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ORIGINAL PAPER

Identifying QTL for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'

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Abstract Over time, many single, all-stage resistance genes to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in wheat (*Triticum aestivum* L.) are circumvented by race changes in the pathogen. In contrast, high-temperature, adult-plant resistance (HTAP), which only is expressed during the adult-plant stage and when air temperatures are warm, provides durable protection against stripe rust. Our objective was to identify major quantitative trait loci (QTL) for HTAP resistance to stripe rust in the spring wheat cultivar 'Louise'. The mapping population consisted of 188 recombinant inbred lines (RIL) from a Louise (resistant) by 'Penawawa' (susceptible) cross. $F_{5:6}$ lines were evaluated for stripe rust reaction under natural infection in replicated field trials at five locations in the US Pacific Northwest in 2007 and 2008. Infection type (IT) and disease severity were recorded for each RIL 2–4 times per location. In all environments, Penawawa, the susceptible parent, was rated with an IT ranging from 6 to 8 at all growth stages evaluated. In contrast, Louise, the resistant parent, was rated with an IT of 2 or 3 across growth stages. Distribution of IT values was bimodal, indicating a single major gene was affecting the trait. The parents and RIL population were evaluated with 295 polymorphic simple sequence repeat and one single nucleotide polymorphism markers. One major QTL, designated *QYrlo.wpg-2BS*, associated with HTAP resistance in Louise, was detected on chromosome 2BS (LOD scores ranging from 5.5 to 62.3 across locations and years) within a 16.9 cM region flanked by *Xwmc474* and *Xgwm148*. SSR markers associated with *QYrlo.wpg-2BS* are currently being used in marker-based forward breeding strategies to transfer the target region into adapted germplasm to improve the durability of resistance in resulting cultivars.

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Introduction

Wheat (*Triticum* spp.) is the primary food grain directly consumed by humans worldwide, and more acreage is dedicated to the commercial production of wheat than any other crop in the world (Briggle and Curtis 1987). As a result of its broad adaptation, numerous pathogens plague the crop. Stripe rust (also known as yellow rust), caused by the obligate biotroph fungus *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*Pst*), is among the most destructive of these pathogens, causing substantial losses to wheat production annually on a global scale (Stubbs 1985; Chen et al. 2002; Chen 2005). Genetic resistance is the most economical and environmentally friendly way to control stripe rust; however, adequate levels of resistance are not currently available in adapted germplasm in all wheat producing regions of the world.

Genetic resistance to stripe rust is either race-specific or non-race-specific. All-stage resistance is typically race-specific and qualitatively inherited, closely adhering to the gene-for-gene interaction model of Flor (1971). This type of resistance can be detected during the seedling stage of the plant, is frequently expressed at high levels, and continues throughout the life cycle of the plant. Due to their specific nature and the strong selection pressure placed on the pathogen, all-stage resistance is frequently overcome by race changes in the pathogen population (Chen and Line 1995a, b). In contrast, high-temperature, adult-plant (HTAP) resistance is non-race-specific, often durable, and generally quantitatively inherited (Qayoum and Line 1985; Line and Chen 1995; Line 2002; Chen 2005). This type of resistance is manifested during later stages of plant development, when temperatures are typically above 21°C (Chen and Line 1995a, b).

More than 30 different genes for all-stage resistance have been identified and mapped to date, allowing wide deployment into germplasm through marker-assisted selection (reviewed in Chen 2005). In contrast, relatively few reports identifying and mapping the more durable HTAP resistance to stripe rust have been published in wheat (Chen and Line 1995a, b; Chen 2005; Uauy et al. 2005; Chicaiza et al. 2006; Lin and Chen 2007, 2008; Santra et al. 2008). Multiple quantitative trait loci (QTL) are typically associated with HTAP resistance; however, in all previous reports, a few major QTL accounted for a significant portion of the variation (Uauy et al. 2005; Lin and Chen 2007).

In the US, stripe rust epidemics are most prevalent in California and the Pacific Northwest (PNW) where mild winters, followed by cool, wet springs, and dry summers are typical (Chen 2005). Many adapted winter wheat cultivars have combinations of HTAP and all-stage resistances to stripe rust. Relatively few spring wheat cultivars carry HTAP resistance, leaving the crop vulnerable to the disease. Louise (Kidwell et al. 2006), a soft white spring wheat cultivar released in 2006 has high levels of HTAP resistance. The objectives of this research were to: (1) identify major QTL for HTAP resistance in Louise through genetic linkage analysis; and (2) identify DNA markers linked to the resistance genes for use in marker-assisted selection.

Materials and methods

Plant materials

One hundred and eighty-eight $F_{5:6}$ recombinant inbred lines (RIL) were developed through single seed descent from F_2 seed harvested from a single F_1 plant of Louise (resistant parent; PI 634865) × Penawawa (susceptible parent; PI 495916). Louise, a soft white spring wheat, has moderate grain volume, low grain protein concentration, and high grain yield potential in non-irrigated production scenarios (Kidwell et al. 2006). Penawawa, also a soft white spring wheat, has moderate grain volume, moderate grain protein concentration, and low grain yield potential without irrigation (unpublished data). Both cultivars are adapted to growing conditions in the PNW.

