japonica cultivar Koshihikari / indica cultivar Kasalath cross were developed in which basically only a partial segment of one chromosome was substituted with Kasalath one in the genetic background of Koshihikari (Fig. 1, Rice Genome Resource Center, 2003; Ebitani et al., 2005).

The detection power of QTLs in CSSLs population is superior to that in primary mapping populations, such as F<sub>2</sub> or recombinant inbred lines (RILs) maintaining the effect of epistasis, but the mapping resolution for QTLs in the former population is inferior than the latter populations, because such resolution depends on the size of substituted chromosome segment (Ebitani et al., 2005). The relatively rough QTLs detected in a CSSLs population have been studied more in detail using reciprocal sets of CSSLs populations in rice (Kubo et al., 2002; Uchimura et al., 2003) or backcross progeny populations in maize (Bouchez et al., 2002).

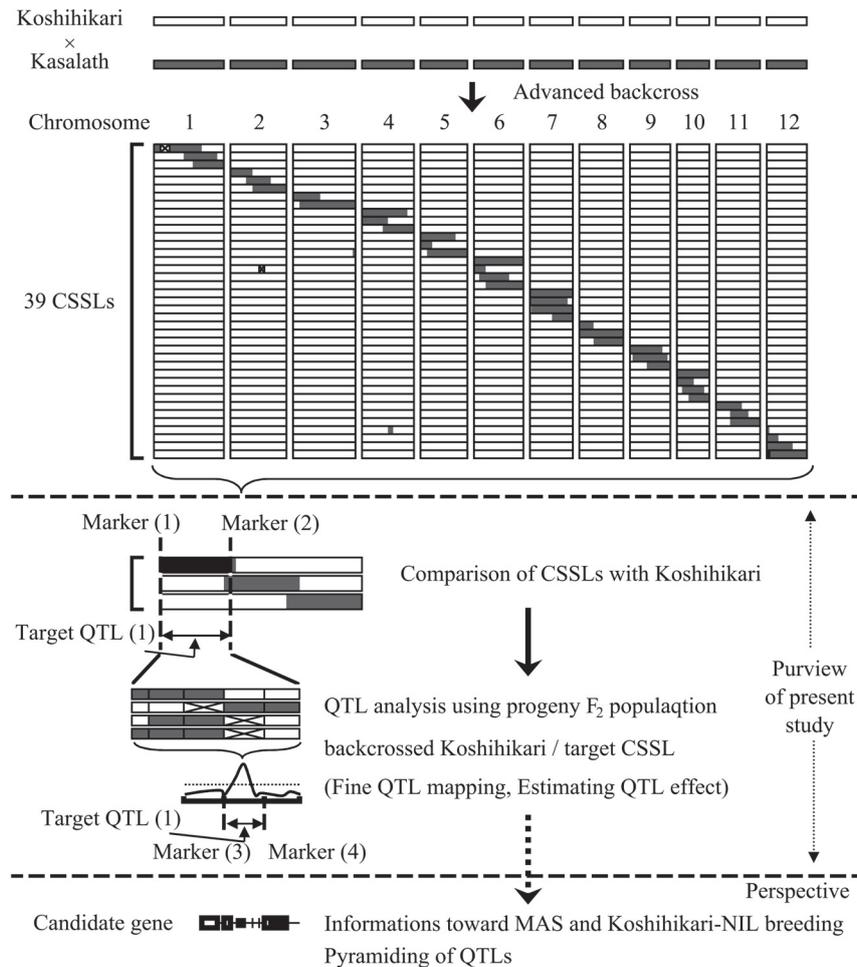


Fig. 1. Conceptual diagram of QTL analysis using CSSLs population and backcross progeny F<sub>2</sub> population derived from Koshihikari / target CSSL in the present study. Twelve chromosomes are layout horizontally and parents or each CSSLs are distributed vertically. White, gray and crossed region indicate Koshihikari homozygous, Kasalath homozygous and heterozygous in the graphical genotypes, respectively. Graphical genotype of 39 CSSLs population is cited from Rice Genome Resource Center (2003).

Ebitani et al. (2005) also recommended that this disadvantage of CSSLs population can be overcome by fine mapping of putative QTLs using backcross progeny populations with CSSLs as the base materials or parents. To date, CSSLs have been used only to verify the ordinary fineness of QTL analysis with the primary mapping population (Yamamoto et al., 2000; Takeuchi et al., 2007). In addition to verify the result of CSSLs, the progeny test can allow to estimate allelic effects on the QTL such as additive effect, dominant effect and phenotypic difference explained with QTLs. Such information is very important because a QTL with only a dominant effect is difficult to apply to the breeding scheme since this genetic effect is not fixable. The use of CSSLs population is also advantageous for rapid construction of linkage mapping on the specific target QTL region among progeny population (Ebitani et al., 2005).

Many researchers have reported QTLs for agronomic traits toward the improvement of plant type such as culm length, plant height, panicle number, chlorophyll content and specific leaf weight etc. (Wu and Luo, 1996; Ishimaru et al., 2001; Yamamoto et al., 2001; Wang et al., 2003; Cui et al., 2004; Dong et al., 2005). For example, Ishimaru et al. (2001) constructed a rice function map for some agronomic characters with Nipponbare / Kasalath BILs and detected 9 QTLs for plant height, 3 QTLs for panicle number per plant, 10 QTLs for chlorophyll content, 5 QTLs for leaf area and 2 QTLs for specific leaf area. Six QTLs for culm length on the chromosome 1, 3, 6 and 12 were detected by Yamamoto et al. (2001) using Koshihikari / Kasalath BILs. Most of the QTL were detected under a single environment without reiterant studies (Yamamoto et al., 2000; Li et al., 2003; Wan et al., 2005). There are few reports on consistently detectable QTLs

toward fine mapping and gene isolation (Wissuwa et al., 1998; 2002; Wissuwa and Ae, 2001). In order to obtain reliable QTLs for breeding, QTL analysis for a wide range of phenotypes should be done for the same genetic resource under a different environment (Paran and Zamir, 2003).

