Mapping QTL for Heat-Tolerance at Grain Filling Stage in Rice

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Abstract: A mapping population of 98 lines (backcross inbred lines, BILs) derived from a backcross of Nipponbare/Kasalath//Nipponbare was planted at two experimental sites, Nanjing and Nanchang, and treated with high and optimal temperature during grain filling, respectively. The grain weight heat susceptibility index \[ GWHSI = \left( \text{grain weight at optimum temperature} - \text{grain weight at high temperature} \right) / \text{grain weight at optimum temperature} \times 100 \] was employed to evaluate the tolerance of rice to heat stress. A genetic linkage map with 245 RFLP markers and a mixed linear-model approach was used to detect quantitative trait loci (QTLs) and their main effects, epistatic interactions and QTL × environment interactions (Q × E). The threshold of LOD score=2.0 was used to detect the significance of association between marker and trait. A total of 3 QTLs controlling heat tolerance during grain filling were detected, on chromosomes 1, 4 and 7, with LOD scores of 8.16, 11.08 and 12.86, respectively, and they explained the phenotypic variance of 8.94, 17.25 and 13.50 %, correspondingly. The QTL located in the C1100–R1783 region of chromosome 4 showed no QTL × environment interaction and epistatic effect, suggesting that it could be stably expressed in different environments and genetic backgrounds, and thus it would be valuable in rice breeding for heat tolerance improvement. This QTL allele, derived from Kasalath reduced 3.31% of the grain weight loss under heat stress. One located between R1613–C970 on chromosome 1 and the other between C1226–R1440 on chromosome 7, with additive effect 2.38 and 2.92%, respectively. The tolerance alleles of both these QTLs were derived from Nipponbare. Both of these QTLs had significant QTL × environment interactions, and the latter was involved in epistatic interaction also. Eight pairs of epistatic effect QTLs were detected, one pair each on chromosomes 1, 2, 3, 5, 7, 8, 10 and 12. The results could be useful for elucidating the genetic mechanism of heat-tolerance and the development of new rice varieties with heat tolerance during grain filling phase.

Key words: rice; grain filling; heat tolerance; quantitative trait locus

Recently, with the radical global climate change and global warming that results from increasing atmospheric concentrations of carbon dioxide and other greenhouse gases, high temperature stress has become one of the major factors exerting serious influence on rice production [1,2]. The most sensitive growth stages of rice to heat stress are booting, heading and grain filling. Heat stress occurring at the booting and heading/flowering stages causes floret sterility, abnormal pollination and ultimately results in low seed setting rate. In contrast, heat stress during grain filling period (GFP) causes the combined economics loss on grain yield and quality. Grain yield loss from the stress is attributed to limited assimilate supply because of the shortening of GFP as there would be lower photosynthesis and higher respiratory consumption. Grain quality loss included overall degradation of grain appearance, milling and cooking quality due to higher percentage of grains with chalkiness, larger square of chalky endosperm, lower head rice recovery, change of fine structure of amylopectin, higher gelatinization temperature, harder gel consistency and elasticity and viscosity, and so on [3-5]. In the single or double-crop rice cropping area around the middle-lower reaches of Yangtze River in China, where the mean daily temperature during mid-summer is 32 to 33 °C or higher, and the highest temperature may reach 35 to 40 °C [6, 7], grain filling of the early-season rice and heading and flowering of the mid-season rice, coinciding with the hot weather period, are particularly subject to heat stress. For example, the continual extremely high temperature in summer of 2003 incurred much heavy loss of rice yield and quality. Hence, it is urgent and need of time to elucidate the genetic mechanism and develop heat-tolerant varieties for improving rice quality, quantity and stability of rice production across wide environmental conditions.

The genetic studies on rice heat-tolerance during heading and flowering stages, where heat injury was
evaluated according to the ratio of seed setting rate under heat stress to that under normal temperature, have been conducted since the 1970s. The results indicated that the heat tolerance of rice was a quantitative trait with high broad-sense and narrow-sense heritabilities. Genotypic differences in heat tolerance of rice were detected [8-11]. However, to date, there were no reports on genetic analysis of heat tolerance during grain filling stage. With the development of molecular technology, it has become possible to detect the quantitative trait locus (QTL) conferring heat-tolerance of rice during grain filling stage and analyze their genetic effects. The QTLs to be detected could facilitate the understanding of genetic mechanism, QTL cloning and marker-assistant selection in rice breeding. Therefore, in this study, we used a permanent backcross inbred lines (BILs) population derived from a cross of indica/japonica and its genetic linkage map to identify QTLs associated with heat-tolerance of rice during grain filling period and to analyse their additive, epistatic interaction and QTL×environment interaction effects by a mixed-linear model approach.

