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ORIGINAL PAPER

Indian rice "Kasalath" contains genes that improve traits of Japanese premium rice "Koshihikari"

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Abstract Rice (*Oryza sativa* L.) chromosome segment substitution lines (CSSLs), in which chromosomal segments of the Indian landrace "Kasalath" replace the corresponding endogenous segments in the genome of the Japanese premium rice "Koshihikari", are available and together cover the entire genome. Chromosome regions affecting a trait (CRATs) can be identified by comparison of phenotypes with genotypes of CSSLs. We detected 99 CRATs for 15 agronomic or morphological traits. "Kasalath" had positively acting alleles in 53 CRATs. Its CRATs increased panicle number per plant by up to 23.3%, grain number per panicle by up to 30.8%, and total grain number by up to 15.1%, relative to "Koshihikari". CRATs were identified for grain size (grain thickness and width), with positive effects of about 5.0%. A CRAT on chromosome 8 almost doubled the weight of roots in uppermost soil layers compared to "Koshihikari". Additionally, "Kasalath" possessed CRATs for higher lodging resistance (reduction in plant height and increase in stem diameter). In some cases, multiple CRATs were detected in the same chromosome regions. Therefore, CSSLs with these chromosome segments might be useful breeding materials for the simultaneous improvement of multiple traits. Five CRATs, one for plant height on chromosome 1, one for stem diameter on chromosome 8, and three for heading date on chromosomes 6, 7, and 8 overlapped with the corresponding QTLs that already had been mapped with back-crossed inbred lines of "Nipponbare" and "Kasalath". In both "Koshihikari" CRATs and "Nipponbare" QTLs, "Kasalath" had similar effects.

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Introduction

The *japonica* "Koshihikari" is a Japanese premium rice (*Oryza sativa* L.) cultivar grown in most areas of Japan and in various other countries (e.g., Australia and USA; http://www.tdb.maff.go.jp/toukei/a02stopframeset) but has a weak root system and a poor lodging resistance (Morita et al. 1995; Kashiwagi et al. 2007). The Indian landrace rice cultivar, *Indica* "Kasalath", is low yielding with poor 1,000-grains weight and a low proportion of filled grains (Ishimaru 2003; Ishimaru et al. 2005; Kojima et al. 2005). The genomic information of "Kasalath" is already available, including a high-density genetic map that compares its genome with that of the common "Nipponbare", and the large-scale sequencing and computer-based chromosomal mapping of bacterial artificial chromosomes derived from the "Kasalath" genome (Katagiri et al. 2004; Harushima et al. 1998).

Plant breeders traditionally have relied on introgressing a single gene at a time. Marker-assisted selection has been used to improve several traits by the accumulation of superior genes from a donor, thus reducing costs and time requirements of breeding efforts. Servin et al. (2004) proposed a new approach, "pyramiding", in which quantitative trait loci (QTLs) are accumulated by crossing after narrowing down the chromosome regions containing desired loci. With this method, Ashikari and Matsuoka (2006) introduced two superior traits—shorter plant height and higher grain number per panicle—from one donor into rice.

Backcross inbred lines (BILs) produced by the single-seed descent method have been used in many QTL analyses

(e.g., Tanksley 1993; Ishimaru et al. 2001). QTL analysis with BILs provides statistical predictions about the existence and position of a gene for a trait. To prove such a gene's existence and to characterize it, comprehensive analysis is needed, using near-isogenic lines (NILs) containing the targeted QTL (Lin et al. 2000, 2003; Ishimaru 2003; Kashiwagi and Ishimaru 2004). To perform comprehensive analyses, new mapping populations were developed by substituting chromosome segments from a donor into the genetic background of the recipient; several lines of this type together cover all chromosomes. Such lines have been called "introgression lines" (ILs) in tomato (*Lycopersicon esculentum*; Eshed and Zamir 1995) and *Brassica napus* (Howell et al. 1996), "chromosome substitution strains" in *Arabidopsis thaliana* (Koumproglou et al. 2002), and "recombinant chromosome substitution lines" in barley (*Hordeum vulgare*; Matus et al. 2003). In rice (*Oryza sativa*), several ILs have been produced (Mei et al. 2006; Tian et al. 2006; Zhang et al. 2006). Additionally, Ebitani et al. (2005) developed chromosome segment substitution lines (CSSLs) in which different chromosomal segments of "Kasalath" are substituted in the genetic background of the Japanese top premium rice "Koshihikari" to cover the entire genome. In contrast to BILs, it is possible with ILs or CSSLs to identify the chromosome region affecting a trait through its effect on the phenotype. This allows a region to be distinguished from existing QTLs. As a consequence, Koumproglou et al. (2002) pointed out that a new notation was needed. However, the term "QTL" was used in analyses of ILs, and Ebitani et al. (2005) and Ishikawa et al. (2005a) named the regions "chromosomal regions containing putative QTLs".