Field experiments

The 188 RIL along with parental controls were evaluated for stripe rust resistance in the field in four locations in 2007. These locations included Pullman, WA (at Spillman Agronomy Farm and Whitlow Agronomy Farm); Mt. Vernon, WA (Northwestern Washington Research and Extension Center); and Genesee, ID (Kambitsch Farm). In 2008, trials were planted at similar locations except the Genesee, ID site was changed to Moscow, ID (Parker Farm). Five grams of seed were planted in rows of 0.5 m length spaced 30 cm apart in a randomized complete block design, with three replications per location. All trials in all locations were evaluated under natural infections of stripe rust. Spreader rows of the susceptible cultivar 'Lemhi' (CI 011415) were planted every 30 rows to increase the uniformity of stripe rust inoculum across the trial. Lemhi contains the all-stage resistance gene *Yr21*, which is ineffective against almost all races identified in the US. In 2007, planting dates were April 19th, 24th, 26th, and 24th for Mt. Vernon, Spillman Farm, Genesee, and Whitlow Farm, respectively. In 2008, planting dates were April 22nd, 21st, May 2nd, and April 17th for Mt. Vernon, Spillman Farm, Moscow, and Whitlow Farm, respectively. Before planting, the field was cultivated and fertilized with nitrogen (formulated as urea) at a rate of 101 kg ha^{-1}. Stripe rust symptoms were rated as infection type (IT) based on a 0–9 scale (Line and Qayoum 1992) and disease severity (DS) as a percentage of plants (0–100) in the row that were infected. Disease ratings were first taken when all plots of the susceptible parent Penawawa had a disease severity of 20% and continued until initiation of senescence (Feekes 11.2; Feekes 1941). Data were collected every 3–7 days depending on disease progression and location.

Greenhouse experiment

Parental lines, as well as the 188 RIL, were planted in a randomized complete block design with three replicates. To determine HTAP resistance levels, three seeds of each line were planted in 3 l pots using #1 Sunshine Mix (Sun Gro Horticulture, Bellevue, WA) and fertilized with

250 mg nitrogen (formulated as ammonium nitrate) per pot over a 1 month period. Plants were grown under a 16 h photoperiod, with daytime temperatures ranging from 21 to 24°C and nighttime temperatures ranging from 15 to 18°C until heading (Feekes 10.2). Light intensity was 400 μmol at bench level. At initiation of heading (Feekes 10.2), plants were placed in a dew chamber (Percival Scientific, Inc., Perry, IA), inoculated with fresh urediniospores of *P. striiformis* f. sp. *tritici* race PST-100, the most predominant race throughout the US since 2003, in a mixture of 1 part spores and 20 parts talc, and incubated for 24 h at 100% humidity in the dark at 10°C. After 24 h incubation in the dew chamber, plants were returned to the greenhouse chamber at a diurnal temperature cycle of 15°C at night and 28°C at day with a daily 16 h photoperiod. The IT data was collected 18–20 days after inoculation as described previously. To determine seedling resistance levels, seeds were planted in 96 well trays, replicated three times. Seedlings were inoculated at the 3–5 leaf stage as described above. After 24 h incubation in the dew chamber, trays were placed in a growth room set at a diurnal temperature cycle of 4°C at night and 20°C at day with a daily 16 h photoperiod (Chen and Line 1992). Disease ratings for seedlings were evaluated 18–20 days after inoculation as previously described.

Statistical analysis for disease ratings

Area under the disease progress curve (AUDPC) was calculated for each RIL and the parental lines using the IT and DS data collected according to the formula: $\text{AUDPC} = \sum_i [(x_i + x_{i+1})/2]t_i$, where x_i is the severity value on date i, t_i the time in days between dates i and $i + 1$ (Chen and Line 1995a). Relative AUDPC (rAUDPC) was calculated for each line as a percentage of the most susceptible AUDPC value in each of the experiments. The statistical analysis of rAUDPC was performed within each environment using the statistical package SAS v. 9.1 (SAS Institute, Raleigh, NC). Genotype and replication * genotype effects were tested using the Proc GLM procedure. Broad-sense heritability (h^2) was calculated for all locations using the formula: $h^2 = \text{Var}(G)/\text{Var}(P)$ (where Var(G) is the variance of the genotypic effect and Var(P) is the variance of the phenotypic effect) using SAS code provided by Holland et al. (2003). The mean rAUDPC values for each line within each environment were used in QTL mapping.

