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ORIGINAL PAPER

Genetic dissection and pyramiding of quantitative traits for panicle architecture by using chromosomal segment substitution lines in rice

Tsuyu Ando · Toshio Yamamoto · Takehiko Shimizu · Xiu Fang Ma · Ayahiko Shomura · Yoshinobu Takeuchi · Shao Yang Lin · Masahiro Yano

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Abstract To understand the genetic basis of yield-related traits of rice, we developed 39 chromosome segment substitution lines (CSSLs) from a cross between an average-yielding japonica cultivar, Sasanishiki, as the recurrent parent and a high-yielding indica cultivar, Habataki, as the donor. Five morphological components of panicle architecture in the CSSLs were evaluated in 2 years, and 38 quantitative trait loci (QTLs) distributed on 11 chromosomes were detected. The additive effect of each QTL was relatively small, suggesting that none of the QTLs could explain much of the phenotypic difference in sink size between Sasanishiki and Habataki. We developed nearly isogenic lines for two major QTLs, *qSBN1* (for secondary branch number on chromosome 1) and *qPBN6* (for primary branch number on chromosome 6), and a line containing both. Phenotypic analysis of these lines revealed that *qSBN1* and *qPBN6* contributed independently to sink size and that the combined line produced more spikelets. This suggests that the cumulative effects of QTLs distributed throughout the genome form the major genetic basis of panicle architecture in rice.

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T. Ando and T. Yamamoto contributed equally to this work.

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T. Ando · T. Shimizu · A. Shomura · Y. Takeuchi · S. Y. Lin
Institute of the Society for Techno-Innovation of Agriculture, Forestry, and Fisheries, Tsukuba, Ibaraki 305-0854, Japan

T. Yamamoto · X. F. Ma · M. Yano (✉)
National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
e-mail: myano@nias.affrc.go.jp

Present Address:
X. F. Ma
North Japonica Hybrid Rice Research and Development Center, Liaoning Rice Research Institution, Shenyang, Liaoning 110101, China

Present Address:
Y. Takeuchi
National Institute of Crop Science, Tsukuba, Ibaraki 305-8518, Japan

Present Address:
S. Y. Lin
Honda Research Institute Japan, Kisarazu, Chiba 292-0818, Japan

Introduction

Rice is one of the most important food crops in the world, feeding over half of the global population. Therefore, the study of rice productivity is vital to ensuring adequate food for all. Rice yield—the total amount of grain harvested—is broadly divided into two components: sink size (panicle architecture or grain size) and source potential (efficiency of photosynthesis). The balance between these two components makes yield one of the most complex traits (Yoshida and Parao 1976). The panicle consists of spikelets and rachillae, which are composed of peduncles and primary, secondary, and tertiary branches. In general, elite *indica* rice cultivars with high yield tend to have more spikelets than *japonica* cultivars, but this is because they also have longer panicles, longer branches, and more primary and secondary branches. In other words, optimization of the balance of panicle components is also necessary for

increasing spikelet numbers, which is the most direct way to increase yield.

Many genetic studies of yield components in rice have used *indica–japonica* crosses (Lu et al. 1996; Redona and Mackill 1998; Sasahara et al. 1999; Nagata et al. 2002; Kobayashi et al. 2003; Mei et al. 2003, 2006). The results of these studies indicate that the genetic basis of panicle architecture is explained by many quantitative trait loci (QTLs) with relatively small phenotypic effects. The phenotypic expression of such traits is generally affected by both environmental effects and pleiotropic effects of genes for non-target traits, such as days-to-heading or culm length. Even though noise from environmental effects can be removed by careful cultivation under uniform conditions with replication, pleiotropic effects of genes for non-target traits cannot be removed. The populations used in the previous studies showed a wide range of phenotypic segregation in days-to-heading, culm length, and target panicle morphology. Thus, the parameters of detected QTLs, such as their chromosomal positions, additive and dominant effects, and percentage of variance explained, are not always evaluated correctly. For that reason, it is still difficult to fully explain the difference in panicle architecture between *indica* and *japonica* cultivars, which is one of the major determinants of their differences in productivity.

To facilitate genetic analysis of complex traits in rice, series of chromosome segment substitution lines (CSSLs) and nearly isogenic lines (NILs) have been developed (Doi et al. 1997; Ebitani et al. 2005; Tian et al. 2006; Xi et al. 2006). These CSSLs and NILs are used to confirm QTLs putatively detected in primary populations, such as F_2s, recombinant inbred lines (RILs), or backcross inbred lines (BILs). Additionally, CSSLs and NILs are directly used in breeding programs when their genetic background is an elite cultivar (Takeuchi et al. 2006). CSSLs and NILs can also contribute to analysis of the physiological functions of QTLs and to identification of the underlying genes by map-based strategies (Yano et al. 2000; Takahashi et al. 2001; Doi et al. 2004; Ashikari et al. 2005; Ren et al. 2005; Fan et al. 2006; Konishi et al. 2006; Song et al. 2007). Some important QTLs for sink size traits, such as number of spikelets (Ashikari et al. 2005), grain length and weight (Fan et al. 2006), and grain width and weight (Song et al. 2007), have recently been identified. Although these studies present useful information on the mechanisms of panicle and grain formation, no study has yet been directed at a comprehensive understanding of the genetic basis of sink size.

In this study, to dissect the genetic basis of panicle architecture, which is tightly related to yield performance, we derived CSSLs from the backcross progeny of a high-yielding *indica* cultivar, Habataki, with large panicles, as the donor parent, and a *japonica* cultivar, Sasanishiki, with a relatively small panicle, as the recurrent parent. By using these plant materials, we mapped QTLs controlling morphological components of panicle architecture. We developed NILs for two of the major QTLs by marker-assisted selection, and we analyzed the independent and combined phenotypic effects of those QTLs.

