A novel quantitative trait locus, *qCL1*, involved in semi-dwarfism derived from Japanese rice cultivar Nipponbare

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To identify quantitative trait loci (QTLs) associated with culm length in Japanese *japonica* rice varieties, we conducted QTL analyses using a set of reciprocal backcrossed inbred lines (BILs) from crosses between Nipponbare and Koshihikari. We detected five QTLs in the two BILs that are involved in the culm-length difference between Nipponbare and Koshihikari. A QTL located on the short arm of chromosome 1, *qCL1*, was commonly detected near the simple sequence repeat (SSR) marker RM8068 in both BILs in three growing seasons. The Nipponbare allele of *qCL1* shortened from 1.9 to 3.0 cm of culm length. Substitution lines (SLs) of Koshihikari in which the QTL was replaced with the Nipponbare allele exhibited decreased lengths of lower internodes. *qCL1* was mapped within about 2.6 Mbp in the region distal from SSR marker RM10132, suggesting *qCL1* differed from the dwarf or semi-dwarf genes that have been previously reported. Japanese *japonica* rice landraces with Nipponbare alleles of DNA markers around *qCL1* had significantly shorter culm length than other landraces. The results suggest that the novel allele for short culm length was originally distributed in Japanese rice landraces and has been used for introducing the semi-dwarf phenotype and improving plant architecture during practical breeding.

Key Words: *Oryza sativa* L., quantitative trait locus, semi-dwarf phenotype, Japanese rice landraces.

Introduction

Dwarfism is an important agronomic trait in rice because it increases lodging resistance and as a result, increases yield (Hedden 2003). More than 60 dwarf and semi-dwarf genes have previously been reported in rice (Kinoshita 1998). These genes are categorized according to the distribution patterns of the elongated internodes (Takahashi and Takeda 1969, Takeda and Takahashi 1969, Kinoshita and Shinbashi 1982). Several dwarf and semi-dwarf mutants are associated with biosynthesis of or sensitivity to two phytohormone groups, namely gibberellins and brassinosteroids (Sakamoto *et al.* 2006, Takeda and Matsuoka 2008). Since most of the dwarf genes exert unfavorable effects on other agronomic traits such as grain length, panicle length, and number of grains per panicle (Sakamoto *et al.* 2004), the only useful source of dwarf genes in practical breeding thus far is considered to be the one recessive gene *semi-dwarf1* (*sd1*; Hedden 2003). The *sd1* gene was isolated by means of positional cloning and encodes a gibberellic acid 20 oxidase enzyme, which controls a step in the biosynthetic pathway of gibberellins (Sasaki *et al.* 2002, Monna *et al.* 2002, Spielmeyer *et al.* 2002).

The development of semi-dwarf varieties using the *sd1* gene has significantly increased world rice production during

the “Green Revolution”. However, the extensive use of this limited dwarfing resource may cause a bottleneck effect in the genetic background of modern rice varieties. Identifying useful new semi-dwarf or dwarf genes is thus an important goal for the development of varieties with short culms while maintaining genetic diversity during practical rice breeding.

Crop landraces are valuable genetic resources because they are considered to contain a large amount of genetic diversity and should therefore complement and diversify the gene pool of advanced cultivars (Frankel and Soule 1981). Rice landraces exhibit extensive variation, but only some of this variation has been introduced into a few elite lines (Ram *et al.* 2007). Ebana *et al.* (2008) evaluated genetic and phenotypic diversity in Japanese *japonica* rice landraces and developed a small core collection. These Japanese rice landraces showed a wide range of variation in culm length, with lengths ranging from 68 to 143 cm (Ebana *et al.* 2008). As a possible explanation for this variation, Asano *et al.* (2007) reported that multiple alleles of *sd1* generated part of the phenotypic variation in Japanese rice varieties. However, allelic differences in *sd1* cannot explain all of the culm-length variation in Japanese rice varieties. Recently, Monden *et al.* (2009) developed *mPing*-SCAR (sequence-characterized amplified region) markers based on transposable element insertion sites derived from Gimbozu EG4, which contains a high frequency of *mPing*. They detected two culm-length quantitative trait loci (QTLs) that were different from *sd1* in recombinant inbred lines (RILs) of Gimbozu EG4 and

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Nipponbare. These QTLs might be partially responsible for culm-length variation in Japanese rice varieties.

The frequency of DNA polymorphisms among Japanese rice cultivars is extremely low due to the narrow range of nucleotide sequence polymorphisms. This formerly prevented genetic analyses, including QTL analysis, using the progeny from Japanese rice cultivars. Recently, a number of simple sequence repeats (SSRs) have been identified after the completion of the rice genome sequencing project (IRGSP 2005). This has allowed effective large-scale screening for polymorphic SSRs between any pair of Japanese cultivars and permitted QTL analyses of their progeny. Using SSRs that are polymorphic between Japanese cultivars, several QTL analyses have been completed for eating quality (Kobayashi and Tomita 2008, Wada *et al.* 2008, Takeuchi *et al.* 2008), grain quality (Tabata *et al.* 2007, Kobayashi *et al.* 2007), and flowering time (Matsubara *et al.* 2008) in a mapping population derived from crosses between Japanese cultivars.

