Theor Appl Genet (2010) 121:1395–1404
DOI 10.1007/s00122-010-1396-4

ORIGINAL PAPER

# Dissection and fine mapping of a major QTL for preharvest sprouting resistance in white wheat Rio Blanco

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Received: 16 April 2010 / Accepted: 17 June 2010 / Published online: 4 July 2010


**Abstract** Preharvest sprouting (PHS) is a major constraint to white wheat production. Previously, we mapped quantitative trait loci (QTL) for PHS resistance in white wheat by using a recombinant inbred line (RIL) population derived from the cross Rio Blanco/NW97S186. One QTL, *QPhs.pseru-3A*, showed a major effect on PHS resistance, and three simple sequence repeat (SSR) markers were mapped in the QTL region. To determine the flanking markers for the QTL and narrow down the QTL to a smaller chromosome region, we developed a new fine mapping population of 1,874 secondary segregating $F_2$ plants by selfing an F6 RIL (RIL25) that was heterozygous in the three SSR marker loci. Segregation of PHS resistance in the population fitted monogenic inheritance. An additive effect of the QTL played a major role on PHS resistance, but a dominant effect was also observed. Fifty-six recombinants among the three SSR markers were identified in the population and selfed to produce homozygous recombinants or QTL near-isogenic lines (NIL). PHS evaluation of the recombinants delineated the QTL in the region close to *Xbarc57* flanked by *Xbarc321* and *Xbarc12*. To saturate the QTL region, 11 amplified fragment length polymorphism (AFLP) markers were mapped in the QTL region with 7 AFLP co-segregated with *Xbarc57* by using the NIL population. Dissection of the QTL as a Mendelian factor and saturation of the QTL region with additional markers created a solid foundation for positional cloning of the major QTL.

Communicated by H. H. Geiger.

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## Introduction

Preharvest sprouting (PHS) occurs when physiologically mature grains germinate in a wheat spike because of continuous wet weather before or during harvest. It can have serious, negative effects on wheat production in many wheat-growing areas worldwide (Imtiaz et al. 2008). Growing PHS-resistant cultivars is the most effective solution to minimize PHS damage.

Seed dormancy (SD) is considered the major factor affecting PHS resistance (Anderson et al. 1993; Mares and Mrva 2001; Ogbonnaya et al. 2008). Because of molecular marker technology, detailed linkage maps are available for many crops, including wheat, and enable genome-wide scan of quantitative trait loci (QTL) and dissection of QTL into single Mendelian factor for breeding manipulation (Monforte and Tanksley 2000; Dong et al. 2002; Olmos et al. 2003; Kulwal et al. 2004). QTL for SD and PHS resistance have been extensively reported in barley (Li et al. 2003; Prada et al. 2004; Ullrich et al. 2008), rice (Lin et al. 1998; Cai and Morishima 2000; Dong et al. 2002; Gu et al. 2004) and sorghum (Lijavetzky et al. 2000). In wheat, QTL for SD or PHS resistance have been reported on most chromosomes (Anderson et al. 1993; Kato et al. 2001; Mares and Mrva 2001; Groos et al. 2002; Osa et al. 2003; Kulwal et al. 2004; Munkvold et al. 2009). The QTL on chromosomes 4A, 2B, 3AL and 3AS were reported to have major effects on both PHS resistance and SD (Mares and Mrva 2001; Kato et al. 2001; Osa et al. 2003; Mares et al. 2005; Mori et al. 2005; Liu et al. 2008a,

b; Munkvold et al. 2009). However, map resolution for most of the QTL was low. Further fine mapping of these QTL may facilitate map-based cloning and improve the efficiency of marker-assisted breeding for PHS resistance in wheat.

PHS resistance is controlled by several QTL with various effects (Gu et al. 2004, 2006; Imtiaz et al. 2008; Ogbonnaya et al. 2008). Expression of an individual QTL in regular mapping populations such as double haploid (DH) or recombinant inbred line populations (RIL) may be confounded by other QTL and their genetic backgrounds if the individual QTL is the major focus of a study. Therefore, estimation of the gene effects of an individual QTL in such a population may not be accurate. Near-isogenic lines (NIL) contrasting only in a single QTL are ideal materials for dissecting individual QTL effects, fine mapping the QTL region, studying interactions between the QTL and environments and determining breeding potential of an individual QTL.

NIL for SD or PHS resistance QTL have been successfully isolated in *Arabidopsis*, barley and rice and used to evaluate component gene effects (Han et al. 1999; Alonso-Blanco et al. 2003; Gao et al. 2003; Takeuchi et al. 2003; Bentsink et al. 2006; Gu et al. 2006; Liu et al. 2008b). In most cases, additive effects were detected for dormancy QTL; however, dominant effects were also reported in some studies (Buraas and Skinnes 1984; Gu et al. 2004). Dissection of multiple QTL for PHS resistance into a single Mendelian factor and studies of QTL effects using NIL have not been reported to date in wheat.