Materials and methods

Development of the CSSLs

The strategy for the development of the CSSLs is shown in Fig. 1. Eighty-five BILs (BC_2F_5) were developed from the cross between Sasanishiki/Habataki//Sasanishiki///Sasanishiki (Nagata et al. 2002). According to the criteria of Ebitani et al. (2005), we selected 31 BC_2F_5 lines from the genotype of 236 restriction fragment length polymorphism (RFLP) markers (Rice Genome Resource Center, http://www.rgrc.dna.affrc.go.jp/ineSHBIL85.html). These plants and their progeny were crossed with Sasanishiki to produce secondary backcrossed F_1s (SBC_1F_1). Three to ten plants of each SBC_1F_1 line were genotyped by the same set of RFLP markers, and 30 SBC_1F_1 plants were selected as candidate CSSLs. One to five PCR-based markers per heterozygous segment remained in the candidate lines were selected from the Rice Genome Research Program (http://rgp.dna.affrc.go.jp/E/publicdata/caps/index.html)

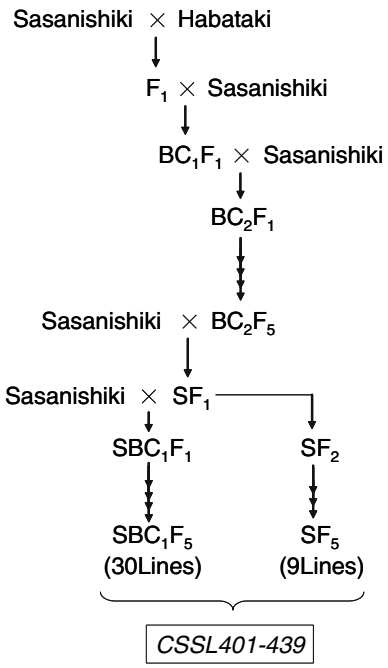

Fig. 1 Strategy for the development of the CSSLs. The number of *arrowheads* in each arrow indicates the number of generations

and simple sequence repeat (SSR) markers (McCouch et al. 2002). To fill the gaps, we developed additional PCR-based markers in four chromosomal regions (Table S1). These markers were used for the genotyping of target (to be substituted) or non-target chromosomal regions in each of the SBC_1F_2, SBC_1F_3, and SBC_1F_4 generations. As a result, we selected 30 lines with a homozygous target region in an otherwise heterozygous. Since the combined substituted chromosome segments did not cover the whole genome, we selected nine additional SF_5 plants derived from some of the SF_2 plants to cover the gaps. The genotypes of the 39 resultant lines were confirmed by using 166 uniformly distributed (~2.3-Mb interval) PCR-based markers (http://www.rgrc.dna.affrc.go.jp/ineSHCSSL39.html).

Phenotypic measurements

All CSSLs were grown, at 22–24 plants per line, in an experimental field at the National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan. In both 2004 and 2005, germinated seeds were sown in mid April, and the seedlings were transplanted into the paddy field about 30 days later in double-row plots, with a distance of 18 cm between plants and 30 cm between rows. The crops were raised according to the standard practices for the time of year in Japan.

At maturity (30–40 days after heading), we sampled 18 panicles—three average panicles from each of six average plants—in each CSSL. We measured five morphological components of panicle architecture—number of spikelets per panicle (SN), number of primary branches per panicle (PBN), average number of secondary branches per primary branch (SBN), panicle length (PL), and primary branch average length (PBL)—as well as culm length (CL) and days-to-heading (HD).

Detection of QTLs

The existence of a QTL was declared when the average value of a trait was significantly different between a CSSL and the recurrent parent, Sasanishiki, according to Dunnett's multiple comparisons test at a probability level of 0.01. From the identity of the CSSLs, QTLs could be assigned to the substituted chromosomal segment. When QTLs were detected on overlapping chromosome segments in multiple CSSLs, their location could be narrowed down.

To validate and finely dissect putative QTLs detected on chromosome 1, QTL analysis of F_2 progeny ($n = 276$) of a cross between SL403 and Sasanishiki was conducted by composite interval mapping with QTL cartographer version 1.17 (Basten et al. 2004). Putative QTLs were estimated based on a threshold of LOD > 2.5.

Development of QTL-NILs and pyramiding of the major QTLs

Some of the lines showed prominent QTLs for secondary branch number on chromosome 1 and primary branch number on chromosome 6 (tentatively called *qSBN1* and *qPBN6*, respectively). Since Nagata et al. (2002) detected QTLs at similar regions in progeny of the same parents, we developed QTL-NILs by marker-assisted selection to confirm their effects.

To develop NIL(*SBN1*), we developed a BC_1F_2 population ($n = 200$) from Sasanishiki/BIL8 (Nagata et al. 2002)//Sasanishiki. One BC_1F_2 plant homozygous for the Habataki allele at *qSBN1* in a Sasanishiki background was selected. One of the remaining heterozygous regions became homozygous for Sasanishiki in the progeny (BC_1F_3), and it became a candidate for NIL(*SBN1*). To develop NIL(*PBN6*), we selected one plant heterozygous for the Habataki allele at *qPBN6* in a Sasanishiki background from BC_2F_1s derived from Sasanishiki/BIL21 (Nagata et al. 2002)//2*Sasanishiki. QTL analysis was carried out in the BC_2F_2 population ($n = 100$) to confirm the effect of *qPBN6*. One plant heterozygous for *qPBN6* in the Sasanishiki background was selected. Marker-assisted selection of the progeny (BC_2F_3) produced one plant as a candidate for NIL(*PBN6*).

