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REVIEW

Analysis of Quantitative Trait Loci for Cold Tolerance at the Booting Stage of Rice

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Abstract

We analyzed the quantitative trait loci (QTLs) for cold tolerance at the booting stage of a cold-tolerant rice variety, Norin-PL8, which harbors cold tolerance genes derived from a cold-tolerant variety, Silewah. We found that 5 segments on chromosomes 1 (2 segments), 3, 4 and 8 were incorporated from Silewah into Norin-PL8. The association between the incorporated segments and cold tolerance was examined using BC_1F_5 lines between Norin-PL8 and Kirara397, which is a cold-sensitive commercial variety. The segment on the short arm of chromosome 3 and that on the long arm of chromosome 4 were associated with cold tolerance, indicating the presence of QTLs for cold tolerance on chromosomes 3 and 4. Interval mapping on the long arm of chromosome 4 suggested that 2 QTLs were located in the centromeric half of the incorporated segment. In order to confirm the results of interval mapping, we developed a set of near-isogenic lines (NILs). Comparison of the levels of cold tolerance of the NILs indicated that either the proximal end or the center of the segment is necessary for cold tolerance. Based on these results, it was concluded that there are at least 3 QTLs for cold tolerance on chromosomes 3 and 4.

Discipline: Biotechnology / Plant breeding
Additional key words: QTL, interval mapping, RFLP, graphical genotype

Introduction

Rice is a cold-sensitive plant that originated in tropical or subtropical areas. Spikelet fertility of rice decreases if rice plants are exposed to low temperature at the booting stage, due to the failure of microspore development under low temperature conditions[6]. Sterile type of cold injury is a very serious problem not only at high latitudes but also in uplands at low latitudes, e.g. Yunnan Province in China, because it inevitably leads to yield reduction. Cold tolerance at the booting stage is controlled by quantitative trait loci (QTLs). Many DNA markers have recently been developed, and they have enabled to analyze QTLs. For example, 2 QTLs for cold tolerance of a japonica variety, Koshihikari, were mapped on chromosomes 7 and 11 using molecular markers[8].

From 1974 to 1977, IRRI selected several cold-tolerant varieties from 17,689 accessions in its germplasm bank. Satake and Toriyama tested their cold tolerance at the booting stage and showed that 2 varieties, Leng Kwang and Silewah, were cold-tolerant[7]. Silewah, which is a tropical japonica variety from Indonesia, was backcrossed to a breeding line, Hokkai241, and a cold-tolerant variety, Norin-PL8, was developed[1]. The number of cold tolerance genes of Norin-PL8 was estimated to be 2 based on statistical analysis in the F_2 population between Norin-PL8 and a cold-sensitive variety, Norin20[1]. How-

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ever, the chromosomal locations of these genes had not been determined. In this study, we analyzed the QTLs for cold tolerance of Norin-PL8 using DNA markers.

Materials and methods

1. Plant materials

Norin-PL8 is a cold-tolerant variety developed from a BC_3F_6 line between Silewah and Hokkai241, which were used as a donor and a recurrent parent, respectively[1]. Hokkai241 is a cold-sensitive breeding line. Kirara397, which is a cold-sensitive commercial variety grown in Hokkaido, was crossed to Norin-PL8 for the genetic analysis of cold tolerance.

2. Evaluation of cold tolerance

We evaluated cold tolerance by the cool-water irrigation method. Plants were treated with cool water controlled at 19ºC from the primordial stage to the completion of heading. The depths of water were about 20 cm and about 24 cm in the paddy field and in the greenhouse, respectively. After ripening of the seeds, cold tolerance was evaluated based on the mean seed fertility.