The aim of this study was to find reliable and valuable QTLs starting with CSSLs toward the improvement of some traits related to plant type in rice because the plant type is an important factor which contributes to the improvement of biomass and/or yield. To this end, we performed multi-year tests using the CSSLs derived from the Koshihikari and Kasalath cross, and we also conducted the validation of detected QTLs and the estimation of allelic effect on the QTL by the QTL analysis using the progeny F<sub>2</sub> population derived from the Koshihikari / target CSSL holding the QTL (Fig. 1).

## Materials and Methods

### 1. Meteorological parameters

Mean air temperature (°C) and total solar radiation (MJ m<sup>-2</sup>) were measured at the Field Production Science Center of the University of Tokyo (UT Farm), Nishitokyo (35°44' N, 139°32' E, ASL 58 m) from 2002 to 2004. Climatic normal values were cited from the homepage data of Japan Meteorological Agency at Nerima (for mean air temperature from 1979 to 2000) and Tokyo (Otemachi, for total solar radiation from 1972 to 2000) observation site (Japan Meteorological Agency, 2002).

### 2. Phenotyping in CSSLs

The 39 Koshihikari / Kasalath CSSLs (SL-201~SL-239) were developed with 130 RFLP markers at the National Institute of Agrobiological Science, Rice Genome Resource Center (RGRC, Tsukuba, Japan) in 2001 (Rice Genome Resource Center, 2003; Ebitani et al., 2005). CSSLs and their parental line, Koshihikari, were grown on the experimental paddy field of the UT Farm by the conventional cultivation method from 2002 to 2004 (SL-209 and SL-238 were not used in 2002 because they were provided in 2003). Seeds were sown in a greenhouse on 19 April 2002, 25 April 2003 and 27 April 2004. Four-leaved seedlings were transplanted (one plant per hill) at intervals of 30 × 30 cm (11.1 hills m<sup>-2</sup>) on 21 May in 2002, 27 May in 2003 and 25 May in 2004. Compound fertilizer for paddy fields (N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O=12: 16: 18%) was applied at 50 g m<sup>-2</sup> as a basal dressing. The 39 CSSLs and parents were planted in single row (65.8 m<sup>2</sup>) with two replications.

At the heading of each CSSL or parent cultivar, culm length, plant height, panicle number per plant and SPAD (Chlorophyll meter SPAD-502, Minolta, Tokyo, Japan) value of flag leaf were measured (n=5). The chlorophyll content was estimated by the regression analysis between specific chlorophyll content

measured by Smith-Benitez's method (1955) and SPAD value of 188 flag leaf blades (chlorophyll content (g m<sup>-2</sup>)=0.011107×SPAD value, R<sup>2</sup>=0.989, P<0.001). Five leaf disks 6 mm in diameter without a midrib were harvested from each flag leaf blade (n=3), and dried at 80°C for 3 days for determination of dry weight and specific leaf weight. In the analysis of F<sub>2</sub> population, to analyze QTL for specific leaf weight in more detail, we also measured flag leaf area by the area meter (AAM-6, Hayashi Denko Co. Ltd., Tokyo, Japan) and dry weight of flag leaf at heading.

### 3. QTL mapping using CSSLs

One-way ANOVA post hoc test (Dunnett's pairwise multiple comparison test using Koshihikari as the control) was used to detect differences between the mean values of the trait in Koshihikari and each CSSL. When we observed a significant difference (P<0.05) between Koshihikari and particular CSSL in each year, putative QTL was assumed to be located in substituted chromosomal segment(s) in that CSSL. CSSL that showed obviously a different tendency among CSSL holding similar chromosome regions was excluded.

### 4. QTL mapping using progeny F<sub>2</sub> population

Three CSSLs containing the valuable QTL for panicle number on chromosome 2 (SL-204) that for chlorophyll content on chromosome 4 (SL-209) and that for specific leaf weight on chromosome 7 (SL-222) were backcrossed with Koshihikari as the pollen parent, respectively. Each 200 F<sub>2</sub> individual seeds derived from a F<sub>1</sub> plant by self-pollination were sown in a greenhouse on 28 April in 2005, transplanted into a paddy field at the UT Farm on 23 May (30×30 cm, 11.1 hills m<sup>-2</sup>), and traits were measured for QTL analysis. F<sub>2</sub> progeny QTL was detected by using QTL Cartographer 2.5 (Wang et al., 2006) with above LOD value 2.5. Correlation and partial correlation analyses were carried out for three traits of flag leaf among the F<sub>2</sub> progeny.

### 5. DNA marker analysis

DNA was extracted from young fresh leaves at the early tillering stage by the alkali extraction method (Wang et al., 1993). Based on the database (Sakata et al., 2000; Jaiswal et al., 2006; Cold Spring Harbor Laboratory and Cornell University, 2007; Rice Genome Research Program, 2007), 4 or 5 SSR markers (McCouch et al., 2002) on the target QTL were chosen. DNA samples were diluted to ×300 and mixed with Smart Taq DNA Polymerase (SP-1000, Nippon Genetics, Tokyo, Japan), 0.1% gelatin, dye, 30% glycerol, and distilled water with SSR marker in a total volume 20 μL. The PCR was conducted with a thermocycler (GeneAmp 9700, Applied Biosystems Japan Ltd., Tokyo, Japan); first extension at 95°C for 10 min, and 35 cycles at 94°C for 1 min, 55°C for

1 min and 72°C for 2 min with a final extension at 72°C for 7 min. PCR products were fractionated by electrophoresis through 3% agarose gel in 0.5% TBE for 2 hr at 150V. Currently detected QTLs were named according to McCouch et al. (1997).

