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ORIGINAL PAPER

Mapping of *Yr62* and a small-effect QTL for high-temperature adult-plant resistance to stripe rust in spring wheat PI 192252

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Abstract

Key message **This manuscript reports a new gene (*Yr62*) and a small-effect QTL for potentially durable resistance to stripe rust and usefulness of *Yr62* markers for marker-assisted selection.**

Abstract Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a devastating disease of wheat worldwide. Spring wheat germplasm PI 192252 showed a high level of high-temperature adult-plant (HTAP) resistance to stripe rust in germplasm evaluation over 8 years in the State of Washington. To elucidate the genetic basis of resistance, PI 192252 was crossed with 'Avocet susceptible'. A mapping population of 150 F_5 recombinant inbred lines was developed using single-seed descent. Stripe rust tests were conducted with selected *Pst* races in a greenhouse and in field conditions under natural infections. The relative area under the disease progress curve (rAUDPC) data showed continuous distributions, indicating that HTAP resistance of PI 192252 was controlled by quantitative trait loci (QTL). Two QTL were identified in PI 192252, explaining 74.2 % of the total phenotypic variation for rAUDPC. These two QTL were mapped to chromosomes 4BL (*QYrPI192252.wgp-4BL*) and 5BS (*QYrPI192252.wgp-5BS*) with SSR and SNP markers and explained 40–60 and 22–27 %, respectively, of the phenotypic variation across the four environments. Because the major-effect QTL on 4BL is different from previously named *Yr* genes and inherited as a single gene, it is named *Yr62*. The SSR marker alleles $Xgwm192_{222}$ and $Xgwm251_{133}$ flanking *Yr62* were different from the alleles in various wheat varieties, suggesting that these markers could be useful in marker-assisted selection for incorporating *Yr62* into commercial cultivars.

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Introduction

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), is one of the most devastating diseases of wheat worldwide (Chen 2005). Resistance is the most effective, economical and environment-friendly method to control stripe rust. Cultivars with high-temperature adult-plant (HTAP) resistance become increasingly resistant from

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about the jointing stage and as the weather becomes warmer (Qayoum and Line 1985; Milus and Line 1986; Chen and Line 1995a, b). HTAP resistance has proven to be non-race specific and durable (Line 2002; Chen 2005, 2013). Warmer weather is necessary for HTAP resistance genes to express under conditions where night temperatures are generally above 10 °C and day temperatures are between 25 and 35 °C (Qayoum and Line 1985; Line and Chen 1995).

High-temperature adult-plant resistance is generally quantitatively inherited (Chen 2005, 2013). The level of HTAP resistance is usually incomplete and is affected by growth stage, temperature, humidity and inoculum load. Low levels of HTAP resistance in some cultivars do not provide adequate control (Chen 2005, 2013). Taking stripe rust epidemics in the US Pacific Northwest (PNW) in 2010 and 2011 as examples, cultivars with low to moderate levels of HTAP resistance had 10–40 % yield losses, better than highly susceptible cultivars with 60–90 % losses, but were not as good as cultivars with high levels of HTAP or effective all-stage resistance without significant yield losses (Chen, unpublished data).

To date, more than 80 genes or quantitative trait loci (QTL) conferring various levels of adult-plant or HTAP resistance have been mapped to all 21 chromosomes except 3D and 5D (Chen 2013). Only a few of these genes provide high levels of resistance (He et al. 2011; Chen 2013). There are two major approaches to achieve high levels of HTAP resistance: one is to accumulate multiple QTL each with a small effect and the other is to identify QTL with a large effect. Because wheat cultivars with HTAP resistance controlled by one or a small number of QTL have shown durability, identifying major-effect QTL which contribute high levels of HTAP resistance is more efficient for use in breeding programs (Chen 2013).

PI 192252, a Portuguese spring wheat variety, was deposited in the USDA-ARS National Small Grains Collection (NSGC) in 1950 (http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=PI+192252). In a previous study, the variety showed a high level of stripe rust resistance in our germplasm evaluation program from 2004 to 2010 (Wang et al. 2012). Greenhouse and field tests showed that PI 192252 was susceptible to predominant *Pst* races at the seedling stage, but was highly resistant at the adult-plant stage under high temperatures. The objectives of the present study were to identify QTL for resistance to stripe rust in PI 192252, and to develop new germplasm lines with the resistance QTL and better plant type.

Materials and methods

Plant materials

The mapping population used in this study comprised of 150 F_5 recombinant inbred lines (RIL) developed through single-seed descent from the cross AvS/PI 192252. Avocet susceptible (AvS), an Australian spring wheat selection, is highly susceptible to most *Pst* races in the US and many other countries (Chen et al. 2010; Wellings et al. 2012; Sharma-Poudyal et al. 2013).

One hundred fifty F_2 plants, obtained from a single F_1 plant grown in the greenhouse, were grown in the field to produce F_3 lines. A single spike from each F_3 RIL was harvested during the 2010 field season and one F_4 seed from each spike was grown in the greenhouse to produce F_5 seeds. F_5 RILs were used in the field experiments in 2011, from which a single spike was separated to obtain F_6 seeds and the remaining seeds from each F_5 line were bulked and used as F_5 RIL in field experiments in 2012 and used for DNA extraction.

Chinese Spring (CS), its nulli-tetrasomic lines and ditelosomic lines (Sears 1966; Sears and Sears 1978) and deletion lines for wheat chromosomes 4B and 5B (Endo and Gill 1996) were used to determine specific positions of the resistance genes in PI 192252. A total of 140 spring and winter wheat cultivars or breeding lines were used to evaluate polymorphisms of molecular markers flanking the resistance genes. CH223, a Chinese winter wheat line with *Yr50* reported on chromosome 4BL (Liu et al. 2013), was used to cross with PI 192252 for allelism test.

