Theor Appl Genet (2001) 103:862–868

K. Saito · K. Miura · K. Nagano · Y. Hayano-Saito
H. Araki · A. Kato

Identification of two closely linked quantitative trait loci for cold tolerance on chromosome 4 of rice and their association with anther length

Received: 17 July 2000 / Accepted: 1 February 2001

Abstract Norin-PL8 is a cold-tolerant variety of rice (*Oryza sativa* L.) that was developed by introgressing chromosomal segments from a cold-tolerant *javanica* variety, Silewah. We previously detected quantitative trait loci (QTLs) for cold tolerance of Norin-PL8 in the introgressions on chromosomes 3 and 4. We provide fine mapping of the QTLs on chromosome 4 and the association between the QTLs and anther length, which has been reported to be a major component of cold tolerance. Interval mapping using a segregating population derived from an advanced backcross progeny indicated that a QTL for cold tolerance is probably located from the center to the proximal end of the introgression. For fine mapping, we developed a set of near-isogenic lines (NILs) from recombinants in the segregating population. Comparison of cold tolerance between the NILs indicated that either the proximal end or the center of the introgression is necessary for cold tolerance. From these results, we concluded that there are at least two QTLs for cold tolerance, tentatively designated as *Ctb-1* and *Ctb-2*, in the introgression on chromosome 4. The map distance between *Ctb-1* and *Ctb-2* is estimated to be 4.7–17.2 cM. In order to investigate the mechanism underlying cold tolerance by the QTLs, we compared anther lengths of the NILs. The results indicate that both *Ctb-1* and *Ctb-2* are associated with anther length.

Communicated by H.C. Becker

K. Saito (✉) · K. Miura · K. Nagano · Y. Hayano-Saito · H. Araki
A. Kato
National Agricultural Research Center for Hokkaido Region,
Hitsujigaoka 1, Toyohira, Sapporo, Hokkaido 062-8555, Japan
e-mail: kjsaito@cryo.affrc.go.jp
Tel.: +81-11-857-9478, Fax: +81-11-859-2178

K. Miura
National Institute of Agrobiological Sciences, Tsukuba,
Ibaraki 305-8602, Japan

K. Nagano
Furukawa Agricultural Experiment Station, Osaki, Furukawa,
Miyagi 989-6227, Japan

Keywords Cold tolerance · Anther length · QTL mapping · Near-isogenic line · Rice

Introduction

Rice is a cold-sensitive plant that originated from tropical or subtropical areas. Recent expansion of rice cultivation into areas with a cool summer has focused attention on the injurious effect of low temperatures on rice. Cold injuries are observed at many growth stages, e.g. failure in germination, slow seedling growth, stunting, discoloration, panicle tip degeneration, prolonged duration of cultivation, sterility and irregular maturity (Kaneda and Beachell 1973). In northern Japan, sterility caused by low temperature is the most serious problem because it inevitably leads to yield reduction. In the sterile-type of cold injury, the growth stage sensitive to low temperature is the booting stage, especially the young microspore stage spanning from the tetrad to the first contraction phase (Satake and Hayase 1970). Low temperature during microsporogenesis causes degeneration of microspores and hypertrophy of tapetal cells in the anther. The female organs retain their ability for successful fertilization, while microsporogenesis is completely inhibited (Hayase et al. 1969). Therefore, the degeneration of microspores and/or tapetal hypertrophy seems responsible for the sterile-type of cold injury (Nishiyama 1976). On the other hand, the mechanism of cold tolerance has not yet been studied in detail. For successful pollination, it is essential for anthers to contain sufficient pollen. The amount of pollen decreases with cold treatment and is highly correlated with fertility (Satake 1991). Cold-tolerant varieties generally produce more pollen than do cold-sensitive varieties (Satake and Shibata 1992). Therefore, it is thought that the amount of pollen is an important factor in the mechanism of cold tolerance. Since the amount of pollen is highly correlated with anther length (Oka and Morishima 1967; Suzuki 1981), it is likely that anther length is correlated with cold tolerance (Tanno et al. 1999). Cold tolerance is a

quantitative trait and no major gene has been identified so far. Futsuhara and Toriyama (1966) estimated the number of cold tolerance genes to be four or more. It has been reported that they are linked with the genes *Pr* (purple hull), *Rc* (brown pericarp), d_2 (dwarf), *gh* (gold hull), *nl* (neck leaf) and *bc* (brittle culm) on chromosomes 3, 4, 5 and 7 (Futsuhara and Toriyama 1966; Takahashi et al. 1973; Sawada 1978).

