Identification of QTLs for Rice Cold Tolerance at Plumule and 3-Leaf-Seedling Stages by Using QTLNetwork Software

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Abstract: A doubled haploid (DH) population consisted of 120 lines, derived from a cross between an indica variety, TN1, and a japonica variety, Chunjiang 06, was used to identify QTLs controlling rice cold tolerance at the plumule and 3-leaf-seedling stages by using the QTLNetwork software. The percentages of normal plumules after 4°C treatments for 7 d, 9 d, 11 d, and 14 d, as well as the cold stress tolerance index (CSTI) and the withering index (WI) of rice seedling were investigated. A total of five single-effect QTLs, each for percentages of normal plumules after 4°C treatments for 9 d, 11 d and 14 d, and CSTI and WI, respectively were identified. The QTLs for the percentages of normal plumules after low temperature treatments for 9 d, 11 d and 14 d were on chromosomes 4, 2 and 11, accounting for 14.1%, 17.3% and 21.5% of the phenotypic variation, respectively. QTLs for CSTI and WI were on chromosomes 10 and 1, respectively. Two pairs of epistatic loci were identified, but none of the epistatic loci involved the single-effect QTLs. The RM528–RM340 interval on chromosome 6 interacted with the RM278–RM3919B interval on chromosome 9 for CSTI, and the epistatic interaction accounted for 17.7% of the phenotypic variation. A pair of epistatic loci was identified for WI, the RM246–RM5461 interval on chromosome 1 interacted with the ISA–RM447 interval on chromosome 8, which accounted for 22.6% of the phenotypic variation.

Key words: rice; cold tolerance; quantitative trait loci; plumule; QTLNetwork software

Cultivated rice is sensitive to temperature below 15–20°C (Yoshida et al, 1996; Nakagahra et al, 1997). The optimum temperature for seed germination and early seedling growth of rice ranges from 25°C to 35°C (de los Reyes et al, 2003). Low-temperature stress is a common phenomenon encountered in temperate or high-altitude rice-growing regions when rice plants are grown in early spring, because water or soil temperature is frequently below 15°C (Zhang et al, 2005). During the early growth stage of rice, the seedlings are often affected and injured by cold temperature. The types of low-temperature effects on seedlings can be manifested as poor germination, slow growth, discoloration or yellowing, withering after transplanting, reduced tillering, stunted growth, delayed heading and sterility (Kaneda et al, 1974; Mackill et al, 1997). The seedling tolerance to cold stress is also very important for direct-seeding cultivation system. Therefore, developing cultivars tolerant to low-temperature stress is of considerable importance to rice production in early spring in temperate or subtropical rice-growing regions.

The tolerance of rice to low-temperature stress is complex and controlled by QTLs. Zhang et al (2005) detected a major QTL (qSCT-11) on chromosome 11 for 10°C/13-day cold treatment at the early seedling stage, which explained 29.8% of the phenotypic variation. Andaya and Mackill (2003a) identified a large-effect QTL (qCTS12a) on chromosome 12 at the RM101–RM292 interval, which accounted for 40.6% of the phenotypic variation. Andaya and Tai (2006) fine mapped this QTL to a 55-kb region containing eight candidate genes, and considered that the most likely candidates for the gene(s) underlying this QTL were OsGSTZ1 and OsGSTZ2. A number of QTLs were detected for seedling cold tolerance (Qian et al, 2000; Qu et al, 2003), cold tolerance at the booting stage (Andaya and Mackill, 2003b), and low-temperature germination ability (Miura et al, 2001; Teng et al, 2001; Gong et al, 2009; Ji et al, 2009) in rice, but most of the QTLs detected had small effect, only a few of them explained more than 20% of
the phenotypic variation. These results confirm the complexity of the genetic mechanism of rice tolerance to low-temperature stress.

