QTL Mapping for Grain Size Traits Based on Extra-Large Grain Rice Line TD70

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Abstract: Grain size traits, including grain length, grain width and grain thickness, are controlled by quantitative trait loci (QTLs). Many QTLs relating to rice grain size traits had been reported, but their control mechanisms have not yet been elucidated. A recombinant inbred line (RIL) population of 240 lines, deriving from a cross between TD70, an extra-large grain size japonica line with 80 g of 1000-grain weight, and Kasalath, a small grain size indica variety, were constructed and used to map grain size QTLs to a linkage map by using 141 SSR markers in 2010 and 2011. Five QTLs for grain length, six for grain width and seven for grain thickness were detected distributing over chromosomes 2, 3, 5, 7, 9 and 12. Seven QTLs, namely qGL3.1, qGW2, qGW2.2, qGW5.1, qGW5.2, qGT2.3 and qGT3.1, were detected in either of the two years and explained for 56.19%, 4.42%, 29.41%, 10.37%, 7.61%, 21.19% and 17.06% of the observed phenotypic variances on average, respectively. The marker interval RM1347–RM5699 on chromosome 2 was found common for grain length, grain width and grain thickness; qGL3.1 and qGT3.1 were mapped to the same interval RM6080–RM6832 on chromosome 3. All 18 QTL alleles were derived from the large grain parent TD70. Most of the QTLs mapped in the present study were found the same as the genes previously cloned (GW2, GS3 or qGL3, GW5 and GS5), and several were the same as the QTLs (GS7 and qGL-7) previously mapped. Three QTLs, qGL2.2 on chromosome 2, qGW9 and qGT9 on chromosome 9, were first detected. These results laid a foundation for further fine mapping or cloning of these QTLs.

Key words: rice; extra-large grain; recombinant inbred line; grain trait; quantitative trait locus
positioned a new QTL \( qGL3.1 \) using large grain rice variety SLG-1 (1000-grain weight 58.8 g). Zhang et al (2011) found a major QTL marker interval using an extra-large grain material GSL156 (1000-grain weight 71.9 g); Zhang et al (2012) found a major grain length QTL \( qGL3 \) from N411, an extra-large grain japonica variety [1000-grain weight = \((72.13 \pm 2.32)\) g]. In this study, an extra-large grain rice material TD70 (1000-grain weight was 80 g in 2011) and small grain indica rice Kasalath were selected to build RILs for mapping grain size traits. The discovery and analysis of the result may be useful for further fine mapping of grain size QTLs and molecular breeding of new grain type varieties.

**MATERIALS AND METHODS**

**Rice materials**

An RIL population of 240 lines was constructed from a cross between extra-large grain japonica line TD70 (derived from Tian-e-gu///9520//(72-496/Yu-nuo)) and indica variety Kasalath. The 240 \( F_2 \) and \( F_2:8 \) RIL populations and their parents were grown in the experimental field of Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, China, in 2010 and 2011, respectively. All the materials were transplanted 30 d after sowing, and each family was planted into a plot of four rows of ten plants each, with no replications. The plant spacing was 26.7 cm × 16.7 cm. Field management followed normal agricultural practice. At harvest, five plants per family were randomly chosen and sampled from the middle two rows of each plot.

**Trait investigation**

Grain length, grain width and grain thickness were measured by using a vernier caliper (accurate to 0.01 mm). Five individual plants were randomly selected from each of 240 lines and parents. Single grain length, width and thickness were measured from ten grains per plant. The average values of five individuals were used as phenotype values for genetic analysis.

**Data analysis**

Molecular linkage map contains 141 molecular markers (Dong et al, 2012). QTL analysis was conducted by the Inclusive Composite Interval Mapping (ICIM) method with the software QTL IciMapping 3.1. QTLs were detected where their LOD values exceeded 2.5. The QTLs were named according to the method described by McCouch et al (1997).

**RESULTS**

**Grain size performance of the RIL and its parents**

The phenotype performance and variations of the grain traits of the RIL populations and its parents in 2010 and 2011 were listed (Table 1, Fig. 1). In 2010, the mean value of grain length was 9.42 mm, ranging from 7.20 mm to 12.89 mm, the grain width mean value 2.99 mm, from 2.28 mm to 4.28 mm, and the grain thickness mean value 2.14 mm, from 1.74 mm to 2.75 mm. The three grain size values in 2011 were generally higher than those in 2010 due to better grain filling. Grain length and grain width may be controlled by major genes because of the continuous multi-peaked distribution of their performances in TD70/Kasalath RILs, while grain thickness showing continuous single-peaked distribution (Fig. 2). Three traits showed similar frequency distribution during the two years.

