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Mapping a Novel Gene of Cold Tolerance at Booting Stage by Using Near-Isogenic Lines in japonica Rice

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Abstract: Genetic analysis showed that cold tolerance at booting stage of near-isogenic lines (NILs) of Kunmingxiaobaigu was controlled by a gene with large phenotypic variance. One hundred and sixty-four simple sequence repeats (SSR) distributed over 12 chromosomes were used to screen polymorphism between Towata (recurrent parent, RP) and near-isogenic line pool (NILP), and two SSR markers at the long arm of chromosome 5 showed polymorphism in comparison with RP genome. Of the two markers, RM31 was found possibly linked with the cold tolerance gene at booting stage through one-way ANOVA. Twelve SSR markers around RM31 were then used to detect polymorphism between RP and NIL, and only RM7452 had polymorphism. The gene of cold tolerance at booting stage was further mapped on chromosome 5 between RM7452 and RM31 with genetic distances of 4.8 cM and 8.0 cM, respectively. This gene explained 10.50% of phenotypic variance and 5.10% of phenotypic variance of fully filled grains, and was tentatively designated as *Ctb*(t).
Key words: booting stage; near-isogenic line; simple sequence repeat; genetic analysis; cold tolerance gene

Cold damage is a common problem in rice cultivation all over the world, especially in Japan, Korea and China, and it is also a main problem in Yunnan Province, China [1]. Cold damage is a restricting factor in rice production, and cold tolerance at booting stage behaves as a quantitative trait controlled by polygenes. Therefore, it is difficult to clone cold tolerance gene [1-4]. Rice is a cold-sensitive plant that originated from tropical or subtropical areas. Low temperatures at micro-sporogenesis stage resulted in degeneration of microspores and hypertrophy of tapetum cells in the anther of rice; However, the mechanism of cold tolerance remained unclear. 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 spikelet fertility. Therefore, 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 and seed setting rate, it is likely that anther length and seed setting rate is correlated with cold tolerance. Muehlbauer et al [5] proposed that there were difference between goal traits in a pair of near-isogenic lines (NIL), if the markers showed polymorphisms between recurrent parent (RP) and NIL, then the markers were possibly linked with the genes of goal traits.

In this study, we analyzed and mapped the gene of cold tolerance at the booting stage, using the NIL population from a cross between KMXBG (donor parent) and Towada (recurrent parent).

MATERIALS AND METHODS

Plant materials

A BC_5F_2 population consisting of 154 individuals was developed from a cross between japonica varieties, Kunmingxiaobaigu (KMXBG, donor parent, Yunnan landrace) and Towada (recurrent parent, Japanese variety). Of the two parents, KMXBG has been cultivated in Kunming, Yunnan Province for more than 300 years and showed high tolerance to low temperatures at the booting and seedling stages, and Towada was a low cold-tolerance variety.

The population and parents were planted in the Experimental Farm of Yunnan Academy of Agricultural Sciences in Kunming (elevation, 1916 m; average temperature, 18–21℃) in 2002. Fully filled

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grain, unfilled grain, anther length, anther volume and seed setting rate were used to evaluate cold tolerance. One hundred and sixty-four simple sequence repeats (SSR) over 12 chromosomes were used to screen polymorphism between Towada (RP), KMXBG and NIL pool (NILP).

SSR analysis

Total DNAs of the 154 individuals and their parental lines were extracted from leaves according to the CTAB method [6]. SSR procedures followed the method described by Temnykh et al [7]. DNA amplification was performed by PCR machine. PCR reaction system(10 μL) included 10×*Taq* buffer 1 μL, ddH_2O 4.8 μL, $MgCl_2$ 0.6 μL, dNTP-mix 0.5 μL, Primer 1(0.5 μL), Primer 2 (0.5 μL), *Taq* polymerase 0.1 μL (5 U/μL), DNA 2 μL. PCR reaction was programmed as follows: pre-denaturated at 94℃ for 5 min, and denaturated for 60 s at 94℃, followed by 35 cycles of 55, 61 or 67℃ for 40 s, 72℃ for 2 min, with a final extension step at 72℃ for 5 min. Amplification products were analyzed on 5% polyacrylamide gels stained with 0.1% silver nitrate.

Linkage mapping

Linkage analysis was performed with MAPMAKER/EXP3.0. To determine linkage between two markers, we used a threshold LOD score of 3.0 and a maximum recombination value of 0.3.

