JARQ 43 (2), 115 – 121 (2009) http://www.jircas.affrc.go.jp

Quantitative Trait Locus Analysis for Cold Tolerance at the Booting Stage in a Rice Cultivar, Hatsushizuku

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Abstract

We evaluated cold tolerance at the booting stage on the basis of seed fertility after cool-water treatment for three years (2005-2007) in 114 recombinant inbred lines (RILs) between temperate *japonica*s, Kirara397 (cold-sensitive) and Hatsushizuku (cold-tolerant). Composite interval mapping (CIM) was performed to identify quantitative trait locus (QTL) for cold tolerance. A QTL for cold tolerance at the booting stage was reproducibly detected in three trials on chromosome 1. Contribution of the QTL to the phenotypic variation ranged from 16.2 to 47.3%, and their additive effects were all towards Hatsushizuku. We also analyzed QTL for heading time and culm length, which are thought to affect cold tolerance. A QTL for heading time and that for culm length was detected every three years on the neighboring regions of chromosome 10, while no QTL for the two traits was detected on chromosome 1. These results suggest that the QTL on chromosome 1 has a major effect on the variation of cold tolerance between Kirara397 and Hatsushizuku without affecting heading time and culm length.

Disciplines: Plant breeding / Biotechnology
Additional key words: composite interval mapping (CIM), QTL, simple sequence repeat (SSR)

Introduction

Rice has its origin in tropical or sub-tropical areas, and it is cold-sensitive. Particularly at the booting stage, low temperature causes spikelet sterility in rice plants due to failure of microspore development under low temperature conditions[13]. Rice production in Hokkaido, which is the northernmost island of Japan, is always at risk for yield reduction by this sterile type of cold injury. Therefore, rice breeders have been making efforts to develop more cold-tolerant cultivars, resulting in the breeding of some superior cold-tolerant lines. Two cold-tolerant lines, Norin-PL8 and Norin-PL11, were developed in the late 1980's via introgression of cold tolerance from tropical *japonica* cultivars, Silewah and Padi Labou Alumbis, respectively[1]. Unfavorable traits of Norin-PL11, such as long duration to heading and inferior eating quality were improved, and a cold-tolerant breeding line, Hokkai-PL9, was developed.

Genetic studies on cold tolerance using these cold-tolerant materials have been carried out. A study on chromosomal location of QTLs for cold tolerance at the booting stage in Norin-PL8 indicated that at least two regions on chromosomes 3 and 4 are responsible for cold tolerance[9]. Moreover, it was revealed that the QTL for

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Received 7 May 2008; accepted 19 August 2008

cold tolerance on chromosome 4 of Norin-PL8 consists of two closely linked genes, *Ctb1* and *Ctb2*[10], and *Ctb1* was delimited to a 56-kb region[11]. Kuroki et al.[6] detected a QTL for cold tolerance of Hokkai-PL9 on chromosome 8, and the region responsible for the QTL was narrowed down to a 193-kb interval. Takeuchi et al.[14] mapped QTLs for cold tolerance in a cross between temperate *japonica* cultivars, Koshihikari (cold-tolerant) and Akihikari (cold-sensitive), on chromosomes 1, 7 and 11. QTLs for cold tolerance were also mapped on chromosomes 1, 2, 3, 5, 6, 7, 9, and 12 in *japonica* × *indica* crosses[2].

The objective of this study was to locate QTLs associated with rice cold tolerance at the booting stage. We used recombinant inbred lines (RILs) developed from a cross between the *japonica*s Kirara397 (cold-sensitive) and Hatsushizuku (cold-tolerant). Heading time and culm length, as relevant traits to cold tolerance, were also analyzed. The reproducibility of the detected QTLs was confirmed by three trials in 2005–2007.

