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Microarray-Assisted Fine-Mapping of Quantitative Trait Loci for Cold Tolerance in Rice

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ABSTRACT Many important agronomic traits, including cold stress resistance, are complex and controlled by quantitative trait loci (QTLs). Isolation of these QTLs will greatly benefit the agricultural industry but it is a challenging task. This study explored an integrated strategy by combining microarray with QTL-mapping in order to identify cold-tolerant QTLs from a cold-tolerant variety IL112 at early-seedling stage. All the early seedlings of IL112 survived normally for 9 d at 4–5°C, while Guichao2 (GC2), an *indica* cultivar, died after 4 d under the same conditions. Using the $F_{2:3}$ population derived from the progeny of GC2 and IL112, we identified seven QTLs for cold tolerance. Furthermore, we performed Affymetrix rice whole-genome array hybridization and obtained the expression profiles of IL112 and GC2 under both low-temperature and normal conditions. Four genes were selected as cold QTL-related candidates, based on microarray data mining and QTL-mapping. One candidate gene, LOC_Os07g22494, was shown to be highly associated with cold tolerance in a number of rice varieties and in the $F_{2:3}$ population, and its overexpression transgenic rice plants displayed strong tolerance to low temperature at early-seedling stage. The results indicated that overexpression of this gene (LOC_Os07g22494) could increase cold tolerance in rice seedlings. Therefore, this study provides a promising strategy for identifying candidate genes in defined QTL regions.

Key words: QTL; fine-mapping; cold tolerance; rice.

INTRODUCTION

Cold stress is one of the major constraints on rice (*Oryza sativa* L.) growth at the early-seedling stage. In south and south-east Asia, modern rice varieties cannot be planted in an estimated 7 million hectares of land because of low-temperature stress (Sthapit et al., 1998). Previous studies have identified several quantitative trait loci (QTLs) for cold tolerance at the early-seedling, seedling, and booting stages using doubled-haploid lines, the backcross population, or the F_2 population, respectively (Li et al., 1997; Qian et al., 1999; Takeuchi et al., 2001; Andaya et al., 2003; Liu et al., 2003; Andaya and Tai, 2006). However, few genes related to these QTLs have been isolated because of their complex genetic basis. With the development of near-isogenic lines (NILs), a single genomic segment, containing the QTL in a relatively uniform genetic background, was identified, in which the molecular basis underlying allelic variation at the QTL was found to be similar to the identified variation for a simple Mendelian locus, namely alterations in gene expression or protein function (Paran and Zamir, 2003). Using NILs, Saito et al. (1995, 2004, 2010) delimited a cold-tolerant QTL at booting stage to a 17-kb region, which contained two genes on chromosome 4 that encoded an F-box protein and a ser/thr protein kinase. They further characterized the target gene (F-box protein gene) by transforming the rice. Fujino et al. (2008) identified a major QTL, *qLTG3-1*, which controlled low-temperature germinability in rice.

Although genetic approaches are extremely powerful and unbiased, delimiting a QTL to a single gene is still a time-consuming and technically demanding process (Fridman et al., 2000, 2004). The increasing availability of genomic resources and rice whole-genome microarray analysis has provided additional information that has helped define the candidate

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genes. A transcriptomic analysis could provide a route to link genetic changes with phenotypes (from genotyping to phenotyping). Additionally, the whole-genome sequences of rice that have already been released could help researchers to compare the genome DNA differences between the two rice subspecies. By combining QTL-mapping with microarray analysis, Wanyne and McIntyre (2002) identified 34 candidate genes within a quantitative trait in *Ovariole number*. Baxter et al. (2005) used a similar strategy to localize the candidate genes to the mapped QTLs by comparing the gene expression changes of tomato fruit in the introgression lines.

In this study, we used IL112, which showed high cold tolerance, as a donor, and mapped seven cold-tolerant QTLs using the $F_{2:3}$ population derived from the cross progeny of GC2 and IL112. Then expression profiles for IL112 and GC2 under cold conditions were created. Finally, by combining QTL-mapping and microarray analysis, we identified one candidate gene (LOC_Os07g22494) that had a high association with cold tolerance. The overexpression of the candidate gene could increase cold tolerance in rice early seedlings.