## Results

### 1. Meteorological parameters

Meteorological data showed marked differences among the three years (Fig. 2). The mean air temperature and the total solar radiation from July to

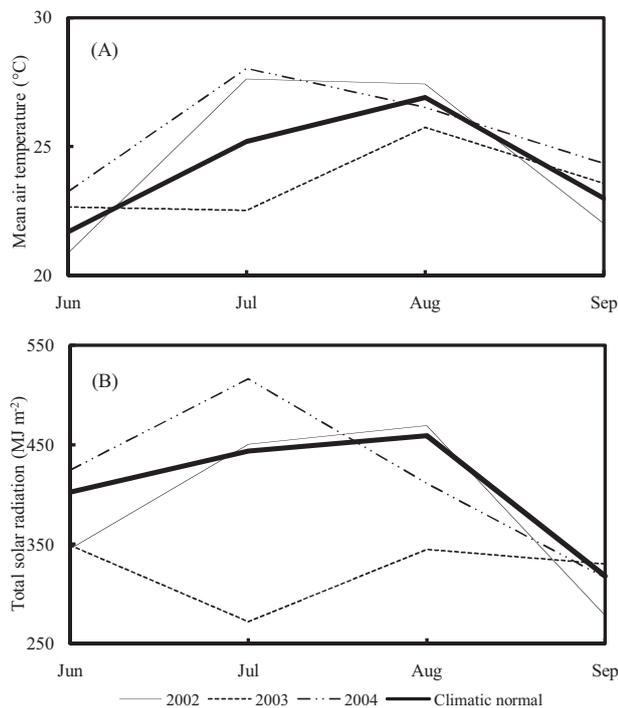


Fig. 2. Changes of meteorological parameters (mean air temperature (A) and total solar radiation (B)) during cultivation period at UT Farm (Nishitokyo) in 3 years with climatic normal. Thin right, dotted, chain double-dashed and heavy line indicate meteorological parameters in 2002, 2003, 2004 and climatic normal, respectively. Climatic normal values were cited from the data of Japan Meteorological Agency (2002) at Nerima (mean air temperature) and Tokyo (Otemachi) (total solar radiation) observation site.

August in 2003 were lower than those in either 2002 or 2004. Furthermore, they were also low, compared with the climatic normal, indicating that the year of 2003 had a typical cold summer with shortage of solar radiation.

### 2. Phenotypic variation in Koshihikari and CSSLs

The values of culm length, plant height and panicle number in Koshihikari was smallest in 2003 possibly due to low temperature and shortage of solar radiation (Table 1). The values of the traits in CSSLs extended widely over the means of Koshihikari, although the highest value of specific leaf weight in CSSLs was lower than the mean of Koshihikari.

### 3. QTLs for culm length, plant height and panicle number

Three QTLs for culm length were detected on chromosomes 1, 2 and 3 (Fig. 3). On chromosome 1, SL-202 and SL-203 showed significant differences in culm length from Koshihikari in each year with an increase of 5.2%~20.0%. Thus, putative QTL *qCL-1* was detected in shared chromosomal segment between C1370 and C742. In a similar manner, *qCL-2* (between C747 and C1470) on chromosome 2 and *qCL-3* (between C515 and S1513) on chromosome 3 were detected.

The QTL for plant height *qPH-3* (C515~S1513) was detected in the same region as in *qCL-3* on chromosome 3 (Fig. 4). The *qPH-8* on chromosome 8 (C390~C1121) was also detected. Although some CSSLs had a shorter culm length and plant height than Koshihikari in each year, no CSSLs were found to show shorter culm length or plant height than Koshihikari for 3 years.

Only one putative QTL for panicle number, *qPN-2* was detected in the interval between C1357 and G132 on chromosome 2 (Fig. 5).

### 4. QTL for chlorophyll content and specific leaf weight

Although data for SL-209 in 2002 was lacking (see Materials and Methods), the *qCHL-4* for chlorophyll content was detected in between C513 and C1016

Table 1. Mean values for the traits of the Koshihikari and the range of CSSLs in 3 years.

Trait	Koshihikari mean $\pm$ SD			Range of CSSLs		
	2002	2003	2004	2002	2003	2004
CL (cm)	81.3 $\pm$ 5.1	73.8 $\pm$ 3.1	85.3 $\pm$ 2.3	71.7~98.3	65.3~83.7	70.5~96.9
PH (cm)	109.7 $\pm$ 3.1	103.2 $\pm$ 2.6	114.2 $\pm$ 2.7	103.7~129.7	92.3~120.7	102.8~129.2
PN (No. m <sup>-2</sup> )	185.4 $\pm$ 45.6	151.0 $\pm$ 22.9	229.8 $\pm$ 24.0	114.3~276.4	106.6~232.0	192.0~305.3
CHL (g m <sup>-2</sup> )	0.45 $\pm$ 0.03	0.38 $\pm$ 0.01	0.39 $\pm$ 0.01	0.33~0.50	0.35~0.51	0.32~0.44
SLW (g m <sup>-2</sup> )	63.6 $\pm$ 3.5	53.5 $\pm$ 2.7	64.0 $\pm$ 7.1	46.3~70.6	47.9~60.4	47.1~63.4

CL; culm length, PH; plant height, PN; panicle number, CHL; chlorophyll content, SLW; specific leaf weight.

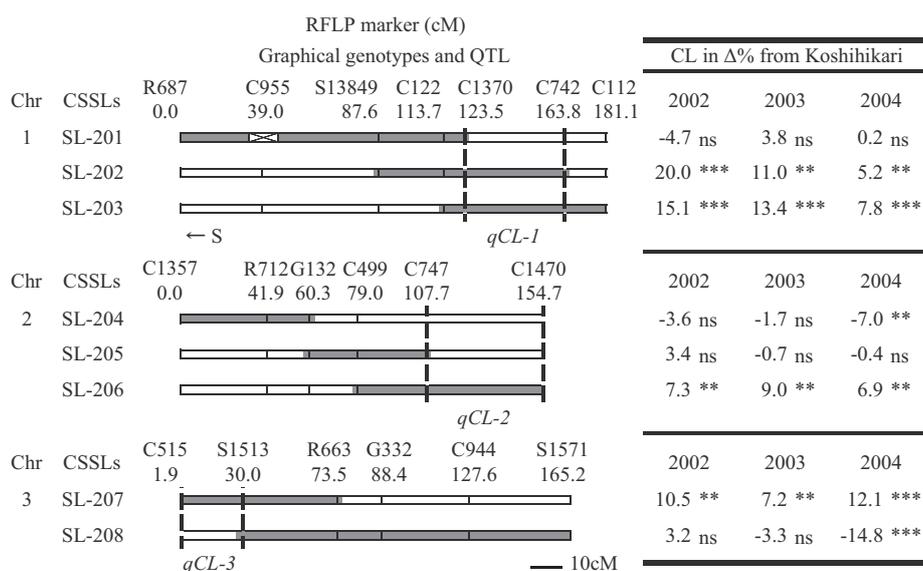


Fig. 3. The putative QTLs for culm length (CL) and graphical genotype and phenotypic variation (trait in Δ% from Koshihikari) of each CSSL in each year. Chr; chromosome number. White, gray and crossed region in graphical genotype indicate Koshihikari homozygous, Kasalath homozygous and heterozygous, respectively. ← S; side of short arm. ns, \*, \*\* and \*\*\*; not significant, significant at 5%, 1% and 0.1% levels, respectively.

