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ORIGINAL PAPER

Identification and mapping QTL for high-temperature adult-plant resistance to stripe rust in winter wheat (*Triticum aestivum* L.) cultivar 'Stephens'

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Abstract High-temperature adult-plant (HTAP) resistance from the winter wheat (*Triticum aestivum*) cultivar 'Stephens' has protected wheat crops from stripe rust caused by *Puccinia striiformis* f. sp. *tritici* for 30 years. The objectives of this study were to identify quantitative trait loci (QTL) for HTAP resistance in Stephens through genetic linkage analysis and identify DNA markers linked to the QTL for use in marker-assisted breeding. Mapping populations consisted of 101 recombinant inbred lines (RILs) through single-seed descent from 'Stephens' (resistant) × 'Michigan Amber' (susceptible). F_5, F_6 and F_7 RILs were evaluated for stripe rust resistance at Pullman, WA in 1996, 1997 and 1998, respectively, whereas F_8 RILs were evaluated at Mt Vernon, WA, USA in 2005. The 101 F_8 RILs were evaluated with 250 resistance gene analog polymorphism (RGAP), 245 simple sequence repeat (SSR) and 1 sequence tagged site (STS) markers for genetic linkage map construction. Two QTL, which explained 48–61% of the total phenotypic variation of the HTAP resistance in Stephens, were identified. *QYrst.wgp-6BS.1* was within a 3.9-cM region flanked by *Xbarc101* and *Xbarc136*. *QYrst.wgp-6BS.2* was mapped in a 17.5-cM region flanked by *Xgwm132* and *Xgdm113*. Both two QTL were physically mapped to the short arm of chromosome 6B, but in different bins. Validation and polymorphism tests of the flanking markers in 43 wheat genotypes indicated that the molecular markers associated with these QTL should be useful in marker-assisted breeding programs to efficiently incorporate HTAP resistance into new wheat cultivars.

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Introduction

Stripe rust (also known as yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is an important disease of wheat (*Triticum aestivum* L.) worldwide (Stubbs 1985; Chen 2005). Since the late 1950s, stripe rust has been one of the most destructive diseases of wheat in the western United States, and in recent years, it also has become a major threat in the Great Plains and southeastern states (Chen 2007). The use of resistant cultivars is the most effective, economical, and environmentally friendly means to control stripe rust. Two major types of resistance to stripe rust, all-stage resistance (also known as overall or seedling resistance) and high-temperature adult-plant (HTAP) resistance have been used in wheat cultivars to control stripe rust (Chen 2005). All-stage resistance, which is often conferred by single genes, can be detected in the seedling stage and remains effective at all stages of plant growth. The resistance phenotype is expressed at a very

high level. This type of resistance is often race-specific, qualitatively inherited, and therefore, not durable. In contrast, High-temperature adult-plant (HTAP) resistance is expressed at later stages of plant growth when the weather becomes warm. This type of resistance is effective when average night temperatures are above 10°C and day temperatures are between 25 and 30°C (Qayoum and Line 1985; Line and Chen 1995). Generally, HTAP resistance is non-race specific, often quantitatively inherited, and, therefore, is durable (Milus and Line 1986a, b). The level of resistance conferred by HTAP resistance is usually incomplete and is affected by plant growth stage, temperature, humidity, and the inoculum load.

More than 70 stripe rust resistance genes with official or provisional symbols have been reported (McIntosh et al. 1998; Chen 2005). Most of these genes confer race-specific all-stage resistance. DNA markers, including simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and resistance gene analog polymorphism (RGAP) markers, have been identified that are linked to all-stage resistance genes *Yr1*, *Yr5*, *Yr7*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr26* and *Yr28* (reviewed by Chen 2005). Compared to the large number of reports on DNA markers associated with all-stage resistance genes, there are relatively few reports identifying genes or QTL and markers for durable resistance to stripe rust in wheat. Multiple QTL in different resistant wheat varieties were reported for durable resistance (Börner et al. 2000; Bariana et al. 2001; Suenaga et al. 2003; Mallard et al. 2005). Recently several genes for HTAP resistance have been identified in wheat, including *Yr36* on chromosome 6BS in chromosomal substitution durum wheat line 'LDN (DIC6B)' from *T. turgidum* var. *dicoccoides* (Uauy et al. 2005), *Yr39* on 7BL in spring wheat cultivar 'Alpowa' (Lin and Chen 2007), three QTL on 1BL, 3BL and 6AS in spring wheat cultivar 'Express' (Chen and Lin 2007), and a QTL on 2D in the 'AVS/6*Yr8' spring wheat genotype (Chen and Zhao 2007).