The frequencies of observed recombination between two markers were converted to genetic distance by the map function of Kosami.

QTL analysis

A single-point analysis was performed to detect putative QTLs by using one-way ANOVA with SPSS 11.0. The threshold probability level was less than 0.01. We also used WQTLCART V2.0 and QTLmapper 2.0 to confirm the presence of putative QTLs and to estimate genetic parameters, such as additive effects and percentage of variance explained, based on the "f2" mode.

RESULTS

Genetic analysis of cold tolerance traits

Under low temperature, seed setting rate of KMXBG, NIL and Towada were 90.8, 80.3 and 16.9%, respectively (Table 1). And these test materials showed similar growth duration. Therefore, it is credible to analyze genetic cold tolerance using the cross of Towada NIL (BC_4F_5) as materials. As shown in Fig. 1, skewness and kurtosis of seed setting rate of BC_5F_2 population were -1.40 and 2.12, respectively, showing skewness distributions with multiple peaks. This result indicated that KMXBG might harbor cold tolerance genes with large phenotypic variation, reflecting the quality-quantitative inheritance nature. Anther length and anther volume both showed continuous distributions, reflecting the quantitative inheritance of anther traits, which were in accordance with the results of Saito et al [8].

SSR analysis

The BC_5F_2 population and their parents were grown in Kunming(2002). One hundred and sixty-four simple sequence repeats (SSR) over 12 chromosomes were tested for polymorphism between Towata, KMXBG and NIL high seed setting rate pool (NILHP). Two SSR markers at the long arm of chromosome 5 had polymorphism in comparison with RP (Towata) genome, and one of them was found

Table 1. Cold tolerance traits of NIL population and parents (Mean±SD).

Character	Population (BC_5F_2)			Parent		
	Value	Skewness	Kurtosis	RP (Towada)	DP (KMXBG)	NIL (BC_4F_5)
Anther length (mm)	1.95±0.23	-0.16±0.12	-0.53±0.40	1.64±0.01	2.52±0.15	2.27±0.19
Anther volume (mm^3)	0.12±0.03	0.05±0.20	0.29±0.40	0.09±0.05	0.19±0.03	0.14±0.03
Fully filled grain	119.70±34.80	-0.53±0.20	0.18±0.40	28.70±17.10	191.20±19.90	128.60±9.60
Unfilled grain	40.00±24.80	1.32±0.20	2.13±0.40	145.60±37.40	20.20±11.50	31.30±9.60
MPSSR	70.90±14.70	-1.40±0.20	2.12±0.40	16.90±9.60	90.80±4.40	80.30±4.90

NIL, Near-isogenic line; MPSSR, Seed setting rate of the main panicle.