Our previous work identified three PHS-resistant QTL from white wheat cultivar Rio Blanco, and one QTL on 3AS explained a large portion of phenotypic variation for PHS resistance (Liu et al. 2008a). The flanking markers were not determined in that study because of the limitations in population size and available polymorphic markers. Objectives of this study are to (1) dissect the QTL into a single Mendelian factor and develop NIL contrasting in the QTL region by using the within-family segregation strategy (Tuinstra et al. 1997), (2) narrow down the QTL location within the three marker intervals, (3) evaluate the gene effects of the QTL, and (4) saturate the QTL region with amplified fragment length polymorphism (AFLP) markers.

## Materials and methods

### Plant materials

Three simple sequence repeat (SSR) markers (*Xbarc12*, *Xbarc57* and *Xbarc321*) linked to *QPhs.pseru-3A* (Liu et al. 2008a) were used to screen $F_6$ RIL. Two RIL (RIL25 and RIL81) segregating for markers in the QTL region were identified. Line RIL25 segregated at all three marker loci, whereas RIL81 segregated only at the *Xbarc12* locus. These two RIL were used to produce two $F_2$ populations as described in Fig. 1. Because these $F_2$ population only segregated at one and three target marker loci, hereafter they were called secondary segregating $F_2$ populations. After 1,874 RIL25-derived secondary $F_2$ plants were genotyped (Table 1; Fig. 1), 56 were identified to have at least one recombination among the three markers. The heterozygous recombinants were continuously selfed, and more homozygous recombinants among the three markers were selected on the basis of marker data from 20 to 120 plants per selected $F_3$ family. The selected homozygous recombinant NIL was used for fine mapping of the QTL region. In the RIL81-derived population, 269 secondary $F_2$ plants were genotyped (Table 1).

### Evaluation of PHS resistance

Two parents, both RIL25- and RIL81-derived secondary segregating $F_2$ populations, and selected RIL25-derived $F_3$ recombinant families were evaluated for PHS resistance in the greenhouses at Kansas State University, Manhattan, KS, from spring 2007 to fall 2008. All materials were transplanted into a 13 cm × 13 cm tora pot (Hummert Int., St. Louis, MO, USA) filled with Metro Mix 360 soil mix (Hummert Int., St. Louis, MO, USA) after vernalization at 4°C for 8 weeks. All plants were grown on a greenhouse bench at 22°C day/15°C night temperature with supplemental light of 16 h. Four spikes per plant were harvested at physiological maturity. Harvested spikes were air-dried for 5 days in the greenhouse at 25 ± 5°C and then stored in a freezer at −20°C to maintain dormancy. After all materials were harvested, the spikes were air-dried again for an additional 3 days in a greenhouse and evaluated for

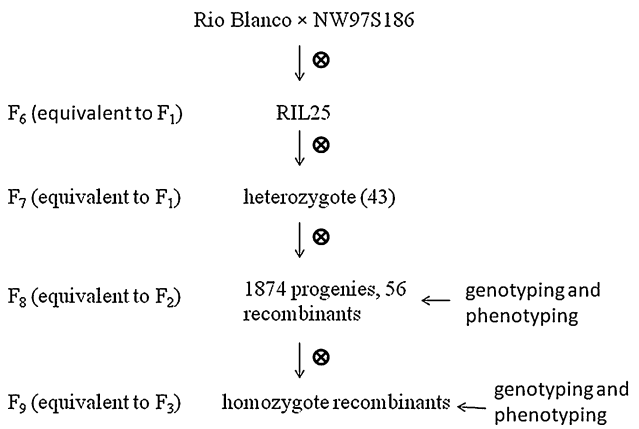


**Fig. 1** Development of the homozygous recombinant population from the heterozygous RIL (RIL25) at the three marker loci linked to the *QPhs.pseru-3A* QTL

**Table 1** Segregation of the three markers in the 3AS PHS resistance QTL region in the two secondary segregating $F_2$ populations derived from heterozygous RIL of Rio Blanco/NW97S186

| Name | No. of secondary $F_2$ progenies | Markers evaluated | Genotype a | Heterozygote h | Genotype b | $\chi^2$(1:2:1) | *P* value |
|---|---|---|---|---|---|---|---|
| RIL25 | 1,874 | *Xbarc12* | 471 | 941 | 462 | 0.12 | 0.94 |
| | | *Xbarc57* | 467 | 925 | 482 | 0.55 | 0.76 |
| | | *Xbarc321* | 473 | 920 | 481 | 0.69 | 0.71 |
| RIL81 | 269 | *Xbarc12* | 77 | 129 | 63 | 1.9 | 0.39 |

*a* Homozygous Rio Blanco genotype, *b* NW97S186 genotype, *h* heterozygote

PHS in a moist chamber as described by Liu et al. (2008a). The percentage of germinated kernels in a spike was calculated to determine PHS resistance. The remaining spikes from each line were harvested as seeds for future planting. PHS resistance of all selected homozygous recombinants from each $F_3$ family was tested in the greenhouse again in spring 2009 with two replicates per line.

