Minor-effect QTL for heading date detected in crosses between indica rice cultivar Teqing and near isogenic lines of IR24

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ABSTRACT

Identification of quantitative trait loci (QTL) having small effects on heading date (HD) is important for fine-tuning flowering time in rice (Oryza sativa L.). In this study, minor-effect QTL for HD were identified using five segregating rice populations, including a recombinant inbred line population derived from crosses between indica cultivar Teqing and near isogenic lines of IR24, and four populations derived from residual heterozygotes identified in the original population. HD data from these populations were obtained in multiple years or at two locations with different photoperiods. A total of 11 QTL were detected; they had small additive effects ranging from 0.21 to 1.63 days. The QTL were all detected in different populations, locations and/or years, having consistent allelic effects across experiments and a stable magnitude across years at the same location. These QTL, and other minor-effect QTL that have been cloned or fine-mapped, generally do not have strong photoperiod sensitivity, and thus can be used in a wide range of ecological conditions. Seven of the 11 QTL were different from those that had been cloned or fine-mapped, providing new candidates for gene cloning and marker-assisted breeding. Allelic effects of QTL corresponding to those that had been cloned or fine-mapped, were much smaller in this study than previously reported. The results supported the assumption that qualitative and quantitative genes may be different alleles at the same loci, suggesting that it may be promising to identify minor-effect QTL from major heading date genes/QTL that have been cloned.

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1. Introduction

Flowering is prerequisite for grain production in cereal crops. Timing of flowering is the key to maintaining an appropriate balance between full use of resources and avoidance of environmental stresses. High yield in rice (Oryza sativa L.) is associated with long growth duration in most growing areas [1–3]; however, early flowering is required for double-season...
cropping and for production in northernmost cultivation areas [4–6]. The wide range of variation in flowering time in rice is controlled by many quantitative trait loci (QTL) that vary greatly in magnitude of additive effects and in association with photoperiod sensitivity [7]. Large-effect QTL often have strong responses to photoperiod and play critical roles in eco-geographical adaptation [8–10]. Small-effect QTL generally adjust heading by several days without obvious photoperiod sensitivity, allowing flowering time to be fine-tuned for optimal use of the available temperature and light [11–13].

Over the past two decades, a complex gene network of regulation of flowering time in rice was unraveled whereby most pivotal genes are large-effect QTL isolated by map-based cloning [7]. In recent years, more attention has been given to QTL with relatively small effects. For example, two minor-effect QTL for flowering time were cloned [11,12], and three others were fine-mapped [13–15]. It was shown that minor-effect QTL also make important contributions to flowering regulation. Nevertheless, many more QTL with small effect were detected as large regions of statistical significance [16,17]. Further genetic analysis including fine-mapping, gene cloning, and functional characterization are necessary. Since minor-effect QTL may have inconsistent additive effects across different genetic backgrounds and environments, selection of reliable candidates for further study remains a challenge. In this regard, specific types of mapping populations could prove useful [16].

A primary mapping population of rice generally has wide phenotypic variation, but only a small number of QTL with relatively large effects are found [18–20]. A common way to detect QTL that failed to show significant effects in original populations has been the use of secondary populations in which previously detected major QTL are fixed [21–23]. The use of a population derived from a cross between two cultivars carrying the same allele at major locus could also facilitate the detection of minor QTL [24]. Presumably, a population derived from a cross between two cultivars of similar ecological adaption could be used to detect minor QTL for flowering time. In a previous study [25], we constructed a recombinant inbred line (RIL) population for which the male and female parents were middle-season indica rice cultivars with similar eco-geological adaptation. As expected, a small range of variation on flowering time was observed. This population and four others derived from its progenies were used in the present study that aimed to identify QTL having consistent small effects on flowering time. QTL analysis was firstly performed using the original RIL population, followed by two runs of validation with increasing homogeneity of the genetic background. A total of 11 QTL were detected. All were detected in different populations, locations and/or years and showed small and stable additive effects.

