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Genetic Aanalyses of Quantitative Trait Loci for Cold Tolerance at the Booting Stage in Rice (*Oryza sativa* L.)

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Summary

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 and severe yield reduction. Genetic analysis has shown that cold tolerance is a complex trait and that many genes are involved in this trait. Therefore, identification of loci involving cold tolerance is essential for genetic improvement of cold tolerance. In the present study, Novel quantitative trait loci (QTLs) associated with cold tolerance at the booting stage (CT) in rice were analyzed using a cold-tolerant breeding line, Hokkai-PL9 and a cold-tolerant cultivar, Hatsushizuku. CT was evaluated on the basis of seed fertility after cold water treatment. QTLs for heading time (HT) and culm length (CL) were also analyzed.

1) A QTL for cold tolerance at the booting stage of a cold-tolerant rice breeding line, Hokkai-PL9, was analyzed. A total of 487 simple sequence repeat (SSR) markers distributed throughout the genome were used to survey for polymorphism between Hokkai-PL9 and a cold-sensitive breeding line, Hokkai287, and 54 markers were polymorphic. Single marker analysis revealed that markers on chromosome 8 are associated with cold tolerance. By interval mapping using an F2 population between Hokkai-PL9 and Hokkai287, a QTL for cold tolerance was detected on the short arm of chromosome 8. The QTL explains 26.6% of the phenotypic variance, and its additive effect is 11.4%. Substitution mapping suggested that the QTL is located in a 193-kb interval between SSR markers RM5647 and PLA61.

2) CT was evaluated on the basis of seed fertility after cool-water treatment for three years (2005-2007) in 114 recombinant inbred lines (RILs) between temperate japonicas, Kirara397 (cold-sensitive) and Hatsushizuku (cold-tolerant). Composite interval mapping was performed to identify a quantitative trait locus for cold tolerance. A QTL for CT 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. A QTL for HT and that for CL were 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 CT between Kirara397 and Hatsushizuku without affecting HT and CL.

3) QTL analysis for CT was performed using 84 RILs derived from a cross combination between Hokkai-PL9 and Hokkai287. Twelve QTLs for CT were detected on chromosomes 1, 2 (two QTLs), 3 (three QTLs), 4, 7, 8, 10 and 11 (two QTLs) by interval mapping. *qCTB8* on the short arm of chromosome 8 was detected in five out of six trials, and its phenotypic variance explained (PVE) ranged from 14.08 to 20.36% . Comparatively large

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PVE values (7.68-15.04%) were obtained for *qCTB1* that was detected on the same region of chromosome 1 as Hatsushizuku, *qCTB10* and *qCTB11.1*. The Hokkai-PL9 alleles on the four QTLs increased CT. The effect of Hokkai-PL9 alleles of *qCTB8* and each of *qCTB1*, *qCTB10* and *qCTB11.1* were additive to increase CT, suggesting that the QTL combination is effective for CT improvement. *qCTB1* was not affected by HT and CL, because no QTL for HT and CL was detected on the neighbouring region of *qCTB1*. The Hokkai-PL9 alleles of *qCTB3.3*, *qHT3.2* and *qCL3* on the same region of chromosome 3 decreased phenotypic values. This result suggests that CT evaluation might be affected by the QTL for HT or CL.

The results of the present study showed that Hokkai-PL9 and Hatsushizuku harbor major and multiple minor QTLs for CT and that the effect of some QTLs for CT is additive. DNA markers flanking QTLs for CT identified here are applicable for marker-assisted selection with higher efficiency and precision than the conventional CT selection and will be useful for breeding of cultivars with improved cold tolerance.