Breeding Science 55 : 65–73 (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'

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To facilitate the genetic analysis of quantitative traits and the use of marker-assisted breeding in rice, we developed a novel mapping population consisting of 39 chromosome segment substitution lines (CSSLs). In each line, a different chromosomal segment of the *indica* cultivar 'Kasalath' was substituted in the genetic background of the *japonica* cultivar 'Koshihikari' (Japanese elite cultivar). The substituted chromosome segments in the 39 CSSLs covered most of the genome, except for small regions at the distal end of the short arm of chromosome 8 and at the distal end of the long arm of chromosome 12. To verify the potential advantages of quantitative trait locus (QTL) detection in these CSSLs, we used the CSSLs to locate QTLs for heading date under three different environmental conditions: a natural summer field in Tsukuba, Japan, long-day conditions (14.5-h light), and short-day conditions (10-h light). The results clearly demonstrated that the use of CSSLs enabled to identify a larger number of QTLs than did a BC_1F_3 population derived from the same cross combination. We examined several advantages of the use of CSSLs in terms of genetic analysis, molecular cloning of QTLs, and marker-assisted selection in rice breeding.

Key Words: *Oryza sativa* L., mapping population, marker-assisted selection, QTL mapping, heading date.

Introduction

During the past 15 years, many genetic studies using quantitative trait locus (QTL) analysis have been performed to detect genes underlying genetically complex traits in rice and other plant species (reviewed by Tanksley 1993, McCouch and Doerge 1995, Paterson 1995, Yano and Sasaki 1997, Mackey 2001). Some QTLs genetically identified have recently been cloned by adopting a map-based strategy in rice (Yano *et al.* 2000, Takahashi *et al.* 2001, Kojima *et al.* 2002, Doi *et al.* 2004) and other plant species, such as tomato, maize and *Arabidopsis* (reviewed by Paran and Zamir 2003). These achievements undoubtedly have enhanced our understanding of complex traits in plant species, and have also improved crop cultivars.

QTLs with relatively large effects can be detected in the genetic analysis of primary mapping populations, such as F_2 and recombinant inbred lines (RILs). However, some QTLs with minor effects and those with epistatic interaction with other loci might not be detected in QTL analysis (Yano and Sasaki 1997). Such QTLs can be detected by using advanced backcross progeny (Yamamoto *et al.* 2000, Lin *et al.* 2002, 2003). Even though QTLs of interest can be identified in existing primary mapping populations, further development of nearly isogenic lines (NILs) is required for fine mapping and cloning of QTLs. Development of these materials is laborious and time-consuming. This problem has prevented many researchers from performing map-based cloning of QTLs as a more general strategy in plant molecular genetics.

To address these problems, researchers have developed novel mapping populations as introgression lines (ILs) in tomato (Eshed and Zamir 1995) and *Brassica napus* (Howell *et al.* 1996), as chromosome substitution strains in *Arabidopsis* (Koumproglou *et al.* 2002), as chromosome segment substitution lines (CSSLs) or ILs in rice (Doi *et al.* 1997, Sobrizal *et al.* 1999, Kubo *et al.* 2002), and recently as recombinant chromosome substitution lines in barley (Matus *et al.* 2003). In these lines, a particular chromosome segment from a donor line is substituted in the genetic background of the recurrent line. The substituted segments cover all chromosomes in a whole set of lines. These materials enable to perform detailed and reliable QTL analyses

Communicated by Y. Furuta
Received July 20, 2004. Accepted November 3, 2004.
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(Eshed and Zamir 1996, Zamir 2001, Koumproglou *et al.* 2002, Kubo *et al.* 2002). It is obvious that genetic allele mining depends on the combination of the parental lines. Therefore, to facilitate genetic mapping and map-based cloning of QTLs, it will be necessary to develop such novel mapping populations for a wide range of cross combinations.

The *japonica* rice cultivar 'Koshihikari' is an elite cultivar in Japan, because it displays several characteristics of economic value, such as good eating quality, high resistance to pre-harvest sprouting, and cool temperature tolerance at the booting stage. Therefore, Koshihikari has often been used as a parental line to develop new cultivars in Japanese rice breeding programs. Although QTL analyses of some traits, such as heading date and cool temperature tolerance, have been conducted in Koshihikari (Yamamoto *et al.* 2001, Takeuchi *et al.* 2001), the genetic basis of such economic characteristics has not been fully elucidated owing to their complex mode of inheritance. Thus, it will be necessary to analyze the genetic basis of several characteristics of Koshihikari in order to improve cultivars by using Koshihikari as a parental line.