2. Materials and methods

2.1. Plant materials

Five mapping populations were used. The first was the TI population reported by Mei et al. [25], consisting of 204 RILs of Teqing/IRBB. The male parent IRBB included six near isogenic lines in the genetic background of IR24, among which IRBB52, IRBB59 and IRBB50 were parents of 122, 77 and two RILs, respectively, and IRBB51, IRBB54 and IRBB55 were each a parent of one RIL. Teqing (TQ) and IR24 are middle-season indica rice varieties and restorer lines of three-line hybrids grown in the middle-lower reaches of the Yangtze River valley.

The other four populations were derived from residual heterozygotes of the cross TQ/IRBB52 as described below and illustrated in Fig. 1. To verify QTL detected in the TI population and identify new QTL in a more homogenous background, two F2 plants carrying a number of heterozygous segments were selected. They were selfed to produce two S1 populations, Ti52-2 and Ti52-3 consisting of 251 and 250 plants, respectively. QTL were determined from data generated from the S1,2 and S1,3 families of each population. Two S2 plants that were heterozygous for one or two QTL of interest but homozygous at other QTL regions were selected from the Ti52-2 population. They were selfed to produce two S2 populations designated ZC9 and ZC12 and consisting of 203 and 241 plants, respectively. QTL were determined using S1 plants and S1,2 families of the two populations.

2.2. Field trials and phenotypic evaluation

Rice populations were grown in paddy fields at the experiment stations of the China National Rice Research Institute located at either Hangzhou in Zhejiang province or Lingshui in Hainan (Table 1). During the floral transition period the day length was longer than 13.5 h at Hangzhou and shorter than 12.5 h at Lingshui, corresponding to natural long-day (NLD) and short-day (NSD) conditions, respectively [26]. There was no replication of S1 plants in the ZC9 and ZC12 populations. For other populations the experiments followed a randomized complete block design with two replications. In each replication, one line was grown as a single row of 12 plants. Seedlings at about 25 days post sowing were transplanted at a planting density of 16.7 cm × 26.7 cm in all the trials. Field management followed the normal agricultural practices.

Heading date (HD) was scored in all populations. For RILs which generally do not have within-line segregation, HD was recorded as days from sowing to the time when one or more panicles on 50% of the 12 plants had emerged from the leaf sheath. For S1,2 and S1,3 lines in which within-line segregation was expected, HD was recorded for each plant at first panicle emergence. In replicated trials, HD values of each line were averaged over the two replications and used for data analysis. In S1 plant trials, HD data was recorded and analyzed on a single-plant basis.

2.3. DNA marker analysis

The linkage map for the TI population constructed previously consisted of 125 simple sequence repeat (SSR) and two sequence tagged site (STS) markers [25]. New markers were developed according to 50× whole genome re-sequencing of TQ, IRBB52 and five bulk samples of the TQ/IRBB progeny. Eight polymorphic markers, including four on chromosome 1,
three on chromosome 2, and one on chromosome 4 (Table S1), were tested using the original DNA samples from the 204 RILs. For Ti52-2 and Ti52-3 leaf samples collected from the middle 10 plants of each S1:2 line were mixed for DNA extraction using a conventional method [27]. For ZC9 and ZC12, a 2 cm leaf sample collected from an S1 plant was used for DNA extraction using a mini-preparation protocol [28]. A total of 152 markers were assayed, including 127 markers in the original TI map [25], eight markers newly integrated into the TI map, 16 other SSRs selected from the Gramene database (http://www.gramene.org), and InDel marker Tv963 (Table S1). PCR amplification was performed according to Chen et al. [29]. The products were visualized on 1.5% and 2.0% agarose gels using Gelred staining for the two STS and two of the new markers, respectively. Other markers were visualized on 6% or 8% non-denaturing polyacrylamide gels by silver staining.

2.4. Data analysis

Basic descriptive statistics, including mean, standard deviation, coefficient of variation, range, skewness and kurtosis, were computed for each population in each trial.