## SSR analysis

Leaf tissue was harvested at the three-leaf stage, stored in 96-wells plates, dried in a freeze dryer (Thermo Savant, Holbrook, NY, USA) for 3 days, and ground to fine powder for 5 min at 20 times per second in a Mixer Mill (Retsch GmbH, Haan, Germany) with the aid of a 3.2 mm metal bead in each well. DNA was isolated by a modified CTAB method (Saghai-Maroof et al. 1984).

To screen the RIL25- and RIL81-derived populations by using markers *Xbarc12*, *Xbarc57* and *Xbarc321*, a 12-μL PCR mixture containing 50 ng of template DNA, 1 mM each of reverse and M13-tailed forward primers, 1 mM fluorescence-labeled M13 primer, 0.2 mM of each dNTP, 1× PCR buffer, 2.5 mM $MgCl_2$, and 0.6 units of *Taq* polymerase was used for PCR analysis. PCR was performed in a DNA Engine Tetrad Peltier Thermal Cycler (Bio-Rad Lab, Hercules, CA, USA). A touch-town program was used for PCR amplification as described by Liu et al. (2008a). PCR products labeled with four different M13 dyes (FAM, VIC, NED and PET) were pooled with the Biomek $NX^P$ liquid handling system (Beckman Coulter Inc., CA, USA) and then separated in the ABI Prism 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). SSR data were analyzed using GeneMarker ver. 1.5 (SoftGenetics LLC, State College, PA, USA).

## AFLP analysis

AFLP analysis was conducted with a LI-COR DNA Analyzer 4300 (LI-COR Inc., Lincoln, NE, USA). A total of 300 ng of genomic DNA for each line was completely digested with *Pst*I and *Mse*I. *Pst*I and *Mse*I adaptors were ligated to the restriction fragments. Pre-amplifications were performed in a total volume of 20 μL containing 3.0 μL of a 1:10 dilution of the digested and ligated DNA, 75 ng of each primer (*Pst*I and *Mse*I adaptors plus 1 additional nucleotide), 0.2 mM dNTP mix, and 1 U *Taq* DNA polymerase. The PCR profile for pre-amplification was as follows: after initial denaturing (94°C for 3 min), 30 cycles of 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min were carried out followed by an additional extension of 5 min at 72°C. PCR product from the pre-amplification was diluted at 1:20 and used as a template for selective amplification that used primers with three additional selective nucleotides. The selective PCR was performed in a total volume of 20 μL comprising 30 ng of *Mse*I primer, 5 ng of 5′-IRD700 labeled *Pst*I primer or 10 ng of 5′-IRD800 labeled *Pst*I primer, 0.2 mM dNTP mix and 1 U *Taq* DNA polymerase and 1× PCR buffer. PCR amplification started with 3 min of initial denaturation at 94°C followed by 13 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 60 s with the annealing temperature decreased by 0.7°C per cycle and 23 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. All PCR reactions were carried out in a DNA Engine Tetrad Peltier Thermal Cycler (Bio-Rad Lab, Hercules, CA, USA). An equal volume of formamide loading buffer [95% formamide (v/v), 10 mM EDTA pH 8, 0.1% basic fuchsin, 0.01% bromophenol blue] was added to the samples and then used for denaturation at 94°C for 5 min. Each sample (1 μL) was loaded onto a 6.5% denaturing polyacrylamide gel for electrophoresis in a 1× TBE buffer at 1,500 V, 40 W, 40 mA at 48°C for 2.5 h. The two parents, Rio Blanco and NW97S186, together with two resistant and two susceptible NIL were screened for polymorphism. All polymorphic AFLP were analyzed across all selected homozygous recombinants from RIL25-derived progenies to construct the linkage map.

## Data analysis

The markers were mapped by using the RIL25-derived homozygote recombinant NIL. Recombination frequency was converted into genetic distance by using the Kosambi

**Table 2** Germination rates of different secondary $F_2$ genotypes in RIL25- and RIL81-derived secondary segregating $F_2$ populations

| Secondary $F_2$ populations | Marker genotypes[a] | No. of secondary $F_2$ plants genotyped | No. of secondary $F_2$ plants phenotyped | Germination rate range (%) | Mean germination rate (%) |
|---|---|---|---|---|---|
| RIL25 | aaa | 457 | 229 | 0–17.2 | $2.92^{A}$ |
| | haa | 10 | 5 | 2.4–7.3 | $4.7^{A}$ |
| | aah | 1 | 1 | – | 3.3 |
| | hhh | 903 | 398 | 5.5–50.7 | $20.5^{B}$ |
| | ahh | 12 | 10 | 12.4–28.6 | $27.7^{B}$ |
| | aha | 2 | 1 | – | 35.3 |
| | bhh | 4 | 2 | 23.0–33.8 | $28.4^{B}$ |
| | bhb | 1 | 1 | – | 32.3 |
| | hbb | 24 | 21 | 76.2–95.7 | $84.6^{C}$ |
| | hbh | 1 | 1 | – | 84.3 |
| | bba | 1 | 1 | – | 91.3 |
| | bbb | 456 | 228 | 55.7–96.1 | $85.0^{C}$ |
| RIL81 | a | 77 | 69 | 0.7–4.2 | 3.11 |
| | h | 129 | 124 | 0–5.1 | 3.06 |
| | b | 63 | 60 | 1.1–6.3 | 3.2 |