Previously, we reported a QTL analysis of heading date, culm length, internode length and panicle length, using BC_1F_3 lines derived from a Koshihikari/Kasalath//Koshihikari cross (Yamamoto *et al.* 2001). Since then, we have developed a series of CSSLs from the same combination. In the present paper, we described the selection and characterization of the substituted chromosome segments in these CSSLs. Furthermore, we located the QTL for heading date by using these CSSLs in order to demonstrate the potential advantages of the use of such CSSLs in QTL analyses.

Materials and Methods

Development of the CSSLs

The multi-step selection process for the CSSLs is shown in Figure 1. Rice cultivar Koshihikari was crossed with Kasalath, and a resultant F_1 plant was backcrossed to Koshihikari to produce BC_1F_1. Then the single-seed-descent method was used to develop the BC_1F_3 generation. To minimize the number of lines to be treated, appropriate BC_1F_3 lines were selected based on the genotype of 116 restriction-fragment-length polymorphism (RFLP) markers in the BC_1F_3 plants (Yamamoto *et al.* 2001). The criteria for selection were as follows: a single, relatively large chromosome segment of Kasalath was substituted in the target chromosome, a high level of homozygosity of Koshihikari alleles remained in non-target chromosomal regions, and target chromosome segments partially overlapped in the selected lines in order to cover all the 12 rice chromosomes. As a result, we selected 49 BC_1F_3 lines as the starting materials for the second round of backcrossing.

A randomly selected plant (as female parent) of each BC_1F_3 line was crossed with Koshihikari (as male parent) to produce secondary F_1 (SF_1). Two or three plants were randomly selected from each SF_1 line and were crossed with

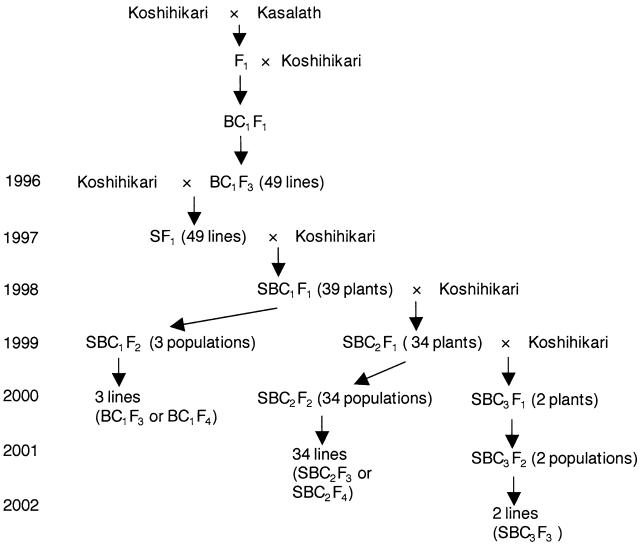

Fig. 1. Flowchart of the process of selection of CSSLs in the present study.

Koshihikari to produce secondary BC_1F_1 (SBC_1F_1). Two plants in which the target chromosome segment was heterozygous were selected from each SBC_1F_1 line, based on the genotype of cleaved amplified polymorphic sequence (CAPS) markers, and were crossed with Koshihikari again to produce SBC_2F_1. Approximately 10 plants of SBC_2F_1 were selected, based on the genotypes of the target chromosome segments. At least two plants in which the target chromosome segments were heterozygous were crossed with Koshihikari to produce SBC_3F_1.

One to three CAPS markers were used in the genotyping of target or non-target regions in accordance with the approximate genetic distance estimated from a linkage map of the BC_1F_3 populations (Yamamoto *et al.* 2001). One marker was used for selection in the target segments less than 20 cM long, two in the segments 20 to 50 cM long, and three in the segments more than 50 cM long. We selected plants carrying a heterozygous chromosome segment for the target region that were as homozygous as possible for Koshihikari alleles in the non-target regions. Genotypes of the plants were determined by using 129 RFLP markers selected from a high-density linkage map to cover the 12 chromosomes (Harushima *et al.* 1998, Rice Genome Research Program 2000). Plants with desired genotypes that carried the target chromosome segment under a heterozygous condition and less than five heterozygous chromosome segments in non-target regions were selected from the SBC_1F_1, SBC_2F_1 and SBC_3F_1 lines. Finally, we cultivated 39 populations (SBC_1F_2, SBC_2F_2 and SBC_3F_2) derived from the self-pollinated progeny of the selected plants. Subsequently, 39 candidate plants carrying homozygous chromosome segments of Kasalath in the target regions were selected. The genotypes of the 129 RFLP markers for the candidate CSSLs selected were determined to confirm the substitution of the target chromosome segments.