The phenotype of one species or cultivar does not necessarily reflect the presence of genes that will lead to superior performance if expressed in a different species or line. Xiao et al. (1996) showed that a wild relative of cultivated rice with low yield, *Oryza rufipogon*, contains genes that can substantially increase the yield of modern high-yield rice. The aim of the present study was to establish whether the Indian landrace "Kasalath" might harbor genes that may be superior compared to those of the Japanese premier rice "Koshihikari" with respect to various traits. We mapped chromosome regions affecting a trait (named CRATs to distinguished them from QTLs) for 15 agronomic or morphological traits through the use of CSSLs.

Materials and methods

Plant materials

Seeds of 39 CSSLs in rice with chromosomal segments of "Kasalath" in the genetic background of "Koshihikari" were sown in a greenhouse on 6th May 2004, and seedlings were transplanted into paddy fields in Tsukuba, Japan (latitude 36°N), on 5th June with single plant per hill spaced at 18 × 30 cm, and grown under natural conditions with 15 plants per line.

Trait characterization and determination of CRATs

We characterized 15 agronomic and morphological traits (listed in Table 1) detectable in the aerial parts of the plants as described previously (Ishimaru et al. 2001; Kashiwagi and Ishimaru 2004). We measured maximum plant height and the height from the ground to the second leaf below the flag leaf (−2 leaf height). The root weight was measured by the modified method by Tanaka et al. (1985). We sampled soil including roots with a 50 mm diameter stainless steel soil sampler (hand sampler HS-25, Fujiwara Co. Tokyo, Japan), separated roots into 0–4 cm (upper) and 4–8 cm (lower) layers, and washed off the soil with water. Root weights were measured after drying at 80°C for 2 days. The traits were measured with six plants in each line. The position of a CRAT was determined by comparing phenotypes and genotypes of RFLP markers in CSSLs according to the methods of Ebitani et al. (2005). A probability level of 0.05 was used as the threshold for the detection of a CRAT. The effect of a CRAT was expressed as a percentage relative to the corresponding value determined in "Koshihikari".

Comparison of CRATs and QTLs

We compared the positions of CRATs and QTLs. The positions of QTLs, which are mapped with BILs in "Nipponbare" and "Kasalath", for plant height, −2 leaf height, and panicle number per plant, had been reported by Ishimaru et al. (2001). Likewise, the positions of QTLs for stem diameter and heading date had been established by Kashiwagi and Ishimaru (2004) and Yano et al. (1997). Comparisons of effects between CRATs and QTLs were carried out using data established for NILs that contain QTLs for plant height on chromosome 1 (Ishimaru et al. 2004), for stem diameter on chromosome 8 (Kashiwagi et al. unpublished data), and for heading date on chromosomes 6, 7 and 8 (Lin et al. 2000, 2003). RFLP markers employed were described by Ebitani et al. (2005).

Results

Phenotypic variation in CSSLs

Chromosome segment substitution lines produced transgressive segregants with better values than "Koshihikari" in all 15 traits (Fig. 1). Compared to "Koshihikari, the CSSLs

Table 1 Correlation coefficients between panicle number (PN), grain number per panicle (GN), total grain number (TGN), plant height (PH), −2 leaf height (−2LH), panicle neck length (PNL), panicle length (PL), crown width (CW), stem diameter (SD), panicle neck diameter (PND), heading date (HD), grain thickness (GT), grain width (GW), upper root weight (URW), and lower root weight (LRW)

Traits	PN	GN	TGN	PH	−2LH	PNL	PL	CW	SD	PND	HD	GT	GW	URW
PN														
GN	NS													
TGN	0.44**	0.86***												
PH	−0.52***	NS	NS											
−2LH	−0.52***	NS	NS	0.77***										
PNL	NS	NS	NS	0.36*	NS									
PL	−0.36*	0.41**	NS	0.41**	NS	NS								
CW	NS	NS	NS	NS	NS	−0.43**	NS							
SD	−0.48**	0.53***	NS	NS	NS	NS	0.73***	NS						
PND	NS	0.70***	0.60***	NS	−0.38*	NS	0.58***	NS	0.64***					
HD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
GT	NS	NS	NS	0.37*	0.33*	NS	NS	−0.34*	NS	NS	NS			
GW	NS	0.44**	0.35*	0.33*	NS	NS	NS	NS	NS	NS	NS	0.38*		
URW	NS	NS	NS	NS	NS	NS	NS	0.35*	NS	NS	0.64***	−0.33*	NS	
LRW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Significant differences at *** $P < 0.001$, ** 0.01, * 0.05, respectively