Within columns, different superscript letters indicate significant difference at $P < 0.01$

[a] Marker order is *Xbarc12*, *Xbarc57* and *Xbarc*321

(1944) function. One-way analysis of variance was conducted using the GLM procedure of SAS Institute (2003) to estimate the contribution of individual QTL to phenotypic variance of PHS resistance and test the phenotypic difference in each heterozygous family by using a linear model in which a phenotypic value was partitioned into mean, genotypic, and error components. The QTL effect ($R^2$) was calculated as the proportion of component type III sum-of-square (SS) to the corrected total SS. Additive ($a$) and dominant ($d$) effects of the QTL were estimated using the RIL25-derived secondary segregating $F_2$ population as described in other studies (Kearsey and Pooni 1996; Monforte and Tanksley 2000; Brouwer and Clair 2004; Gu et al. 2004) by using the following formula:

$$a = 1/2M_{SS} - 1/2M_{EE}$$

$$d = M_{ES} - 1/2M_{EE} - 1/2M_{SS}$$

where $M_{EE}$, $M_{SS}$ and $M_{ES}$ are means of homozygous Rio Blanco-type, homozygous NW97S186-type, and heterozygous genotypes, respectively, for the marker locus in the QTL region. Standard errors for the parameters $a$ and $d$ were estimated as:

$$S_a = 1/2\left(S_{EE}^2 + S_{SS}^2\right)^{1/2}$$

$$S_d = \left(S_{ES}^2 + 1/4S_{EE}^2 + 1/4S_{SS}^2\right)^{1/2}$$

where $S_{EE}^2$, $S_{SS}^2$ and $S_{ES}^2$ are variances of the means $M_{EE}$, $M_{SS}$ and $M_{ES}$, respectively. Significance of the estimates for $a$ and $d$ were determined by the Student's $t$ test.

## Results

### Isolation of recombinant NIL

After progenies of RIL25 were screened with the 3 SSR markers in the QTL region, 43 heterozygotes were identified to start a new population for fine mapping. A total of 1,874 secondary $F_2$ progenies were produced from those heterozygotes and genotyped again with the three markers. In RIL81, 269 secondary $F_2$ progenies segregating at the *Xbarc12* locus were produced and genotyped (Table 1).

Marker alleles segregating at each marker locus in the two populations were scored as three genotypes: homozygous Rio Blanco allele (a), heterozygote (h) and homozygous NW97S186 allele (b). The segregation ratio fit 1(a):2(h):1(b) Mendelian single gene segregation in both populations derived from the two RILs (Table 1). Among the 1,874 secondary $F_2$ plants derived from RIL25, 1 homozygous recombinant plant and 55 heterozygous recombinant plants of 8 haplotypes were identified (Table 2). Those heterozygous recombinants had at least one recombination at the three marker loci in the QTL region and were selfed again to produce homozygous recombinants. The marker order for the three marker loci was consistent (*Xbarc12*, *Xbarc57* and *Xbarc321*).

In the RIL25-derived secondary segregating $F_2$ population, 863 plants were randomly selected across all genotypes to evaluate PHS resistance (Table 2). The spike germination rate in the population showed two peaks: one

