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Detection of a novel quantitative trait locus for cold tolerance at the booting stage derived from a *tropical japonica* rice variety Silewah

Masahiko Mori[†1,2], Kazumitsu Onishi[*†1,2,3], Yoshiro Tokizono[3], Hiroshi Shinada[2], Toru Yoshimura[2], Yoshinori Numao[2], Hideho Miura[1] and Takashi Sato[2]

[1] *Obihiro University of Agriculture and Veterinary Medicine*, 2-11 Nishi, Inada, Obihiro, Hokkaido 080-8555, Japan
[2] *Rice Breeding Group, Kamikawa Agriculture Experimental Station, Local Independent Administrative Agency Hokkaido Research Organization*, 1-5 Minami sen, Pippu, Hokkaido 078-0397, Japan
[3] *Graduate School of Agriculture, Hokkaido University*, Kita 9 Nishi 9, Sapporo, Hokkaido 060-8589, Japan

Cold stress at the booting stage in rice induces spikelet sterility because of aberrant microspore development, which often seriously damages seed production. Some breeding lines with high cold tolerance were developed by using *tropical japonica* variety Silewah as a donor of cold tolerance; however, the genetic factors that confer cold tolerance of this variety have not been comprehensively analyzed. In this study, phenotypic and molecular characterization of novel cold-tolerant strains derived from crosses with Silewah was performed to identify quantitative trait loci (QTLs) responsible for cold tolerance. Molecular marker analysis revealed that 2 cold-tolerant strains carried chromosomal segments of Silewah at the same genomic regions on chromosomes 3, 4 and 11. Single marker analysis in segregating population confirmed that the allele of Silewah on chromosome 3 (*qCTB3-Silewah*) conferred cold tolerance. The effect of *qCTB3-Silewah* was supported by the fact that this allele had been a target of selection during developing a breeding line by phenotypic selection from backcrossed progenies with an elite variety as a recurrent parent. *qCTB3* is a different QTL from those reported for Silewah previously, suggesting that different QTLs might be exploited in different breeding programs depending on the genetic backgrounds and environmental conditions.

Key Words: *Oryza sativa*, cold tolerance, booting stage, QTL, phenotypic selection, marker-assisted selection, exotic germplasm.

Introduction

Low environmental temperature is one of the limiting factors in the agricultural productivity of Asian rice (*Oryza sativa* L.) (Mackill and Lei 1997, Vergara 1976). At the booting stage, low temperatures induce spikelet sterility because of aberrant microspore development, which often results in seriously damaged rice production (Satake 1976). Therefore, breeding efforts look to improve the cold tolerances of rice varieties, especially in the northern areas. Tolerance to cold at the booting stage is one of the most genetically complex traits in rice. To date, about 40 quantitative trait loci (QTLs) located on all chromosomes have been reported in various cross combinations (Andaya and Mackill 2003, Dai *et al.* 2004, Kuroki *et al.* 2007, 2009, Li *et al.* 1997, Oh *et al.* 2004, Saito *et al.* 1995, Suh *et al.* 2010, Takeuchi *et al.* 2001, Xu *et al.* 2008). Detection of QTLs with large effects has made it possible to transfer the target QTL (or gene) into elite varieties by marker-assisted selection (MAS); however, breeding for cold tolerance has been mainly by artificial selection with conventional breeding methods because of its complex pattern of inheritance. Using these techniques, plant breeders have successfully made many beneficial changes in cold tolerance at the booting stage of rice.

On the Hokkaido island of Japan (41°N–45°N)—one of the most northern areas for rice production—genetic improvements in rice varieties and modernization of the cultivation systems has enabled rice production during the last century. The cold tolerance of Hokkaido varieties has been progressively increased by intensive breeding efforts; however, cold damage still periodically occurs. Therefore, further development of varieties with improved cold tolerance is needed for stable rice production. In Hokkaido, breeding programs that use exotic germplasms as sources of cold-tolerance genes has been conducted over the last two decades. The Indonesian *tropical japonica* variety Silewah is one of the varieties having highest cold tolerances at the booting stage; this strain was selected from more than 17000 varieties or strains in the International Rice Research Institute in the Philippines (International Rice Research Institute 1978, Satake and Toriyama 1979). Abe *et al.* (1989) developed a cold-tolerant strain, Norin-PL8, from Silewah by

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*Corresponding author (e-mail: onishi@obihiro.ac.jp)
† These authors contributed equally to this work

backcrossing it with a strain in Hokkaido as the recurrent parent. Recently, other cold-tolerant strains (Joukei-04501 and Joukei-04502), derived from crosses between Silewah and strains (varieties) in Hokkaido, were developed by conventional methods of phenotypic selection (Yoshimura *et al.* 2006); however, the QTLs responsible for cold tolerance in these cold-tolerant strains were remained to be studied.