Many wheat cultivars in the Pacific Northwest (PNW) of the United States have combinations of HTAP and all-stage resistances to stripe rust (Line 2002; Chen 2005). 'Stephens', a soft white winter wheat cultivar released in 1978 (Kronstad et al. 1978), has both HTAP and all-stage resistance genes (Chen and Line 1995a, b). Stripe rust races with virulence to the all-stage resistance in Stephens were first detected in 1977 and have been prevalent in the PNW since that time (Line and Qayoum 1992; Chen 2007). Stephens is still resistant to stripe rust in the field, and it has been grown extensively across the PNW without significant damage by this pathogen for 30 years. Stripe rust resistance inherited from Stephens is effective over a wide geographical area including the PNW and Great Plains regions of the United States, Central Asia and the Caucasus (K. Garland Campbell, unpublished data), and southern Russia (X. M. Chen 2006, personal observations). Chen and Line (1995a, b) used biometric analyses to determine the genetic basis of durable resistance in Stephens in crosses with Michigan Amber, a susceptible cultivar without all-stage or HTAP resistance. Two to three loci were estimated to be responsible for HTAP resistance in Stephens (Chen and Line 1995b). However, chromosomal locations of these QTL were not determined and markers for the QTL were not identified. The objectives of this study were to map QTL for HTAP resistance in Stephens through genetic linkage analysis and identify molecular markers linked to the resistance genes for use in marker-assisted breeding.

Materials and methods

Plant materials

One hundred and one F_5, F_6, F_7 and F_8 recombinant inbred lines (RILs) were all developed from 101 F_2 plants of a single F_1 plant of Stephens (resistant parent) × Michigan Amber (susceptible parent) by single-seed descent (Chen and Line 1995a). Each of the F_3 line consisted of seeds from a single F_2 plant and each of the F_4 to F_8 lines consisted of seeds from a single plant of previous generation. Bulked seeds from each F_8 line were used for the field evaluation in 2005 and for genotyping with DNA markers.

Chinese Spring nulli-tetrasomic lines for the 21 wheat chromosomes and ditelosomic and deletion lines for wheat chromosome 6B, kindly provided by Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, KS, USA, were used to determine chromosomal locations of the markers linked to the QTL for HTAP resistance in Stephens.

To determine polymorphism at the marker loci, a total of 43 wheat genotypes, 23 winter and 20 spring accessions, were amplified with four SSR markers flanking two QTL for HTAP resistance in Stephens.

Field evaluation of adult-plants for stripe rust resistance and data analysis

F_5, F_6 and F_7 RILs and parents were evaluated in field nurseries at Pullman, WA in 1996, 1997 and 1998, respectively. The nurseries were inoculated with race PST-25 of *P. striiformis* f. sp. *tritici* that is virulent on seedlings of Stephens following the procedures described by Chen and Line (1995a). In 2005, F_8 RILs and parents were evaluated at Mt Vernon under natural infections of the stripe rust population, in which races with virulence on seedlings of Stephens were predominant (Chen, unpublished data). At each site, the experiment was conducted using a randomized

complete block design with three replications. About 5 g of seed for each of 101 RILs and the parents were planted in 1 m rows planted 20 cm apart in each replication. The nursery was surrounded by PS 279, a susceptible genotype used as a spreader. The cultural practices commonly used in wheat production in these regions for fertilization and weed control were used to manage the nurseries. Infection type (IT) based on the 0–9 scale (Line and Qayoum 1992) and disease severity (DS, percentage of infected leaf area) were visually recorded as average for each row at tillering/boot, heading-flowering, and dough growth stages, when the severity on PS 279 and Michigan Amber had reached approximately 30, 60 and 95%, respectively.

The area under the disease progress curve (AUDPC) was calculated for each of the RILs and the parents using all three sets of DS data according to the formula: 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). For easy comparison of RILs with the susceptible parent, a relative AUDPC (rAUDPC) value for each line was calculated as a percentage of the Michigan Amber AUDPC value. After we determined that there were no RIL X replication interactions using the SAS program (SAS Institute, Raleigh, NC, USA), rAUDPC values of the three replications were averaged for each RIL and the mean rAUDPC values from each year's trial were used in QTL mapping. Because the IT data recorded at the heading-flowering stage best represented the IT phenotypes of HTAP resistance in the fields, this data set was used for most of the IT analyses.

Molecular marker analysis

Leaf tissue of ten individual seedlings of each F_8 RIL or parent was used to extract genomic DNA using the CTAB method described by Anderson et al. (1992). The resistance gene analog polymorphism (RGAP) method described previously (Chen et al. 1998; Shi et al. 2001) was followed. Sequences and chromosomal locations of simple sequence repeat (SSR) markers were obtained from the Graingenes website (http://wheat.pw.usda.gov/) and their primers were synthesized by MWG-Biotech (High Point, NC, USA). Testing of SSR markers were conducted using the PCR conditions as described by Röder et al. (1998) with following modifications according to Oetting et al. (1995). An additional 19-bp sequence of M13, which has no sequence homology with eukaryotic genome, was added to each forward primer. The M13 tail, labeled with fluorescence label IRD-700 or IRD-800, was added to each PCR to the final concentration of 0.05 μM. Thus, amplified DNA bands were tagged with fluorescence labeled M13 tail for detection in LiCor 4300 DNA analysis system (Li-Cor Biosciences, Lincoln, NE, USA). The Global IR^2 analysis system and the GENESCAN software were used for SSR data collection. The sequence tag site (STS) marker analysis was done according to Distelfeld et al. (2004).

