Introduction

Seed dormancy is an important trait in programs for the breeding of rice and other cereal crop species because it confers resistance to pre-harvest sprouting (PHS). To detect quantitative trait loci (QTLs) for pre-harvest sprouting resistance, we used chromosome segment substitution lines (CSSLs) derived from a cross between the Japanese upland rice cultivar ‘Owarihatamochi’ and the lowland rice cultivar ‘Koshihikari’. In the CSSLs, several chromosomal regions were associated with PHS resistance. Among these, the chromosome 9 segment from ‘Owarihatamochi’ had the greatest association with increased PHS resistance. Further QTL analysis using an advanced backcross population (BC4) from a ‘Koshihikari’ × ‘Owarihatamochi’ cross revealed two putative QTLs, here designated qSDR9.1 (Seed dormancy 9.1) and qSDR9.2, on chromosome 9. The ‘Owarihatamochi’ alleles of the two QTLs reduced germination. Further fine mapping revealed that qSDR9.1 and qSDR9.2 were located within 4.1-Mb and 2.3-Mb intervals (based on the ‘Nipponbare’ reference genome sequence) defined by the simple sequence repeat markers RM24039 and RM24260 and Indel_2 and RM24540, respectively. We thus identified two QTLs for PHS resistance in ‘Owarihatamochi’, even though resistance levels are relatively low in this cultivar. This unexpected finding suggests the advantages of using CSSLs for QTL detection.

Key Words: seed dormancy, germination, upland rice, QTL, pre-harvest sprouting resistance, PHS resistance.
Time (MFT) has been reported (Nakamura et al. 2011). Recently, a wheat PHS resistance gene, PHS1, and a barley PHS resistance gene, SD2, were isolated, and it was revealed that a mitogen-activated protein kinase cascade controls seed dormancy (Nakamura et al. 2016, Torada et al. 2016). Furthermore, map-based cloning of barley Qsd1 has revealed that an aminotransferase encoded by Qsd1 controls seed dormancy (Sato et al. 2016).

QTLs for seed dormancy or PHS resistance have also been mapped in rice. So far, more than 165 QTLs associated with seed dormancy have been identified (reviewed by Magwa et al. 2016). These QTLs have been reported in the progeny of crosses between japonica and indica cultivars (Dong et al. 2003, Gao et al. 2008, Guo et al. 2004, Lin et al. 1998, Wan et al. 2005, You et al. 2006), in weedy rice (Gu et al. 2004), and in the wild relatives of rice Oryza rufipogon and Oryza nivara (Cai and Morishima 2000, Lee et al. 2005, Thomson et al. 2003). Moreover, many QTL candidates have been detected by using population genomics methods such as genome-wide association study (Magwa et al. 2016). Of these QTLs, Seed dormancy 4 (Sdr4), a major regulator of dormancy in rice, was the first to be molecularly identified in crops. It was identified through map-based cloning using backcross inbred lines (BILs) derived from crosses between the japonica cultivar ‘Nipponbare’ and the indica cultivar ‘Kasalath’ (Sugimoto et al. 2010). A major QTL for PHS resistance has been identified on the short arm of chromosome 3 in BILs derived from crosses between the lowland japonica rice cultivars ‘Nipponbare’ and ‘Koshihikari’ (Hori et al. 2010). Among the japonica cultivars, ‘Koshihikari’ has relatively strong PHS resistance. A strong candidate PHS resistance gene from weedy rice, qSD7-1, is present at the Rc locus, which encodes a transcription factor that controls both pigmentation and abscisic acid biosynthesis (Gu et al. 2011). Map-based cloning of qSD1-2 of weedy rice has shown that this gene region includes OsGA20ox2, also known as semidwarfl (sd1), and controls seed dormancy through gibberellic acid biosynthesis (Ye et al. 2015).