NS No correlation (not significant)

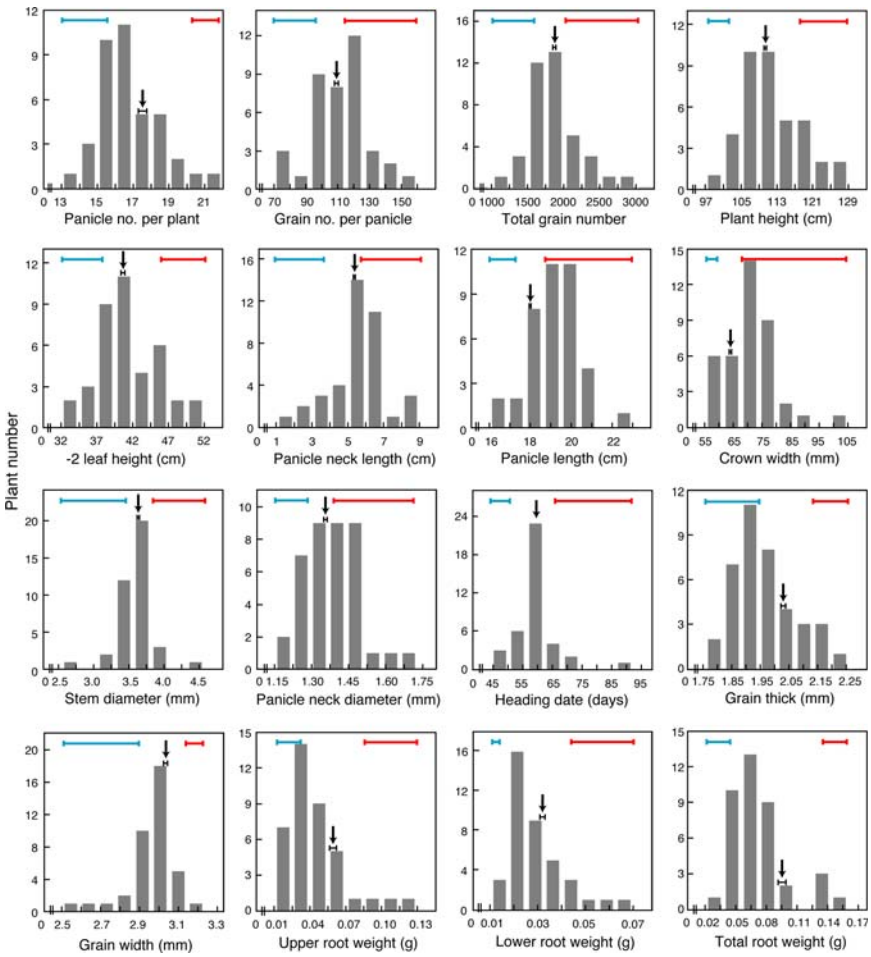

Fig. 1 Frequency distributions of 15 traits in 39 CSSLs. *Arrows* indicate the mean of "Koshihikari" and *black cross bars* indicate the standard error of the mean. *Red* and *blue cross bars* show CSSLs included in bars which traits were significant higher and lower compared to "Koshihikari", respectively

whose traits were significantly higher and lower were indicated in red and blue lines, respectively. Concerning yield components, 23.1, 46.2, and 26.6% of the CSSLs outperformed "Koshihikari" in panicle number per plant, grain number per panicle, and total grain number, respectively. The "Kasalath" segment increased the maximum total grain number by up to 50% over that of "Koshihikari". Plant height and −2 leaf height of CSSLs ranged from 97 to 129 cm and 32 to 52 cm, respectively. Panicle neck length, panicle length, and crown width increased by 36.0, 69.2, and 69.2%, respectively, of the "Koshihikari" values. Half of all CSSLs had a greater panicle neck diameter than "Koshihikari". The heading date of "Koshihikari" was 68 days after planting, and those of the CSSLs ranged from 15 days earlier to 33 days later. Nineteen percent of the CSSLs outperformed "Kasalath" in grain thickness, and 12.8% in grain width. Upper and lower root weights showed improvements in 10.3 and 25.6%, respectively, of lines.