Molecular marker analysis

Fresh leaf tissue of three individuals from each $F_{5:6}$ RIL or parent was collected at the five leaf stage, and used to extract genomic DNA using the CTAB method as described by Anderson et al. (1992). Sequences of available SSR markers along with their previously determined chromosomal locations were obtained from Graingenes (http://wheat.pw.usda.gov/). SSR marker analyses were conducted using the PCR conditions described by Röder et al. (1998) except that primers were synthesized to include the M13-tail (Oetting et al. 1995). The 10 μl reaction mixture consisted of 50 ng of template DNA, 1.0 μl Mg-free 10× PCR buffer, 0.5 units of Taq DNA polymerase, 1.5 mM of $MgCl_2$ (Promega, Madison, WI, USA), 200 μM each of dCTP, dGTP, dTTP, and dATP (Fermentas, Glen Burnie, MD) and 0.25 μM of each primer pair synthesized by MWG-Biotech (High Point, NC, USA). Appropriate fluorophores for either the Global IR^2 analysis system (LiCor Biosciences, Lincoln, NE, USA) or the ABI 3130xl (Applied Biosystems, Foster City, CA) fragment detection systems were included in the PCR mix. Amplification conditions were an initial 5 min denaturation at 94°C, followed by 41 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 50–65°C (depending on primers), and a 1 min extension at 72°C. The final extension step was 10 min at 72°C.

Identification and sequences of SNP markers were obtained from Dr. Shiaoman Chao at the USDA-ARS Biosciences Research Laboratory, Fargo, ND. SNP marker analyses were conducted according to PCR conditions provided by Dr. Shiaoman Chao (personal communication). Pre-amplification of DNA was conducted with the forward and reverse primer corresponding to each SNP using the PCR conditions described above. After pre-amplification, SNP detection was completed following protocols outlined using the AcycloPrime II Combination SNP Detection Kit (PerkinElmer, Boston, MA, USA).

Linkage map construction and QTL analysis

Segregation of marker loci was tested for goodness-of-fit to the expected 1:1 ratio using the χ^2 test. Linkage maps were constructed using Mapmaker V3.0 (Lander et al. 1987). Linkage groups were established using the "group" command with a recombination value of 0.5 and a constant LOD score of 3.0. Three-point linkage analyses were performed using the "compare" command to determine the most likely order of markers with the shortest genetic distance within each group. For large linkage groups, a framework order was established using the above procedure and additional markers were mapped using the "try" command and verified using the "ripple" command. The Kosambi map function was applied to calculate the genetic distances in centiMorgans (cM) between the ordered markers (Kosambi 1944). Each linkage group was assigned to a wheat chromosome based on previously published wheat genome maps available at Graingenes.

Single marker analysis using one-way ANOVA with a comparison-wise probability level of $P < 0.01$ was used to identify markers with significant effects on HTAP resistance. The QTL detection was performed using composite interval mapping (CIM) (Zeng 1993, 1994) with the software WinQTLCart V2.5 (Basten et al. 1997) and was targeted toward linkage groups previously identified through the one-way ANOVAs. In order to detect significant QTL, a critical LOD threshold value of 3.0 was used. Percentage of phenotypic variation (R^2) explained by the whole model was determined using multiple interval mapping (MIM).

QTL validation

One F_5 breeding population was used to validate the expression of the Louise HTAP QTL (Pumphrey et al. 2007). The F_5 population was a cross between Louise and Nick, a cultivar developed by Westbred, LLC, that is rated as susceptible to prevalent PNW races of stripe rust. Six separate F_4 headrows from this cross were growing in non-replicated F_5 yield plots at the Spillman Agronomy Farm in Pullman, WA. Tissue was harvested from 12 plants selected at random from each plot for marker analysis. DNA was extracted from each plant as described above. IT and DS values were recorded for each plant from each plot as described earlier.

Five F_2 breeding populations were used to identify the utility of markers flanking the HTAP resistance QTL for marker-assisted selection. The five populations consisted of crosses between Louise and various other cultivars and experimental breeding lines, all of which were susceptible to stripe rust. The pedigrees of the five populations were: (1) Alpowa (PI 566596)/CItr14689//Louise; (2) Louise/CItr14734//Louise; (3) Louise/CItr14689//Louise; (4) Louise//Blanca Grande (PI 631481)//Otis (PI 634866)*2/P985RE1-16 (Purdue University); and (5) Louise//WA7919 (Washington State University)/WA7921 (Washington State University)//IDO000586 (PI 632713). Sixteen plants from each population were selected at random and allowed to self-pollinate in the field, and resulting F_3 seed was collected. The 16 plants per population were genotyped for the presence or absence of *Xwmc474* and *Xgwm148*. Plants were then phenotyped in the greenhouse using the methods previously described for HTAP resistance screening. Race PST-100 was used to inoculate the flag leaf of plants at Feekes 10.2, and IT and DS readings were collected as described previously.

To identify polymorphisms between the HTAP flanking markers in other germplasm, a panel of 45 lines was evaluated including currently grown spring (30) and winter (15) wheat cultivars, stripe rust differentials, and advanced breeding lines. Level of polymorphism in current germplasm determines uniqueness and current deployment of the QTL, as well as usefulness of markers for marker-assisted selection (MAS). DNA was extracted as previously described when plants reached the five leaf stage. Allele band size was determined using a 50–350 bp ladder (LiCor Biosciences, Lincoln, NE, USA).