Greenhouse tests

To characterize HTAP resistance of PI 192252, the parents of the cross AvS/PI 192252 and nine homozygous resistant F_6 RIL lines were planted in a greenhouse for the four-way tests (Chen and Line 1995a; Chen 2013). For seedling tests, both the low and high temperature cycles were used. Five to seven seedlings of each line were grown in a 7 × 7 × 7 cm pot filled with a potting mixture (24 L of peat moss, 8 L of perlite, 12 L of sand, 12 L of commercial potting soil, 16 L of vermiculite and 250 g of 14-14-14 Osmocote) in a rust-free greenhouse. *Pst* races PST-116 and PST-127 were selected based on their recent predominance in the US and unique and broad virulence patterns (Chen et al. 2010). At the two-leaf stage, seedlings were inoculated with fresh urediniospores of each race following the method described by Chen and Line (1992a, b). Inoculated plants were placed in a dew chamber without light at 10 °C for 24 h and then moved to separate greenhouse chambers set at a diurnal 16 h light/8 h dark cycle, one at a low diurnal temperature cycle (4–20 °C) and the other at a high diurnal temperature cycle (10–30 °C) for rust development (Chen and Line 1995a; Chen 2013). Infection types (IT) based on a 0–9 scale were recorded for each plant 20–25 days after inoculation (Line and Qayoum 1992).

For the adult-plant tests, three plants for each line were grown in a pot (diameter 16 cm by height 20 cm). Plants at

the heading stage were inoculated with urediniospores of each selected race about 40 days after planting. Inoculated plants were kept in a dew chamber for 24 h at 10 °C without light, and then moved to growth chambers set at a diurnal 16 h light/8 h dark cycle and either a low-temperature or high-temperature profile as described above.

Field tests

The parents and F_5 RIL population were evaluated for stripe rust reaction in the experimental fields at Pullman and Mount Vernon in 2011 and 2012. In 2011, the nurseries were planted on 6th May near Pullman in eastern WA and on 10th May at Mount Vernon in western WA. In 2012, the nurseries were planted on 10th May at Pullman and on 17th May at Mount Vernon. The two sites are about 500 km from each other and have different weather conditions and *Pst* race compositions. The F_6 RIL population was evaluated only at Pullman in 2012. At each site, the experiment was conducted in a randomized complete block design with three replications. Each replication comprised of one row of each parent and 150 rows of RILs. About 30 seeds from each line were planted in a 1 m row with 20 cm space between rows. F_2 seeds were space planted to determine the segregation ratio at Pullman in 2012. The trials were subjected to natural infection. Susceptible spring wheat variety 'Lemhi' was planted around each plot and after every 19 rows to increase the uniformity of infection throughout the field. The nurseries were managed using common practices for the regions.

Seeds of F_2 and the parents of the PI192252/CH223 cross were sown in small pots in a greenhouse on 1st April, 2013. After first leaf emerged, the plants were moved to a chamber set at 4 °C for vernalization. On 15th May 15, 261 F_2 plants and about 20 plants for each of the parents were transplanted in a field near Pullman, WA for evaluation of stripe rust response under natural infection of the pathogen. IT and severity data were recorded in July when the plants were at heading (Zadoks 55) and milk (Zadoks 75) stages.

Infection type and disease severity (DS) data were recorded at the booting, flowering and soft dough stages when the severity on AvS was about 40, 70 and more than 95 %, respectively. ITs were recorded based on the 0–9 scale described by Line and Qayoum (1992). Disease severities were assessed visually using percentages of infected leaf areas. For the parents and F_5 RILs, IT was recorded as a single value for homozygous lines and as two or more values for segregating lines, but later analyzed as a mean value for each line.

Genetics and statistical analyses

To estimate the number of genes for HTAP resistance in PI 192252, F_1, F_2, F_5 and F_6 generations of the cross AvS/PI 192252 were analyzed. The goodness of fit of observed numbers to expected frequencies for the phenotypic IT and DS data was calculated with the 'Chitest' function in Microsoft Office Excel 2007 (Version 12), and reaction means were compared using least significant difference criteria ($P < 0.05$). Severity data were used to calculate the area under the disease progress curve (AUDPC) for each F_5 RIL and the parents according to the formula: $\text{AUDPC} = \sum \left[(x_i + x_{i+1})/2\right] t_i$, where x_i is the DS value on date i, t_i is the time in days between dates i and $i + 1$ (Chen and Line 1995a, b). Relative AUDPC (rAUDPC) values were calculated for each of the RILs and parents as a percentage of the mean AUDPC value of the susceptible parent, AvS (Lin and Chen 2007). The rAUDPC values were used for the subsequent analysis of variance (ANOVA) and QTL analysis.

To determine genetic and environmental effects on stripe rust response, a site–year combination was treated as an environment, and ANOVA was performed for each combination of environments using the PROC GLM program of SAS Statistical Software v9.1 (SAS Institute Inc., Cary, NC).

Broad-sense heritabilities (h^2) of phenotypic traits were calculated using the formula $h^2 = \sigma_G^2 / \left[\left(\sigma_G^2 + \sigma_{GE}^2/e + \sigma_E^2/(e \times r)\right)\right]$, where σ_G^2, σ_{GE}^2 and σ_E^2 are estimates of genotypic, genotype × environment interaction and error variances, respectively, and e and r are the numbers of environments and replications per environment, respectively (Yang et al. 2005).

For the allelism test using the stripe rust IT data of the F_2 population from the PI 192252 × CH233 cross, a map distance was calculated from recombination values using the Kosambi function $d = 0.25 \ln[(1 + 2r)/(1 - 2r)]$, where r is the recombination frequency (Kosambi 1944).

Identification of molecular markers

To extract DNA, about 10–15 seeds for each of the RILs and parents were sown in a 7 × 7 × 7 cm pot in a greenhouse and leaf tissues were collected from all plants at the two-leaf stage and dried in an evacuated silica gel desiccator for 3 days. The dried samples were ground using a Mini BeadBeater (Biospec Products, Inc., Bartlesville, OK, USA). Genomic DNA was extracted from plant samples using the CTAB method (Riede and Anderson 1996), dissolved in 1 × TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) and stored at −20 °C. DNA of each sample was adjusted to the same concentration (25 ng/μL) and quality was assessed by electrophoresis in 1 % (wt/vol) agarose gels.