A cold-tolerant rice variety, Norin-PL8, harbors cold tolerance genes derived from a *javanica* variety, Silewah. We previously showed that two chromosomal segments introgressed from Silewah, which are located in the short arm of chromosome 3 and in the long arm of chromosome 4, are responsible for the cold tolerance of Norin-PL8 (Saito et al. 1995). In the present study, we fine-mapped QTLs for cold tolerance on chromosome 4 using advanced backcross progenies and near-isogenic lines (NILs), and we also investigated the relationship between QTLs and anther length.

Materials and methods

Plant materials

Norin-PL8 is a cold-tolerant variety that was developed by backcross breeding in which a cold tolerant *javanica* variety, Silewah, and a *japonica* breeding line, Hokkai241, were used as a donor and recurrent parent, respectively (Satake and Toriyama 1979). Kirara397 is a cold-sensitive commercial variety grown in northern Japan. We selected a line, Syo6, that contains introgressed segments on chromosomes 3 and 4 from BC_1F_5 lines derived from the cross Kirara397/Norin-PL8//Kirara397 (Saito et al. 1995). In order to eliminate the effects of genetic factors other than those on chromosomes 3 and 4 as much as possible, an F_1 plant between Syo6 and Kirara397 was backcrossed with Kirara397. After two backcrosses, we selected a single plant that is heterozygous for the introgression on chromosome 4 but does not contain the introgression on chromosome 3. By self-pollination of the plant, we developed a segregating population, designated as BT4, and 117 plants of BT4 were used in interval mapping. We developed a set of NILs derived from 12 recombinants in BT4 by marker-assisted selection in order to fine-map the QTLs and examine the effect of the QTLs on anther length. Fifty nine F_7 lines between Hokkai241 and Norin-PL8 were used for the purpose of testing the association between anther length and the introgressed segments on chromosomes 3 and 4.

Molecular analysis

Total DNA was isolated from leaves according to the modified CTAB method (Murray and Thompson 1980). Two micrograms of DNA were digested with restriction endonucleases, electrophoresed on 0.8% (w/v) agarose gels, and transferred to Hybond-N+ nylon membranes (Amersham Pharmacia). Restriction fragment length polymorphism (RFLP) markers with the prefix 'XNpb' were developed by Saito et al. (1991). RFLP markers with the prefix 'R' or 'C' were obtained from the Rice Genome Research Program, Japan (RGP) (Kurata et al. 1994; Harushima et al. 1998). RFLP probes were non-radioactively labelled using the ECL system (Amersham Pharmacia) and hybridized with the DNA on the blots, which were then washed at 42°C in 6 M urea, 0.5× SSC and 0.4% SDS, rinsed with the detection reagent of the ECL system, and exposed to X-ray films for 10 to 60 min. Amplification of sequence-characterized amplified region (SCAR) markers, SCAB11 and SCAM20, was performed according to the method of Williams et al. (1990) using the GeneAmp PCR System 9600 (Perkin Elmer). The thermal cycles used were: one cycle of 4 min at 94°C followed by 45 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C, and then finally one cycle of 7 min at 72°C. The amplification products were resolved by electrophoresis in a 1.2% (w/v) agarose gel.

Evaluation of cold tolerance and anther length

We evaluated cold tolerance according to the cool-water irrigation method (Futsuhara and Toriyama 1964), which is a general method used in cold tolerance breeding. Although natural cold injury is caused by low atmospheric temperature, deep cool water is used to cool young panicles and cause artificial cold injury. Plants were treated with cool water controlled at 19°C from the primordial stage to the completion of heading. The depth of water was 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 on the basis of mean seed fertility. Cold tolerance evaluation of BT4 was conducted in the paddy field. Cold tolerance evaluation of the NILs was conducted both in the paddy field and in the greenhouse.

Anthers from spikelets in the middle of panicles were harvested before flowering and their length was measured using a microscope. Six anthers from two spikelets were measured for each F_7 line between Hokkai241 and Norin-PL8 grown in the greenhouse. Eighty anthers from 40 spikelets were measured for each NIL between Kirara397 and Norin-PL8 grown under field conditions. Since anther length is affected by environmental conditions, anthers produced in the greenhouse were generally larger than those produced under field conditions.

Mapping and QTL analysis

We used the computer program MAPL97 (Ukai et al. 1991; Hayashi and Ukai 1994) for the calculation of genetic distances between RFLP markers and interval mapping of the QTL. The computer program StatView 5.0 (SAS Institute) was used for the analysis of variance between marker classes.