Usually, QTL analysis for PHS resistance is done by using populations derived from crosses between cultivars with high and low levels of PHS resistance. Previously, we attempted to identify PHS resistance QTLs in crosses between ‘Nipponbare’ and PHS-resistant ‘Kasalath’ (Lin et al. 1998), ‘Koshihikari’ and resistant ‘Nona Bokra’ (Marzogui et al. 2012), and ‘Nipponbare’ and the more resistant ‘Koshihikari’ (Hori et al. 2010). In these genetic analyses, the donor cultivars showed higher PHS resistance than the recurrent parent cultivar and PHS resistance QTLs were identified. However, we made a very interesting observation during the development of CSSLs by using the Japanese upland rice ‘Owarihatamochi’. In general, upland rice cultivars, including ‘Owarihatamochi’, have high germinability rates upon direct seeding to overcome competition from weeds (Sasaki 1974). Although ‘Owarihatamochi’ exhibits low levels of PHS resistance (lower than in ‘Koshihikari’), here we observed some progeny with relatively high levels of PHS resistance among advanced backcross progeny of a cross between these ‘Owarihatamochi’ and ‘Koshihikari’ as the recurrent parent. These progeny, unexpectedly, had higher PHS resistance than ‘Koshihikari’. This interesting observation suggests that some genetic factor in ‘Owarihatamochi’ increases the level of PHS resistance.

We then performed further analyses, including validation of the effects of the QTLs found on chromosome 9, by using an advanced backcross population, BC,F2, derived from crosses between ‘Owarihatamochi’ and ‘Koshihikari’. Fine mapping narrowed down the candidate genomic regions of the QTLs. For further characterization of the identified QTLs, we investigated the mRNA levels of dormancy-related genes in substitution lines stacking each QTL, and we discuss the possible mechanisms of action of the genes associated with the QTLs.

### Materials and Methods

#### Plant materials

To detect the chromosomal regions involved in PHS resistance, we used CSSLs developed from a cross between ‘Owarihatamochi’ (very weak dormancy) and ‘Koshihikari’ (weak dormancy). The procedure used to develop the CSSLs is summarized in Fig. 1. We began with an F1 population derived from ‘Koshihikari’ and ‘Owarihatamochi’, and we repeatedly backcrossed the progeny with ‘Koshihikari’ to produce BC,F1 plants. We conducted a whole-genome survey using 121 simple sequence repeat (SSR) markers in each backcross generation (BC,F1 to BC,F4) to select target chromosome segments in each CSSL and to minimize the number of non-target chromosome segments from the donor. Selected BC,F1 plants were self-pollinated to produce 29 BC,F2 populations. Forty-four plants were selected from

![Fig. 1. Process of development of the plant materials used. CSSL, chromosome segment substitution line; MAS, marker-assisted selection; QTL, quantitative trait locus.](image-url)
among 1400 BC<sub>1</sub>F<sub>2</sub> individuals by using marker-assisted selection. Although heterozygous segments remained in the non-target regions, almost all target regions were homozygous. The self-pollinated progeny of all 44 plants gave 44 CSSLs in the BC<sub>4</sub>F<sub>5</sub> generation. The genotypes of the CSSLs, which we characterized by using 339 SNP markers (Supplemental Table 1), were determined by using 768-plex single nucleotide polymorphisms for the Illumina Golden Gate Bead Array technology platform (Illumina, Inc., San Diego, CA, USA) on the basis of previously reported information (Nagasaki et al. 2010).

To measure the levels of PHS resistance, the 44 CSSLs and their background controls (‘Koshihikari’ and ‘Owarihatamochi’) were grown in a paddie field at the National Institute of Agrobiological Sciences (NIAS), in Tsukuba, in 2011. For QTL analysis, 189 BC<sub>4</sub>F<sub>2</sub> plants, which were produced by crossing ‘Owarihatamochi’ with ‘Koshihikari’, were grown in the experimental field at NIAS in 2012. The plants were genotyped by using 11 SSR markers. For fine mapping of the putative QTLs detected on chromosome 9, the self-pollinated BC<sub>4</sub>F<sub>5</sub> progeny were genotyped by using 13 DNA markers, and 7 pairs of recombinant BC<sub>4</sub>F<sub>5</sub> lines were selected. The BC<sub>4</sub>F<sub>5</sub> plants were used to verify the allelic effects of the QTLs detected. To measure the expression levels of Sdr<sub>4</sub>, OsDOG1L-2, which is regulated by Sdr<sub>4</sub> (Sugimoto et al. 2010), and OsHB20, an ortholog of AtHB20 (Barrero et al. 2010), from among the BC<sub>4</sub>F<sub>5</sub> plants we selected two substitution lines (SLs) for the QTLs detected; in these lines, recombination occurred between the SSR marker loci RM24260 and RM24540 (4.0 Mb) and RM24039 and RM24260 (4.1 Mb), respectively.