3. Molecular analysis

Two microgram of total DNA isolated from leaves was digested with restriction endonucleases, electrophoresed on 0.8% agarose gels, and transferred to nylon membranes. Restriction fragment length polymorphism (RFLP) markers with the prefix 'XNpb' were developed by Saito et al[5]. RFLP markers with the prefix 'R' or 'C' were obtained from the Rice Genome Research Program, Japan (RGP)[2,4]. RFLP probes were non-radioactively labeled and hybridized using an ECL system (Amersham Biosciences), according to the manufacturer's instructions. Sequence-characterized amplified region (SCAR) markers, SCAB11 and SCAM20, were amplified in a mixture containing 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, 0.001% gelatin, 0.1 mmol/L of each deoxyribonucleotide, 0.2 µmol/L of each primer, 1 ng/µL template DNA and 0.02 unit/µL AmpliTaq gold DNA polymerase (Applied Biosystems). The thermal cycles used were as follows: 1 cycle at 94ºC for 4 min, followed by 45 cycles at 94ºC for 1 min, 55ºC for 1 min and 72ºC for 2 min, and finally 1 cycle at 72ºC for 7 min.

4. Mapping and QTL analysis

We used the computer program MAPL97[3,9] for the calculation of genetic distances and interval mapping of the QTLs. The computer program StatView 5.0 (SAS Institute) was used for the analysis of variance between marker classes.

Results

1. Graphical genotype of Norin-PL8

We compared the RFLP patterns of Silewah, Hokkai241 and Norin-PL8. Out of the 240 RFLP markers we used, 102 markers gave polymorphic bands. All of the polymorphic markers could be divided into 2 groups, those monomorphic between Silewah and Norin-PL8 (S-type) and those monomorphic between Hokkai241 and Norin-PL8 (H-type). Chromosomal segments of Norin-PL8 around the S- and H-types of markers were derived from Silewah and Hokkai241, respectively. The S-type polymorphism was detected by the use of 12 markers (XNpb346 and XNpb368 on chromosome 1; XNpb100, XNpb279 and XNpb345 on chromosome 3; XNpb102, XNpb177, XNpb235, XNpb264 and XNpb267 on chromosome 4; XNpb379 on chromosome 7 and XNpb278 on chromosome 8). Although XNpb379 had been mapped on chromosome 7[5], the polymorphism observed in this study was linked to the marker on chromosome 4 (data not shown). XNpb379 gave 2 bands in Southern hybridization. It is likely that XNpb379 hybridizes with the DNA sequences on chromosomes 4 and 7. On the basis of these results, we represented the genotype of Norin-PL8 in a graphical form (Fig. 1). Five segments on chromosomes 1, 3, 4 and 8 were found to be incorporated from Silewah to Norin-PL8.

2. Association between the incorporated segments and cold tolerance

In order to examine the association between the incorporated segments and cold tolerance, 92 BC_1F_5 lines of the cross Kirara397/Norin-PL8//Kirara397 were tested for cold tolerance and RFLP genotypes. Cold tolerance of the BC_1F_5 lines and their parental varieties was evaluated based on the seed fertility of rice plants irrigated with cool water in the paddy field. The seed fertilities of Norin-PL8 and Kirara397 were 82.4 and 26.8%, respectively. The seed fertilities of the BC_1F_5 lines were distributed between those of Norin-PL8 and Kirara397. Three markers on chromosomes 1 and 3, out of the 11 markers on the incorporated segments, gave monomorphic bands between Norin-PL8 and Kirara397. The other 8 markers, which gave polymorphic bands, were used for genotyping of the BC_1F_5 lines. Table 1 shows the association between the RFLP markers and QTLs for cold tolerance. For the markers on chromosome 3, the mean seed fertility of the Norin-PL8 type was significantly higher than that of the Kirara397 type. Similar results were