By crossing the two candidates for QTL-NILs, we purified and pyramided the two QTLs in the F_2 population ($n = 120$). On the basis of the genotypes of the nearest DNA markers (RM3360 for *qSBN1* and RM3430 for *qPBN6*), we selected three genotypic classes—homozygous for Habataki RM3360 and Sasanishiki RM3430, homozygous for Sasanishiki RM3360 and Habataki RM3430, and homozygous for Habataki RM3360 and Habataki RM3430. These progeny were self-pollinated and called NIL(*SBN1*), NIL(*PBN6*), and NIL(*SBN1* + *PBN6*), respectively. They were grown in the experimental field at NIAS. Ten plants of each line were measured as described above.

Results

Characteristics of the CSSLs

Figure 2 graphs the genotypes of 166 markers in the 39 CSSLs. Each line contained a substituted segment of a particular chromosomal region and additional small segments in non-target regions. If we assume that recombination occurred midway between two adjacent markers, the target chromosomal segments substituted from Habataki in each CSSL ranged from a minimum 3.3 Mb to a maximum 34.1 Mb, and averaged 12.3 Mb. Although several non-target chromosomal regions from

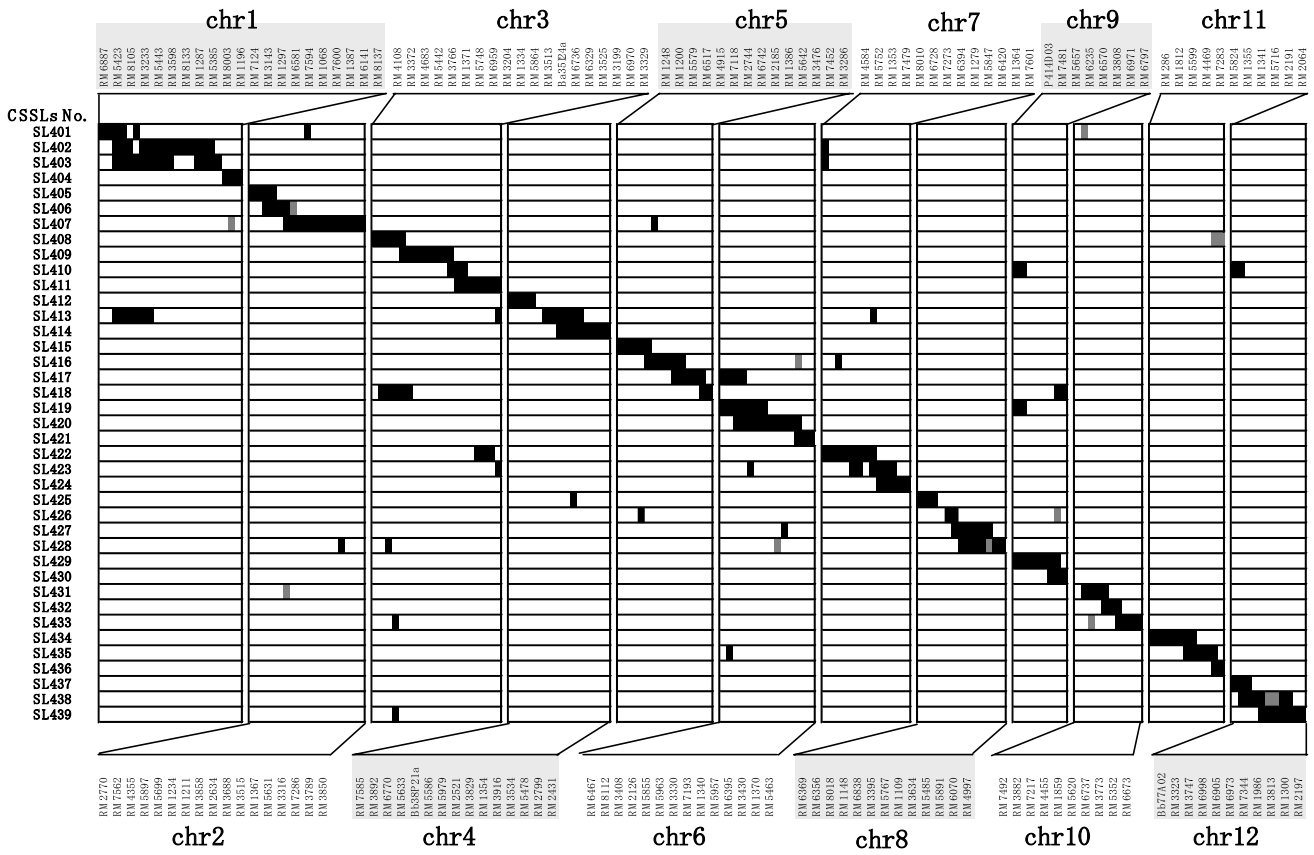

Fig. 2 Graphical representation of genotype of whole chromosomes in the CSSLs developed. *White*: homozygous for Sasanishiki alleles; *black*: homozygous for Habataki alleles; *shaded*: heterozygous. Genotype data for SSR markers can be obtained on the website of the Rice Genome Resource Center (http://www.dna.affrc.go.jp/ine39.html) at NIAS

Habataki remained (3.3 Mb on average), each CSSL had more than 90% of the Sasanishiki genome. All substituted Habataki segments covered most of the genome, except for three small regions on chromosomes 4 (defined by Bb38P21a), 8 (defined by RM1148), and 10 (defined by RM7492). One of the CSSLs (SL407) possessing a Habataki segment on the long arm of chromosome 2 was excluded from the analysis because of its poor growth (Matsubara et al. 2007).