Correlation between traits

Panicle number per plant was negatively correlated with plant height ($r = -0.52$, $P < 0.001$) and −2 leaf height ($r = -0.52$, $P < 0.001$; Table 1). Grain number per panicle showed significant positive correlations with total grain number per plant ($r = 0.86$, $P < 0.001$), stem diameter ($r = 0.53$, $P < 0.001$), and panicle neck diameter ($r = 0.70$, $P < 0.001$). Total grain number per plant correlated positively with panicle neck diameter ($r = 0.60$, $P < 0.001$). There were significant correlations between plant height and −2 leaf height ($r = 0.77$, $P < 0.001$), and between panicle length and stem diameter ($r = 0.73$, $P < 0.001$) as well as panicle neck diameter ($r = 0.58$, $P < 0.001$). Stem diameter showed a significant correlation with panicle neck diameter ($r = 0.64$, $P < 0.001$), and heading date was positively correlated with upper root weight ($r = 0.64$, $P < 0.001$).

Position and effects of CRATs

For the 15 traits, 99 CRATs were identified on the genetic map (Fig. 2). Among them, we detected 53 with positive effects exerted by "Kasalath" alleles (Table 2). We found four CRATs for panicle number per plant. One of them was located on chromosome 4, and the positive "Kasalath" alleles induced a 23.3% increase relative to the "Koshihikari" genotype. Six CRATs derived from "Kasalath" had positive effects on grain number per panicle; two on chromosomes 1 and 7 increased grain number by up to 30% relative to "Koshihikari". Six CRATs for total grain number per panicle were detected; the "Kasalath" allele of one located on chromosome 1 caused a 15.1% increase. "Kasalath" alleles in five CRATs had positive effects on plant height. We detected six CRATs for −2 leaf height; four of them found on chromosomes 1, 2, 3 and 8, respectively, increased height when carrying "Kasalath" alleles. Nine CRATs for panicle neck length were found, of which six were responsible for length increases by 9.9–40.1% when harboring "Kasalath" alleles. These six CRATs were located on chromosomes 2, 3, 5, 6, 10 and 12. Eight of ten CRATs controlling panicle length evoked positive effects when occupied by alleles from "Kasalath". Nine CRATs for crown width were detected. "Kasalath" alleles on chromosomes 1, 3, 4, 5, 7, 9, and 10 gave positive effects, increasing crown width by up to 46.9%. "Kasalath" alleles in three of the seven CRATs for stem diameter had positive effects. Seven CRATs for panicle neck diameter were detected, and four of them increased diameter with alleles from "Kasalath". Four CRATs with effects on heading date were identified. When carrying "Kasalath" alleles, three of them located on chromosomes 3, 7, and 8, delayed heading by 8.3–19.3% relative to "Koshihikari". Eight CRATs controlled grain thickness, three of which on chromosomes 2 and 3 were beneficial when occupied by alleles from "Kasalath". Seven CRATs for grain width were detected, but only one CRAT on chromosome 1 gave positive effects with the "Kasalath" allele. Six CRATs for upper root weight were detected, of which only one, on chromosome 8, led to an improvement with the allele from "Kasalath", increasing weight by 95.8% relative to "Koshihikari". Only one CRAT for lower root weight was detected in which the "Koshihikari" allele had a negative effect.

On chromosome 1, several CRATs for various traits with positive "Kasalath" effects overlapped between R607 and C178 (Fig. 2). CSSLs with this region occupied by "Kasalath" alleles outperformed "Koshihikari" in grain number per panicle, total grain number, panicle neck diameter, and grain width, but panicle neck length and upper root weight were reduced. Substitution of the region between R2171 and R2549 on chromosome 6 increased grain number per panicle, panicle length, and panicle neck diameter, and decreased −2 leaf height, days to heading, and upper root weight.

Comparison of CRATs and QTLs

We analyzed the overlapping between CRATs and QTLs, which were detected with BILs between "Nipponbare" and "Kasalath" (Fig. 3). Overlap between a CRAT and a locus for plant height was detected on chromosome 1. A CRAT for stem diameter overlapped with a locus on chromosome 8, and overlaps for heading date were detected on chromosomes 3, 6, 7, and 8. The effect of the CRAT for plant height on chromosome 1 was +18% relative to "Koshihikari", and the effect of the corresponding locus in "Nipponbare" was +13% (Ishimaru et al. 2004; Table 3).

