Mapping of Seed Shattering Loci Provides Insights into Origin of Weedy Rice and Rice Domestication

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Abstract

Seed shattering is an important trait that distinguishes crop cultivars from the wild and weedy species. The genetics of seed shattering was investigated in this study to provide insights into rice domestication and the evolution of weedy rice. Quantitative trait locus (QTL) analysis, conducted in 2 recombinant inbred populations involving 2 rice cultivars and a weedy rice accession of the southern United States, revealed 3–5 QTLs that controlled seed shattering with 38–45% of the total phenotypic variation. Two QTLs on chromosomes 4 and 10 were consistent in both populations. Both cultivar and weedy rice contributed alleles for increased seed shattering. Genetic backgrounds affected both QTL number and the magnitude of QTL effects. The major QTL qSH4 and a minor QTL qSH3 were validated in near-isogenic lines, with the former conferring a significantly higher degree of seed shattering than the latter. Although the major QTL qSH4 overlapped with the sb4, the presence of the nonshattering single nucleotide polymorphism allele in the weedy rice accession suggested involvement of a linked locus or an alternative molecular genetic mechanism. Overlapping of several QTLs with those from earlier studies indicated that weedy rice may have been derived from the wild species Oryza rufipogon. Natural hybridization of rice cultivars with the highly variable O. rufipogon present in different geographic regions might be responsible for the evolution of a wide range of phenotypic and genotypic variabilities seen in weedy rice populations worldwide.

Key words: evolution, Oryza rufipogon, Oryza sativa, quantitative trait locus, red rice, sh4

Wild and weedy species were transformed into domesticated crops through the modification of a suite of morphological and physiological attributes, collectively referred to as the “domestication syndrome” (Harlan 1975), to improve crop productivity and facilitate harvesting. The study of the evolutionary genetic changes associated with the domestication process offers new insights into past human interventions, as well as new clues for crop improvement. Limited seed shattering (SH) is a signature domestication trait that distinguishes crop cultivars from their wild ancestors. Heavy shattering, which has been eliminated during the domestication process, is an important adaptive attribute for successful expansion and persistence of all wild and weedy species (Warwick and Stewart 2005). Although a moderate degree of seed shattering in cultivated species is usually preferred, the degree of seed shattering in rice cultivars depends on the harvesting methods followed in different geographic regions. Moderate shattering rice varieties are preferred for both hand and combine harvesting, but harvesting by small head-feeding combines requires hard-to-thresh or nonshattering varieties (Kobayashi 1990). Shattering is routinely evaluated in breeding programs to reduce crop loss. Because progress in exploiting wild rice species for rice improvement is often delayed or restricted due to linkage of seed shattering genes with desirable traits, precise localization of SH loci will positively impact rice breeding programs.

Cultivated rice exhibits a wide range of seed shattering. Generally, indica varieties shatter more easily than japonica cultivars. Four shattering genes, sb1, sb2, Sh3, and sb4, were identified and localized on chromosomes 11, 1, 4, and 3, respectively, in rice, using the classical genetics approach (Eiguchi and Sano 1990; Oba et al. 1990; Fukuta 1995; Nagai et al. 2002). A number of quantitative trait locus (QTL) mapping studies conducted in wild species (Oryza rufipogon (Xiong...
et al. 1999; Cai and Morishima 2000; Thomson et al. 2003; Lee et al. 2005; Ishikawa et al. 2010), *Oryza nivara* (Li et al. 2006a), weedy rice (Bres-Patry et al. 2001; Gu et al. 2005; Thurber et al. 2013), and cultivated rice (Qin et al. 2010) demonstrated complex polygenic control for SH.

To date, 4 shattering genes with large effects have been cloned: *sh4* (Li et al. 2006b; Lin et al. 2007), *qSH1* (Konishi et al. 2006), *sb-b* (Ji et al. 2010), and *SHAT1* (Zhou et al. 2012). Seed shattering in rice is controlled by the formation of the abscission layer between the grain and pedicel. The *sh4* gene encodes a transcription factor with a Myb3 DNA binding domain and a nuclear localizing signal and was isolated from the wild species *O. nivara* (Li et al. 2006b). It is reported that a single nucleotide substitution of G to T at the *sh4* locus is responsible for reduced SH in cultivated rice. The QTL *qSH1* was identified by using a population developed from a cross between a shattering *indica* type, Kasalath, and a nonshattering *japonica* cultivar, Nipponbare (Konishi et al. 2006). A single nucleotide polymorphism (SNP) in the 5’ regulatory region of a BEL1-type homeobox gene is responsible for reduced SH in most *japonica* rice cultivars. The *sb-b* gene, cloned from a shattering mutant line, encodes a nuclear phosphatase, which represses the formation of the abscission layer (Ji et al. 2010). To investigate the molecular regulation of seed shattering, Zhou et al. (2012) mutagenized an easy shattering line with an introgression of a chromosome segment carrying the *sb4* locus from *O. rufipogon* and identified 2 nonshattering mutants. Using the map-based cloning approach, they identified an AP2 domain containing the transcription factor gene, *SHAT1*, in 1 mutant, affecting the abscission zone development. The other was a null allele of *sb4* with a much stronger nonshattering phenotype. Red pericarp (*Rc*), a distinguishing characteristic of weedy red rice, is believed to be closely associated with *SH* and is eliminated during rice domestication (Sweeney et al. 2007).

