Quantitative Trait Loci for Sink Size and Ripening Traits in Rice (*Oryza sativa* L.)

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Quantitative trait loci (QTLs) that affect sink size and ripening, which are often negatively correlated, were analyzed in two inbred lines of rice (*Oryza sativa* L.) derived from crosses between the semi-dwarf *indica* and *japonica* cultivars. Recombinant inbred lines (RILs) from Milyang23 (*indica*)/Akihikari (*japonica*), and back-crossed inbred lines (BCILs) from Sasashihiki (*japonica*)/Habataki(*indica*)//Sasanishiki//Sasanishiki were studied over a period of two years. A major QTL that was related to the number of spikelets per panicle was found in the same region of chromosome 1 in both populations. The *indica* allele increased the number of spikelets and reduced the ripening percentage. The *indica* allele of the QTL, which was found in both populations but at different locations on chromosome 6, also increased the number of spikelets per panicle, although to a lesser extent than in the case of the QTL on chromosome 1. In this instance, the number of spikelets per panicle did not have a negative effect on the ripening percentage. The increased number of spikelets produced by the QTL on chromosome 6 was due mainly to an increase in the number of primary rachis branches. In contrast, the effect of the QTL on chromosome 1 relied on an increase in the number of secondary rachis branches. In addition, dry matter production tended to increase when the QTL region of chromosome 6 belonged to the *indica* genotype. Therefore, both panicle structure and source productivity might contribute to the increase of the sink size without reducing the ripening percentage. We also found two loci on chromosomes 11 and 12 of the RILs that were associated with the percentage of incompletely filled spikelets, but which did not affect the sink size. It is considered that these QTLs could contribute significantly to the breeding of cultivars with increased sink size and increased ripening.

Key Words: QTL, rice, ripening percentage, sink size, rachis branch.

Introduction

Sink size, which is often represented by the number of spikelets per plant, and ripening percentage are two of the major determinants of rice yield (Matsushima 1957). Although an increase in either, or both, of these parameters increases crop yield, the two components are generally negatively correlated (Matsushima 1957). It is important to overcome the negative correlation between sink size and ripening in order to achieve higher yields.

Therefore, we investigated the possibility that sink size and ripening were not linked genetically. A technique for analyzing quantitative trait locus (QTL) has been developed using restriction fragment length polymorphism (RFLP) markers (Soller et al. 1976, Lander and Botstein 1986, Nienhuis et al. 1987, Lander and Botstein 1989, Haley and Knott 1992). This technique can be applied to analyze loci that affect the sink size and ripening traits, and to determine whether ripening can be improved without affecting the sink size or vice versa. Xiao et al. (1998) reported that the pleiotropic effects of QTLs on negatively correlated, yield-determining traits were significant in an inter-subspecific cross, although inter-specific crossing with a wild rice relative revealed several beneficial alleles that were free of the deleterious effects on other characteristics (Xiao et al. 1996). It is important to distinguish loci that independently affect sink size and ripening from those that show pleiotropic effects on these two traits.

QTL analysis has been used to study many agronomic traits in rice (Yano et al. 1995, Lin et al. 1996, Lu et al. 1996, Wu et al. 1996, Xiao et al. 1996, Zhaung et al. 1997, Redoña and Mackill 1998, Xiao et al. 1998, Moncada et al. 2001). Although sterile spikelets were investigated in those reports, very few studies have dealt with the ripening percentage, which is important for determining economical grain yields. For the analysis of ripening, it is essential to distinguish carefully between the grain filling ability, which is affected by the sink-source relationship, and sterility, which might be associated with hybrid sterility. Although Zhaung et al. (1997) investigated the number of filled grains, their materials for the *F*₂ and/or *F*₃ populations showed a high frequency of sterility, and the grain filling ability may not have been analyzed properly. Many QTLs have been reported for the number of spikelets per panicle and the number of panicles per plant (Lin et al. 1996, Lu et al. 1996, Wu et al. 1996, Xiao et al. 1996, Zhaung et al. 1997).
1997, Xiao et al. 1998), these two parameters are combined to determine the number of spikelets per plant in rice (Khush 1996). However, the effect of these QTLs on the ripening percentage has not been investigated thoroughly.

In this study, we conducted QTL analysis for the sink size and ripening percentage using recombinant inbred lines (RILs) and back-crossed inbred lines (BCILs) of rice that were developed from inter-specific crosses between indica and japonica cultivars, and we determined whether these were separate genetic traits. One difficulty with QTL analysis is the stability of the locus peaks, which often lack significance from one year to the next. Mackill (1999) suggested that analysis over several years and the use of different population combinations are necessary for accuracy. Therefore, we conducted our analysis over a period of two years with two different combinations of inbred lines, and we confirmed the consistency of our results.