Materials and methods

1. Plant materials

Hatsushizuku is a cold-tolerant rice cultivar in Hokkaido and was selected from the cross of Matsumae/Jou116//Hokkai258. Matsumae and Hokkai258 are temperate *japonica*s in Hokkaido, while Jou116 is an *indica* breeding line. Kirara397 is a cold-sensitive temperate *japonica* and is a leading cultivar in Hokkaido. The F_2 plants between Kirara397 and Hatsushizuku were advanced through the single seed descent (SSD) method, and 114 recombinant inbred lines (RILs) were established for analysis of cold tolerance. The F_6, F_7 and F_8 generations were used in 2005, 2006 and 2007, respectively.

2. Evaluation of cold tolerance

Cold tolerance was evaluated by the cool water irrigation method[4,12] in a paddy field (National Agricultural Research Center for Hokkaido Region, Sapporo, Japan). The field was irrigated with cool water controlled at 19.4ºC from the primordial stage to the completion of heading (from June 28 to September 8 in 2005, from June 28 to September 4 in 2006, and from June 29 to August 31 in 2007). The depth of water was about 20 cm. RILs were seeded and five seedlings of each of the RILs per hill were transplanted in the field. Dates of seeding were April 15 in 2005, April 20 in 2006 and April 20 in 2007, and dates of transplanting were May 27 in 2005, May 26 in 2006 and May 28 in 2007. After ripening of the seeds, cold tolerance at the booting stage (CT) was evaluated on the basis of mean seed fertility of five panicles per line. Heading time (HT) was recorded as the number of days from seeding until the first panicle had started to emerge, and culm length (CL) was measured in centimeters from the soil to the panicle neck of the longest culm.

3. Molecular marker analysis

DNA was extracted from leaves following the method described by Monna et al.[8] with minor modifications. Simple sequence repeat (SSR) markers were amplified in 10 µL of mixture containing 10 mM Tris-Cl (pH 8.3), 1.5 mM $MgCl_2$, 0.001% gelatin, 0.1 mM of each deoxyribonucleotide, 0.2 µM of each primer, and 0.02 units/µL AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). The thermal cycles used were as follows: 1 cycle at 94ºC for 4 min, followed by 45 cycles at 94ºC for 1 min, 55ºC or 60ºC for 1 min and 72ºC for 2 min, and finally 1 cycle at 72ºC for 7 min. PCR products were separated by electrophoreses in 4% (w/v) MetaPhor agarose (Cambrex, East Rutherford, NJ).

4. QTL analysis

A linkage map was constructed using the computer program MAPL97[16,17], and composite interval mapping (CIM) was employed to detect QTLs affecting cold tolerance at the booting stage. The arcsine transform of mean seed fertility percentage after cool-water treatment was used as an index of cold tolerance for CIM. Significance thresholds were evaluated by 1,000 permutations, and an experiment-wise error threshold of 0.05 was retained. CIM and permutation tests were implemented in the program Windows QTL Cartographer version 2.5[18].

Results

1. Phenotypic variation

Cold tolerance at the booting stage (CT) of RILs between Kirara397 and Hatsushizuku was evaluated as seed fertility after cool-water treatment. Seed fertility of Kirara397 under normal temperature condition was 94.1% in 2005, 96.5% in 2006 and 91.4% in 2007, suggesting the variation in seed fertility after cool-water treatment largely reflects the genetic variation of cold tolerance among RILs. CT of Hatsushizuku was 82.6% in 2005, 74.7% in 2006 and 72.1% in 2007, while Kirara397 showed significantly lower CT than Hatsushizuku (45.9% in 2005, 35.0% in 2006 and 35.6% in 2007, Fig. 1). RIL values continuously ranged between or over the parental values (from 32.1% to 89.3% in 2005, from 9.4% to 94.1% in 2006 and from 9.3% to 86.4% in 2007). Continuous distributions were similarly observed for heading time (HT) and culm length (CL), indicating quantitative inheritance of all three traits. HT showed a transgressive inheritance and its distribution was skewed towards late-