Bulked segregant analysis (BSA) was used to identify markers linked with stripe rust resistance genes. Resistant and susceptible bulks were constructed by mixing

equal amounts of DNA from ten highly resistant and ten highly susceptible RILs. A total of 700 simple sequence repeat (SSR) markers were screened in the BSA tests. These markers covered all 21 wheat chromosomes according to the locations reported by Somers et al. (2004). Sequences of available SSR markers along with their previously determined chromosomal locations were obtained from GrainGenes (http://wheat.pw.usda.gov). SSR marker PCR was performed using the conditions: 12 μL reaction mixtures containing 4.56 μL ddH_2O, 1.2 μL Mg-free 10 × PCR reaction buffer, 1.0 unit of *Taq* DNA polymerase, 0.1 mM of $MgCl_2$, 0.2 mM of dNTP, 0.6 mM of forward primer (M13 tailed at 5′), 0.25 μM of reverse primer, 0.2 μM of appropriate fluorescence-M13 primer dyes and 4 μL template DNA (25 ng/μL). PCR products were separated on an ABI3730 DNA fragment analyzer (Applied Biosystems, Grand Island, NY), with alleles scored using software GeneMarker v4.0 (SoftGenetics, LLC., State College, PA, USA).

Simple sequence repeat markers showing associations with stripe rust resistance in the BSA were used to genotype all F_5 RILs. Additional markers for enriching the chromosomal regions linked to resistance genes were selected from the consensus maps. To locate the resistance loci to specific wheat chromosomes, three SSR markers closely linked to the resistance genes were tested on each of the chromosome 4B and 5B deletion lines.

To obtain more markers, PI 192252 and AvS were screened with the 9 K SNP chip at the USDA-ARS Biosciences Research Laboratory, Fargo, North Dakota using the method described by Cavanagh et al. (2013). From a total of 3,000 polymorphic SNP markers, 65 specific to chromosomes 4BL and 5BS were chosen to genotype F_5 RILs. The genotyping of RILs with the selected SNP markers was conducted at the USDA-ARS Wheat Genetics, Quality, Physiology and Disease Research Unit, Pullman, Washington. The SSR and SNP markers linked to resistance genes were used to construct linkage maps.

Molecular mapping

Software JoinMap 4.0 (Voorrips 2002) was used to construct linkage groups including the molecular markers and stripe rust response as a qualitative locus. A minimum LOD (Logarithm of odds) threshold of 2.0 was used for grouping markers into linkage groups. Three-point linkage analysis was carried out to determine the most likely order of linked markers. The Kosambi mapping function (Kosambi 1944) was used to convert recombination values to genetic distances. Linkage groups were assigned to chromosomes according to the consensus maps of Somers et al. (2004) and the wheat chromosome information available at the GrainGenes website (http://wheat.pw.usda.gov/GG2/index.shtml). Resistance genes were assigned to chromosomal bins using tightly flanking markers based on the deletion maps of Sourdille et al. (2004).

QTL mapping was performed with WinQTLCart V2.5 (Wang et al. 2010) using a composite interval mapping (CIM) method with a window size of 10 cM and a walk speed of 1 cM. The rAUDPC scores for each line from the two locations and two years were used in the QTL analysis. To detect QTL, a LOD threshold value of 2.0 based on a 1,000-permutation test was used ($P = 0.05$). The percentages of phenotypic variance (R^2) explained by individual QTL were also carried out using CIM.

Results

Characterization of HTAP resistance in PI 192252

In the greenhouse experiments with *Pst* races PST-116 and PST-127, PI 192252 was susceptible (IT 8) in both low- and high-temperature seedling tests and also susceptible (IT 8) at the adult-plant stage in low-temperature profile tests, whereas it was highly resistant (IT 2) at the adult-plant stage in the high-temperature profile (Table 1). In contrast, the susceptible parent, AvS, had a susceptible reaction (IT 8) in all four-way tests with both races. In field tests, PI 192252 began showing resistance at the jointing stage and became highly resistant (IT 1–2) after flag leaf emergence in all field tests. In contrast, AvS was highly susceptible (IT 8–9) in the field tests. These results clearly showed that PI 192252 has a high level of HTAP resistance.

Seven F_1 plants and 170 F_2 plants were tested under field conditions in 2012 at the Pullman location. All F_1 plants showed IT 6–7 and F_2 population segregated 36 resistant (IT 1–4) and 134 susceptible (IT 6–9), complying with a 1:3 ratio ($\chi^2 = 1.33$, $P = 0.25$) (Table 2). The F_5 RILs had IT ranging from 1 to 9, and were classified into resistant (IT 1–4), segregating (IT 1–9) and susceptible (IT 7–9). These classes fit a 7 (43.75 %):2 (12.5 %):7 (43.75 %) ratio expected for single gene segregation among F_5 lines in all location–year experiments ($\chi^2 = 1.38$–2.21, $P = 0.33$–0.50) (Table 2). The qualitative analysis of the arbitrary classes indicated that the HTAP resistance in PI 192252 was mainly controlled by a single partially dominant gene.