**Evaluation for pre-harvest sprouting resistance**

Levels of PHS resistance were evaluated by measuring germination rates at seed maturity. The time when each panicle protruded from the leaf sheath was defined as the heading date. To synchronize conditions after the ripening period, three panicles sampled at 6 or 8 WAH (weeks after heading) from each of three plants in the CSSLs, or at 9 WAH in the case of fine mapping and BC<sub>4</sub>F<sub>5</sub> progeny-testing for the putative QTLs qSDR9.2 and qSDR9.1, were wrapped in paper towels and dipped in water. Samples taken at 6 WAH were used to find CSSLs with lower levels of PHS resistance, whereas those taken at 8 WAH were used to find those with higher levels of PHS resistance. The harvested panicles were immediately incubated in the dark at 30°C and 100% humidity for 7 days. In the case of CSSL and QTL analyses, the number of germinated seeds was then counted as a percentage of the total number of seeds on each panicle. In the case of fine mapping and BC<sub>4</sub>F<sub>5</sub> progeny testing of qSDR9.2 and qSDR9.1, 50 seeds from each of three panicles from each of three plants from each line were collected, and the number of seeds that germinated was counted and indicated as a percentage (i.e., average germination rate). To evaluate the PHS resistance of the parental cultivars, 50 seeds collected from each line at 4 to 8 WAH were placed on a filter paper in an 11-cm Petri dish, and 5 mL of distilled water was added. The dishes were then placed in an incubator at 30°C and 100% humidity. These experiments were performed twice, and the results represent the mean values from three experiments. Because we were focusing on PHS resistance in plants in the paddy field before harvesting, we used fresh seeds and did not control their water content.

**DNA extraction and SSR marker analysis**

Genomic DNA was extracted as follows: leaves (<2.5 cm long) were homogenized in 40 μL of 0.5 M NaOH with stainless-steel beads, and 160 μL of 1 M Tris-HCl (pH 9.0) was then added. Supernatants were isolated by centrifugation (3100 g). For PCR, a fivefold dilution of the supernatant was used. SSR markers showing polymorphism between ‘Koshihikari’ and ‘Owarihatamochi’ were obtained by investigating SSR motifs in the target chromosomal regions (IRGSP ver. 1, http://rapdb.dna.affrc.go.jp/) and used to genotype the BC<sub>2</sub> to F<sub>3</sub> plants. PCR was performed with initial denaturation for 2 min at 95°C and 35 cycles (1 min at 95°C, 1 min at 60°C, and 2 min at 72°C), followed by final extension for 3 min at 72°C. PCR products were electrophoresed on 3% agarose gel to detect polymorphisms.

**Linkage mapping and QTL analysis**

Linkage maps were constructed from the genotypes by using version 3.0 of MAPMAKER/EXP (Lander et al. 1987). Genetic distances were estimated by using the software’s Kosambi map function (Kosambi 1944). For QTL analysis, we used composite interval mapping, as implemented in the MapQTL program (model 6) provided in version 2.5 of QTL Cartographer software (Wang et al. 2005). Genome-wide threshold values (α = 0.05) used to detect putative QTLs were based on the results of 1000 permutations.

**RNA preparation and real-time PCR**

RNA was purified by using an RNaseasy Plant Kit with on-column DNase I treatment (Qiagen, Hilden, Germany). cDNA was synthesized from total RNA (2.5 μg) by using a ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan; FSQ-201), in accordance with the manufacturer’s instructions. The RT-PCR analysis was conducted with an ABI7900HT Fast real-time PCR system and Thunderbird SYBR qPCR Mix (Toyobo; QPS-201). To quantify Sdr<sub>4</sub> and Act-1 mRNA levels, specific primers were used (Table 1). PCR was performed for 2 min at 50°C, then 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The quantified fragments of cloned cDNAs were used as copy-number standards. The results are represented as the means of at least three biological replicates.
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The germination rate of ‘Koshihikari’ increased to 59.3% in the period from 5 to 7 WAH, indicating that a transition from the dormant to the non-dormant state had occurred. At 8 WAH, the germination rates of both ‘Koshihikari’ and ‘Owarihatamochi’ were 92%. These results confirmed that the dormancy level of ‘Owarihatamochi’ was lower than that of ‘Koshihikari’.