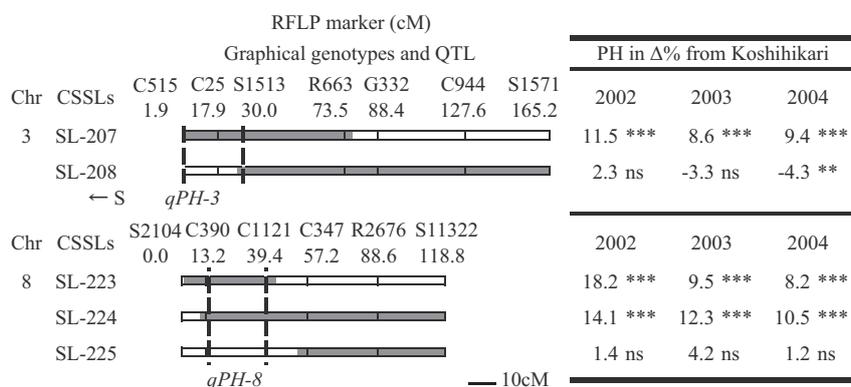


Fig. 4. The putative QTLs for plant height (PH) and graphical genotype and phenotypic variation of each CSSL in each year. See the details in Fig. 3.

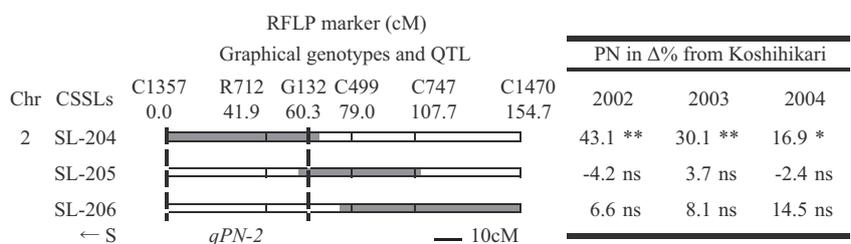


Fig. 5. The putative QTL for panicle number (PN) and graphical genotype and phenotypic variation of each CSSL in each year. See the details in Fig. 3.

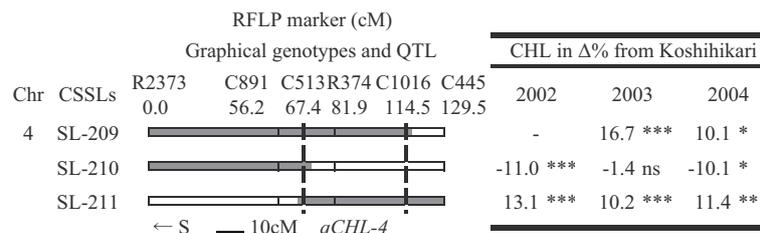


Fig. 6. The putative QTL for chlorophyll content (CHL) and graphical genotype and phenotypic variation of each CSSL in each year. See the details in Fig. 3.

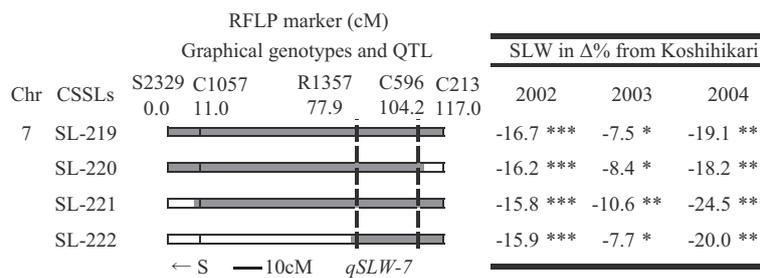


Fig. 7. The putative QTL for specific leaf weight (SLW) and graphical genotype and phenotypic variation of each CSSL in each year. See the details in Fig. 3.

Table 2. QTL analysis for the traits using progeny  $F_2$  population derived from Koshihikari / the target CSSL holding QTL for PN (No.  $m^{-2}$ ) on chromosome 2, CHL ( $g\ m^{-2}$ ) on chromosome 4 and SLW ( $g\ m^{-2}$ ) on chromosome 7.

Chr	Trait	Progeny QTL	Marker interval	Map location (cM)	LOD	Effects on the phenotype			
						<i>a</i>	<i>d</i>	<i>d/a</i>	$R^2\%$
2	PN (No. $m^{-2}$ )	<i>qPN-2</i>	RM3865~RM6378	21.9	2.7	22.9	-	-	8.6
4	CHL ( $g\ m^{-2}$ )	<i>qCHL-4-1</i>	RM241~RM255	96.5	5.9	0.02	-	-	17.1
		<i>qCHL-4-2</i>	RM255~RM349	110.7	13.5	0.02	-	-	28.6
7	SLW ( $g\ m^{-2}$ )	<i>qSLW-7</i>	RM2752~RM234	83.9	4.1	-0.6	-1.6	2.4	14.9
	DWFL (mg)	<i>qDWFL-7-1</i>	RM2752~RM234	85.9	4.0	25.6	-48.6	-1.9	12.7
		<i>qDWFL-7-2</i>	RM234~RM429	96.9	3.4	20.0	-43.7	-2.2	8.3
	FLA ( $cm^2$ )	<i>qFLA-7-1</i>	RM2752~RM234	87.9	7.5	6.1	-7.9	-1.3	20.4
<i>qFLA-7-2</i>		RM234~RM429	97.9	7.5	4.6	-9.0	-2.0	17.4	

Chr; chromosome number, DWFL; dry weight of flag leaf, FLA; flag leaf area, *a*; additive effect of Kasalath allele on each traits, *d*; dominant effect of the Kasalath allele, *d/a*; degree of dominance, -; hypothesis of  $d=0$  was adopted,  $R^2\%$ ; percentage of phenotypic variance explained by each QTL.

on chromosome 4 (Fig. 6). The *qSLW-7* for specific leaf weight was detected between R1357 and C596 on chromosome 7 (Fig. 7).