DNA marker analysis

RFLP analysis was performed according to the method described by Kurata *et al.* (1994) by using the ECL Direct Nucleic Acid Labeling and Detection System (Amersham Pharmacia, Uppsala, Sweden). Total DNA of the plants was extracted by the CTAB method (Murray and Thompson 1980). CAPS analysis was performed according to the method of Lin *et al.* (2003). In brief, a small piece of rice leaf was crushed in a 1.5-mL microfuge tube containing 300 µL of 100 mM Tris·HCl, 1 M KCl, and 10 mM EDTA. The DNA in the centrifuged supernatant was precipitated with isopropanol, and the pellet was redissolved in 50 µL of 0.1 × TE buffer (10 mM Tris·HCl, 1 mM EDTA). A 1-µL aliquot of this DNA extract was used as the template for PCR amplification. The 10-µL reaction volume contained 1 µL template DNA, 1 µL of 10 × PCR buffer, 25 mM of $MgCl_2$, 2 mM of each dNTP, 2 µL of 50% glycerol, 0.1 µL *Taq* DNA polymerase (5 U/µL), 0.2 µL of a 20 pM solution of each primer, and 3.7 µL of H_2O. Amplification was performed for 30 cycles (30 s at 94°C, 1 min at 60°C and 1 min at 72°C) followed by 7 min at 72°C. To detect polymorphism, the amplified product was digested overnight with an appropriate restriction enzyme and then electrophoresed on a 2% agarose gel.

Phenotyping and mapping of QTLs for days-to-heading

Five plants from each CSSL were grown under three different conditions: a natural summer field in Tsukuba, Japan; and long-day (14.5-h light: LD, 28°C for 12 hours and 24°C for 12 hours) and short-day conditions (10-h light: SD) in a controlled growth cabinet. Plants were raised twice and scored under the LD and SD conditions, but only once in the field, in 2002. The number of days required from sowing to heading of the first panicle (days-to-heading: DTH) was scored for each plant, and mean values were calculated for each CSSL. QTLs were detected based on the *t*-test of the difference between the mean of each CSSL and Koshihikari. A probability level of 0.001 was used as the threshold for the detection of a putative QTL.

Results

Characteristics of the CSSLs

Graphical genotypes of 39 CSSLs were determined by using 129 RFLP markers distributed evenly across the 12 rice chromosomes (Fig. 2). The substituted chromosome segments in the CSSLs covered most of the 12 chromosomes, except for small regions at the distal end of the short arm of chromosome 8 (defined by the RFLP marker S2104) and at the distal end of the long arm of chromosome 12 (defined by R2706A) (Fig. 2). Basically, in each CSSL, only one chromosome segment was substituted with Kasalath in the genetic background of Koshihikari. However, a small chromosomal segment of Kasalath was substituted in the non-target region in SL-216 (heterozygous at R1826 on