Materials and Methods

Plant materials and field experiments

The two populations of inbred lines that were used in this study were developed from different cross combinations: 191 RILs were derived from the cross Milyang23/Akihikari (Fukuta et al. 1997), and 85 BCILs were derived from the cross Sasanishiki/Habataki/Sasanishiki//Sasanishiki (Sasanishiki × Habataki) (Hirayama et al. 1999). Milyang23, which is an indica semi-dwarf Korean cultivar, is often used in Japan as the parental strain for the breeding of high-yielding rice cultivars. Akihikari is a japonica cultivar with a large number of spikelets per plant and a relatively high yield. Habataki is a semi-dwarf indica cultivar with a large number of spikelets per panicle (Kobayashi et al. 1990). Sasanishiki is a japonica cultivar with a large number of panicles per plant and properties that make it more suitable for consumption in Japan.

All of the 191 RILs from Milyang23/Akihikari (F1) were cultivated in a paddy field at the Hokuriku National Agricultural Experiment Station (Joetsu, Japan) in 1997. One plant per line was sampled after maturation, and the number of panicles per plant, the average number of spikelets per panicle, the ripening percentage, and the plant dry weight were determined for QTL analysis of the sink size and ripening. The sink size was evaluated from the number of spikelets per plant, which was calculated by multiplying the number of panicles per plant by the average number of spikelets per panicle. The ripening percentage was calculated from the ratio of the number of spikelets with a specific gravity above 1.0 to the total number of spikelets. To distinguish the sterility from the analysis of grain filling, we separated the spikelets with a specific gravity less than 1.0 from those with a specific gravity above 1.0. Spikelets that had developed a grain whose specific gravity was less than 1.0 due to incomplete grain filling associated with the sink-source relationship. In 1998, 72 F2 lines were selected mainly to eliminate the lines that showed a high percentage of sterility or poor plant growth. Segregative distortion of selected lines was checked using the Chi-square test. The plants were grown as in 1997, and three plants per line were sampled. The measurement of each trait was performed in the same way as in 1997, and the number of primary and secondary rachis branches was also counted.

Seeds of the BC3F4, which were developed using the single seed descent method after the production of BC3F3 from Sasanishiki × Habataki, were supplied by the National Institute of Agrobiological Sciences of Japan, and 85 each of the BC3F4 and BC3F5 lines were grown in a paddy field at the Hokuriku National Agricultural Experiment Station in 1998 and 1999, respectively. Five plants per line with two replications in 1998 and five plants per line in 1999 were sampled. The same measurements were taken as in the 1997 Milyang23/Akihikari RILs experiment. In 1999, counts of the number of primary and secondary rachis branches were added.

QTL mapping

The RIL mapping data from Milyang23/Akihikari with 165 RFLP markers (Fukuta et al. 1997), and the BCIL mapping data from Sasanishiki × Habataki with 238 RFLP markers (Hirayama et al. 1999), were used for the QTL analysis, which was conducted using the simple interval mapping procedure with QGene (ver. 3.06v; Nelson 1997). Since QGene does not compute BC3F4 or BC3F5 in the analysis of the BCILs, we designated this population as “BC3S2” for the purpose of calculation, as recommended by the program developer (Nelson, personal communication). A LOD score of 3.0 was used to detect putative QTLs. QGene was also used to estimate the additive effect and the percentage of phenotypic variance for each QTL determination. Since dominance effect in the BCIL analysis was not properly simulated using this population type, it was therefore not listed.

Results

Phenotypic variation

Fig. 1 shows the frequency distribution of the traits that were associated with the sink size and ripening in the RILs from Milyang23/Akihikari in 1997 and 1998. Akihikari had more panicles per plant than Milyang23 (Fig. 1 A1 and A2), while the number of spikelets per panicle was almost the same in both cultivars (Fig. 1 B1 and B2); thus the number of spikelets per plant was higher for Akihikari (Fig. 1 C1 and C2). The ripening percentage was also slightly higher for Akihikari (Fig. 1 D1 and D2). All the measured traits showed a higher segregation in the RILs than in the parental cultivars in 1998, and transgressive segregations in both directions were observed for the measured traits in both years.

Fig. 2 shows the frequency distribution of the BCIL traits from Sasanishiki × Habataki. The number of panicles per plant was higher for Sasanishiki than for Habataki (Fig. 2 A1 and A2), whereas the number of spikelets per panicle
was higher for Habataki (Fig. 2 B1 and B2). Consequently, the number of spikelets per plant was similar in the parents (Fig. 2 C1 and C2). The ripening percentage was almost the same or slightly higher for Habataki (Fig. 2 D1 and D2). Although the average value of each BCIL trait was similar to that of Sasanishiki because Sasanishiki was back-crossed twice, large segregations were also observed in the BCILs, which were similar to those observed for the RILs of Milyang23/Akihikari.