The mean rAUDPC values in the F_5 population obtained from both 2011 and 2012 experiments at Pullman and Mount Vernon were also continuous (Fig. 1), indicating that HTAP resistance in PI 192252 was quantitatively inherited. PI 192252 had rAUDPC values of <10 %, compared to the 100 % for AvS in all experiments (Fig. 1). The rAUDPC data of the RIL population were highly correlated with the IT data ($r = 0.95$–0.98, $P < 0.01$) in both locations and years. No significant differences were detected among

Table 1 Infection types of wheat genotypes PI 192252, Avocet Susceptible (AvS) and three homozygous resistant F_6 lines derived from the AvS/PI 192252 cross to races PST-116 and PST-127 of *Puccinia striiformis* f. sp. *tritici* at the seedling and adult-plant stages under low- and high-temperature conditions

Line (*Yr* gene chromosome)	Infection type		
	Seedling	Adult-plant	
	LT or HT[a]	LT	HT
PI 192252 (4BL + 5BS)	8	8	2
AvS (none)	8	8	8
F_6-93 (4BL)	8	8	2
F_6-171 (4BL)	8	8	3
F_6-185 (4BL)	8	8	2
F_6-96 (5BS)	8	8	5
F_6-97 (5BS)	8	8	5
F_6-176 (5BS)	8	8	5
F_6-83 (4BL + 5BS)	8	8	2
F_6-143 (4BL + 5BS)	8	8	2
F_6-218 (4BL + 5BS)	8	8	1

[a] *LT* low diurnal temperature cycle (4–20 °C) and high diurnal temperature cycle (10–30 °C). Both tests of LT and HT produced the identical infection type data on all wheat genotypes with both races PST-116 and PST-127

replications, experiments and for interactions between experiments and RILs; and RILs were the only significant source of the phenotypic variation observed in the study (Table 3). Clearly, HTAP resistance in PI 192252 was consistently expressed in the different environments. Therefore, the mean rAUDPC values of three replications in each experiment were used for detecting QTL.

The broad-sense heritability (h^2) based on the mean rAUDPC value in the F_5 population was estimated as 0.85, suggesting that the QTL controlling HTAP resistance had very large effects on the stripe rust phenotype.

Construction of linkage groups and identification of stripe rust response-linked molecular markers

Thirteen SSR markers, selected from the BSA, based on their association with IT and rAUDPC data and repeatability, were used to genotype 150 F_5 RILs; 12 were specific to chromosome 4B and one was specific to chromosome 5B.

All nulli-tetrasomic lines of Chinese Spring and the ditelosomic lines for chromosomes 4B and 5B were used to determine the chromosomal locations of resistance genes. Two closely linked SSR markers, *Xgwm192* and *Xgwm251*, amplified fragments of 212 and 117 bp in CS, respectively; and all tested aneuploid lines, except N4BT4D and Dt4BS, produced fragments of these markers. Similarly, SSR marker *Xgwm335* showed the 234 bp band in CS and all tested aneuploid lines, except N5BT5A and Dt5BL. These results indicated that the SSR markers were located on chromosomes 4BL and 5BS.

To develop high resolution maps, 42 SNP markers specific to chromosome 4BL and 23 specific to 5BS were screened in BSA tests, and 23 of the 4BL markers and 15 of the 5BS markers were polymorphic and associated with the resistance loci. Using these markers, together with the 12 SSR markers on 4BL and 1 SSR marker on 5BS, two linkage groups spanning 78.4 and 29.3 cM, respectively, were constructed (Fig. 2a, b).

Using the IT data of the F_5 RILs and the JoinMap program, a resistance locus was mapped at 3.3 cM from SSR marker *Xgwm251* and 2.0 cM from *Xgwm192* on 4BL (Fig. 2a). The order of the markers in both sides of the gene is: *IWA1923*–1.2 cM–*Xgwm251*–3.3 cM–*Yr62*–2.0 cM–*Xgwm192*–0.6 cM–*Xgwm495*. No resistance gene was mapped to 5BS using the IT data.

Six SSR markers, *Xgwm251*, *Xgwm192*, *Xgwm495*, *Xwmc349*, *Xcfd39* and *Xgwm375*, were further used in testing a set of five CS deletion lines of 4BL to assign the 4BL resistance locus to a chromosomal region. One of the flanking markers, *Xgwm192*, was assigned to chromosome bin 0.58–0.68 and the other, *Xgwm251*, to 0.68–0.70 (Fig. 2a). Other four markers were located to the same chromosomal arm, but in same or different bins, which were consistent with the deletion map of Sourdille et al. (2004). Thus, the 4BL resistance gene was located to the region between 0.58 and 0.70, within 12 % of the chromosomal arm.

Table 2 Segregation of stripe rust response in F_2 and F_5 progeny of AvS/PI 192252 under natural infection in field in Washington during the 2011 and 2012 growing seasons

Environment (year, location)	Generation	No. of F_2 plants or F_5 lines			Expected ratio	χ^2	*P*
		Res.	Seg.	Sus.			
2012, Pullman	F_2	36		134	1:3	1.33	0.25
2011, Pullman	F_5	68	14	68	7:2:7	1.38	0.50
2011, Mount Vernon	F_5	67	14	69	7:2:7	1.41	0.50
2012, Pullman	F_5	70	13	67	7:2:7	2.08	0.35
2012, Mount Vernon	F_5	71	13	66	7:2:7	2.21	0.33