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 MacIntosh V2.0 (Lander et al. 1987). A two-point linkage analysis was conducted to determine maximum likelihood recombination frequency and the LOD score for each possible pair of loci. Linkage groups were established using the "group" command on the two-point data with a recombination value (θ) of 0.25 and constant LOD score of 5.0. Three-point linkage analyses were then conducted to determine the most likely order of loci within groups using the "compare" command for obtaining the group with the shortest genetic distance. For large linkage groups, a framework was established based on two-point linkage analysis and additional markers were mapped using the "try" command and linkage order was verified using the "ripple" command. The Kosambi mapping recombination function was used to determine distance in centimorgan (cM) between markers (Kosambi 1944). Each linkage group was assigned to a wheat chromosome based on SSR markers common in our linkage group and on previously published wheat genome map available at the Graingenes site (http://wheat.pw.usda.gov/).

The statistical analysis of traits was performed using the SAS statistics package (SAS Institute, Raleigh, NC, USA). Genotype and year/generation effects were tested using the analysis of variance (ANOVA) and PROC GLM procedure. A one-way ANOVA with a probability level of $P < 0.001$ was employed to identify markers with significant effects on resistance. The QTL detection was performed using interval mapping (IM) (Lander and Botstein 1989) and composite interval mapping (CIM) (Zeng 1993, 1994) with the WinQTLCart software version 2.5 (Basten et al. 1997). In order to detect significant QTL, a critical LOD threshold was established for rAUDPC and IT by conducting a test of 1,000 permutations. Six co-factors were taken into account and a window size of 10 cM around the test interval was chosen for CIM analysis. For each QTL, the position corresponding to the maximum LOD and the part of the phenotypic variation it explained was estimated. The percentage of phenotypic variation explained by the whole model (total R^2) was determined using multiple interval mapping (MIM).

In order to determine relative effect of each QTL on stripe rust resistance, the RILs were classified into different groups by substitution of the marker allele flanking the QTL. Two-tailed Student's t-test was used to determine if

means of rAUDPC and IT of each group were significantly different from that of other group.

Results

Stripe rust evaluation in the fields

The frequency distributions of infection type (Fig. 1) and mean rAUDPC values (Fig. 2) of the 101 RILs obtained from 4 years indicated that HTAP resistance in Stephens was quantitatively inherited. When the rAUDPC value for Michigan Amber was treated as 100% at each year, the values for Stephens were 4.4, 2.3, 2.9 and 14.8% at Pullman in 1996, 1997 and 1998 and at Mt Vernon in 2005, respectively. The susceptible parent Michigan Amber had IT 8 or 9 at heading-flowering stages, whereas the resistant parent Stephens had IT 2 or 3 in all tests. Significant genotype effects for each year (Table 1) and a significant year effect (data not shown) were found for the rAUDPC, but not for the IT data. Total phenotypic variations explained by the ANOVA model were 0.86, 0.69, 0.89 and 0.63 based on rAUDPC values in 1996, 1997, 1998 and 2005, respectively.

Linkage map construction

Of 496 segregating markers (250 RGAP, 245 SSR and 1 STS), 452 segregated in expected 1:1 ratio based on chi-squared analyses and 44 markers that did not fit the 1:1 ratio were discarded from analyses. Linkage analysis of the 452 markers at LOD of 5.0 established 46 linkage groups consisting of 425 markers and the remaining 27 markers were unlinked. All these 46 linkage groups were assigned to 21 wheat chromosomes, which covered a total genetic distance of 1830.5 cM. The shortest chromosome was 1A (20.3 cM) and longest chromosome was 7D (155.2 cM). The seven A-genome chromosomes ranged from 20.3 cM (1A) to 148.3 cM (5A) and covered 540.3 cM. The seven B-genome chromosomes ranged from 37.3 cM (2B) to 126.4 cM (1B) and covered 627.3 cM. The seven D-genome chromosomes ranged from 60 (4D) to 155.2 cM (7D) and covered 662.9 cM. Average distances between two markers in the A-, B- and D-genome were 7.4, 8.3 and