##### 5. QTL analysis using progeny $F_2$ population

In order to validate QTLs detected in the CSSL analysis (*qPN-2*, *qCHL-4* and *qSLW-7*), we developed  $F_2$  populations derived from crosses between Koshihikari / target CSSL (SL-204, SL-209 and SL-222). Resultant  $F_2$  population was used for QTL analysis (Table 2). The putative QTL *qPN-2* for panicle number at heading was mapped between RM3865 and RM6378

on chromosome 2, which was within the region of QTL using CSSLs, though the genetic effect was low (LOD value 2.7 and  $R^2\%$  value 8.6) (Table 2, Fig. 8). Two QTLs for chlorophyll content of flag leaf blade at heading (*qCHL-4-1* and *qCHL-4-2*) were detected on chromosome 4 only with an additive effect. The QTL *qCHL-4-2*, which was located adjacent to RM349 on chromosome 4, had a high LOD value of 13.5 and  $R^2\%$  value of 28.6. The QTL *qSLW-7* for specific leaf weight of flag leaf blade at heading was detected between RM2752 and RM234. In addition, QTLs for dry weight of flag leaf (*qDWFL-7-1* and *qDWFL-7-2*) and for flag leaf

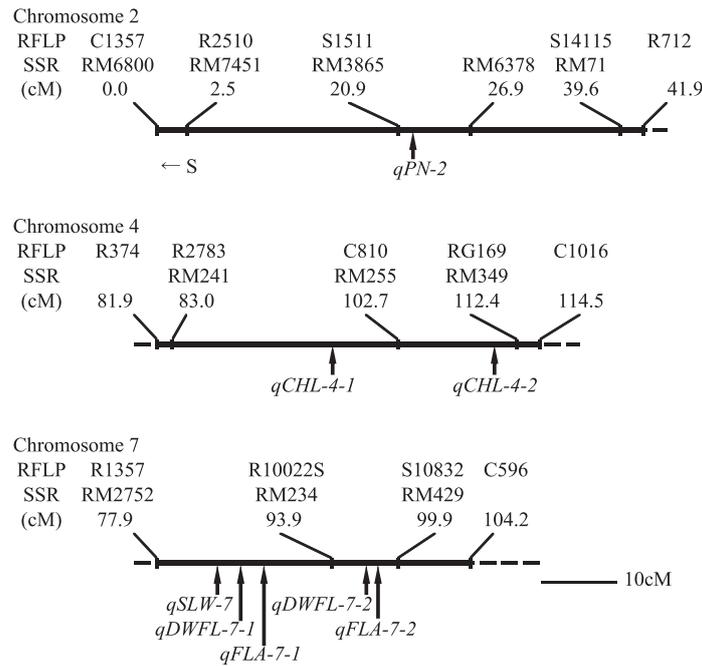


Fig. 8. Mapping progeny QTLs for panicle number, chlorophyll content, specific leaf weight, dry weight of flag leaf and flag leaf area in the enlargement of chromosome region containing QTL in CSSLs. Arrows indicate the position of QTL. Alternative SSR markers of RFLP in the present study are also shown.

Table 3. Correlation and partial correlation analysis for three traits of flag leaf among the progeny F<sub>2</sub> population derived from Koshihikari / SL-222 cross.

Source	Traits	SLW	DWFL
Pearson's product moment correlation coefficient	DWFL	0.63***	–
	FLA	0.25***	0.90***
Partial correlation coefficient	DWFL	0.97***	–
	FLA	–0.95***	0.99***

DWFL; dry weight of flag leaf, FLA; flag leaf area, SLW; specific leaf weight, \*\*\*; significant correlation (two-tailed test) at 0.1% level.

area (*qFLA-7-1* and *qFLA-2*) were detected in a similar region. LOD value and *R*<sup>2</sup> % value of *qFLA-7-1* (7.5 and 20.4) were higher than that of *qSLW-7* (4.1 and 14.9). The additive effect on the *qSLW-7* was negative. In contrast, QTLs for dry weight of flag leaf and flag leaf area showed positive additive effects.

We measured dry weight of flag leaf and flag leaf area in addition to specific leaf weight on QTL analysis using progeny F<sub>2</sub> population. As a result, QTLs for specific leaf weight, dry weight of flag leaf and flag leaf area were detected. To clarify the relationships among these QTLs, we conducted correlation and partial correlation analyses. The correlation analysis showed a close relation of the traits of flag leaf at the 0.1% level (Table 3). The partial correlation analysis revealed that

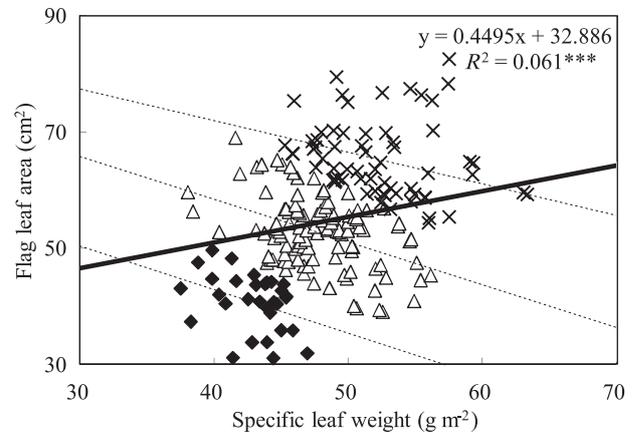


Fig. 9. Spurious correlation of specific leaf weight and flag leaf area from a view point of 3 classes of dry weight of flag leaf. Each plots show a value of each progeny F<sub>2</sub> individual. Black lozenges, white triangles and cross marks indicate the class of dry weight of flag leaf 200<, 200~300 and <300 (mg), respectively. Heavy line and dotted lines indicate regression line of whole population and each 3 classes of dry weight of flag leaf, respectively. \*\*\* shows significant correlation (two-tailed test) at 0.1% level.

the decrease in specific leaf weight leads to an increase in flag leaf area (*r* = –0.95, *P* < 0.001). When the scatter plots were divided into 3 levels of dry weight of flag leaf (200<, 200~300 and <300 mg), the distribution among component classes showed downward-sloping

and characteristics of spurious correlation (Fig. 9).