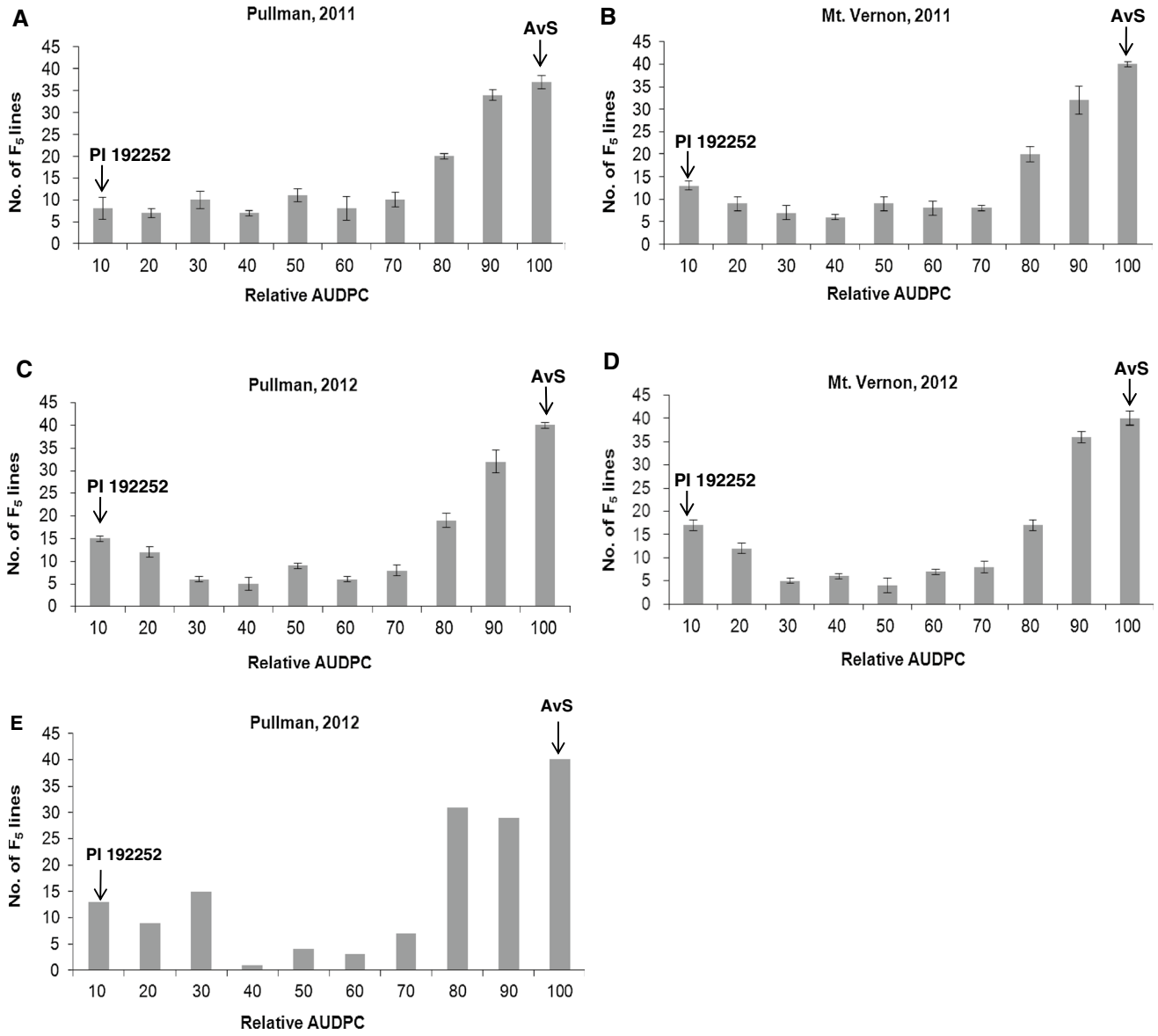

Fig. 1 Frequency distribution of relative area under the disease progress curve (rAUDPC) for 150 F_5 recombinant inbred lines (RILs) for high-temperature adult-plant resistance to stripe rust at Pullman and Mount Vernon in Washington State in 2011 and 2012. The rAUDPC data of F_5 RILs are shown in **a** (Pullman, 2011), **b** (Mount Vernon, 2011) in Pullman, 2012 (**c**) and **d** (Mount Vernon, 2012); and of F_6 RILs in **e** (Pullman, 2012)

Table 3 Variance components of relative area under the disease progress curve for the F_5 recombinant inbred line population derived from AvS/PI 192252

Source of variation	*df*	Mean square	*F*	*P*
RILs	149	1,689.69	35.15	<0.0001
Replication	2	33.27	0.69	0.50
Experiment	3	148.04	1.69	0.20
Experiment × RILs	447	49.58	0.36	1.00
Error	1,350	138.27		

QTL mapping of resistance genes

When the F_5 rAUDPC data from the various experiments were used in QTL mapping by CIM method of WinQTLCart V2.5, QTL for resistance were detected on chromosomes 4BL and 5BS (Fig. 3). The QTL on 4BL had LOD values from 11.8 to 20.5 and explained 40–60 % of the phenotypic variation of rAUDPC. The 4BL QTL region was flanked by SSR marker *Xgwm495* and SNP marker *IWA2171* covering a 7-cM interval containing four other