Fig. 3 shows the relationship between the number of spikelets per plant and the ripening percentage in the two populations. The higher the number of spikelets per plant, the lower the ripening percentage in both populations. In some lines, particularly the RILs, the ripening percentage was less than 50%, which might have been due to hybrid sterility. However, even if these lines were excluded from the analysis, there were still large differences in the ripening percentage when the number of spikelets per plant was similar. These results indicate that the ripening percentage in the two populations was influenced not only by the sink size, but also by other factors.
Mapping QTLs for traits that are related to sink size and ripening

Table 1 and Fig. 4 show the results of interval mapping of the QTL analysis of the RILs from Milyang23/Akihikari. Eleven putative QTLs were detected on chromosomes 1, 2, 7 and 8 in the 1997 trial, whereas in 1998, 9 putative QTLs were detected on chromosomes 1, 6, 7, 8, 11 and 12 (Table 1). Three QTLs, for the number of spikelets per panicle, the number of spikelets per plant and the ripening percentage were detected in both years in approximately the same region of the short arm of chromosome 1 between markers \( N079A \) and \( XNpb90 \) (Fig. 4). The Milyang23 allele in this region increased the number of spikelets per panicle and the number of spikelets per plant, but decreased the ripening percentage (Table 1). In this region, QTLs for the number of panicles per plant, the percentage of both sterile and incompletely filled spikelets and the number of secondary rachis branches were also detected, although they were prominent for only one year (Fig. 4). The following QTLs (with their associated genetic loci) were also detected for only one year: number of panicles per plant (chromosome 1 between \( C122 \) and \( XNpb201 \)), number of spikelets per panicle (chromo-
Quantitative trait loci for sink size and ripening traits in rice

some 1 between XNpb92 and C86 and chromosome 7 between XNpb338C and XNpb33; number of spikelets per plant (chromosome 6 between C235 and XNpb172); total dry weight (chromosome 2 between N162A and XNpb199).

Fig. 3. Relationship between the number of spikelets per plant and the ripening percentage in: (A) the RILs developed from the cross Milyang23/Akihikari; (B) the BCILs from the cross Sasanishiki/Habataki/Sasanishiki, and of the parents. Data shown are derived from the 1997 RIL experiment and from the 1999 BCIL experiment. The regression lines and correlation coefficients are indicated.

Table 1. Putative quantitative trait loci (QTLs) detected for the traits related to sink size and ripening in the rice RILs developed from the cross Milyang23/Akihikari

<table>
<thead>
<tr>
<th>Traits 1)</th>
<th>Chromosome number</th>
<th>Location between</th>
<th>1997 Distance LOD Additive effect Variance explained (%)</th>
<th>1998 Distance LOD Additive effect Variance explained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of panicles per plant (npp)</td>
<td>1 N079A XNpb359</td>
<td>2.0 3.2 −1.3 7.1</td>
<td>(not detected)</td>
<td></td>
</tr>
<tr>
<td>Number of spikelets per panicle (nsp)</td>
<td>1 R210B XNpb90</td>
<td>6.3 17.6 27.3 29.5</td>
<td>5.3 8.5 33.8 37.2</td>
<td></td>
</tr>
<tr>
<td>Number of spikelets per plant (spl)</td>
<td>1 R210B XNpb90</td>
<td>5.3 3.6 198.8 8.9</td>
<td>7.3 5.3 223.0 23.1</td>
<td></td>
</tr>
<tr>
<td>Ripening percentage (rp)</td>
<td>1 N079A XNpb359</td>
<td>6.0 6.4 −5.2 15.1</td>
<td>2.0 3.6 −4.7 19.5</td>
<td></td>
</tr>
<tr>
<td>Total dry weight (tdw)</td>
<td>2 N162A XNpb199</td>
<td>5.1 3.6 −5.6 10.1</td>
<td>8 XNpb104</td>
<td></td>
</tr>
<tr>
<td>Number of primary rachis branches (rb1)</td>
<td>7 XNpb338C XNpb33</td>
<td>(not measured)</td>
<td>3.8 3.4 0.7 18.2</td>
<td></td>
</tr>
<tr>
<td>Number of secondary rachis branches (rb2)</td>
<td>8 XNpb187 XNpb56</td>
<td>23.4 4.2 −1.0 24.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of sterile spikelets (ss)</td>
<td>1 N079A XNpb359</td>
<td>5.0 3.3 3.2 3.0</td>
<td>(not detected)</td>
<td></td>
</tr>
<tr>
<td>Percentage of incompletely filled spikelets (ifs)</td>
<td>1 R210B XNpb90</td>
<td>4.3 3.7 2.1 8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 XNpb335A NSSK190</td>
<td></td>
<td>0.0 3.0 −2.7 17.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 XNpb124 G2140A</td>
<td></td>
<td>0.0 3.4 −2.8 19.8</td>
<td></td>
</tr>
</tbody>
</table>

1) Abbreviations are the same as those in Fig. 4.
2) Distance from left markers.
3) + and − signs indicate the positive and negative additive effect of the Milyang23 allele, respectively.
Fig. 4. RFLP linkage map showing the locations of QTLs for the traits related to sink size and ripening for the RILs of rice derived from the cross Milyang23/Akihikari. Markers are shown on the right side of the chromosomes. Arrowheads indicate the peak positions of the LOD, and dark and black bars indicate the regions with one LOD support interval in the 1997 and 1998 experiments, respectively. Trait designations are abbreviated as follows: npp, number of panicles per plant; nsp, number of spikelets per panicle; spl, number of spikelets per plant; rp, ripening percentage; tdw, total dry weight at maturity; ss, percentage of sterile spikelets; ifs, percentage of incompletely filled spikelets; rb1, number of primary rachis branches; rb2, number of secondary rachis branches. Abbreviations enclosed in boxes indicate the traits that increased in the Milyang23 alleles, and the others in the Akihikari alleles. The chromosomes are arranged so that the short arm is at the bottom.
Quantitative trait loci for sink size and ripening traits in rice and chromosome 8 between XNpb38 and XNpb104); number of primary rachis branches (chromosome 7 between XNpb338C and XNpb33 and chromosome 8 between XNpb187 and XNpb56); and percentage of incompletely filled spikelets (chromosome 11 between XNpb335A and NSSK190 and chromosome 12 between XNpb124 and G2140A).