Eight new markers were integrated into the TI map using Mapmaker/Exp 3.0 [30]. The updated map consisted of 135 markers and spanned 1345.3 cM (Fig. S1). Detection of QTL in multiple environments was performed using QTLNetwork 2.0 [31], taking different years as different environments. QTL having significant additive effects were determined and genotype-by-environment (GE) interactions were analyzed. Critical F-values for genome-wide type I error were calculated by the 1000 permutation test and used for claiming a significant event. The significance level used was P < 0.05 for candidate interval selection, and P < 0.01 for putative QTL detection and QTL effect estimation. The proportion of phenotypic variance explained (R2) was calculated by the Markov Chain Monte Carlo algorithm. A test window of 10 cM, filtration window of 10 cM and walk speed of 1 cM were chosen for the genome scan.

For other populations, linkage maps of segregating regions were constructed using Mapmaker/Exp 3.0 [30]. Genetic distances between markers were in cM derived with the Kosambi function. QTL were determined using the Composite Interval Mapping (CIM) approach in Windows QTL Cartographer 2.5 [32]. The analysis was performed using the default setting except that a walk speed of 0.5 cM was chosen. A threshold of LOD > 2.0 was used for claiming a putative QTL. QTL detected in this study were designated following the rules proposed by McCouch and CGSNL [33].

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**Table 1 – Heading dates (HD) in the rice populations.**

<table>
<thead>
<tr>
<th>Trial site</th>
<th>Year</th>
<th>Population</th>
<th>n</th>
<th>Mean HD</th>
<th>SD</th>
<th>CV</th>
<th>Range</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>Parental mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Name</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hangzhou</td>
<td>2009</td>
<td>TI RIL</td>
<td>204</td>
<td>83.8</td>
<td>2.03</td>
<td>0.024</td>
<td>18.8</td>
<td>-0.26</td>
<td>0.12</td>
<td>83.5 84.3</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>TI RIL</td>
<td>204</td>
<td>81.7</td>
<td>2.23</td>
<td>0.027</td>
<td>75.5</td>
<td>-0.39</td>
<td>0.73</td>
<td>84.0 81.0</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>TI RIL</td>
<td>204</td>
<td>84.7</td>
<td>2.33</td>
<td>0.028</td>
<td>75.5</td>
<td>-0.14</td>
<td>0.53</td>
<td>87.0 83.3</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>Ti52-2 S1</td>
<td>251</td>
<td>84.7</td>
<td>1.27</td>
<td>0.015</td>
<td>81.6</td>
<td>-0.08</td>
<td>-0.29</td>
<td>86.7 90.6</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>Ti52-3 S1</td>
<td>250</td>
<td>86.6</td>
<td>1.35</td>
<td>0.016</td>
<td>86.2</td>
<td>-0.19</td>
<td>-0.21</td>
<td>87.0 84.3</td>
</tr>
<tr>
<td></td>
<td>2017</td>
<td>ZC9 S1</td>
<td>203</td>
<td>83.3</td>
<td>0.95</td>
<td>0.011</td>
<td>81.8</td>
<td>0.11</td>
<td>-0.34</td>
<td>82.4 87.1</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>ZC12 S1</td>
<td>241</td>
<td>82.6</td>
<td>0.93</td>
<td>0.011</td>
<td>81.4</td>
<td>-0.08</td>
<td>-0.18</td>
<td>99.7 104.8</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>Ti52-2 S1</td>
<td>251</td>
<td>96.5</td>
<td>1.99</td>
<td>0.021</td>
<td>92.2</td>
<td>-0.04</td>
<td>-0.51</td>
<td>95.6 100.2</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>Ti52-3 S1</td>
<td>250</td>
<td>101.9</td>
<td>2.28</td>
<td>0.022</td>
<td>96.2</td>
<td>0.08</td>
<td>0.05</td>
<td>104.8 108.5</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>ZC9 S1</td>
<td>203</td>
<td>99.7</td>
<td>3.67</td>
<td>0.037</td>
<td>92.1</td>
<td>0.52</td>
<td>0.37</td>
<td>95.9 102.1</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>ZC12 S1</td>
<td>241</td>
<td>97.8</td>
<td>4.11</td>
<td>0.042</td>
<td>89.1</td>
<td>0.21</td>
<td>-0.53</td>
<td>99.7 102.1</td>
</tr>
</tbody>
</table>

SD, standard deviation; CV, coefficient of variation.

* Plants were grown from May to Sep. at Hangzhou, and from Dec. to Apr. of the next year in the following year at Lingshui.

