

## Research Paper

# Detection of QTLs for traits associated with pre-harvest sprouting resistance in bread wheat (*Triticum aestivum* L.)

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Pre-harvest sprouting (PHS) is one of the serious problems for wheat production, especially in rainy regions. Although seed dormancy is the most critical trait for PHS resistance, the control of heading time should also be considered to prevent seed maturation during unfavorable conditions. In addition, awning is known to enhance water absorption by the spike, causing PHS. In this study, we conducted QTL analysis for three PHS resistant related traits, seed dormancy, heading time and awn length, by using recombinant inbred lines from ‘Zenkouji-komugi’ (high PHS resistance) × ‘Chinese Spring’ (weak PHS resistance). QTLs for seed dormancy were detected on chromosomes 1B (*QDor-1B*) and 4A (*QDor-4A*), in addition to a QTL on chromosome 3A, which was recently cloned as *TaMFT-3A*. In addition, the accumulation of the QTLs and their epistatic interactions contributed significantly to a higher level of dormancy. *QDor-4A* is co-located with the *Hooded* locus for awn development. Furthermore, an effective QTL, which confers early heading by the Zenkouji-komugi allele, was detected on the short arm of chromosome 7B, where the *Vrn-B3* locus is located. Understanding the genetic architecture of traits associated with PHS resistance will facilitate the marker assisted selection to breed new varieties with higher PHS resistance.

**Key Words:** wheat, pre-harvest sprouting, seed dormancy, heading time, awn length.

## Introduction

Pre-harvest sprouting (PHS) in bread wheat (*Triticum aestivum* L.) is characterized by the germination of seeds in the spikes before harvest. PHS is a serious problem that decreases yields and end use quality of grains, particularly in regions with wet and humid weather conditions at the harvest season. Therefore, resistance to PHS has been one of the major breeding objectives in the many countries, including Japan.

Resistance to PHS is known to be associated with various physiological, morphological and developmental characteristics of the seed and the spike (Groos *et al.* 2002, King and Richards 1984, Munkvold *et al.* 2009). In wheat breeding programs, the most attention has been paid to selection for seed dormancy, which is one of the most critical factors for PHS resistance. The control of phenology or heading time also should be considered important to prevent seed maturation during the wet and humid season (Derera 1989, Mares and Mrva 2014). In Japan, the early heading trait is preferable because late heading results in the maturation of

seeds in the rainy season. In addition, ear morphology, such as awning and ear nodding angle are associated with water absorption by the spike, affecting the degree of PHS (King 1989). The awnless characteristic causes less water uptake of the spike, resulting in decreased PHS (King and Richards 1984). The understanding of the genetic architecture of various traits associated with PHS resistance should contribute the breeding of new varieties more resistant to PHS.

Regarding the genetic control of PHS resistance, a number of studies for quantitative trait locus (QTL) analysis for seed dormancy have been done, indicating that seed dormancy is controlled by multiple genes or QTLs, which are distributed on all 21 chromosomes of the wheat genome (see review in Mares and Mrva 2014). Major genes or QTLs were reported on chromosome 2B (Chao *et al.* 2010, Munkvold *et al.* 2009, Somyong *et al.* 2014), 3A (Liu *et al.* 2013, Mori *et al.* 2005) and 4A (Chen *et al.* 2008, Mares *et al.* 2005, Mohan *et al.* 2009, Ogbonnya *et al.* 2008, Rasul *et al.* 2009, Torada *et al.* 2008.). Recently, one of the major QTLs on chromosome 3A, *QPhs.ocs-3A.1*, was molecularly characterized, revealing that a causal mutation in the promoter region of *MOTHER OF FT and TFL 1 (MFT)* gene affects seed dormancy (Nakamura *et al.* 2011). In addition, multi-allelic variation of the *TaMFT-3A* locus was found to cause diverse phenotype for PHS resistance (Liu *et al.*

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2013); however, a single major gene or QTL cannot fully explain the genetic diversity of wheat varieties. In addition, seed dormancy is a complex trait, in which the effects of QTLs depend on the genetic background or gene combinations, as well as environmental conditions (Jaiswal *et al.* 2012, Kulwal *et al.* 2004, 2012). Therefore, it is important for breeding to explicitly identify the genetic network among QTLs, not only for seed dormancy, but also for various other traits associated with PHS resistance in wheat.

Previously, we reported the QTL analysis for seed dormancy using recombinant inbred lines (RILs) from a cross between ‘Zenkouji-komugi’ (Zen), a Japanese variety with higher PHS resistance, and ‘Chinese Spring’ (CS), a strain susceptible to PHS (Mori *et al.* 2005, Osa *et al.* 2003). One major QTL on chromosome 3A (*QPhs.ocs-3A.1*) in addition to the two minor QTLs were detected; however, the analysis was limited to only three chromosomes, 3A, 4A and 4B (Mori *et al.* 2005). In this study, genome-wide QTL analysis for seed dormancy, as well as awning and heading time were conducted using RILs from Zen × CS. We focused on three QTLs on chromosomes 1B, 3A and 4A and how their epistatic interactions contribute to higher PHS resistance.

## Materials and Methods

### Plant materials

Recombinant inbred lines (RILs) were developed from the cross between ‘Zenkouji-komugi’ (Zen) and ‘Chinese spring’ (CS) by the single-seed descent method described in Osa *et al.* (2003). Zen is a Japanese spring wheat variety showing an extremely high level of seed dormancy (Miura *et al.* 1997, Osanai and Amano 1993) and CS is a strain with weak seed dormancy (Flintham 2000, Warner *et al.* 2000). For the experiments 127 RILs in F<sub>9</sub> or later generations were used.

For the analysis of the *Vrn-B3* locus, KT742, a chromosomal substitution line of the chromosome 7B of CS to that of ‘Hope’ were used. KT742 was provided by the National BioResource Project-Wheat with support in part by the National BioResource Project of the Ministry of Education, Culture, Sports, Science & Technology (MEXT) Japan.

