Identification of QTLs controlling heading date on the short arm of chromosome 3 in rice (\textit{Oryza sativa} L.)

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Heading date (flowering time) in rice is a major factor in regional and seasonal adaptation. The shaping of adaptability to local areas has advantages for rice growth. In this study, we identified a quantitative trait locus (QTL) controlling heading date with a small effect in advanced backcrossed progenies. A near isogenic line (NIL) of rice variety Hoshinoyume for a major QTL controlling low-temperature germinability on chromosome 3, qLTG3-1, from Italica Livorno was developed. This NIL showed not only vigorous low-temperature germinability but also early heading with short culms compared with Hoshinoyume. QTL analysis revealed that a QTL controlling heading date, qDTH3, is located in the same chromosomal region as qLTG3-1. The late heading parent Italica Livorno-derived allele at qDTH3 showed early heading. Substitution mapping using a series of introgression lines carrying chromosomal segments from Italica Livorno revealed that qDTH3 involves multiple sub-QTLs, which have different genetic effects on the control of heading date. Recombination within the target region of qDTH3 provides new opportunities for altering heading date in local populations to shape adaptability.

Key Words: allelic variation, breeding program, flowering time, heading date, qLTG3-1, rice.

Introduction

Flowering-time control in plants is a major determinant of local adaptation. Heading date (flowering time) in rice is a major factor in regional and seasonal adaptation. Because of the importance of understanding its genetic control using natural variation in rice (Izawa 2007a, 2007b, Yano et al. 2001). Furthermore, molecular genetic analyses have facilitated our understanding of the mechanisms regulating heading date in rice (Hayama and Coupland 2004, Izawa 2007b, Tsuji et al. 2008). Therefore, it is clear that heading date is a quantitative trait under complex genetic control with numerous interactions between genes and response to environmental conditions including daylength and temperature.

Cultivated rice, \textit{Oryza sativa} L., originated from tropical regions, but rice has been adapted to diverse environmental conditions from 53°N to 40°S with considerable variation in adaptive traits. A wide range of natural variation in heading date is involved in the genetic diversity of cultivated rice. Recent progress in quantitative trait locus (QTL) analysis has revealed the genetic basis of quantitative traits underlying natural variation and the number and effects of genes/QTLs and interaction between them. QTL analyses using multiple crosses of Koshihikari with 12 diverse varieties originating from various regions in Asia revealed a comprehensive series of loci involved in natural variation in heading date (Eba et al. 2011). Variation of heading date among these varieties could be generated by combinations of different alleles of eight loci. As a short-day plant, rice has sensitivity to photoperiod. Long daylength conditions prolong heading date. For rice varieties grown in the northern-limit of rice cultivation, extremely low photoperiod sensitivity has been selected for the adaption to such long day-length conditions (Fujino and Sekiguchi 2005a, 2005b, 2008, Nonoue et al. 2008). This is just one example of the shaping of adaptability to local areas for rice growth. These studies have suggested that allelic variations contributing to the wide adaptability of rice may have occurred during the diversification of cultivated rice.

In plant breeding programs, the target gene/locus is introgressed into the recipient as a chromosomal segment linked to the target gene/locus by marker-assisted selection (MAS). To perform MAS efficiently, it is important to identify genes or traits on the chromosomal segment linked to the target gene. When exotic germplasm are used as a genetic resource, recombination close to the target gene is desirable to eliminate the introduction of undesirable traits, which is also known as linkage drag. Only molecular mapping strategies can identify such novel recombinants.

In temperate rice-growing areas and at high altitudes in tropical and sub-tropical areas, improvement of tolerance to...
low-temperature during rice growth is important in rice breeding programs. In this study, we tried to identify traits and genes linked with gene for tolerance to low-temperature, qLTG3-1, which is a major QTL controlling tolerance to low-temperature at the seed germination stage, termed low-temperature germinability (Fujino et al. 2004). qLTG3-1 encodes a protein of unknown function (Fujino et al. 2008, Fujino and Matsuda 2010, Fujino and Sekiguchi 2010). Moreover, qLTG3-1 shows a large genetic effect and may be useful for the improvement of low-temperature germinability in rice breeding programs (Iwata and Fujino 2010).