We recently released a set of reciprocal backcrossed inbred lines (BILs) derived from a cross between the Japanese cultivars Nipponbare and Koshihikari (Matsubara *et al.* 2008). Nipponbare and Koshihikari originated from crosses among Japanese *japonica* rice landraces and their progenies during the 1950s in Japan. Koshihikari has weak lodging resistance due to its slender and slightly long culm, whereas Nipponbare has a shorter culm than Koshihikari and exhibits stronger lodging resistance. Sasaki *et al.* (2002) and Ashikari *et al.* (2005) reported that Nipponbare and Koshihikari both commonly possess a functional (normal) *sdl* allele. This suggests that loci other than *sdl* might be involved in the differences in culm length between Nipponbare and Koshihikari. However, it is not yet clear whether the culm-length QTLs in Nipponbare and Koshihikari populations are the same QTLs reported by Monden *et al.* (2009).

The objective of the present study was to detect novel QTLs for culm length in Japanese rice varieties. We found QTLs for culm length and lower-internode lengths using a set of reciprocal BILs derived from crosses between Nipponbare and Koshihikari. We defined the genomic region of the QTLs, including that of one novel QTL, *qCL1*, on chromosome 1 that was stably detected in multiple seasons and reciprocal populations, by means of substitution mapping using a series of substitution lines (SLs). *qCL1* appears to be different from several previously reported dwarf and semi-dwarf genes. We also investigated the distribution of the SSR alleles near the QTL region and variations in culm length in many Japanese rice landraces. Based on these results, we discuss the importance of the novel QTL for introducing short culm length and improving plant architecture during practical rice breeding in Japan.

Materials and Methods

Plant materials

Two populations of BILs were used for the QTL analysis: 79 BILs derived from the Nipponbare/Koshihikari//

Nipponbare cross (N-BILs) and 127 from the Nipponbare/Koshihikari//Koshihikari cross (K-BILs) (Matsubara *et al.* 2008).

We used four semi-dwarf mutants and *sdl* isogenic lines of Koshihikari, Hokuriku 100 (Samoto and Kanai 1975), Saga 1 (Nonaka *et al.* 1991), Koshihikari Tsukuba SD1 (Wang *et al.* 2005), and Hikaishinseiki (Tomita 2009), to compare culm and internode lengths with those of Koshihikari.

To evaluate the distribution of the QTL alleles in Japanese *japonica* rice landraces, we used the small core collection developed by Ebana *et al.* (2008), but we excluded two landraces (Akage and Fukoku) due to their aberrant short-culm phenotypes, which result from extremely early heading. We also used 11 additional Japanese landraces obtained from the National Institute of Agrobiological Science (NIAS) Genebank: Asahi (JP7416), Kamenoo (JP6521), Mii (JP7582), Ouba (JP7084), Sekitori (JP7911), Sen-ichi (JP6028), Shiratama (JP7846), Takenari (JP8043), Jukkoku (JP10577), Asahi (JP6761), and Awaakamai (JP7998). We used a total of 61 Japanese rice landraces in this study.

QTL analysis in the N-BILs and K-BILs

We cultivated the N-BILs in 2005 and the K-BILs in 2006 in an experimental field at the National Institute of Crop Science (NICS; Yawara, Tsukuba, Ibaraki, Japan). We also cultivated the N-BILs in 2006 and 2007 and the K-BILs in 2007 in an experimental field at NIAS (Kannondai, Tsukuba, Ibaraki, Japan). At the NICS field, seeds were sown in early May, and seedlings were transplanted into the paddy field at the end of May in plots with one row per line with a distance of 15 cm between plants and 30 cm between rows. At the NIAS field, seeds were sown in mid-April, and seedlings were transplanted into the paddy field in mid-May in plots with double row per line with a distance of 18 cm between plants and 30 cm between rows. We measured the mature lengths of the main culm (from the soil surface to the neck of the panicle) in five plants for each line.

We performed linkage mapping using version 3.0 of MAPMAKER/EXP (Lander *et al.* 1987), and used the Kosambi function to calculate genetic distances. We performed QTL analyses using composite interval mapping, as implemented by the Zmapqtl program (model 6) provided by version 2.5 of the QTL Cartographer software (Basten *et al.* 2005). We used genome-wide threshold values ($\alpha=0.05$) to detect putative QTLs based on the results of 1000 permutations.