### Cultivation

RILs and their parental strains (CS and Zen) were cultivated on the experimental farm of Obihiro University of Agriculture and Veterinary Medicine, in Obihiro, Japan. Seeds were sown in late April with 10 cm between plants and 30 cm between rows, and were grown under standard field management. Twelve plants each of RIL and 72 plants for each parental strain were grown. After anthesis, the experimental plots were covered with a transparent plastic roof to prevent rain damage. Spikes were harvested at physiological maturity (loss of green color in the seeds and spikes, and the moisture content of the grains was approximately 20–22%). Harvested spikes were allowed to air-dry for about 4 days until the moisture content of the grain was

approximately 14%. Dried spikes were gently hand-threshed and seeds were used for the germination test described below. Because of disease damage and poor growth, 117 out of 127 RILs were used for evaluation of seed dormancy.

For the evaluation of heading time and awn length, 127 RILs and their parental strains were grown in a plastic box (60 × 15 cm). Twelve plants per plastic box were raised. Seeds were sown at the late April and seedlings were grown in a greenhouse for 3 weeks. After that, plastic boxes were placed outside until heading. Four plants (replicates) for each RIL and 12 plants (replicates) for parental strains were raised.

### Phenotypic evaluation

The degree of seed dormancy was evaluated by germination tests on petri dishes (90 mm diameter) under two germination temperatures: 15°C and 20°C. About 30–40 grains from one or two spikes were placed on a single layer of filter paper wetted with water. The dishes were incubated under darkness for 10 days. Each dish was examined daily, and grains where the seed coat was ruptured by the plumule or radicle were counted as germinated seeds. The degree of seed dormancy was checked during 10 days after incubation and expressed as a cumulative percentage of germination (GR: germination rate). The degree of seed dormancy was evaluated as follow: Seed dormancy rate (%) = 100 – GR (%). The experiment was conducted with two replicates for each RIL and six replicates for parental strains.

For the evaluation of awn length, three spikes for each RIL were harvested at maturity and the longest awn of each spike was measured.

### Marker analysis and linkage map construction

The total DNA was extracted from fresh leaves collected from a single plant of each RIL. Small pieces of wheat leaf were crushed in a 2.0 ml tube, and 500 µl of the DNA extraction buffer reported by Monna *et al.* (2002) was added. Four hundred microliter of chloroform : isoamyl alcohol (24:1) was added, and mixed with the solution by inversion. After mixing, the tube was spun at 13000 rpm for 15 min. Crude DNA in the centrifuged supernatant was precipitated by isopropanol, and the pellet was re-dissolved in TE. For the construction of genome-wide linkage map, 15 restriction fragment length polymorphism (RFLP) markers, 158 simple sequence repeat (SSR) markers, and one derived cleaved amplified polymorphic sequence (dCAPS) marker and three gene specific markers were used. Previously, Mori *et al.* (2005) created a linkage map of chromosomes 3A, 4A, and 4B in the same population and the genotype data of markers were re-analysed for this study. SSR markers were selected from a public database of polymorphism of markers (<http://www.shigen.nig.ac.jp/wheat/komugi/strains/about/NbrpMarker.jsp> and Somers *et al.* 2004). The dCAPS marker *UCW99*, which is known to be tightly linked to the *Vrn-B3* locus on chromosome 7B (Yan *et al.* 2006), was also added. Three gene specific markers for *TaMFT-3A*, *Ppd-D1*

and *R-B1* were used for map construction. For the *TaMFT-3A* loci, the cleaved amplified polymorphic marker (CAPS) marker reported in Nakamura *et al.* (2011) was used. For the series of *Ppd-1* loci, the allelic difference between CS and Zen was reported at the *Ppd-1B* and *Ppd-1D* loci (Nishida *et al.* 2013, Tanio and Kato 2007). A polymorphism was found for the marker of the *Ppd-1D* locus developed by Tanio and Kato (2007) and used for the map construction. Red grain color is controlled by the three dominant *R-1* genes located on the long arm of chromosomes 3A (*R-A1*), 3B (*R-B1*), and 3D (*R-D1*) and they were cloned as *Tamyb10-A1*, *Tamyb10-B1*, and *Tamyb10-D1* genes, respectively (Himi *et al.* 2011). Both Zen and CS showed red grains and the genotypes of Zen and CS were assumed to be *R-A1a R-B1b R-D1b* and *R-A1a R-B1a R-D1b*, respectively (Himi *et al.* 2011, Miura *et al.* 2002). The *Tamyb10-B1* (*R-B1*) marker developed by Himi *et al.* (2011) was added.

The linkage map was constructed from marker genotypes of 127 RILs by using JoinMap<sup>®</sup> 4 (van Ooijen 2006). The map distance in centimorgans (cM) was determined using the Kosambi map function (Kosambi 1943).

The insertion of retrotransposon in the promoter region of the *Vrn-B3* (*TaFT1*), was investigated based on Yan *et al.* (2006). Three primer-pairs developed by Yan *et al.* (2006), FT-B-INS-F (CATAATGCCAAGCCGGTGAGTAC) and FT-B-INS-R (ATGTCTGCCAATTAGCTAGC), FT-B-NOINS-F (ATGCTTTCGCTTGCCATCC) and FT-B-NOINS-R (CTATCCCTACCGGCCATTAG), FT-B-NOINS-F2 (GCTGTGTGATCTTGCTCTCC) and FT-B-NOINS-R, were used for the analysis.

### QTL analysis

QTL analysis was conducted by simple interval mapping and MQM (multiple QTL model) mapping methods by using MapQTL<sup>®</sup> 6 (van Ooijen 2002). In the first step, putative QTLs were identified by interval mapping. Then, one marker at each putative QTL was selected as a cofactor, and the selected markers were used as genetic background controls in an approximate multiple QTL model of Map QTL. The markers closest to the significant QTL, which maximized the LOD score, were finally selected as cofactors. The genome-wide LOD thresholds for a significant QTL were estimated by performing a permutation test for each trait (Churchill and Doerge 1994) in MapQTL. The each trait data of RILs were permuted 1000 times over the genotypes, and the empirical LOD thresholds corresponding to the genome-wide significance at 5% were estimated for the all traits examined. The additive effect and percentage of phenotypic variance explained by each QTL were obtained using MapQTL in the final multiple QTL model in which one cofactor marker was fixed per QTL.