Results

Stripe rust evaluation

Significant genotype effects ($P < 0.0001$) for each environment were observed for both IT and DS values (Supplementary Table 1). In 2007, race PST-114 was the most predominant race in all locations. In 2008, race PST-54 was the most predominant race in Mt. Vernon, whereas race PST-114 was still the most predominant races in the other locations. In all environments, Penawawa, the susceptible parent, was rated with an IT ranging from 6 to 8 at all growth stages evaluated. In contrast, Louise, the resistant parent, was rated with an IT of 2 or 3 across growth stages. The rAUDPC values for IT ranged from 1 to 100 for the entire population across locations. The frequency distribution of mean rAUDPC values for IT of the 188 RIL obtained from each environment was bimodal (Fig. 1), indicating a single major gene confers HTAP resistance in this population. Under greenhouse conditions, Louise and Penawawa were both susceptible to race PST-100 of *P. striiformis* f. sp. *tritici* at the seedling stage indicating the absence of effective all-stage resistance genes to this race.

Louise, the resistant parent, was rated with a DS of 1–15%, whereas Penawawa, the susceptible parent, was rated with a DS ranging from 20 to 70%, at all growth stages evaluated. The rAUDPC values for DS for the entire RIL population ranged from 1 to 100 across locations. The frequency distribution of rAUDPC values for DS was continuous and skewed toward resistance (Fig. 1). Although the IT and DS values were correlated ($R^2 = 0.48$, $P < 0.0001$), the bimodal nature of the IT data was not evident in the DS data suggesting that DS is impacted by more genes than IT.

Linkage map construction

Over 1000 SSR and 250 SNP markers were tested for polymorphism between Louise and Penawawa. Of the markers tested, 322 SSR and 40 SNP markers were polymorphic between the parental lines. Of the 322 SSR markers, 295 produced distinguishable polymorphism among RIL and were used for genetic linkage map construction. Due to difficulties with the SNP protocol and lack of distinguishable polymorphism, only one of the 40 SNP markers was used for linkage map construction. Linkage analysis of the 296 segregating markers at LOD 3.0 established 29 linkage groups

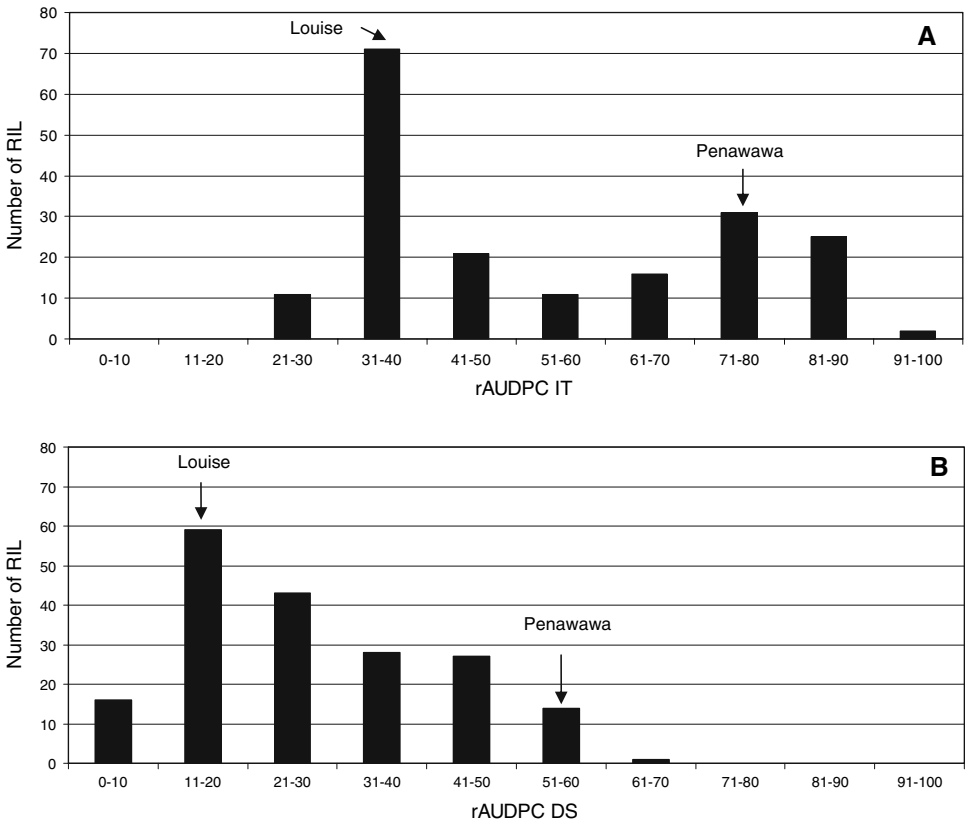

Fig. 1 Distribution of relative area under the disease progress curve (rAUDPC) values from the Louise by Penawawa mapping population averaged across locations and years for: **a** infection type (Average IT rAUDPC value for Louise and Penawawa are 33 and 85, respectively, with an LSD value of 4.0) (IT) values and **b** disease severity (Average DS rAUDPC value for Louise and Penawawa are 13 and 52, respectively, with an LSD value of 3.5) (DS) values