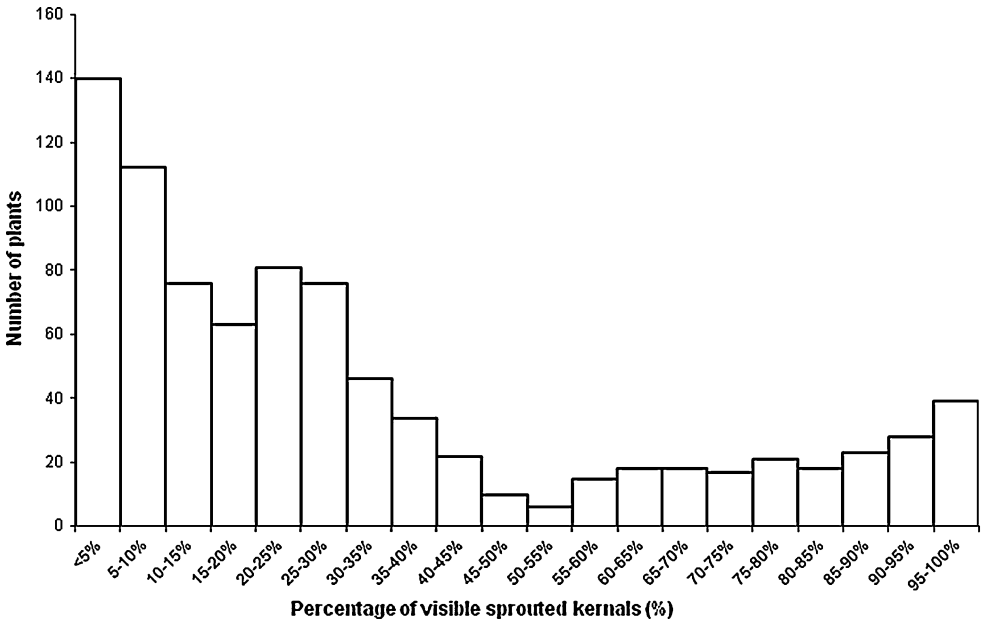


**Fig. 2** Distribution of the percentage of sprouted kernels in the secondary segregating $F_2$ population developed from RIL25

major peak toward a low germination rate and a minor peak toward a high germination rate (Fig. 2). A total of 229 secondary $F_2$ plants with the same allelic pattern as Rio Blanco at the three marker loci had a very low average germination rate of 2.9%, whereas the 228 lines with the same allelic pattern as NW97S186 had a high average germination rate of 85.0%. PHS resistance was significantly different between the two groups contrasting in the three marker loci, confirming that *QPhs.pseru-3A* is located in the contrasting region of the two groups. The two groups of lines are considered NIL contrasting in the *QPhs.pseru-3A* QTL region because they were derived from the same genetic background of the same $F_6$ plant.

However, 398 heterozygous secondary $F_2$ plants at all three marker loci in the QTL region showed a significantly lower average germination rate compared with the susceptible group with the similar allelic pattern as NW97S186 (Table 2). Although the heterozygous group showed a significantly higher average germination rate (20.5%) than the resistant group (2.9%), its germination rate was significantly lower than that of the mid-parent (44%), suggesting the QTL was incomplete dominant. Nonetheless, the additive effect (*a*) still played a major role in enhancing PHS resistance with a decrease of 43.6% in germination rate, whereas the dominant effect (*d*) decreased 13.9% of germination rate. The QTL explained 74.3% of total phenotypic variation in this secondary segregation population.

## Delineation of the QTL near marker *Xbarc57*

In the RIL81-derived population, all 253 secondary $F_2$ plants tested had a low germination rate (about 3.0%) despite segregation at *Xbarc12* locus, suggesting *Xbarc12* did not co-segregate with *QPhs.pseru-3A*. Thus, this population was not analyzed further for fine mapping of the QTL. The same result was obtained in the RIL25-derived secondary segregating $F_2$ population, in which all recombinants of haa (5) showed a low germination rate (4.7%) and hbb (21) showed a high germination rate (84.6%); h represents heterozygous alleles for *Xbarc12* locus (Table 2). Low germination rates similar to that of $F_1$ (hhh) ranging from 27.7 to 35.3% for haplotypes ahh, aha, bhh and bhb indicates that allelic substitution at the *Xbarc321* locus did not significantly change PHS resistance of the recombinants. However, the recombinant became highly susceptible when the Rio Blanco allele of *Xbarc57* was replaced with the NW97S186 allele (hbh and bba). These results suggest *QPhs.pseru-3A* is flanked by markers *Xbarc12* and *Xbarc321* and closely linked with *Xbarc57*.

To validate the result from the secondary $F_2$ data, the eight types of secondary $F_2$ recombinants with heterozygous haplotypes of ahh, aha, aah, bhh, haa, hbh, hbb and bhb were selfed, and their $F_3$ families were genotyped and evaluated for PHS resistance. Five haplotypes of homozygous recombinants (aab, aba, abb, baa and bab) were obtained from these families (Table 3). The $F_3$ homozygous recombinants were all resistant to PHS when the Rio Blanco allele of *Xbarc57* was present (aab, baa and bab) and all susceptible when the NW97S186 allele of *Xbarc57* was present (aba and abb). These results confirmed that *Xbarc57* was the closest marker to *QPhs.pseru-3A* and *Xbarc12* and *Xbarc321* were flanking markers for the QTL.

After 600 AFLP primer combinations were screened, 11 showed polymorphism between the 2 parents and NIL contrasting in PHS resistance. These polymorphic primers

**Table 3** Homozygous recombinants among markers *Xbarc12*, *Xbarc57* and *Xbarc321* developed from $F_3$ families and germination rate

| Secondary $F_2$ genotypes[a] | No. of $F_3$ families tested | $F_3$ homozygous recombinants[a] | No. of lines | No. of lines PHS resistance tested | Germination rate range (%) | Mean of Germination rate (%) | No. of homozygous recombinants |
|---|---|---|---|---|---|---|---|
| aah | 1 | aab | 5 | 5 | 0–3.1 | $1.8^{A}$ | 1 |
| bhb | 1 | bab | 9 | 6 | 0–4 | $1.1^{A}$ | 1 |
| bhh | 4 | baa | 17 | 17 | 1.3–14.1 | $4.7^{A}$ | 4 |
| haa | 4 | baa | 13 | 13 | 5.3–17.7 | $8.5^{A}$ | 4 |
| aha | 2 | aba | 42 | 42 | 84.3–93.9 | $91.1^{B}$ | 2 |
| ahh | 12 | abb | 36 | 33 | 86.9–100 | $95.8^{B}$ | 12 |
| hbb | 4 | abb | 6 | 6 | 93.7–99.1 | $96.9^{B}$ | 4 |
| hbh | 1 | aba | 13 | 13 | 68.6–94.4 | $84.1^{B}$ | 1 |