To identify genes linked to the target gene/locus for MAS, advanced backcrossed progenies are useful, which have advantages for detecting QTLs with small effects and for high-resolution mapping. In this study, a near isogenic line (NIL) for qLTG3-1 was developed and its agronomic traits were compared with the recipient. The NIL showed not only vigorous low-temperature germinability but also early heading with short culms. QTL analysis revealed that a QTL controlling heading date, qDTH3, was co-located in the same chromosomal region as qLTG3-1. The late heading parent-derived allele at qDTH3 showed early heading. Substitution mapping revealed that qDTH3 involves multiple sub-QTLs, which had different genetic effects on the control of heading date. qDTH3 provides new opportunities for altering heading date in local populations.

Materials and Methods

Plant materials

Several kinds of backcrossed progenies, backcrossed inbred lines (BILs), a near isogenic line (NIL), and introgression lines derived from crosses between Hoshinoyume and Italica Livorno were used. Hoshinoyume is a temperate japonica variety from Japan, while Italica Livorno is a temperate japonica variety from Italy. A BIL population (BC1F5) derived from the cross Hoshinoyume/Italica Livorno//Hoshinoyume was developed using a single-seed descent (SSD) method in our previous study (Iwata et al. 2010). Development of NIL and introgression lines are described below. These backcrossed progenies with the parental varieties were cultivated in an experimental paddy field at HOKUREN Agricultural Research Institute (Naganuma, Hokkaido, Japan, 43°03′ N latitude) in 2003, 2004, 2005, 2007 and 2008. The cultivation conditions were described in Fujino and Sekiguchi (2005a, 2005b, 2008). Sowing and transplanting were performed in late April and late May, respectively. Days to heading (DTH) of the earliest heading panicle among individuals was recorded for each plant as the number of days required from sowing to heading. Leaf samples from each plant were collected for DNA extraction.

Development of NIL and introgression lines

To develop NIL and introgression lines, backcrossing of Italica Livorno with Hoshinoyume was conducted by MAS following the scheme in Fig. 1. First, Hoshinoyume was crossed with Italica Livorno. The resultant F1 plants were backcrossed with Hoshinoyume to obtain BC1F1 seeds. In each generation, MAS for qLTG3-1 was conducted using the marker GBR3001 (Fujino et al. 2004). In addition, the whole genome genotype of each individual was surveyed using 19 restriction fragment length polymorphism (RFLP) and 80 simple sequence repeat (SSR) markers. Among 32 BC1F3 plants, a single plant, #HS12, carrying the minimum introgressed chromosome segments was selected. This plant was backcrossed to obtain BC1F3 seeds. Among nine BC1F3 plants, a single plant, #HS12-1, carrying the minimum introgressed chromosome segments was selected. After self-pollination, a BC2F1 line carrying the fixed target region was selected as a NIL. The F2 population derived from the cross between the NIL and Hoshinoyume was used as a mapping population for low-temperature germinability and heading date. Four individuals, which had different recombination points within the target region between GBR3001 and SSRAC90485, were selected among 84 plants from the F2 population. Two F1 lines from each individual were developed as introgression lines. Genetic maps constructed in this study were assigned using the high-density rice genetic map.
Table 1. Comparison of agronomic traits between Hoshinoyume and the NIL

<table>
<thead>
<tr>
<th>Year</th>
<th>Variety</th>
<th>DTH</th>
<th>CL</th>
<th>PL</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Hoshinoyume</td>
<td>107.4</td>
<td>60 ± 2.2</td>
<td>15.3 ± 0.5</td>
<td>22.0 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>NIL</td>
<td>98.7***</td>
<td>45.2 ± 3.6***</td>
<td>12.4 ± 1.8**</td>
<td>25.8 ± 3.7*</td>
</tr>
<tr>
<td>2004</td>
<td>Hoshinoyume</td>
<td>91.3</td>
<td>62.9 ± 2.3</td>
<td>16.4 ± 0.8</td>
<td>24.4 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>NIL</td>
<td>87.0***</td>
<td>51.3 ± 2.2***</td>
<td>13.0 ± 0.8***</td>
<td>26.4 ± 3.0</td>
</tr>
<tr>
<td>2005</td>
<td>Hoshinoyume</td>
<td>97.8</td>
<td>72.7 ± 3.4</td>
<td>16.8 ± 0.9</td>
<td>30.9 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>NIL</td>
<td>89.9***</td>
<td>57.5 ± 3.1***</td>
<td>14.9 ± 1.0***</td>
<td>30.1 ± 3.2</td>
</tr>
</tbody>
</table>