The replacement of the shattering allele of the *sb4* gene with the mutant nonshattering allele has been widely viewed as a major event in the evolution of the cultivated rice. This claim has been contested by several researchers as more diverse wild, weedy, and cultivated samples were analyzed (Izawa 2008; Thurber et al. 2010; Zhu et al. 2012). Due to sharing of similar haplotypes of *sb4* associated with reduced seed shattering in both cultivated and weedy rice, Thurber et al. (2010) concluded that the single nucleotide mutation in the *sb4* gene alone may not be sufficient to reduce SH. Similarly, Zhu et al. (2012) reported the presence of the nonshattering *sb4* allele in all weedy rice varieties and in wild species with heavy shattering phenotype in high frequency. They hypothesized that there are still unidentified shattering loci, which may have played important roles in the initial domestication of cultivated rice. On the other hand, Izawa (2008) suggested the involvement of multiple loci in the rice domestication process. Therefore, QTL mapping studies involving diverse materials are needed for understanding the rice domestication process.

Weedy red rice is traditionally classified as *Oryza sativa* based on its morphological attribute and easy crossability with the cultivated rice (Langevin et al. 1990). Due to its high genetic and phenotypic variability (Oka 1988), it has been a major weed problem for rice growers in the southern United States, as well as other parts of the world. Due to its closeness with cultivated rice and availability of abundant rice genomic resources, including whole genome sequences (IRGSP 2005), weedy red rice represents an excellent experimental organism to investigate the genetic mechanisms of evolution in red rice and molecular basis of weedy attributes. Application of genomics tools has been beneficial in understanding the processes of crop evolution as well as crop improvement (Paterson 2002). QTL mapping and comparative mapping has been instrumental in advancing our understanding of the domestication process in many field crops. This has been done through the elucidation of genetic control of domestication traits with an emphasis on number, chromosomal location, phenotypic effects, and genetic interactions (Paterson 2002; Peng et al. 2003). *Oryza rufipogon*, which is considered as a wild ancestor, has been used in numerous studies, but the weedy rice varieties, which shed seeds easily, have not been looked at extensively (Bres-Patry et al. 2001; Gu et al. 2005; Thurber et al. 2013) in elucidating the genetic basis of SH. In a population involving temperate weedy rice, Bres-Patry et al. (2001) identified a single QTL for SH, whereas Gu et al. (2005) reported 4 QTLs located on chromosomes 3, 4, 7, and 8 in a population involving an *indica* weedy rice from Thailand.

The evolution of red rice in many geographic regions of the world, where no wild relatives of rice occur, continues to be a genetic mystery. Several hypotheses have been advocated regarding the origin of weedy rice: 1) Selection and adaptation of wild rice to disturbed habitats (De Wet and Harlan 1975; Harlan 1975); 2) reversion of the crop species to a wild form; 3) hybridization between cultivated rice and its wild ancestor, *O. rufipogon* (Londo and Schaal 2007); and 4) unintentional introduction of red rice as contaminants during the colonial period based on the anecdotal evidence (Craigmiles 1978). Molecular examination of cultivated rice and Korean weedy rice revealed that weedy rice could be classified into *O. sativa*, ssp. *japonica*, or *indica*-like groups (Cho et al. 1995; Suh et al. 1997). However, the weedy rice collections of the southern United States are highly diverse, and some are closely related to *O. nivara* or *O. rufipogon* (Vaughan et al. 2001; Gealy et al. 2002). Genetic analysis of domestication traits will be helpful in understanding the origin and evolution of US red rice.

The overall goal of this study is to determine the genetic architecture for SH using 2 recombinant inbred line (RIL) populations, from crosses between 2 rice cultivars and a red rice accession, in order to enhance our understanding of the evolution of weedy rice. The variation and consistency of these QTLs in 2 different genetic backgrounds, as well as the reported QTLs in wild and weedy rice, were examined. Because data from several studies involving wide range of wild, weedy, and cultivated species have challenged the major role of *sb4* gene in the rice domestication process (Izawa 2008; Thurber et al. 2010; Zhu et al. 2012), we examined *sb4* gene sequence variation to determine whether it can be correlated to the variability in SH in weedy rice accession used in our study.
Materials and Methods

Mapping Populations and Evaluation of Seed Shattering

Two RIL populations were developed from the crosses involving 2 rice cultivars (Bengal and Cypress) and a weedy rice accession PSRR-1. Bengal is an early maturing, high yielding medium grain cultivar that originated from the cross MARS//M201/MARS (Linscombe et al. 1993a). Cypress is a high yielding, early maturing long grain cultivar developed from the cross L-202/Lemont (Linscombe et al. 1993b). The weedy rice accession is highly prone to seed shattering compared with Bengal and Cypress. The RIL population, developed from the cross “Bengal × PSRR-1” (named as BR), comprised 198 individuals in the F$_{7.8}$ generation, whereas the population developed from the cross “Cypress × PSRR-1” (named as CR) had 174 RILs in the F$_{8.9}$ generation. Two near-isogenic lines (NILs) for 2 QTLs (qSH3BR and qSH4BR) were developed in Bengal background by repeated backcrossing coupled with marker-assisted selection.

The parents and both RIL populations were grown at the Central Research Station of the LSU Agricultural Center in Baton Rouge, LA. Each line was sown in a 2-m row of 20 plants with a row spacing of 20 cm. Standard cultural practices were followed (Linscombe et al. 1999). Due to variation in heading dates in the mapping populations, heading date was recorded to ensure proper timing of harvest for phenotypic evaluation. Mean temperature between heading date and harvesting ranged from 23 °C to 34 °C. Plants were considered to reach physiological maturity 30–35 days after flowering. Five plants were randomly sampled from each line in order to assess SH, and 2 panicles from each plant of each RIL or parent were picked by hand at physiological maturity, and a shattering score was given for each line on a scale of 1–9: 1 = no shattering, 3 = low shattering, 7 = average shattering, and 9 = heavy shattering.