Within same secondary $F_2$ genotype derived $F_3$ families, different superscript letters indicate significant difference at $P < 0.01$

[a] Marker order is *Xbarc12*, *Xbarc57* and *Xbarc321*

were used to analyze the homozygous recombinant QTL-NIL obtained in this study. Eleven AFLP markers together with the three SSR were mapped in one linkage group and covered a genetic distance of 2.1 cM (Fig. 3). Seven AFLP markers co-segregated with *Xbarc57*, and all recombinants with Rio Blanco alleles at these marker loci were resistant, whereas all recombinants with the opposite alleles were susceptible (Table 4). Three AFLP markers showed recombinations between either *Xbarc12* and *Xbarc57* or *Xbarc57* and *Xbarc321* (Fig. 3). Therefore, the PHS-resistant QTL was delimited in the 1.4 cM region between AFLP markers *XpAGG-mCTA320* and *XpCAG-mTCGA145*.

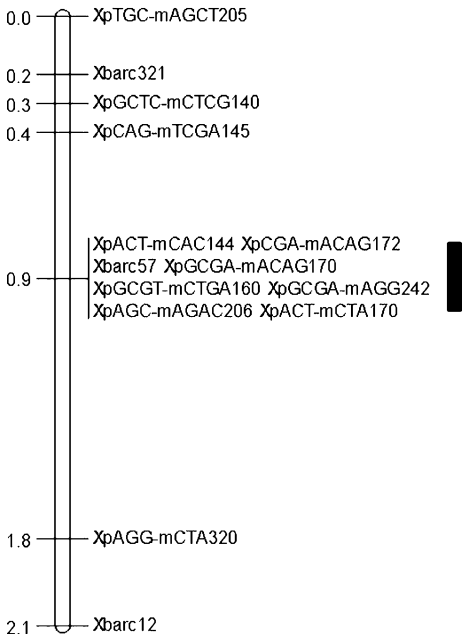


**Fig. 3** Genetic linkage map saturated with the AFLP markers. The *filled square* showed *QPhs.pseru-3A* located on this *bar*

## Discussion

### Evaluation of PHS for fine mapping

Accurate phenotyping to obtain repeatable phenotypic data is a very critical step in narrowing down the major QTL for PHS resistance to a small chromosome region and isolating a QTL for PHS resistance as a single Mendelian factor. PHS resistance in wheat is a complicated trait, and many factors may contribute to overall PHS resistance, including SD (Mares and Mrva 2001; Imtiaz et al. 2008; Ogbonnaya et al. 2008), germination-inhibiting substances residing in chaff tissue, physical barriers to water penetration in a spike, and spike morphologies (King and Richards 1984; Gatford et al. 2002). In this study, wheat was grown under controlled greenhouse conditions, harvested at physiological maturity, and dried at a constant temperature for a fixed period (8 days) before phenotyping. During the germination test, all spikes were soaked in distilled water overnight and then incubated at 100% humidity for germination. This protocol excludes physical or morphological barriers that may prevent water penetration in spikes. Therefore, the difference in PHS phenotypes among genotypes observed in this study is most likely due to SD or other genetic factors.

For the initial secondary segregating $F_2$ population, the phenotypic result was determined from a single secondary $F_2$ plant. However, we evaluated four spikes from tillers from each plant, and germination rates among the four spikes were similar. Furthermore, 229 Rio Blanco (aaa) type secondary $F_2$ plants, 228 NW97S186 (bbb) type plants and 398 heterozygous (hhh) type plants were evaluated, and germination rates were consistent within each genotype and large variation was observed among the three types (Table 2), which also indicates that environmental