* The TI population was constructed from crosses between Teqing and near isogenic lines of IR24; the Ti52-2 and Ti52-3 populations were derived from two F1 plants of Teqing/IRBB52; the ZC9 and ZC12 populations were derived from two S1 plants of Ti52-2.

* Measured as the mean value of IRBB52 and IRBB59 in the TI population, and as the value of IRBB52 for other populations.
3. Results

3.1. Heading date

Descriptive statistics of the HD data collected from each population in each trial are presented in Table 1. In all the trials, heading date was continuously distributed with low skewness and kurtosis, a typical pattern of quantitative variation.

The Ti population was tested for three years in replicated trials at Hangzhou, and the other four populations were tested for one year. Differences between the minimum and maximum HD in the Ti population were 10.5, 14.0, and 14.5 days in 2009, 2010, and 2011, respectively. In the Ti52-2 and Ti52-3 populations derived from two lines of the Ti population, the differences were reduced to 6.5 and 7.5 days, respectively. In the ZC9 and ZC12 populations derived from two individuals in the Ti52-2 population, the differences were further reduced to 4.8 and 4.7 days, respectively. In the same order the standard deviations declined from 2.03–2.33 to 1.27 and 1.35, and then to 0.95 and 0.93; and the coefficients of variation decreased from 0.024–0.028 to 0.015 and 0.016, and then to 0.011. These results are in agreement with the expectation that the background variations were being increasingly fixed from Ti to Ti52-2 and Ti52-3, and from Ti52-2 to ZC9 and ZC12.

3.2. QTL for heading date detected in the Ti population

Three years of HD data for the Ti population were used for QTL analysis in multiple environments with QTLNetwork 2.0 [31]. Four QTL were detected (Table 2); all had significant additive effects and none showed significant GE interaction. Alleles derived from TQ delayed heading at qHD3 and qHD5, and promoted heading at qHD10 and qHD12. These QTL were each detected at high significance (P < 0.0001), but the effects were all small. The additive effect and R² of QTL ranged from 0.39 days and 1.9% at qHD5 to 0.82 days and 7.9% at qHD3, respectively.

The absence of any significant GE interaction indicated that all four QTL had consistent effects across the three years. However, these QTL explained only a small proportion of the phenotypic variance, suggesting that other QTL were also segregating in the Ti population, and might be detected in a more homogeneous background. Two new populations were thus constructed, of which Ti52-2 and Ti52-3 segregated for 38 and 39 of the original 152 DNA markers, respectively. Segregating regions were distributed on all 12 chromosomes, including the regions covering qHD3, qHD5, and qHD12 (Fig. S2).

<table>
<thead>
<tr>
<th>QTL</th>
<th>Interval</th>
<th>P</th>
<th>A</th>
<th>R² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qHD3</td>
<td>RM1430D–RM14383</td>
<td>&lt;0.0001</td>
<td>−0.82</td>
<td>7.9</td>
</tr>
<tr>
<td>qHD5</td>
<td>RM3321–RM274</td>
<td>&lt;0.0001</td>
<td>−0.39</td>
<td>1.9</td>
</tr>
<tr>
<td>qHD10</td>
<td>RM3773–RM3123</td>
<td>&lt;0.0001</td>
<td>0.56</td>
<td>3.2</td>
</tr>
<tr>
<td>qHD12</td>
<td>RM20–RM27610</td>
<td>&lt;0.0001</td>
<td>0.66</td>
<td>4.5</td>
</tr>
</tbody>
</table>

A, additive effect measured as the genetic effect when the Teqing allele is replaced with the IR24 allele; R², proportion of phenotypic variance explained by the QTL effect.

3.3. QTL for heading date detected in the Ti52-2 and Ti52-3 populations

HD data collected from S12 lines in the Ti52-2 and Ti52-3 populations tested under NLDs at Hangzhou and S13 lines under NSDs in Lingshui were separately used for QTL analysis with Windows QTL Cartographer 2.5 [32]. A total of ten QTL were detected, among which qHD5, qHD9.1, and qHD12.2 were detected only at Hangzhou and the others were identified at both locations (Table 3). Additive effects ranged from 0.21 to 1.63 days, with the R² ranging from 2.3% to 24.2%.