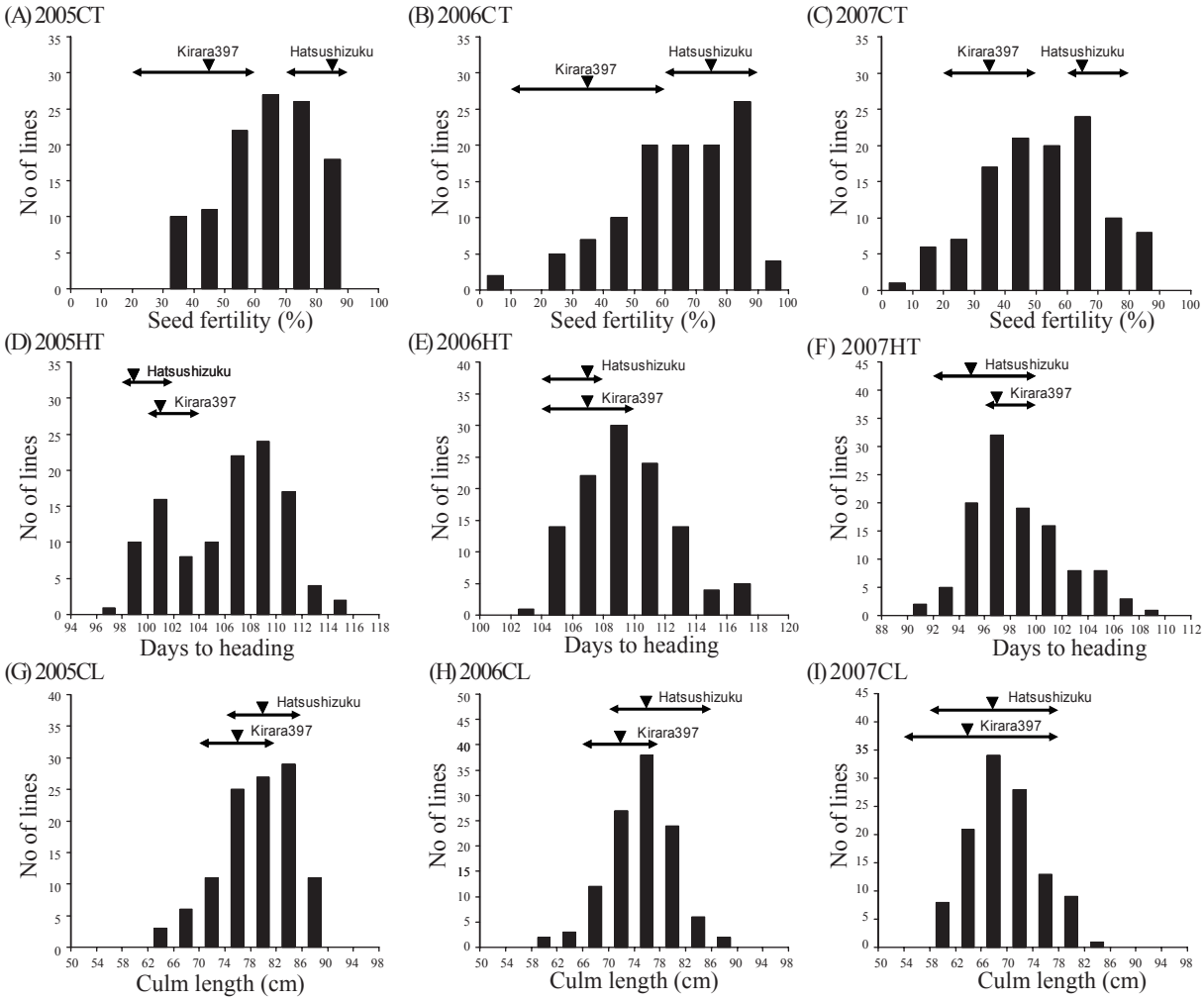

Fig. 1. Frequency distribution of cold tolerance at the booting stage (CT), heading time (HT) and culm length (CL) in RILs
CT was evaluated as seed fertility after cool-water treatment. The ranges and means of the parents are represented by arrows and solid triangles, respectively.

heading.