MATERIALS AND METHODS

Plant materials

A mapping population with ninety-eight BILs was used. The BILs, kindly provided by Dr. Yano at Rice Genome Research Program, National Institute of Agrobiological Resources, Tsukuba, Japan, were derived from a cross of indica/japonica and its genetic linkage map to identify QTLs associated with heat-tolerance of rice during grain filling period and to analyse their additive, epistatic interaction and QTL×environment interaction effects by a mixed-linear model approach.

Evaluation of rice heat-tolerance at grain filling stage

The experiments were carried out at two locations, Nanchang, Jiangxi Province and Nanjing, Jiangsu Province in 2003. The seeds of all the lines (including two parents) were sown every 10 days from May 1 to June 1 at Nanchang and May 15 to June 15 at Nanjing. Twenty-eight-day-old seedlings were transplanted to pots, with four seedlings per pot. Each line of every sowing was planted in four pots. The fertilizer and water management followed the normal practice in the region. Short-daylight treatment was performed on the photosensitive lines during their vegetative growth phase to ensure all the lines headed at the same dates approximately. Due to damage by insects or with too fewer filled grains, two and three lines at Nanchang and Nanjing, respectively, were treated as data absence.

At Nanchang, five days after heading, two of the four pots for each line were transferred to a growth chamber with high temperature treatment (6:00–12:00, 36 ℃; 12:00–18:00, 39 ℃; 18:00–24:00, 34 ℃; 24:00–6:00, 27 ℃; 34 ℃ mean daily) and a growth chamber with optimal temperature treatment (6:00–12:00, 24 ℃; 12:00–18:00, 27 ℃; 18:00–24:00, 22 ℃; 24:00–6:00, 19 ℃; 23 ℃ mean daily), respectively, to perform temperature treatments until they maturated. Both treatments were illuminated with natural light.

At Nanjing, two of the four pots for each line were transferred to greenhouse for heat stress treatment from the 5th day after heading till maturity. The other two pots remained outdoors as normal temperature treatment. By window-opening with electric fan-aided ventilation or supplying electric heat when necessary, the mean daily temperature in greenhouse during treatment was maintained at 31.5–37.0 ℃, whereas the outdoor temperature was 22.5–30.0 ℃. Statistics analysis showed that the difference of temperatures between the two environments was remarkable.

Rice grains were harvested at ripening and dried under natural conditions. Five hundred filled grains were randomly selected and then weighed. Mean of the two replications was used to calculate the grain weight heat susceptibility index (GWHSI): GWHSI = ([Grain weight at optimum temperature − Grain weight at high temperature) / Grain weight at optimum temperature] × 100]. The rice tolerance to heat stress at grain filling stage was expressed as GWHSI.

QTL analysis

A set of molecular data was provided by Yano. A linkage map covering 1179.9 cM was constructed. With an average distance of 4.8 cM between two markers, 245 markers were well distributed on 12 chromosomes with the same order as those of previous studies. The chromosomal locations and the number of putative QTLs with additive and epistatic
effects as well as QTL × environment (Q × E) interaction effects were determined by using a mixed linear-model and the corresponding computer software QTLMapper 1.6. A LOD threshold of 2.0 and a significance level of 0.005 were used. Nomenclature for QTLs was followed as described by McCouch et al. [12].

RESULTS

Phenotypic variation of the BILs

The grain weight heat susceptibility indices (GWHSI) of the BIL lines and their parents in the two locations had been listed in Table 1. The heat-tolerances during grain filling were different between the two parents, Nipponbare and Kasalath. Heat tolerance in the BIL population showed a continuous variation, indicating polygenic involvement (Fig. 1). Both skewness and kurtosis values were less than 1.0, suggesting that the segregation of heat tolerance during grain filling in BIL population fitted normal distribution and was suitable for QTL analysis.