The NILs along with parents were evaluated in a greenhouse, and 10 panicles from 10 plants were evaluated for shattering using a digital force gauge (Imada, Northbrook, IL). Breaking tensile strength (BTS) was determined as the weight required to release the seed from the pedicel. A digital force gauge was suspended from a stand with panicles attached to it. Individual seed from the panicle was detached by holding the seed with a clip, and the peak measurement on grain removal was recorded.

QTL Mapping and Statistical Analysis

DNA from each RIL and parent was isolated by the potassium acetate method (Dellaporta et al. 1983), and genotyping of both mapping populations was done using simple sequence repeat (SSR) markers. Primers were synthesized by Alpha DNA, Canada, using the available primer information (McCouch et al. 2002; http://www.gramene.org/microsat/RM_primers.html). SSR analysis was conducted following the protocol outlined by Chen et al. (1997). The polymerase chain reaction (PCR) products were separated on a 4.5% superfine resolution agarose (Amresco) gel and visualized under UV light after ethidium bromide staining.

Genetic linkage maps for both RIL populations were generated (Subudhi et al. 2012) using MAPMAKER/EXP (Lander et al. 1987) with the Kosambi function (Kosambi 1944).

QTL Cartographer V 2.5 (Wang et al. 2011) was used for QTL analysis following composite interval mapping (CIM) procedure. Logarithm of odds (LOD) threshold values for CIM were determined based on 1000 permutations to declare significant QTLs at $P < 0.01$. For both BR and CR populations, these LOD values were 3.73 and 3.36, respectively. The QTLs identified at LOD 2.4 were included as suggestive QTLs. CIM was performed by using the standard model (model 6) in the forward and backward regression method, which included the selection of 15 markers as cofactors with window size of 10 cM to identify QTLs with 1 LOD confidence interval. The total phenotypic variation explained by all putative QTLs was estimated by fitting a model in the multiple interval mapping procedure of QTL Cartographer. QTLs were named after the nomenclature of McCouch et al. (1997), with some modification to distinguish QTLs detected for the same trait in both RIL populations. A superscript of “BR” or “CR” was added after the QTL to indicate a QTL identified in BR or CR populations, respectively. Interactions among the identified QTLs were detected using the multiple interval mapping method in the QTL Cartographer.

Analysis of variance was conducted using the seed shattering score collected on each RIL. Broad-sense heritability was calculated as $H = \frac{\sigma_e^2}{\sigma_g^2 + \sigma_e^2}$, where $H$ is broad-sense heritability, $\sigma_g^2$ is the genetic variance, and $\sigma_e^2$ is the error variance from the trial. Frequency distributions for both traits were implemented using SAS (SAS Institute Inc. 2006).

Sequencing of sh4

The region with an SNP responsible for SH in the first exon of the $sh4$ was amplified from the genomic DNA of PSRR-1 using Phusion High Fidelity DNA Polymerase (New England Biolab, MA) and cloned into the pGEM-T Easy vector system I (Promega Corp., WI), followed by sequencing. Primers were designed based on the available sequences in the database (SH4F1: 5′-ATGTCGGGTCTCCTGCGGA-3′; SH4RGD: 5′-TCAGCTCGCTGGAGCTG-3′). DNA sequencing was performed in an ABI Prism 3130 sequencer (Applied Biosystem) at the Gene Lab of the School of Veterinary Medicine, Louisiana State University. Three independent PCR products were sequenced. The available $sh4$ nucleotide sequences for Bengal, Cypress, and other accessions were downloaded from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) for comparison. DNA and protein sequences were aligned using MegaAlign module of the Lasergene genomics suite 11.0 (DNAStar, Madison, WI). The partial nucleotide sequence of $sh4$ of red rice accession PSRR-1 was submitted to GenBank under accession number JN014834.

Results

Phenotypic Variation

Both rice cultivars, Bengal and Cypress, differed significantly from PSRR-1 with respect to SH (Figure 1). The SH
score of Bengal, Cypress, and PSRR-1 was 5.0, 7.0, and 9.0, respectively. The intensity of SH in both hybrids was similar to PSRR-1. The mean SH score was 7 in both RIL populations. There was a skewed distribution for SH toward the weedy rice. There were transgressive segregants for extreme nonshattering. A full spectrum of variation, from extreme shattering to nonshattering in RILs with red and white pericarp, was observed in both populations. The broad-sense heritabilities for SH were 0.89 and 0.61 in the BR and CR population, respectively.

**QTL Analysis**

Because the transformation of SH score did not improve the trait distribution, original SH scores were used for QTL analysis. The BR linkage map consisted of 212 markers with a total distance of 1410 cM, whereas the CR linkage map consisted of 189 markers with a total distance of 1574 cM (Subudhi et al. 2012). The marker order in both linkage maps was similar to those published earlier (McCouch et al. 2002; Temnykh et al. 2001). CIM detected 5 QTLs for seed shattering in the BR population on 5 chromosomes (Table 1, Supplementary Figure 1 online). A wide range of variation was observed with respect to the magnitude of additive effects and percentage of the phenotypic variation explained by these QTLs. The largest QTL on chromosome 4 explained 22% of the phenotypic variance, and the remaining QTLs had smaller effects on phenotypic variation ranging from 4% to 9%. In the multilocus model implemented in QTL Cartographer, all 5 QTLs accounted for 45% of the phenotypic variation. Weedy rice alleles were responsible for increased SH in 3 QTLs on chromosomes 2, 3, and 4, whereas the Bengal alleles contributed to increased shattering in the rest 2 QTLs located on chromosomes 7 and 10.