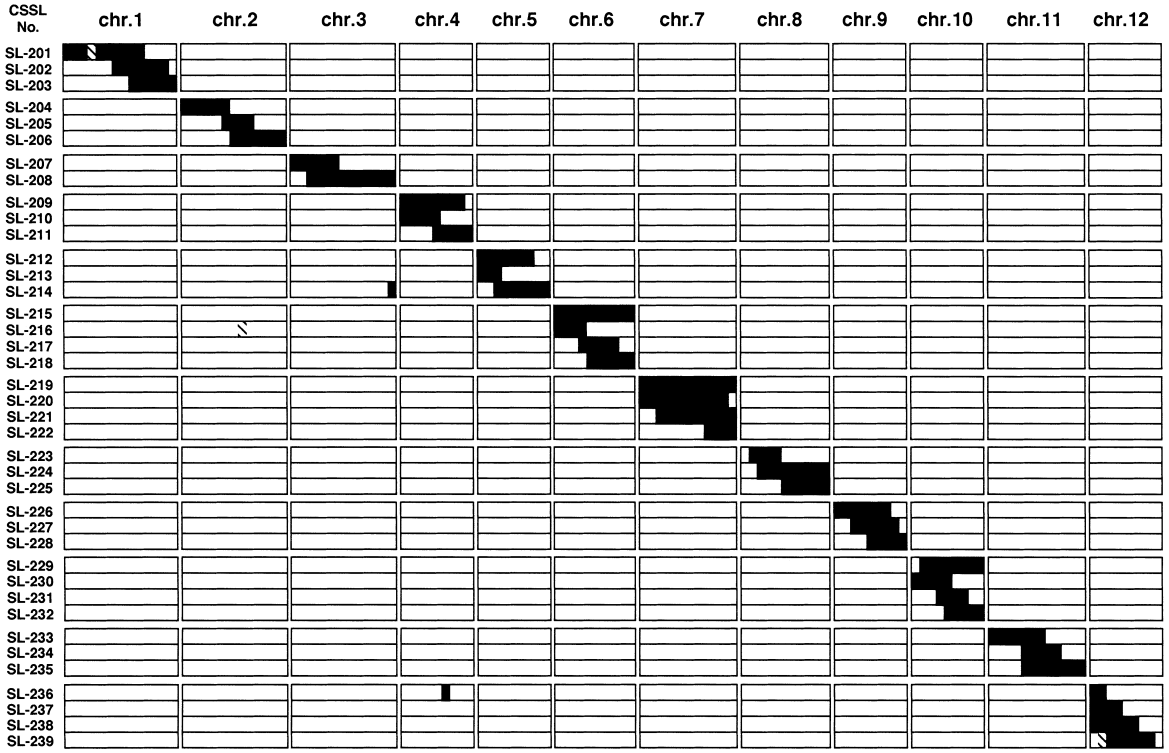

Fig. 2. Graphical representation of genotypes of the CSSLs developed in the present study. The black regions indicate the regions homozygous for Kasalath alleles; the white regions indicate the regions homozygous for Koshihikari alleles; the hatched regions indicate heterozygous regions. Genotype classes of the 129 RFLP markers for the CSSLs can be obtained at the Web site of the Rice Genome Resource Center (URL: http://www.rgrc.dna.affrc.go.jp/ine39.html).

chromosome 2), in SL-214 (Kasalath homozygous at S1571 on chromosome 3) and in SL-236 (Kasalath homozygous at R374 on chromosome 4) (Fig. 2). In addition, one small region of chromosome 1 (defined by C955) could not be fixed as homozygous for Kasalath and remained heterozygous owing to segregation distortion (Fig. 2). The entire region of chromosomes 6 and 7 was substituted in two lines, SL-215 and 219, respectively. Based on the genetic distance in the linkage map of Harushima *et al.* (1998), the percentage of substituted segment(s) in each CSSL ranged from 2.3% to 9.4%.

QTL mapping for DTH

Under natural field conditions, the DTH value was 101 ± 0.8 days in Koshihikari and 104 ± 5.7 days in Kasalath. It ranged from 89.4 to 125.0 days among the 39 CSSLs. Significant differences ($P < 0.001$) in the DTH values were detected between 18 CSSLs and Koshihikari (Fig. 3). The DTH value was lower than that of Koshihikari in lines SL-215–218 and SL-225, but higher in lines SL-207–209, 219–224, 231 and 237–SL-239 (Fig. 3).

Under LD conditions, the DTH value was 77.0 ± 3.1 days in Koshihikari and 87.3 ± 1.4 days in Kasalath. It ranged from 68.6 to > 122 days among the 39 CSSLs (Fig. 3). The differences in the DTH values between each CSSL and