Development of substitution lines (SLs) and characterization of the QTL on chromosome 1

We developed three SLs to define the genomic region of the QTL on chromosome 1, *qCL1*, and characterize their effects. An F₁ plant of Nipponbare/Koshihikari and its progenies were consecutively backcrossed three or four times with Koshihikari. In every backcrossed generation, plants heterozygous at the target region were selected using SSR markers (as described below). In the BC₃F₃ and BC₄F₃

generations, we performed whole-genome genotyping to select plants in which the target regions were homozygous for the Nipponbare allele and other regions were homozygous for the Koshihikari allele. For marker-assisted selection to develop the three SLs, we selected 102 SSR markers that covered the 12 chromosomes from the lists provided by McCouch *et al.* (2002), IRGSP (2005) and Matsubara *et al.* (2008). We added seven SSR markers (RM6887, RM10081, RM7278, RM1320, RM10132, RM3740, and RM10183) that showed polymorphisms between Nipponbare and Koshihikari on the short arm of chromosome 1 from IRGSP (2005). The DNA extraction and polymorphism detection procedures for the SSR markers are described by Matsubara *et al.* (2008).

The Koshihikari SLs were cultivated in an experimental field at NIAS in 2007 and 2008. The difference in culm length between Nipponbare and Koshihikari was small, and culm length and internode length were both affected by differences in environmental conditions. Thus, we planted each SL in one row and Koshihikari as the control line in another row in plots with double-row per line. At maturity, the main culms of 12 individuals from each line were collected. All internodes lengths longer than 0.5 cm and panicle length were measured, and mean values were calculated for each line. We then compared the mean internode and panicle lengths with those of the neighboring Koshihikari control line using the unequal-variance *t*-test procedure provided by the JMP 6.0 software (SAS Inc., Cary, NC).

Sequence analysis of the dwarf genes d18 and d2

To verify sequence differences between two dwarf genes (*d18* and *d2*) that are located in the same genomic region with the target QTL on chromosome 1, we amplified genomic fragments of Nipponbare and Koshihikari by means of polymerase chain reaction (PCR) using primers designed from the genomic sequences of the two genes (Itoh *et al.* 2001, Hong *et al.* 2003). We directly sequenced the amplified fragments (0.5 to 1.0 kbp) from both sides with appropriate primers using an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA).

Distribution of SSR alleles near the QTL and phenotypic evaluation of Japanese rice landraces

We genotyped the Japanese rice landraces using SSR markers located on the short arm of chromosome 1. The DNA extraction and PCR amplification procedures for the SSR markers are described by Matsubara *et al.* (2008). We fluorescently labeled the PCR products, and determined their fragment sizes using the ABI 3100 DNA sequencer and the Data Collection 2.0 and GeneMapper 3.7 software (Applied Biosystems). We cultivated the Japanese rice landraces in an experimental field at NIAS in 2007. At maturity, we measured all internode lengths of the main culms of five individuals per line. We then compared the mean culm length and internode lengths between the Nipponbare allele group and the group with other alleles at each DNA marker

around the QTL using the unequal-variance *t*-test procedure of the JMP 6.0 software.

Results

Detection of QTLs for culm length in the N-BILs and K-BILs

Figure 1 shows the phenotypic variation in culm length of the N-BILs and K-BILs. The culm lengths (mean $\pm$ SD) of Nipponbare were 79.1 $\pm$ 2.7 cm at NICS in 2005, 86.1 $\pm$ 3.9 cm at NIAS and 93.7 $\pm$ 3.4 cm at NICS in 2006, and 74.5 $\pm$ 2.5 cm at NIAS in 2007. The culm lengths of Koshihikari were 83.6 $\pm$ 2.9 cm at NICS in 2005, 91.2 $\pm$ 3.4 cm at NIAS and 97.1 $\pm$ 2.5 cm at NICS in 2006, and 78.8 $\pm$ 1.7 cm at NIAS in 2007. Nipponbare thus showed a shorter culm length than Koshihikari in both fields and in all three seasons. Culm lengths varied among years and between fields in both N-BILs and K-BILs. Culm lengths of the N-BILs ranged from 68 to 94 cm in 2005, from 73 to 100 cm in 2006, and from 61 to 90 cm in 2007. Culm lengths of the K-BILs ranged from 75 to 106 cm in 2006 and from 59 to 88 cm in 2007. Both populations showed a continuous distribution for culm length and contained transgressive segregants with values larger and smaller than those of the crossing parents, Nipponbare and Koshihikari. More number of transgressive lines had a culm length shorter than that of Nipponbare. The culm lengths (mean $\pm$ SD) of BILs for all seasons were 77.3 $\pm$ 4.6 cm, 82.0 $\pm$ 4.5 cm, and 73.5 $\pm$ 5.2 cm in the N-BILs, and 90.1 $\pm$ 5.4 cm and 74.9 $\pm$ 5.0 cm in the K-BILs. All of the Pearson's correlation coefficients for the culm lengths between years were significant: 0.59 (2005–2006), 0.80 (2005–2007), and 0.64 (2006–2007) in the N-BILs, and 0.81 (2006–2007) in the K-BILs.