In the CR population, 3 QTLs were detected on chromosomes 4, 6, and 10 with a total phenotypic variation of 38% (Table 2, Supplementary Figure 1 online). The contribution of the QTL on chromosome 4 was higher than the BR population with 30% of the phenotypic variation. The effect of other 2 QTLs on chromosomes 6 and 10 was substantially smaller, explaining 3% and 4% of the phenotypic variation. Cypress was the donor for SH increasing allele in 1 QTL (qSH6CR), and PSRR-1 allele of the remaining 2 QTLs (qSH4CR, qSH10CR) was responsible for increased SH.

The linkage groups of each RIL population were aligned using common markers, and comparison of the QTL positions revealed that 2 QTLs were consistent in both populations (Figure 2). For qSH4BR and qSH4CR, the QTL peak was near the same marker RM127. Although there was congruency in the position of the SH QTL on chromosome 10, cultivated allele contributed to enhanced SH in BR population, but weedy rice allele was responsible for increased SH in CR population.

**Epistatic QTLs**

In BR population, 5 epistatic QTLs affecting SH were identified, whereas only one epistatic QTL was identified in CR population (Table 3). Most of these QTL interactions accounted for a small amount of phenotypic variation with a range of 3–5%. The QTL interaction between the consistent major QTL on chromosome 4 and minor QTL on chromosome 10 was identified in both populations. Another interesting observation was in the BR population; the QTL on chromosome 2 (qSH2BR) interacted with QTLs localized on chromosome 3 (qSH3BR) and chromosome 10 (qSH10BR). The qSH4BR interacted with qSH7BR and qSH10BR. All SH QTLS in BR population were involved in epistatic
interactions, with the QTL $qSH10^{BR}$ being consistent in interacting with all QTLs.

**Sequence Variation in sh4**

The comparison of the partial nucleotide sequence for the first exon of $sh4$ from weedy rice PSRR-1 with those of Bengal, Cypress, 2C05 (wild Asian rice O. rufipogon), IRGC80470 ($O. nivara$), and CL 16 (traditional tall indica cultivar) revealed 6 SNPs at the 114th, 147th, 237th, 455th, 656th, and 677th nucleotide positions (Supplementary Figures S2 and S3 online). Nucleotide substitution of G with T at the 114th position was earlier reported to contribute to the non-shattering phenotype ($sh4$) and 656th position was numerically smaller and significant compared with the other 4 accessions. At the 455th and 677th nucleotide positions, IRGC80470 and 2C05 differed from others, having C instead of T. The above 6 SNPs resulted in 4 amino acid substitutions, and CL 16 differs from others having 2 amino acid differentiations PSRR-1 from other accessions (glutamine in PSRR-1 and proline in BR-RIL population) and 677th nucleotide positions, the weedy rice accession has C and A, respectively, in the other 4 accessions. At the 455th and 677th nucleotide positions, IRGC80470 and 2C05 differed from others, having C instead of T. The above 6 SNPs resulted in 4 amino acid substitutions, and CL 16 differs from others having 2 amino acid differentiations PSRR-1 from other accessions (glutamine in PSRR-1 and proline in the others) (Supplementary Figure S3 online).

**Validation of Two QTLs in NILs**

We chose a major QTL ($qSH-4^{BR}$) and a minor QTL ($qSH-3-1^{BR}$) from the BR-RIL population to develop NILs by marker-assisted selection and repeated backcrossing to the recurrent parent Bengal. The IL4-1 had a red rice–derived segment between markers RM348 and RM127, whereas the red rice segment between RM3203 and RM5819 was introgressed in IL3-1 (Figure 3a). The comparison of BTS values among the parents and the NILs (Figure 3b) indicated that the mean BTS value of Bengal was significantly higher than both NILs. The BTS value of IL4-1 harboring the major QTL $qSH-4^{BR}$ was numerically smaller and significant compared with the IL3-1 with the introgressed minor QTL $qSH-3-1^{BR}$.

**Discussion**

**Genetics of Seed Shattering in Weedy Rice**

An important objective of this study was to identify QTLs for SH, which is a major domestication attribute responsible for transformation of wild species to cultivated forms. Contrary to the results of classical studies that supported simpler genetic models involving few genes (Kadam 1936; Eiguchi and Sano 1990; Paterson et al. 1995), this study suggests complex genetic control with the involvement of both major and minor QTLs whose expression was modulated by epistasis (Ishikawa et al. 2010) and genetic backgrounds (Onishi et al. 2007b). Seed shattering is a key trait that differentiates weedy rice from cultivated rice. Elucidation of genomic differences between weedy rice and cultivated rice could be helpful for tracing the origin and evolution for weedy rice, as well as providing important genetic targets to develop NILs by marker-assisted selection and repeated backcrossing.

**Table 1** Quantitative trait loci for seed shattering in BR-RIL population detected using composite interval mapping procedure