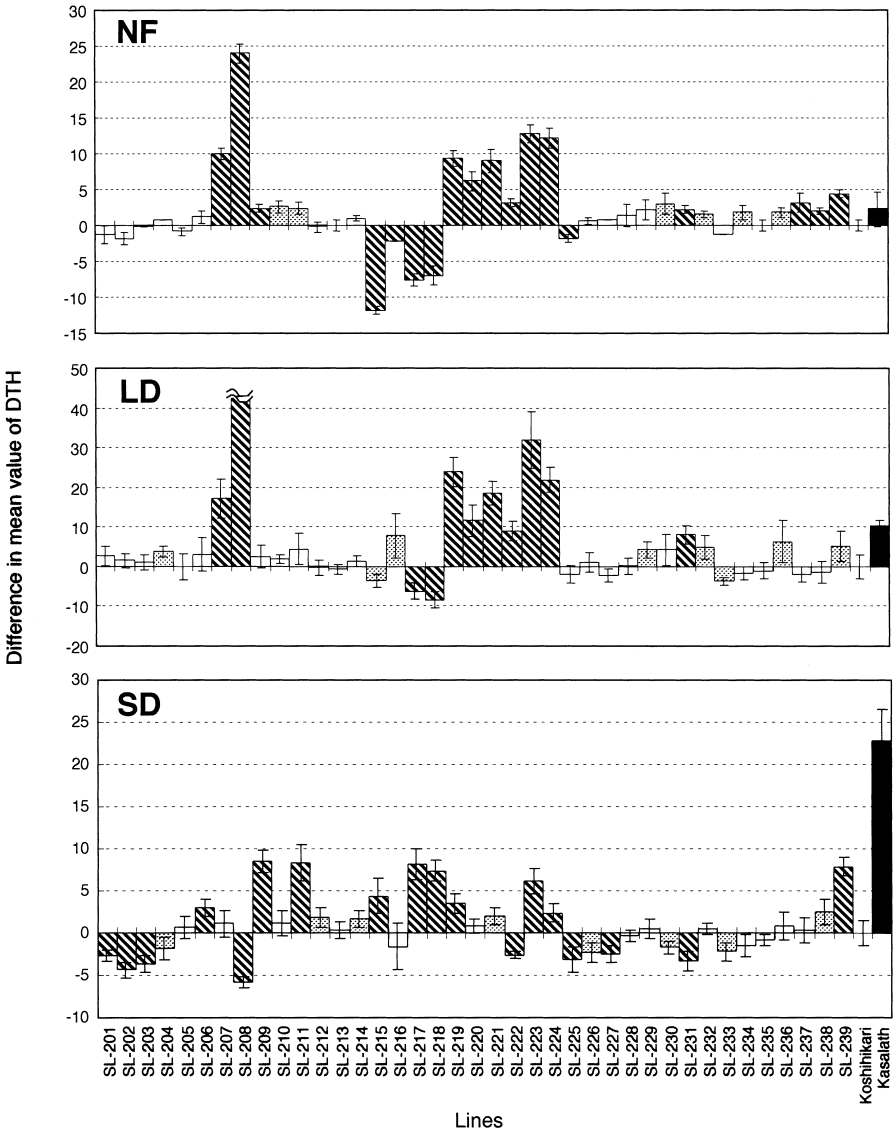

Fig. 3. Difference in mean value of DTH between CSSLs and the recurrent parent, Koshihikari, under three different environmental conditions: natural summer field (NF), long-day (LD) and short-day (SD). In each CSSL, bars denote the difference in the mean value of DTH with standard deviation. Hatched bars: significant at 0.1%; shaded bars: significant at 1%; open bars: not significant. Black bar denotes the difference between the donor cultivar Kasalath and Koshihikari.

Koshihikari showed the same tendency as that under natural field conditions, but the degree of difference was much larger under LD. Significant differences ($P < 0.001$) in the DTH values were detected between 11 CSSLs and Koshihikari (Fig. 3). The DTH value was lower than that of Koshihikari in only two lines, SL-217 and SL-218, but higher in the other nine. In particular, SL-208 did not flower by 122 days, and a significant increase in the DTH value (>20 days) was detected in SL-219, 223 and 224 (Fig. 3).

Under SD conditions, the DTH value was 53.6 ± 1.5 days in Koshihikari and 76.5 ± 3.5 days in Kasalath. It ranged from 49.2 to 62.1 days among the 39 CSSLs. Significant differences in the DTH values were detected between 18 CSSLs and Koshihikari (Fig. 3). The DTH value was lower than that of Koshihikari in lines SL-201–203, 208, 222, 225, 227 and SL-231, but higher in lines SL-206, 209, 211, 215, 217–219, 223, 224 and SL-239 (Fig. 3).

Once we detected significant differences between the CSSLs and the recurrent parent Koshihikari, comparison of the size of the substituted segments enabled to delineate candidate chromosomal regions of QTLs (substitution mapping). For example, a significant difference in the DTH value was detected in lines SL-209 and SL-211, but not in SL-210, under SD conditions (Fig. 3). These results clearly suggest that a putative QTL was located on the long arm of chromosome 4 (defined by R374 and C1016) (Fig. 4). This segment was shared in two lines, SL-209 and SL-211, but not in SL-210 (Fig. 2). In another example, under natural field and LD conditions, a significant difference was detected in lines SL-207, SL-208 and Koshihikari, and also between lines SL-207 and SL-208. Considering the size of the substituted chromosome segments, it is likely that at least two putative QTLs (defined by C515 and G332, and by R250 and S1571) were located on chromosome 3 (Fig. 4). Although some chromosomal regions could not be defined by substitution mapping (for example, all the CSSLs for chromosome 7 showed a significant difference in mean values from Koshihikari under natural field and LD conditions), at least eight chromosomal regions were likely to be associated with the heading date under natural field conditions, five under LD, and nine under SD (Fig. 4).

Discussion

Sequencing of the entire rice genome has made remarkable progress (Sasaki 2003). This achievement has significantly contributed to the molecular analysis of complex traits (Yano 2001, Izawa *et al.* 2003). Although sequence information and molecular tools have already been rapidly accumulated, plant materials for genetic analysis are still being developed, because material development usually requires a long period of time and much labor. Thus, a lack of genetic