We detected two, one, and two QTLs for culm length (*qCL*) in the N-BILs in 2005, 2006, and 2007, respectively (Fig. 2 and Table 1). The QTLs were mapped on chromosomes 1 (*qCL1*; near SSR marker RM8068) and 4 (*qCL4*; near RM7181) in 2005, on chromosome 1 (*qCL1*; near RM8068) in 2006, and on chromosomes 1 (*qCL1*; near RM8068) and 3 (*qCL3-2*; near S87C10-17) in 2007. In the K-BILs, four and three QTLs for culm length were detected in 2006 and 2007, respectively. The QTLs were mapped on chromosomes 1 (*qCL1*; near RM8068), 3 (*qCL3-1*; near S0040-07), 3 (*qCL3-2*; near S87C10-17), and 7 (*qCL7*; near RM1364) in 2006, and on chromosomes 1 (*qCL1*; near RM8068), 3 (*qCL3-2*; near S87C10-17) and 7 (*qCL7*; near RM1364) in 2007. *qCL1* was detected in the same chromosomal region in both BILs in all growing seasons and most likely represents the same locus. The additive effects and proportion of the variances explained by *qCL1* (Table 1) were –2.6 cm and 18.4% (2005), –3.0 cm and 27.9% (2006), and –2.1 cm and 10.6% (2007) in the N-BILs, and they were –2.3 cm and 12.3% (2006) and –1.9 cm and 10.0% (2007) in the K-BILs. The Nipponbare allele of *qCL1* therefore decreased culm length.

Using RM8068, we divided both BILs into three subgroups: homozygous for the Nipponbare allele, homozygous

Fig. 1. Frequency distributions for culm length in 2005, 2006, and 2007 in the Nipponbare/Koshihikari/Nipponbare backcrossed inbred lines (N-BILs), and in 2006 and 2007 in the Nipponbare/Koshihikari/Koshihikari BILs (K-BILs). Black and white arrows indicate the mean values for Nipponbare and Koshihikari, respectively. Horizontal lines under the arrows indicate the standard deviations. Black, white, and shaded bars indicate the number of lines homozygous for the Nipponbare allele, homozygous for the Koshihikari allele, and heterozygous, respectively, using SSR marker RM8068, which is the marker nearest to *qCL1*, the culm-length QTL on the short arm of chromosome 1.

for the Koshihikari allele, and heterozygous (Fig. 1). The Nipponbare-homozygous group exhibited decreased culm length in both BILs in all seasons, although BILs that possessed the homozygous Nipponbare alleles showed a wide range of culm lengths. *qCL3-2* was detected in both BILs (in 2007 in the N-BILs, and in 2006 and 2007 in the K-BILs), and the Nipponbare allele increased culm length in both BILs. *qCL7* was only detected in the K-BILs but was found in both 2006 and 2007. *qCL4* was detected in 2005 in the N-BILs and *qCL3-1* was detected in 2006 in the K-BILs. The Nipponbare alleles of *qCL7*, *qCL4*, and *qCL3-1* all decreased culm length. Detection of these minor QTLs depended on differences in genetic backgrounds or seasons.

Fine mapping of qCL1 using substitution lines

qCL1 was stably detected in both BILs in all seasons, but the estimated proportions of the variances explained and the additive effects were too small to explain the total phenotypic variation of the two BILs. To confirm the genetic effect of *qCL1* and its chromosome location, we adopted a more accurate evaluation method: each line was planted in one row

and Koshihikari was planted as a control line in the adjacent row of double-row plots. We developed three Koshihikari SLs that possessed different Nipponbare segments in chromosome 1 by means of marker-assisted selection using 109 SSRs covering the rice genome. Koshihikari SL-1, SL-2, and SL-3 were substituted for Nipponbare homozygous at the marker intervals RM6887-RM3873 (0.2 to 6.2 Mbp in Rice Genome Sequence build 4.0; IRGSP 2005), RM6887-RM10132 (0.2 to 2.6 Mbp), and RM10132-RM3873 (2.6 to 6.2 Mbp) on the short arm of chromosome 1 (Fig. 3). The culm lengths of Nipponbare, Koshihikari SL-1, and Koshihikari SL-2 were significantly shorter than those of the control lines (Koshihikari), whereas Koshihikari SL-3 did not differ significantly from the control line. Koshihikari SL-1 and SL-2 had culm lengths 6.0 and 4.1 cm shorter than the corresponding control line (Koshihikari), which supports the additive effects that were estimated using the BILs. The lower-internode lengths were also shorter in Koshihikari SL-1 and SL-2 (data not shown). Thus, *qCL1* appears to be localized within 2.6 Mbp on the rice genome sequence in a region of the short arm of chromosome 1 distal from RM10132.

N-BILs

K-BILs

Fig. 2. Putative QTLs for culm length detected in Nipponbare/Koshihikari/Nipponbare backcrossed inbred lines (N-BILs) and Nipponbare/Koshihikari/Koshihikari BILs (K-BILs). Parallel lines under the chromosome number denote the linkage map constructed for the two BILs (Matsubara *et al.* 2008). Marker names and genetic distance for each marker are indicated on the left and right sides of the linkage groups, respectively. Physical positions of dwarf and semi-dwarf genes *d18*, *d2* and *sd1* were shown on the left side in parentheses on the linkage groups of chromosome 1. Vertical bars to the right of each linkage map indicate the 95% confidence intervals for the QTLs with a one-LOD reduction, and triangles indicate the positions of the LOD peaks at each QTL. Black and white triangles indicate that the Nipponbare alleles decrease and increase the trait score, respectively.