Of the two QTL with largest effects in the Ti population, qHD3 was segregating in both the Ti52-2 and Ti52-3 populations, and qHD12 was located in a segregating region of Ti52-3 (Fig. 2). Their effects, including the allelic direction and magnitude, were well validated in the new populations. At qHD3, the TQ allele in the Ti52-2 and Ti52-3 populations delayed heading by 0.61 and 0.56 days at Hangzhou and 1.28 and 1.03 days at Lingshui, respectively. The TQ allele at qHD12 advanced heading by 0.66 days at Hangzhou and 1.63 days at Lingshui in the Ti52-3 population. In addition, a QTL with an allelic effect in the opposite direction to qHD12 was detected in the interval Pita–RM511 downstream of qHD12 in the Ti52-2 population (Fig. 2). Thus qHD12 was renamed as qHD12.1 and the new QTL was named qHD12.2 (Table 3).

QTL qHD5, with the smallest effect in the Ti population, had segregating regions in both the Ti52-2 and Ti52-3 populations. It was detected only at Hangzhou with a LOD score of 2.0 in the Ti52-2 population, but the TQ allele continued to delay heading (Table 3). This indicated that qHD5 had a stable effect that was too small for readily achieving statistical significance. The last QTL, qHD10, detected in the Ti population was located downstream of the segregating RM6704–RM7300 region in the Ti52-2 and Ti52-3 populations. A new QTL with an allelic effect in the opposite direction to qHD10 was detected in RM6704–RM7300 (Fig. 2), and was named qHD10.1; the original qHD10 was renamed as qHD10.2.

Another QTL, qHD9.1, was detected in the segregating region RM219–RM1896 in both the Ti52-2 and Ti52-3 populations (Table 3, Fig. 2). It showed significant effects with the TQ allele promoting heading in both populations at Hangzhou, but no significant effects were found at Lingshui. The remaining four QTL were each located in regions that segregated in only one population, including qHD6 detected in Ti52-2, and qHD7, qHD9.2 and qHD11 in Ti52-3.

A total of 11 QTL for HD were detected in the Ti, Ti52-2 and Ti52-3 populations; four were located in regions covering HD genes that were previously cloned or fine-mapped, including qHD3 in the Ehd4-DTH3 region [34,35], qHD6 in the Hd17-RFT1-Hd3a region [36,37], qHD10.2 in the OsMADS56 region [38], and qHD12.1 in the DTH12 region [14]. To verify the remaining QTL, plants heterozygous for one or two QTL and homozygous in other QTL regions were selected and selfed to produce new populations. Two populations, ZC9 segregating for qHD9.1 and qHD10.1, and ZC12 segregating for qHD12.2, were used in this study.

3.4. Effects of qHD9.1, qHD10.1, and qHD12.2 in isogenic backgrounds

HD data collected from S1 plants in the ZC9 and ZC12 populations grown at Lingshui and S12 lines tested at
Hangzhou were used to analyze the effects of qHD9.1, qHD10.1, and qHD12.2 in more isogenic backgrounds. The analysis was performed with Windows QTL Cartographer 2.5 [32]. Effects are presented in Table 4 irrespective of whether statistical significance was reached.

In the ZC9 population that was segregating for qHD9.1 and qHD10.1, the effects were significant only for qHD10.1 at Hangzhou. LOD scores estimated for qHD9.1 at the two locations were 1.7 and 1.9, and the direction of allelic effect remained unchanged with the TQ allele promoting heading.