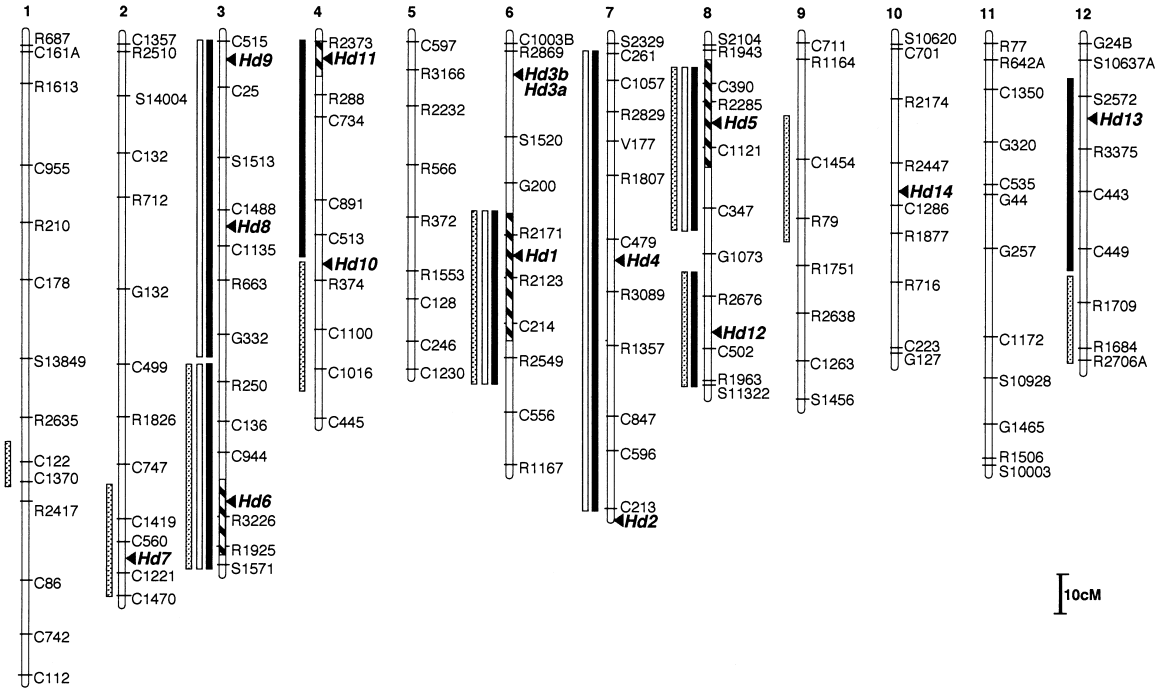

Fig. 4. Chromosome regions showing the association with the variation in DTH in the CSSLs. The vertical open bars numbered 1–12 denote the 12 rice chromosomes; the names of the RFLP markers and their chromosomal positions are indicated on the right side of each bar. Black bars denote the most likely chromosomal regions containing putative QTLs for DTH under natural field conditions, open bars under LD conditions, and shaded bars under SD conditions. Hatching denotes the most likely position of putative QTLs for DTH in an analysis of the BC_1F_3 population of the same cross combination (Yamamoto *et al.* 2001). Arrowheads denote the map locations of the QTLs detected in previous analyses of the progeny derived from a cross between Nipponbare and Kasalath (summarized in the report of Yano *et al.* 2001).

materials would limit our comprehensive understanding of quantitative traits.

Series of CSSLs or ILs have been developed in several plant and animal species (Eshed and Zamir 1995, Nadeau 2000, Kubo *et al.* 2002). Their potential for genetic analysis has been demonstrated in plants (Eshed and Zamir 1996, Kubo *et al.* 2002, Koumproglou *et al.* 2002) and mouse (Cowley *et al.* 2003). For example, CSSLs or ILs can be used in genetic analysis to associate QTLs with particular chromosome regions and to quickly develop NILs of target regions containing QTLs of interest. In general, when an association is detected between a chromosomal region and a trait, it is often difficult to validate the QTLs, especially those with very small genetic effects. In such a case, NILs are required to analyze genetic effects in detail. Because CSSLs normally have one chromosomal region substituted, they can be used as NILs themselves or as starting material to develop NILs.

In this regard, it will be necessary to develop CSSLs from a wide range of cross combinations to facilitate the genetic analysis of quantitative traits in rice. The primary objective of the present study was to develop a series of CSSLs with substituted chromosome segments of Kasalath in the genetic background of Koshihikari. Although two small chromosomal regions could not be covered by Kasalath segments, we successfully developed 39 CSSLs in which the substituted Kasalath chromosome segments covered most of the genome (Fig. 2). As some lines carried relatively large chromosome segments, the 12 rice chromosomes could be covered by at least 23 CSSLs (Fig. 2). To retain substituted chromosome segments as large as possible, we conducted marker-assisted selection of the target chromosome segment by using CAPS markers during the backcrossing step. CAPS marker analysis enabled to select appropriate plants with desirable genotype (heterozygous) for target chromosome segments before backcrossing was performed. Before this stage, labor and time requirements prevented us from determining the genotype of non-target chromosome segments remaining in each selected plant. This was achieved by RFLP analysis after backcrossing. Although this selection required additional labor, it enabled to develop CSSLs with single substituted chromosome segments.