Sequence analysis for *d18* and *d2*

Several dwarf or semi-dwarf genes were reported on chromosome 1 by Ogi *et al.* (1993) and Yoshimura *et al.* (1997). The genes *d18* and *d2* are located at 4.0 and 5.2 Mbp from the distal end of the short arm of chromosome 1, respectively (Itoh *et al.* 2001, Hong *et al.* 2003). Although *qCL1* is apparently located at a different genome position from these genes, we determined genomic sequences of about 8.6 kbp of *d18* and 8.1 kbp of *d2* in Nipponbare and Koshihikari to exclude the possibility of additive effects by these genes. We found one synonymous substitution in the first exon and one SSR polymorphism in the upstream sequence of *d18* between Nipponbare and Koshihikari. Genomic sequences at all exons and introns of *d2* were the same in both Nipponbare and Koshihikari. We observed no

nonsynonymous substitutions, insertions, or deletions in the genome sequences of *d18* and *d2* between Nipponbare and Koshihikari.

Culm lengths and internode lengths of the Koshihikari SLs, mutants, and isogenic lines

Figure 4 illustrates the distribution patterns of all internode lengths and panicle length for the main culm of Koshihikari, Nipponbare, Koshihikari SL-1, three *sd1* isogenic lines of Koshihikari (Koshihikari Tsukuba SD1, Hikarishinseiki, and Saga 1), and the semi-dwarf mutant cultivar Hokuriku 100 in 2007. Semi-dwarfism of Hokuriku 100 is controlled by the single recessive gene, *d60* (Tanisaka *et al.* 1990). Culm length of Nipponbare, Koshihikari SL-1, the *sd1* isogenic lines of Koshihikari and the semi-dwarf

Table 1. Quantitative trait loci (QTLs) for culm length detected in two groups of backcrossed inbred lines (BILs): Nipponbare/Koshihikari//Nipponbare (N-BILs) and Nipponbare/Koshihikari//Koshihikari (K-BILs)

Population	Year	QTL	Marker interval	Nearest marker	Chromosome	LOD	PVE	AE
N-BILs	2005	<i>qCL1</i>	RM3252-RM3453	RM8068	1	5.2	18.4	-2.6
		<i>qCL4</i>	RM7472-RM7113	RM7181	4	3.0	10.6	-1.7
	2006	<i>qCL1</i>	RM3252-RM3453	RM8068	1	6.4	27.9	-3.0
	2007	<i>qCL1</i>	RM3252-RM3453	RM8068	1	3.3	10.6	-2.1
		<i>qCL3-2</i>	RM2593-RM1038	S87C10-17	3	3.4	13.2	2.0
K-BILs	2006	<i>qCL1</i>	RM3252-RM3453	RM8068	1	5.2	12.3	-2.3
		<i>qCL3-1</i>	RM5849-RM7365	S0040-07	3	4.6	13.6	-2.4
		<i>qCL3-2</i>	RM2593-RM1038	S87C10-17	3	9.3	24.1	3.4
		<i>qCL7</i>	RM2752-RM5720	RM1364	7	3.2	6.3	-1.8
	2007	<i>qCL1</i>	RM3252-RM3453	RM8068	1	4.0	10.0	-1.9
		<i>qCL3-2</i>	RM2593-RM1038	S87C10-17	3	5.3	13.6	2.4
		<i>qCL7</i>	RM2752-RM5720	RM1364	7	3.4	10.8	-2.1

All genetic parameters were calculated using version 2.5 of QTL Cartographer (Basten *et al.* 2005).

^a log-likelihood value.

^b percentage of total phenotypic variance explained by the QTL.

^c additive effect of the Nipponbare allele.

LOD thresholds used for the detection of the QTL in composite interval mapping: 2.6 in 2005, 2.8 in 2006, and 2.7 in 2007 for the N-BILs; 3.0 in 2006 and 2.8 in 2007 for the K-BILs.

Fig. 3. Illustration of loci on chromosome 1 and of the culm lengths of Nipponbare, of Koshihikari (control), and of three Koshihikari substitution lines (SLs). Marker positions of the simple sequence repeat (SSR) markers, and dwarf genes *OsGA3ox2* (*d18*) and *OsCYP90D2* (*d2*) in the rice genome sequence (Rice Genome Sequence build 4.0; IRGSP 2005) are oriented from the short arm (left) to the long arm (right). Black and white bars indicate homozygous for the Nipponbare and Koshihikari alleles, respectively. * and ** indicate significantly different culm lengths ($P < 0.05$ and $P < 0.01$, respectively) between Nipponbare or the Koshihikari SLs and the corresponding control value (for Koshihikari).