### Discussion

Many CSSLs populations have been used for the rough mapping on the basis of the comparison with the parental cultivar (Kubo et al., 2002; Ebitani et al., 2005; Ishikawa et al., 2005) and to verify a result of another mapping population (Yamamoto et al., 2000; Takeuchi et al., 2007). In this study, we used CSSLs and backcross progeny  $F_2$  population to detect valuable and reliable QTLs for plant type and the Dunnett's pairwise multiple test as a statistical method. As a result, we found 8 QTLs for culm length, plant height, panicle number, chlorophyll content and specific leaf weight using CSSLs (Figs. 3–7). In addition, by QTL analysis using backcross progeny  $F_2$  population, we estimated allelic effect on QTLs for these traits. Progeny QTLs for PN ( $qPN-2$ ) and CHL ( $qCHL-4-1$  and  $qCHL-4-2$ ) had only additive effect. On the other hand, other QTLs for leaf traits showed over-dominance and over-recessiveness. Therefore, it is possible to narrow QTL regions and estimate allelic effects by using progeny  $F_2$  population, although further analysis with much more DNA markers is necessary to detect accurate QTL and allelic effects.

The  $qCL-1$  (Fig. 3) obtained here was corresponded to the genetic effect of the Kasalath allele in the QTL for increasing culm length reported by other researchers (Ishimaru et al., 2001; Yamamoto et al., 2001), suggesting that this Kasalath allele has high generality and stability regardless of cross combination, generation and environmental condition. In addition, the other QTLs for culm length ( $qCL-2$  and  $qCL-3$ ) or plant height ( $qPH-3$  and  $qPH-8$ ) were similar to several QTLs detected using  $F_2$  and  $F_3$  populations derived from Tesanai 2 / CB (Zhuang et al., 1997),  $BC_1F_7$  derived from Nipponbare / Kasalath (Ishimaru et al., 2001) and doubled haploid (DH) lines derived from IR64 / Azucena (Li et al., 2003). These QTLs could be useful to change the plant type and light-intercepting characteristics that may lead to improvement of biomass in combination with a strong culm.

The  $qPN-2$  for panicle number detected in our study was different from the previous report in which QTLs for panicle number were detected on the chromosome 4, 7 and 8, and not detected on chromosome 2 (Ishimaru et al., 2001). This may be partly due to the fact that we used the single segment substitution which may elicit this QTL. However, it was difficult to compare our results with their reports directly because the cultivation conditions such as planting density and fertilizer application in their study were unknown. Keeping the necessary number of panicles is one of the most basic prerequisites for some theories of the ideal rice production (Inaba, 1991; Matsushima, 1995). Therefore, the QTL  $qPN-2$  constantly detected both in CSSLs and  $F_2$  populations would be useful for increas-

ing panicle number.

The improvements of flag leaf traits through breeding have also led to an increase in rice production (Samejima and Kumura, 1971; Evans and Terashima, 1987; Kobayashi et al., 2003). The QTL,  $qCHL-4$  for increasing chlorophyll content with the allelic effect by Kasalath was similar to the QTL previously reported (*i.e.* RG143-RG329 by Wu and Luo (1996) using  $F_2$  derived from IR42 / Palawan, and R896-P19 / M76-2 by Wang et al. (2003) using recombinant inbred lines (RILs) derived from Acc8558 / H359). One QTL with the allelic effect for decreasing chlorophyll content was already found near  $qCHL-4$ , but did not overlap with  $qCHL-4$  (Ishimaru et al., 2001). Their QTL might be included in the Kasalath region of SL-210 because SL-210 showed lower chlorophyll content than Koshihikari (Fig. 6). Dong et al. (2005) detected three QTLs for chlorophyll content at maximum tillering stage using Koshihikari / Kasalath RILs with high planting density ( $10 \times 15$  cm) in Miyazaki, Japan. However, they were different from our QTL detected at heading. These differences might be attributed to the differences in sampling period (maximum tillering stage or heading). Judging from our result together with the previous papers, we suggested that the detected  $qCHL-4$  would be a promising target to improve the plant type.

The QTL  $qSLW-7$  for specific leaf weight was detected in all 3 years. The QTL  $qFLA-7-1$  and  $qDWFL-7-1$  were detected near  $qSLW-7$  in the progeny  $F_2$  test. Specific leaf weight is the principal index of flag leaf area expansion in rice. In spite of similar marker interval, the  $qSLW-7$  showed the opposite additive effect from the  $qFLA-7-1$ . Our result showed that the decrease in specific leaf weight brought an increase in flag leaf area (Table 3). Partial correlation analysis and reference scatter diagram clearly showed spurious correlation relationship between specific leaf weight and flag leaf area under the strong influence of dry weight of flag leaf (Table 3, Fig. 9). Based on the correlation analyses, three QTLs might be correlated with each other and one of these QTLs might influence strongly the other two QTLs, although further analysis is required. Ishimaru et al. (2001) firstly measured specific leaf area (a reciprocal of specific leaf weight) in the QTL analysis of rice and detected two QTLs on the chromosome 3 and 11. They also investigated flag leaf area, but these correlations were not mentioned.  $qFLA-7-1$  with over-recessiveness may be additive and also available in breeding.

The results presented in this study clearly showed the importance of the combination of CSSLs and their progeny  $F_2$  population to detect QTLs including minor ones, such as  $qPN-2$ , which cannot be detected in the  $F_2$  or RILs. By using powerful genetic tools such as CSSLs, which isolate a single QTL region, the transition from a QTL to a Mendelian gene is feasible

not only for major QTL but also for minor ones (Paran and Zamir, 2003). In this study, several valuable QTLs for plant type were detected. These QTLs are expected to be used for breeding in the future because CSSLs containing target QTLs can be used as parental lines for a backcross with Koshihikari and also as candidates for new cultivars, for example NIL of Koshihikari. In addition, due to smaller number of lines compared to BILs and NILs (39 lines in this study), CSSLs can facilitate genetic analysis of crop physiological traits such as photosynthetic rate, content of RuBisCO or nonstructural carbohydrate that require a lot of efforts and time for analysis.