![Fig. 1. Grain size of TD70 and Kasalath.](image)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Year</th>
<th>Parent</th>
<th>Average</th>
<th>Range</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain length (mm)</td>
<td>2010</td>
<td>13.22 ± 0.36</td>
<td>8.03 ± 0.22</td>
<td>9.42</td>
<td>7.20–12.89</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>13.70 ± 0.10</td>
<td>8.09 ± 0.06</td>
<td>9.92</td>
<td>7.53–13.69</td>
</tr>
<tr>
<td>Grain width (mm)</td>
<td>2010</td>
<td>4.18 ± 0.27</td>
<td>2.54 ± 0.10</td>
<td>2.99</td>
<td>2.28–4.28</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>4.65 ± 0.11</td>
<td>2.55 ± 0.04</td>
<td>3.17</td>
<td>2.39–4.42</td>
</tr>
<tr>
<td>Grain thickness (mm)</td>
<td>2010</td>
<td>2.86 ± 0.11</td>
<td>1.91 ± 0.10</td>
<td>2.14</td>
<td>1.74–2.75</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>3.01 ± 0.04</td>
<td>1.91 ± 0.04</td>
<td>2.23</td>
<td>1.85–2.86</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SD.
QTL mapping for grain traits in RIL populations

QTL mapping in 2010
Eleven QTLs affecting grain length, grain width and grain thickness were detected on chromosomes 2, 3, 5, 7 and 12 (Table 2, Fig. 3) with the LOD values of these QTLs ranging from 2.68 to 31.25, and they explained 3.39% to 57.22% of the phenotypic variations.

Three QTLs, qGL2.2, qGL3.1 and qGL7.2, affecting grain length were detected. Among them, qGL3.1 was a major QTL with grain length phenotype performance contribution of 57.22% and additive effect of 0.89 cm; it was located between simple sequence repeat (SSR) marker interval RM300 and RM145, and its positive allele was from TD70.

Four QTLs (qGW2.1, qGW2.2, qGW5.1 and qGW5.2) controlling grain width were detected on chromosomes 2 and 5 with the additive effect values ranging from 0.08 cm to 0.20 cm, and the contribution rate and the LOD values ranged from 4.70% to 29.43% and 3.23 to 13.35, respectively. The total grain width phenotype performance contribution was 49.26%. The positive alleles of the four QTLs were all from the large grain parent TD70.

Four QTLs controlling grain thickness were detected, two on chromosome 2 and one each on chromosomes 3 and 12, with the phenotypic contributions ranging from 5.97% to 16.48% and the LOD values ranging from 2.93 to 7.42. The positive alleles of them were all from the large grain parent TD70.

QTL mapping in 2011
A total of 14 QTLs affecting grain length, grain width and grain thickness were detected on chromosomes 2, 3, 5, 7 and 9 (Table 2, Fig. 3). These QTLs explained 3.24% to 55.16% phenotypic variation of grain size traits. The detected QTLs were three to six for each trait with the LOD values ranging from 2.82 to 30.70. Each trait involved a major QTL with LOD value more than 10.

Three grain length QTLs were detected, one each on chromosomes 2, 3 and 7. With a single QTL contribution ranging from 3.97% to 55.16%, they had a joint contribution of 65.52% to grain length phenotype performance. The additive effects were all positive, indicating that the positive alleles were all derived from TD70.

Six grain width QTLs were detected, three locating on chromosome 2, two on chromosome 5 and one on chromosome 9. Among them, qGW2.2 is a major QTL with the highest LOD value of 15.29, and it had a contribution of 29.38% to grain width phenotype.

Five grain thickness QTLs were detected on chromosomes 2, 3, 5 and 9. The LOD values ranged from 3.88 to 14.40 and from 5.07% to 25.89% for the contributions to grain thickness phenotype. The positive alleles of the five QTLs were all derived from TD70.