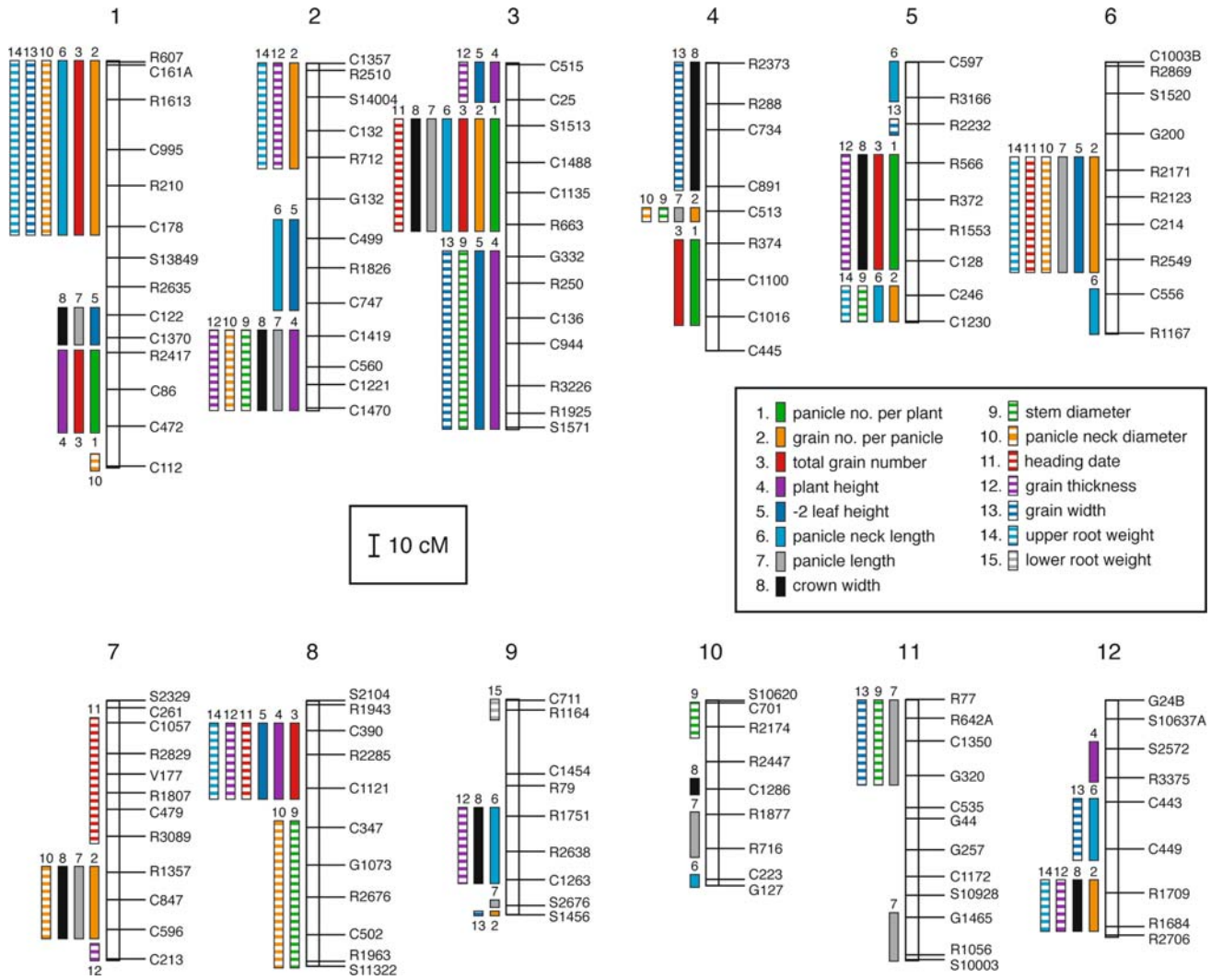

Fig. 2 Positions of CRATs on the rice genetic map. RFLP markers were defined by Ebitani et al. (2005)

The CRAT for stem diameter on chromosome 8 increased the diameter by 6.9%, and the corresponding locus in "Nipponbare" increased it by 7.0% (Kashiwagi et al. unpublished data). A CRAT on chromosome 6 decreased the days to heading in "Koshihikari" by 13.5% (12.5 days earlier) while the corresponding locus caused heading to commence 17 days earlier (−14.4%) in "Nipponbare" (Lin et al. 2000). CRATs on chromosomes 7 and 8 increased the number of days to heading in "Koshihikari" by 8.3 and 9.9% (7 and 11 days earlier), respectively, and the corresponding loci increased it by 6 and 13.4 days (increasing by 5.3 and 11.8%) in "Nipponbare" (Lin et al. 2003).