DTH, days to heading; CL, culm length (cm); PL, panicle length (cm); NP, number of panicles.

Data represent the mean ± SD (n = 10).

*, **, and *** indicate significance levels of 5%, 1%, and 0.1 %, respectively, compared with Hoshinoyume tested by ANOVA.

(Harushima et al. 1998). Primer sequences of the markers used in this study are listed in Supplemental Table 1.

Evaluation of photoperiod sensitivity

Hoshinoyume, NIL, and three introgression lines were grown under three different daylength conditions: 14-h day (14D; 14-h light; 27°C for 12 h and 23°C for 12 h), 12-h day (12D; 12-h light; 27°C for 12 h and 23°C for 12 h) and 10-h day (10D; 10-h light; 27°C for 12 h and 23°C for 12 h) in a controlled growth cabinet (Nihonika, Japan). DTH required for sowing to heading was scored for 10 plants per line.

DNA analysis

Total DNA was extracted from the leaves of each plant using the CTAB method (Murray and Thompson 1980). Genotyping with SSR and RFLP markers was performed as described in Fujino et al. (2004). For QTL analyses for heading date in the F2 population derived from the cross between NIL and Hoshinoyume, 11 markers representing all the introgressed chromosome segments from Italica Livorno were genotyped.

QTL analysis

Analysis of QTLs controlling heading date was performed by composite interval mapping using the software package QTL CARTOGRAFER with forward-backward regression (Wang et al. 2010). The significance level of the log of the odds (LOD) ratio threshold was estimated by computing 1000 permutation tests using QTL CARTOGRAFER.

Results

Evaluation of agronomic traits in the NIL

A NIL for qLTG3-I was developed in the genetic background of Hoshinoyume (as described in the Materials and Methods). A graphical representation of the genotype of the BC1F2 NIL is shown in Supplemental Fig. 1. The NIL carries an Italica Livorno segment around the qLTG3-I region between the markers GBR3001 and RM5928 on chromosome 3, which equates to 56.3 cM at most. In addition to the target chromosome region of qLTG3-I on chromosome 3, the NIL had three chromosomal segments on chromosomes 3, 4 and 12. As expected in Iwata and Fujino (2010), an increase in low-temperature germinability was observed in the NIL (Supplemental Fig. 2).

To identify genes linked to qLTG3-I, four traits in the NIL and Hoshinoyume were evaluated over three years; heading date, culm length, panicle length, and number of panicle (Table 1). The NIL clearly showed early heading and short culms and panicles compared with Hoshinoyume. The result suggested strongly that the NIL carried a gene(s) for early heading on the introgressed segments from Italica Livorno.

Mapping of early heading in the NIL

Using BILs derived from the cross between Italica Livorno and Hoshinoyume, only a single QTL for heading date with a large effect was detected near the marker RM1306 located on the distal end of the long arm of chromosome 7 (Supplemental Fig. 3). This QTL was identical to qDTH7 (Fujino and Sekiguchi 2005a). The Italica Livorno allele at this QTL delayed heading. To identify QTLs controlling early heading in the NIL, QTL analysis was performed using the F2 population derived from the cross between the NIL and Hoshinoyume. The means DTH was 97.8 days for Hoshinoyume and 89.9 days for the NIL. In the F2 population, DTH showed continuous variation and ranged from 89 days to 97 days (Fig. 2A). Using 11 markers located in all the introgressed segments from Italica Livorno among the NIL, only a single QTL with a relatively small effect was detected near marker SSRAC105363-14 on chromosome 3, named qDTH3. This QTL explained 16.4% of total phenotypic variation, and the Italica Livorno allele at qDTH3 headed earlier. No QTLs were detected on the non-target chromosomal regions. Early heading in the NIL may be controlled by qDTH3. This result indicated that qDTH3 was linked to qLTG3-I on the short arm of chromosome 3.