### Data analysis

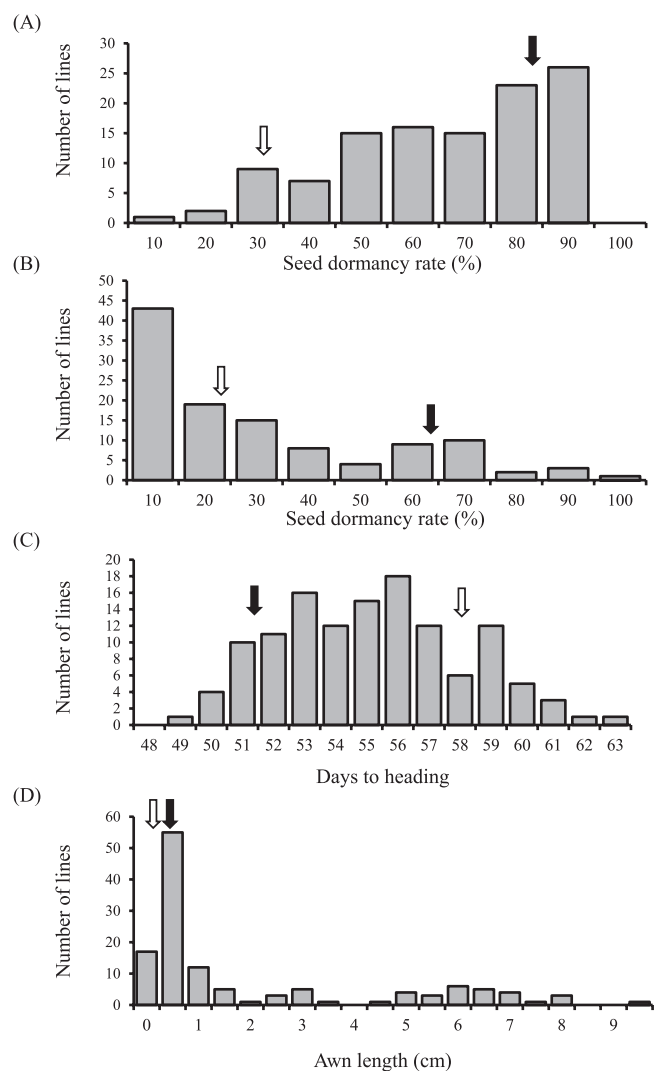
For the detection of epistatic interactions among the three QTLs for seed dormancy, the eight classes of genotypes consisting of different combinations of alleles were

analyzed by three-way analysis of variance (ANOVA). The differences among genotypes were tested by Tukey-Kramer test for multiple comparisons. All statistical analyses were conducted using PASW<sup>®</sup> statistics 18 (SPSS Inc.). For three-way ANOVA and QTL analyses, the data of germination rate were arcsin-transformed to improve normality and heteroscedasticity.

## Results

### Segregation of three traits associated with PHS resistance

Three traits associated with PHS resistance (seed dormancy, heading time and awn length) were evaluated in RILs (Fig. 1). Seed dormancy rate (Dor) was examined under two temperature conditions (15°C and 20°C). The Dor of Zen was significantly higher than that of CS and RILs had a continuous frequency distribution of Dor under both



**Fig. 1.** Frequency distributions for three traits in RILs. (A) Seed dormancy rate at 20°C, (B) Seed dormancy rate at 15°C, (C) Days to heading, (D) Awn length. Black and white arrows indicate the mean values of Zen and CS, respectively.

**Table 1.** Correlation coefficients among the traits associated with PHS resistance in the RILs

|        | Dor 15   | Awn     | Ht    |
|--------|----------|---------|-------|
| Dor 20 | 0.69 *** | 0.25 ** | -0.14 |
| Dor 15 |          | 0.29 ** | -0.21 |
| Awn    |          |         | -0.17 |

Ht: Heading time (Days to heading), Awn: Awn length, Dor 20: Seed dormancy rate at 20°C, Dor 15: Seed dormancy rate at 15°C.

\*\* and \*\*\* indicate the significance at the 1 and 0.1% levels, respectively.

conditions, whereas Dor at 20°C in the RILs and their parental strains was higher than at 15°C. Significant transgressive segregations in the RILs were observed in both directions. Heading time of Zen was about one week earlier than CS. The RILs showed continuous frequency distributions between parental values, although a small part of the RILs transgressed the parental strains. Regarding awn length, both parental strains either had no awns or very short ones; however, segregants showing a long awn phenotype appeared in the RILs.

Phenotypic correlations among traits are shown in **Table 1**. A high positive correlation was observed between the Dor at 15°C and 20°C. The Dor and awn length did not correlate with heading time (days to heading), indicating an independent genetic base. In contrast, significant positive correlations were observed between Dor (both at 15°C and 20°C) and awn length while correlation coefficients were relatively low, indicating a partial genetic relationship between the two traits.

### QTL analysis

To understand the genetic control for traits associated with PHS resistance, QTL analysis for three traits (seed dormancy, heading time and awn length) was conducted. A genome-wide linkage map was constructed on the basis of genotypes of 127 RILs. A total of 177 polymorphic marker loci consisting of 158 SSR, 15 RFLP, one dCAPS and three gene specific markers, were distributed on 21 chromosomes, which covered the total chromosome of the wheat genome; however, markers were scarce in some regions (**Fig. 2**).

Three significant QTLs (*QDor-1B*, *QDor-3A* and *QDor-4A*) were detected for seed dormancy (**Fig. 2**, **Table 2**). *QDor-3A* and *QDor-4A* were detected under both temperature conditions, whereas *QDor-1B* had the effect only at 20°C. *QDor-3A* and *QDor-4A* had relatively large effects in conferring high seed dormancy (decreasing the GR) by the Zen allele. In contrast, *QDor-1B* had the opposite effect (conferring high seed dormancy by the CS allele). A QTL with the large effect on heading time was detected on chromosome 7B (*QHt-7B*), which conferred early heading by the Zen allele (**Fig. 2**, **Table 2**). Two QTLs for awn length (*QAwn-4A* and *QAwn-6B*) were found on chromosome 4A and 6B (**Fig. 2**, **Table 2**). *QAwn-4A* and *QAwn-6B* suppressed awn development by the CS allele.