Main-effect QTLs and their interactions with environments

Combined data of the two locations were analyzed by QTLMapper 1.6. One QTL was detected on each of chromosomes 1, 4 and 7, with LOD score of 8.16, 11.08 and 12.86, respectively. Their total contribution to total phenotypic variance was 39.69%. Three QTLs, two located in the region of R1613–C970 on chromosome 1 and C1226–R1440 on chromosome 7, respectively, had significant QTL × environment interaction effects, and the latter was also involved in epistatic interaction. The Nipponbare alleles of both QTLs could reduce 2.38 and 2.92% of the grain weight loss under heat stress. The environment of Nanchang was beneficial to expression of heat-tolerance for these two QTLs. The third QTL located between C1100–R1783 of chromosome 4 showed no QTL × environment or epistatic interaction. The tolerance allele derived from Kasalath could reduce 3.31% of the grain weight loss (Table 2, Fig. 2).

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Table 1. Variation of grain weight susceptibility index among the BIL lines and two parents.

<table>
<thead>
<tr>
<th>Trial Location</th>
<th>Parent</th>
<th>BIL Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Nanchang</td>
<td>4.50</td>
<td>11.58</td>
</tr>
<tr>
<td>Nanjing</td>
<td>6.43</td>
<td>12.61</td>
</tr>
</tbody>
</table>

Table 2. Putative main-effect QTL and QTL × environment interactions characterized by QTLMapper combining the data of Nanjing and Nanchang.

<table>
<thead>
<tr>
<th>QTL</th>
<th>Marker interval</th>
<th>Distance (cM) *</th>
<th>LOD score</th>
<th>Additive effect (H²_A, %)</th>
<th>AE (H²_AE, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qHT1</td>
<td>R1613–C970</td>
<td>8</td>
<td>8.16</td>
<td>2.38 (8.94)</td>
<td>1.25 (4.93)</td>
</tr>
<tr>
<td>qHT4</td>
<td>C1100–R1783</td>
<td>0</td>
<td>11.08</td>
<td>-3.31 (17.25)</td>
<td></td>
</tr>
<tr>
<td>qHT7</td>
<td>C1226–R1440</td>
<td>0</td>
<td>12.86</td>
<td>2.92 (13.50)</td>
<td>2.76 (24.09)</td>
</tr>
</tbody>
</table>

*Indicates the distance of QTL from the left marker.
Epistatic interaction analysis

Eight pairs of epistatic interaction QTL which involved 15 loci were detected, one pair each on chromosomes 1, 2, 3, 5, 7, 8, 10 and 12, with epistatic effect from -1.87 to 2.21%. Variation explained by single interaction pair ranged from 2.45 to 5.29%. Four pairs of the eight increased while the others reduced heat tolerance (Table 3, Fig. 2). No epistatic QTL by environment effect was detected.

DISCUSSION

High-temperature stress affected almost all the processes of crop growth and development. A number of physiological, biochemical, morphological, grain yield and quality traits that associated with heat stress had been widely used for heat tolerance evaluation [13-16]. During grain filling period of rice, heat stress mainly leads to produce small and
deformed grains, and decreases fully filled grain number and weight. Hence stability of grain weight under heat stress environment is an important trait in determining the heat tolerance of different rice varieties. Considering the difference of grain weight existed among genotypes, the grain weight heat susceptibility index (GWHSI), i.e. relative reduction in grain weight between normal environment and heat stress environment would be a simple, fast and reliable criterion for heat tolerance evaluation.

One of the main objectives for QTL mapping was to explore the beneficial alleles and detect closely linked molecular markers which can be used for crop breeding strategies. Since the expression of many quantitative traits is influenced by both the environment and interaction among non-allelic genes, the results of QTL mapping under different genetic backgrounds and environments may be different [17–19]. In the present study, the obvious difference in environmental conditions between the two locations had significant impact on expression of heat tolerance. Of the three main-effect QTLs detected, two had significant QTL by environment interaction effect, showing that the heat tolerance of rice was significantly influenced by environment. In addition, among eight epistatic QTL pairs, only one pair involved main-effect QTL, suggesting that the epistatic interactions played an important role in heat tolerance. Thus, effects of environment and genetic background should be considered in varietal improvement for heat tolerance. The QTL located in the region of C1100–R1783 on chromosome 4 showed no QTL×environment interaction or epistatic effect, suggesting it could be stably expressed in different environments and genetic backgrounds, and would be valuable in rice breeding for heat tolerance. Therefore, it is necessary to develop substitution or near-isogenic lines, and then precisely to map this QTL.