**Table 4** Graphical presentation of haplotypes of selected homozygous lines used for fine mapping of the QTL *QPhs.pseru-3A* for PHS resistance

| Lines | *Xbarc12* | *XpAGG-mCTA320* | *XpACT-mCTA170* | *XpAGC-mAGAC206* | *XpGCGA-mAGG242* | *XpGCGT-mCTGA160* | *XpGCGA-mACAG170* | *Xbarc57* | *XpCGA-mACAG172* | *XpACT-mCAC144* | *XpCAG-mTCGA145* | *XpGCTC-mCTCG140* | *Xbarc321* | *XpTGC-mAGCT205* | Germination rate (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 08s29 | a | b | b | b | b | b | b | b | b | b | b | b | b | b | 94.3 |
| 08s419 | a | a | b | b | b | b | b | b | b | b | b | b | b | a | 96.1 |
| 08s80 | a | a | b | b | b | b | b | b | b | b | b | b | b | b | 93.3 |
| 08s296 | a | a | b | b | b | b | b | b | b | b | a | a | a | a | 93.8 |
| 08f577 | a | a | b | b | b | b | b | b | b | b | b | a | a | a | 86.7 |
| 08s341 | b | b | a | a | a | a | a | a | a | a | a | a | a | b | 5.5 |
| 08s276 | b | a | a | a | a | a | a | a | a | a | a | a | a | a | 2.5 |
| 08f901 | b | b | a | a | a | a | a | a | a | a | a | a | a | a | 3.7 |
| 08f736 | b | b | a | a | a | a | a | a | a | a | b | b | b | b | 0 |
| 08f145 | a | a | a | a | a | a | a | a | a | a | b | b | b | b | 0 |
| Rio Blanco | a | a | a | a | a | a | a | a | a | a | a | a | a | a | 1.4 |
| nw97s186 | b | b | b | b | b | b | b | b | b | b | b | b | b | b | 98.3 |
| 07f45-8-R-NIL | a | a | a | a | a | a | a | a | a | a | a | a | a | a | 1.2 |
| 07f11-8-S-NIL | b | b | b | b | b | b | b | b | b | b | b | b | b | b | 95.3 |

*a* Homozygous Rio Blanco segments, *b* homozygous NW97S186 segments

variation was well controlled in this study. The PHS resistance of the selected recombinants was also validated by evaluating their homozygous recombinants in the $F_3$ families with 20–79 plants per family (data not shown). Results from the repeated $F_3$ family tests were the same as those from secondary $F_2$ plants. The phenotypic data were highly repeatable, as seen from the very low variation among secondary $F_2$ plants with the same marker alleles and within a homozygous family; thus, PHS data from this study are reliable for fine mapping of the QTL.

## Isolation of QTL-NIL

Studies on mapping QTL for PHS resistance have been reported in wheat (Anderson et al. 1993; Kato et al. 2001; Kulwal et al. 2004, 2005; Groos et al. 2002; Imtiaz et al. 2008). In these studies, QTL were detected by using $F_2$, RIL or DH populations. To fine map a QTL, eliminating the complication of other QTL by using a population segregating for only one single QTL may provide better resolution than using a population segregating for several QTL (Zamir 2001). Therefore, further separating QTL from other background effects to develop NIL contrasting in only the target QTL in an identical genetic background and studying the single QTL effect will provide a solid foundation for fine mapping and map-based cloning of the QTL (Olmos et al. 2003; Brouwer and Clair 2004; Goodstal et al. 2005; Gu et al. 2006; Xie et al. 2006, 2008).

Two common methods have been used to develop NIL: continuous backcross (Alonso-Blanco et al. 2003; Gao et al. 2003; Olmos et al. 2003; Brouwer and Clair 2004; Xie et al. 2006) and selecting segregated plants from within a heterogeneous RIL (Takeuchi et al. 2003; Tuinstra et al. 1997; Liu et al. 2006). Continuous backcross is time consuming and may take several years to obtain the desired NIL. Selecting segregating progenies from a heterogeneous RIL at the target QTL region by marker-assisted selection can speed up the process if the RIL are available. This method has been used to develop NIL for Fusarium head blight resistance QTL in wheat (Liu et al. 2006), seed weight QTL in sorghum (Tuinstra et al. 1997), chilling tolerance QTL in tomato (Goodstal et al. 2005) and SD QTL in rice (Takeuchi et al. 2003). This approach has proven to be an effective approach for isolating NIL in crops if a RIL segregates at the target QTL region.

On the basis of previously reported map information (Liu et al. 2008a), two $F_6$ RILs, RIL25 and RIL81, were identified to be heterogeneous in at least one marker locus in the QTL region. Progenies of RIL81 segregated for marker *Xbarc12* but did not segregate in germination rate; therefore, this population was not an informative population for fine mapping of the QTL and was not analyzed further. The population derived from RIL25 segregated at

all three marker loci in the QTL region, and PHS resistance of a randomly selected set of 863 plants from this population showed Mendelian single gene segregation for PHS resistance. Genotypic and phenotypic data showed that progenies with Rio Blanco alleles at the three marker loci in the QTL region were all resistant but those with NW97S186 alleles were susceptible. Therefore, the population was ideal for NIL isolation and fine mapping of the major QTL.