Results

QTL mapping

It was previously reported that the QTLs for cold tolerance of Norin-PL8 are located on chromosomes 3 and 4 (Saito et al. 1995). In order to determine the precise map position of the QTL on chromosome 4, we developed advanced backcross progenies by using Kirara397 as a recurrent parent. Furthermore, we added five RFLP markers (R738, R740, R1427, R2737 and C1016) and two SCAR markers (SCAB11 and SCAM20) to the analysis. All markers were mapped in the order shown in Fig. 1B. We used 117 individuals of the segregating population BT4 for the interval-mapping of cold tolerance. Figure 1 A shows the log-likelihood (LOD) score plot in the introgressed segment on chromosome 4. LOD scores were relatively high (LOD>25) from the center to the proximal end of the introgression, and the maximum LOD score was observed between R2737 and XNpb102, indicating that the QTL is most likely positioned between R2737 and XNpb102.

For fine mapping of the QTL, we developed a set of NILs and compared their cold tolerance. We selected 12

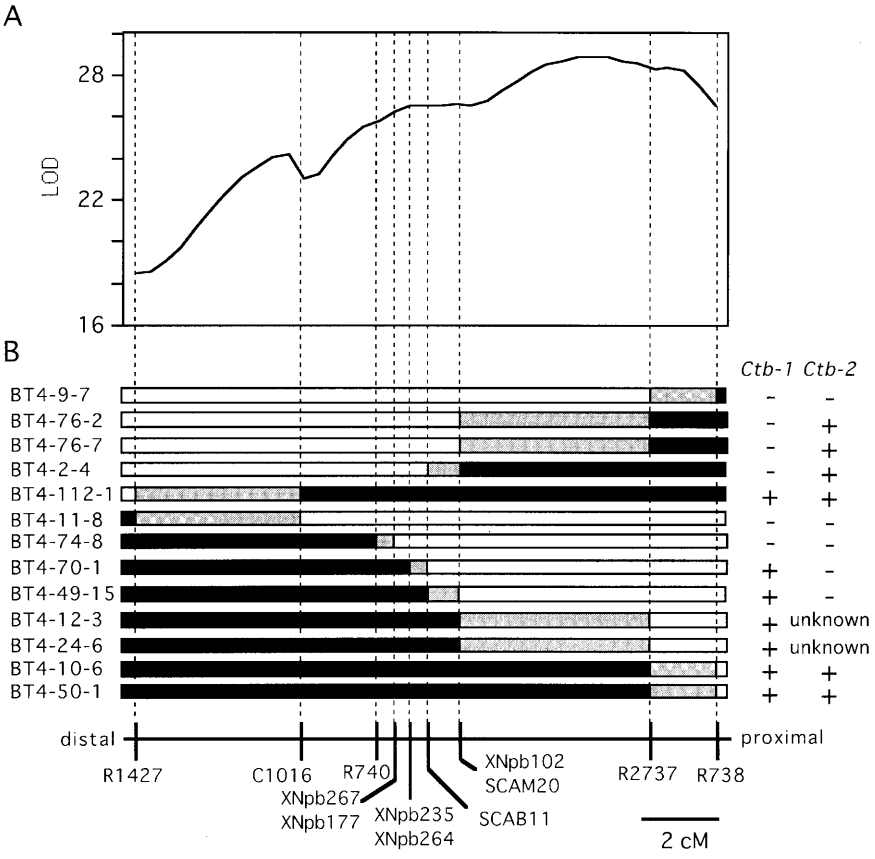

Fig. 1 Interval mapping of the QTL for cold tolerance on the long arm of chromosome 4 using BT4 (**A**) and genotypes of the NILs (**B**). *Solid bars* and *open bars* represent the Norin-PL8 type and Kirara397 type, respectively. *Dotted bars* represent the interval in which recombination has occurred. Genotypes of *Ctb-1* and *Ctb-2* estimated by marker genotypes and cold tolerance are also shown

Table 1 Cold tolerance, heading date and anther length of the NILs and parental varieties