Between-year comparison
In 2010 and 2011, a total of 18 QTLs affecting grain length, grain width and grain thickness were detected
Table 2. Identification of QTLs for three traits in RIL population and their genetic parameters estimated.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Locus</th>
<th>Marker interval</th>
<th>LOD value</th>
<th>Variation (%)</th>
<th>Additive effect (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain length</td>
<td>qGL2.1</td>
<td>RM1347–RM5699</td>
<td>3.58</td>
<td>6.39</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>qGL2.2</td>
<td>RM341–RM262</td>
<td>2.68</td>
<td>6.21</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>qGL7.1</td>
<td>RM11–RM455</td>
<td>2.82</td>
<td>3.97</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>qGL7.2</td>
<td>RM1209–RM1335</td>
<td>2.84</td>
<td>3.39</td>
<td>0.21</td>
</tr>
<tr>
<td>Grain width</td>
<td>qGW2.1*</td>
<td>RM109–RM3264</td>
<td>3.23</td>
<td>4.70</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>qGW2.2*</td>
<td>RM341–RM262</td>
<td>2.68</td>
<td>4.70</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>qGW3.1*</td>
<td>RM11–RM455</td>
<td>2.82</td>
<td>3.97</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>qGW3.2*</td>
<td>RM1209–RM1335</td>
<td>2.84</td>
<td>3.39</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>qGW9</td>
<td>RM1328–RM7038</td>
<td>2.88</td>
<td>3.24</td>
<td>0.21</td>
</tr>
<tr>
<td>Grain thickness</td>
<td>qGT2.1</td>
<td>RM109–RM3264</td>
<td>3.38</td>
<td>8.09</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>qGT2.3</td>
<td>RM3264–RM279</td>
<td>5.17</td>
<td>8.93</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>qGT3.1*</td>
<td>RM1347–RM5699</td>
<td>7.42</td>
<td>14.40</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>qGT3.2</td>
<td>RM341–RM262</td>
<td>14.40</td>
<td>25.89</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>qGT5</td>
<td>RM11–RM455</td>
<td>3.88</td>
<td>5.07</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>qGT9</td>
<td>RM19–RM247</td>
<td>4.03</td>
<td>5.62</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* indicates locus detected both in 2010 and 2011. Positive additive effect value indicates that the allele is contributed by TD70.

locating on chromosomes 2, 3, 5, 7, 9 and 12 (Fig. 3). Seven of them, locating on chromosomes 2, 3 and 5, were detected in either of the two years.

Three QTLs controlling grain length were detected in 2010 and 2011, but only qGL3.1 was detected in either year. With LOD values of 31.25 in 2010 and 30.70 in 2011 (Table 2), qGL3.1 explained phenotypic contributions of 57.22% and 55.16%, respectively.
Four QTLs for grain width, namely qGW2.1, qGW2.2, qGW5.1 and qGW5.2, were detected in either year, indicative of stable inheritance of this character between years. In 2010, qGW2.1 and qGT2.1 were detected and located on the same interval RM109–RM3264, and in 2011, qGW5.2 and qGT3 were detected and located on the same interval RM169–RM1237.

Seven QTLs controlling grain thickness were detected, and among them, qGT2.3 and qGT3.1 were detected in either year, locating on chromosomes 2 and 3, respectively. Three QTLs detected in 2011, namely qGT2.3, qGW2.2 and qGL2.1, all located on the same interval (RM1347–RM5699), and qGT3.1 and qGL3.1 located on the same interval (RM6080–RM6832). These results showed that some major genes or major QTLs may exit on the chromosomes 2, 3 and 5.

### DISCUSSION

#### Hotspot intervals of grain size QTLs

We found five QTLs for grain length, six QTLs for grain width and seven QTLs for grain thickness, and these QTLs were detected locating on chromosomes 2, 3, 5, 7, 9 and 12. A single QTL explained approximately from 3.39% to 57.22% of the phenotypic variations of grain size traits in the population. Our study found some hot intervals of the grain trait QTLs on chromosomes 2, 3, 5 and 7, which was consistent with previous reports (Lin et al, 1995; Li et al, 1997; Redoña and Mackill, 1998; Xu et al, 2002; Lin and Wu, 2003; Thomson et al, 2003; Ma et al, 2004; Li et al, 2010; Gao et al, 2011).