Phenotypic variations in panicle architecture in the CSSLs

Table 1 shows the values of several components of panicle architecture in the CSSLs, Sasanishiki, and Habataki in 2004 and 2005. Since the lines with significant deviation from the value of Sasanishiki and trends of distribution were similar in both years, except for days-to-heading, on account of the cool temperature in early summer in 2005 (Fig. S1), we give details for 2004 only. The culm length of the CSSLs ranged from 66.5 cm (SL403) to 91.5 cm (SL417), and that of Sasanishiki was 79.9 cm. Days-to-heading of the CSSLs ranged from 92.7 days (SL435) to 98.5 days (SL422), and that of Sasanishiki was 94.5. SN was 113 in Sasanishiki and 219 in Habataki. The frequency distribution of the CSSLs showed a normal distribution around the SN value of Sasanishiki, ranging from 165 seeds (SL401) to 83 (SL414). The distribution and relative ratios of the highest and lowest values of SBN, PBL, and PL were similar to SN. However, the highest value of PBN was 12.1 branches (SL420), almost the same as in Habataki (12.0).

Chromosomal location of QTLs for panicle architecture

The numbers of lines showing a significant difference ($P < 0.01$, either increase, ↑, or decrease, ↓) in any trait in either year are as follows: SN, 10↑/6↓; PBN 6↑/4↓; SBN, 10↑/5↓; PL, 5↑/2↓; PBL 9↑/8↓.

The chromosomal locations of the QTLs detected in the 2 years are shown in Fig. 3. In total, there were 21 regions where the Habataki allele increased the value and 17 regions where it decreased it. The numbers of QTLs detected in both years were as follows: SN, 1↑/2↓; PBN, 1↑/2↓; SBN, 2↑/1↓; PL, 1↑/0↓; PBL, 3↑/0↓. However, when the level of significance was changed to $P < 0.05$, almost all QTLs were detected in both years (data not shown). The

Table 1 Measurements of phenotypic traits for panicle architecture in CSSLs derived from Sasanishiki and Habataki