In this study, we characterized the cold tolerance of 4 strains (Joukei-04501, Joukei-04502, Joukei-06214, and Joukei-06215) derived from crosses with Silewah. We used molecular markers to determine whether the same QTLs as those detected by Saito *et al.* (1995) were responsible for cold tolerance. The present study identified a novel QTL derived from Silewah, which was different from the QTLs detected by Saito *et al.* (1995), suggesting that different QTLs might be exploited in different breeding programs depending on the genetic backgrounds and environmental conditions.

Materials and Methods

Strains used in this study

Cold-tolerant strains Joukei-04501 (J501) (Silewah/Kita-ake//Yukihikari), Joukei-04502 (J502) (Silewah/2*Kita-ake//Dohoku 50), Joukei-06214 (J214) and Joukei-06215 (J215) (Joukei-04501/3*Hoshinoyume) were developed by the breeding programs at Hokkaido Kamikawa Agricultural Experimental Station in Pippu, Hokkaido, Japan (43°N) (Yoshimura *et al.* 2006). In the development of strain J501, the selection of cold tolerance was initiated in 1988 using about 2200 plants of the F_2 generation; these plants were randomly produced from 410 F_1 plants by self-pollination. In the development of strain J502, the selection of cold tolerance was initiated in 1991 using about 4000 plants from the F_4 generation, which were randomly produced from 110 F_1 plants by self-pollination. Evaluation and selection of cold tolerance were repeatedly conducted in every generation by the cool water irrigation method as described below, and strains with cold tolerance were selected as J501 from the F_{17} generation and as J502 from the F_{16} generation. Furthermore, J214 and J215 were developed by backcross breeding using Hoshinoyume, which is one of the leading varieties in Hokkaido, as a recurrent parent. The selection of cold tolerance and some agronomic traits (yield and yield components, eating quality, days to heading, and so on) was conducted from the BC_2F_4 to BC_2F_6 generations, and 2 strains with cold tolerance as well as superior agronomic traits were selected as J214 and J215 from the BC_2F_6 generation in 2006. The cold-tolerant strains (J501, J502, J214 and J215) and their parental strains (Kita-ake, Yukihikari, Dohoku50 and Hoshinoyume) were used in the comparison of cold tolerance at the booting stage. Silewah was not evaluated because of its extremely late heading in Hokkaido, Japan.

Evaluation of cold tolerance at the booting stage

Cold tolerance was evaluated by the cool water irrigation method (Futsuhara and Toriyama 1964) in a paddy field of the Kamikawa Agricultural Experimental Station. The field was irrigated with flowing cool water at about 19.0°C from the panicle initiation stage to the completion of heading (late-June to mid-August) to cause cold injury during the booting stage. The water was kept at about 25 cm depth. Heading date was recorded for each plant. At maturity, 3 to 6 panicles per plant were harvested and were measured for seed fertility, which was the percentage of spikelets setting seed in a panicle. Cold tolerance was evaluated on the basis of mean seed fertility.

In 2007, 2 cold-tolerant strains (J501 and J502), their parental strains (Kita-ake, Yukihikari and Dohoku50) and Hoshinoyume were compared for cold tolerance at the booting stage. Four cold-tolerant strains (J501, J502, J214 and J215) and Hoshinoyume were evaluated in 2008. In total, 6 plants per plot with 2 replicates were used in 2007 and 4 plants per plot with 4 replicates were used in 2008.

Examination of segregating population

To examine the inheritance of cold tolerance and detect responsible QTLs, two segregating populations developed from crosses of J501 × Daichinohoshi and J502 × Hoshimaru were evaluated for cold tolerance at the booting stage by the same cool water irrigation method as described above. For the cross of J501 × Daichinohoshi, bulked seeds from more than 500 plants in F_2 and F_3 populations were used to produce the next generation. In 2004, 204 F_4 plants were raised from bulked F_3 seeds and 3 F_5 plants from each F_4 plant were raised in 2005. Four to 15 plants were raised for parental strains in both years. For the cross of J502 × Hoshimaru, 150 F_2 plants were raised in 2006. In 2007, 12 F_3 plants were raised from each F_2 plant and were planted in 2 replicates (6 plants per plot). For parental strains, 4 to 6 plants were raised in 2006 and 6 plants per plot with 8 replicates were raised in 2007.