### Epistatic interaction among QTLs for seed dormancy

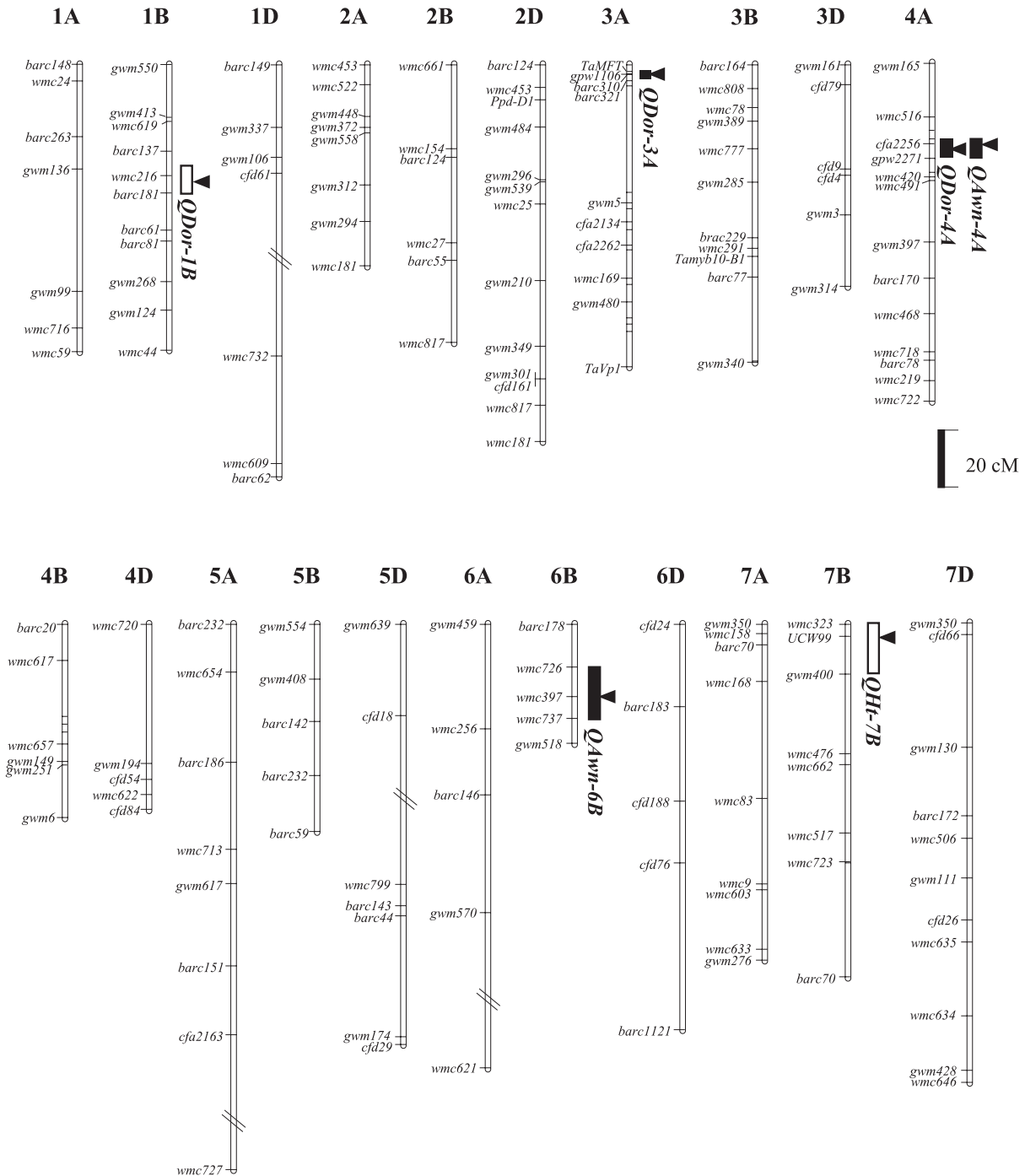
To elucidate the genetic interaction among QTLs for seed dormancy, RILs were classified into eight genotypes having different combinations of alleles at three QTLs (**Fig. 3**). The alleles of QTLs were estimated by two flanking markers for *QDor-1B* (*barc137* and *barc181*) and *QDor-4A* (*cfa2256* and *gpw2271*), and a CAPS marker for the *TaMFT-3A* gene. The seed dormancy rate (Dor) was compared among genotypes. Three-way ANOVA indicated that all the main effects of the three QTLs were significant in both condition (15°C and 20°C) (**Table 3**). The effect of *QDor-1B* was not significant at 15°C in QTL analysis, which might be due to the higher resolution of ANOVA. The interactions among the two (*QDor-1B* × *QDor-4A*) and three QTLs (*QDor-1B* × *QDor-3A* × *QDor-4A*) at the 20°C condition were significant ( $P = 0.006$  and  $P = 0.001$ , respectively) (**Table 3**), indicating epistatic interactions among QTLs for seed dormancy. The Dor of the Type II–IV having one of the dormant-alleles at three QTLs (CS allele for *QDor-1B*, and Zen allele for *QDor-3A* and *QDor-4A*) showed no significant differences from the Type I having no dormant-alleles at all; however, RILs having the combinations of two (Type V–VII at 20°C and Type VII at 15°C) or three (Type VIII) dormant-alleles showed significantly higher Dor than Type I (**Fig. 3**).

### Comparison of the chromosomal locations among QTLs and genes

The chromosomal locations of the QTLs detected in this study were compared with previously reported QTLs or genes. The location of *QDor-3A* was in the region including *TaMFT-3A* (Nakamura *et al.* 2011), which is the causal gene of *QPhs.ocs-3A.1* found in the same mapping population by Mori *et al.* (2005). On chromosome 1B, a few QTLs have been reported, so far (**Fig. 4A**). On the chromosome 4A, a number of QTLs for seed dormancy were detected in the region including the centromere and on the long arm of the chromosome (**Fig. 4B**). A major gene (*Phs-1*; Torada *et al.* 2008) and QTLs with large effects (Chen *et al.* 2008, Mares *et al.* 2005, Mohan *et al.* 2009, Ogbonnaya *et al.* 2008, Tan *et al.* 2006) were frequently found in the region between *Xbarc170* and *Xgwm397*, indicating that *QDor-4A* should be a different locus.