<table>
<thead>
<tr>
<th>QTLs</th>
<th>Marker interval</th>
<th>Position</th>
<th>Position</th>
<th>LOD</th>
<th>AE</th>
<th>$R^2$</th>
<th>Increasing effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$qSH2^{BR}$</td>
<td>RM13910-RM13938</td>
<td>114.9</td>
<td></td>
<td>7.31</td>
<td>-0.412</td>
<td>8.9</td>
<td>PSRR-1</td>
</tr>
<tr>
<td>$qSH3^{BR}$</td>
<td>RM3203-RM3372</td>
<td>1.1</td>
<td></td>
<td>3.18</td>
<td>-0.271</td>
<td>4.4</td>
<td>PSRR-1</td>
</tr>
<tr>
<td>$qSH4^{BR}$</td>
<td>RM5506-RM127</td>
<td>112.6</td>
<td></td>
<td>12.65</td>
<td>-0.623</td>
<td>22.3</td>
<td>PSRR-1</td>
</tr>
<tr>
<td>$qSH7^{BR}$</td>
<td>RM429-RM3555</td>
<td>92.4</td>
<td></td>
<td>2.37</td>
<td>0.243</td>
<td>3.6</td>
<td>Bengal</td>
</tr>
<tr>
<td>$qSH10^{BR}$</td>
<td>RM6100-RM1146</td>
<td>63.4</td>
<td></td>
<td>3.29</td>
<td>0.317</td>
<td>5.3</td>
<td>Bengal</td>
</tr>
</tbody>
</table>

* QTL peak position on the linkage map.
* Additive effects of Bengal allele.
* Phenotypic variation (%) explained by each QTL.
* Source of allele increasing the trait value.
* Estimate of the total phenotypic variation explained by the QTL from a multiple QTL model fit in QTL Cartographer (Wang et al. 2011).

**Table 2** Quantitative trait loci for seed shattering in CR-RIL population detected using composite interval mapping procedure

<table>
<thead>
<tr>
<th>QTLs</th>
<th>Marker interval</th>
<th>Position</th>
<th>Position</th>
<th>LOD</th>
<th>AE</th>
<th>$R^2$</th>
<th>Increasing effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$qSH4^{CR}$</td>
<td>RM5506-RM127</td>
<td>106.5</td>
<td></td>
<td>13.27</td>
<td>-1.033</td>
<td>30.3</td>
<td>PSRR-1</td>
</tr>
<tr>
<td>$qSH6^{CR}$</td>
<td>RM8225-RM4924</td>
<td>57.3</td>
<td></td>
<td>2.58</td>
<td>0.402</td>
<td>3.2</td>
<td>Cypress</td>
</tr>
<tr>
<td>$qSH10^{CR}$</td>
<td>RM171-RM1146</td>
<td>60.2</td>
<td></td>
<td>3.06</td>
<td>-0.416</td>
<td>4.0</td>
<td>PSRR-1</td>
</tr>
</tbody>
</table>

* Peak position of the QTL on the linkage map.
* Additive effects of Cypress allele.
* Phenotypic variation (%) explained by each QTL.
* Source of allele increasing the trait value.
* Estimate of the total phenotypic variation explained by the QTL from a multiple QTL model fit in QTL Cartographer (Wang et al. 2011).
The weedy rice accession differed from cultivated rice with respect to at least 2 QTLs for SH that was consistent in both RIL populations (Figure 2). However, in the case of the QTL on chromosome 10, the Bengal allele increased SH in BR population as opposed to the weedy allele in CR population. Other QTLs present in either population could be responsible for the phenotypic differences between weedy and cultivated rice, but their expression tended to vary with genetic backgrounds.

Comparing positions of SH QTLs with those from earlier studies (Bres-Parry et al. 2001; Gu et al. 2005) revealed clear differences in the genetic makeup of the weedy rice accessions. Seed shattering in our study showed complete dominance, and in both populations, the trait distribution was skewed toward higher SH scores. This is in clear contrast to

Table 3  Epistatic QTLs for seed shattering identified in RIL populations developed from the Bengal × PSRR-1 and Cypress × PSRR-1 populations

<table>
<thead>
<tr>
<th>Population</th>
<th>QTL</th>
<th>Marker interval</th>
<th>QTL</th>
<th>Marker interval</th>
<th>LOD</th>
<th>Additive effect</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal × PSRR-1</td>
<td>(q_{SH4}^{BR})</td>
<td>RM13910-RM13938</td>
<td>(q_{SH3}^{BR})</td>
<td>RM3203-RM3372</td>
<td>2.46</td>
<td>−0.235</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>(q_{SH4}^{BR})</td>
<td>RM5508-RM127</td>
<td>(q_{SH7}^{BR})</td>
<td>RM6100-RM1146</td>
<td>1.44</td>
<td>0.194</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>(q_{SH4}^{BR})</td>
<td>RM13910-RM13938</td>
<td>(q_{SH10}^{BR})</td>
<td>RM6100-RM1146</td>
<td>1.51</td>
<td>0.216</td>
<td>4.0</td>
</tr>
<tr>
<td>Cypress × PSRR-1</td>
<td>(q_{SH4}^{CR})</td>
<td>RM5508-RM127</td>
<td>(q_{SH10}^{CR})</td>
<td>RM171-RM1146</td>
<td>2.49</td>
<td>−0.391</td>
<td>4.6</td>
</tr>
</tbody>
</table>

\(a\) Phenotypic variation (%) explained by the QTL interaction.
the observation of Gu et al. (2005), who observed incomplete dominance and normal distribution of the trait values. Despite the high intensity of SH in all weedy rice accessions, the genetic architecture for SH was quite distinct in each of these weedy accessions. For example, none of the QTLs from this study overlapped with the QTLs identified by Bres-Patry et al. (2001) in a population involving temperate japonica weedy rice. There was no congruence between SH QTLs identified in our study and those from a recent study involving US weedy rice (Thurber et al. 2013). The major SH QTL corresponding to the sh4 locus was identified in our study and many other studies involving O. rufipogon and O. nivara, and it was not detected by the above 3 studies involving weedy rice. These observations suggest that genetic loci other than sh4 may be responsible for the high degree of SH. A wide range of genetic divergence observed among worldwide weedy rice populations could be dependent on the geographic regions where they evolved through hybridization with highly variable O. rufipogon, as suggested by Gu et al. (2005). Contrary to earlier studies involving wild and weedy species, the cultivar alleles in some QTLs enhanced SH in our study.