Molecular marker analysis

Total DNA was extracted from fresh leaves according to the method of Monna *et al.* (2002) with slight modifications. For the analysis of the segregating population from the cross of J502 × Hoshimaru, pooled DNA was collected from more than 7 F_3 plants for each F_3 line. To examine the genomic region of introgressed segments derived from Silewah, polymorphic markers were selected on the basis of reported simple sequence repeat (SSR) markers (http://www.gramene.org). The SSR markers were amplified by polymerase chain reaction (PCR) in a 20.0 μL reaction mixture containing 25 ng total DNA, 0.4 mM of each dNTP, 0.5 μM of each forward and reverse primer, 2 units of HOT Goldstar™ DNA polymerase and 1 × PCR buffer (EUROGENTEC Bel S.A.), and 1.5 mM $MgCl_2$. The samples were prepared in a 96-well plate for amplification using a thermal cycler (iCycler, Bio-Rad Laboratories, Inc., Germany). The PCR conditions were as follows: a 1-min hold at 95°C; 30 cycles of 30 sec at 95°C, 30 sec at the annealing temperature and 40 sec at 72°C; and a final extension step at 72°C

for 7 min. The PCR products were separated by electrophoreses in 10 or 12% acrylamide gels (acrylamide : *N,N'*-methylenebisacrylamide = 29 : 1) and stained with ethidium bromide.

Data analysis

The heritability of cold tolerance at the booting stage was estimated based on parent-offspring regression using data of seed fertility in J501 × Daichinohoshi and J502 × Hoshimaru populations. The estimate of heritability was obtained from the linear regression coefficient (*b*) calculated by regressing means of seed fertility in F_5 lines on that of F_4 plants in J501 × Daichinohoshi population whereas *b* was calculated by regressing means of seed fertility in F_3 lines on that of F_2 plants in J502 × Hoshimaru population. Data were transformed with arcsin transformation before calculation. All statistical analysis were conducted using STATVIEW (SAS Institute, Cary NC).

Results

Phenotypic and molecular characterization of J501 and J502 derived from crosses with Silewah

Two cold-tolerant strains (J501 and J502) derived from crosses with Silewah were compared with their parental strains (Kita-ake, Yukihikari and Dohoku50) for cold tolerance at the booting stage (Table 1). J501 and J502 showed significantly higher seed fertility than did their parental strains, and there was no significant difference in fertility between J501 and J502. J501 and J502 showed higher seed fertility for 2 years than did Hoshinoyume, which is currently one of the leading varieties in Hokkaido. Although Silewah showed extremely late heading under field conditions in Hokkaido (data not shown), the heading time of J501 and J502 were not significantly different from that of Hoshinoyume (Table 1).

Because the 2 cold-tolerant strains (J501 and J502) showed significantly higher cold tolerance than their parental strains (Yukihikari, Kita-ake and Dohoku50) (Table 1), we expected that their cold tolerance should be caused by the genes derived from Silewah. To survey for QTLs responsible for cold tolerance, we investigated the genomic regions introgressed from Silewah using molecular markers (Fig. 1). In total, we used 234 markers for J501 and 122 markers for J502 that could distinguish Silewah alleles from the alleles of the other parental strains; these markers were used for genotyping. J501 carried segments of Silewah on chromosomes 1, 2, 3, 4, 5, 10 and 11, whereas J502 carried segments on chromosomes 3, 4, 7, 8 and 11. Two strains had chromosomal segments of Silewah at the same genomic regions on chromosomes 3, 4 and 11. Previously, Saito *et al.* (1995, 2004) identified QTLs for cold tolerance on the distal end of the short arm of chromosomes 3 and the long arm of chromosome 4 from Norin PL-8, which was a cold-tolerant strain derived from crosses with Silewah (Fig. 1). The genomic regions introgressed from Silewah into J501 and J502 were different from the QTL regions of Norin PL-8 (Fig. 1).