NIL/parent	Mean seed fertility (%)			Mean date to heading[a] (days)	Mean anther length[b] (mm)
	Paddy field (A)	Green house (B)	Average between A and B		
BT4–9–7	13.7	47.8	30.8	113.6	1.83
BT4–76–2	26.3	70.3	48.3	117.8	2.06
BT4–76–7	14.6	70.6	42.6	116.0	2.02
BT4–2-4	23.0	58.4	40.7	116.2	1.81
BT4–112–1	26.4	79.1	52.8	120.6	2.16
BT4–11–8	10.9	55.3	33.1	119.8	1.91
BT4–74–8	16.4	56.7	36.5	119.6	1.94
BT4–70–1	32.5	78.3	55.4	119.6	2.03
BT4–49–15	20.3	66.3	43.3	122.0	2.17
BT4–12–3	14.2	74.7	44.5	123.6	2.09
BT4–24–6	25.9	68.6	47.2	121.0	2.10
BT4–10–6	22.2	62.2	42.2	122.0	2.12
BT4–50–1	20.9	67.0	44.0	124.0	2.07
Kirara397	12.8	59.3	36.0	113.2	1.82
Norin-PL8	53.0	91.3	72.2	120.0	2.36

[a] Evaluated in a field irrigated with cool water
[b] Evaluated under field conditions without cool-water treatment

recombinants between R738 and R1427 from BT4. From progenies of each recombinant, we developed fixed lines by marker-assisted selection. Figure 1B shows the genotype of each NIL. We evaluated the cold tolerance of the NILs by mean seed-fertility (Table 1). The genotype of BT4–9-7 is the Norin-PL8 type for R738 and the Kirara397 type for the other markers, and their cold tolerance was the same level as that of Kirara397. On the other hand, the cold tolerance of BT4–76–2 and BT4–76-7, which are Norin-PL8 type for R738 and R2737, was higher than that of Kirara397. This result suggests the presence of a QTL for cold tolerance in the region between R738 and XNpb102. The result coincides with the LOD peak observed in the interval mapping. Therefore, it is likely that there is a QTL for cold tolerance between R2737 and XNpb102. Cold tolerance of BT4–74–8 was similar to that of Kirara397, indicating that there is no QTL for cold tolerance in the region from R740 to the distal end. However, the cold tolerance of B T4–70–1 was higher than

that of BT4–74–8 and Kirara397. The genotype is different between BT4–70–1 and BT4–74–8 only in the region from SCAB11 to R740. The LOD curve is even in this region, suggesting the presence of a hidden LOD peak. These results indicate the presence of another QTL for cold tolerance between R740 and SCAB11. Furthermore, cold tolerance of the NILs that are of the Norin-PL8 type for R2737–XNpb102 and/or SCAB11–R740 was higher than that of Kirara397 (Fig. 1B, Table 1).

We found that there are two separate QTLs for cold tolerance in the long arm of chromosome 4, and we tentatively designate the distal one and the proximal one as *Ctb-1* and *Ctb-2*, respectively. Although three NILs (BT4–112–1, BT4–10–6 and BT4–50–1) harbor both *Ctb-1* and *Ctb-2*, their cold tolerance was indistinguishable from that of the NILs that harbor only *Ctb-1* or *Ctb-2*, such as BT4–76–2, BT4–76–2 and BT4–70–1. This result suggests that the interaction of *Ctb-1* and *Ctb-2* is not additive.

Map positions of *Ctb-1* and *Ctb-2*

SCAB11 and SCAM20 are SCAR markers developed from random amplified polymorphic DNAs (RAPDs) (data not shown). Although the RFLP markers used in this study had already been mapped on the genetic linkage map by the RGP (Kurata et al. 1994; Harushima et al. 1998), the positions of SCAB11 and SCAM20 on the map had not been determined. Furthermore, there was severe recombination shrinkage in BT4. Therefore, we could not estimate the genetic distance between *Ctb-1* and *Ctb-2* exactly. To determine the map positions of the SCAR markers in the genetic linkage map by the RGP, we mapped the markers on the yeast artificial chromosome (YAC) physical map by the RGP (Koike et al. 1997). SCAM20 was on the YAC clone Y1676 covering the region from 102.0 cM to 102.6 cM in the map. SCAB11 was mapped on the YAC clone Y1955, which is at 106.7 cM. Figure 2 shows the determined *Ctb-1* and *Ctb-2* regions. *Ctb-1* is located in a 6.7-cM region spanning from 106.7 cM to 113.4 cM. *Ctb-2* is located in a 5.8-cM region spanning from 96.2 cM to 102.0 cM. The map distance between *Ctb-1* and *Ctb-2* is estimated to be 4.7–17.2 cM.