2. Polymorphism detection and linkage map construction

A total of 676 SSR markers[7,15] distributed throughout the genome were used to survey polymorphism between Kirara397 and Hatsushizuku, and 117 markers were polymorphic (Fig. 2). The percentage of polymorphism ranged from 12.2 to 35.7%, and its mean was 17.3% over 12 chromosomes. The polymorphism frequency was much lower than that of a *japonica* × *indica* cross[2] but was slightly higher than that of a *japonica* × *japonica* cross[6,14]. Heterozygosities at polymorphic markers ranged from 0 to 0.035, and their average is 0.006. Segregation distortions were observed for 7 markers RM1334, RM6832, RM3513, RM3601, and RM3525 on chromosome 3, RM1328 on chromosome 9 and RM5716 on chromosome 11. A linkage map consisting of 18 linkage groups and covering 1174.2 cM with a total number of 111 markers was constructed (Fig. 3). Few markers were observed on chromosomes 4 and 10, and there were several gaps even on the other chromosomes. The orders of the markers on the linkage map almost coincided with those estimated from the IRGSP genome sequence[5].

3. QTL analysis

The estimated experimental LOD threshold values for QTL detection ($p > 0.05$) were 4.2 in 2005, 4.4 in 2006 and 3.6 in 2007 for CT. A total of five QTLs for CT were detected in the three years, and three QTLs were mapped on the region flanked by RM1003–RM3482 on the long arm of chromosome 1 (Fig. 3). The directions of the phe-