Chi-square testing for markers revealed that the original 191 RILs contained a total of 61 segregatively distorted markers. This high frequency of segregative distortion in the RILs was previously reported by Fukuta et al. (1999). The QTLs for the total dry weight (chromosome 2), number of spikelets per plant (chromosome 6) and percentage of incompletely filled spikelets (chromosome 12) in the RILs were located close to the distorted segregation markers XNpb199, C235 and XNpb124, respectively (Table 1 and Fig. 4), and were possibly affected by segregative distortion. The number of markers with distorted segregation was reduced to 30 in 72 RILs. Whereas the number of distorted markers on chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 10 and 12 was reduced without the introduction of any new distortions, new distortions were introduced on chromosome 4 (0 to 1 marker) and chromosome 11 (2 to 8 markers). In particular, on chromosome 11, the QTL for the percentage of incompletely filled spikelets was possibly affected by a newly introduced segregatively distorted marker (NSSK190) in the RIL population of 72 lines in 1998 (Table 1 and Fig. 4). A simulation to reduce the number of lines from 191 to 72 in the 1997 experiment revealed that the introduction of segregative distortion reduced the LOD score for the location of NSSK190. Therefore, it was assumed that the presence of the QTL on chromosome 11 was not an artifact of segregative distortion that was introduced by decreasing the number of lines. However, this distortion possibly affected the position of the LOD peak between XNpb335A and NSSK190.

Table 2 and Fig. 5 show the results obtained for the BCILs of Sasanishiki × Habataki. Eight putative QTLs were detected on chromosomes 1, 6 and 12 in the 1998 trial (Table 2). The 1999 analysis revealed the presence of 13 putative QTLs on chromosomes 1, 5, 6 and 12 (Table 2). Five QTLs in the four regions on chromosomes 1 and 12 were detected frequently in both years: one QTL for the number of panicles

<table>
<thead>
<tr>
<th>Traits</th>
<th>Chromosome number</th>
<th>Location between</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance(2)</td>
<td>LOD</td>
<td>Additive effect(3)</td>
<td>Variance explained (%)</td>
</tr>
<tr>
<td>Number of panicles per plant (npp)</td>
<td>1</td>
<td>C86H</td>
<td>G2200</td>
<td>0.0</td>
</tr>
<tr>
<td>Number of spikelets per panicle (nsp)</td>
<td>1</td>
<td>R1613</td>
<td>S14085E</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>S14085E</td>
<td>S11122EH</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>R372EH</td>
<td>G1458</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>R6988H</td>
<td>R2549EH</td>
<td>(not measured)</td>
</tr>
<tr>
<td>Number of spikelets per plant (spl)</td>
<td>1</td>
<td>R1613</td>
<td>C470</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>C955</td>
<td>S11122EH</td>
<td>5.6</td>
</tr>
<tr>
<td>Ripening percentage (rp)</td>
<td>1</td>
<td>R1613</td>
<td>C470</td>
<td>(not detected)</td>
</tr>
<tr>
<td>Total dry weight (tdw)</td>
<td>1</td>
<td>G2200</td>
<td>R3203</td>
<td>1.1</td>
</tr>
<tr>
<td>Number of primary rachis branches (rb1)</td>
<td>5</td>
<td>R372EH</td>
<td>G1458</td>
<td>(not measured)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>R566M</td>
<td>G329</td>
<td>5.8</td>
</tr>
<tr>
<td>Number of secondary rachis branches (rb2)</td>
<td>1</td>
<td>R1613</td>
<td>C470</td>
<td>(not measured)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>C955</td>
<td>S11122EH</td>
<td>0.0</td>
</tr>
<tr>
<td>Percentage of sterile spikelets (ss)</td>
<td>12</td>
<td>R1709H</td>
<td>G1069</td>
<td>(not detected)</td>
</tr>
<tr>
<td>Percentage of incompletely filled spikelets (ifs)</td>
<td>1</td>
<td>SI4064EH</td>
<td>R1613</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>R1709H</td>
<td>C1069</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1,2) See Table 1.
3) + and – signs indicate the positive and negative additive effect of the Habataki allele, respectively.
per plant between the markers $C86H$ and $G2200$ on chromosome 1, two QTLs for the number of spikelets per panicle and the number of spikelets per plant between $R1613$ and $S1408SE$ on chromosome 1, one QTL for the number of spikelets per panicle between $S1408SE$ and $S11122EH$ on chromosome 1, and one QTL for the percentage of incompletely filled spikelets between $R1709H$ and $C1069$ on chromosome 12 (Fig. 5). Of these, the QTLs on chromosome 1 increased the trait value in the indica genotype, whereas the QTL on chromosome 12 increased the trait value in terms of incompletely filled spikelets for the japonica allele (Table 2). The region on chromosome 1 between $S14064EH$ and $C470$ also contained QTLs for the ripening percentage, number of secondary rachis branches, and percentage of incom-