To identify candidate chromosomal regions for seed dormancy, the germination rates of the parents and the 44 CSSLs were evaluated at 6 and 8 WAH (Fig. 4A, 4B). We first assigned putative QTLs for low and high PHS resistance on the donor’s chromosomes of a certain CSSL when germination rates significantly differed between the CSSL and the recurrent parent at \( P < 0.05 \), \( P < 0.01 \), or \( P < 0.001 \). Then we predicted the candidate regions for the PHS resistance QTLs on the basis of the association, among CSSLs, between the phenotype and the genotype within a certain chromosomal region. Putative QTLs were assigned in such a way as to maximize the number of CSSLs in which the phenotype could be explained by the genotype of that small chromosome segments of ‘Owarihatamochi’ in the genetic background of ‘Koshihikari’ (Fig. 2, Supplemental Table 1). In each CSSL, a particular target chromosomal region was substituted with that from ‘Owarihatamochi’, and in some CSSLs one or two small untargeted chromosomal regions were also substituted. The target chromosomal regions covered most of the 12 chromosomes, with the exception of some small regions. In addition, some lines contained small heterozygous chromosomal regions. Although the set of CSSLs did not cover all the chromosomes, the CSSLs developed were applicable to comparative QTL surveys of any trait between ‘Koshihikari’ and ‘Owarihatamochi’.

Identification of putative chromosomal regions for seed dormancy

We investigated the germination rates of ‘Koshihikari’ and ‘Owarihatamochi’ every week from 4 to 8 WAH. Between 4 and 5 WAH the seeds of ‘Koshihikari’ went from dormancy to less than 20% germination, whereas those of ‘Owarihatamochi’ already had a high germination rate at 4 WAH (51%, Fig. 3). The germination rate of ‘Koshihikari’ increased to 59.3% in the period from 5 to 7 WAH, indicating that a transition from the dormant to the non-dormant state had occurred. At 8 WAH, the germination rates of both ‘Koshihikari’ and ‘Owarihatamochi’ were 92%. These results confirmed that the dormancy level of ‘Owarihatamochi’ was lower than that of ‘Koshihikari’.

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At 8 WAH, the germination rates of ‘Koshihikari’ and ‘Owarihatamochi’ were 83.3% and 87.9%, respectively. At this time, a wide range of variation in germination rates was observed among the CSSLs, ranging from 3.4% to 95.1%. The germination rates of 27 CSSLs were significantly lower than that of the ‘Koshihikari’ control (Fig. 4B). PHS resistance QTL candidates were detected on all chromosomes except chromosome 10. The chromosome segment substitutions in these lines suggested that 16 chromosomal regions—two QTLs on chromosome 1, two QTLs on chromosome 2, one QTL on the end of the long arm of chromosome 3, one QTL on the end of the long arm of chromosome 4, one QTL in the central part of chromosome 5, two QTLs on chromosome 6, two QTLs on chromosome 7, one QTL on the end of the long arm of chromosome 8, two QTLs in the central part of chromosome 9, one QTL on the end of the long arm of chromosome 11, and one QTL on the long arm of chromosome 12 (Supplemental Fig. 1)—were associated with relatively high levels of PHS resistance. Among the CSSLs showing significantly higher levels of PHS resistance than in the recurrent parent, we were unable to find the regions explaining the effects of the substitutions in SL2327 and SL2336. The region substituted in SL2327 overlapped with those in SL2329. However, significantly higher levels of PHS resistance than in the recurrent parent was not found in SL2329. The region substituted in SL2336 overlapped with those in SL2338. However, significantly higher levels of PHS resistance than in the recurrent parent was not found in SL2338.
Among the QTL candidates detected, the putative QTLs on chromosome 9 exhibited the largest difference in allelic effects between ‘Koshihikari’ and ‘Owarihatamochi’ (Fig. 4); this and the results above suggested that the ‘Owarihatamochi’ allele increased the level of seed dormancy, thus increasing the level of PHS resistance.

**Validation and fine mapping of QTLs for PHS resistance**

We then performed further analyses (fine mapping and characterization) of the putative QTLs on chromosome 9 to validate the allelic effect. To validate the existence of the putative QTLs, we conducted a QTL analysis by using the BC\(_2\)F\(_2\) population. A wide range of variation in germination rates was observed, and two QTLs were detected—in the intervals between SSR markers RM24103 and RM24126 and RM24353 and RM11189—on the long arm of chromosome 9 (Fig. 5A). These QTLs explained 6.1% and 9.2%, respectively, of the phenotypic variance in the F\(_2\) plants, and the presence of the ‘Owarihatamochi’ allele decreased the germination rate by 7.3% and 8.9%, respectively. These results verified the presence of the QTLs on the long arm of chromosome 9 and showed that the presence of the ‘Owarihatamochi’ allele at the QTLs decreased the germination rate. We designated the two QTLs, qSDR9.1 (SEED DORMANCY 9.1) and qSDR9.2.