Table 1. Comparison of seed fertility and days to heading (mean ± SD) among 4 cold tolerant strains and their parental strains evaluated by cool water irrigation method in two years

Strain or variety	2007		2008	
	Seed fertility (%)	Days to heading	Seed fertility (%)	Days to heading
J501	$70.3^{a}\pm5.2$	$86.3^{b}\pm2.6$	$68.6^{a}\pm10.4$	$92.8^{c}\pm1.0$
J502	$63.8^{a}\pm8.0$	$87.3^{b}\pm2.0$	$72.1^{a}\pm8.0$	$94.4^{bc}\pm1.2$
J214	N.D.	N.D.	$57.4^{b}\pm8.5$	$95.1^{ab}\pm1.5$
J215	N.D.	N.D.	$36.5^{c}\pm11.6$	$97.1^{a}\pm3.0$
Kita-ake	$12.0^{c}\pm6.5$	$83.5^{c}\pm1.7$	N.D.	N.D.
Yukihikari	$3.1^{d}\pm2.6$	$93.6^{a}\pm2.6$	N.D.	N.D.
Dohoku50	$25.7^{b}\pm13.0$	$87.1^{b}\pm2.9$	N.D.	N.D.
Hoshinoyume	$26.5^{b}\pm12.8$	$86.4^{b}\pm2.0$	$23.7^{d}\pm8.7$	$93.5^{bc}\pm2.0$

Different letters followed by mean values indicate significant differences between strains ($P < 0.05$, protected Fisher's least significant difference test). N.D., not determined.

Inheritance of cold tolerance and detection of QTLs

Regression coefficients (*b*) as estimates of heritability were 0.728 in J501 × Daichinohoshi population (Fig. 2A) and 0.601 in J502 × Hoshimaru population (Fig. 2B). This result indicated that cold tolerance of J501 and J502 was highly heritable traits under different genetic backgrounds.

To detect the QTLs responsible for cold tolerance that were derived from Silewah, we focused J502 because backcrossing with varieties (or strains) in Hokkaido was more advanced in J502 and the chromosomal segments introgressed from Silewah was smaller in J502 than in J501. Single marker analysis was performed in F_3 population from J502 × Hoshimaru. The frequency distribution of seed fertility showed continuous distribution that ranged between the values of the 2 parental strains (Fig. 3). The genotypes were determined using 144 lines at 8 marker loci at which Silewah segments were introgressed in J502. The genotypes at each marker locus were classified into 2 classes: homozygotes of Silewah or Hoshimaru alleles. Seed fertility and days to heading were compared between the 2 groups (Table 2). For seed fertility, only 2 marker loci (RM3180 and RM6974) on chromosome 3 showed significant differences. At locus RM5824 on chromosome 11, the Silewah allele slightly increased seed fertility although the difference between the genotypes was marginally significant ($P = 0.088$). For days to heading, only chromosome 8 had a large effect, showing that the Silewah allele caused late heading. There were no regions in which the genetic effects were significant for both seed fertility and days to heading.

To examine the genetic effects of putative QTLs region more closely, the genotypes of 144 lines were classified into 4 homozygous groups depending on the genotype of chromosome 3 (RM6974) and chromosome 11 (RM5824) (Fig. 4). Results confirmed that the effect of chromosome 3 was highly significant. There was no significant effect on