chr.	CSSL	Spiklet numbers per panicle: SN		Primary branch numbers per panicle: PBN		Secondary branch numbers per panicle: SBN		Panicle length: PL (cm)		Primary branch length: PBL (cm)		Culm length: CL (cm)		Days to heading: HD (days)	
		2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
1	SL401	165.3[a]	153.3[a]	11.8[a]	10.8[a]	2.8[a]	2.7[a]	18.1	18.2	6.4	6.5	78.0	7.1	96.2	101.0
	SL402	117.3	110.6	9.1[a]	9.2	2.4[a]	2.2	18.5	17.9	7.5[a]	7.1	69.7[a]	63.2[a]	97.3[a]	100.3
	SL403	130.1[a]	132.8	8.9[a]	9.6	2.8[a]	2.7[a]	7.8	18.2	7.6[a]	7.5[a]	66.5[a]	62.8[a]	95.2	102.3
	SL404	118.1	122.4	10.2	11.0	2.1	1.9	19.3	18.7	7.0[a]	6.7	81.2	80.1	95.8	103.3
2	SL405	117.7	128.4	9.9	10.7	2.1	2.1	19.0	18.6	6.7	6.4[a]	79.0	76.6	96.5[a]	102.3
	SL406	128.6[a]	126.1	10.4	10.1	2.4[a]	2.3	19.5[a]	18.9	6.8	6.7	78.9	77.7	96.0	104.0
	SL407[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	SL408	124.9[a]	133.5	10.1	10.4	2.3[a]	2.3[a]	19.1	19.4	6.9	6.9	81.6	82.4[a]	93.7	100.0
	SL409	112.6	125.9	11.2[a]	11.2	1.7	1.8	19.2	19.2	6.4	6.5	86.2[a]	85.6[a]	97.5[a]	104.0
	SL410	107.8	120.8	10.2	10.4	1.7	2.0	18.7	19.1	6.8	7.2	80.9	78.6	95.5	102.7
	SL411	91.0[a]	100.4[a]	9.3	9.6	1.7	1.8	17.5	17.5[a]	6.3	6.5[a]	79.3	76.4	97.3[a]	103.3
4	SL412	112.8	114.8	10.2	10.2	1.8	1.9	18.1	18.4	6.6	6.8	77.1[a]	77.3	93.5	102.7
	SL413	117.2	136.2	9.8	10.1	1.9	2.3	18.0	19.2	6.9	7.3[a]	80.6	80.9[a]	95.2	101.7
	SL414	83.4[a]	92.4[a]	9.4	9.7	1.1[a]	1.3[a]	17.7	18.5	6.2 [a]	6.6	79.4	81.2[a]	94.7	102.3
5	SL415	108.3	106.7	9.9	10.1	1.9	1.7[a]	18.1	18.4	6.5	6.6	76.5[a]	76.3	95.2	101.0
	SL416	131.1[a]	138.3[a]	11.7[a]	11.6[a]	2.0	2.1	20.6[a]	20.3[a]	7.3[a]	7.3[a]	80.1	79.6	95.2	103.3
	SL417	105.1	101.4[a]	10.1	9.8	1.7	1.6[a]	19.1	19.0	6.6	6.6	91.5[a]	87.9[a]	94.8	00.3
	SL418	113.1	99.4[a]	9.6	9.4	2.1	1.8	18.3	18.1	6.6	6.3[a]	78.8	75.9[a]	96.7[a]	101.0
6	SL419	130.5[a]	123.2	10.9	10.5	2.2[a]	2.0	17.5	18.4	6.2 [a]	6.6	82.8[a]	81.5[a]	92.8[a]	98.7[a]
	SL420	139.6[a]	117.6	12.1[a]	11.1	2.0	1.7[a]	19.9[a]	18.8	6.9	6.7	88.6[a]	80.7[a]	96.5[a]	100.0
	SL421	139.9[a]	139.1[a]	11.7[a]	11.2	2.2[a]	2.3	20.2[a]	20.5[a]	7.0[a]	7.2[a]	76.8[a]	74.5[a]	93.7	99.7
7	SL422	120.9	110.7	10.1	9.2[a]	2.2[a]	2.2	19.0	18.2	6.9	6.9	78.8	74.9[a]	98.5[a]	104.0
	SL423	13.9	113.7	10.1	9.9	2.0	1.9	18.2	19.3	6.8	7.0	80.1	77.5	94.5	101.0
	SL424	108.1	105.9	9.9	9.7	1.9	1.8	19.1	19.5	6.8	6.9	80.2	78.2	94.7	100.7
8	SL425	116.2	119.0	10.1	10.8	2.0	1.8	18.5	19.3	6.8	6.8	79.1	78.3	93.0	101.0
	SL426	111.2	111.3	10.0	9.4	1.9	2.0	18.2	18.5	6.5	6.9	78.8	79.4	94.0	101.0
	SL427	126.7[a]	121.8	11.0[a]	10.9	2.0	1.8	19.4[a]	19.6	6.5	6.4[a]	81.0	78.9	95.0	102.7
	SL428	131.3[a]	138.9[a]	10.1	10.9	2.5[a]	2.3	17.9	17.9	6.6	6.6	75.6[a]	77.6	96.3[a]	104.7[a]
9	SL429	119.1	130.9	10.3	10.9	2.0	2.1	17.6	18.2	6.9[a]	6.7	74.7[a]	75.3[a]	95.5	104.0
	SL430	118.7	111.4	10.3	10.1	2.0	1.8	18.3	7.8[a]	6.6	6.5[a]	73.7[a]	74.3[a]	95.5	103.0
10	SL431	116.8	122.9	10.7	10.6	1.8	2.0	18.6	19.1	6.6	6.8	81.7	78.7	96.2	103.3
	SL432	121.3	128.3	10.6	11.0	2.0	2.1	18.4	18.8	6.6	6.8	76.7[a]	73.7[a]	92.7[a]	100.0
	SL433	122.6	127.9	10.0	10.1	2.4[a]	2.4[a]	18.3	18.9	6.8	6.9	72.7[a]	69.9[a]	95.5	101.3
11	SL434	116.7	127.1	10.2	10.5	1.9	2.1	18.3	18.1	6.6	6.6	76.9[a]	76.6	94.0	101.3
	SL435	110.9	125.3	10.4	11.1	1.7	1.9	18.9	19.6	6.6	6.9	81.0	78.2	92.7[a]	101.0
	SL436	117.3	120.4	10.0	10.2	2.1	2.1	18.8	19.2	6.8	6.9	81.1	75.8[a]	95.0	102.7
12	SL437	116.1	112.8	10.5	10.3	2.0	1.8	18.2	18.3	6.5	6.5[a]	82.9[a]	78.0	95.8	100.2
	SL438	98.4[a]	116.1	9.8	9.9	1.6[a]	2.0	18.7	19.7	7.2[a]	7.8[a]	88.1[a]	82.5[a]	97.2[a]	104.2[a]
	SL439	95.6[a]	108.6	8.6[a]	8.7[a]	2.0	2.2	18.0	19.0	7.3[a]	7.7[a]	90.4[a]	82.1[a]	97.0[a]	103.3
	Sasanishiki	112.6	121.1	10.1	10.3	1.9	2.0	18.4	18.9	6.6	6.9	79.9	78.3	94.5	101.9
	Habataki[c]	218.7	218.5	12.0	12.8	4.4	3.5	22.6	22.2	11.0	10.5	76.9	73.2	99.6	102.3

[a] Significantly different from Sasanishiki within year

[b] SL407 was excluded from the evaluation because of its very poor growth

[c] Habataki was evaluated in a different place from the CSSLs in the same experimental field