Although there have been some reports about the QTLs affecting rice cold tolerance, the studies on the epistatic loci involved in the low-temperature response were scarce. Ji et al (2008) identified some digenic epistatic loci for rice low-temperature germination ability using QTLMapper, a software for identifying digenic epistatic loci (Wang et al., 1999). Four pairs of digenic epistatic loci for rice seedling cold tolerance were also detected using QTLMapper (Qu et al., 2003). Zhang et al. (2005) detected nine digenic epistatic loci for cold tolerance at the early seedling stage by using the same software. To date, a more advanced version of this software named QTLNetwork was distributed by Zhu’s laboratory in 2007 (Yang et al., 2008). QTLNetwork reduces the false positive QTLs and increases the accuracy of the mapping results compared to QTLMapper.

In the present study, a doubled haploid (DH) population derived from a cross between an indica rice variety (TN1) and a japonica rice variety (Chunjiang 06) was used in QTL analysis. The 1670.92 cM genetic map developed using this population had an average marker interval of 9.44 cM, and has been used to identify QTLs for tolerance to alkali (Cheng et al., 2008).

**MATERIALS AND METHODS**

**Plant materials**

A DH population consisted of 120 lines, derived from a cross between an indica rice variety (TN1) and a japonica rice variety (Chunjiang 06) was used to detect single-effect (single-locus effect) and digenic epistatic QTLs at the plumule and seedling stages by using the QTLNetwork software.

**Low-temperature treatment to plumules**

About 20 plumules (at 2–3 days after germination) for each DH line were treated at 4°C constant temperature for 7 d, 9 d, 11 d and 14 d, respectively, then recovered at normal temperature (28°C/24°C day/night temperature) under a photoperiod of 12 h light/12 h dark for 7 d. The frequencies of normal plumules (with healthy green color) were calculated after treatments.

**Low-temperature treatment to seedlings at the 3-leaf stage**

Since stunted growth is one of the important characteristics of the seedlings injured by cold, we separated seedlings at the 3-leaf stage for each DH line into two groups (10 seedlings in each group): one under the normal temperature conditions (28°C/24°C day/night temperature, 12 h light/12 h dark photoperiod), and the other under the low-temperature conditions (12°C/10°C day/night temperature, 12 h light/12 h dark photoperiod). The two groups were kept under each temperature condition for 4 d, then the cold stress tolerance index (CSTI) of seedlings was calculated using the following formula: CSTI = (seedling height under the normal conditions – seedling height under the low-temperature conditions)/seedling height under the normal conditions×100%. Seedling height is the mean height of the seedlings under each temperature condition (the highest and the lowest seedlings were excluded).

To examine the leaf withering or yellowing in each DH line after the low-temperature treatments, the seedlings at the 3-leaf stage for each DH line were treated at a 12°C/10°C of day/night temperature (12 h light/12 h dark photoperiod) for 8 d, followed by a 10°C/8°C of day/night temperature (12 h light/12 h dark photoperiod) for 4 d. Afterward, the withering index (WI) was recorded using the scales of 1.0 (highly tolerant to cold, healthy leaf color), 1.5 and 2.0 (moderately tolerant to cold, the leaf color is between green and yellow), 2.5 and 3.0 (susceptible to cold, yellow leaf), 3.5 and 4.0 (leaf withered and the seedling died).

All plumules and seedlings were placed in transparent bottles with nutritional solution (0.01 mol/L urea). The low-temperature treatments were done in a SANYO Versatile Environmental Test Chamber (Model MLR-351H, SANYO Electric Co., Ltd.).

**QTL analysis**

QTL analysis was performed for six traits, i.e. percentages of normal plumules under 4°C low-temperature treatments for 7 d, 9 d, 11 d and 14 d, as well as CSTI (cold stress tolerance index) and WI (withering index). Before QTL analysis, the ‘PROC
UNIVARIATE’ procedure in SAS software (version 8.1) was used to check whether the trait data were normal distribution. Those trait data which were not normal distribution were subjected to arcsine square-root transformation. The transformed data were used for QTL analysis. The software QTLNetwork (version 2.0) was used to detect main effect loci and putative digenic epistatic loci. The mapping procedures used in QTLNetwork were based on the mixed linear model (Yang et al, 2008). The nomenclature of QTL followed McCouch et al (1997).