The recombination between markers shrunk in BT4. The degree of shrinkage was higher between XNpb102 and R740 than between R738 and XNpb102 and between R740 and R1427 (Fig. 2). Recombination shrinkage has been reported in some interspecific crosses (Bonierbale et al. 1988; Paterson et al. 1988; Causse et al. 1994) and in advanced generations (Alpert and Tanksley 1996; Monforte and Tanksley 2000). Low homology between DNA strands is generally related to recombination shrinkage (Silver and Artzt 1981). Recombination shrinkage between XNpb102 and R740 might be due to lower homology in the region between the *japonica* and *javanica* varieties.

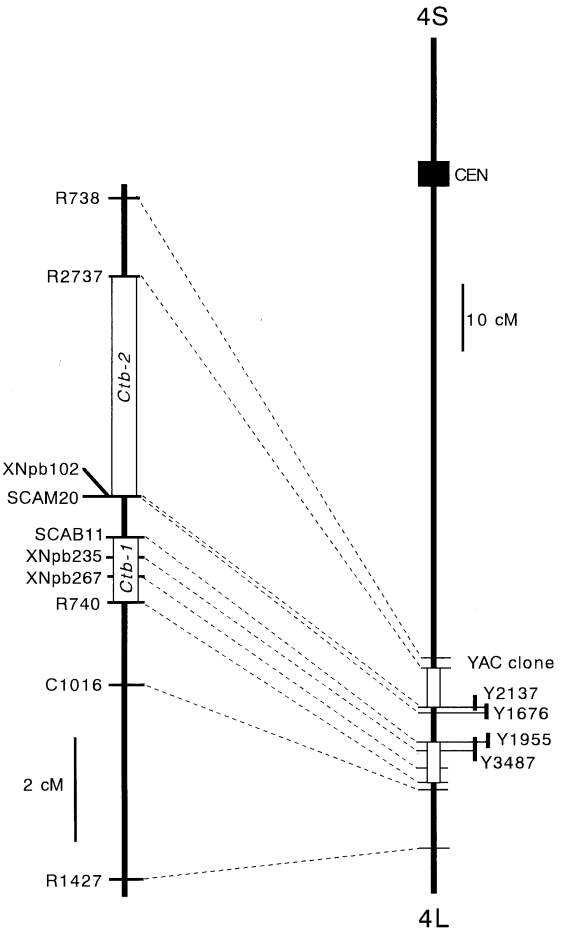

Fig. 2 Map positions of the *Ctb-1* and *Ctb-2* regions: the map on the left is based on the recombination in BT4, and the map on the right is based on the genetic linkage map of the Rice Genome Research Program, Japan (Harushima et al. 1998). YAC clones were positioned according to Koike et al. (1997)

Cold tolerance and anther length

The anther lengths of Norin-PL8 and Hokkai241 were 2.85 mm and 2.13 mm, respectively, and the anther lengths of F_7 line s were distributed between those of the parents (Fig. 3). Because multiple peaks were not observed in the frequency distribution, it is unlikely that anther length is controlled by a major gene. The association of anther length with the introgressed segments on chromosomes 3 and 4 was tested by analysis of variance procedures using each marker as a treatment. Although the marker R2737 on chromosome 4 was significantly related to anther length, the significance level of association between XNpb345 on chromosome 3 and anther length was low (Table 2). The results indicate the presence of a QTL for a large anther in the long arm of chromosome 4.

There are two QTLs for cold tolerance in the introgressed segment on chromosome 4. In order to examine the association between each locus and anther length, we measured the length of 80 anthers from each

Table 2 Association of the introgressed segments on chromosomes 3 and 4 with anther length

Chromosome	Marker	Anther length (mm)		*t*	*P*
		Kirara397 type [*n*]	Norin-PL8 type [*n*]		
3	XNpb345	2.42 (22)	2.51 (33)	2.048	0.0455
4	R2737	2.38 (26)	2.55 (27)	4.257	<0.0001

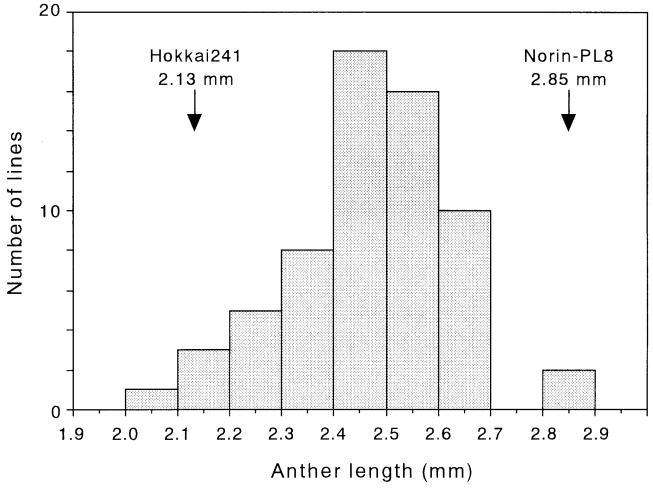

Fig. 3 Frequency distribution of anther lengths in the F_7 family between Norin-PL8 and Hokkai241