consisting of 1 SNP and 242 SSR markers and the remaining 53 SSR markers were unlinked. The LOD value was lowered to 1.8, which reduced the number of linkage groups to 25. These 25 linkage groups were assigned to 21 wheat chromosomes, which covered a total genetic distance of 2,181.8 cM. Chromosomes 1A, 3A, 4A, and 5B each were comprised of two linkage groups. The shortest chromosome was 4D (27.6 cM) and the longest chromosome was 5D (237.1 cM). The seven A-genome chromosomes ranged from 47.9 cM (4A) to 118.0 cM (5A) and covered a total of 587.7 cM. The seven B-genome chromosomes ranged from 73.4 cM (7B) to 137.8 cM (2B) and covered a total of 662.4 cM. The seven D-genome chromosomes ranged from 27.6 cM (4D) to 237.1 cM (5D) and covered a total of 931.7 cM. The average distances between markers on the A-, B-, and D-genome were 8.6 cM, 7.8 cM, and 10.4 cM, respectively. The genome-wide average distance between markers was 9.0 cM.

HTAP resistance QTL identification

Based on single marker analysis, six markers were significantly associated with the rAUDPC values derived from the IT and DS data in each of the eight environments. One major QTL was identified on chromosome 2B based on composite interval mapping and was significantly associated with the HTAP resistance in Louise for both IT and DS readings. This QTL was consistently detected using rAUDPC values from each of the eight environments (Fig. 2) and was designated *QYrlo.wpg-2BS* (Louise designated as lo). This QTL mapped to the short arm of chromosome 2B and explained 19–68% of the phenotypic variation for IT and 11–57% of the phenotypic variation for DS, depending upon year and location (Table 1). *QYrlo.wpg-2BS* is located within a 27 cM region spanned by the six markers identified using single marker analysis. The most likely location of *QYrlo.wpg-2BS* is within a 16.9 cM region flanked by SSR markers *Xwmc474* and *Xgwm148* (Fig. 2). Resistance was always associated with the allele derived from Louise; however, variation in significance levels associated with *QYrlo.wpg-2BS* and stripe rust resistance was detected across years and locations. LOD values ranged from 7.5 to 62.3 for IT and 5.5 to 30.0 using DS depending on environment (Table 1). Heritability values ranged from 0.50 to 0.89 for IT and 0.22 to 0.84 for DS across environments.

Flanking markers for HTAP resistance QTL

Molecular markers *Xwmc474* and *Xgwm148* flank *QYrlo.wpg-2BS*. The peak of the QTL mapped between these two markers, 2.4 cM from *Xwmc474* and 14.5 cM

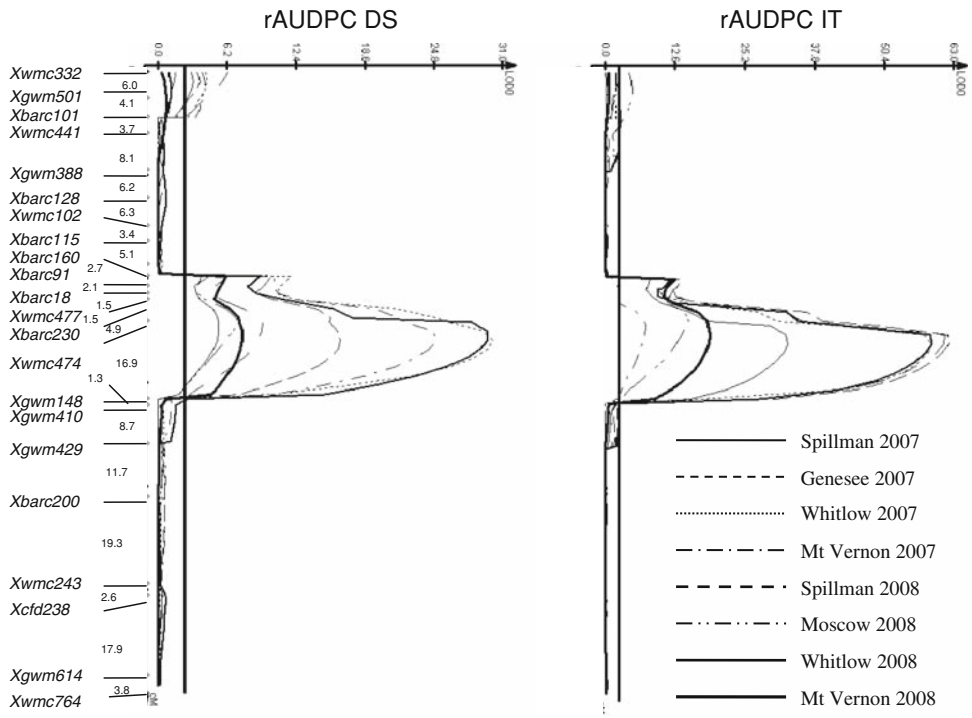

Fig. 2 High-temperature adult-plant resistance quantitative trait locus on chromosome 2B identified by composite interval mapping. Positions (cM) of the molecular markers along the chromosome are on the vertical axis