Fig. 4. Diagram of the panicle, 1st internode, 2nd internode, 3rd internode, 4th internode, and 5th-6th internode lengths of Koshihikari, Nipponbare, Koshihikari SL-1, and Koshihikari isogenic lines with a semi-dwarf phenotype. *, **, and *** indicate significantly different lengths from the corresponding length for the control (Koshihikari) at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

mutant cultivar were shorter than Koshihikari. The 3rd and 4th internode lengths of Nipponbare were 14.0 ± 0.9 cm and 9.3 ± 1.8 cm long, and they were significantly shorter than those of Koshihikari. The 3rd, 4th, and 5th-6th internode lengths of Koshihikari SL-1 with the Nipponbare allele of *qCL1* were 14.1 ± 1.6 cm, 10.5 ± 1.8 cm, and 3.4 ± 1.9 cm, and they were significantly shorter than those of Koshihikari. Koshihikari Tsukuba SD1, Saga 1, and Hokuriku 100 all had significantly shorter internodes than those of Koshihikari. Hikaishinseiki had significantly shorter 3rd to 6th internodes than Koshihikari. The dwarfism effect of Koshihikari SL-1 with the Nipponbare allele of *qCL1* was weak compared to the mutant gene effects of the semi-dwarf mutant cultivar and the *sd1* isogenic lines. However, *qCL1* only shortened the lower internodes, and it didn't significantly decrease the 1st and 2nd internode lengths or the panicle length.

Correlation between genotypes around the QTL and culm lengths in Japanese rice landraces

To reveal how much of the total phenotypic variance for culm length can be explained by *qCL1* in the Japanese landraces, we investigated the distribution of the Nipponbare *qCL1* allele for short culm length in 61 Japanese landraces. Since landraces with the Nipponbare *qCL1* allele show Nipponbare alleles at DNA markers near *qCL1* on the short arm of chromosome 1, we surveyed the genotypes of the 61 landraces using 11 SSR markers and one nucleotide substitu-

tion in *d18*, which is located near *qCL1*. All of the SSR markers exhibited multiple alleles; the fewest number of alleles was two at RM3252 and the largest number of alleles was eight at RM3873. When the landraces were divided into two groups based on the genotype (i.e., the Nipponbare allele group and the "other alleles" group), the mean culm lengths for the Nipponbare allele group and the other alleles group differed significantly at five SSR markers (Table 2): by 7.0 cm at RM6887, 10.9 cm at RM8068, 8.1 cm at RM7278, 8.8 cm at RM1320, and 11.0 cm at RM10132. The average culm lengths in the Nipponbare allele group were shorter than those in the other alleles group. When the average culm lengths of all landraces in the group with no Nipponbare allele were compared, there was no significant difference for any of the SSR markers (data not shown).

Differences in the lengths of the 3rd to 6th internodes between Nipponbare and the other allele group were also significant at the same five SSR markers (Table 2): by 5.5 cm at RM6887, 6.1 cm at RM8068, 4.3 cm at RM7278, 4.8 cm at RM1320, and 5.2 cm at RM10132. Figure 5 shows the variation in culm length among the 61 Japanese rice landraces plus Nipponbare and Koshihikari in 2007. The 11 landraces with the Nipponbare allele at RM8068 had culm lengths that ranged from 76.1 to 95.5 cm, whereas those of the other landraces ranged from 75.1 to 119.8 cm. Mean culm lengths in the Nipponbare allele group were clearly smaller than that in the other alleles group.

Table 2. Comparison of average culm length and the length of the 3rd to 6th internodes between the Nipponbare allele group and the other allele group in 61 Japanese rice landraces

Marker name	Position (Mbp)	Number of landraces		Culm length (cm)			Length of 3rd to 6th internodes (cm)		
		Nipponbare allele	Other alleles	Nipponbare allele	Other alleles	<i>t</i> -value	Nipponbare allele	Other alleles	<i>t</i> -value
RM6887	0.2	15	46	89.3±2.7	96.3±1.5	2.28 *	28.4±3.6	33.9±6.0	3.31 **
RM3252	0.3	21	40	95.6±2.1	92.6±2.2	0.97	32.7±5.5	31.1±4.1	1.04
RM10081	1.5	31	30	93.1±1.9	96.1±2.0	1.10	32.4±7.0	32.6±4.8	0.09
RM8068	1.7	11	50	85.6±2.8	96.5±1.4	3.47 ***	27.6±4.1	33.7±5.8	3.48 ***
RM7278	1.8	12	49	88.6±3.1	96.7±1.8	2.28 *	29.2±6.5	33.5±5.9	2.13 *
RM1320	2.2	9	52	87.1±3.4	95.9±1.4	2.36 *	28.4±5.2	33.2±5.9	2.34 *
RM10132	2.6	9	52	85.4±3.9	96.4±1.5	2.62 *	28.2±4.5	33.4±6.1	2.18 *
RM3740	2.8	14	47	92.1±3.4	95.0±1.7	0.75	29.2±4.4	33.2±6.3	1.92
RM10183	3.7	33	28	93.9±1.9	96.7±2.3	0.92	32.3±5.3	33.2±7.1	0.53
<i>OsGA3ox2(d18)</i>	4.0	57	4	95.9±1.5	85.4±5.3	1.91	33.2±6.2	28.1±1.6	1.63
RM3453	4.8	22	39	92.2±2.3	96.9±2.0	1.51	30.7±5.0	34.2±6.7	2.01
RM3873	6.2	15	46	91.8±2.2	94.2±1.8	0.66	31.6±5.5	32.6±6.2	0.54