### Acknowledgements

We greatly thank RGRC for providing seeds of 39 CSSLs and parents. We also thank chief engineer N. Washizu, H. Kimura, S. Chikazawa and R. Soga for their growing rice plants and management of paddy field, and H. Kubota for collecting up meteorological parameters (UT Farm). We would like to thank Drs. K. Nemoto and O. N. Lee for their suggestions about polymorphic analysis and QTL analysis.

### References

- Bouchez, A., Hospital, F., Causse, M., Gallais, A. and Charcosset, A. 2002. Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. *Genetics* 162 : 1945-1959.
- Cold Spring Harbor Laboratory and Cornell University. 2007. GRAMENE Genome Browser. [Online]. Available at [www.gramene.org/](http://www.gramene.org/) (accessed 1 April 2006; verified 21 April 2008). GRAMENE Database, Ithaca.
- Cui, K. H., Peng, S. B., Ying, Y. Z., Yu S. B. and Xu C. G. 2004. Molecular dissection of the relationships among tiller number, plant height and heading date in rice. *Plant Prod. Sci.* 7 : 309-318.
- Dong, Y., Ogawa, T., Lin, D., Kamiunten, H., Terao, H. and Matsuo, H. 2005. Detection of quantitative trait loci for leaf chlorophyll content at maximum tillering. *Int. Rice Res. Notes* 30 : 16-17.
- Ebitani, T., Takeuchi, Y., Nonoue, Y., Yamamoto, T., Takeuchi, K. and Yano, M. 2005. Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of indica rice cultivar Kasalath in a genetic background of japonica elite cultivar Koshihikari. *Breed. Sci.* 55 : 65-73.
- Evans, J. R. and Terashima, I. 1987. Effects of nitrogen on electron transport components and photosynthesis in spinach. *Aust. J. Plant Physiol.* 14 : 59-68.
- Fridman, E., Liu, Y. S., Carmel-Goren, L., Gur, A., Shoshani, M., Pleban, T., Eshed, Y. and Zamir, D. 2002. Two tightly linked QTLs modify tomato sugar content via different physiological pathways. *Mol. Genet. Genom.* 266 : 821-826.
- Inaba, M. 1991. Yield increase with the improvement leaf lamina structure. In Rural Culture Association Ed., The outline of agricultural technology. Crop. 2-1. Rice; Basic technology (1). Theory for the high-yielding in rice. Nobunkyo, Tokyo, Japan. 104. 2-21\*\*.
- Ishikawa, S., Ae, N. and Yano, M. 2005. Chromosomal regions with quantitative trait loci controlling cadmium concentration in brown rice (*Oryza sativa*). *New Phytol.* 168 : 345-350.
- Ishimaru, K., Yano, M., Aoki, N., Ono, K., Hirose, T., Lin, S., Monna, L., Sasaki, T. and Ohsugi, R. 2001. Toward the mapping of physiological and agronomic characters on a rice function map : QTL analysis and comparison between QTLs and expressed sequence tags. *Theor. Appl. Genet.* 102 : 793-800.
- Jaiswal, P., Ni, J. J., Yap, I., Ware, D., Spooner, W., Youens-Clark, K., Ren, L. Y., Liang, C. Z., Zhao, W., Ratnapu, K., Faga, B., Canaran, P., Fogleman, M., Hebbard, C., Avraham, S., Schmidt, S., Casstevens, T. M., Buckler, E. S., Stein, L. and McCouch, S. 2006. Gramene : a bird's eye view of cereal genomes. *Nucleic Acids Res.* 34 D : 717-723.
- Japan Meteorological Agency. 2002. Past meteorological data retrieval. [Online]. Available at [www.data.jma.go.jp/obd/stats/etrn/index.php](http://www.data.jma.go.jp/obd/stats/etrn/index.php) (Accessed 7 April 2007). Japan Meteorological Agency homepage, Tokyo\*.
- Kobayashi, S., Fukuta, Y., Morita, S., Sato, T., Osaki, M. and Khush, G. S. 2003. Quantitative trait loci affecting flag leaf development in rice (*Oryza sativa* L.). *Breed. Sci.* 53 : 255-262.
- Kubo, T., Aida, Y., Nakamura, K., Tsunematsu, H., Doi, K. and Yoshimura, A. 2002. Reciprocal chromosome segment substitution series derived from japonica and indica cross of rice. *Breed. Sci.* 52 : 319-325.
- Li, Z. K., Yu, S. B., Lafitte, H. R., Huang, N., Courtois, B., Hittalmani, S., Vijayakumar, C. H. M., Liu, G. F., Wang, G. C., Shashidhar, H. E., Zhuang, J. Y., Zheng, K. L., Singh, V. P., Sidhu, J. S., Srivantaneeyakul, S. and Khush, G. S. 2003. QTL × environment interactions in rice. I. Heading date and plant height. *Theor. Appl. Genet.* 108 : 141-153.
- Liu, Y. S., Gur, A., Ronen, G., Causse, M., Damidaux, R., Buret, M., Hirschberg, J. and Zamir, D. 2003. There is more to tomato fruit colour than candidate carotenoid genes. *Plant Biotech. J.* 1 : 195-207.
- Matsushima, S. 1995. III Physiological mechanism of dry matter production. Chapter 8 physiology of high-yielding rice plants from the view point of yield components. In T. Matsuo, K. Kumazawa, R. Ishii, K. Ishihara and H. Hirata Eds., Science of the rice plant Vol. 2 physiology. Nobunkyo, Tokyo. 737-766.
- McCouch, S. R., Cho, Y. G., Yano, M., Paul, E., Blinstrub, M., Morishima, H. and Kinoshita, T. 1997. Report of the committee on gene symbolization, nomenclature and linkage groups II. Report from coordinators 1) Suggestions for QTL nomenclature for rice. *Rice Genet. Newsl.* 14 : 11-13.
- McCouch, S. R., Teytelman, L., Xu, Y. B., Lobos, K. B., Clare, K., Walton, M., Fu, B. Y., Maghirang, R., Li, Z. K., Xing, Y. Z., Zhang, Q. F., Kono, I., Yano, M., Fjellstrom, R., DeClerck, G., Schneider, D., Cartinhour, S., Ware, D. and Stein, L. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res.* 9 : 199-207.
- Paran, I. and Zamir, D. 2003. Quantitative traits in plants : beyond the QTL. *Trends Genet.* 19 : 303-306.
- Rice Genome Research Program. 2007. Generic genome browser version 1.66. [Online]. Available at [rgp.dna.affrc.go.jp/whoga/index.html.en](http://rgp.dna.affrc.go.jp/whoga/index.html.en) (accessed 1 April 2006; verified 21 April 2008). WhoGA Database, Tsukuba.
- Rice Genome Resource Center. 2003. Koshihikari/Kasalath Chromosome Segment Substitution Lines (CSSLs) 39 lines.