Potential of CSSLs for QTL analysis for heading date

To demonstrate the potential power of CSSLs for QTL analysis, we used CSSLs to detect QTLs controlling heading date. Usually, it is very difficult to design phenotype assays in a small growth chamber using a primary mapping population with a large number of lines, such as RILs. The small number of CSSLs we developed enabled to conduct such an experiment.

In a previous study, Yamamoto *et al.* (2001) had detected four putative QTLs for DTH on chromosomes 3, 4, 6 and 8 by using a BC_1F_3 population derived from a Koshihikari/Kasalath//Koshihikari cross. In our study, eight chromosomal regions were associated with DTH under natural field conditions (Fig. 4). Although we could not detect any association between the distal-end region of the short arm of chromosome 4 and DTH, three chromosomal regions (chromosomes 3, 6 and 8) coincided with those of the QTLs reported by Yamamoto *et al.* (2001). We compared the eight chromosomal regions for DTH with those of QTLs previously detected in a cross of Nipponbare and Kasalath (summarized by Yano *et al.* 2001). As a result, we considered that seven contained *Hd1*, *Hd4*, *Hd5*, *Hd6*, *Hd8*, *Hd9*, *Hd10*, *Hd12* and *Hd13* (Fig. 4). In addition, we searched for QTLs for DTH under controlled day-length conditions. Of nine chromosomal regions associated with DTH under SD conditions, five were associated with DTH under SD conditions only (Fig. 4). Among them, four chromosomal regions, the long arms of chromosomes 1,4, 9 and 12, may contain new QTLs for DTH. Nevertheless, these relationships should be verified by fine mapping of the QTLs detected in the present study. This detection of additional QTLs clearly demonstrates that the potential of CSSLs for QTL detection is higher than that of a BC_1F_3 population.

In previous study using progenies between Nipponbare and Kasalath, two QTLs, *Hd3a* and *Hd3b*, had been detected on the short arm of chromosome 6 (Monna *et al.* 2002). In the present study, SL-216 carrying the distal end region of chromosome 6 of Kasalath exhibited a higher DTH value than that of Koshihikari under LD, but a lower DTH value under SD, even though statistical significance was not satisfactory in both cases. These results indicate that two different QTLs, *Hd3a* and *Hd3b*, were also detected in the present study. Fine mapping will be required to confirm the existence of these two loci, *Hd3a* and *Hd3b*. In addition, *Hd14* has been detected on chromosome 10 in a cross of Nipponbare and Kasalath (Yano *et al.* 2001). SL-231 carrying the chromosomal region of Kasalath corresponding *Hd14* showed a higher DTH value than that of Koshihikari under natural field and LD conditions, while a lower DTH value under SD conditions. These results may also support the existence of *Hd14*. However, other three lines, SL-229, SL-230 and SL-232, which might carry the chromosomal region of *Hd14*, did not show consistent results with high statistical reliability. Thus, additional analysis should be carried out to confirm the existence of *Hd14*.

In general, when QTLs with large effects are segregated, it is very difficult to detect QTLs with minor effects in primary mapping populations. In CSSLs, it is possible to compare phenotypic effects between alleles on defined substituted chromosome segments. This is the main reason why the detection power of CSSLs is much higher than that of primary mapping populations.

CSSLs as experimental materials

Development of NILs for QTLs is necessary for the verification and characterization of the QTLs detected (Tanksley 1993, Yano and Sasaki 1997). Once a QTL for a particular trait is detected, a CSSL can be used as NIL itself or as a base material for NIL development. As only one

chromosome fragment was substituted, only one additional backcross should be performed for NIL development. Such NILs enabled to combine two or three QTLs in one genetic background, in order to clarify the epistatic interaction among the QTLs (Yamamoto *et al.* 2000, Lin *et al.* 2000, 2003).

Although the detection power of QTLs in CSSLs is higher than that in primary mapping populations, such as F_2 and RILs, as mentioned above, the mapping resolution for QTLs in CSSLs may be lower than that in primary mapping populations, because it depends on the size of the substituted chromosome segments in CSSLs. However, this disadvantage can be easily overcome by fine mapping of putative QTLs using the CSSLs as base materials. In a primary mapping population, developing NILs for target QTLs is required in order to map QTLs precisely as single Mendelian factors. In contrast, the uniformity of the genetic background of each CSSL enabled to rapidly proceed to the linkage mapping of target QTLs. We have already revealed the advantages of these CSSLs in the analysis of resistance to ultraviolet-B radiation (Sato *et al.* 2003, Ueda *et al.* 2004), the response of rice to Al (Ma *et al.* 2002), and seed longevity (Miura *et al.* 2002).