RESULTS

Phenotypic data of the DH population

The percentages of normal plumules after the low temperature treatment for 7 d and recovery for 7 d in the DH population varied from 0% to 100%, with most of the DH lines >90% (Fig. 1-A). The distributions of the percentage of normal plumules after the low temperature treatments for 9 d, 11 d and 17 d and recovery for 7 d were apparently different from the low temperature treatment for 7 d (Fig. 1-B, C and D). The longer the low temperature treatment was, the less the percentage of normal plumules was (Fig. 1). The percentage of normal plumules after the low temperature treatment for 11 d showed continuous distribution (Fig. 1-C), and most of the DH lines exhibited <10% normal plumule frequency after the treatment for 14 d (Fig. 1-D).

Of the 120 lines examined in the DH population, only one line was found with the CSTI score below zero, i.e. -1.66%. This indicates that the seedlings in this DH line grew higher under the low-temperature conditions than under the normal temperature conditions. The CSTI scores of the other 119 lines ranged from 11.34% to 58.01%. Furthermore, CSTI and WI displayed normal distribution (Fig. 2).

Single-effect QTL

A total of five single-effect QTLs were detected for the percentages of normal plumules after the low temperature treatments for 9 d, 11 d and 14 d, and for CSTI and WI (Fig. 3). The QTL q9d-4 for the percentage of normal plumules after the low temperature treatment for 9 d was detected at the RM3735–RM252 interval on chromosome 4, explaining 14.1% of the phenotypic variation (Table 1). While the QTLs for the percentage of normal plumules after the low temperature treatments for 11 d and 14 d were located
on chromosomes 2 and 11, respectively. These indicate that the trait of the percentage of normal plumules under the low temperature treatments for various days was controlled by different loci on different chromosomes in this mapping population. A relatively large-effect QTL for the percentage of normal plumules after the low temperature treatment for 14 d, q14d-11, was identified on chromosome 11, which explained 21.5% of the phenotypic variation. A QTL for CSTI, qCST-10, was detected on chromosome 10, explaining 11.9% of the phenotypic variation. The QTL qWI-1 for WI identified on chromosome 1, accounting for 11.8% of the phenotypic variation. No QTL was detected for the percentage of normal plumules after the low temperature treatment for 7 d.

**Putative digenic epistatic QTL**

Epistatic effects were detected for two pairs of loci (Table 2), but none of the epistatic loci involved the single-effect QTLs. For CSTI, the RM528–RM340 interval on chromosome 6 interacted with the RM278–RM3919B interval on chromosome 9 (Fig. 4), and the epistatic interaction accounted for 17.7% of the phenotypic variation. For WI, the RM246–RM5461 interval on chromosome 1 interacted with the ISA–RM447 interval on chromosome 8 (Fig. 4), and the

**Table 1. Main effect QTLs for the percentages of normal plumules after the low temperature treatments for 9 d (4°C/9 d), 11 d (4°C/11 d) and 14 d (4°C/14 d), and for cold stress tolerance index (CSTI) and withering index (WI) detected using QTLNetwork (version 2.0).**

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL</th>
<th>Chromosome</th>
<th>Marker interval</th>
<th>F</th>
<th>P-value</th>
<th>h² (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C/9 d</td>
<td>q9d-4</td>
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<td>RM3795–RM252</td>
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<tr>
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<td>RM286–RM1812</td>
<td>16.24</td>
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<td>0.215</td>
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<tr>
<td>CSTI</td>
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<td>RM271–RM258</td>
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<td>RM1282–RM428</td>
<td>12.19</td>
<td>0.00013</td>
<td>0.118</td>
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</table>
epistatic interaction accounted for 22.6% of the phenotypic variation. Digenic epistatic QTLs were not detected for the percentage of normal plumules after the low temperature treatments.