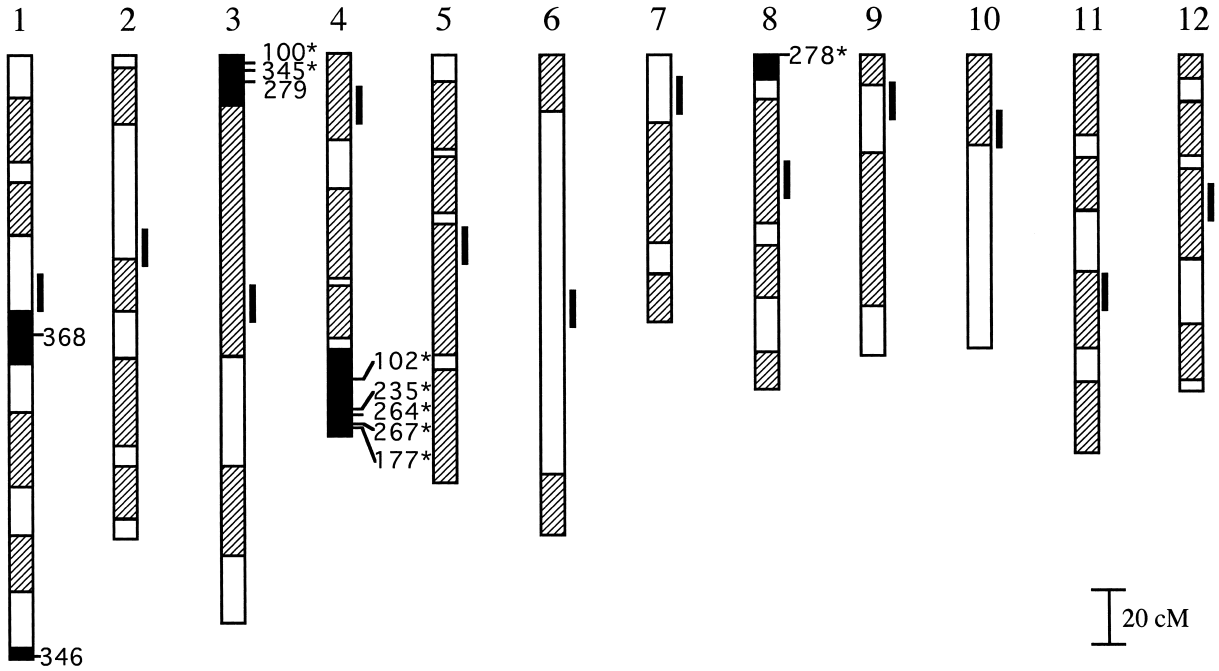

Fig. 1. Graphical genotype of Norin-PL8

Black portions and shaded portions indicate the segments derived from Silewah and Hokkai241, respectively. White portions indicate unidentified segments. Numbers in the map indicate the loci of RFLP markers with the prefix 'XNpb'. Asterisks indicate the markers polymorphic between Norin-PL8 and Kirara397. Vertical bars indicate the positions of the centromeres.

obtained for the markers on chromosome 4. These results indicated that the incorporated segments on chromosomes 3 and 4 were associated with cold tolerance. On the other hand, XNpb278 on chromosome 8 was not associated with the cold tolerance, indicating that there was no QTL for cold tolerance on chromosome 8.

3. Fine mapping of the QTLs on chromosome 4

We selected a line, Syo6, which contained the incorporated segments on chromosomes 3 and 4 from the BC_1F_5 lines. In order to eliminate as much as possible the effects of genetic factors other than those on chromosomes 3 and 4, Syo6 was backcrossed 3 times to Kirara397. Finally, we eliminated the effect of the incorporated segment on chromosome 3 by selecting a plant heterozygous for the incorporated segment on chromosome 4 but which did not contain the segment on chromosome 3. By self-pollination of the plant, we developed a segregating population, designated as BT4, and 117 plants of BT4 were used for interval mapping.

Table 1. Association between cold tolerance and RFLP markers on the incorporated segments

RFLP marker	Mean seed fertility in each genotype (%)			t-value[a)]	*P*
	Kirara397 type	Heterozygote	Norin-PL8 type		
XNpb100 (3)	49.7 [48]	51.7 [14]	56.8 [30]	2.407	0.0185
XNpb345 (3)	49.8 [47]	51.4 [13]	56.8 [30]	2.339	0.0220
XNpb102 (4)	50.5 [53]	52.1 [9]	58.5 [20]	2.487	0.0152
XNpb235 (4)	49.9 [67]	59.9 [9]	58.3 [16]	2.510	0.0141
XNpb264 (4)	49.8 [66]	59.6 [10]	58.3 [16]	2.527	0.0135
XNpb267 (4)	49.9 [67]	59.9 [9]	58.3 [16]	2.510	0.0141
XNpb177 (4)	49.9 [67]	59.9 [9]	58.3 [16]	2.510	0.0141
XNpb278 (8)	53.0 [39]	49.8 [17]	52.8 [36]	0.077	0.9391

a): Between Kirara397 type and Norin-PL8 type. ( ): Chromosome number. [ ]: Number of lines.