In this study, we further validated the effect of the major QTL qSH4BR and 1 minor QTL qSH3-1BR in near-isogenic backgrounds of a rice cultivar Bengal. As expected, the BTS was significantly lesser in qSH4BR NIL compared with the qSH3-1BR NIL. Because the effect of the minor QTL on SH could be quantified in NIL through measuring the BTS, it would be now possible to fine map and clone this minor QTL. The validation of the major QTL would provide an opportunity to clone this QTL and to determine whether it is another locus linked to the sh4, which did not reveal sequence polymorphism among the parents used in this population.

Comparison of QTL Architecture in Different Genetic Backgrounds

Several QTLs discovered in this study (Table 4) were consistent with those from earlier studies involving O. rufipogon (Xiong et al. 1999; Cai and Morishima 2000; Thomson et al. 2003; Gu et al. 2005; Lee et al. 2005; Onishi et al. 2007a) or O. nivara (Li et al. 2006a). The qSH4 has been detected in 4 different wild rice populations (Xiong et al. 1999; Cai and Morishima 2000; Li et al. 2006a; Onishi et al. 2007a) but not in studies involving weedy rice (Brespatry et al. 2001; Gu et al. 2005; Thurber et al. 2013). Similarly, QTLs on chromosomes 3 and 6 were mapped in similar chromosomal locations in populations involving O. rufipogon (Xiong et al. 1999; Thomson et al. 2003), and the QTL on chromosome 7 was congruent with the map location in weedy rice population (Gu et al. 2005) (Table 4). The only discrepancy compared with earlier studies was with respect to 2 QTLs (qSH6CR and qSH7BR) in which cultivar alleles were responsible for increased SH in our study. There was no overlapping of any of our QTLs with those reported in a recent study involving US weedy rice accessions (Thurber et al. 2013). Many QTLs might have escaped detection in their study due to use of a low resolution linkage map.

In most modern rice cultivars, SH has been eliminated due to the combined effect of domestication and the breeder’s selection process for efficient crop production. Seed shattering needs to be optimized for successful crop production. Of the 2 rice cultivars used in this study, Cypress exhibited a higher degree of shattering compared with relatively hard to shatter cultivar Bengal, but it was still lower.
### Table 4  
Comparison of QTLs for seed shattering identified in this study with the earlier studies involving wild and weedy rice

<table>
<thead>
<tr>
<th>Populations involving wild or weedy rice</th>
<th>Chr 1</th>
<th>Chr 2</th>
<th>Chr 3</th>
<th>Chr 4</th>
<th>Chr 6</th>
<th>Chr 7</th>
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<tr>
<td><strong>A. Wild rice</strong></td>
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<tr>
<td>AijiaoNante (indica) × P16 (Oryza rufipogon) (Xiong et al. 1999)</td>
<td>$^{1}sh_{1}^{W}$ (32.67)</td>
<td>$^{2}sh_{3}^{F}$ (4.77)</td>
<td>$^{3}sh_{4}^{W}$ (34.69)</td>
<td>$^{4}sh_{6}^{C}$ (9.53)</td>
<td>$^{5}sh_{8}^{W}$ (4.10)</td>
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<td>Pei-kub (indica) × P1944 (O. rufipogon) (Cai and Morishima 2000)</td>
<td>$^{1}qSHT-1^{W}$ (31.04)</td>
<td>$^{2}qSHT-4^{W}$ (31.60)</td>
<td>$^{3}qSHT-5^{W}$ (22.88)</td>
<td>$^{4}qSHT-11^{W}$ (0.26)</td>
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<td>Jefferson (japonica) × IRGC105491 (O. rufipogon) (Thomson et al. 2003)</td>
<td>$^{1}sh_{1.1}^{W}$ (40.56)</td>
<td>$^{2}sh_{3.1}^{W}$ (0.10), $^{3}sh_{3.2}^{W}$ (12.40)</td>
<td>$^{4}sh_{4.1}^{W}$ (21.24)</td>
<td>$^{5}sh_{7.1}^{W}$ (12.78)</td>
<td>$^{6}sh_{8.1}^{C}$ (20.09)</td>
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<td>Hwayeongbyeo (japonica) × W1944 (O. rufipogon) (Lee et al. 2005)</td>
<td>$^{1}sh_{1}^{W}$ (35.19)</td>
<td>$^{2}sh_{3}^{W}$ (12.15)</td>
<td>$^{3}sh_{6}^{W}$ (13.88)</td>
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<td>A58 (japonica) × W107 (O. rufipogon) (Onishi et al. 2007a)</td>
<td>$^{1}qSHT-1^{W}$ (36.62)</td>
<td>$^{2}qSHT-3^{W}$ (23.12)</td>
<td>$^{3}qSHT-4^{W}$ (33.97)</td>
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<td>CL16 (indica) × IRGC80470 (Oryza nivara) (Li et al. 2006)</td>
<td>$^{1}sh_{3}^{W}$ (31.64)</td>
<td>$^{2}sh_{4}^{W}$ (34.04)</td>
<td>$^{3}sh_{8}^{W}$ (22.46)</td>
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<td><strong>B. Weedy rice</strong></td>
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<td>Miara (japonica) × C6 (Bres-Paty et al. 2001)</td>
<td>$^{1}sh_{2}^{C}$ (33.05)</td>
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<td>EM93-1 (japonica) × SS18-2 (Gu et al. 2005)</td>
<td>$^{1}qSH1^{W}$ (22.01)</td>
<td>$^{2}qSH4^{W}$ (18.82)</td>
<td>$^{3}qSH5^{W}$ (26.63)</td>
<td>$^{4}qSH8^{W}$ (22.46)</td>
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<td>SH-RR09 × Dee Geo Woo Gen (indica) (Thurber et al. 2013)</td>
<td>$^{1}qSS1^{C}$ (2.11)</td>
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<td>BHA-RR20 × Dee Geo Woo Gen (indica) (Thurber et al. 2013)</td>
<td>$^{2}qSS1^{W}$ (40.16)</td>
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<td>Bengal (japonica) × PSRR-1 (this study)</td>
<td>$^{1}qSH2^{W}$ (30.69)</td>
<td>$^{2}qSH3.1^{W}$ (0.79)</td>
<td>$^{3}qSH4^{W}$ (34.53)</td>
<td>$^{4}qSH5^{C}$ (27.89)</td>
<td>$^{5}qSH10^{C}$ (19.61)</td>
<td>$^{6}qSH10^{W}$ (19.04)</td>
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<tr>
<td>Cypress (japonica) × PSRR-1 (this study)</td>
<td>$^{1}qSH2^{W}$ (30.69)</td>
<td>$^{2}qSH3.1^{W}$ (0.79)</td>
<td>$^{3}qSH4^{W}$ (34.53)</td>
<td>$^{4}qSH5^{C}$ (27.89)</td>
<td>$^{5}qSH10^{C}$ (19.61)</td>
<td>$^{6}qSH10^{W}$ (19.04)</td>
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</table>