- [Online]. Available at [www.rgrc.dna.affrc.go.jp/index.html.en](http://www.rgrc.dna.affrc.go.jp/index.html.en) (accessed 1 April 2006). National Institute of Agrobiological Sciences, Tsukuba.
- Sakata, K., Antonio, B. A., Mukai, Y., Nagasaki, H., Sakai, Y., Makino, K. and Sasaki, T. 2000. INE : a rice genome database with an integrated map view. *Nucleic Acids Res.* 28 : 97-101.
- Samejima, M. and Kumura, A. 1971. Pigment content and kind in leaf and photosynthesis. In Y. Togari Ed., *Photosynthesis and matter production of crops*. Yokendo, Tokyo. 85-86\*\*.
- Serraj, R., Hash, C. T., Rizvi, S. M. H., Sharma, A., Yadav R. S. and Bidinger F. R. 2005. Recent Advances in marker-assisted selection for drought tolerance in pearl millet. *Plant Prod. Sci.* 8 : 334-337.
- Smith, J. H. C. and Benitez, A. 1955. Chlorophylls : analysis in plant materials. In K. Paech and M. V. Tracey Eds., *Modern methods of plant analysis*. Vol.4. Springer-Verlag, Berlin. 143-196.
- Takeuchi, Y., Nonoue, Y., Ebitani, T., Suzuki, K., Aoki, N., Sato, H., Ideta, O., Hirabayashi, H., Hirayama, M., Ohta, H., Nemoto, H., Kato, H., Ando, I., Ohtsubo, K., Yano, M. and Imbe T. 2007. QTL detection for eating quality including glossiness, stickiness, taste and hardness of cooked rice. *Breed. Sci.* 57 : 231-242.
- Tanksley, S. D. and Nelson, J. C. 1996. Advanced backcross QTL analysis : a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* 92 : 191-203.
- Tuinstra, M. R., Ejeta, G. and Goldsbrough, P. B. 1997. Heterogeneous inbred family (HIF) analysis : a method for developing near-isogenic lines that differ at quantitative trait loci. *Theor. Appl. Genet.* 95 : 1005-1011.
- Uchimura, Y., Sato, H., Ogata, T., Matsue, Y. and Yoshimura, A. 2003. Selection of the DNA markers for rice amylose content in japonica-indica cross variety. *Bull. Nat. Agr. Res. Cent. Kyushu Okinawa Region* 18 : 59-60\*\*\*.
- Wan, X. Y., Wan, J. M., Weng, J. F., Jiang, L., Bi, J. C., Wang, C. M. and Zhai, H. Q. 2005. Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. *Theor. Appl. Genet.* 110 : 1334-1346.
- Wang, H., Qi, M. Q. and Cutler, A. J. 1993. A simple method of preparing plant samples for PCR. *Nucleic Acids Res.* 21 : 4153-4154.
- Wang, B., Lan, T., Wu, W. R. and Li, W. M. 2003. Mapping of QTLs controlling chlorophyll content in rice. *Acta Genet. Sin.* 30 : 1127-1132\*\*\*\*.
- Wang, S., Basten, C. J. and Zeng, Z. B. 2006. Windows QTL Cartographer 2.5. [Online]. Available at [statgen.ncsu.edu/qtlcart/WQTLCart.htm](http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) (accessed 1 April 2007). Department of Statistics, North Carolina State University, Raleigh.
- Wissuwa, M., Yano, M. and Ae, N. 1998. Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 97 : 777-783.
- Wissuwa, M. and Ae, N. 2001. Further characterization of two QTLs that increase phosphorus uptake of rice (*Oryza sativa* L.) under phosphorus deficiency. *Plant Soil* 237 : 275-286.
- Wissuwa, M., Wegner, J., Ae, N. and Yano, M. 2002. Substitution mapping of *Pup1* : a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. *Theor. Appl. Genet.* 105 : 890-897.
- Wu, P. and Luo, A. C. 1996. Investigation on genetic background of leaf chlorophyll content variation in rice under nitrogen stressed condition via molecular markers. *Acta Genet. Sin.* 23 : 431-438\*\*\*\*.
- Yamamoto, T., Lin, H., Sasaki, T. and Yano, M. 2000. Identification of heading date quantitative trait locus *Hd6* and characterization of its epistatic interactions with *Hd2* in rice using advanced backcross progeny. *Genetics* 154 : 885-891.
- Yamamoto, T., Taguchi-Shiobara, F., Ukai, Y., Sasaki, T. and Yano, M. 2001. Mapping quantitative trait loci for days-to-heading, and culm, panicle and internode lengths in a BC<sub>1</sub>F<sub>3</sub> population using an elite rice variety, Koshihikari, as the recurrent parent. *Breed. Sci.* 51 : 63-71.
- Zhuang, J. Y., Lin, H. X., Lu, J., Qian, H. R., Hittalmani, S., Huang, N. and Zheng, K. L. 1997. Analysis of QTL×environment interaction for yield components and plant height in rice. *Theor. Appl. Genet.* 95 : 799-808.

---

\* In Japanese.

\*\* Translated from Japanese by the present authors.

\*\*\* Translated from Japanese expect journal title by the present authors.

\*\*\*\* In Chinese with English summary.