To further narrow down the candidate genomic regions of qSDR9.1 and qSDR9.2 on the long arm of chromosome 9, we performed fine mapping using a BC\(_3\)F\(_3\) lines (Fig. 5B). At 8.7% to 30.7%, the germination rates of six pairs—#5478, #5379, #5388, #5404, #5418, and #5389—harboring different ‘Owarihatamochi’ segments were significantly lower than those of the equivalent lines homozygous for the ‘Koshihikari’ allele (32.2% to 61.3%) at 9 WAH. However, #5400 harboring ‘Owarihatamochi’ segments had a significantly higher germination rate than the equivalent line homozygous for the ‘Koshihikari’ allele in the entire candidate region (* Student’s t-test, \(P < 0.05\)). Together, these results clearly delimited the candidate genomic region of qSDR9.1 to between SSR marker loci RM24039 and RM24260 (a 4.1-Mb interval in the ‘Nippobare’ genome reference sequence) on chromosome 9 and that of qSDR9.2 to between Indel_2 and RM 24540 (2.3 Mb).

**Characterization of qSDR9.1 and qSDR9.2**

To elucidate the molecular functions of qSDR9.1 and qSDR9.2 within the known seed dormancy mechanism, we investigated the mRNA levels of dormancy-related genes (Sdr4 and OsDOG1L-2) in the embryo. To reduce noise from other chromosomal regions, we developed SLs carrying the ‘Owarihatamochi’ alleles of qSDR9.1 (SL[qSDR9.1]) and qSDR9.2 (SL[qSDR9.2]) in a ‘Koshihikari’ genetic background (Fig. 6A). The germination rates of SL[qSDR9.1] and SL[qSDR9.2] were lower than that of ‘Koshihikari’ at 8 and 9 WAH (Fig. 6B). Expression analysis revealed that the mRNA level of Sdr4 was significantly higher in SL[qSDR9.1] than in ‘Koshihikari’ 3 h after imbibition at 4 WAH (Fig. 6C). Likewise, OsDOG1L-2 mRNA was upregulated in SL[qSDR9.1] 9 h after imbibition. The expression of OsHB20, an ortholog of the Arabidopsis germination-promoting gene AtHB20, was reduced in SL[qSDR9.1] 9 h after imbibition (Fig. 6C). In contrast, the mRNA levels of Sdr4, OsDOG1L-2, and OsHB20 were not significantly altered in SL[qSDR9.2], except for a small but significant effect on Sdr4 gene expression 3 h after imbibition. It is therefore difficult to predict the probable function of qSDR9.1.
found here that was predicted to be located between 14.0 and 15.1 Mb. We detected at least six putative QTL regions determined PHS resistance in the two cultivars. ‘Owarihatamochi’, exhibits weak PHS resistance (see Fig. 3). Here, we used an advanced backcross population derived from a cross between ‘Koshihikari’ and ‘Owarihatamochi’ (Fig. 3). We detected at least six putative QTL regions determining PHS resistance in the two cultivars. ‘Owarihatamochi’ had alleles with putative QTLs on chromosomes 4, 8, 10, and 11 associated with reduced PHS resistance; these alleles were likely dormant in ‘Koshihikari’. Sdr5 and qPHS-8 have been reported on chromosome 8 (Dong et al. 2003, Takeuchi et al. 2003) and qDOR11-3 to -6 and qSD-1 have been reported on chromosome 11 (Cai and Morishima 2000, Miura et al. 2002). The germination rates of 27 CSSLs were significantly (Student’s t-test, \( P < 0.05 \)) lower than that of the ‘Koshihikari’ at 8 WAH (Fig. 4B). The candidate QTL region on chromosome 10 was predicted to be located between 14.0 and 15.1 Mb. We found here that OsHB20 was located in this region.