* The approximate physical positions on the chromosome in parentheses were estimated based on the Gramene Annotated Nipponbare Sequence 2009 (http://gramene.org/ accessed on 9 August 2012) in megabase corresponding to the closest marker linked to the seed shattering QTL. Congruent QTLs between different populations on same chromosome are underlined. For distinguishing congruent QTLs at multiple map locations on chromosome 1, QTLs are prefixed with 1, 2, and 3. Wild or weedy allele and cultivar allele increasing seed shattering were denoted by superscripts, W and C, respectively. The nomenclature of QTLs reported in this table is same as in the corresponding manuscripts to avoid confusion.
Molecular Basis of Seed Shattering and Rice Domestication

Four shattering loci, \( sh4, gSH1, SHAT1 \), and \( sb-h \), have been cloned to unravel the molecular basis of SH and the domestication process. Among these loci, \( sh4 \) is widely accepted as the most significant gene involved in the evolution of cultivated rice (Li et al. 2006b; Purugganan and Fuller 2009). Detecting QTLs in the same location and the finding that the weedy rice allele enhanced SH at \( sb4 \) loci prompted us to analyze the key segment of this gene in all 3 parents to shed further light on the molecular basis of SH. A single nucleotide substitution from G to T in the first exon of \( sb4 \) locus was responsible for nonshattering or seed persistence phenotype (Li et al. 2006b).

The weedy rice accession PSRR-1 used in our study shared the nonshattering T mutation with Bengal and Cypress, which supported the observation of Thurber et al. (2010), who reported that the \( sb4 \) haplotype of the majority of the weedy rice samples were identical to most common haplotypes of nonshattering rice cultivars. Analysis of \( sb4 \) sequence data from a larger and geographically more diverse weedy rice accessions with heavy seed shattering phenotype by Zhu et al. (2012) revealed fixation of the nonshattering T allele of \( sb4 \) locus. Zhu et al. (2012) further observed the presence of T type allele of the \( sb4 \) in high frequency in shattering wild species (\( O. rufipogon \) complex), which is in agreement with earlier studies (Izawa 2008; Thurber et al. 2010). All these results suggest that the presence or absence of the G mutation has little influence on the SH phenotype in wild and weedy rice, and the presence of T mutation alone may not be sufficient to reduce SH. This contradicts the widely held notion that a single nucleotide mutation (G/T) of the \( sb4 \) locus was a major event in the rice domestication process. Our sequencing result is in agreement with the above observations. On the other hand, the present QTL study in 2 different genetic backgrounds clearly demonstrated that the chromosomal region in the vicinity of the \( sb4 \) allele from US weedy rice was responsible for SH. Recently, Zhou et al. (2012) identified a seed shattering \( abortion1 \) (\( shat1 \)) gene encoding an APETALA2 transcription factor, which affected the abscission zone (AZ) development in a rice mutant of a wild rice introgression line. Because the SH gene \( SHAT1 \) is located on chromosome 4 near the \( sb4 \) locus, Zhu et al. (2012) compared the \( SHAT1 \) DNA sequences of wild and cultivated species but did not find any sequence polymorphism. Based on this finding, \( SHAT1 \) might not be the locus subject to artificial selection during domestication. Zhu et al. (2012) hypothesized that the \( sb4 \) gene may be a modifier locus to an unidentified nonshattering allele or alleles fixed at one or more loci, which is consistent with the report of Li et al. (2006b), who associated \( sb4 \) locus to SH phenotype based on cosegregation and transformation results.

Ishikawa et al. (2010) investigated the allelic interaction between 2 SH loci \( gSH1 \) and \( sb4 \) from nonshattering cultivar Nipponbare in the genetic background of wild rice \( O. rufipogon \). The backcross plants with Nipponbare homozygous alleles at either \( gSH1 \) or \( sb4 \) showed extreme shattering, like wild or weedy species, but plants with Nipponbare homozygous alleles at both \( gSH1 \) and \( sb4 \) did not shatter seeds easily. This study concluded that the nonshattering habit in cultivated rice did not result from a single mutation in the wild species allele, and interactions involving some minor genes may be more likely. Because the rice cultivar Cypress shatters seeds easily despite the presence of the nonshattering \( gSH4 \) allele, we suggest that effect of multiple minor QTLs together could lead to the shattering phenotype and therefore might have a role in rice domestication process as suggested by Izawa (2008). The involvement of other unidentified loci closely linked to \( sb4 \) in this region should be investigated to provide further insights into the rice domestication process (Zhu et al. 2012).