RESULTS

IL112 Cold Tolerance

In order to evaluate the cold tolerance of IL112 at the early-seedling stage, several analyses on IL112 and GC2 were performed. The results showed that the GC2 early seedlings could not recover after the seedlings were grown at 4–5°C for 4 d. However, the IL112 early seedlings could survive 9 d of cold treatment (Figure 1A), but 40% died after 10 d of cold treatment. Furthermore, the fresh weights of 10 seedlings of IL112 were 11.6%, 10.7%, 27.4%, and 96.6% more than that of GC2 after 12, 36, 72, and 96 h, respectively, of treatment at 4–5°C and with 7 d of recovery. The fresh weight of GC2 became 0 because of their complete death after 120 h (5 d) treatment, while the fresh weight of IL112 seedlings was still 0.48 g (Figure 1B). No significant fresh-weight difference was observed between IL112 and GC2 under normal conditions (Figure 1B). These results indicated that IL112 was more cold-tolerant than GC2 at the early-seedling stage.

QTL Analysis

In an attempt to map the QTLs for cold tolerance at the early-seedling stage, we crossed IL112 with GC2 and developed an $F_{2:3}$ population, which included 394 F_2 plants and 394 F_3 families that were derived from self-pollinated F_2 plants. Based on the survival seedling rate of the $F_{2:3}$ population and 176 polymorphic simple-sequence repeat (SSR) markers, putative QTLs were analyzed for cold tolerance at the early-seedling stage. Seven cold-tolerant QTLs were detected on chromosomes 1, 2, 5, 6, 7, and 10 (Table 1 and Supplemental Figure 1). Each QTL could explain 8%~20% of total phenotypic variation. With the exception of *qCST6*, the additive effect of the other QTLs was positive, which suggested that the IL112-derived alleles could increase the cold tolerance of the population (Table 1).

Exploring Candidate Genes Related to Cold Tolerance

In order to further explore the candidate genes related to cold tolerance, microarray hybridization analysis was performed using RNAs extracted from GC2 and IL112. The Affymetrix rice whole-genome array, containing 51 279 transcripts, was chosen. After 1 week's growth, the early seedlings were subjected to 4–5°C for 12 h and 36 h and the whole seedlings were then harvested. Seedlings grown under normal conditions were harvested at the same time and used as a control. Based on the results of the microarray, the expression patterns of all the genes were analyzed within the whole genome. As shown in Figure 2A, there were 2304 and 1937 probe sets showing expression changes between IL112 and GC2 under cold treatment and normal conditions, respectively. There were also 3700 probe sets changed in expression patterns between the normal and cold conditions in IL112. The Venn diagram shows that 139 probe sets (Supplemental Table 1) exhibited significant expression changes under all the above conditions.

To narrow down the number of target candidate genes, a comparative genomics approach was applied. A search was undertaken for genomic sequence variation in these 139 probe sets between Nipponbare (*O. sativa* ssp. *japonica*), which is

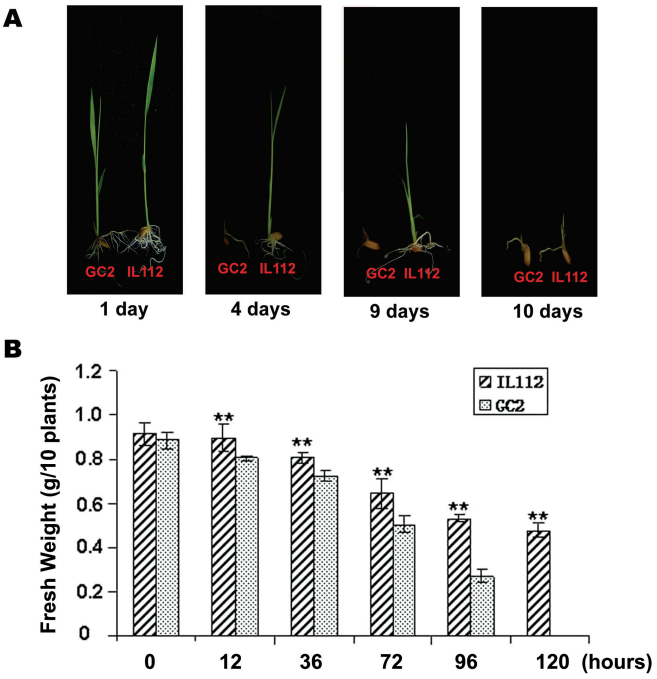

Figure 1. Effects of Low Temperature on GC2 and IL112.
(A) Early seedlings of GC2 and IL112 were treated for 1, 4, 9, and 10 d at 4–5°C, with a recovery period of 7 d.
(B) Fresh weight of 10 seedlings of IL112 and GC2 after different time treatment at 4–5°C and with a recovery period of 7 d.