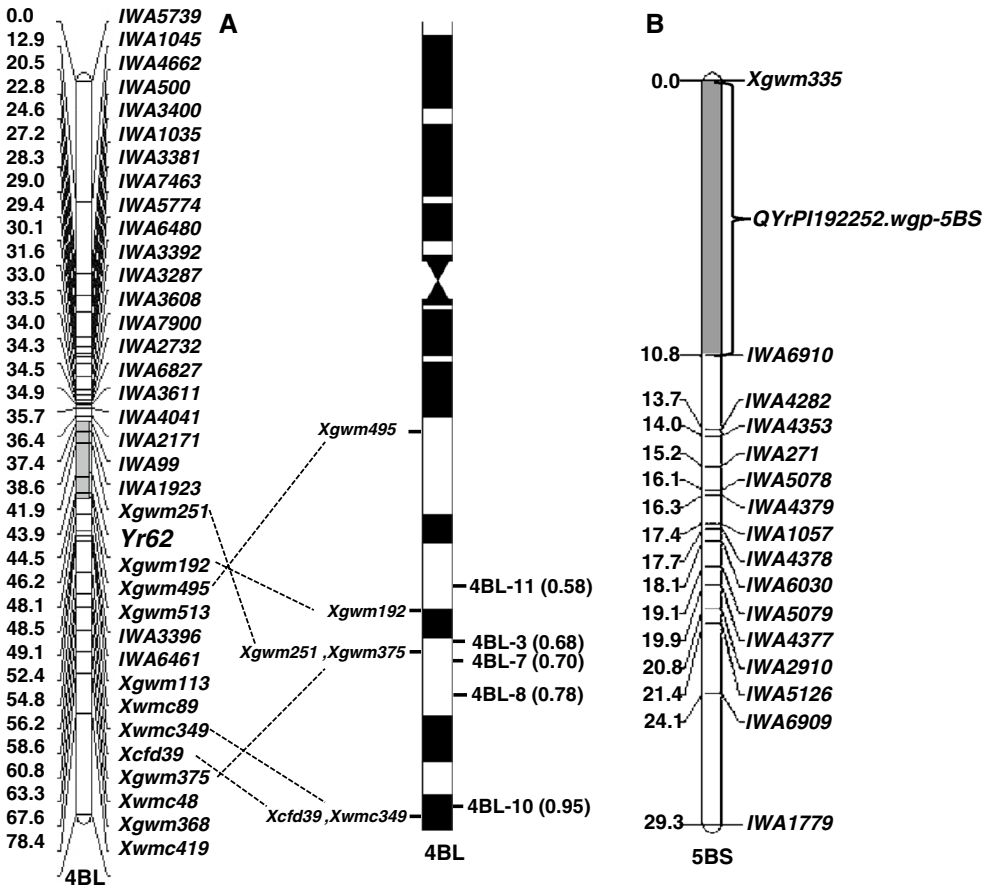

Fig. 2 Linkage maps of *Yr62* and *QYrPI192252.wgp-5BS* on the long arm of chromosome 4B (**a**) and the short arm of chromosome 5B (**b**). The QTL region for *Yr62* indicated in *gray* was determined by the QTL mapping using the rAUDPC data, and the gene was mapped qualitatively between SSR markers *Xgwm251* and *Xgwm192* using infection type data (**a**, *left*). The deletion bin location of *Yr62* in 4BL indicated by SSR markers (**a**, *right*) is based on the deletion map at Wheat Genetic and Genomic Center of Kansas State University (http://www.k-state.edu/wgrc/Germplasm/Deletions/grp4.html)

markers (*IWA99*, *IWA1923*, *Xgwm251* and *Xgwm192*) (Fig. 3a), and the peak of the QTL region corresponded to the gene mapped qualitatively using the JoinMap program (Fig. 2a). The second QTL, *QYrPI192252.wgp-5BS*, was mapped between *Xgwm335* and *IWA6910* covering a 10.8 cM region on chromosome 5BS (Figs. 2b, 3b), and explained 22.1–27.4 % of the phenotypic variation in rAUDPC. Both QTL were contributed by PI 192252 and explained 74.2 % of the phenotypic variation for rAUDPC.

Phenotypic values of QTL represented by flanking markers

Based on the genotypes of markers flanking the QTL, the F_5 RILs were classified into four genotypic groups to examine the genetic effects of the QTL on rAUDPC. Lines with marker alleles flanking the 4BL and/or 5BS resistance QTL had rAUDPC values significantly ($P < 0.05$) lower than the susceptible parent or the RILs with the alternative marker alleles, but similar to the resistant parent (Fig. 4). The mean rAUDPC value (11.5 %) for RILs with the 4BL QTL was much lower than the 27.2 % mean value of lines with the 5BS QTL. Although lines with both QTL had lower rAUDPC values than those with only the 4BL QTL, the differences were not statistically significant ($P = 0.32$).

Similar results were obtained for the QTL categories using the IT data. Lines with marker alleles flanking both the 4BL and 5BS QTL, e.g., F_6-83, F_6-143 and F_6-218, had IT values (1–3) similar to the resistant parent (Table 1). Lines with only the 4BL QTL marker alleles (e.g., F_6-93, F_6-171 and F_6-185) were resistant (IT 2 and 3), similar to the resistant parent and lines with both the 4BL and 5BS QTL. Lines with only the 5BS QTL (e.g., F_6-96, F_6-97 and F_6-176) had IT 5. Thus, the 4BL QTL provide a higher level HTAP resistance than the 5BS QTL.

Relationship of the 4BL QTL with *Yr50*

As the major-effect gene earlier named *Yr50* is located on the long arm of chromosome 4B (Liu et al. 2013), a cross was made between PI 192252 and CH223 to conduct an allelism test for determining if the two genes are at different loci. Responses of space-planted F_2 plants, together with the parents, were evaluated for stripe rust response in a naturally infected field near Pullman, WA in 2013. The