*, **, and *** indicate markers that differ significantly between the Nipponbare allele group and the other alleles group at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively (*t*-test).

Discussion

Identifying useful new semi-dwarf genes is important for the genetic manipulation of plant architecture in practical rice breeding. Previously, it has been difficult to perform genetic analysis among Japanese cultivars due to the low frequency of DNA polymorphism. Using information on more than 18,000 SSR sequences identified by the rice genome sequencing project (IRGSP 2005), we detected five QTLs in the two reciprocal BILs between the Japanese rice cultivars Nipponbare and Koshihikari (Table 1 and Fig. 2). A QTL for culm length on chromosome 1 (*qCL1*) was detected in all three years (Fig. 2), indicating that *qCL1* is a stable QTL. The Nipponbare allele at this QTL shortened from 1.9 to 3.0 cm of culm length, and proportion of phenotypic variance explained were from 10.0 to 27.9% in the QTL analysis. Because culm length varied among years and between fields, we adopted a more accurate two-row evaluation method for culm and internode lengths. As a result, we could finely map *qCL1* within about 2.6 Mbp at marker interval RM6887-RM10132 using the Koshihikari substitution lines (SLs) and explain some of the difference in culm length between Nipponbare and Koshihikari (Fig. 4). It is important to note that the Nipponbare allele of *qCL1* shortened only the lower internode lengths.

qCL3-2, which is located on the long arm of chromosome 3, increased culm length as a Nipponbare allele (Table 1 and Fig. 2). Matsubara *et al.* (2008) reported a QTL with a large effect for heading date (*Hd16*) in the same marker interval between RM2593 and RM1038. The Nipponbare allele of *Hd16* increased the number of days to heading. This suggests that *qCL3-2* is affected pleiotropically by *Hd16*. *qCL7* was only detected in the K-BILs but was detected in both years. The expression of *qCL7* might depend on differences in the rice genetic backgrounds. *qCL3-1* and *qCL4* were

found in one year or one population. The difference in culm length between Nipponbare and Koshihikari might thus result from a combination of five QTLs: *qCL1*, *qCL3-1*, *qCL3-2*, *qCL4*, and *qCL7*.

Kobayashi *et al.* (2003) reported a minor QTL for culm length or plant height near *qCL1*. They used recombinant inbred lines (RILs) derived from an *indica* cultivar (Milyang 23) and a *japonica* cultivar (Akihikari), and found that the Akihikari allele shortened culm length. Since Akihikari shares some of its ancestral varieties with Nipponbare, it is possible that Akihikari possesses the same alleles as Nipponbare at *qCL1*. However, the Akihikari QTL allele has not yet been proven to be identical to either *d18* or *d2*. Thus, we hypothesize that the *qCL1* detected in the present study is a novel QTL. Monden *et al.* (2009) used RILs derived from a cross between the Japanese *japonica* lines Gimbozu EG4 and Nipponbare. They reported a culm-length QTL on the short arm of chromosome 3 and found that the Nipponbare allele decreased culm length. This QTL might thus be the same as *qCL3-1*, because the Nipponbare allele of *qCL3-1* also decreased culm length. Xiao *et al.* (1996) found one plant height QTL on the long arm of chromosome 7 in RILs of *indica* 9024/*japonica* LH422. This QTL might be the same as *qCL7* in the present study.

To reveal the relationship between *qCL1* and the dwarf and semi-dwarf genes on chromosome 1, we determined the genomic sequences of *d18* and *d2* in Nipponbare and Koshihikari. Itoh *et al.* (2001) mapped *d18* to the short arm of chromosome 1, where it encodes gibberellin 3 β -hydroxylase (*OsGA3ox2*). Hong *et al.* (2003) mapped the brassinosteroid-deficient dwarf mutant *d2* to the short arm of chromosome 1, where the gene, *CYP90D2*, encodes a cytochrome P450. There were no nonsynonymous polymorphisms of the genomic sequences of *d18* and *d2* between Nipponbare and Koshihikari in the present study. Substitution mapping using

Koshihikari SLs showed that the position of *qCL1* had different marker intervals from those of *d18* and *d2* (Fig. 3), suggesting that *qCL1* is a novel culm-length QTL but that the *d18* and *d2* alleles are not. *sd1* is located on the long arm of chromosome 1 and encodes gibberellin 20-oxylase, *OsGA20ox2* (Sasaki *et al.* 2002). Sasaki *et al.* (2002) and Ashikari *et al.* (2005) indicated that the *sd1* alleles of Nipponbare and Koshihikari were the functional (normal) type. No QTLs were detected near *sd1* in both the BILs in the present study. Based on these results, we conclude that *sd1* was not responsible for the culm length differences between Nipponbare and Koshihikari.