However, PHS resistance QTL candidates were detected on all chromosomes except chromosome 10. The reported QTLs qSD-1 and Sdr6 are colocalized on the short arm of chromosome 1; OsDOG1L-1, OsDOG1L-2, and qSD-1 are also found in this region (Gu et al. 2004, Marzougui et al. 2012, Sugimoto et al. 2010, Ye et al. 2015). SD1, qPHS1, and qRGV1 have been found on the long arm of chromosome 1 (Dong et al. 2003, Lee et al. 2005). The QTL qRGV2 is colocalized with central part of chromosome 2 (Lee et al. 2005). The QTLs SD-3.2 and qPHS3-2, for which the most probable candidate gene is OsGA20ox-1 (Abe et al. 2012, Dong et al. 2003), are colocalized on the long arm of chromosome 3. The QTLs qPHS-4 and qSD4 (Dong et al. 2003, Gu et al. 2004) are located on chromosome 4. qDOR6-2, qSD6, Sdr9, and Sdr10 (Cai and Morishima 2000, Gu et al. 2006, Marzougui et al. 2012) are located on chromosome 6. The QTL qSD7-1/Rc (Gu et al. 2011) is located on the short arm of chromosome 7, and qSD-7, qSDn-7, qRVG1, and Sdr4 (Gu et al. 2004, Miura et al. 2002, Sugimoto et al. 2010, Wan et al. 2005, You et al. 2006) are found on the long arm. qDOR8 and qPSR8 have been reported on the long arm of chromosome 8 (Cai and Morishima 2000, Dong et al. 2003). Until now, no QTLs for seed dormancy had been reported on chromosomes 5 and 9. qSDn-11 (Wan et al. 2005), on the end of the long arm of chromosome 11, and qSD12 (Gu et al. 2010) on chromosome 12, have been reported. Since, SL2332 showed lowest germination rate, 3.4%, we considered that the priority of the QTLs on chromosome 9 were greater than that of other QTL candidates detected in the ‘Owarihatamochi’ genome. We therefore focused on the QTLs on chromosome 9 in our further analysis.

**Discussion**

**Genetic dissection of seed dormancy in the Japanese upland rice ‘Owarihatamochi’**

Because Japanese upland rice is planted by direct seeding, its breeding has been focused on germinability and seedling vigor to overcome competitors such as weeds. Furthermore, the field conditions for upland rice are drier than those in the paddies used for lowland rice. Under these environmental conditions and requirements, upland rice has not been reported on chromosome 11, and qPHS-3.2, for which the most probable candidate gene is OsGA20ox-1 (Abe et al. 2012, Dong et al. 2003), are colocalized on the long arm of chromosome 3. The QTLs qPHS-4 and qSD4 (Dong et al. 2003, Gu et al. 2004) are located on chromosome 4. qDOR6-2, qSD6, Sdr9, and Sdr10 (Cai and Morishima 2000, Gu et al. 2006, Marzougui et al. 2012) are located on chromosome 6. The QTL qSD7-1/Rc (Gu et al. 2011) is located on the short arm of chromosome 7, and qSD-7, qSDn-7, qRVG1, and Sdr4 (Gu et al. 2004, Miura et al. 2002, Sugimoto et al. 2010, Wan et al. 2005, You et al. 2006) are found on the long arm. qDOR8 and qPSR8 have been reported on the long arm of chromosome 8 (Cai and Morishima 2000, Dong et al. 2003). Until now, no QTLs for seed dormancy had been reported on chromosomes 5 and 9. qSDn-11 (Wan et al. 2005), on the end of the long arm of chromosome 11, and qSD12 (Gu et al. 2010) on chromosome 12, have been reported. Since, SL2332 showed lowest germination rate, 3.4%, we considered that the priority of the QTLs on chromosome 9 were greater than that of other QTL candidates detected in the ‘Owarihatamochi’ genome. We therefore focused on the QTLs on chromosome 9 in our further analysis.