The LOD peak of *QHt-7B* was detected around marker *UCW99* and Yan *et al.* (2006) found it to be closely linked to the *Vernalization-B3* (*Vrn-B3*) locus (**Fig. 5A**). The *Vrn-B3* locus was cloned as the *FLOWERING LOCUS T1* (*FT1*) gene (*TaFT1*) and the insertion of retrotransposon in the promoter region was found to cause early heading in ‘Hope’ whereas there is no insertion in CS (Yan *et al.* 2006). The insertion of retrotransposon was examined based on the analysis of the PCR-product size by using primers in Yan *et al.* (2006) (**Fig. 5B**). For the primer pairs FT-B-INS-F and FT-B-INS-R, a 1.2-kb fragment is amplified when the insertion is present, and no fragment is found when the insertion is absent (Yan *et al.* 2006). In contrast, for the primers pairs





**Fig. 2.** Linkage map showing the positions of putative QTLs detected in the RILs. SSR and gene specific markers are shown on the left of chromosomes. QTL positions are represented as boxes with two-LOD support intervals with the LOD peaks indicated by arrowheads. Filled and white boxes indicate the effects of the QTLs to increase the trait values of the Zen and CS alleles, respectively.

FT-B-NOINS-F and FT-B-NOINS-R or FT-B-NOINS-F2 and FT-B-NOINS-R, the fragments of 1,140 bp or 691 bp are amplified, respectively, when the insertion is absent, and no fragment is found when the insertion is present (Yan *et al.* 2006). The results indicate that Zen has no-insertion of retrotransposon as shown in CS.

Regarding awn development, three dominant inhibitor gene, *Hd* (*Hooded*) on chromosome 4A, *B1* on chromosome

5A, and *B2* on chromosome 6B, were reported (McIntosh *et al.* 2013). CS and Zen show awnless and very-short awn phenotypes, respectively, which suggests that both strains have inhibitor gene(s). CS is known to have *Hd* and *B2* (Sourdille *et al.* 2002), and based on the location and the direction of effects, two QTLs detected in this study assumed to correspond to *Hd* and *B2* carried by CS. Although no significant QTL was found on chromosome 5A, a weak

QTLs for traits associated with pre-harvest sprouting resistance in wheat

**Table 2.** Results of MQM mapping for three traits associated with PHS resistance in the RILs

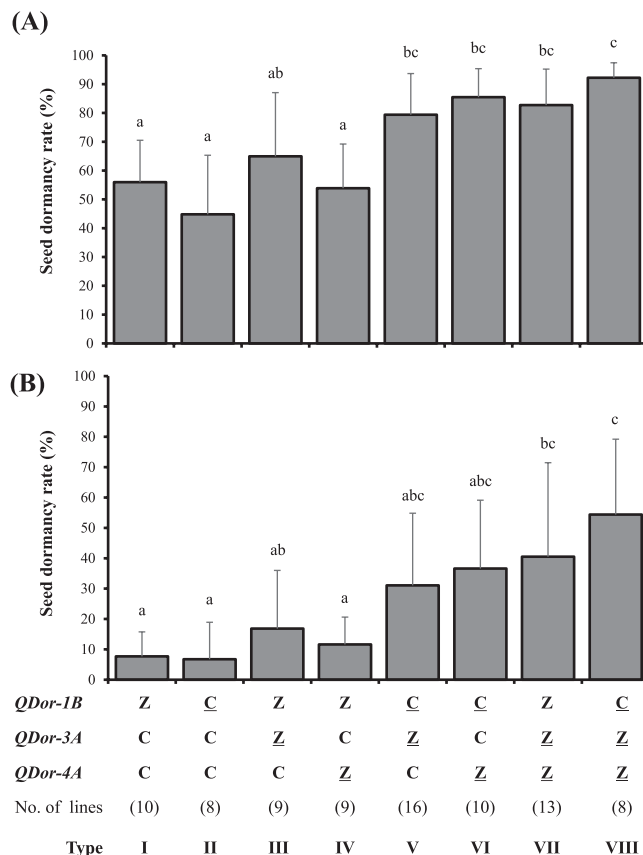
| Trait                | QTL            | Nearest marker | LOD  | PVE (%) | Add  |
|----------------------|----------------|----------------|------|---------|------|
| Seed Dormancy (20°C) | <i>QDor-1B</i> | <i>barc181</i> | 3.0  | 8.4     | 4.5  |
|                      | <i>QDor-3A</i> | <i>TaMFT</i>   | 6.3  | 18.9    | -6.2 |
|                      | <i>QDor-4A</i> | <i>cfa2256</i> | 5.0  | 14.3    | -5.7 |
| Seed Dormancy (15°C) | <i>QDor-3A</i> | <i>TaMFT</i>   | 4.6  | 15.3    | -7.3 |
|                      | <i>QDor-4A</i> | <i>cfa2256</i> | 3.8  | 12.4    | -7.1 |
| Heading time         | <i>QHt-7B</i>  | <i>UCW99</i>   | 12.5 | 36.5    | 5.7  |
| Awn length           | <i>QAwn-4A</i> | <i>cfa2256</i> | 7.8  | 19.8    | -1.1 |
|                      | <i>QAwn-6B</i> | <i>wmc397</i>  | 5.8  | 14.6    | -1.0 |

Nearest markers, LOD values, PVE (phenotypic variance explained by each QTL), and Add (additive effect) are listed. Positive and negative values of Add indicate the effects increasing trait values by the CS and Zen alleles, respectively.

LOD score (LOD = 2.3) detected in the terminal region of chromosome 5AL suggests the Zen allele decreases awn length, and might correspond to the *B1* gene carried by Zen. The *QDor-4A* was co-located with *QAwn-4A* (Fig. 4B).