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Fig. 5. RFLP linkage map showing the locations of QTLs for the traits related to sink size and ripening in the BCILs developed from the cross Sasanishiki/Habataki/Sasanishiki/Sasanishiki. The black and dark bars indicate the regions with one LOD support interval obtained in the 1998 and 1999 experiments, respectively. Abbreviations enclosed in boxes indicate the traits that increased in the Habataki alleles, and the others in the Sasanishiki alleles. Other explanations are the same as those in Fig. 4.
pletely filled spikelets. QTLs for the number of spikelets per plant and the number of secondary rachis branches were also detected in the region between C955 and SI1122EH on chromosome 1 (Fig. 5). The following additional QTLs (with their associated genomic locations) were detected: total dry weight (chromosome 1 between G2200 and R3203); number of spikelets per panicle and number of primary rachis branches (chromosome 5 between R372EH and G1438); number of spikelets per panicle and number of primary rachis branches (chromosome 6 between R688H and R2549EH and between R566M and G329, respectively); and percentage of sterile spikelets (chromosome 12 between R1709H and G1069) (Fig. 5).

Effects of QTLs that control the number of spikelets per panicle on ripening

The main focus of this study was to determine the effect of the QTLs that control the sink size on the ripening percentage. QTLs that controlled the number of spikelets per panicle were detected frequently on the short arm of chromosome 1 in both the RIL and BCIL populations (Fig. 4 and Fig. 5). A Peak of LOD for the number of spikelets per panicle was also found on the long arm of chromosome 6 in both populations, although only the locus for the BCILs was significant (Fig. 5) and the locations were different in the two populations. To investigate these regions in detail, the results for chromosomes 1 and 6 were combined so that the direction and strength of the effects of the sink size and ripening percentage in the RILs and BCILs could be represented (Fig. 6 and Fig. 7, respectively). The indica (Milyang23 or Habataki) allele of the QTL on the short arm of chromosome 1 markedly increased the number of spikelets per panicle (Fig. 6 B1 and Fig. 7 B1) and the number of spikelets per plant (Fig. 6 C1 and Fig. 7 C1), while simultaneously reducing the number of panicles per plant (Fig. 6 A1) and the ripening percentage (Fig. 6 D1 and Fig. 7 D1). The QTL on chromosome 6 also increased the number of spikelets per panicle with the indica genotype, although the effect was not as pronounced as that mediated by the QTL on chromosome 1 (Fig. 6 B6 and Fig. 7 B6). However, it should be noted that the QTL on chromosome 6 did not affect appreciably either the number of panicles per plant (Fig. 6 A6 and Fig. 7 A6) or the ripening percentage (Fig. 6 D6 and Fig. 7 D6). This suggests that the QTL on chromosome 6 increased substantially the number of well-filled grains, and could thus increase crop yield.

Since the number of spikelets per panicle is determined mainly by the structure of the panicle, we conducted an analysis of the rachis branches. The indica allele of the QTL on chromosome 1 increased significantly the number of secondary rachis branches, whereas the number of primary rachis branches was not significantly affected, in both RILs and BCILs (Fig. 6 E1 and Fig. 7 E1, respectively). As a result, the ratio of the number of secondary rachis branches to that of primary rachis branches was extremely high with the indica genotype at this locus (Fig. 6 E1 and Fig. 7 E1). In contrast, the QTL on chromosome 6 in the RILs and BCILs affected the number of primary rachis branches (Fig. 6 E6 and Fig. 7 E6, respectively). The QTL on chromosome 6 increased the number of primary rachis branches in the indica genotype, and the number of secondary rachis branches peaked due to the increase in the number of primary rachis branches, from which secondary rachis branches were derived. This phenomenon was more obvious in the BCILs, in that the ratio of secondary:primary rachis branches was scarcely affected (Fig. 7 E6). The effect of the QTL on chromosome 6 in the RILs was similar to that in the BCILs, although the peaks for the primary and secondary rachis branches somewhat shifted (Fig. 6 E6).

It is noteworthy that the QTL on chromosome 6 exerted some effect on the plant dry weight at maturity. The dry weight of both populations increased when the allele of the QTL on chromosome 6 belonged to the indica genotype, although the increase was not statistically significant (Fig. 6 F6 and Fig. 7 F6). In contrast, such a peak was not observed consistently in the region on the short arm of chromosome 1.

Putative QTLs that were related to the number of panicles per plant were also identified on the long arm, but at different locations of chromosome 1 in the RILs (Table 1, Fig. 4 and Fig. 6 A1) and the BCILs (Table 2, Fig. 5 and Fig. 7 A1). Although the indica genotypes of these QTLs showed an increase in the number of panicles, they also reduced the number of spikelets per panicle (Fig. 6 B1 and Fig. 7 B1). In the BCILs, a significant QTL for total dry weight was detected for the same locus (Fig. 7 F1), which also seemed to be related to the number of primary rachis branches (LOD = 2.7) (Fig. 7 E1). However, while the indica allele at this locus increased the number of panicles, it also reduced the total dry weight and the number of primary rachis branches.