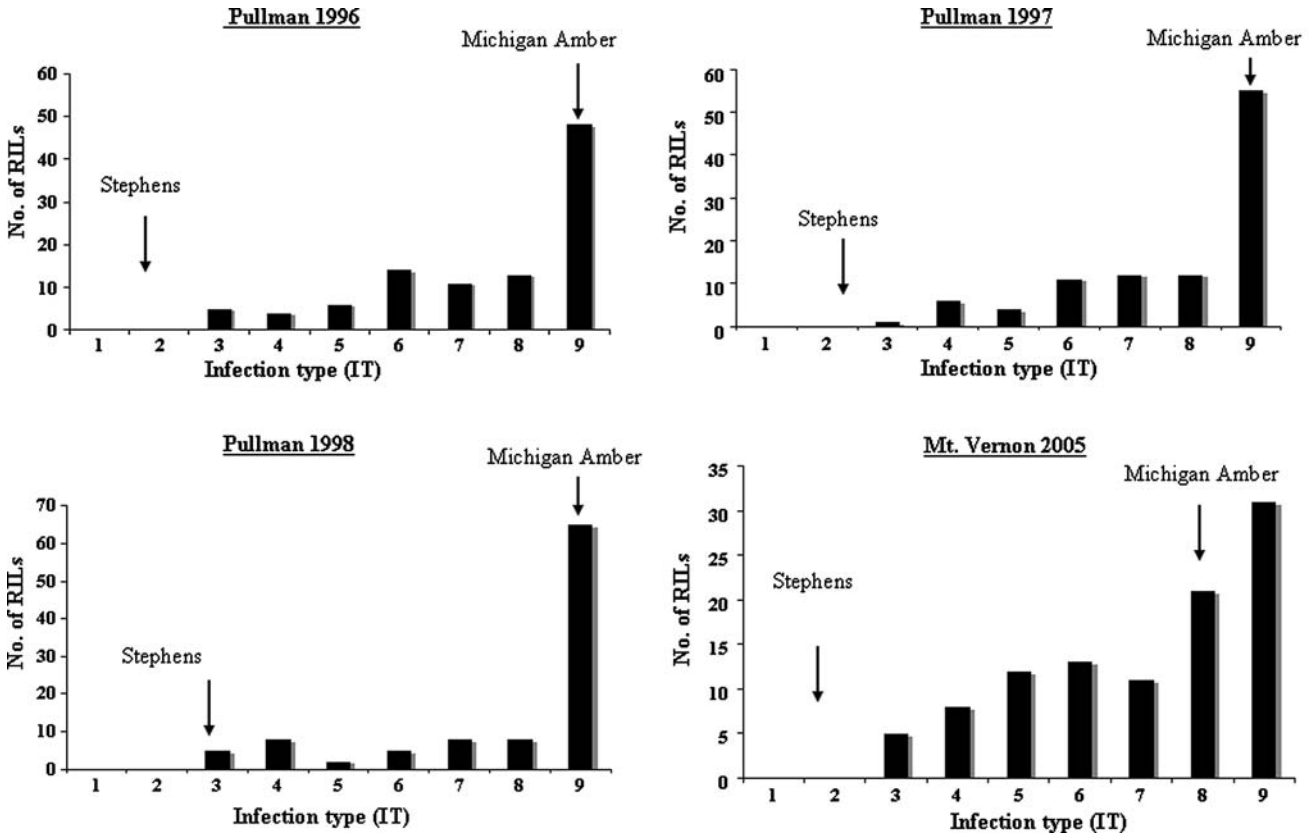

Fig. 1 Frequency distributions of infection type (IT) values (scored at heading-flowering stages) averaged over three replications of 101 recombinant inbred lines (RILs) derived from Stephens (resistant)/Michigan Amber (susceptible) in 1996, 1997 and 1998 at Pullman and in 2005 from Mt Vernon, WA, USA. *Arrows* indicate IT values for Stephens and Michigan Amber