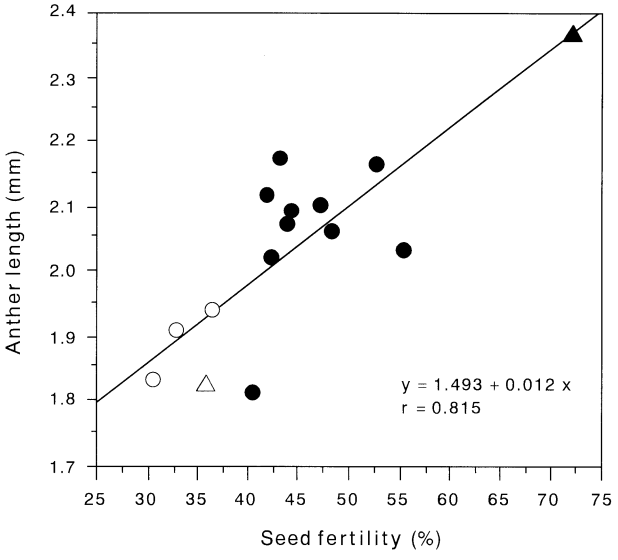

Fig. 4 Relation between cold tolerance and anther length shown in Table 1. *Circles* represent the NILs: *open circles* represent the NILs without either *Ctb-1* or *Ctb-2*, and *closed circles* represent the NILs with *Ctb-1* and/or *Ctb-2*. *Open* and *closed triangles* represent Kirara397 and Norin-PL8, respectively

NIL between Norin-PL8 and Kirara397 (Table 1). Anther lengths of Norin-PL8 and Kirara397 were 2.36 mm and 1.82 mm, respectively. The anthers of BT4–70–1, BT4–76–2 and BT4–76–7 were about 0.2-mm longer than that of Kirara397. On the other hand, the anther lengths of BT4–9-7, BT4–11–8 and BT4–74–8 were similar to that of Kirara397. The NILs harboring *Ctb-1* and/or *Ctb-2* produced larger anthers than did the NILs without *Ctb-1* and *Ctb-2* (Fig. 4). This result suggests that both *Ctb-1* and *Ctb-2* express cold tolerance by producing a large anther. The anther length of BT4–2-4 was shorter than that expected from its genotype. This might be due to environmental variance because anther length is controlled by both genetic and environmental factors.

Discussion

We previously showed that the QTLs for cold tolerance of Norin-PL8 are located on the short arm of chromosome 3 and on the long arm of chromosome 4 (Saito et al. 1995). We found that a cold-tolerant variety, Hokkai-PL4, which is a sister line of Norin-PL8, contains the introgression on chromosome 4 but lacks the introgression on chromosome 3 (data not shown). The QTL for cold-tolerance on chromosome 4 was also detected in a cold-tolerant variety, Hokkai-PL5, whose cold tolerance was introduced from a cold-tolerant *javanica* variety, Lambayque 1 (unpublished data). Therefore, the QTL on chromosome 4 is possibly more important for the expression of cold tolerance than is the QTL on chromosome 3. We developed advanced backcross progenies and a set of NILs in order to determine the precise location of the QTL on chromosome 4, and we identified two closely linked QTLs for cold tolerance.

The sterile type of cold injury is due to suppressed pollen maturation under a low temperature condition. The amount of mature pollen necessary for successful pollination is estimated to be 640 pollen grains/anther (Nishiyama 1983). Rice varieties are variable in the amount of pollen produced under normal conditions, ranging from 1,000 to 2,500 pollen grains/anther (Suzuki 1981). These observations suggest that a variety with less pollen tends to become sterile when pollen maturation is inhibited by low temperature. Therefore, the amount of pollen is an important factor in cold-tolerance. Furthermore, it was shown that the amount of pollen is highly correlated with anther length (Oka and Morishima 1967; Suzuki 1981). A variety with a large anther could therefore be cold-tolerant. In fact, the anther of the cold-tolerant variety Norin-PL8 is larger than that of the cold-sensitive varieties Hokkai241 and Kirara397. Two introgressed segments of Norin-PL8 on chromosomes 3 and 4 are responsible for cold tolerance (Saito et al. 1995). In this study, we showed that the introgressed segment on chromosome 4 is associated with anther length more significantly than is that on chromosome 3.