Comparing with the other related mapping results, all of the three QTLs detected in the present study are different with the six QTLs for heat tolerance during flowering and grain filling period detected by Cao, using a DH population derived from the cross between an indica variety, IR64, and a japonica variety, Azucena [11]. This result indicated that the genetic mechanism of heat tolerance of rice is quite complex. One reasonable explanation for the complexity may lie in substantial difference for heat tolerance between flowering-setting and grain filling stages. The other plausible reason may arise from the differences of genetic and environmental backgrounds in environmental conditions, due to different mapping populations and experimental locations.

Heat tolerance is the synthetic expression of numerous physiological and biochemical processes of plant [13-16]. For example, several reports have showed that nonstructural carbohydrate accumulation in leaf sheaths and culm was closely associated with yield under heat stress. Photosynthesis provides 90–95% of the carbohydrates in wheat grain under optimum temperature conditions. However, high temperature directly damages the photosynthetic apparatus and reduces the rate and duration of assimilation. Depending on the reduction in photosynthesis, stress

<table>
<thead>
<tr>
<th>QTL</th>
<th>Marker interval</th>
<th>Distance (cM)</th>
<th>QTL</th>
<th>Marker interval</th>
<th>Distance (cM)</th>
<th>LOD score</th>
<th>Epistasis effect (H², %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qHT 1-1</td>
<td>C1370–C122</td>
<td>8</td>
<td>qHT 7-1</td>
<td>C1226–R1440</td>
<td>0</td>
<td>11.16</td>
<td>-1.77 (3.41)</td>
</tr>
<tr>
<td>qHT 1-2</td>
<td>R1928–C178</td>
<td>16</td>
<td>qHT 8</td>
<td>R1963–R2662</td>
<td>4</td>
<td>6.53</td>
<td>-1.87 (3.81)</td>
</tr>
<tr>
<td>qHT 2</td>
<td>C560–C1408</td>
<td>0</td>
<td>qHT 7-2</td>
<td>C1057–R565</td>
<td>10</td>
<td>5.22</td>
<td>2.21 (5.29)</td>
</tr>
<tr>
<td>qHT 2</td>
<td>C560–C1408</td>
<td>0</td>
<td>qHT 12-2</td>
<td>R3375–R2672</td>
<td>0</td>
<td>6.02</td>
<td>-1.72 (3.22)</td>
</tr>
<tr>
<td>qHT 3</td>
<td>C1677–C361</td>
<td>0</td>
<td>qHT 7-3</td>
<td>C451–R1357</td>
<td>0</td>
<td>7.68</td>
<td>2.03 (4.47)</td>
</tr>
<tr>
<td>qHT 5</td>
<td>R830–R3166</td>
<td>0</td>
<td>qHT 12-3</td>
<td>R617–R3375</td>
<td>2</td>
<td>6.79</td>
<td>1.96 (4.16)</td>
</tr>
<tr>
<td>qHT 10-1</td>
<td>C1369–R1877</td>
<td>0</td>
<td>qHT 10-2</td>
<td>G127–C223</td>
<td>0</td>
<td>5.97</td>
<td>1.50 (2.45)</td>
</tr>
<tr>
<td>qHT 12-1</td>
<td>G2140–C443</td>
<td>0</td>
<td>qHT 12-4</td>
<td>C732–G193</td>
<td>0</td>
<td>6.77</td>
<td>-1.67 (3.02)</td>
</tr>
</tbody>
</table>

*a* indicates the distance of QTL from the left marker.
may increase the contribution of stem reserves from 6 to 100% of the mass of grain \[16\]. By comparison with the other mapping results of QTL for related traits associated with heat tolerance, it revealed that the QTLs in the region of R1613–C970 on chromosome 1 and C1100–R1783 on chromosome 4 are identical with that of the QTL for total amount of nonstructural carbohydrate accumulation in leaf sheaths and culm per plant, and for that per spikelet, respectively, which were detected by Nagata \[20\]. The relationships among these QTLs need to be investigated further.

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**REFERENCES**