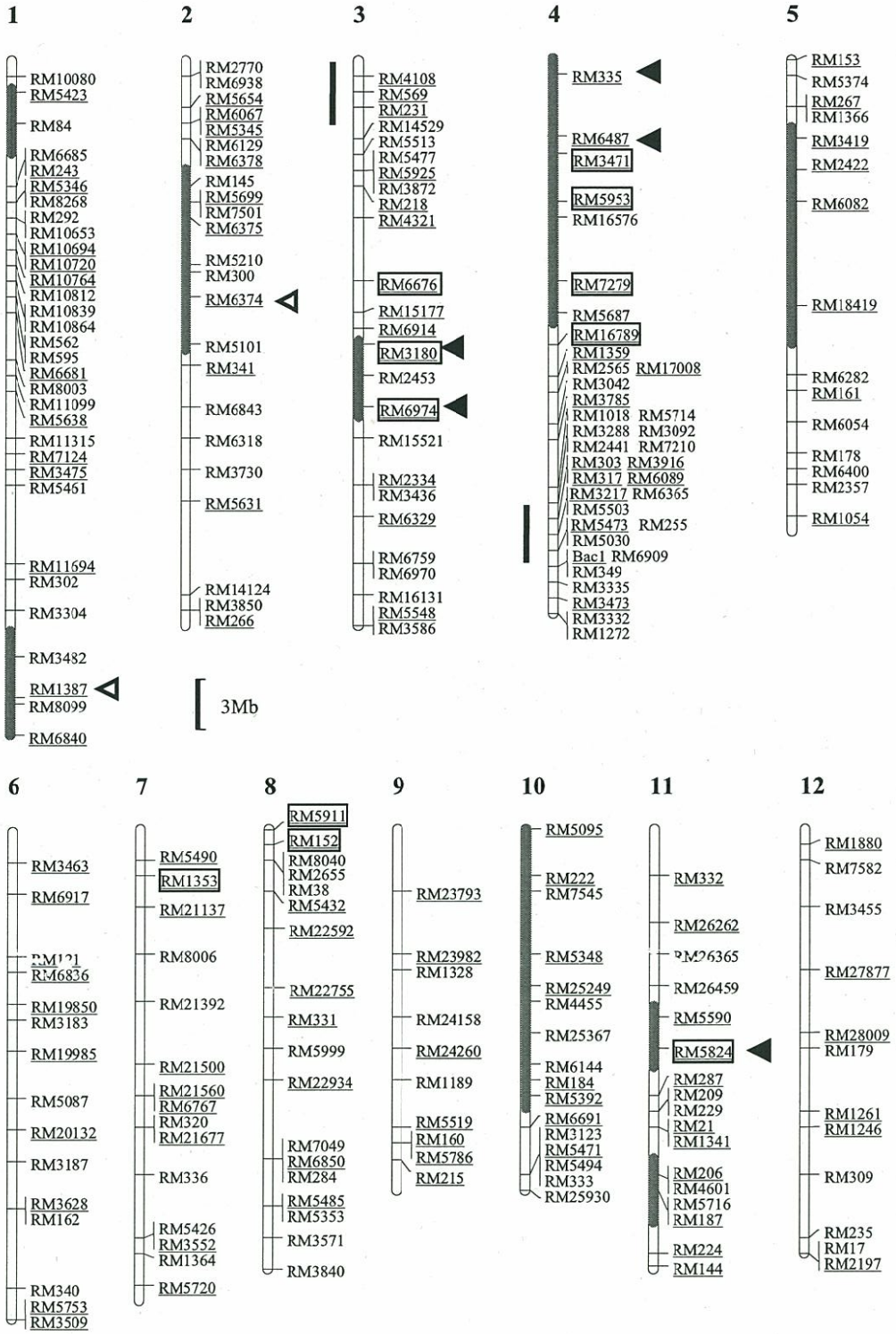

Fig. 1. Graphical representation of the genotypes of 4 cold-tolerant strains. For J501, 234 markers could be used to determine the chromosomal region derived from Silewah. Filled regions on each chromosome indicate the estimated segments derived from Silewah in J501. For J502, J214 and J215, 122 markers with underlines were used for examination. Markers in boxes indicate the chromosomal regions derived from Silewah in J502. Filled and open arrow heads indicate chromosomal regions derived from Silewah in J214 and J215, respectively. The bars on the left of chromosomes 3 and 4 indicate QTL regions for cold tolerance at the booting stage detected from Norin-PL8, which was the cold-tolerant strain derived from crosses with Silewah (Saito *et al.* 1995, 2004, 2010). The chromosomal location of the markers was based on the genomic sequence of a cultivar Nipponbare (http://rgp.dna.affrc.go.jp).