Fig. 2A shows the log-likelihood (LOD) score plot in the incorporated segment on chromosome 4. The maximum LOD score was found between R2737 and XNpb102, indicating that the QTL was most likely positioned between R2737 and XNpb102.

For fine mapping of the QTL, we developed a set of near-isogenic lines (NILs) from 13 recombinants in BT4 by marker-assisted selection. Fig. 2B shows the genotype of each NIL. We evaluated the cold tolerance of the NILs and parental varieties in the paddy field and in the greenhouse. Mean seed fertilities in both tests are shown in Fig. 2B. The genotypes of BT4-9-7 were Norin-PL8 type for R738 and Kirara397 type for the other markers, and their levels of cold tolerance were the same as that of

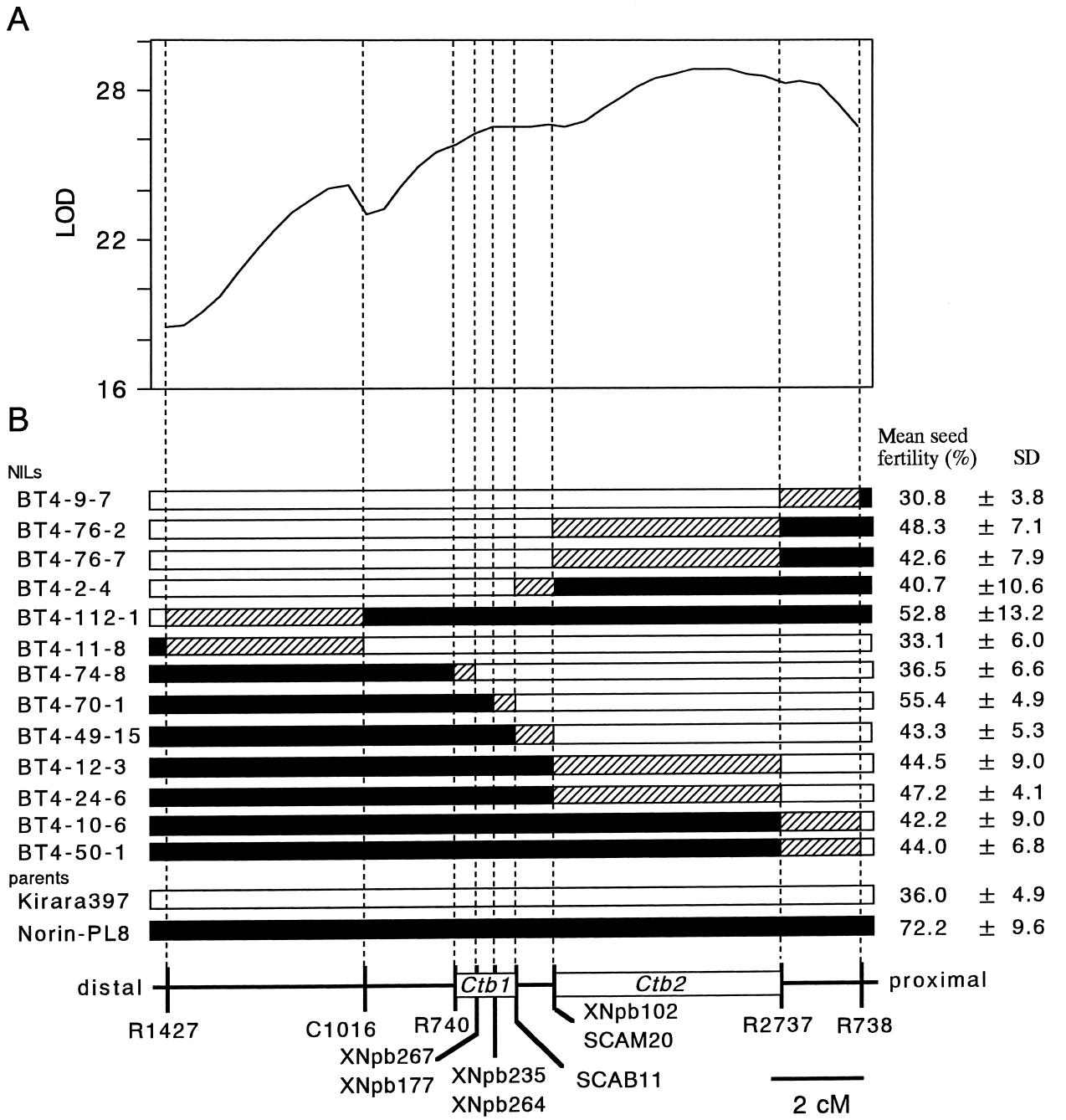

Fig. 2. Interval mapping of the QTL for cold tolerance on the long arm of chromosome 4 (A) and genotypes of the NILs (B)

Solid bars and open bars represent the Norin-PL8 type and Kirara397 type, respectively. Shaded bars represent the interval at which recombination had occurred. Map positions of *Ctb1* and *Ctb2* are also shown. Standard deviations were calculated based on the seed fertilities in the field test.

Kirara397. On the other hand, the cold tolerance of BT4-76-2 and BT4-76-7, which belonged to the Norin-PL8 type for R738 and R2737, was higher than that of Kirara397. These findings suggest the presence of a QTL for cold tolerance between R738 and XNpb102. The results coincided with the LOD peak observed in interval mapping. Cold tolerance of BT4-74-8 was similar to that of Kirara397, indicating that there was no QTL for cold tolerance in the region from R740 to the distal end. However, the cold tolerance of BT4-70-1 was higher than that of BT4-74-8 and Kirara397. The genotypes of BT4-70-1 and BT4-74-8 were different only in the region from SCAB11 to R740. The LOD curve was even in this region, suggesting the presence of an unidentified LOD peak. These results indicated the presence of another QTL for cold tolerance between R740 and SCAB11.

As a result, we identified 2 separate QTLs for cold tolerance on the long arm of chromosome 4, and we tentatively designated the distal one and the proximal one as *Ctb1* and *Ctb2*, respectively.