In this study, qGL2.1, qGW2.2 and qGT2.3 were located in the same interval between RM1347–RM5699 on chromosome 2, qGL3.1 and qGT3.1 between RM6080–RM6832 on chromosome 3 and qGW5.2 and qGT5 between RM169–RM1237 on chromosome 5. These hotspot intervals showed a gene cluster distribution and simultaneous control of multiple grain traits, which may be due to significant correlations among grain length, grain width and grain thickness traits. Further research is needed to determine which factor, pleiotropic effect, overlapping gene or gene linkage, results in the QTL cluster distribution presented in this study.

#### Between-year stability of grain size QTLs

Combined effects of gene, environment and gene-environment interaction result in the performance of quantitative traits in rice. Some minor QTLs may not be detected under different planting environments and in different years (Paterson et al, 1991; Guo et al, 2003).

Seven QTLs, qGL3.1, qGT3.1, qGW2.2, qGT2.3, qGW2.1, qGW5.1 and qGW5.2, were detected in either year locating on chromosomes 2, 3 and 5 in this study. The research results had proved that the QTLs with greater contribution were relatively stable in different environments and in different years. Three QTLs, qGL3.1, qGW2.2 and qGT2.3, were detected contributing over 50.00%, near 30.00% and over 16.00%, respectively, of the phenotypic variations in either year. Their additive effects were at the same direction.

#### Comparison with previous results

Grain size is an important factor for rice yield. The mapping of grain size QTLs has been well documented. Most QTLs detected in this study located at the same or similar intervals to those previously reported (Gao et al, 2011). Our grain size QTL mapping was successful in both years because many important QTLs previously reported were detected with similar results. The interval RM6080–RM6832 contained a major QTL qGL3.1 controlling grain length, which was in agreement with the results by previous researchers (Redoña and Mackill, 1998; Li et al, 2004; Wan et al, 2008; Hu et al, 2012; Qi et al, 2012). In this region, Fan et al (2006) cloned a gene GS3, and Zhang et al (2012) cloned a gene qGL3. Three QTLs qGL2.1, qGW2.2 and qGT2.3 in this study located in the same interval (RM1347–RM5699), and at this interval a grain width gene GW2 was successfully cloned by Song et al (2007). The interval of qGW5.2 and qGT5.1 contained a grain thickness GW5 (Weng et al, 2008). qGW5.1 was a GS5 gene cloned by Li Y B et al (2011). Cai and Morishima (2002) and Li S Q et al (2011) found several grain QTLs located in the same or similar region to qGW2.1 and qGW2.3. In this study, qGL7-2 was located at the same interval on chromosome 7 as qGL7, which had been fine mapped (Bai et al, 2010), and qGL7.1 located at the same site as fine mapped GS7 (Shao et al, 2012).

There had been the least research conducted on grain thickness (Lin et al, 1995; Xu et al, 2002; Lin and Wu, 2003; Ma et al, 2004). We found seven grain thickness QTLs locating on chromosomes 2, 3, 5, 9 and 12. qGT2.1 and qGW2.1 were located in the same interval where another grain QTL was detected by Cai and Morishima (2002). qGT2.3 was located near or at the same site as GW2. The interval of qGL2.2 was also found by Zhang et al (2004), Wang et al (2003) with
the similar results, and qGT12 site was also found by Li et al (2010). Xie et al (2008) had fine mapped a grain locus on chromosome 9, but it was in a different interval from that of qGW9 or qGT9 in this study.

**Larger grain material advantage**

Previous studies have cloned six grain size genes deriving from different genetic background. Except GS5 and GW8, GW2, GS3, GW5 and qGL3 controlled negative regulation, or referred to as missing gene, indirectly increasing grain size due to gene dysfunction. The genetic difference in grain size between parents was essential for associated QTL positioning, and therefore, application of extra-large grain material will contribute to a better QTL mapping. In this study, 18 grain size traits QTLs were detected in two years, including GW2, GS3, qGL3, GW5 and GS5, which have been cloned, and several fine mapped loci. qGT2.1, qGL2.2 and qGT12 locations had been previously reported, and three QTLs qGW9, qGT2.2 and qGT9 were first reported in this study. These results indicated that extra-large grain materials favored the construction of genetic population, and tended to produce multiple QTL mapping of grain size trait. Although this study did not detect GW8 locus, the grain thickness QTL qGT12, detected on chromosome 12, deserves further investigation.

**ACKNOWLEDGEMENTS**

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