Table 1 Quantitative trait locus and heritability (h^2) analysis for relative area under the disease progress curve (rAUDPC) values of both infection type (IT) and disease severity (DS) for five locations across 2 years

Location	Year	IT rAUDPC values				DS rAUDPC values			
		LOD	R^2	h^2	Confidence interval (h^2)	LOD	R^2	h^2	Confidence interval (h^2)
Spillman Farm[a]	2007	34.2	0.62	0.50	0.46–0.54	25.0	0.39	0.22	0.17–0.27
	2008	61.5	0.67	0.76	0.73–0.79	9.4	0.18	0.31	0.25–0.37
Whitlow Farm	2007	59.9	0.68	0.89	0.88–0.90	30.0	0.47	0.84	0.82–0.86
	2008	59.3	0.58	0.85	0.83–0.87	29.6	0.57	0.74	0.71–0.77
Genesee, ID	2007	62.3	0.68	0.92	0.91–0.93	16.4	0.35	0.67	0.64–0.70
Moscow, ID	2008	15.0	0.24	0.58	0.54–0.62	7.2	0.14	–[b]	–
Mount Vernon, WA	2007	7.5	0.19	0.82	0.80–0.84	7.6	0.16	0.81	0.79–0.83
	2008	19.0	0.38	0.65	0.62–0.68	5.5	0.11	0.69	0.66–0.72
Range		7.5–62.3	0.19–0.68	0.50–0.92		5.5–30.0	0.11–0.57	0.22–0.84	

LOD values are those given for chromosome 2B

[a] Spillman Farm and Whitlow Farm are located in Pullman, WA

[b] Could not be calculated due to missing values

from *Xgwm148*. *Xwmc474* is a co-dominant marker (annealing temperature 51°C) represented by a 154 bp band in Louise and a 150 bp band in Penawawa. *Xgwm148* also is a co-dominant marker (annealing temperature 60°C), represented by a 178 bp band in Louise and a 160 bp band in Penawawa. Both marker loci were verified to be on chromosome 2B based on analysis using Chinese Spring nulli-tetrasomic lines (data not shown).

QTL validation

The selected F_5 populations from the Louise by Nick cross were used to validate the expression of *QYrlo.wpg-2BS* in advanced breeding lines. Nick is susceptible to stripe rust and does not contain the Louise alleles for either *Xwmc474* or *Xgwm148*. Marker analysis confirmed the presence of both the Louise alleles for *Xwmc474* and

Xgwm148 in all selected plants of all six F_5 populations evaluated. Under field conditions, the 12 selected lines from five of the six populations had IT values of 2–3 and DS values of 5–15%. Selected plants from the other population had IT values ranging from 2 to 5 and DS values from 20 to 30%. Even though the IT and DS values are slightly higher in this population, a resistant reaction was present on the plants selected for marker analysis.

Evaluation of F_2 breeding populations were used to validate the usefulness of *Xwmc474* and *Xgwm148* in MAS. In all five breeding populations tested, lines homozygous for the presence of the Louise allele for both markers resulted in a resistant phenotype, whereas lines homozygous for the absence of the Louise alleles demonstrated a susceptible phenotype. In addition, one population contained lines that were homozygous for one but not both markers. The presence of the Louise allele for *Xwmc474* and the absence of *Xgwm148* resulted in a resistant phenotype. The absence of the Louise allele for *Xwmc474* and the presence of *Xgwm148* demonstrated a susceptible phenotype. Based on the map distances between these markers and the peak of the QTL, *Xwmc474* appears to be more diagnostic in determining the presence of this QTL.

A total of 45 wheat cultivars were tested for polymorphism in comparison to the flanking markers. Based on marker phenotypes, *QYrlo.wpg-2BS* has not been widely deployed in current cultivars (Table 2). *Xwmc474*, the closest flanking marker for *QYrlo.wpg-2BS*, detected differences from Louise in 87% of the genotypes tested, whereas *Xgwm148* detected differences in only 69% of the tested genotypes. Both the Louise alleles for *Xwmc474* and *Xgwm148* were present in three of the 20 spring lines but none of the winter lines tested (Table 2). The presence of these alleles in Wakanz, WA8089, and WA8090 were expected since Wakanz is a parent of Louise and WA8089, and WA8090 are progeny from a Louise cross. These three lines are confirmed to have HTAP (using the rating system previously described) based on multiple year and field testing locations under natural inoculation (Supplementary Table 2). Two other cultivars, Kelse (Kidwell et al. 2009) and Lee, were positive for the Louise allele for *Xwmc474* but not for *Xgwm148*. Kelse is a hard red spring line confirmed to have HTAP based on 4 years of field testing and 1 year of greenhouse testing, although it is unknown what the sources of this resistance is (Kidwell et al. 2009). Lee, another spring line, carries three all-stage resistance genes (*Yr7*, *Yr22*, and *Yr23*) and no HTAP resistance genes (Chen 2005). Excluding the three lines mentioned above, nine spring and one winter line contained the Louise allele for *Xgwm148*.