QTLs for the number of spikelets per panicle and the number of primary rachis branches were also detected, albeit for only one year, on chromosome 7 between the XNpb383C and XNpb33 markers in the RILs (Fig. 4), and on chromosome 5 between R372EH and G1438 in the BCILs (Fig. 5). The indica alleles of these QTLs increased the number of spikelets and the number of primary rachis branches (Table 1, Table 2, Fig. 4 and Fig. 5), while concomitantly reducing the number of panicles per plant (data not shown).

QTLs that control the percentage of sterile and incompletely filled spikelets

Another objective of this study was to identify QTLs for the ripening percentage that were controlled independently of the sink size. To distinguish the effect of sterility from poor grain filling, interval mapping was conducted on the QTL analysis of the traits related to the percentage of sterile and incompletely filled spikelets (Table 1, Table 2, Fig. 4 and Fig. 5). Among the detected loci, QTLs for the percentage of sterile and incompletely filled spikelets on chromosome 1 displayed the effect of the aforementioned QTL (for the number of spikelets per panicle), in that they also reduced the ripening percentage (Fig. 4 and Fig. 5). In
Fig. 6. Interval mapping of the QTL analysis for sink size and ripening traits on chromosomes 1 and 6 for RILs from the cross Milyang23/Akihikari. A1 and A6, number of panicles per plant; B1 and B6, number of spikelets per panicle; C1 and C6, number of spikelets per plant; D1 and D6, ripening percentage; E1 and E6, number of primary and secondary rachis branches and their ratios; F1 and F6, total dry weight at maturity. The suffixes 1 and 6 denote chromosomes 1 and 6, respectively. The solid and broken lines in A-D and F indicate LOD scores for the 1997 and 1998 experiments, respectively. The lines in E1 and E6 are indicated in the figure. LOD scores of loci that showed positive and negative additive effects in the presence of the Milyang23 allele are indicated above and below, respectively. Chromosomes are arranged so that the short arm is on the left side. The locations of the markers on each chromosome are shown at the bottom. Thin lines indicate significant levels for LOD score = 3.0. Ticks on the x-axis represent 20cM.
the RILs, the *indica* allele increased the percentage of both sterile and incompletely filled spikelets (Fig. 4), whereas the *indica* allele mainly increased the percentage of incompletely filled spikelets in the BCILs (Fig. 5).

Putative QTLs for incompletely filled spikelets were detected on chromosomes 11 and 12 in the RILs (Table 1, Fig. 4, Fig. 8 B11 and B12), and for sterile and incompletely filled spikelets on chromosome 12 in the BCILs (Table 2, Fig. 5, Fig. 8 C12 and D12). These loci did not affect appreciably the number of spikelets (Fig. 4 and Fig. 5). Among these QTLs, the QTL on chromosome 12 in the BCILs showed consistent peaks in both years, although the value

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**Fig. 7.** Interval mapping of the QTL analysis for sink size and ripening traits on chromosomes 1 and 6 for the BCILs from the cross Sasanishiki/Habataki/Sasanishiki///Sasanishiki. The solid and broken lines indicate the LOD scores for the 1998 and 1999 experiments, respectively. LOD scores of the loci that showed positive and negative additive effects in the presence of the Habataki allele are indicated above and below, respectively. The remaining abbreviations are identical with those listed in Fig. 6.
for the sterile spikelets in 1998 was not significant (Fig. 8 C12 and D12). However, no QTLs were detected at this locus on chromosome 12 in the RILs (Fig. 8 A12 and B12). The QTL on chromosome 12 in the BCILs seemed to have pleiotropic effects, in that the percentage of sterile spikelets for the Habataki allele increased, while the percentage of incompletely filled spikelets decreased (Fig. 8 C12 and D12). QTLs for incompletely filled spikelets on chromosomes 11 and 12 in the RILs (Fig. 8 B11 and B12, respectively) lacked pleiotropic effects for the traits measured. However, these properties were observed less consistently over the two-year period, and were not observed in the BCILs. Therefore, these loci could become potential candidates for improving the ripening traits of rice, and they require further investigation.

Fig. 8. Interval mapping of QTL analysis of the percentage of sterile and incompletely filled spikelets on chromosomes 11 and 12 in the RILs developed from Milyang23/Akihikari and in the BCILs developed from Sasanishiki/Habataki//Sasanishiki//Sasanishiki. The solid and broken lines indicate the LOD scores from the 1997 and 1998 RIL experiments and from the 1998 and 1999 BCIL experiments, respectively. The suffixes 11 and 12 represent chromosomes 11 and 12, respectively. The LOD scores of the loci that showed positive and negative additive effects in the presence of the *indica* (Milyang23 or Habataki) allele are indicated above and below, respectively. The remaining abbreviations are identical with those listed in Fig. 6.
Discussion

The objective of this study was to identify QTLs that independently control the sink size and ripening traits in rice. For improving crop yields, it is important to identify QTLs that lack pleiotropic effects, i.e., QTLs that do not mediate simultaneously increases in certain traits and reductions in other traits, although pleiotropic effects have been observed occasionally in the QTL analysis of agronomic traits in rice (Xiao et al. 1996, Zhuang et al. 1997).