## Discussion

Pre-harvest sprouting (PHS) resistance is a complex trait involving various physiological, morphological and developmental characteristics in cereal crops (Mares and Mrva 2014). Seed dormancy is the most critical factor for PHS resistance in wheat. Previously, Mori *et al.* (2005) detected a major QTL for seed dormancy on chromosome 3A (*QPhs.ocs-3A.1*) with two minor QTLs on chromosome 4A and 4B in RILs from the cross between Zen × CS, although QTL analysis was limited on the three chromosomes (chromosome 3A, 4A and 4B). In this study, we constructed a linkage map of 21 chromosomes and did a genome-wide QTL analysis using the same mapping population. In addition, we also conducted a QTL analysis for awn length and heading time, which could be the traits associated with PHS resistance. The understanding of the genetic architecture not only of



**Fig. 3.** Interactions among three QTLs for seed dormancy on chromosomes 1B (*QDor-1B*), 3A (*QDor-3A*) and 4A (*QDor-4A*). (A) Seed dormancy rate at 20°C, (B) Seed dormancy rate at 15°C. The alleles of the QTLs were estimated by using their flanking markers (see text). C and Z indicate the CS and Zen alleles, respectively, and the alleles with underline indicate the dormant-alleles. The bar plots show seed dormancy rate (%) ± SD. Different letters above the bars indicate a significant difference by Tukey-Kramer test.

seed dormancy but also of other traits associated with PHS resistance will contribute to breeding new varieties with higher PHS resistance.

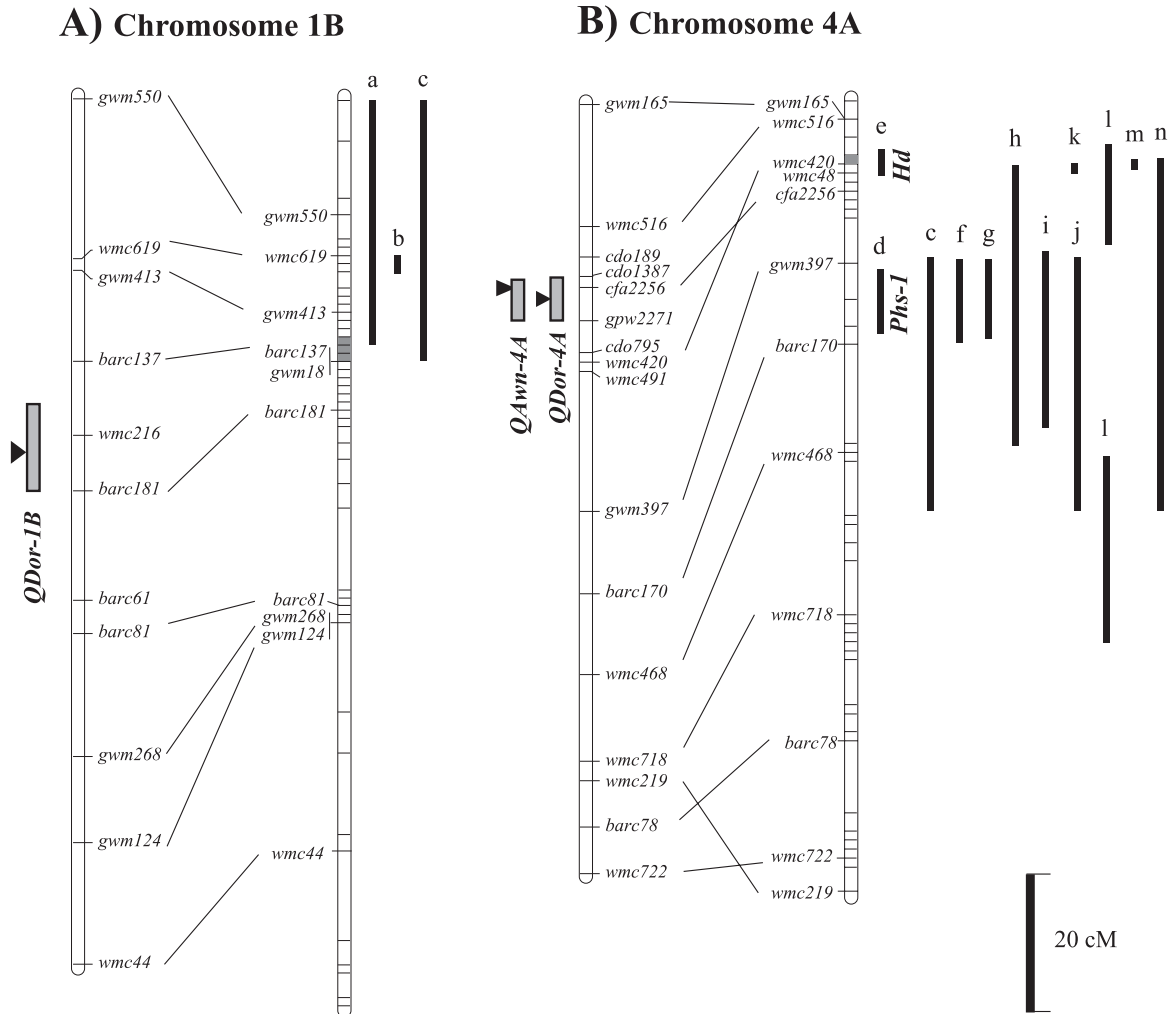
The ability to synchronize the heading time with favorable environmental conditions is the one of the key factors for PHS resistance as well as creating regional adaptability

**Table 3.** Three-way ANOVA for the effects of three QTLs for seed dormancy in the RILs

| Factor   | d.f | Dor 20  |         |           | Dor 15  |         |           |
|--|-----|---------|---------|-----------|---------|---------|-----------|
|  |     | MS      | F-value | P         | MS      | F-value | P         |
| <i>QDor-1B</i>                                   | 1   | 2425.70 | 10.92   | 0.001 **  | 2653.72 | 5.50    | 0.022 *   |
| <i>QDor-3A</i>                                   | 1   | 7719.48 | 34.74   | 0.000 *** | 6823.33 | 14.14   | 0.000 *** |
| <i>QDor-4A</i>                                   | 1   | 5905.99 | 26.58   | 0.000 *** | 6940.32 | 14.39   | 0.000 *** |
| <i>QDor-1B</i> × <i>QDor-3A</i>                  | 1   | 15.13   | 0.07    | 0.795 ns  | 3.50    | 0.01    | 0.932 ns  |
| <i>QDor-1B</i> × <i>QDor-4A</i>                  | 1   | 1765.83 | 7.95    | 0.006 **  | 485.72  | 1.01    | 0.319 ns  |
| <i>QDor-3A</i> × <i>QDor-4A</i>                  | 1   | 77.91   | 0.35    | 0.556 ns  | 69.33   | 0.14    | 0.706 ns  |
| <i>QDor-1B</i> × <i>QDor-3A</i> × <i>QDor-4A</i> | 1   | 2803.24 | 12.62   | 0.001 *** | 1266.70 | 2.63    | 0.109 ns  |
| Error  | 75  | 222.19  |         |           | 482.41  |         |           |

Dor 20: Seed dormancy rate at 20°C, Dor 15: Seed dormancy rate at 15°C.