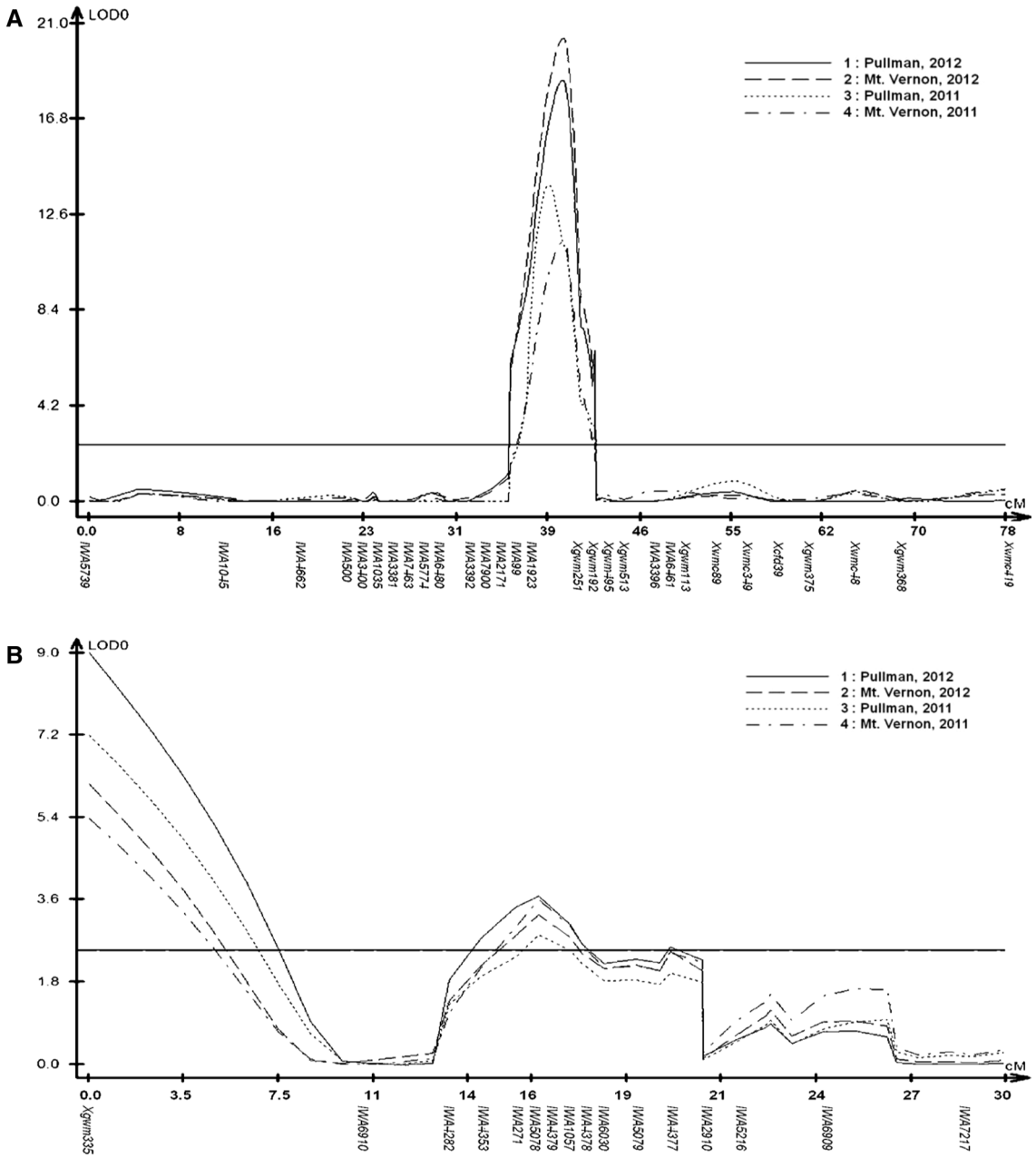

Fig. 3 QTL for high-temperature adult-plant resistance to stripe rust were mapped with composite interval mapping on chromosomes 4BL (**a**) and 5BS (**b**), respectively, using rAUDPC data from four experiments (Pullman 2011, Mt. Vernon 2011, Pullman 2012 and Mt. Vernon 2012)

response of CH233 plants was IT 0 or 1 and PI 192252 was IT 1 or 2. Of 261 F_2 plants, 247 had resistant reactions (IT 0–3), 10 had moderately resistant to moderately susceptible reactions (IT 4–7) and four plants were susceptible (IT 8). Assuming single major genes in both parents, two linked genes were at different loci, and the genetic distance was estimated as 27.1 ± 8.6 cM. Because the major-effect gene/QTL was mapped to 4BL and was different from *Yr50*, it was designated *Yr62*.

Polymorphisms of molecular markers flanking *Yr62* in wheat genotypes

Because *Yr62* provides a high level of HTAP resistance and is more useful than the 5BS QTL, SSR markers *Xgwm192* and *Xgwm251* flanking *Yr62* were tested on 70 spring and 70 winter wheat cultivars and breeding lines developed in the US PNW (Supplementary Table 1). All of the 140 wheat entries had alleles of different sizes from those of

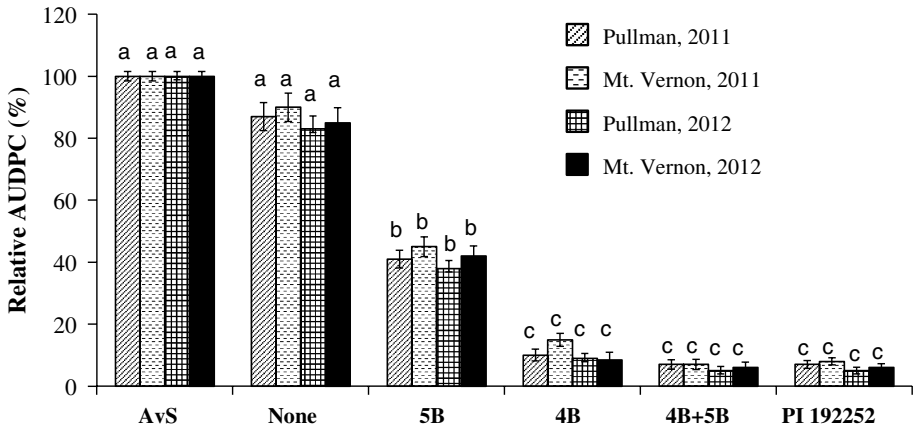

Fig. 4 Comparison of mean rAUDPC among the parents and F_5 recombinant inbred lines derived from AvS/PI 192252 with both-, single- and no QTL

the marker alleles in PI 192252. Thus, the flanking markers were 100 % polymorphic across the tested wheat cultivars and breeding lines, indicating that markers can be used for marker-assisted selection of *Yr62*.

Discussion

According to Johnson (1981), durable resistance is the resistance that remains effective when deployed over an extensive area and time, in an environment favorable for the disease. Due to its non-race specific nature, HTAP resistance has been durable in many wheat cultivars grown in the US PNW, where stripe rust can cause significant damage every year (Qayoum and Line 1985; Line 2002; Chen 2005, 2007, 2013; Wan and Chen 2012). In the present study, we confirmed that spring wheat germplasm PI 192252 possesses HTAP resistance that by its nature may be durable.