We found two important regions on chromosomes 1 and 6 that increased the sink size, particularly in terms of number of spikelets per panicle (Fig. 4 and Fig. 5). The QTL on chromosome 1 markedly increased the number of spikelets per panicle when the allele belonged to the indica type (Milyang23 or Habataki), but at the same time, it reduced the number of panicles per plant and the ripening percentage (Fig. 6 and Fig. 7). Since the effect on the reduction of the number of panicles per plant was not as pronounced as the effect on the increase of the number of spikelets per panicle, the number of spikelets per plant still increased. However, the effect on the reduction of the ripening percentage decreased the effect of elevated sink size on grain yield. These two deleterious effects seemed to be pleiotropic, and were probably not separable. On the contrary, the QTL on chromosome 6 increased the number of spikelets per panicle with little or no influence on other traits, such as the number of panicles per plant and the ripening percentage (Fig. 6 and Fig. 7). These effects of the QTLs on chromosomes 1 and 6 were consistent for the two different populations of the RILs and the BCILs, and for the two-year period of this study. The results indicate that the QTL on chromosome 6 may be useful in increasing yields, since it is likely that the sink size can be increased without a concomitant reduction of ripening.

Several studies have been carried out to analyze QTLs that are related to rice yields (Yano et al. 1995, Lin et al. 1996, Lu et al. 1996, Wu et al. 1996, Xiao et al. 1996, Zhuang et al. 1997, Redoña and Mackill 1998, Xiao et al. 1998, Moncada et al. 2001). Zhuang et al. (1997) identified a QTL on chromosome 1 for the number of spikelets per panicle that was located near the sd-1 locus, which is the major gene for semi-dwarfism in rice. However, this locus was located on the long arm of the chromosome, and was different from the locus detected in our study. The QTL on chromosome 1 that was detected by Xiao et al. (1998) appears to lie close to the locus that we identified, although precise comparisons are difficult because of the difference in the markers used. Moreover, the effect of their QTL was not very strong [percentage of variance explained (PVE)=4.45%], compared to ours (PVE = 37.2% for the RILs of Milaying23/Akihikari in 1998). Recently, Yagi et al. (2001) have identified a QTL that lies close to the R3192 marker on chromosome 1 using the same RILs from Milaying23/Akihikari; this QTL appears to be identical with that described in our study. In addition, we found that this QTL markedly increased the number of spikelets per panicle (PVE = 34.3% in 1998) in the BCILs from Habataki × Sasanishiki. This locus is ubiquitous in high-yielding indica cultivars; it is derived from IR8, and has been used to increase the number of spikelets in recently bred high-yielding indica-japonica crossed cultivars in Japan, as described by Yagi et al. (2001).

It might be possible to divide this region on chromosome 1 into segments that include one or two peaks: (1) the regions between N079A and XNpb90, and between XNpb90 and R210A in the RILs (Fig. 6 B1); and (2) the regions between SI4064EH and C955, and between C955 and S11122EH in the BCILs (Fig. 7 B1). The effects of these two segments were almost equivalent in terms of sink size. However, in terms of ripening percentage, only the former region was highly significant in the BCILs (Fig. 7 D1), and the effect of the former region was more pronounced than that of the latter region in the RILs (Fig. 6 D1). These data suggest that there are two different QTLs for the sink size, although more precise experiments should be conducted to determine the exact number of QTLs in this region.

Lu et al. (1996) and Xiao et al. (1998) reported the presence of QTLs on chromosome 6 that modulated the number of spikelets per panicle. These QTLs appear to be located close to each other, and lie close to the QTL in the BCILs from the Sasanishiki × Habataki cross described in this study. However, it is not clear whether the QTLs identified in other studies have the same characteristics as those observed in our experiments. The locations of the QTLs on chromosome 6 in the RILs and the BCILs were slightly different (Fig 4, Fig. 5, Fig. 6 and Fig. 7). It remains to be determined whether there are two different QTLs with a similar function on chromosome 6, either of which can be expressed in the RILs or BCILs, or whether one QTL is present at different locations. A more precise determination of the location using heterozygote segregation and the production of near isogenic lines is underway. However, the prominent characteristic of the QTLs reported here is consistent: the increase in the number of spikelets was independent of both the number of panicles per plant and the ripening percentage. Therefore, the QTLs detected in these two populations may be equally useful in increasing crop yields. Should two different QTLs exist, we might expect that the cumulative effect would also lead to increase yields.