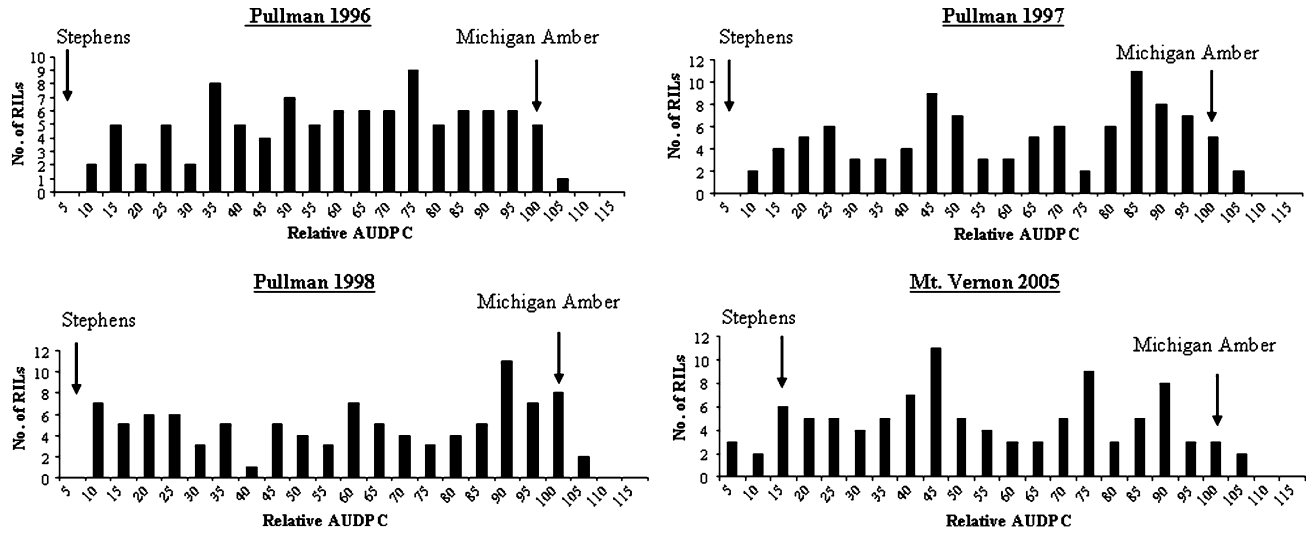

Fig. 2 Frequency distributions of relative area under the disease progress curve (rAUDPC) values averaged over three replications based on disease severity data of 101 recombinant inbred lines (RILs) derived from Stephens (resistant)/Michigan Amber (susceptible) in 1996, 1997 and 1998 at Pullman and at in 2005 Mt Vernon, WA, USA. *Arrows* indicate rAUDPC values for Stephens and Michigan Amber

Table 1 Analysis of variance of genotypes and replication effects on resistance to stripe rust based on relative AUDPC values obtained for each year-location among recombined inbred lines from the Stephens/Michigan Amber cross

Year, location Effect	*df*	MS	F	*P*	R^{2a}	Coefficient of variance
1996, Pullman					0.86	20.87
Genotype effect	100	2249.56	14.1	<0.0001		
Replication effect	2	180.74	1.03	0.32		
Error	200	215.98	1.03			
1997, Pullman					0.69	7.54
Genotype effect	100	2509.77	137.09	<0.0001		
Replication effect	2	36.69	2.00	0.14		
Error	197	365.35				
1998, Pullman					0.89	22.86
Genotype effect	100	2631.60	16.31	<0.0001		
Replication effect	2	410.33	2.54	0.08		
Error	197	161.40				
2005, Mt Vernon					0.63	27.59
Genotype effect	100	1340.84	3.46	<0.0001		
Replication effect	2	23.96	0.06	0.94		
Error	200	387.75				

[a] The coefficient of determination, R^2, value was obtained from the ANOVA analysis using the SAS program (SAS Institute, Raleigh, NC, USA), indicating the fitness of the data to the model

9.7 cM, respectively, and the genome-wide average distance between two markers was 8.47 cM.

Identification of QTL for HTAP resistance

Based on single marker analysis, 23 markers were significantly associated with the IT and rAUDPC data in each of the 4 years. Sixteen of the 23 significant markers were mapped on a single linkage group, which corresponds to the wheat chromosome 6B based on previously published map of bread wheat (Somers et al. 2004; Song et al. 2005). Remaining 7 of the 23 significant markers was mapped on chromosome 3B (2 markers), 5D (3 markers), 6A (1 marker), and 7B (1 marker). However, none of these putative QTL was detected above the minimum threshold LOD in interval mapping. Therefore, we did not consider these QTL significant.

Two QTL, which were significantly associated with HTAP resistance in Stephens, were identified on chromosome 6B based on composite interval mapping using both rAUDPC and IT data. The first QTL, *QYrst.wgp-6BS.1*, was consistently detected using rAUDPC and IT data of all 4 years, whereas the second QTL, *QYrst.wgp-6BS.2* was detected using stripe rust data of all years except 1996 (Fig. 3). *QYrst.wgp-6BS.1* explained a large proportion of the phenotypic variance (R^2 = 32–45%, depending on the year), whereas *QYrst.wgp-6BS.2* explained from 25% to 43% of the phenotypic variance depending on the year (Table 2). *QYrst.wgp-6BS.1* was located within a 3.9 cM region flanked by *Xbarc101* and *Xbarc136* (Fig. 3). *QYrst.wgp-6BS.2* was located within a 17.5 cM region spanned by the SSR markers *Xgwm132*, *Xgwm705*, *Xgwm508* and *Xgdm113* (Fig. 3). Resistance was always conferred by alleles from Stephens. Variation in the level of significance of associations of the two QTL indicated by LOD values and portion of the phenotypic variations (PVE) explained was detected among locations and across years. *QYrst.wgp-6BS.1* was detected at LOD values ranging from 3.89 to 6.83, and *QYrst.wgp-6BS.2* was detected at LOD values ranging from 1.4 to 8.31 depending on the trial (Table 2). The two QTL together explained 48 to 61% of the total variation in stripe rust resistance based on multiple interval mapping.