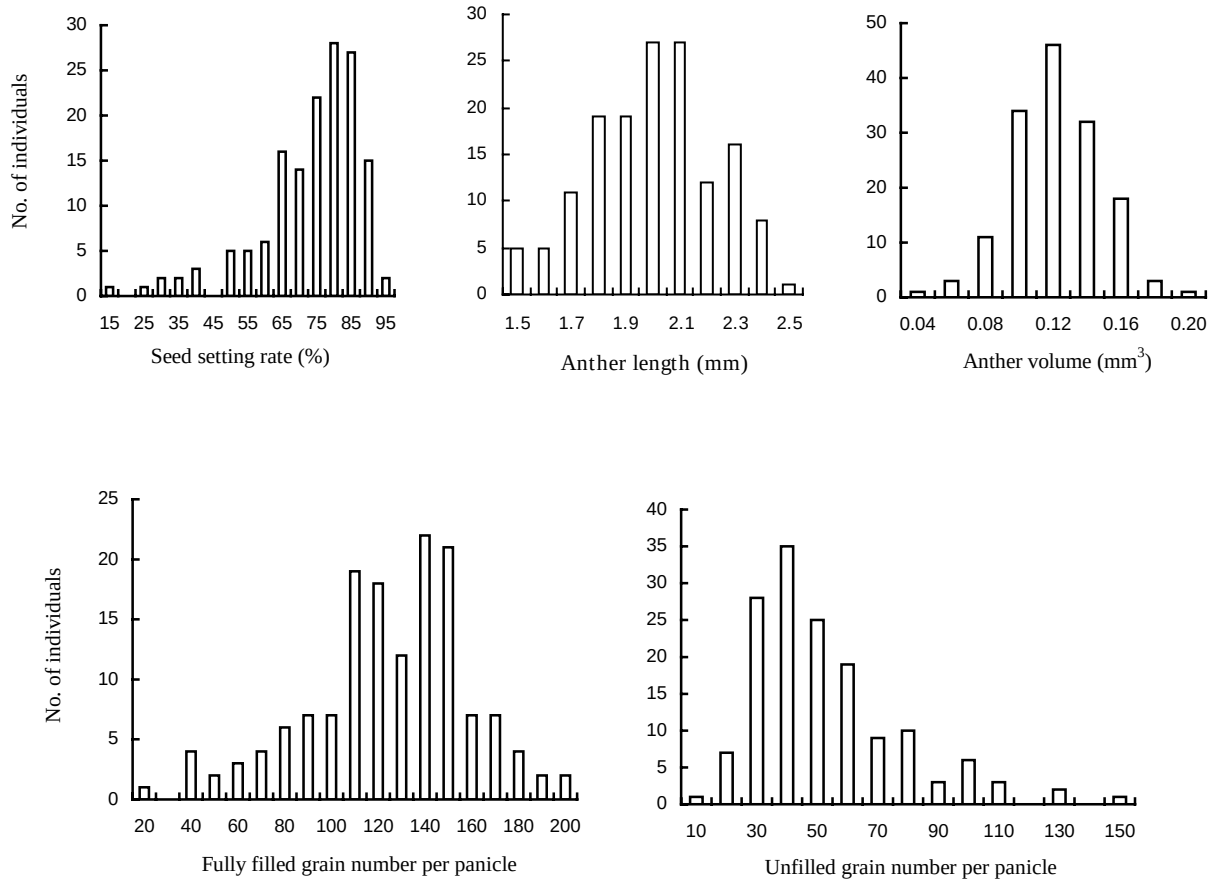

Fig. 1. Frequency distribution of NIL traits.

possibly linked with the cold gene at booting stage through one-way ANOVA, then 12 SSR markers around this SSR (RM31) were used to detect polymorphism between RP and NIL, and it was found that RM7452 displayed polymorphism. So two SSR markers were linked with the cold tolerance gene at booting stage.

One-way ANOVA of SSR and traits of cold tolerance

The traits of cold tolerance with SSR makers were tested by analysis of variance procedures using each marker as a treatment by SPSS software (Fig. 2). The makers RM31, RM7452 were strongly and significantly related to filled grain number per panicle and seed setting rate (Table 2). The results indicated the presence of the gene for high seed setting rate at the long arm of chromosome 5 (http://www.gramene.org).

Estimate effect and location of cold tolerance gene at booting stage

By using these markers, the gene of cold tolerance at booting stage was mapped on

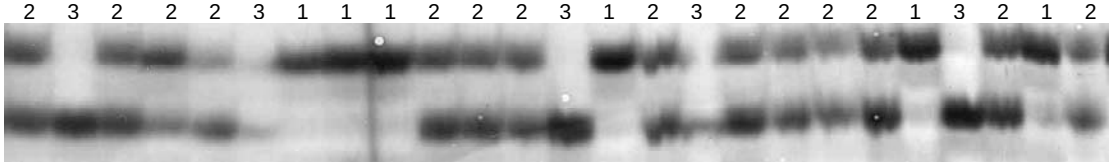

Fig. 2. Segregation of RM7452 at BC_5F_2 population.

1, Stands for Towada; 2, Stands for heterozygous bands; 3, Stands for KMXMG and NIL.

Table 2. One-way ANOVA of traits about cold tolerance among SSR genotype in BC_5F_2 population.

Marker	Anther length		Anther volume		Fully filled grain number per panicle		Unfilled grain number per panicle		Seed setting rate of the main panicle	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
RM31	0.31	0.73	1.41	0.25	5.56**	0.00	3.84*	0.02	10.84**	0.00
RM164	0.20	0.82	0.66	0.52	0.05	0.96	1.21	0.30	0.92	0.40
RM7452	0.19	0.83	1.07	0.35	8.16**	0.00	3.59*	0.03	12.90**	0.00

Table 3. Composite interval mapping of cold tolerance gene at booting stage in BC_5F_2 population.