### Table 3 – QTL for heading date detected in the Ti52-2 and Ti52-3 populations.

<table>
<thead>
<tr>
<th>QTL</th>
<th>Interval</th>
<th>Trial site</th>
<th>Ti52-2</th>
<th>Ti52-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LOD</td>
<td>A</td>
</tr>
<tr>
<td>qHD3</td>
<td>RM14302–RM14383</td>
<td>Hangzhou</td>
<td>8.1</td>
<td>−0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lingshui</td>
<td>13.7</td>
<td>−1.28</td>
</tr>
<tr>
<td>qHD5</td>
<td>RM3321–RM274</td>
<td>Hangzhou</td>
<td>2.0</td>
<td>−0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lingshui</td>
<td>n.s.</td>
<td>−</td>
</tr>
<tr>
<td>qHD6</td>
<td>RM589–RM587</td>
<td>Hangzhou</td>
<td>2.6</td>
<td>−0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lingshui</td>
<td>6.5</td>
<td>−0.87</td>
</tr>
<tr>
<td>qHD7</td>
<td>RM10–RM70</td>
<td>Hangzhou</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>Lingshui</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>qHD9.1</td>
<td>RM219–RM1896</td>
<td>Hangzhou</td>
<td>5.9</td>
<td>0.50</td>
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<td>Lingshui</td>
<td>n.s.</td>
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<td>RM107</td>
<td>Hangzhou</td>
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<td>qHD10.1</td>
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<td>Hangzhou</td>
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<td></td>
<td>Lingshui</td>
<td>n.s.</td>
<td>—</td>
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<td>Lingshui</td>
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<td>—</td>
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<tr>
<td>qHD12.1</td>
<td>TV963–RM27610</td>
<td>Hangzhou</td>
<td>—</td>
<td>—</td>
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<td></td>
<td>Lingshui</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>qHD12.2</td>
<td>Pita–RM511</td>
<td>Hangzhou</td>
<td>5.5</td>
<td>−0.47</td>
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<tr>
<td></td>
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<td>Lingshui</td>
<td>n.s.</td>
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</table>

A, additive effect measured as the genetic effect when the Teqing allele is replaced with the IRBB52 allele; D, dominance effect; R², proportion of phenotypic variance explained by the QTL effect; n.s., not significant; —, homozygous region.

Hangzhou were used to analyze the effects of qHD9.1, qHD10.1, and qHD12.2 in more isogenic backgrounds. The analysis was performed with Windows QTL Cartographer 2.5 [32]. Effects are presented in Table 4 irrespective of whether statistical significance was reached.

In the ZC9 population that was segregating for qHD9.1 and qHD10.1, the effects were significant only for qHD10.1 at Hangzhou. LOD scores estimated for qHD9.1 at the two locations were 1.7 and 1.9, and the direction of allelic effect remained unchanged with the TQ allele promoting heading.

Fig. 2 – Chromosomal regions covering QTL for heading date detected in this study. For each chromosome, the Ti52-2 and Ti52-3 maps are shown on the left and right sides, respectively; qHD3, qHD5, qHD10, and qHD12 detected in the Teqing/IRBB RIL population are indicated on the far left; and cloned or fine-mapped heading date genes located in the QTL regions are indicated on the far right.
The effects of five minor QTL that have been cloned or fine-mapped in earlier studies, hence providing new candidates for controlling heading date in rice had been cloned or fine-mapped heading date genes/QTL, seven QTL were located in regions where no genes/QTL had been detected. Additive effects of these QTL were small but consistent minor-effects but a precise experimental design was required in order to reach statistical significance.

4. Discussion

Optimizing heading date to maximize grain yield is an important task in rice breeding. It requires a better understanding of the major and minor genes controlling the trait [2,4–16]. In the present study, 11 minor-effect QTL for HD were detected. Additive effects of these QTL were small but generally stable. The initial four QTL detected in the Ti population all had stable effects in the three years of trials. Three of them were further validated in new populations with more homogeneous backgrounds. The other seven QTL were each detected in two locations and/or two populations. The directions of allelic effect of the QTL remained consistent across each detected in two locations and/or two populations. The LOD score and additive effect of a QTL were always larger at Lingshui than Hangzhou as long as the QTL was detected at both locations (Table 3). To determine possible causes for the variation, differences in days to heading between the two locations were calculated for each line and used for QTL analysis using Windows QTL Cartographer 2.5 [32]. Three QTL, were detected in each of the Ti52-2 and Ti52-3 populations (Table S2). The LOD scores, additive effects, dominance effects, and \( R^2 \) values were in general agreement with corresponding QTL detected using the separate datasets from the two locations (Table S3). These results suggest that differences in effects of these QTL between the two locations were largely due to the difference in accumulated daily temperature.