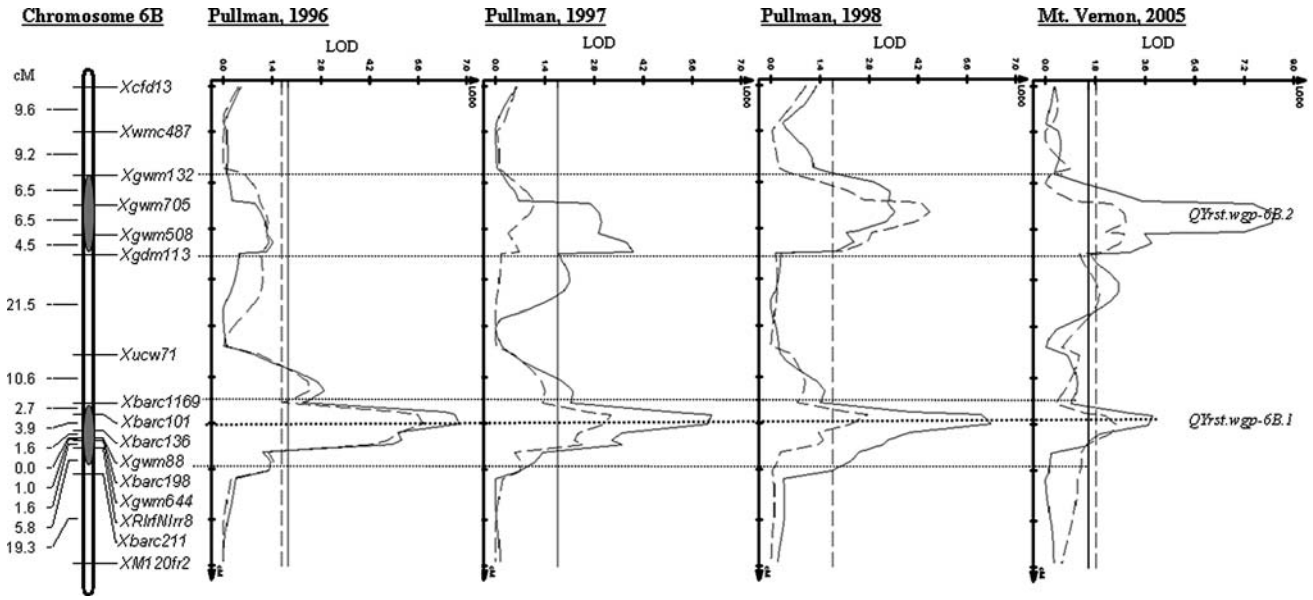

Fig. 3 Quantitative trait loci (QTL) for high-temperature adult-plant (*HTAP*) resistance to stripe rust on wheat chromosome 6B detected by composite interval mapping (*CIM*) based on stripe rust evaluation of the 101 recombinant inbred lines (*RILs*) of Stephens (resistant)/Michigan Amber (susceptible). The map of QTL for each year (1996, 1997, 1998 and 2005) is represented separately. The positions in cM and names of the molecular markers are shown on the chromosome 6B along the vertical axis. Critical LOD threshold scores for relative area under the disease progress curve (*rAUDPC*) and infection type (*IT*) are represented in each map. Positions of *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* are indicated by *gray area* on chromosome 6B and by *horizontal dotted line* on each map. Most likely position of *QYrst.wgp-6BS.1* is indicated by *bold dotted line*

Table 2 Summary of quantitative trait loci (QTL) for high-temperature adult-plant (HTAP) resistance to stripe rust in wheat detected by composite interval mapping (CIM): chromosome location and position, QTL effects in terms of phenotypic variance explained (R^2), maximum LOD scores based on individual relative area under disease progress curve (rAUDPC) data of the Stephens/Michigan Amber RIL population at 4-year locations

QTL	Chromosome arm	Position (cM)	rAUDPC							
			Pullman, 1996		Pullman, 1997		Pullman, 1998		Mt Vernon, 2005	
			R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD
QYrst.wgp.6BS.1[a]	6BS	68.4–85.0	0.45	6.83	0.43	6.18	0.32	6.30	0.36	3.89
QYrst.wgp.6BS.2[a]	6BS	18.8–36.6	0.25	1.40	0.35	3.92	0.30	3.54	0.43	8.31
Critical LOD[b]				1.90		1.80		1.80		1.60

[a] Two QTLs together explained 48–61% of the total variation based on multiple regression analyses

[b] Critical LOD threshold for each AUDPC data set was established by conducting a permutation test with 1,000 permutation