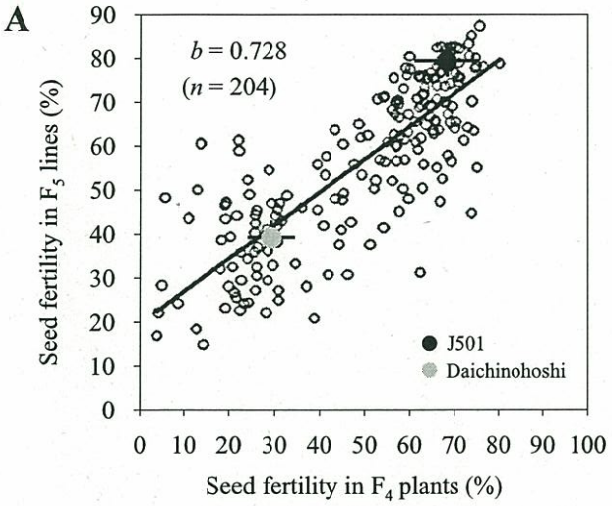

Fig. 2. Regression for seed fertility of F_5 lines on F_4 plants in the cross of J501 × Daichinohoshi (A) and that of F_3 lines on F_2 plants in the cross of J502 × Hoshimaru (B). Closed circles with error bars represent means ± standard deviations of the 2 parental strains. Regression coefficients (b) were calculated from the data after arcsin transformation.

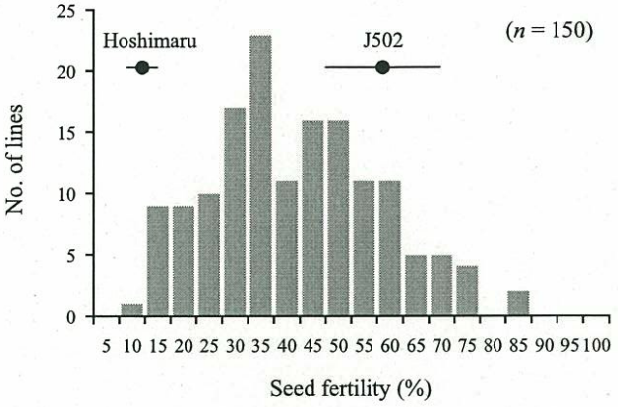

Fig. 3. Frequency distribution of seed fertility in F_3 lines of J502 × Hoshimaru. Closed circles with error bars represent means ± standard deviations of the 2 parental strains.

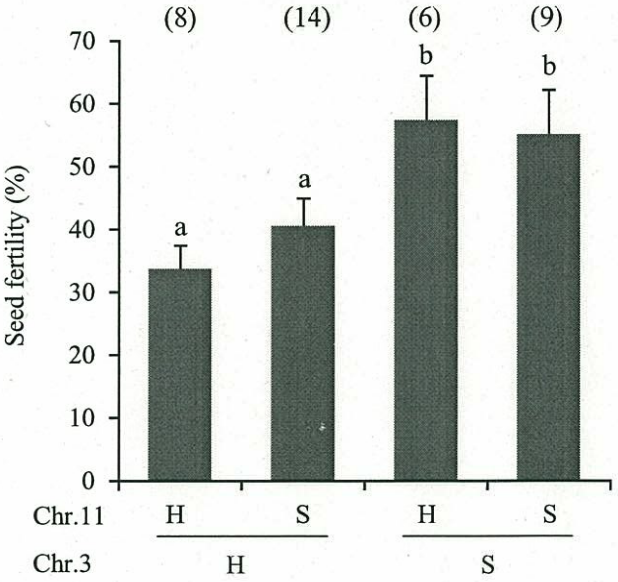

Fig. 4. Effects of putative quantitative trait loci (QTL) on chromosomes 3 and 11. Alleles of each QTLs were estimated by use of the genotypes of RM6974 on chromosome 3 and RM5824 on chromosome 11. S and H represent homozygotes of Silewah- and Hoshimaru-derived alleles, respectively. Each histogram bar represents the mean seed fertility (%) for each genotype, and the error bars represent standard deviation. Bars with different letters above them indicate significant differences between genotypes (protected Fisher's least significant difference test, $P < 0.05$). The number of lines for each genotype is shown in parentheses.

chromosome 11 whereas Silewah allele slightly increased seed fertility in the homozygote of Hoshimaru allele at RM6974 on chromosome 3. The F_3 lines with the Silewah allele on chromosome 3 had more than 20% greater seed fertility than the lines with the Hoshimaru alleles on both chromosomes 3 and 11, indicating that the QTL responsible for cold tolerance is located on the segment introgressed from Silewah on chromosome 3. The allele conferring cold tolerance derived from Silewah was tentatively designated *qCTB3-Silewah*.