Therefore, it is likely that the cold tolerance genes on chromosome 4 express cold tolerance by producing more pollen. Two QTLs for cold tolerance, *Ctb-1* and *Ctb-2*, are present on chromosome 4. A comparison of anther lengths between the NILs indicated that both genes are associated with anther length. Interestingly, Norin-PL8 was superior to the NILs not only in cold tolerance but also in anther length (Fig. 4). Since there are many QTLs for anther length (Xiong et al. 1999), it is likely that Norin-PL8 harbors genes for anther length other than *Ctb-1* and *Ctb-2*. Although the cold tolerance gene on chromosome 3 possibly affects anther length, its effect is small (Table 2). Therefore, Norin-PL8 probably harbors undetected cold tolerance genes that affect anther length.

There was no additive interaction between *Ctb-1* and *Ctb-2*. Two possible hypotheses account for this observation: (1) *Ctb-1* and *Ctb-2* are complementary, and (2) a gene that decreases cold tolerance lies between *Ctb-1* and *Ctb-2*. The result showing that both *Ctb-1* and *Ctb-2* are possibly associated with anther length supports the first hypothesis. In fact, with respect to anther length, the interaction between the genes was not additive. However, we observed that the cold tolerance of BT4–70–1 was higher than that of BT4–49–15, BT4–12–3 and BT4–24–6. The difference in seed fertility was about 10%, which is equivalent to the effect of *Ctb-2*. Therefore, the second hypothesis is also likely. A candidate for the gene that has a negative effect on cold tolerance is a gene for late heading. In the cool-water irrigation method, cold treatment to young anthers automatically ends when panicles emerge from the cool water. Therefore, as heading is later, cold treatment of the anthers becomes longer. The cold tolerance of late heading lines might, therefore, have been underestimated (Table 1). Further study is needed to clarify the interaction between *Ctb-1* and *Ctb-2*.

Sato et al. (1994) suggested the possibility that the earliness gene *Ef-1* pleiotropically shortens the anther. Since early heading is also important in areas with a cool and short summer, the relation between heading date and anther length is notable. The heading date of BT4–70–1 was almost the same as that of BT4–74–8, indicating that *Ctb-1* is not associated with heading date. On the other hand, the heading date of BT4–76–2 was about 3 days later than that of BT4–9-7. Therefore, we could not eliminate the possibility of an association between heading date and *Ctb-2*.

In this study, we identified two linked QTLs for cold tolerance on chromosome 4. Our ultimate goal is to develop cold-tolerant varieties using cold tolerance genes. In this context, it is notable that the genes increased the cold tolerance of Kirara397, which is a commercial variety with high grain quality. In spite of breeders' efforts, Norin-PL8 retains some unfavorable traits such as late heading. If there are loci for unfavorable traits between *Ctb-1* and *Ctb–2*, it is difficult to remove such loci retaining both *Ctb-1* and *Ctb-2* by usual breeding procedures. Marker-assisted selection might be effective in removing unfavorable trait loci. In addition, the NILs, BT4–70–1 and BT4–76–2, can be new donor parents of each cold tolerance gene. Therefore, the use of NILs is effective not only for genetic and physiological analysis of QTLs but also for the improvement of agricultural traits (Touzet et al. 1995).

Acknowledgements We thank the Rice Genome Research Program, Japan, for providing RFLP probes and YAC clones. This work was supported by the Rice Genome Project sponsored by the Ministry of Agriculture, Forestry and Fisheries of Japan. The experiments presented here comply the current laws of Japan.