Substitution mapping

The precise location of qDTH3 was unclear because the shape of the interval plot for this QTL was broad, ~15 cm (Fig. 2B). To identify the precise location of qDTH3, substitution mapping was performed using eight introgression lines selected from the F2 population derived from the cross between the NIL and Hoshinoyume. All introgression lines showed significantly earlier heading in different degrees,
QTLs for heading date in rice

<87.0-90.4 in 2007, compared with a Hoshinoyume control (Fig. 3). Similar results were obtained in the experiment in 2008 (Supplemental Table 2).

These eight introgression lines were classified into three significantly different heading dates (Fig. 3 and Supplemental Table 2). These introgression lines carried different introgressed chromosomal regions across the target region along with non-target chromosomal regions (Fig. 3). Two introgression lines, subA42 and subA1, had no detectable Italica Livorno-derived segments other than the target region. The other six introgression lines had fixed and heterozygous Italica Livorno segments of non-target regions on chromosomes 4 and 12. These lines showed a small variation in DTH within each line. In addition, no QTLs other than qDTH3 did not detected in QTL analysis using the F2 population derived from the cross between the NIL and...
Hoshinoyume. These results suggested that multiple sub-QTLs for heading date were involved in the \textit{qDTH3} locus.

\textbf{Characterization of photoperiod sensitivity in introgression lines}

Three introgression lines, subA1, subA12 and subA61, the NIL and Hoshinoyume were grown under three different daylength conditions. Although these introgression lines showed similar DTH in field conditions (Fig. 3 and Supplemental Table 2), these introgression lines exhibited photoperiod sensitivity to differing degrees (Fig. 4). The NIL showed earlier heading than Hoshinoyume under all conditions, and especially in 14D conditions. All the introgression lines showed similar photoperiod sensitivity to the NIL except for 14D conditions of introgression line subA61. These results indicated that \textit{qDTH3} is involved in the photoperiod sensitivity pathway and the difference in introgression line subA61 might be caused by sub-QTLs in the introgressed segment.

\textbf{Discussion}

Heading date is a genetically well-characterized trait in rice breeding programs. Many QTLs have been identified and some have been cloned. These findings have contributed to the elucidation of the conserved mechanisms of flowering pathways between short-day and long-day plants (Hayama and Coupland 2004, Izawa 2007b, Tsuji \textit{et al.} 2008). However, the complexity of the genetic basis of this trait, number of genes and the interaction between them, makes the control of heading date difficult to analyze. To facilitate the efficient control of heading date in rice breeding programs, identification of the genetic basis of the target gene is important including the precise chromosomal location, gene effect, interaction with other loci, and allelic variation. In this study, we identified a QTL controlling heading date, \textit{qDTH3}, using advanced backcrossed progenies derived from crosses between the \textit{japonica} varieties Hoshinoyume and Italica Livorno. Although Italica Livorno shows later heading than Hoshinoyume, the Italica Livorno-derived allele at \textit{qDTH3} promoted early heading in the genetic background of Hoshinoyume. Furthermore, substitution mapping using introgression lines carrying chromosome segments from Italica Livorno demonstrated that \textit{qDTH3} involves multiple sub-QTLs.

Genetic relationships with tight linkage between QTLs controlling heading date in rice have been reported previously (Hagiwara \textit{et al.} 2009, Maas \textit{et al.} 2010, Monna \textit{et al.} 2002, Thomson \textit{et al.} 2006). A QTL for heading date, \textit{Hd3}, was detected in the \textit{F2} population derived from a cross between \textit{japonica} and \textit{indica} varieties (Yano \textit{et al.} 1997). High-resolution mapping genetically dissected \textit{Hd3} into two tightly linked loci, \textit{Hd3a} and \textit{Hd3b} (Monna \textit{et al.} 2002). In the same chromosomal region, at least three QTLs controlling heading date have been identified using introgression lines from \textit{indica} and a wild accession, \textit{O. rufipogon} (Hagiwara \textit{et al.} 2009). A QTL for heading date, \textit{dth1.1}, was detected originally in an advanced backcrossed population derived from the cross between \textit{japonica} and a wild accession, \textit{O. rufipogon} (Thomson \textit{et al.} 2003). Substitution mapping demonstrated that \textit{dth1.1} involves multiple sub-QTLs controlling heading date (Maas \textit{et al.} 2010, Thomson \textit{et al.} 2006).