Selection of qCTB3-Silewah during breeding program

Although J501 and J502 had high cold tolerance, these strains still had inferior agronomic traits, such as lower yield and eating qualities. To introgress cold tolerance into the elite variety, J501 was backcrossed (BC_2) with Hoshinoyume as a recurrent parent and two strains (J214 and J215) with high cold tolerance was developed by phenotypic selection. The seed fertility of J214 and J215 was significantly higher than that of Hoshinoyume. Strains J214 and J215 showed similar levels of eating quality and yield as a recurrent parent Hoshinoyume (data not shown), whereas the fertility of J215 was about 20% lower than that of J214 (Table 1).

To examine whether *qCTB3-Silewah* was a target of

Table2. Single marker analysis for seed fertility and days to heading evaluated by cool water irrigation method in F_3 populations from J502 × Hoshimaru

Chromosome	Marker	Seed fertility (%)[a]		*t* test[b] (*P* value)	Days to heading[a]		*t* test[b] (*P* value)
		Silewah type	Hoshimaru type		Silewah type	Hoshimaru type	
3	RM6676	46.2 ± 18.5 (38)	40.6 ± 13.7 (33)	ns (0.159)	86.3 ± 4.2 (38)	84.7 ± 2.8 (33)	ns (0.070)
	RM3180	48.8 ± 17.1 (34)	40.9 ± 13.6 (41)	* (0.028)	86.1 ± 4.2 (34)	84.9 ± 3.3 (41)	ns (0.140)
	RM6974	49.0 ± 17.1 (32)	40.3 ± 13.4 (46)	* (0.014)	86.0 ± 4.2 (32)	85.1 ± 2.7 (46)	ns (0.241)
4	RM5953	40.1 ± 17.6 (25)	39.1 ± 17.2 (28)	ns (0.834)	85.6 ± 4.1 (25)	86.2 ± 3.2 (28)	ns (0.521)
	RM7279	40.1 ± 17.5 (25)	38.8 ± 16.7 (25)	ns (0.790)	85.5 ± 4.1 (25)	86.3 ± 2.6 (25)	ns (0.400)
7	RM1353	41.0 ± 18.1 (28)	35.9 ± 18.2 (21)	ns (0.333)	85.1 ± 3.0 (28)	85.5 ± 4.3 (21)	ns (0.715)
8	RM152	44.1 ± 14.7 (35)	39.1 ± 16.2 (42)	ns (0.159)	86.8 ± 3.1 (35)	83.5 ± 3.9 (42)	*** (< 0.001)
11	RM5824	43.0 ± 17.8 (41)	35.8 ± 17.2 (31)	ns (0.088)	85.3 ± 3.9 (41)	85.0 ± 2.7 (31)	ns (0.763)

[a] Mean ± SD. Silewah and Hoshimaru types indicate homozygotes of Silewah and Hoshimaru derived alleles, respectively. Homozygotes were selected from 144 F_3 lines except for RM 5953, RM7279 and RM1353 which were selected from 96 lines. Numbers of selected lines are shown in parentheses.

[b] * and *** indicate significant differences between genotypes at the 5% and 0.1% levels, respectively. ns indicates non-significance. *P* values are shown in parentheses.

artificial selection during breeding of J214 and J215, the chromosomal segments of Silewah introgressed from J501 were determined by using molecular markers. These 2 strains had Silewah segments on different chromosomal regions: J214 had segments on chromosomes 3, 4 and 11, whereas J215 had segments on chromosomes 1 and 2 (Fig. 1). J214 had *qCTB3-Silewah* and showed higher cold tolerance than J215, indicating *qCTB3-Silewah* might be target of selection during the breeding for J214.

Discussion

As described above, Saito *et al.* (1995) identified QTLs that confer cold tolerance at the booting stage on chromosomes 3 and 4 from the cold-tolerant strain Norin-PL8, which was derived from crosses with Silewah. Furthermore, the fine mapping of QTLs on chromosome 4 revealed that the QTL comprised 2 closely linked genes (*Ctb1* and *Ctb2*, Saito *et al.* 2001). Recently, *Ctb1* was isolated as the F-box protein gene by map-based cloning (Saito *et al.* 2010), which will facilitate the practical breeding for cold tolerance at the booting stage by MAS. However, our results indicate that *Ctb1* and *Ctb2* had not been targets of selection during breeding of J501 and J502. Instead, we detected a QTL for cold tolerance derived from Silewah (*qCTB3-Silewah*) on chromosome 3 near the centromeric region. *qCTB3-Silewah* was also different from the QTL on chromosome 3 detected by Saito *et al.* (1995), which was located at the distal end of the short arm of chromosome 3 (Fig. 1), indicating that *qCTB3-Silewah* was a novel QTL derived from Silewah. Although Andaya and Mackill (2003) detected a QTL near the centromeric region of chromosome 3 (*qCTB3*) in the population derived from a cross between ssp. *japonica* and ssp. *indica*, *qCTB3-Silewah* was the allele derived from a ssp. *tropical japonica* strain.