Discussion

The number of cold tolerance genes of Norin-PL8 was estimated to be 2 based on statistical analysis[1]. In this study, we showed that 2 chromosomal segments on chromosomes 3 and 4 were associated with cold tolerance at the booting stage of Norin-PL8, coinciding with the statistical estimation. However, the cold tolerance of Norin-PL8 was not fully explained by the QTLs on chromosomes 3 and 4. It is possible that there are other QTLs in the incorporated segments on chromosome 1 or unknown incorporated segments.

Out of the cold-tolerant varieties selected by IRRI from 1974 to 1977, 4 varieties, Silewah, Padi Labou Alumbis, Lambayque 1 and Mitak, had been used as donor parents for cold tolerance breeding at our institute and the breeding lines Norin-PL8, Norin-PL11, Hokkai PL5 and Hokkai PL6, respectively, were developed. Cold tolerance of the F_1 plants between Norin-PL8 and Hokkai PL5 was the same as that of their parents (H. Shimizu, personal communication). The long arm end of chromosome 4 was also incorporated from Lambayque 1 into Hokkai PL5 (unpublished data). These results suggest that Hokkai PL5 may harbor the same QTL as that of Norin-PL8 on chromosome 4. On the other hand, the cold tolerance of the F_1 plants between Norin-PL8 and Norin-PL11 was higher than that of their parents (H. Shimizu, personal communication), indicating that there is an interlocus or an intralocus interaction between the QTL of Norin-PL8 and that of Norin-PL11. The long arm end of chromosome 4 was not incorporated from Padi Labou Alumbis into Norin-PL11 (unpublished data). Norin-PL11 may harbor a QTL different from that of Norin-PL8, although the possibility of intralocus interaction has not yet been ruled out. A major QTL for cold tolerance on chromosome 7 was identified in 2 japonica varieties Koshihikari[8] and Kunmingxiaobaigu, which is a landrace from Yunnan Province in China (data submitted). These results indicate that it is possible to develop an extremely cold-tolerant variety by accumulating cold tolerance genes using molecular markers.

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