Physical mapping of the HTAP QTL in Stephens

The two QTL for HTAP resistance in Stephens were mapped physically to wheat chromosome 6BS based on the associated SSR markers using Chinese Spring nulli-tetrasomic and ditelosomic lines. Most of the SSR markers, which were associated with the HTAP QTL, were mapped to chromosome 6B in previously reported maps of the wheat genome (http://wheat.pw.usda.gov/). However, two SSR markers *Xbarc101* and *Xbarc136*, flanking *QYrst.wgp-6BS.1* were not mapped in previously reported maps. Using Chinese Spring nulli-tetrasomic lines, *Xbarc101* and *Xbarc136* were mapped to chromosome 6B. The flanking markers for both QTL were mapped using ditelosomic lines Dt 6BS and Dt 6BL of Chinese Spring chromosome 6B. Therefore, *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* were mapped to the short arm of wheat chromosome 6B based on both genetic and physical mapping. To distinguish the two QTL mapped on chromosome 6BS, Chinese Spring deletion lines 6BS-2.3 and 6BS 5 were amplified with the flanking markers for the two QTL. SSR markers *Xbarc101* and *Xbarc136* for *QYrst.wgp-6BS.1* were mapped to chromosomal bin 6BS 5-0.76 and *Xgwm132*, *Xgwm705*, and *Xgwm508* for *QYrst.wgp-6BS.2* on bin 6BS-Satellite (Fig. 4). The former was close to the centromere and the latter close to the telomere.

Molecular markers for HTAP resistance QTL

Molecular markers flanking *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* were identified. The peak of *QYrst.wgp-6B.1* was precisely mapped between SSR markers *Xbarc101* and *Xbarc136*. *Xbarc101* (175 bp) was a dominant marker present in Stephens but absent in Michigan Amber. *Xbarc136* is a co-dominant marker represented by a 275 bp band in Stephens and a 270 bp band in Michigan Amber. The precise location of *QYrst.wgp-6BS.2* could not be detected on chromosome 6BS. However, the SSR markers (*Xgwm132*, *Xgwm705*, *Xgwm508* and *Xgdm113*), which spanned the QTL region, could be used in MAS for *QYrst.wgp-6BS.2*.

In order to estimate effect of HTAP QTL markers in MAS the 101 RILs were classified into four groups by substitutions of *Xbarc101* and *Xbarc136* alleles for *QYrst.wgp-6BS.1* and of *Xgwm132*, *Xgwm705*, and *Xgwm508* alleles for *QYrst.wgp-6BS.2*. The groups were: (1) $RIL_{Q1Q1Q2Q2}$—41 RILs carrying the Stephens alleles of *QYrst.wgp-6BS.1 (Q1)* and *QYrst.wgp-6BS.2* (*Q2*); (2) $RIL_{Q1Q1q2q2}$—11 RILs carrying the Stephens allele of *QYrst.wgp-6BS.1* and the Michigan Amber allele of *QYrst.wgp-6BS.2* (*q2*); (3) $RIL_{q1q1Q2Q2}$—13 RILs carrying the Michigan Amber allele of *QYrst.wgp-6BS.1* (*q2*) and the Stephens allele of *QYrst.wgp-6BS.2*; and (4) $RIL_{q1q1q2q2}$—36 RILs carrying the Michigan Amber alleles of *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2*. Accumulated effect of both QTL on stripe rust resistance in the RILs was significantly higher than that of either QTL alone. RILs with both QTL had a mean rAUDPC value of 42.80% and a mean IT value of 5.20, significantly lower than the rAUDPC (80.81%) and IT (7.73) values of those without any QTL ($P < 0.00001$) for both rAUDPC and IT). Although, the effects of *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* were similar ($P = 0.27$ for rAUDPC and 0.34 for IT) but the effect of either QTL in RILs was significantly higher than the RILs without any QTL ($P = 0.0007$ for rAUDPC and 0.0038 for IT of *QYrst.wgp-6BS.1*; $P = 0.02$ for rAUDPC and 0.04 for IT of *QYrst.wgp-6BS.2*).

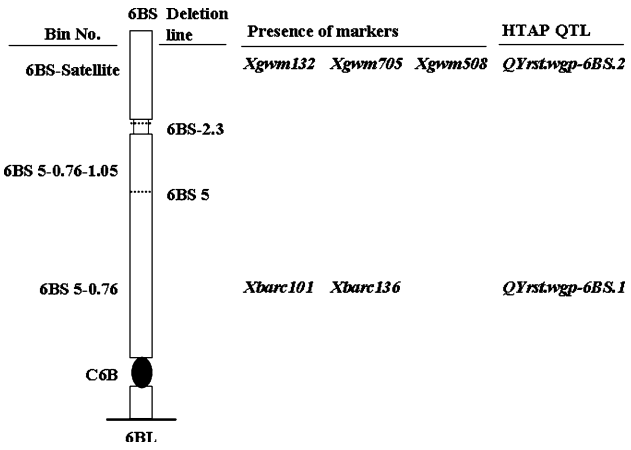

Fig. 4 Diagram showing presence of SSR markers for high-temperature adult-plant (HTAP) resistance QTL, *QYrst.wgp.6BS.1* and *QYrst.wgp.6BS.2*, in chromosomal bins 6BS 5-0.76 and 6BS-satellite using the markers to amplify genomic DNA of Chinese Spring deletion lines 6BS-2.3 and 6BS 5

Validation and polymorphism of molecular markers

In addition to Stephens and Michigan Amber, a total of 43 wheat genotypes (23 winter and 20 spring) were tested to validate and determine polymorphism of SSR markers *Xbarc101* and *Xbarc136* for *QYrst.wgp-6BS.1* and *Xgwm 132* and *Xgwm508* for *QYrst.wgp-6BS.2* (Supplementary Table 1).