The different effects of the QTLs on chromosomes 1 and 6 may be due to morphological differences in the pani
cles. The increased number of spikelets per panicle, which occurs in plants with the indica allele in the QTL on chromosome 1, is related to the increase in the number of secondary rachis branches (Fig. 6 E1 and Fig. 7 E1). The spikelets on the secondary rachis branches often showed a poor grain filling ability, compared with those on the primary rachis branches (Wada 1969, Chaudhry and Nagato 1970). Therefore, the locus that increased the number of secondary rachis branches might reduce the ripening percentage. On the other hand, the QTLs on chromosome 6 probably led mainly to the increase of the number of primary rachis branches (Fig. 6 E6 and Fig. 7 E6). Sasahara et al. (1999) reported that the QTL
for the number of primary rachis branches and major vascular bundles in a peduncle was located on chromosome 6 for the RILs that were derived from the cross Asominori (japonica)/IR24 (indica). Although they did not report the number of spikelets or the ripening percentage, the location of the QTL was close to that observed in our experiment for the number of primary rachis branches, both in the RILs and BCILs. Accordingly, the increase in the number of spikelets per panicle and in the number of primary rachis branches that were controlled by this locus was probably accompanied by an increase in the number of major vascular bundles. If this assumption could be verified, the translocation across section for the source supply to the increased number of spikelets would be maintained, and thus the ripening percentage may not decrease when the source production itself is not limited (Kamejima et al. 1987). Source ability could also increase by the region that included the QTL (Fig. 6 F6 and Fig. 7 F6). Therefore, both panicle structure and source ability may contribute to the increase in the sink size without a reduction of the ripening percentage. The QTLs that were found on chromosome 5 in the BCILs and on chromosome 7 in the RILs increased the number of primary rachis branches and the number of spikelets per panicle (Fig. 4 and Fig. 5). However, these QTLs may not be very useful in improving the sink size, since they also reduced the number of panicles per plant.

The QTL for total dry weight on the long arm of chromosome 1 seems to increase the source ability and the number of primary rachis branches when the japonica allele is present in the BCILs (Fig. 7 E1 and F1). However, this QTL appears to reduce the number of panicles per plant in plants with the same japonica allele, without inducing a significant increase in the number of spikelets (Fig. 7 A1 and B1). We conclude that this QTL stimulates vegetative growth and the production of thin panicles, which may not be very useful in enhancing crop yields.

Improving the ripening ability without reducing the sink size is an alternative way to increase rice yield. Therefore, it is important to detect QTLs for ripening that are independent of the sink size. For the analysis of ripening, it is essential to distinguish the grain filling ability, which is affected by the sink-source relationship, from sterility, which might be affected by hybrid sterility. Therefore, we categorized carefully the spikelets with a specific gravity less than 1.0 into those due to sterility and those due to an insufficient filling ability associated with the sink-source relationship, and identified QTLs that affected each of these categories. We identified a QTL, at the same location on chromosome 1 as the QTL for the number of spikelets per panicle, which increased the percentage of sterile and/or incompletely filled spikelets with indica genotypes (Fig. 4 and Fig. 5). This appears to be a pleiotropic QTL. Increase of the sink size, in the absence of an increase in both the translocation cross-section and source ability, may lead to a deficiency in source supply, and thus increase the frequency of incompletely filled spikelets. Another QTL on chromosome 12 in the BCILs did not affect the sink size (Fig. 5 and Fig. 8 D12). However, this QTL also seemed to be pleiotropic, in that it reduced the number of incompletely filled spikelets but increased the number of sterile spikelets, or vice versa (Fig. 8 C12). Some of the other QTLs for incompletely filled spikelets on chromosomes 11 and 12 in the RILs were independent of the sink size, and showed negligible pleiotropic effects on the sterile spikelets (Fig. 8 A11, A12, B11 and B12). Although these QTLs were not consistently observed over two-year period, and thus need to be confirmed, they might be useful for independently improving incompletely filled spikelets without reducing the overall number of spikelets.

In these experiments, the number of plants per line subjected to measurements was small, particularly in the case of the RILs (1 in 1997 and 3 in 1998). This factor, in addition to the presence of markers that showed segregative distortion in the RILs, might reduce both the efficiency of detection of significant QTLs and the PVE values for the detected QTLs (Table 1). Cheng and Ukai (1995) simulated the effect of segregative distortion and showed that it did not affect appreciably the estimation of QTL positions that were located close to the distorted markers, but that it gave rise to an underestimation of both the LOD scores and the additive and dominance effects of the QTLs. Reduction in the number of lines from 191 to 72 in 1998 effectively eliminated the segregative distortion, except on chromosomes 4 and 11. These chromosomes were investigated further to confirm that the QTL found in this region was not an artifact due to a distortion that was introduced by decreasing the number of lines. In the BCILs, 10 and 5 plants per line were used in 1998 and 1999, respectively. The number of plants in 1999 was not sufficient, but comparison with the data from 1998 may give more reliable data than those obtained for the RILs. The BCILs also displayed segregative distortions on chromosomes 3, 4, 6 and 9 (Hirayama et al. 1999). However, no QTL was linked to the distorted segregation markers in the BCILs.

In this study, two QTLs were identified that affected the sink size by increasing the number of spikelets per panicle. We found that the QTL on chromosome 6 increased the sink size without reducing the ripening percentage, which enables to overcome a major obstacle to increasing yields. In addition, we identified QTLs that controlled the percentage of incompletely filled spikelets without affecting the sink size. It may thus be possible to breed cultivars that both display an increased sink size and increased ripening percentage by combining these QTLs.

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**Literature Cited**