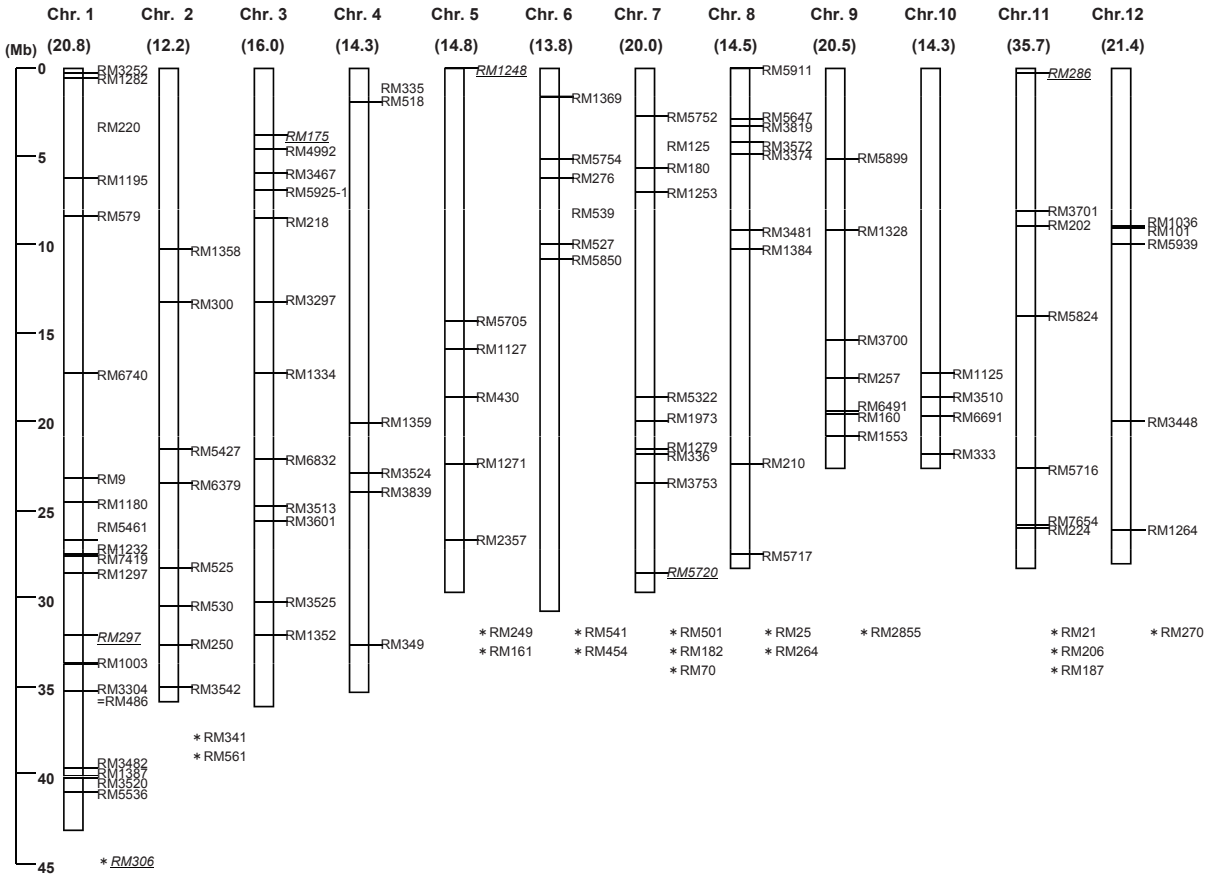

Fig. 2. Polymorphic markers between Kirara397 and Hatsushizuku on the IRGSP genome sequence[5]

Positions of the polymorphic SSR markers between Kirara397 and Hatsushizuku are shown on the basis of the IRGSP genome sequence. Markers with asterisks are not located on the IRGSP genome sequence. Underlined markers are not mapped to any linkage group in this study. The percentage of polymorphic markers on each chromosome is indicated in parenthesis.

notypic effects of the three QTLs for CT on chromosome 1 were all towards Hatsushizuku and accounted for 16.2–47.3% of the phenotypic variance (Table 1). On the other QTLs for CT that was detected on the long arm of chromosome 10 in 2005 and 2007, the Kirara397 allele increased the trait value and its PVE was 35.4% in 2005 and 20.4% in 2007.

The LOD thresholds for QTL detection ($p > 0.05$) were estimated to be 7.6 in 2005, 4.1 in 2006 and 6.3 in 2007 for HT, and 3.8 in 2005, 3.4 in 2006 and 3.7 in 2007 for CL. Four QTLs for HT were detected on chromosomes 2 and 10. Three QTLs for HT located on the region flanked by RM1125–RM333 on the long arm of chromosome 10 in the three years accounted for 32.7–36.7% of the phenotypic variance. Hatsushizuku provided alleles contributing lower values for HT, and their additive effects ranged from 2.1 to 4.4 days. The additional QTL for HT was detected on chromosome 2, and it accounted for 10.5% of the phenotypic variance. Three QTLs for CL were also detected on chromosomes 2 and 10.

Discussion

The QTL for CT was identified in the three-year trials on the interval between SSR markers RM1003 and RM3482 of chromosome 1, and its additive effect and PVE were in the range of 0.09–0.11 and 16.2–47.3%, respectively. We grouped RILs into the two classes (Kirara397 and Hatsushizuku groups) by genotype of RM3304 on the QTL region of chromosome 1. Significant differences in mean seed fertility were observed between the two groups for three years (12.6–16.5%), contributing to 34.4–45.2% of the differences between the parents. No QTL for HT or that for CL was detected on chromosome 1, and differences in the means of culm length between the Kirara397 and Hatsushizuku groups were small but significant (1.9–2.9 cm) whereas those of heading time