The use of CSSLs may be disadvantageous for the detection of QTLs. When we assume that the complex inheritance of a particular trait is controlled by multiple factors with epistatic interaction, we should consider a potential risk. Because of the single segment substitution, it would be very difficult to observe phenotypic effects generated by the combination of two or more chromosome segments from the donor cultivar. In fact, phenotypic variation in DTH in the CSSLs was smaller than that in BC_1F_3 (Yamamoto *et al.* 2001). In such a case, F_2 and RILs may be more suitable for QTL detection than CSSLs. Backcross inbred lines (BILs) derived from the Koshihikari//Kasalath/Koshihikari cross have also been developed and are available at the Rice Genome Resource Center (http://www.rgrc.dna.affrc.go.jp/index.html.en). Thus, in order to avoid such a risk, we recommend the use of both BILs and CSSLs in QTL analysis.

In the map-based cloning of mutant genes in a *japonica* background, a mutant line is usually crossed with a distantly related cultivar or line, for example, *indica* cultivars or wild relatives, to obtain high-level sequence polymorphism between parental lines. In such a cross, a wide range of phenotypic segregations that often affect the expression of a mutant phenotype also occur in addition to those at the target loci. This often prevents researchers from conducting a precise classification of the mutant phenotype in the mapping populations. Furthermore, distant crosses produce several types of phenotypic segregations, including hybrid weakness, pollen sterility, and seed shattering, which may also cause difficulties for progeny testing in linkage mapping. To address these problems, CSSLs that carry substituted chromosome segments containing target loci can be used as parental lines for the production of F_2 populations (Ashikari *et al.* 1999, Yamanouchi *et al.* 2002).

Implication of use of CSSLs for rice breeding

The advent of DNA marker technology has enabled to develop new breeding strategies, such as marker-assisted breeding (Ribaut and Hoisington 1998, Peleman and van der Voort 2003). That strategy requires comprehensive dissection and understanding of complex traits of interest. To that end, even if a large number of DNA markers are available, novel plant materials (mapping populations) such as ILs or CSSLs are necessary to adopt marker-assisted strategies can be used in practical breeding (Zamir 2001, Peleman and van der Voort 2003). In rice, three series of ILs in the genetic background of two *japonica* cultivars, Asominori and Taichung 65, have been developed by using *indica* cultivars (Aida *et al.* 1997, Kubo *et al.* 2002), other cultivated species, *O. glaberrima* Steud. (Doi *et al.* 1997) or a wild relative, *O. glumaepatula* (Sobrizal *et al.* 1999), as donor strains. As these background materials are not elite cultivars, we used Koshihikari as the recurrent parent to develop CSSLs. Koshihikari displays several characteristics of economic value. Thus, the CSSLs developed in the present study could be very useful for the genetic analysis of important traits.

Once favorable alleles in Kasalath are identified, systematic and rapid development of NILs of Koshihikari can be achieved. In addition, such NILs could be used as new cultivars and as promising parental lines in rice breeding. Furthermore, by using various NILs and DNA markers, we may introduce genes derived from different donor cultivars into elite rice cultivars (trait pyramiding). This strategy has not yet been adopted in phenotype-based selection in rice breeding. New series of CSSLs from different donors in a Koshihikari background will be necessary in order to enhance variations of economic interest, and the development of these materials should facilitate the improvement of Japanese rice cultivars. In addition, we have already developed CSSLs from a Nipponbare (recurrent) and Kasalath (donor) cross (http://www.rgrc.dna.affrc.go.jp/index.html.en). We are currently developing new CSSLs from crosses between Sasanishiki and Habataki, and Koshihikari and Nona Bokra (unpublished data). To enhance the exploitation and utilization of novel alleles for rice breeding, these CSSLs should be grown under different environmental conditions, and comprehensive phenotyping will be necessary.

Seeds and genotype data of the CSSLs developed in this study are available at the Rice Genome Resource Center, National Institute of Agrobiological Sciences (URL: http://www.rgrc.dna.affrc.go.jp/ine39.html).

Acknowledgments

We thank Drs. K. Eguchi and Y. Ohtsuki, managing directors of the Rice Genome Research Program (Institute of the Society for Techno-innovation of Agriculture, Forestry, and Fisheries, Japan) for their advice and encouragement. This work was supported by the Rice Genome Project (GR-4001), Ministry of Agriculture, Forestry and Fisheries of Japan.

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