Discussion

We identified 99 CRATs for 15 agronomic or morphological traits (Fig. 2). In all traits expect for lower root weight, "Kasalath" had alleles with positive effects and a total of 53 superior CRATs as compared to "Koshihikari" (Table 2). Especially, in traits related to yield, CRATs from "Kasalath" increased panicle number per plant by up to 23.3%, grain number per panicle by up to 30.8%, and total grain number by up to 15.1%. CRATs were identified for two traits related to grain size, grain thickness and width, with positive effects from "Kasalath" of 5.9 and 5.0%, respectively. Additionally, our analysis showed that one chromosomal region was responsible for multiple superior traits. One CRAT between R607 and C178 on chromosome 1 increased grain number per panicle, total grain number, and grain width (Table 2). These results suggested that this CRAT would increase yield through increases of grain number as well as size. Unlike existing QTL analyses with BILs, CRAT analysis determines the location of chromosomal regions containing several genes for superior traits. "Koshihikari" is a high-yielding rice (Uchiyamada 1995) whereas "Kasalath" is not (Ishimaru 2003; Ishimaru et al. 2005; Kojima et al. 2005). Our results lead to the surprising

Table 2 Characteristics of CRATs for the traits examined and their effects in the "Koshihikari" background

Trait	Number of CRATs detected	Chromosome number	Markers at both ends of putative CRATs[a]	CRAT-dependent change (%)[b]
Panicle number per plant	4	1	R2417–C472	−19.0
		3	S1513–R663	−15.7
		4	R374–C1016	+23.3
		5	R566–C128	−11.4
Grain number per panicle	9	1	R607–C178	+30.8
		2	C1357–R712	+8.1
		3	S1513–R663	−20.5
		4	C513	−25.0
		5	C246–C1230	−14.5
		6	R2171–R2549	+16.8
		7	R1357–C596	+29.7
		9	S1456	+4.7
		12	R1709–R1684	+6.2
Total grain number	6	1	R607–C178	+15.1
		1	R2417–C472	−24.3
		3	S1513–R663	−33.0
		4	R374–C1016	−15.8
		5	R566–C128	−19.4
		8	C390–C1121	−12.5
Plant height	6	1	R2417–C472	+16.0
		2	C1419–C1470	+9.8
		3	C515–C25	+7.7
		3	G332–S1571	−8.5
		8	C390–C1121	+10.6
		12	S2572–R3375	+9.5
−2 Leaf height	6	1	C122–C1370	+18.0
		2	C499–C747	+8.4
		3	C515–C25	+16.1
		3	G332–S1571	−12.3
		6	R2171–R2549	−15.9
		8	C390–C1121	+18.9
Panicle neck length	9	1	R607–C178	−64.5
		2	C499–C747	+24.6
		3	S1513–R663	+9.9
		5	C597–R3166	+25.8
		5	C246–C1230	−47.5
		6	C556–R1167	+40.1
		9	R1751–C1263	−39.6
		10	C223–G127	+18.3
		12	C443–C449	+10.1
Panicle length	10	1	C122–C1370	+11.6
		2	C1419–C1470	−3.5
		3	S1513–R663	+10.5
		4	C513	−6.4
		6	R2171–R2549	+15.2
		7	R1357–C596	+10.9
		9	S2676	+9.5
		10	R1877–R716	+9.2
		11	R77–G320	+5.7
		11	G1465–S10003	+7.9

Table 2 continued

Trait	Number of CRATs detected	Chromosome number	Markers at both ends of putative CRATs[a]	CRAT-dependent change (%)[b]
Crown width	9	1	C122–C1370	+17.0
		2	C1419–C1470	−10.3
		3	S1513–R663	+23.1
		4	R2373–C891	+16.8
		5	R566–C128	+15.3
		7	R1357–C596	+15.4
		9	R1751–C1263	+46.9
		10	C1286	+19.2
		12	R1709–R1684	−10.4
Stem diameter	7	2	C1419–C1470	−6.6
		3	G332–S1571	−5.2
		4	C513	−15.7
		5	C246–C1230	−4.1
		8	C347–S11322	+6.9
		10	C701–R2174	+5.4
		11	R77–G320	+4.1
Panicle neck diameter	7	1	R607–C178	+8.1
		1	C112	−8.1
		2	C1419–C1470	−6.6
		4	C513	−9.6
		6	R2171–R2549	+17.8
		7	R1357–C596	+10.4
		8	C347–S11322	+6.7
Heading date	4	3	S1513–R663	+19.3
		6	R2171–R2549	−13.5
		7	C1057–R3089	+8.3
		8	C390–C1121	+9.9
Grain thickness	8	2	C1357–R712	+5.0
		2	C1419–C1470	+5.9
		3	C515–C25	+4.0
		5	R566–C128	−6.6
		7	C213	−5.6
		8	C390–C1121	−9.2
		9	R1751–C1263	−8.3
		12	R1709–R1684	−4.0
Grain width	7	1	R607–C178	+5.0
		3	G332–S1571	−10.3
		4	R2373–C891	−5.0
		5	R2232	−10.9
		9	S1456	−3.7
		11	R77–G320	−3.7
		12	C443–C449	−3.3
Upper root weight	6	1	R607–C178	−68.2
		2	C1357–R712	−48.8
		5	C246–C1230	−63.2
		6	R2171–R2549	−77.6
		8	C390–C1121	+95.8
		12	R1709–R1684	−61.3
Lower root weight	1	9	C711–R1164	−56.2