Using the rAUDPC data, we identified a QTL making a large contribution to stripe rust resistance on chromosome 4BL and a second QTL conferring a small but significant effect on chromosome 5BS. Both QTL were detected over consecutive years 2011 and 2012, when severe epidemics caused more than 90 % yield losses in susceptible genotypes in 2011 and more than 50 % yield losses in 2012 (http://striperust.wsu.edu). In these epidemics, PI 192252 and its resistant progeny lines remained highly resistant, similar to the responses of PI 192252 in all our germplasm screening nurseries since 2004. When the IT data were classified as resistant (IT 1–4) and susceptible (5–9) groups, only one gene was mapped between SSR markers *Xgwm251* and *Xgwm192* on chromosome 4BL using the JoinMap program. The QTL mapping using the IT data also mapped the gene in the same chromosomal region (data not shown). The location of the gene corresponded the peak of the major-effect QTL region identified using the QTL mapping approach with the rAUDPC data. These results indicated that the major-effect QTL is inherited as a single gene.

Liu et al. (2013) recently mapped *Yr50*, putatively derived from *Thinopyrum intermedium*, to chromosome 4BL in Chinese winter wheat variety CH223. Our tests confirmed that *Yr50* in CH223 confers all-stage resistance in seedling tests conducted in the greenhouse (data not shown). CH223 was highly resistant (IT 1–2) to all eight US *Pst* races tested, including PST-116 and PST-127, which were used to characterize HTAP resistance in PI 192252. CH223 was also highly resistant (IT 1–2) at the adult-plant stage when evaluated in field tests in 2013. *Yr50* was flanked by SSR markers *Xbarc1096* and *Xwmc47* with genetic distances of 8.0 and 7.2 cM, respectively (Liu et al. 2013). However, none of the markers in the *Yr50* linkage group were polymorphic in our mapping population. The exact chromosomal location of *Yr50* is not clear. Based on the linkage to *Xbarc1090*, *Yr50* appears to be located in deletion bin C-4BL5-0.71 (Liu et al. 2013), but it is also possibly in the telomere of 4BL based on their genome in situ hybridization (GISH) result of CH223 and our result of assigning the closest marker *Xwmc47* of *Yr50* to bin 4BL10-0.95-1.0 (data not shown). In contrast, *Yr62* is clearly located in the chromosomal region 0.58–0.70 (Fig. 2a). This suggests that *Yr62* is proximal to *Yr50*. If *Yr50* is truly from *Th. intermedium*, the location of the translocated chromatin in 4BL needs to be determined in a future study. To determine if *Yr62* and *Yr50* were at different loci, we made a cross between PI 192252 and CH223. The F_2 population showed clear segregation in the field indicating that the gene in PI 192252 and *Yr50* are different, but likely linked genes. Because the 4BL gene in PI 192252 had a different origin, confers a different type of resistance and at a different locus to *Yr50*, we designated it as *Yr62*. PI 660060 (AvS/PI192252 F_4-103), previously developed from the AvS/PI 192252 cross, was registered as a single gene reference line for *Yr62* (Wang et al. 2012).

The locus *QPst.jic-4B* in Alcedo was flanked by AFLP marker *S24M37_130* and SSR marker *Xcfd39* on chromosome 4BL with SSR *Xgwm692* as the closest marker to the QTL (Jagger et al. 2011). In our study, *Xgwm692* was not polymorphic between PI 192252 and AvS, and *Xcfd39* was 16.5 cM distal to *Yr62*. In addition to these major-effect genes, several small-effect QTL on chromosome 4BL were reported. Suenaga et al. (2003) detected a QTL for stripe rust severity in cultivar Oligoculm; William et al. (2006) identified a QTL in Avocet; Melichar et al. (2008) identified a QTL in Guardian; Lu et al. (2009) found QTL in Libellula and Strampelli; Zwart et al. (2010) reported a QTL in Janz; and Agenbag et al. (2012) detected a QTL in Palmiet. In contrast to *Yr62*, these small-effect QTL explained only 4–17 % of the phenotypic variance and some of them were not detected across years.

QYr.PI192252-5BS had a small effect on HTAP resistance as seen by IT scores and DS values that were significantly lower than the susceptible parent. Resistance to stripe rust in chromosome 5BS has been found in other studies. *Yr47*, which confers seedling resistance, was located on chromosome 5BS in common wheat landraces AUS28183 and AUS28187; and was flanked by *Xgwm234* and *Xcfb309* (Bansal et al. 2011). Markers *Xgwm234* and *Xcfb309* were not polymorphic between PI 192252 and AvS in the present study. Hao et al. (2011) reported a small effect QTL, *QYr.uga-5B* on 5BS, for stripe rust resistance in US wheat cultivar AGS2000. The QTL explained only 5 % of the phenotypic variation and was detected in only one of the three field tests. Based on the origin, effect, and linked markers, *QYr.PI192252-5BS* is unlikely to be the same as the QTL in AGS2000.

Although not significant, an additive effect of the two QTL in PI 192252 was seen for rAUDPC in lines with both *QYr.PI192252-5BS* and *Yr62* when compared with lines having only *Yr62* (Fig. 4). Statistical analyses of the rAUDPC scores of the RILs within each QTL group indicated significant interactions between *Yr62* and *QYrPI192252.wgp-5BS*, indicating that the addition of the 5BS QTL to *Yr62* may enhance resistance.

To determine the usefulness of the flanking markers for introgressing *Yr62* into various wheat backgrounds, we assayed them on 140 wheat cultivars and breeding lines. The alleles of these markers in PI 192252 were different from those in the test wheat panel, indicating their potential value for incorporating *Yr62* into adapted wheat cultivars and elite lines. F_6 lines with *Yr62* or both *Yr62* and the 5BS QTL (Table 1) selected for agronomic traits superior to those in PI 192252, as well as PI 660060 (Wang et al. 2012), should be more useful for breeding programs targeting stripe rust resistance.

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Conflict of interest None.

Ethical standards All experiments were conducted in Pullman, Washington, the USA, and part of data analyses and manuscript development were done at Northwest A&F University. All authors have contributed to the study and approved the version for submission. The manuscript has not been submitted to any other journal.

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