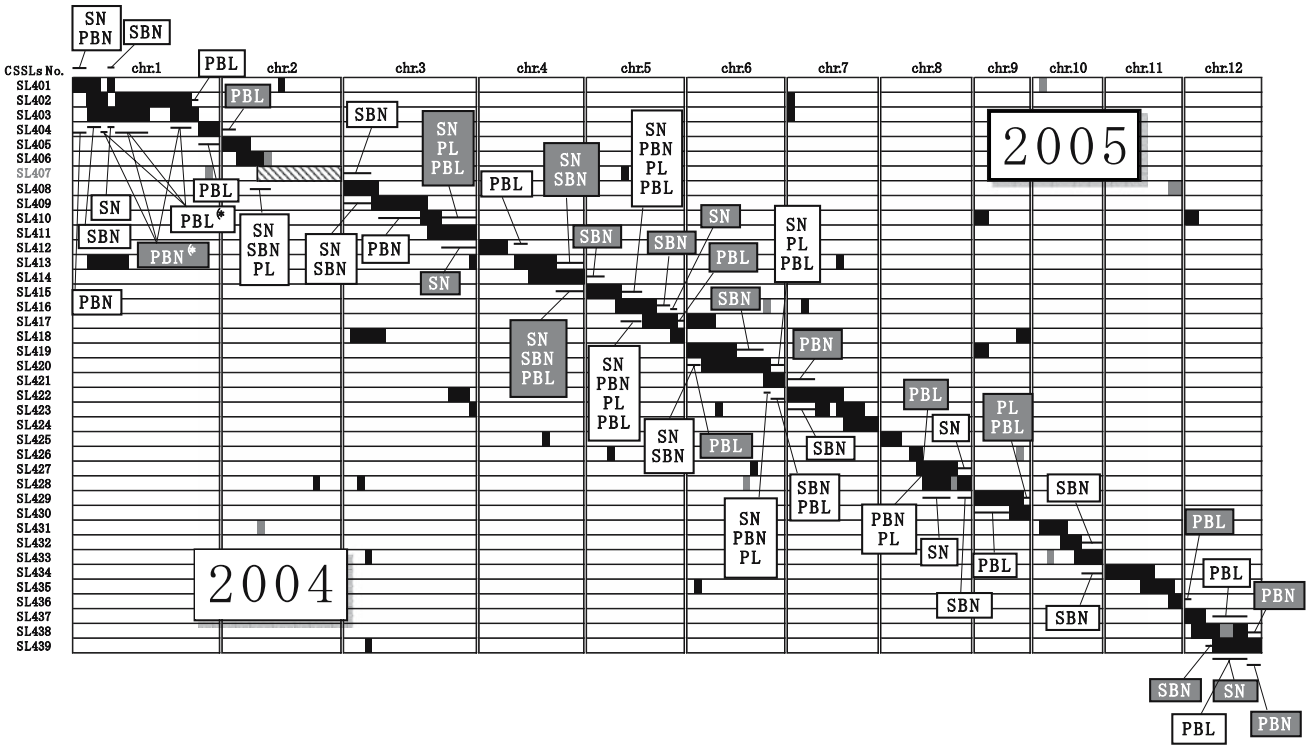

Fig. 3 Chromosomal locations of QTLs for panicle architecture. QTLs detected in 2004 and 2005 are labeled in the lower left and upper right corners, respectively. SN: spikelet number per panicle; PBN primary branch number per panicle; SBN secondary branch number per panicle; PL panicle length; PBL primary branch length. *Black letters* on white box indicate traits increased by Habataki allele, and *white letters* on shaded box indicate traits reduced by Habataki allele. The numbers of the QTLs can be counted by numbers of the connecting line from each box and horizontal area bar. *PBN and PBL, on chromosome 1, indicate three candidate regions because of multiple overlapping segments in SL402 and SL403

distribution of the QTLs was not even; for example, we detected a region on chromosome 5 where the Habataki alleles increased four traits (between markers RM7118 and RM6742), and a region on chromosome 4 where the Habataki alleles decreased three traits (between RM3534 and RM2431). Similar clusters were found on chromosomes 6 (between RM3430 and RM5463) and 1 (between RM6887 and RM3598). Regions increasing some traits but decreasing others were also detected. For example, the Habataki allele in a region on chromosome 6 between RM6467 and RM8112 increased SN and SBN but decreased PBL, and a region on chromosome 12 between RM6905 and RM3813 increased PBL but decreased SN. Regarding the possibility of pleiotropic effect of CL or HD to panicle architecture, positive correlation was implied between CL and PBL on chromosome 12 estimated by both SL438 and SL439. But no correlation was observed between HD and any other traits (Table 1).

Ten QTLs were identified on chromosome 1 by comparison of the CSSLs and Sasanishiki (Fig. 3). To finely dissect QTLs on this chromosomal region, we performed QTL mapping by using the F_2 progeny ($n = 276$) of a cross between SL403 and Sasanishiki (Fig. 4). QTL mapping confirmed the location and direction of effect of nine of the ten QTLs, but not the QTL for PBN around RM5443. Moreover, four new QTLs were detected: for PL around RM3233 and RM7594, for SBN around RM1068, and for SN around RM7600. The most prominent was a QTL for SBN and SN between RM8105 and RM3233.

Development of QTL-NILs and evaluation of their pyramiding effects

Figure 5a shows the graphical genotypes of the QTL-NILs. According to the information of Nipponbare whole genome sequence (IRGSP 2005), the lengths of the substituted regions were narrowed down to physical distances of about 3.35 Mb (at least between RM5302 and RM3877) in NIL(*SBN1*) and about 390 kb (at least between S1306 and RM3430) in NIL(*PBN6*). Figure 5b shows the phenotypic effects of the QTLs produced by pyramiding in the Sasanishiki genetic background. NIL(*PBN6*) and NIL(*SBN1* + *PBN6*) exhibited larger PBNs than that of Sasanishiki. Similarly, NIL(*SBN1*) and NIL(*SBN1* + *PBN6*) exhibited larger SBNs than that of Sasanishiki. On the other hand, all three lines produced more spikelets than Sasanishiki, and NIL(*SBN1* + *PBN6*) produced more spikelets