[a] Putative CRATs data were analyzed with measurements of traits and genotype data of RFLP marker by Ebitani et al. (2005), a probability level of 0.05 was used as the threshold for this value

[b] CRAT-dependent changes expressed as percentage change of the value determined in "Koshihikari"

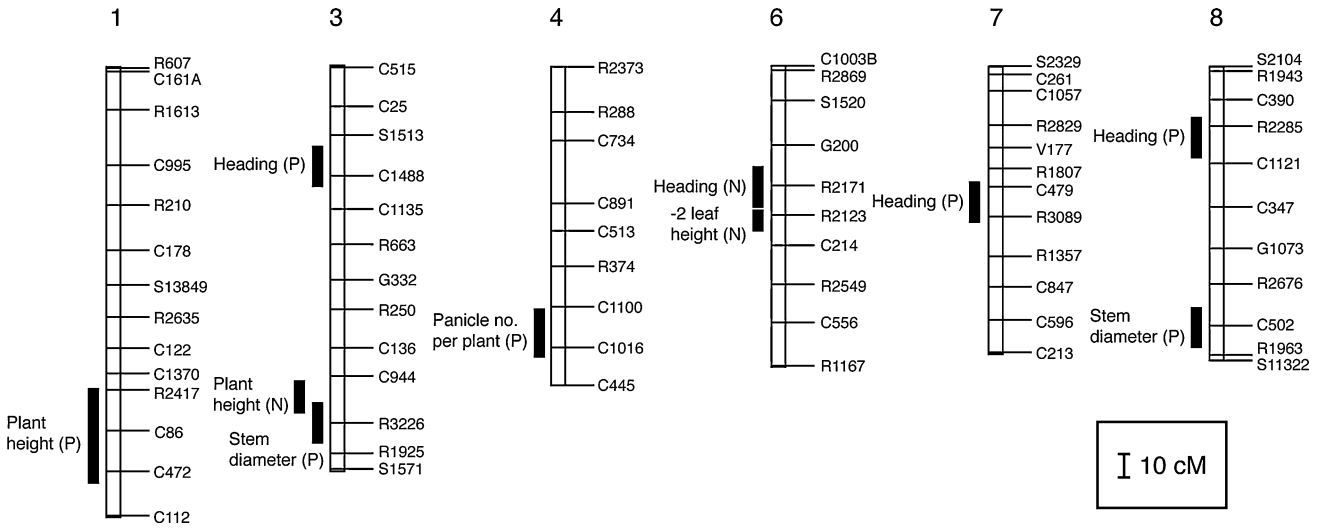

Fig. 3 Chromosomal region overlap between "Kasalath" CRATs detected in this study and "Nipponbare" QTLs. *P* and *N* signify positive and negative phenotypic effects of "Kasalath" alleles. The positions of QTLs were published by Ishimaru et al. (2001; for plant height, panicle number, −2 leaf height), Yano et al. (1997; heading), and Kashiwagi and Ishimaru (2004; stem diameter)

Table 3 Effects of "Kasalath" alleles in CRATs for plant height, stem diameter and heading date that overlapped with corresponding QTLs

Trait	Chr. no.	Background	Percent change (%)
Plant height	1	Koshihikari	+18.0
		Nipponbare	+13.0
Stem diameter	8	Koshihikari	+6.9
		Nipponbare	+7.0
Heading date	6	Koshihikari	−13.5
		Nipponbare	−14.4
	7	Koshihikari	+8.3
		Nipponbare	+5.3
	8	Koshihikari	+9.9
		Nipponbare	+11.8

The effects of CRATs were characterized in the "Koshihikari" background, and those of QTLs in the *japonica* cultivar "Nipponbare" background. Effects are given as relative change in percent. The effects of QTLs on plant height and stem diameter were provided by our group (Ishimaru et al. 2004; Kashiwagi et al. unpublished data), and heading data were calculated from results by Lin et al. (2000, 2003)

conclusion that the low-yielding "Kasalath" possesses a number of genes that could be used to improve yield in "Koshihikari". The present work was based on a single field experiment, which does not exclude the possibility that the mapping results could be affected by environmental conditions. The interaction of the CRATs with environment remains to be revealed by further studies.

The root system determines the efficiency of water and nutrient absorption in plants (Debi et al. 2003). Kondo et al. (2003) suggested that the roots in the upper soil layer have an important role in water and nutrient acquisition, particularly during phases of recovery after dry periods. "Koshihikari" has a weak root system of relatively low weight and a shallow growth pattern (Morita et al. 1995). We detected six CRATs for upper root weight, and the one on chromosome 8 increased upper root weight by 95.8% (Fig. 2; Table 2). This superior CRAT from "Kasalath" could be used for improving nutrient and water uptake in "Koshihikari".