Evolution of US Weedy Rice

Although most studies involved the wild ancestor \( O. rufipogon \) or \( O. nivara \) to understand the domestication process, weedy rice varieties were rarely used for these studies despite their propensity to shatter and higher level of seed dormancy. It could be due to the fact that weedy rice varieties were never considered as wild species, and until now, their origin and evolution have not been unambiguously elucidated. Our study demonstrated that QTL mapping could be an efficient tool to trace the footprints of crop domestication and to provide insights into the origin and evolution of weedy species (Paterson 2002). The detection of QTLs with major effects in wild and weedy species (Xiong et al. 1999; Cai and Morishima 2000; Thomson et al. 2003; Gu et al. 2005; Lee et al. 2005; Li et al. 2006a) suggests that the selection against major loci such as \( sb4 \) might have occurred at the initial stages of domestication, resulting in significant reduction in SH. However, most modern cultivars still retained loci with minor effects (Ishikawa et al. 2010) that exhibit variable degree of SH depending on the combination of these QTLs incorporated during the breeding process.
Most of the QTLs identified in this study were minor QTLs, with the exception of qSH4\textsuperscript{Br}, which overlapped with \textit{sb4} (Li et al. 2006b). However, no SH QTL was detected in both populations in the regions harboring 2 other SH loci—\textit{qSH1} (Konishi et al. 2006) and \textit{sh-h} (Ji et al. 2010)—cloned from cultivated rice and a mutant line, respectively. None of the QTL studies involving weedy rice or intraspecific crosses reported QTLs closer to these SH loci, which suggests that \textit{qSH1} and \textit{sh-h} loci may not be involved in evolution of US weedy rice (Zhang et al. 2009; Thurber et al. 2010).

Several hypotheses have been proposed regarding the origin of weedy rice on a global scale. But in the case of US weedy rice, hybridization and reversion were suggested as the most likely events (Londo and Schaal 2007). The reversion hypothesis was supported by studies on genomewide variation in weedy rice (Londo and Schaal 2007; Gealy et al. 2009; Reagon et al. 2010) and the documentation of the spontaneous reversion of the \textit{Rc} allele (Brooks et al. 2008; Lee et al. 2009). On the other hand, weedy rice was suggested as possible descendant of \textit{indica} or \textit{aus} cultivated rice (Londo and Schaal 2007). Sequencing results of 2 major loci of domestication—\textit{Rc}, \textit{sb4}, and the surrounding regions in weedy, domesticated, and wild species samples of rice by Gross et al. (2010) and Thurber et al. (2010)—did not support the reversion hypothesis because the weedy \textit{Rc} haplotypes were not derived from the US \textit{japonica} cultivars and were distinct from that of red pericarp landraces (Gross et al. 2010). Our results are in agreement with the earlier findings (Thurber et al. 2010; Zhu et al. 2012); weedy rice and cultivated rice share the same single nucleotide substitution associated with reduced SH at the \textit{sb4} locus (Li et al. 2006b). However, the suggestion of Thurber et al. (2010) that weedy rice lost and then reacquired shattering after divergence from its progenitors through an alternative genetic mechanism is not supported by the findings from our study. Because the major QTL \textit{qSH4} was detected consistently in both populations, it is highly unlikely that weedy rice ever lost the shattering habit, thus warranting further investigation of this QTL region.

Despite the suggestion of a selective sweep of the \textit{Rc} genomic regions in the cultivated rice, Gross et al. (2010) could not rule out involvement of \textit{O. rufipogon} in the origin of weedy rice due to an inadequate sample size. Our study suggests that hybridization involving the highly diverse, widely distributed outcrossing species \textit{O. rufipogon} could be responsible for the evolution of weedy rice. Overlapping of several QTLs identified in this study with those from earlier studies involving \textit{O. rufipogon} (Table 4) lends further evidence that weedy traits were probably derived from \textit{O. rufipogon}. Particularly, the major QTL \textit{qSH4} was consistently identified in both RIL populations in this study, as well as in earlier studies involving wild species including \textit{O. rufipogon} (Xiong et al. 1999; Cai and Morishima 2000; Onishi et al. 2007a) but not in cultivated rice, suggesting inheritance of this QTL region from \textit{O. rufipogon}. Because the cultivated rice retained QTLs with minor effects, it is highly unlikely that weedy rice reacquired the shattering habit from cultivated rice as suggested by Thurber et al. (2010); hence, the involvement of minor QTLs is unlikely to result in such extreme shattering phenotypes as seen in weedy rice.

Our earlier study (Subudhi et al. 2012) demonstrated that the red rice accession PSRR-1 has the ancestral functional \textit{Rc} allele like \textit{O. rufipogon}, but it was deleted in both cultivars used in this study. When land races with red pericarp were ruled out as the donor for the red pericarp in red rice (Gross et al., 2010), involvement of \textit{O. rufipogon} may be most likely. High levels of segregation distortion, sterility, and occurrence of segregants with many weedy characteristics—response to photoperiod, awned spikelets, variable tiller angle, and profuse tillering in segregating generations from the crosses involving weedy rice and cultivated rice (Subudhi P, unpublished data)—further lend support for the origin of weedy rice through the introgression of genes from \textit{O. rufipogon}.

**Supplementary Material**

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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**References**


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