Detection of different QTLs in this study and Saito *et al.* (1995) indicate that Silewah has multiple QTLs that confer cold tolerance and different QTLs had been exploited in different breeding programs. For complex traits, it is universally accepted that the environmental factors influences the QTL effects (genotype-by-environment interaction; Lynch and Walsh 1998). In addition, the QTL effects are sensitive to the genetic background or to other genes, indicating the importance of epistasis or gene interaction (Ungerer *et al.* 2003, Ungerer and Riseberg 2003). The number of QTLs responsible for cold tolerance at the booting stage reported in rice might indicate that the allelic effects of QTLs differ depending on the genetic background and environmental conditions. Indeed, the cold tolerant strains used in this study and Norin-PL8 have different genetic backgrounds and these strains were selected under different environmental conditions. This might result in the selection of different QTLs for developing cold tolerant strains in different breeding programs, providing caution for the use of genetic resources in breeding of rice.

In breeding for complex traits, MAS is useful for the selection of major QTLs with large effects; however, this strategy is much less powerful if a trait is controlled by many QTLs (genes) with small effects that are likely to be influenced by environmental and genetic conditions (Laurie *et al.*

2004, Yamamoto *et al.* 2009). The potential of exotic germplasms, such as the landrace of Indonesia, Silewah, to serve as sources of genetic variation for crop improvement has been recognized; however, there may be great difficulty in the use of exotic germplasms because they often carry many agriculturally undesirable genes in addition to the beneficial genes. Thus, simultaneous selection of various genes or traits would be needed for breeding. Furthermore, exotic germplasms often exhibit differential maturity or growth duration, which might prevent the evaluation of agronomic traits such as yield and regional adaptability, including cold tolerance. In contrast to MAS, conventional breeding methods tests the genes of exotic germplasms for their agronomic value in various genetic backgrounds and conducted phenotypic selection for several years, although it requires laborious process and longer periods of time. In fact, plant breeders have successfully developed a number of varieties with superior traits by conventional methods.

In the present study, Silewah had agriculturally undesirable phenotypes compared with modern rice varieties, and it could not adapt to the environmental conditions in Hokkaido, Japan. The segregating populations from crosses with Silewah had broad genetic variation for agriculturally important traits, such as heading time, eating quality, and yield, in addition to cold tolerance, which is likely controlled by many genes. Strains J501 and J502 have been developed by intensive selection during the last two decades, which has made it possible to eliminate the undesirable genes and to test agronomic values of genes for cold tolerance of Silewah under the environmental conditions in Hokkaido; however, these strains still had inferior agronomic traits. Therefore, J214 was developed from J501 by backcross breeding, resulting in the development of a strain with higher cold tolerance and superior phenotypes for agriculturally important traits such as eating quality and yield.

qCTB3-Silewah has been selected by phenotypic selection and introduced into J214 during breeding programs. This indicates that *qCTB3-Silewah* may be useful for practical breeding; however, this QTL did not fully explain the genetic variation observed between the cold-tolerant strains (J501, J502 and J214) and their parental strains. Interestingly, the chromosomal segment of Silewah near the centromeric region of chromosome 11 was co-introgressed with *qCTB3-Silewah* into three cold tolerant strains (J501, J502 and J214). Although in this study a significant QTL for cold tolerance was not detected at this region of chromosome 11, QTLs for cold tolerance were reported on similar genomic regions not only at the reproductive stage (Oh *et al.* 2004) but also at the vegetative stage (Oh *et al.* 2004, Zhang *et al.* 2005). The chromosomal segment of Silewah on chromosome 11 might have minor effect for cold tolerance at the booting stage and might be selected during breeding of cold tolerant strains. This fact may imply that simultaneous selection of cooperatively interacting genes or gene complexes, which might involve multiple genes with minor effects, is necessary for developing cold-tolerant varieties. The present results suggest that combined strategies including both MAS and conventional methods for phenotypic selection should be used to accelerate the breeding of complex traits including cold tolerance at the booting stage in rice.

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