In this study, substitution mapping revealed that \textit{qDTH3} involves multiple sub-QTLs, which showed different genetic effects on heading date. This QTL has great potential to create novel genetic combinations exhibiting novel phenotypes for improved adaptability to local environmental conditions. Further fine mapping should be possible to identify the exact number of sub-QTLs, their precise chromosomal locations, their genetic effects, and interactions between them. A series of introgression lines, which carry a non-overlapped single introgression segment, should be useful for the identification of each gene underlying the \textit{qDTH3} locus and to combine the alleles at these loci to generate novel phenotypes. In addition, it is important to remove all non-target introgressions in the background. Due to the importance of heading date for local adaptability in rice, the genetic diversity of heading date have been reduced in local populations (Fujino 2003, Fujino and Sekiguchi 2005a, 2008). \textit{qDTH3} provides new opportunity for altering heading date in local populations.

\textit{qDTH3} has been identified on the short arm of chromosome 3 as a genetic difference between \textit{japonica} varieties in this study. It exhibits a common chromosomal region with \textit{O. rufipogon} QTLs (Moncada \textit{et al.} 2001, Tan \textit{et al.} 2008, Thomson \textit{et al.} 2003, Xiao \textit{et al.} 1998) and \textit{O. sativa} QTLs (Hittalmani \textit{et al.} 2003, Li \textit{et al.} 1995, Lin \textit{et al.} 2002, Xiao \textit{et al.} 1996). In addition, we identified previously a QTL for

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Days to heading under three different daylength conditions: 10D (white), 12D (gray) and 14D (black). Data represents the means and SD (n = 10).}
\end{figure}
heading date in a common region using populations derived from crosses between Hoshinoyume and landrace type varieties from Hokkaido (Fujino and Sekiguchi 2008). The landrace type alleles of the QTL showed early heading compared with Hoshinoyume. Furthermore, genes controlling flowering time are located in this region; OsMADS50 (Lee et al. 2004), OsPPTR73 (Murakami et al. 2003) and PhyB (Takano et al. 2005). It was unclear whether these QTLs are allelic with the sub-QTLs of the qDTH3 locus identified in this study.

Allelic variation is a major interest in breeding programs. Allelic variation of genes controlling heading date has demonstrated that photoperiod sensitivity plays an important role in adaptability during diversification of cultivated rice. Ghd7 for grain number, plant height, and heading date has a significant role in wide-ranging adaptation across Asia (Xue et al. 2008). Hd1 is the rice orthologue of Arabidopsis CO, a key regulator of floral promotion (Yano et al. 1997, 2000). The diversity of Hd1 and multiple introgression events between subgroups of cultivated rice clearly indicated that allelic variation of Hd1 has played an important role in adaptability to local areas during the domestication process (Fujino et al. 2010). Natural variation of qDTH3 among cultivated rice will provide a novel source of allelic diversity.

In breeding programs, the target gene/locus is introgressed into the recipient as a chromosomal segment linked to the target gene/locus by MAS. To perform MAS efficiently, it is important to identify genes or traits on the chromosomal segment linked to the target gene. The results of this study revealed that a QTL for heading date, qDTH3, was linked to qLTG3-1. Furthermore, QTLs for agronomically important traits, eating quality (Kobayashi et al. 2008, Takeuchi et al. 2008, Wada et al. 2008) and pre-harvest sprouting resistance (Hori et al. 2010), have been identified in this chromosomal region. For the manipulation of these traits, targeted recombination within this chromosomal region should be useful for creating novel genotypes, which may provide novel advantages for adaptation for local environmental conditions.

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Literature Cited