Table 2 Polymorphism in selected 30 spring and 15 winter wheat genotypes for *Xwmc474* and *Xgwm148*, the flanking markers for the major quantitative trait locus for high-temperature adult-plant resistance to stripe rust in Louise

Genotype	Growth habit	Presence of Louise HTAP resistance	*Xwmc474*	*Xgwm148*
Wawawai (PI 574598)	Spring	−	150	178
Wakanz (PI 506352)	Spring	+	154	178
Louise (PI 634865)	Spring	+	154	178
Penawawa (PI 495916)	Spring	−	150	156
WA8089 (WSU[a])	Spring	+	154	178
WA8090 (WSU)	Spring	+	154	178
Alpowa (PI 566596)	Spring	−	150	178
WA8039 (WSU)	Spring	−	150	178
WA7985 (WSU)	Spring	−	150	156
WA8058 (WSU)	Spring	−	152	176
WA8043 (WSU)	Spring	−	150	178
WA8045 (WSU)	Spring	−	152	161
Otis (PI 634866)	Spring	−	150	175
Hollis (PI 632857)	Spring	−	150	178
Kelse (PI 653842)	Spring	−	154	175
Tara2002 (PI 617073)	Spring	−	150	178
Hank (PI 613581)	Spring	−	150	178
Macon (PI 617072)	Spring	−	150	156
Whit (PI 653841)	Spring	−	150	156
Zak (PI 607839)	Spring	−	150	175
Farnum (PI 638535)	Winter	−	150	175
Stephens (GSTR 11901)	Winter	−	150	178
Bauermeister (PI 634717)	Winter	−	150	175
Centurk (CI 015075)	Winter	−	152	141
Norstar (CI 017735)	Winter	−	152	174
Karl (PI 527480)	Winter	−	152	148
Fielder (CI 017268)	Spring	−	146	141
Moro (CI 013740)	Winter	−	156	170
Chinese166 (CI 011765)	Winter	−	152	170
Yr9 (YR 000009)	Spring	−	150	147
Hyak (PI 511674)	Winter	−	150	170
Druchamp (CI 013723)	Winter	−	152	147
Produra (CI 017460)	Spring	−	150	143
Express (PI 573003)	Spring	−	152	178
Lemhi (CI 011415)	Spring	−	159	178
Clement (PI 518799)	Winter	−	152	173
Compair (PI 325842	Spring	−	144	152
Yr8 (YR 000008)	Spring	−	150	171
Tres (CI 017917)	Winter	−	156	170
Paha (CI 014485)	Winter	−	156	170
Tyee (CI 017773)	Winter	−	150	170
Riebesel (YR 000004)	Spring	−	152	170
Lee (CI 012488)	Spring	−	154	147
HeinesVII (PI 201195)	Winter	−	152	162

Table 2 continued

Genotype	Growth habit	Presence of Louise HTAP resistance	*Xwmc474*	*Xgwm148*
Avocet (PI 464644)	Spring	–	151	174
Polymorphism			87%	69%

[a] Washington State University Breeding Line

Discussion

Of the 32 all-stage resistance genes officially identified to date, only two (*Yr5* and *Yr15*) are still resistant to all known races in the US. It is predicted that, with time, these two genes also will be circumvented by new races. HTAP resistance genes have proven durable over many years (Chen 2005). The concern with HTAP resistance is that the mechanism is only active during the adult-plant growth stage, leaving seedlings susceptible to infection unless adequate all-stage resistance genes also are present (Chen et al. 1998, 2002). Cool summer temperatures also can extend the length of susceptible reactions on wheat leaves exacerbating the risk. The ability to deploy both all-stage and HTAP resistance genes into a single cultivar would provide a more effective and durable source of stripe rust resistance. Since all-stage resistance masks the phenotypic expression of HTAP resistance, molecular markers provide the only efficient way of pyramiding all-stage resistance with HTAP (Lin and Chen 2007).

Currently, molecular markers have been associated with several genes and QTL for HTAP resistance (Chen 2005; Lin and Chen 2007, 2008; Guo et al. 2008). The gene *Yr18* is located on chromosome 7DS (Suenaga et al. 2003), *Yr36* on chromosome 6BS (Uauy et al. 2005), *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* also on chromosome 6BS (Santra et al. 2008), three QTL (*QYrex.wgp-6AS*, *QYrex.wgp-3BL*, and *QYrex.wgp-1BL*) on chromosomes 6AS, 3BL, and 1BL, respectively (Lin and Chen 2008), and *Yr39* on chromosome 7BL (Lin and Chen 2007). Other genes or QTL, reported to confer adult-plant and presumably HTAP resistance, have been located on chromosomes other than 2B. These genes or QTL include *Yr16* on 2D (Worland and Law 1986); *Yr29* on 1BL (McIntosh et al. 2001); *Yr30* on 3BS (McIntosh et al. 2001); *Yrns-B1* on 3BS (Börner et al. 2000); *YrCK* on 2DS (Navabi et al. 2005); *QPst.jic-1B*, *QPst.jic-2D*, and *QPst.jic-4B* on 1BL, 2D and 4B, respectively (Melichar et al. 2008); *QYrtm.pau-2A* and *QYrtb.pau-5A* on 2A and 5A, respectively (Chhuneja et al. 2008); and *QYr.inra-2AL* on 2AL, *QYr.inra-2DS* on 2DS, and *QYr.inra-5BL.1* and *QYr.inra-5BL.2* on 5BL (Mallard et al. 2005).