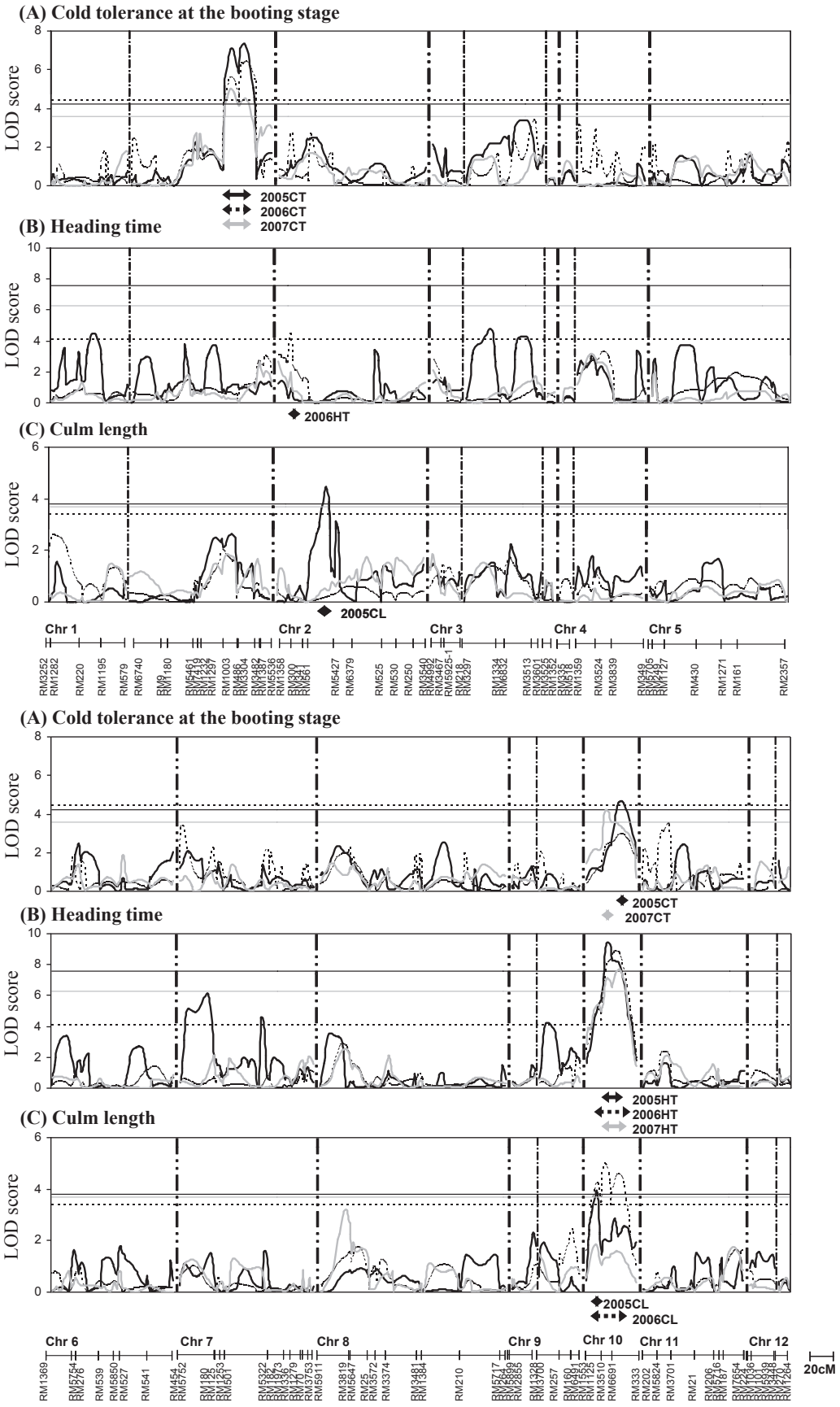

Fig. 3. Composite interval mapping for cold tolerance at the booting stage (A), heading time (B) and culm length (C)
The horizontal lines denote the threshold levels of the LOD scores. The two-headed arrows indicate the intervals recording LOD > thresholds. The linkage map constructed in this study is shown on the bottom. —— :2005, - - - - :2006, —— :2007.