![Graphical genotypes of ‘Koshihikari’, SL[qSDR9.1], and SL[qSDR9.2]. A) Graphical genotypes of ‘Koshihikari’, SL[qSDR9.1], and SL[qSDR9.2] derived from the population used in the F2 analysis. White and black bars indicate ‘Koshihikari’ and ‘Owarihatamochi’ regions, respectively. B) Temporal changes in germination rates after heading. Germination rates were evaluated at 4, 5, 6, 7, 8, and 9 weeks after heading. Means ± SD are shown. C) Temporal changes in mRNA levels of OsHB20 during imbibition. Expression levels in the embryo 4 weeks after heading are shown as ratios to Actin-1 gene expression (* Student’s t-test, \( P < 0.05 \), ** \( P < 0.01 \)).
Of the 16 QTL candidates associated with PHS resistance that we detected across the genome, 4 candidates were not colocalized with reported QTLs as mentioned before, suggesting that ‘Owarihatamochi’, a tropical japonica upland rice, has large amount of natural variations in PHS resistance, including not yet been reported.

**Comparison between previously reported QTLs and \textit{qSDR9.1} and \textit{qSDR9.2}**

The candidate genomic regions of \textit{qSDR9.1} and \textit{qSDR9.2} were mapped to the intervals between marker loci RM24039 and RM24260 (4.1 Mb) and \textit{Indel}_2 and RM24540 (2.3 Mb), respectively (Fig. 7). On the basis of data obtained from the QTL Annotation Rice Online Database [Q-TARO, http://qtaro.abr.affrc.go.jp/ (Yonemaru \textit{et al.} 2010)], the candidate region of \textit{qSDR9.2} overlapped with the region in which \textit{Oryza sativa Delayed Seed Germination 1 (OsDSG1)} is located (Park \textit{et al.} 2010, Fig. 7). The \textit{osdsg1} mutant was originally identified by its delayed-germination phenotype from a population of T-DNA-tagging lines generated from the \textit{OsDSG1} phenotype from a population of T-DNA-tagging lines generated from the \textit{Oryza sativa} Delayed Seed Germination 1 (\textit{OsDSG1}) is located (Park \textit{et al.} 2010, Fig. 7). The \textit{osdsg1} mutant was originally identified by its delayed-germination phenotype from a population of T-DNA-tagging lines generated from the \textit{Oryza sativa} chromosome. Location of each simple sequence repeat marker is shown by physical distance based on the Rice Annotation Project Database (IRGSP ver. 1). Dotted lines indicate positions of flanking markers. Gray boxes display QTLs reported in this study. Arrows pointing to gray boxes display markers nearest to QTLs.

**Characterization of detected QTLs**

Expression analysis of several dormancy- and germination-related genes using SL[\textit{qSDR9.1}] revealed that the mRNA levels of \textit{Sdr4} and \textit{OsDOG1L-2} were higher in SL[\textit{qSDR9.1}] than in ‘Koshihikari’ 1 and 9 h, respectively, after imbibition at 4 WAH (Fig. 6C). This result suggests that the gene underlying \textit{qSDR9.1} acts upstream of \textit{Sdr4} and \textit{OsDOG1L-2} and provides PHS resistance by positively regulating these genes upon imbibition. In Arabidopsis, DOG1 protein levels are stably high in freshly harvested and after-ripened seeds, although the abundance of active DOG1 protein is potentially reduced in after-ripened seeds through a post-translational modification (Nakabayashi \textit{et al.} 2012). Dormancy release is expected to involve DOG1 inactivation, probably through oxidation of the protein (Nakabayashi \textit{et al.} 2012). We can therefore speculate that, if there is increased production of \textit{Sdr4} and \textit{OsDOG1L-2} in seeds that are dormant at 4 WAH, then imbibition will contribute to dormancy-related protein accumulation and elongation of the dormancy period after ripening. Unlike the case with SL[\textit{qSDR9.1}], with SL[\textit{qSDR9.2}] there was no significant difference in \textit{Sdr4} and \textit{OsDOG1L-2} mRNA levels compared to those in the ‘Koshihikari’ control. Therefore, it is difficult to speculate on a putative model involving \textit{qSDR9.2}.

To our knowledge, this report is the first example of PHS resistance QTL identification in Japanese upland rice, which showed shallow dormancy. The reason why so many PHS resistance QTLs, including \textit{qSDR9.1} and 9.2, were detected in the Japanese upland rice used here needs to be clarified by analyses using other upland rice cultivars. Accumulation of information on the genetic effects of PHS resistance QTLs, along with the cloning of \textit{qSDR9.1} and \textit{qSDR9.2}, may uncover the reason behind this abundance in QTLs and shed light on the stacking of the many relevant alleles. Ultimately it will help us to effectively fine-tune PHS resistance in practical rice breeding.

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Pre-harvest sprouting resistance QTLs in upland rice

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