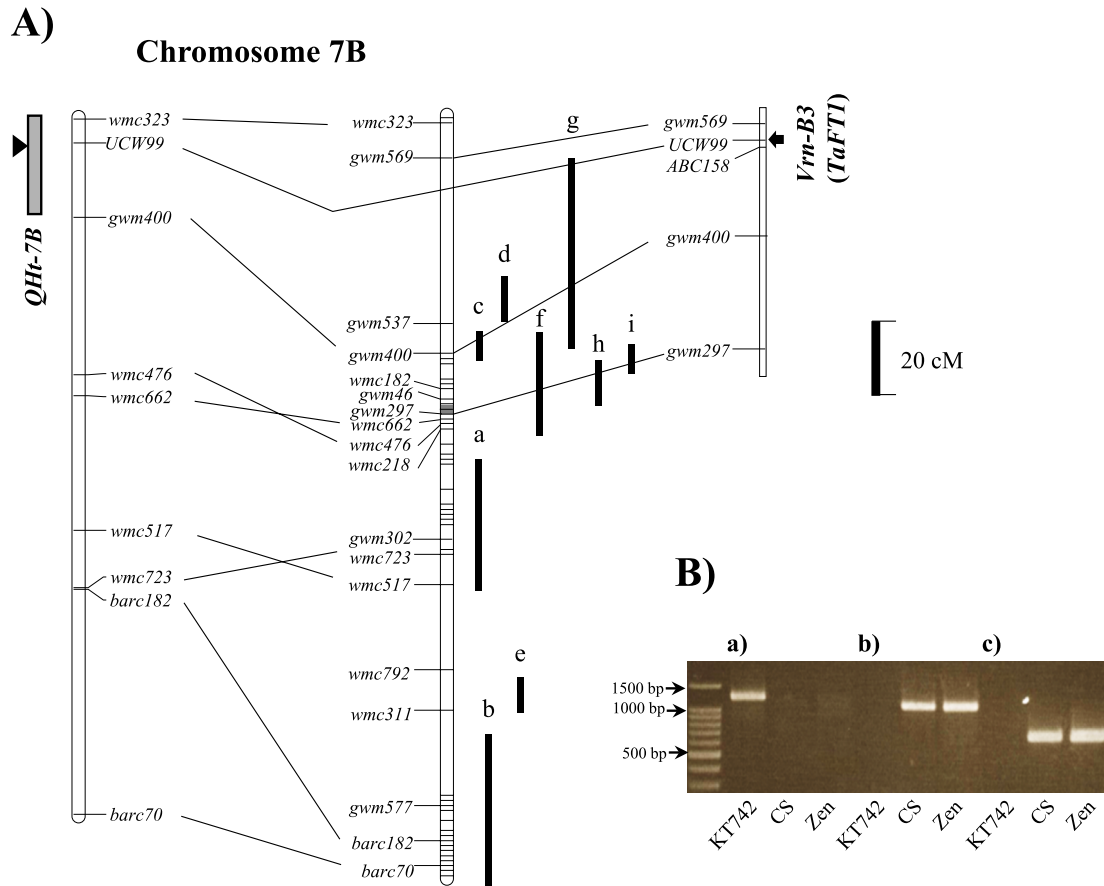
\*, \*\* and \*\*\* indicate the significance at the 5, 1 and 0.1% levels, respectively.



**Fig. 4.** Comparative maps of locations of QTLs or the gene on chromosomes 1B (A) and 4A (B). The linkage map constructed in this study is shown on the left and the framework map based on Somers *et al.* (2004) is shown on the right. QTLs detected in this study are shown on the left linkage map as boxes with two-LOD support intervals with the LOD peaks indicated by arrowheads. The locations of reported QTLs or genes are shown on the right linkage map. Bars represent the regions including the LOD peaks of QTLs. a) Flintham *et al.* (2002), b) Singh *et al.* (2014), c) Zanetti *et al.* (2000), d) Torada *et al.* (2008), e) Sourdille *et al.* (2002), f) Chen *et al.* (2008), g) Imtiaz *et al.* (2008), h) Kato *et al.* (2001), i) Mares and Mrva (2001), j) Mares *et al.* (2005), k) Mohan *et al.* (2009), l) Ogonnaya *et al.* (2008), m) Rasul *et al.* (2009), n) Tan *et al.* (2006). A shaded zone on each chromosome indicates the approximate position of the centromere based on Somers *et al.* (2004).

(Kamran *et al.* 2014). In Japan, harvesting before the rainy season is necessary to prevent PHS. Allelic variation at the *Photoperiod-1* (*Ppd-1*) locus on the group 2 chromosomes and the *Vernalization-1* (*Vrn-1*) locus on the group 5 chromosomes are the major sources for the variation of heading time in diverse germplasm in bread wheat (Kamran *et al.* 2014). The genotype at the *Ppd-1* series loci of Zen and CS are *Ppd-A1b Ppd-B1a Ppd-D1b* and *Ppd-A1b Ppd-B1b Ppd-D1a*, respectively (Nishida *et al.* 2013, Tanio and Kato 2007), whereas Zen and CS have the same alleles (*vrn-A1 vrn-B1 Vrn-D1*) at the *Vrn-1* series loci (Bentley *et al.* 2013, Tanio *et al.* 2005). However, no QTL was detected on chromosomes 2B and 2D under the present environmental conditions, despite the allelic differences at the *Ppd-B1* and *Ppd-D1* loci between Zen and CS. Instead, a major QTL for

heading time was detected on chromosome 7B (*QHt-7B*), which causes early heading by the Zen allele. *QHt-7B* was found in the region where *Vrn-B3* is known to be located. Yan *et al.* (2006) reported that the causal gene of the *Vrn-B3* locus is *TaFT1*, which encodes the protein known as florigen in plants. The insertion of retrotransposon in the promoter region of *TaFT1* causes early heading in ‘Hope’ whereas there is no insertion in CS (Yan *et al.* 2006). In hexaploid wheat strains surveyed, the insertion of retrotransposon has been found in only a few strains, so far (Chen *et al.* 2013, Yan *et al.* 2006). Our results indicate that Zen also has no-insertion, in spite of the Zen allele at *QHt-7B* conferring earlier heading compared to the CS allele. A number of QTLs for traits associated with heading time, such as vernalization and photoperiod responses, were