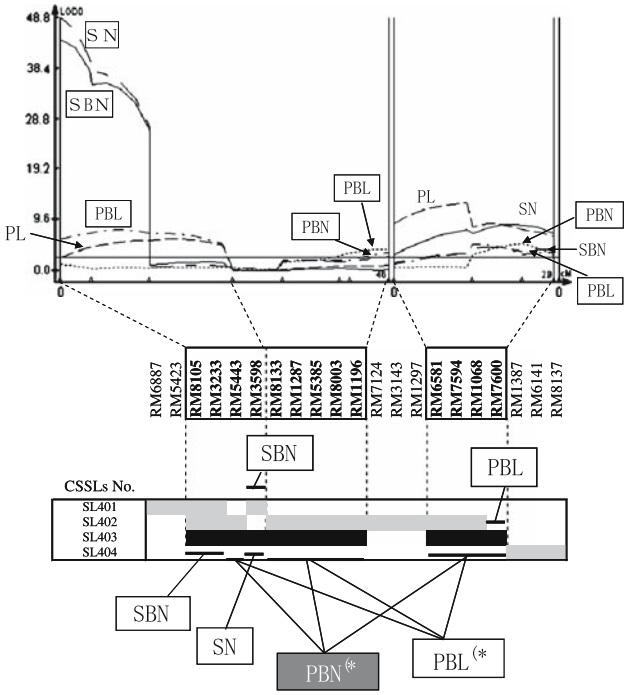

Fig. 4 QTL mapping of panicle architecture on chromosome 1. Aligned DNA markers ("RM" labels) in the center of the figure are connected to the LOD graph produced by QTL Cartographer (*upper figure*) and to the graphical genotypes of chromosome 1 of the four corresponding CSSLs (*lower figure*). Boxed letters on the LOD graph indicate QTLs detected in Fig. 3. The dotted line connecting PBN (*lower figure*) and the region designated by RM5443 in the graphical genotype (*center figure*) indicates that no significant differences were estimated by F_2 analysis. No significant differences were estimated in any traits on chromosome 7 where SL403 possesses a small segment (Figs. 2,3)

than the other two NILs. These results clearly validate the existence of *qSBN1* and *qPBN6* and suggest their additive effect.

To confirm whether such an additive effect is common or whether epistatic interaction is involved in the expression of traits, we evaluated the same traits in F_1 progeny of NIL(*SBN1*) and the 37 CSSLs. If a specific region where a trait value of one F_1 progeny show significant difference to that of F_1 of NIL(*SBN1*) and Sasanishiki are detected, this region might have an epistatic interaction with *qSBN1*. Even though all regions were heterozygous, which may be undervalued by half of dosage effect, most of the detected regions were the same as those detected in the CSSLs (Table S2; Fig. S2).

Discussion

CSSLs suitable for genetic analysis of complex traits

When we try to clarify the genetic basis of complex traits in which many genes are involved, such as yield or

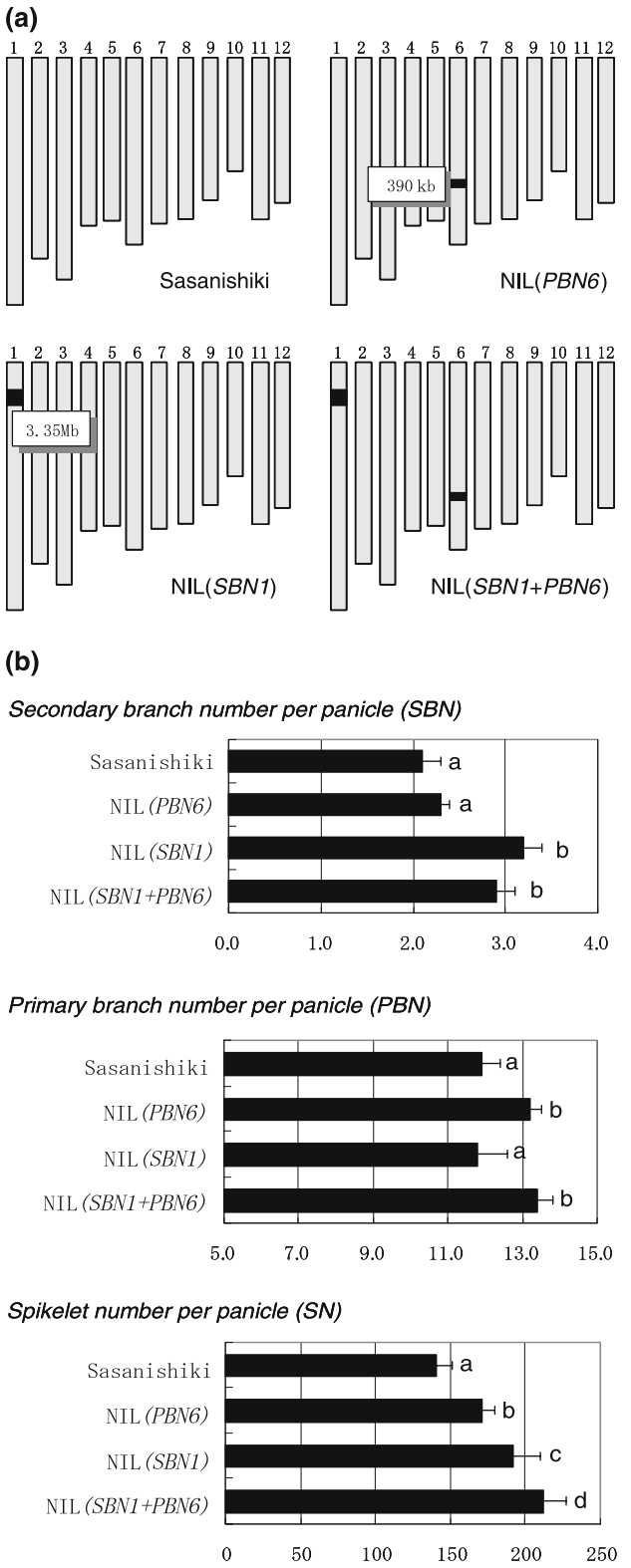

Fig. 5 **a** Graphical genotypes of Sasanishiki and QTL-NILs developed in the pyramiding experiment. The lengths of the substituted regions were 3.35 Mb in NIL(*SBN1*) and 390 kb in NIL(*PBN6*). **b** Phenotypic performances of the QTL-NILs. Bars followed by different letters are significantly different by multiple comparison (Tukey's test)