References

Alpert KB, Tanksley SD (1966) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw2.2*: a major fruitweight quantitative trait locus in tomato. Proc Natl Acad Sci USA 93:15503–15507

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Futsuhara Y, Toriyama K (1964) Studies on the testing methods of cold resistance in rice. Jpn J Breed 14:166–172

Futsuhara Y, Toriyama K (1966) Genetic studies on cool tolerance in rice. III. Linkage relations between genes controlling cool tolerance and marker genes of Nagao and Takahashi. Jpn J Breed 16:19–30

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Hayase H, Satake T, Nishiyama I, Ito N (1969) Male sterility caused by cooling treatment at the young microspore stage in rice plants. II. The most sensitive stage to cooling and the fertilizing ability of pistils. Proc Crop Sci Soc Japan 38:706–711

Hayashi T, Ukai Y (1994) Detection of additive and dominance effects of QTLs in interval mapping of F_2 RFLP data. Theor Appl Genet 87:1021–1027

Kaneda C, Beachell HM (1973) Response of indica-japonica rice hybrids to low temperatures. SABRAO J 6:17–32

Koike K, Yoshino K, Sue N, Umehara Y, Ashikawa I, Kurata N, Sasaki T (1997) Physical mapping of rice chromosomes 4 and 7 using YAC clones. DNA Res 4:27–33

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimao Y, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyano A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300-kilobase-interval genetic map of rice including 883 expressed sequences. Nature Genet 8:365–372

Monforte AJ, Tanksley SD (2000) Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor Appl Genet 100:471–479

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nishiyama I (1976) Male sterility caused by cooling treatment at the young microspore stage in rice plants. XIII. Ultrastructure of tapetal hypertrophy without primary wall. Proc Crop Sci Soc Japan 45:270–278

Nishiyama I (1983) Male sterility caused by cooling treatment at the young microspore stage in rice plants. XXVI. The number of ripened pollen grains and the difference in susceptibility to coolness among spikelets on the panicle. Jpn J Crop Sci Soc Japan 52:307–313

Oka H, Morishima H (1967) Variations in the breeding systems of a wild rice, *Oryza perennis*. Evolution 21:249–258

Paterson AH, Lander ES, Hewitt JD, Paterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Saito A, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhara S, Ukai Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsawa R, Hayano Y, Iwata N, Sugiura M (1991) Linkage map of restriction fragment length polymorphism loci in rice. Jpn J Breed 41:665–670

Saito K, Miura K, Nagano K, Hayano-Saito Y, Saito A, Araki H, Kato A (1995) Chromosomal location of quantitative trait loci for cool tolerance at the booting stage in rice variety 'Norin-PL8.' Breed Sci 45:337–340

Satake T (1991) Male sterility caused by cooling treatment at the young microspore stage in rice plants. XXX. Relation between fertilization and the number of engorged pollen grains among spikelets cooled at different pollen developmental stages. Jpn J Crop Sci 60:523–528

Satake T, Hayase H (1970) Male sterility caused by cooling treatment at the young microspore stage in rice plants. V. Estimation of pollen developmental stage and the most sensitive stage to coolness. Proc Crop Sci Soc Japan 39:468–473

Satake T, Shibata M (1992) Male sterility caused by cooling treatment at the young microspore stage in rice plants. XXXI. Four components participating in fertilization. Jpn J Crop Sci 61: 454–462

Satake T, Toriyama K (1979) Two extremely cool-tolerant varieties. Int Rice Res Newslett 4:9–10

Sato S, Tamaki A, Shinjyo C (1994) Genetic relation between an earliness gene *Ef-1* and anther length in rice, *Oryza sativa* L. Breed Sci 44:127–132

Sawada S (1978) Study of sterile-type cool injury in rice plants with special reference to the mechanism and inheritance of sterility. Res Bull Obihiro Univ 10:837–883

Silver LM, Artzt K (1981) Recombination suppression of mouse *t*-haplotypes due to chromatin mismatching. Nature 290:68–70

Suzuki S (1981) Cold tolerance in rice plants with special reference to the floral characters. I. Varietal differences in anther and stigma lengths and the effects of planting densities on these characters. Jpn J Breed 31:57–64

Takahashi M, Kinoshita T, Takeda K (1973) Character expression of some major genes in rice and their agronomic application. J Facul Agr Hokkaido Univ 57:275–293

Tanno H, Xiong J, Dai L, Ye C (1999) Some characteristics of cool weather-tolerant rice varieties in Yunnan Province, China. Jpn J Crop Sci 68:508–514

Touzet P, Winker RG, Helentjaris T (1995) Combined genetic and physiological analysis of a locus contributing to quantitative variation. Theor Appl Genet 91:200–205

Ukai Y, Ohsawa R, Saito A (1991) MAPL: a package of microcomputer programs for RFLP linkage mapping. Rice Genet Newslett 8:155–158

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Xiong LZ, Liu KD, Dai XK, Xu CG, Zhang Q (1999) Identification of genetic factors controlling domestication-related traits of rice using an F_2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor Appl Genet 98:243–251