**Fig. 5.** (A) Comparative maps of locations of QTLs or the gene on the chromosome 7B. The linkage map constructed in this study is shown on the left and the framework map based on Somers *et al.* (2004) is shown in the center. The linkage map including the *Vrn-B3* (*TaFT1*) locus (Yan *et al.* 2006) is shown on the right. The QTL detected in this study is shown on the left linkage map as a box with two-LOD support interval with the LOD peak indicated by an arrowhead. The locations of reported QTLs are shown on the central linkage map. Bars represent the regions including the LOD peaks of QTLs. a) Bennett *et al.* (2012), b) Griffiths *et al.* (2009), c) Hanocq *et al.* (2007), d) Khlestkina *et al.* (2009), e) Kulwal *et al.* (2003), f) Lin *et al.* (2008), g) Maccaferri *et al.* (2008). h) Manickavelu *et al.* (2011). i) Sourdille *et al.* (2003). A shaded zone on the chromosome indicates the approximate position of the centromere based on Somers *et al.* (2004). (B) PCR analysis for detection of the insertion of retrotransposon in the promoter region of the *Vrn-B3* (*TaFT1*) gene based on Yan *et al.* (2006). PCR products in K742, CS and Zen by using primer pairs (a) FT-B-INS-F and FT-B-INS-R, (b) FT-B-NOINS-F and FT-B-NOINS-R, and (c) FT-B-NOINS-F2 and FT-B-NOINS-R (see text).

reported on the short arm of chromosome 7B (see review in Kamran *et al.* 2014, **Fig. 5**), whereas it is still controversial whether these QTLs were caused by the same gene or multiple genes. Regarding *QHt-7B*, there are two possibilities that *QHt-7B* is a novel allele at the *Vrn-B3* locus or different locus linked to *Vrn-B3*. Further fine-mapping is needed to delimitate the location of *QHt-7B*.

The retention of water in the spike is one of the factors causing PHS and awns trap more water and thereby accelerate PHS as well as other disease problems (King and Richards 1984). The awnless characteristic may cause less water absorption of the spike, resulting in a decrease in PHS (King 1989, King and Richards 1984, Mares and Mrva 2014). In addition, awns sometimes impede harvesting and processing. However, awns may enhance spike photosynthesis and provide a source of assimilates to developing seeds (Maydup *et al.* 2014). Three dominant inhibitors for awn development, *Hooded* (*Hd*), *B1* and *B2* on chromo-

somes 4A, 5A and 6B, respectively, have been well analyzed in wheat (McIntosh *et al.* 2013). From the results of this study, the genotypes of CS and Zen were tentatively assumed to be *Hd b1 B2* and *hd B1 b2*, respectively, whereas allelic states of *B1* locus are uncertain because of multiple allelic variation at the *B1* locus (Watkins and Ellerton 1940). In Japan, awn length has not been the target of intensive selection during the recent breeding program of wheat; however, caution is needed in the breeding of awnless varieties with high PHS resistance because *QDor-4A* was co-located with *QAwn-4A* (*Hd* locus) and the dormant-allele at *QDor-4A* might be closely linked to the allele enhancing awn development (*hd* gene) in Zen. The dissection of the *QDor-4A-QAwn-4A* regions is needed to utilize the useful combinations of alleles for breeding, whereas there remains a possibility that the awning gene pleiotropically affects seed dormancy.

Recent progress in our understanding of genes associated



with seed dormancy in various plant species reveals that seed dormancy is a complex phenomenon involving various physiological and molecular mechanisms (Gao and Ayele 2014, Graeber *et al.* 2012). In natural variation for the levels of seed dormancy in wheat, a number of QTLs were reported in all 21 chromosomes of the genome. Among them, *QPhs.ocs-3A.1*, a QTL detected on chromosome 3A (the same locus as *QDor-3A* in this study), was molecularly characterized as *TaMFT-3A* (Nakamura *et al.* 2011). The *MFT* gene is one of the key factors in the genetic control for seed dormancy in plants (Gao *et al.* 2014, Graeber *et al.* 2012) and multi-allelic variation at the *TaMFT-3A* locus was revealed to cause diverse phenotype of seed dormancy (Liu *et al.* 2013, Nakamura *et al.* 2011). Although Zen has the strongest allele for seed dormancy reported at the *TaMFT-3A* locus, so far (Liu *et al.* 2013, Nakamura *et al.* 2011), the *TaMFT-3A* locus could explain only the small part of the phenotypic variation observed in RILs. The present study detected two other QTLs on chromosomes 4A and 1B, and high level of seed dormancy was achieved by the accumulation of dormant-alleles at these three QTLs.

Epistasis, which describes the non-additivity of effects between loci, has been known to play important roles in phenotypic variation in plants including crop species (Cooper *et al.* 2009, Lynch and Walsh 1998). Regarding PHS resistance, some evidences of epistatic interactions among QTLs have been reported in wheat (Imtiaz *et al.* 2008, Jaiswal *et al.* 2012, Kulwal *et al.* 2004, 2012); however, it remains unclear to what extent the epistasis contributes the diversification of PHS resistance. Present studies have detected the epistatic interaction among three QTLs, *QDor-3A* (*TaMFT-3A*), *QDor-4A* and *QDor-1B*, which suggests that it is important to introduce useful QTLs, combined with proper genes and genetic background, to breed varieties with higher PHS resistance. Although the epistatic interactions among the QTLs detected in this study do not mean interactions at the molecular and physiological levels, these results imply a complex gene network for seed dormancy in wheat. Further studies to delimitate the chromosomal location and map-base cloning of *QDor-4A* and *QDor-1B* will provide new insights for the genetic control of seed dormancy in wheat, which will facilitate the marker assisted selection to breed new varieties with higher PHS resistance.

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