There was a significant negative correlation between panicle number per plant and plant height (Table 1). CRATs for both traits were mapped to the same region on chromosome 1 in which the "Kasalath" alleles increased plant height and decreased grain number (Fig. 2; Table 2). Hittalmani et al. (2003) analyzed the correlations between various traits in *indica* and *japonica* rice in nine Asian locations. They also found a negative relationship between plant height and panicle number per plant. In five rice dwarf mutants, plant stature showed a negative correlation with tiller number (Ishikawa et al. 2005b). These findings suggested that panicle number per plant and plant height might be controlled by the same genetic elements. However, a variety of different genes exist on this chromosome segment which has a considerable size of 35.2 cM. Further analysis is needed to narrow down and characterize the chromosome regions related to each trait in order to clarify this point.

"Koshihkari" has low lodging resistance (Kashiwagi et al. 2007) and the reduction of plant height is the main target for its improvement (Ashikari and Matsuoka 2006). Stem thickness is strictly correlated to lodging resistance in rice and wheat (Tripathi et al. 2003; Won et al. 1998; Zuber et al. 1999). "Kasalath" had beneficial alleles in CRATs for

both relevant traits, lower plant height as well as higher stem diameter (Table 2). Additionally, CRATs for reduced cadmium contents of grains and various characteristics of the heading process have been reported from the same plant materials as in this study (Ebitani et al. 2005; Ishikawa et al. 2005a). In "Kasalath", several loci already have been identified for the improvement of the proportion of ripened grains (Ishimaru et al. 2005). Since a wealth of information about the "Kasalath" genome is already available (Katagiri et al. 2004; Harushima et al. 1998), the cultivar has the potential to provide an excellent resource for the genetic improvement of "Koshihikari".

In many cases, the effects of QTLs have been limited by the genetic background in which they were analyzed (McKendry et al. 1996; Toojinda et al. 1998). Manipulation of a locus can be problematic owing to the loss of target loci though recombination, incorrect information on the location of the QTLs, or altered expression of the QTLs in the new genetic background. We compared the position and effects of "Kasalath" CRATs detected in this study to those of QTLs whose effects were determined by using NILs which contain "Kasalath" chromosome segments underlying loci in "Nipponbare" background. Five CRATs for three traits (one for plant height on chromosome 1, one for stem diameter on chromosome 8, and three for heading date on chromosomes 6, 7, and 8) overlapped with the corresponding loci (Fig. 3). In both the CRATs in the "Koshihikari" and QTLs in the "Nipponbare" background, the additive effect of "Kasalath" was similar on plant height, stem diameter, and heading (Table 3). These results suggested that the overlapping between CRATs and QTLs might indicate the existence of gene(s) with similar effects in "Koshihikari" and "Nipponbare". Unfortunately, the chromosome regions of the CRATs are huge, which complicates the comparative analysis of gene function in these CRATs and QTLs. Previously, our group has shown that the locus for plant height on chromosome 1 coincided with a sucrose-phosphate synthase gene (Ishimaru et al. 2004), and Yano et al. (2000) have identified the gene (*Hd1*) corresponding to a locus for heading on chromosome 6. Further expressional studies and comparison of genome regions of "Koshihikari" and "Nipponbare" will reveal whether identical genes function in both cultivars.

A novel breeding approach named "pyramiding" has been developed in which QTLs are accumulated by crossing after narrowing down the chromosome regions harboring the QTLs (Servin et al. 2004). However, possible interactions between introduced QTLs do not become evident before the last step. We detected multiple CRATs in the same chromosome region and could observe their concurrent phenotypical effects in the field. For example, two CRATs for total grain number per plant and grain width were mapped to the region between R607 and C178 on chromosome 1; the "Kasalath" alleles increased the values by 15.1 and 5.0%, respectively (Table 2). The simultaneous effects of both CRATs were already confirmed in CSSL 201 (data not shown), with a chromosome segment including these CRATs. By a combination of phenotyping and marker-assisted selection in the progeny lines created by backcrossing between these CSSLs and "Koshihikari", we can replace unnecessary regions from "Kasalath" by "Koshihikari" regions while narrowing down the desired region. Ultimately, new cultivars with superior traits can be developed.

In summary, we showed that "Kasalath" contains genes that could be used to improve various traits (e.g., yield and root characters) in the Japanese premium rice "Koshihikari". Several chromosome segments were detected with multiple superior genes, and CSSLs with these segments can be used for the simultaneous improvement of multiple traits.

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