environmental stress tolerance, certain factors such as duration of cultivation or plant architecture indirectly affect the phenotypic performance of target traits, creating considerable noise in the detection of QTLs for target traits. Therefore, phenotypic variation in non-target traits should be minimized in mapping populations. However, many mapping populations for QTL analysis are developed from distantly related cross combinations, such as *indica–japonica* crosses, and the diverse allelic differences result in a wide range of variation in many agronomic traits, such as heading date and plant stature. In fact, several mapping populations used in previous studies of yield-related traits showed a large variation in heading date and culm length. To obtain more reliable results for QTL mapping of yield-related traits, we developed a novel CSSL population from an *indica–japonica* cross combination. Even though Sasanishiki (*japonica*) and Habataki (*indica*) are different ecotypes, the CSSLs derived from them showed low variation in days-to-heading (from 2 days later than Sasanishiki in SL409, SL428, and SL438, to 2 days earlier in SL419) and culm length (from 10 cm shorter than Sasanishiki in SL402 and SL403, carrying the Habataki allele of *sd1* (Asano et al. 2007), to 10 cm longer in SL417). In fact, a large number of QTLs that were not reported from BILs derived from the same cross combination (Nagata et al. 2002) were successfully detected in this analysis. We suggest that this CSSL population is highly suitable for the genome-wide surveying of complex traits that should be evaluated in the absence of pleiotropic effects from non-target traits, such as days-to-heading or culm length.

QTLs for panicle architecture scattered throughout the rice genome

Through evaluations of several morphological components of panicle architecture, we found at least 38 QTLs scattered throughout the rice genome. None of the traits in any CSSL exceeded the phenotypic value of Habataki, except for the PBN of 12.1 in SL420, which is the same as in Habataki. This implies that the effects of the individual QTLs on panicle architecture are generally small.

We detected several clusters of QTLs (Fig. 3). Since SN, SBN, and PBN are similar in being related to the number of branches, their QTLs on chromosome 5 (between markers RM7118 and RM6742) and chromosome 4 (between RM3534 and RM2431), respectively, might have a pleiotropic relationship. Similarly, since PBL and PL are similar in being related to the length of branches, their QTLs on chromosome 5 (between markers RM7118 and RM6742) might have a pleiotropic relationship also.

We confirmed all three QTL regions detected in BILs derived from the same parents (Nagata et al. 2002): one each for number of spikelets per panicle (*nsp*) on the short arm of chromosome 1 and the short arm of chromosome 5, and one for number of primary rachis branches (*rb1*) on the long arm of chromosome 6. However, most of the other QTLs were newly detected by using CSSLs in this study. This result clearly demonstrates that QTL detection can be improved by the removal of noise due to other genetic factors in CSSLs.

Since the substitution status of three of the CSSLs for chromosome 1 (SL402, SL403, and SL404) were too intricate for us to estimate the actual location of the corresponding QTLs (Fig. 3), we performed QTL analysis using F_2 progeny of a cross between SL403 and Sasanishiki (Fig. 4). Comparative cultivation allowed us to confirm nine of the ten QTLs detected. We predict one QTL for SN and SBN, showing the highest LOD peak between RM8105 and RM3233, to be *grain number 1* (*Gn1*), isolated by Ashikari et al. (2005). QTL analysis found four additional QTLs in the same region. This suggests that further QTLs involved in panicle architecture are concealed in the genome.

Additive genetic effect is a major determinant of variation in panicle architecture

SN is one of the most important components of sink size in rice. Our pyramiding experiment revealed that *qSBN1* and *qPBN6* act additively and independently (Fig. 5b). *qSBN1* from Habataki increased SN by up to 30%, *qPBN6* by 15% to 20%, and both by about 50%. We didn't plant the donor parent Habataki along with the four lines in the same experimental design. But judging from the data of Habataki planted at a nearby site, the SN of NIL(*SBN1* + *PBN6*) was not as high as that of Habataki (data not shown). Therefore additional QTLs might be necessary to explain the large panicle of Habataki. The phenotypic values of the CSSLs (Table 1) suggest candidate QTLs in SL408 (chromosome 3) and SL433 (chromosome 10) for SBN, and in SL401 (chromosome 1) for PBN.

Previous studies have suggested the involvement of epistatic interaction among QTLs in yield-related traits (Li et al. 1997; Yu et al. 1997; Mei et al. 2006; You et al. 2006). Since synergistic effects of other chromosomal regions outside the major QTLs may contribute to differences (Kroymann and Mitchell-Olds 2005), we evaluated the same traits in F_1 progeny of crosses between NIL(*SBN1*) and 37 CSSLs. We could not detect any chromosomal region showing epistatic interaction with *qSBN1* (Table S2; Fig. S2). Although we cannot fully rule out the possibility of epistatic interactions between other chromosomal regions, the results show that no

chromosomal region has a large synergistic effect on panicle architecture.

PL is another factor that can be improved to increase sink size. We identified one QTL with a relatively large effect on PL on chromosome 5. However, we have not tried to combine QTLs for PL or PBL into a QTL-NIL. Because larger number of spikelets were densely branched on Sasanishiki-like panicle in NIL(*SBN1* + *PBN6*), it is hard to say that the sink size of this NIL was truly enlarged as preparation of full of source potential. Therefore, we are now developing a pyramiding line combining the PL QTL on chromosome 5 with NIL(*SBN1* + *PBN6*) to clarify its contribution to the final yield.

Toward the pyramiding of genes improving yield potential

At least 38 QTLs are involved in panicle architecture, 21 of which from Habataki increase the phenotype (Fig. 3). Moreover, no specific QTL explains the large sink size of Habataki. Thus, to improve the panicle architecture (sink size), it will be necessary to pyramid favorable alleles of many QTLs in a particular background. This cannot be achieved by using mass pedigree breeding. Even by marker-assisted selection it would be difficult to combine all factors. To accumulate a large number of desirable alleles in short period of time, it will be necessary to consider another strategy that facilitates genome-wide recombination, such as recurrent selection.

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