Some gibberellin-related mutants, including those of *d18* and *sd1*, show dwarfism, with short lower internodes (Sakamoto *et al.* 2004), whereas brassinosteroid-related mutants, including *d2*, show inhibition of elongation at the second internode, erect leaves, and photomorphogenesis in the dark (Hong *et al.* 2003). Koshihikari SLs with the Nipponbare genomic segment around *qCL1* exhibited shorter lower internodes than those of Koshihikari (Fig. 4). The phenotypic appearance and distribution pattern of *qCL1* are thus more similar to those of the *d18* and *sd1* mutants associated with the gibberellin pathway than to those of *d2* mutants associated with the brassinosteroid pathway. There are no metabolic enzyme genes and signaling pathway genes for gibberellins and brassinosteroids on the short arm of chromosome 1 (Sakamoto *et al.* 2004, 2006). Fine mapping of *qCL1* is currently in progress. Cloning and molecular identification of *qCL1* would provide an opportunity to understand a novel factor involved in the phytohormone biosynthesis and signaling pathways for the phytohormones.

The mean culm length of the Japanese landraces differed significantly between the Nipponbare allele group and the other alleles group at SSR markers RM8068, RM7278, RM1320, and RM10132 (Table 2). Japanese rice landraces with the Nipponbare allele at the four SSR markers were collected from various geographical regions of Japan (Ebana *et al.* 2008). The results suggest that the Nipponbare allele at *qCL1* was originally distributed in the Japanese rice landraces and has subsequently been used to develop Nipponbare and other short-culm cultivars in Japan. Four landraces (Asahi (JP7416), Asahi (6761), Kyoutoasahi, and Aichiasahi) have the Nipponbare alleles at all the four SSRs (data not shown), indicating that these landraces might be ancestors of the short-culm allele of Nipponbare. Investigation of SSR alleles of recently released rice cultivars will help us to understand the importance of *qCL1* in practical rice breeding in Japan.

The semi-dwarf *qCL1* allele of Nipponbare could explain part of the culm-length variation in Japanese landraces (Fig. 5). However, the landraces without the Nipponbare allele at RM8068 showed a wide range of culm lengths. QTLs other than *qCL1* should thus be responsible for some of the variation in culm length in Japanese rice landraces. Asano *et al.* (2007) reported multiple dwarf alleles of *sd1* in Japanese rice cultivars. Monden *et al.* (2009) reported two culm-

Fig. 5. Variation in the culm lengths of 61 Japanese rice landraces in 2007. The Japanese rice landraces were divided into two groups: those with the Nipponbare allele and those with other alleles at the SSR marker RM8068. Horizontal range bars indicate standard deviations. Black bars indicate the culm lengths of Nipponbare and Koshihikari.

length QTLs on chromosomes 3 and 10 in RILs of Japanese rice lines. Differences in the heading dates of these landraces also influence the culm-length variation. In the present study, one SSR marker (RM6887) at the distal region of the short arm of chromosome 1 differed significantly between the Nipponbare allele group and the other alleles group (Table 2). These loci would therefore contribute to the variation in culm length among the landraces that lacked the Nipponbare allele at RM8068. Moreover, several dwarf mutants and cultivars have been generated from natural mutations (Sasaki *et al.* 2002, Itoh *et al.* 2004). Association mapping or linkage disequilibrium mapping would be a promising tool to identify the QTLs and genes responsible for natural variation in the germplasm accessions because it does not require crossed populations or pedigree information. To reveal how many QTLs are associated with the variation in culm length in Japanese rice landraces, genome-wide association mapping (Flint-Garcia *et al.* 2003, Aranzana *et al.* 2005, Iwata *et al.* 2007) using the Japanese rice landraces will be necessary.

The identification of agronomically important QTLs and pyramiding of these QTLs represents a useful strategy for efficient crop breeding. Several QTLs with relatively large phenotypic effects have been cloned by following a map-based strategy (reviewed by Yamamoto *et al.* 2009). The combination of desirable alleles of the genes isolated by this analysis could improve defective plant architectures and may have increased grain filling (Ashikari *et al.* 2005, Sakamoto *et al.* 2006, Takeda and Matsuoka 2008). Although the phenotypic effect of *qCL1* was small compared with that of *sd1* alleles, the short lower internodes produced by the Nipponbare allele of *qCL1* should contribute to strong lodging resistance in rice. The combination of *qCL1* with other morphogenetic genes, including genes for semi-dwarfism, number of grains, grain width, and tiller angle, will provide useful information for fine-tuning of plant architecture in most Japanese rice varieties.

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