In chromosomal regions for qHD3, qHD6, and qHD12.1 detected in this study, QTL DTH3 and Hd17 had been cloned and DTH12 had been fine-mapped. For QTL located in the same region, the additive effects were about three times larger at DTH3 [35] and DTH12 [14] than at qHD3 and qHD12.1, respectively, and about 11 times larger at Hd17 [36] than at qHD6. We sequenced the DTH3 and Hd17 coding regions in TQ and IRBB52. Substitution of five amino acids was detected in the HD17 protein, among which the L558S substitution was reported to delay heading [36]. These results support the assumption that qualitative and quantitative genes are different alleles at the same loci [41] and suggest an efficient way to identify QTL having small effects. To date, a large number of heading date genes in rice have been isolated as single Mendelian factors [7]. Determination of different alleles at these loci may provide a rich resource of minor-effect QTL controlling this important trait.

It has long been recognized that genes for heading date, photo-sensitivity and thermo-sensitivity are different [39,40]. Ten of the QTL identified in this study were detected in NLD conditions at Hangzhou and under NSDs at Lingshui. The effects were significant for seven QTL at both locations and for the three other QTL only under NLDs at Hangzhou. This is similar to the five minor QTL that have been cloned or fine-mapped [11–15]. The effects of qHd1 and Hd18 were significant in all natural and controlled LD and SD conditions, and those of DTH2, DTH3b, and DTH12 were significant under natural and controlled LDs but not significant under SDs. These results indicate that QTL showing small-effects for heading date are photoperiod insensitive, and thus could be used for fine-tuning heading date under a wide range of eco-geographical conditions.

Temperatures are lower in the winter growing season at Lingshui than in the summer-autumn season at Hangzhou. Accordingly, for the Ti52-2 and Ti52-3 populations tested in replicated trials at both locations, all lines had longer pre-heading phases at Lingshui than Hangzhou. It was also shown that the LOD score and additive effect of a QTL were always larger at Lingshui than Hangzhou as long as the QTL was detected at both locations (Table 3). To determine possible causes for the variation, differences in days to heading between the two locations were calculated for each line and used for QTL analysis using Windows QTL Cartographer 2.5 [32]. Three QTL, were detected in each of the Ti52-2 and Ti52-3 populations (Table S2). The LOD scores, additive effects, dominance effects and \( R^2 \) values were in general agreement with corresponding QTL detected using the separate datasets from the two locations (Table S3). These results suggest that differences in effects of these QTL between the two locations were largely due to the difference in accumulated daily temperature.

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It has been commonly observed that heading date differences predicted from the sum of additive effects of each QTL deviate from actual data due to complex relationships among different genes [10,16,42]. Four QTL that were in close proximity to cloned or fine-mapped heading date genes/QTL, qHD3, qHD6, qHD10.2, and qHD12.1, were used to analyze the combined effects of different QTL. Based on DNA markers that are closest to the QTL,
the TI population was classified into five groups that carried late-heading alleles at 0, 1, 2, 3, and 4 loci, respectively. The largest difference was observed between groups carrying late-heading alleles at zero and one locus, and the smallest difference was found between groups carrying late-heading alleles at three or four loci (Fig. S3). This result has two implications. Firstly, in constructing populations for fine-mapping a minor QTL for HD, it is preferable to select an isogenic background with other minor QTL having early-heading alleles than late-heading alleles. Secondly, in utilizing minor heading date QTL for maximizing the yield potential, selection of a small number of QTL would be sufficient.

5. Conclusions

A total of 11 minor-effect QTL for HD were detected. The additive effects of these QTL were small but stable across genetic backgrounds and environmental conditions. These QTL and other minor-effect QTL that have been cloned or fine-mapped, generally do not have strong photoperiod sensitivity, indicating that minor-effect QTL can be used to fine-tune heading date under a wide range of eco-geographical conditions. Seven of the QTL detected in this study were different from those that have been cloned or fine-mapped, allelic effects estimated in this study were much smaller than those previously reported. This supported the assumption that qualitative and quantitative genes are different alleles at the same loci and suggests a promising new way to identify minor-effect QTL.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cj.2018.01.002.

REFERENCES