In the RIL25 derived secondary segregating $F_2$ population, we identified 55 heterozygous and one homozygous recombinant for the three SSR marker loci in the QTL region, and the heterozygous plants were continuously selfed to obtain more homozygous recombinants. PHS evaluation identified homozygous recombinant haplotype bab as highly PHS resistant; therefore, the recombinants are good PHS-resistant NIL harboring a small Rio Blanco segment in the QTL region and can be ideal materials for gene expression study and fine mapping of the QTL. Isolation of NIL for a single QTL with different lengths of segments from a PHS-resistant parent via a heterozygous RIL at the QTL region to narrow down the QTL by simultaneous genotyping and phenotyping has not been reported to date in wheat. Our result showed that this method can effectively enlarge the fine mapping population in a reasonably short time; thus, it will be very useful for map-based cloning of QTL in crops.

## Delineation and fine mapping of the QTL

To fine map the QTL, a set of recombinant NIL can be used to narrow down the QTL to a smaller region in which altered phenotype is associated with loss of a specific chromosomal region (Paterson et al. 1990; Monforte and Tanksley 2000). By using this approach, the borders of an interval flanking the QTL can be identified by comparing the homozygous recombinants that carry contrasting alleles of the two parents and phenotypic data of these recombinants. This procedure has been very successful in fine mapping QTL in tomato, rice, wheat and barley (Paterson et al. 1990; Gao et al. 2003; Olmos et al. 2003; Brouwer and Clair 2004; Tian et al. 2006; Andaya and Tai 2007).

In our previous report (Liu et al. 2008a), flanking markers for the QTL were not determined because the likelihood ratio (LR) peak curve of the QTL from the composite interval mapping did not drop down even in the end of the linkage map. To further validate the QTL location and pinpoint the QTL to a smaller region, the recombinant NIL population derived from RIL25 was used. PHS data from the secondary $F_2$ recombinants and their $F_3$ families showed that substitution of Rio Blanco alleles with NW97S186 alleles of *Xbarc12* and *Xbarc321* did not change the PHS resistance of these lines, but did for marker *Xbarc57*. Marker *Xbarc57* was flanked by *Xbarc12* and *Xbarc321*; therefore, QTL *QPhs.pseru-3A* was pinpointed to the region near *Xbarc57* (Tables 2, 3; Fig. 3) flanked by *Xbarc12* and *Xbarc321*.

To saturate the QTL region, 11 AFLP markers were mapped in the linkage map. The new map contained 14 markers covering 2.1 cM. Three AFLP markers showed recombination among *Xbarc12*, *Xbarc57* and *Xbarc321*, and seven co-segregated with *Xbarc57* in the population. PHS data showed that all homozygous recombinants with the Rio Blanco haplotype at the seven AFLP loci were resistant to PHS, whereas those with the NW97S186 haplotype at these markers were all susceptible. However, genotypic change of the other three AFLP markers, *XpAGG-mCTA320*, *XpCAG-mTCGA145* and *XpGCTC-mCTCG140*, was not associated with the PHS resistance; thus, the PHS resistance QTL was tightly linked to *Xbarc57* and the seven AFLP markers (Fig. 3) and delineated to a 1.4 cM region between AFLP markers *XpAGG-mCTA320* and *XpCAG-mTCGA145* (Table 4).

## QTL effect on PHS resistance

Additive effect is a heritable genetic component that can stably transmit from generation to generation; thus, it is a major genetic component that can be fixed in breeding materials to develop pure cultivars. In contrast, dominant or dominance-related epistatic effects that express in heterozygous genotypes cannot be fixed in homozygous breeding materials through conventional breeding. Populations derived from a heterozygous RIL segregating only in the QTL region have been successfully used to estimate gene effects in several crops. In *Arabidopsis*, large additive effects were detected for QTL of dormancy (Alonso-Blanco et al. 2003). In rice, QTL for SD, high yield and panicle number showed additive effects (Gu et al. 2006; Liu et al. 2008b; Xie et al. 2008). In this study, by comparing phenotypic effects of heterozygous and homozygous secondary $F_2$ plants derived from within-family segregation, we found that genotypes heterozygous at the QTL region had a much lower germination rate than the susceptible genotypes and mid-parental value but a significantly higher germination rate than resistant genotypes, suggesting that *QPhs.pseru-3A* was incomplete dominant. However, additive effect was still the predominant genetic component of the QTL, which decreased 43.6% of the germination rate. These results agree with Buraas and Skinnes (1984) and Gu et al. (2004). Therefore, the QTL is highly inheritable and can be stably transmitted in breeding materials for improvement of PHS resistance.

Because of the increased demand for white wheat production, breeding white grain wheat cultivars with PHS resistance is a very urgent task for breeders worldwide. The

QTL detected in this study showed a significant additive effect and, therefore, should be a good source to use for improving PHS resistance of white wheat. SSR marker *Xbarc57* and seven AFLP markers are closely linked to the QTL; thus, *Xbarc57* is a breeder-friendly marker for marker-assisted selection of the QTL. The AFLP markers can be further converted into sequence tag site (STS) markers to add additional markers for cases in which *Xbarc57* is not polymorphic between parents.

**Acknowledgments** Research is partly funded by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, Coordinated Agricultural Project. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. This is contribution No. 10-285-J from the Kansas Agricultural Experiment Station, Manhattan, KS, USA.

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