**DISCUSSION**

It has been shown that the chilling sensitivity in rice varied during the life cycle and the cold tolerance at the vegetative growth stage appeared to be independent of that at the reproductive growth stage (Zhang et al., 2005). In the present study, QTLs for the percentage of normal plumules after the low temperature treatments for 9 d, 11 d and 14 d were detected on chromosomes 4, 2 and 11, respectively, whereas QTL for the percentage of normal plumules after the low temperature treatment for 7 d was not identified. These results suggest that the trait of the percentage of normal plumules after the low temperature treatment for various days is controlled by different loci in the present mapping population.

In previous studies, some major QTLs explaining large proportion of the phenotypic variation for rice cold tolerance were detected. Yan et al (1999) detected a major QTL on chromosome 7 for rice cold tolerance at the early seedling stage, which explained 39% of the phenotypic variation. Andaya and Mackill (2003a) identified a major QTL (qCTS12a) on chromosome 12, which accounted for 40.6% of the phenotypic variation. Fujino et al (2004) identified a QTL (qLTG-3-1) on chromosome 3 controlling low-temperature germinability, which accounted for 35.0% of the phenotypic variation. Zhang et al (2005) identified a major QTL (qSCT-11) on chromosome 11 at the RM202–RM209 interval for seedling cold tolerance, which explained 29.8% of the phenotypic variation. In the present study, we detected a relatively large-effect QTL (q14d-11) on chromosome 11 at the RM286–RM1812 interval, which accounted for 21.5% of the phenotypic variation. The q14d-11 identified in the present study is apparently different from the qSCT-11 detected by Zhang et al (2005) when comparing their locations. The QTL q14d-11 may have potential in rice breeding due to its large effect. The validation and fine mapping of the q14d-11 should be the first step before it can be used in production.

It has been known that the software QTLMapper can be used to identify digenic epistatic QTLs. Although there have been some reports on the epistatic loci involved in low-temperature tolerance in rice, most of the digenic epistatic QTLs detected with the QTLMapper software had small effect. For instance, each of the total nine digenic epistatic QTLs controlled seedling cold tolerance identified by Zhang et al (2005) explained less than 10% of the phenotypic variation. Ji et al (2008) detected 10 pairs of digenic epistatic QTLs for low-temperature germinability using QTLMapper, but only 3 of the epistatic QTLs explained more than 10% of the phenotypic variation. The QTINetwork software is an advanced version of the QTLMapper. It reduces the false positive QTLs detected and increases the accuracy of the mapping results. In the present study, we identified two pairs of epistatic QTLs for seedling cold response using QTINetwork. A pair of digenic

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chromosome-a</th>
<th>Marker interval for QTL-a</th>
<th>Chromosome-b</th>
<th>Marker interval for QTL-b</th>
<th>Epistasis (AA)</th>
<th>SE</th>
<th>P-value</th>
<th>h²(aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSTI</td>
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<td>RM528–RM340</td>
<td>9</td>
<td>RM278–RM391B</td>
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<td>0.012</td>
<td>0.0001</td>
<td>0.177</td>
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<tr>
<td>WI</td>
<td>1</td>
<td>RM246–RM5461</td>
<td>8</td>
<td>ISA–RM447</td>
<td>0.308</td>
<td>0.058</td>
<td>&lt;0.0001</td>
<td>0.226</td>
</tr>
</tbody>
</table>

QTL-a and QTL-b, Two loci involved in epistatic interaction. Chromosome-a and chromosome-b, Two chromosomes on which QTL-a and QTL-b located, respectively. h²(aa), The heritability of additive by additive effect. SE, The standard error of estimated QTL effect.
epistatic loci was detected for CSTI which accounted for 17.7% of the phenotypic variation. Another digenic interaction was identified for WI, explaining 22.6% of the phenotypic variation. The epistatic QTLs for seedling cold response identified in the present study were different from those of Qu et al (2003b) and Zhang et al (2005), who also examined rice cold tolerance at the early seedling stage. The potential of these epistatic QTLs in rice production is still need to be studied in further works.

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