Table 1. QTLs for cold tolerance, heading time and culm length

Year	Chr	Interval[1)]	LOD	Additive effect[2)]	PVE
Cold tolerance at the booting stage (CT)					
2005	1	RM1003-RM3482	7.4	0.09	0.252
2006	1	RM1003-RM3482	6.4	0.11	0.473
2007	1	RM1003-RM3482	5.0	0.09	0.162
2005	10	RM6691-RM333	4.7	−0.09	0.354
2007	10	RM3510-RM6691	4.2	−0.08	0.204
Heading time (HT)					
2006	2	RM300-RM341	4.5	−0.8	0.105
2005	10	RM3510-RM333	9.5	−2.7	0.336
2006	10	RM1125-RM333	8.9	−2.1	0.327
2007	10	RM3510-RM333	7.7	−4.4	0.367
Culm length (CL)					
2005	2	RM561-RM5427	4.5	1.1	0.446
2005	10	RM1125-RM3510	4.0	−1.8	0.390
2006	10	RM1125-RM333	5.0	−2.2	0.224

1): Markers flanking intervals in which LOD values were above the thresholds.
2): Additive effect of the Hatsushizuku allele.

between the groups were not significant (0.6–1.3 day) in all three trials. These results suggest that the QTL on chromosome 1 has a major effect on the variation of CT between Kirara397 and Hatsushizuku without affecting HT and CL. Takeuchi et al.[14] reported that *qCT-1*, a QTL for CT at the booting stage, was mapped to the interval between RFLP markers R494 and R858 on chromosome 1. The region of the QTL for CT on chromosome 1 was overlapped with *qCT-1* on the basis of the positions on the IRGSP sequence[5] of the flanking markers of the two QTLs. Fine mapping of the QTL for CT on chromosome 1 would be useful not only for clarifying the identity of the two QTLs but also for dissecting the function of the QTL.

We detected a QTL for heading time on chromosome 10 in every year in the three-year period (2005–2007). Their additive effects were all towards Hatsushizuku (2.0–4.4 days), and comparatively large PVE values were observed (24.4–36.7%). On the same region, QTL for culm length (in 2005 and 2006) and QTL for cold tolerance (in 2005 and 2007) were also detected. Furthermore, LOD peak was observed for CT in 2006 (LOD = 3.0), while they were below the threshold (LOD = 4.4). We observed significant differences not only in HT ($p < 0.001\%$) but also in CL and CT ($p = 0.1–1\%$) between the genetic classes of RILs that grouped by genotype of RM6691 on chromosome 10. The alleles derived from Hatsushizuku contributed to early heading, short culm length, and cold sensitivity, and their direction did not change during the three-year period. Narrowing down these QTLs on chromosome 10 by a survey and use of additional polymorphic markers will reveal the relationship among them, while in this study we could identify only four polymorphic markers on that chromosome.

The percentage of polymorphism between Kirara397 and Hatsushizuku was higher than that between other *japonicas*[6,14]. This may be partly because Hatsushizuku is derived from an *indica* line. Several large genomic regions without polymorphic markers between Kirara397 and Hatsushizuku were observed (Fig. 2), indicating that the linkage map constructed in this study do not cover the whole genome. Since the QTLs detected in this study did not fully explain the phenotypic variance, other QTLs might remain to be detected. Several LOD peaks were observed at other regions than the QTLs identified in this study, while they were below the estimated thresholds (Fig. 3). Other QTLs for CT, HT and CL should be found using a reconstructed linkage map with higher coverage than that used in this study.

Hatsushizuku is characterized by higher productivity and lower protein content in brown rice than Kirara397 besides its superior CT[3]. RILs established in this study were also available for the analyses of these useful traits. Hatsushizuku is thought to be suitable for use as a parent in breeding, because its agronomical traits are so excellent that it was recommended as the first cultivar for 'sake' (Japanese rice wine) brewing in Hokkaido. In this study, we confirmed the reproducibility of the QTL for

CT on chromosome 1 by three trials in 2005–2007. It is hoped that this QTL for CT will contribute to the breeding of cold-tolerant rice cultivars by its introgression from Hatsushizuku via marker-assisted selection.

Acknowledgment

The authors wish to thank Ms. Yaeko Nishizawa for her technical assistance.

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