Several genes for stripe rust resistance (*Yr5*, *Yr7*, *Yr27*, *Yr31*, and *Yr41*) have been reported on chromosome 2BS, all of which confer all-stage resistance (Macer 1966; McDonald et al. 2004; McIntosh et al. 2006; Lou et al. 2008). *YrSp* (McIntosh et al. 1995), *YrSte* (Chen et al. 1998) and *YrV23* (Chen et al. 1998) have been reported on chromosome 2B, but their locations need to be identified to particular arms, and all of which confer race-specific all-stage resistance. Mallard et al. (2005) identified two QTL, in a French winter wheat cultivar 'Camp Remy' (PI 452119), on both long and short arms of chromosome 2B. *QYr.inra-2BL* confers all-stage resistance and *QYr.inra-2BS* confers adult-plant resistance. Interestingly, *QYr.inra-2BL* is linked to *Xgwm148* and *QYr.inra-2BS* is 45 cM away from *QYr.inra-2BL* and further away from the marker. Because of the significant difference in genetic distance between *Xgwm148* and *QYr.inra-2BS* and between *QYrlo.wgp-2BS* and the markers identified in the present study, these two QTL are unlikely to represent the same locus.

Rosewarne et al. (2008) detected a QTL on 2BS for slow rusting resistance to stripe rust in 'Attila' (PI 35159) spring wheat. The relationship between *QYrlo.wgp-2BS* and the Attila 2BS slow rusting QTL could not be determined because different types of markers were used in these studies. However, *QYrlo.wgp-2BS* was consistently detected across locations and years in the present study, whereas the Attila 2BS QTL was not consistently detected in their study and was considered a minor QTL to the *Lr46/Yr29* complex on chromosome 1BL (Rosewarne et al. 2006, 2008). Guo et al. (2008) identified two QTL for HTAP resistance on chromosome 2BS from the winter wheat 'Luke' (CItr 14586). Although both *QYrlu.cau-2BS2* and *QYrlo.wpg-2BS* are linked to *Xgwm148*, linkage map comparisons localized *QYrlu.cau-2BS2* more distal to the centromere than *QYrlo.wpg-2BS*. Thus, it appears that the Louise QTL for HTAP resistance found on chromosome 2BS is likely novel to currently known HTAP resistance genes.

Many QTL identified for HTAP resistance are not consistently detected in all years and locations and are identified as multiple QTL (Santra et al. 2008; Lin and Chen 2007, 2008; Rosewarne et al. 2008). The ability to detect *QYrlo.wpg-2BS* consistently over locations and years will have significant impact on breeding programs focused on stripe rust resistance. *QYrlo.wpg-2BS* has consistently demonstrated high level of expression in all germplasm it has been incorporated into (Supplementary Table 2), and it accounts for large portion of the phenotypic variation, indicating that environmental variation will have minimal effects on expression levels. Most interesting is the observation that *QYrlo.wpg-2BS* acts as a single gene, similar to the adult plant resistance gene *Yr18* and the slow-rusting gene *Yr36* (Spielmeyer et al. 2008; Fu et al. 2009). This simplifies the process of incorporating *QYrlo.wpg-2BS* into other germplasm through MAS. Although it is not known why this form of HTAP resistance

manifests as a single gene, it has continued to demonstrate non-race specific resistance to stripe rust.

Since circumvention of all stage resistance is a significant problem in wheat, along with the lack of diversity for HTAP resistance in current cultivars, combining different sources of resistance within a single genotype will enhance the durability of resistance in cultivars, and will prolong the life (i.e., utility) of individual resistance genes (Smith et al. 2002). Of the germplasm tested, 64% had neither Louise allele and 94% had only one Louise allele, suggesting that these markers will be useful in MAS strategies designed to pyramid *QYrlo.wpg-2BS* with other stripe rust resistance genes, due to the availability of useful polymorphism in targeted cultivars. Based on validation studies it appears *QYrlo.wpg-2BS* will continue to provide high levels of expression when incorporated into other genetic backgrounds, thus broadening deployment into new adapted cultivars. The uniqueness of *QYrlo.wpg-2BS* compared to other HTAP resistance genes, and the opportunity to combine *QYrlo.wpg-2BS* with all-stage resistance genes, creates the opportunity for wheat breeders worldwide to enhance the genetic diversity and durability of stripe rust resistance in adapted cultivars.

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