Traits of the main panicle	Gene	Marker interval	NML	Distance interval (cM)	Peak LOD	Additive (%)	PVE (%)
MPSSR	*Ctb*(t)	RM31–RM7452	RM7452 (4.8 cM)	12.8	6.80	16.85	10.50
NFG	*Ctb*(t)	RM31–RM7452	RM7452 (4.8 cM)	12.8	3.48	6.82	5.10

MPSSR, Seed setting rate of the main panicle; NFG, No. of fully-filled grains per panicle; NML, Nearest marker locus to the gene; PVE, Phenotypic variance explained by the gene.

chromosome 5 between SSR marker RM7452 and RM31 with g enetic distances of 4.8 cM and 8.0 cM, respectively, and tentatively designated as *Ctb*(t) (Table 3, Fig. 3). Seed setting rate of the main panicle explained 10.50% of phenotypic variance, with a peak LOD of 6.80, and the additive effect of KMXBG at this locus increased seed setting rate by 16.85%; and fully filled grain number per panicle explained 5.10% of phenotypic variance, with a peak LOD of 3.48, and had the additive effect of increasing fully filled grain by 6.82%.

DISCUSSION

In this study, we mapped the gene responsible for cold tolerance at booting stage located on the long arm of chromosome 5 by using SSR analysis. The gene detected in this study did not correspond with any of those previously identified. This gene accounted for 10.5% of the total phenotypic variation, and will be useful for enhancing cold tolerance. The SSR markers linked with the gene conferring cold tolerance will be the effective tools for marker-assisted breeding. Therefore, it should be necessary to determine more precise chromosome locations and to identify more tightly linked DNA markers.

Saito et al [8, 9] detected two QTLs controlling anther length at booting stage in the tropical japonica cultivar, Silewah, on chromosome 3 and 4 by using RFLP analysis. The map distance is estimated to be 4.7–17.2 cM. Kato[10] detected one QTL controlling seed setting rate at booting stage in the japonica cultivar, Hokkai PL5, on chromosome 11 using One-way ANOVA, the marker D181 was linked with the QTL. Anday et al [11] identified two QTLs controlling seed setting rate at booting stage with RILs(M202/IR50), on chromosome 2 and 3 using SSR analysis, which explained about 11% and 17% of the total phenotypic variation, respectively. Yoshinobu [12] found three QTLs controlling seed setting rate at booting stage with DHLs (Akihikari/Koshihikari), on chromosome 1,7 and 11 using RFLP and RAPD analysis; *qCT-7* explained about 22.1% of the total phenotypic variation, and the other two QTLs

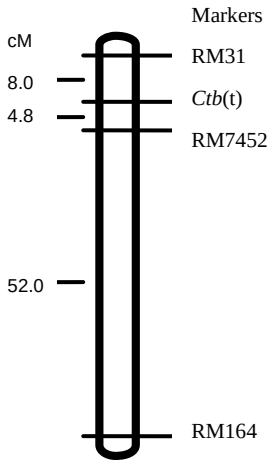

Fig. 3. Linkage map of cold tolerance gene *Ctb*(t) at the booting stage on chromosome 5.

each accounted for approximately 5% of the total phenotypic variation. Liu et al [13] detected three QTLs controlling seed setting rate at booting stage on chromosome 1, 6 and 11, which explained about 5% of the total phenotypic variation. Li [14] identified three QTLs controlling sterile under low temperance. Ye et al [15] detected two QTLs controlling seed setting rate at booting stage on chromosome 3 and 7. Dai [3] located seven QTLs controlling seed setting rate at booting stage on chromosomes 1, 3, 6, 7, 10 and 12 using F_2 population, found four QTLs on chromosome 2, 6, 7 and 8 using F_3 population, and detected three QTLs on chromosome 1, 8 and 10 using F_4 population using SSR and RFLP analysis. Those results indicated that the mechanism of cold tolerance at booting stage was very complex. In this study, we identified one linked gene for cold tolerance on chromosome 5 using PCR-based SSR marker. Our ultimate goal is to develop cold-tolerant varieties utilizing these cold tolerance genes; so marker-assisted selection might be effective by NIL analysis in removing unfavorable trait loci. Most of the permanent mapping population developed have been based on indica / japonica cross. However, it is often difficult to evaluate traits of cold tolerance due to the wide variation of heading dates in the progeny, therefore, development of NILs might be more effective not only for genetic analysis of such complex traits but also for the improvement of agricultural traits.

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