Of eleven winter wheat genotypes that had Stephens or Stephens-related genotypes in their pedigree, two (WA7697 and KS01HW163-4) had the flanking markers for both two QTL; four (Lambert, Brundage 96, Madsen and Hill 81) had the two flanking markers for *QYrst.wgp-6BS.1* and only one (*Xgwm508*) of the flanking markers for *QYrst.wgp-6BS.2*; one (Chukar) had the two flanking markers for *QYrst.wgp-6BS.2* and one (*Xbarc136*) of the flanking markers for *QYrst.wgp-6BS.1*; one (Eltan) only had the flanking markers for *QYrst.wgp-6BS.2*; two (Druchamp and

Finch) had one of the markers for *QYrst.wgp-6BS.2*; and only one (Tubbs) did not have any of the flanking markers. These cultivars or genetic stocks have shown HTAP resistance to stripe rust (Chen unpublished data). The presence of the markers in the most of these HTAP resistant genotypes validated the association of these markers with the HTAP resistance QTL from Stephens. Among the remaining 32 genotypes that did not have Stephens or Stephens-related genotypes in their pedigree, 24 (75%), 22 (69%), 20 (63%) and 23 (72%) did not have the Stephens marker type for *Xbarc136*, *Xbarc101*, *Xgwm132* and *Xgwm508*, respectively. The relatively high polymorphism rates indicated that the markers should be useful in marker-assisted selection to incorporate the Stephens HTAP QTL into other wheat genotypes.

Discussion

The soft white winter wheat cultivar Stephens has remained resistant to stripe rust for 30 years since its release in 1978 under frequent epidemics caused by the pathogen populations virulent on its seedlings (Chen 2005). As a genotype for differentiating races of *P. striiformis* f. sp. *tritici* in the United States, Stephens is susceptible in the seedling stage to 54 of 126 races that have been identified thus far in the United States (Chen 2007; Chen unpublished data). Over the last 30 years since the first race, PST-15, virulent on seedlings of Stephens in 1977, Stephens-virulent races have been frequently predominant in the western United States, especially in the PNW. In recent years, when race PST-100 that is virulent on seedlings of Stephens was predominant in the PNW and throughout the United States, Stephens was still protected by HTAP resistance. It was estimated that Stephens had yield losses of 3% in 2004 and 4% in 2006 at Pullman, WA, USA and these yield losses were not significantly different between fungicide treated and non-treated plots (Chen, unpublished), whereas the yield losses in susceptible genotype PS 279 were 36% and 40% in the same trials during 2004 and 2006, respectively. Stephens is susceptible at seedling stage and it did not have significant stripe rust infection during 2005, when stripe rust was unusually severe. This suggests that the Stephens crops have been protected from stripe rust damage during last 30 years mainly because of its non-race specific HTAP resistance. Stephens was the most widely grown cultivar in Washington, Oregon and Idaho for many years in 1980s and 1990s. It is still the number one cultivar grown in Oregon and the fourth most widely grown soft white winter wheat cultivar in Washington in the recent years (http://www.nass.usda.gov). These clearly demonstrate that Stephens has durable HTAP resistance according to the definition of durable resistance (Johnson 1983).

In general, the Pullman field plots had higher severity levels of stripe rust than those at the Mt Vernon location. This was mainly because the Pullman plots were artificially inoculated in early spring with PST-25 that is virulent on seedlings of Stephens. The early inoculation preoccupied the field, which reduced effects of naturally later occurred races avirulent on Stephens. In contrast, the Mt Vernon field was under natural infection by various races, of which some were virulent and others were avirulent on seedlings of Stephens (data not shown). Mix infection of both virulent and avirulent races tended reducing rust severity. Thus, we found significant interactions between location and the rAUDPC data, but not between location and the IT data. The severity difference between the locations affected consistent detection of QTL with small effects (data not shown), but did not affect the detection of the two QTL with relatively large effects.

Quantitative trait locus mapping identified two QTL conferring HTAP resistance based on both rAUDPC and IT data. These two major QTL together explained 48–61% of the total variation of mean rAUDPC. This result is similar to the earlier report that two to three QTL were estimated to be responsible for HTAP resistance in Stephens (Chen and Line 1995b). Recently in spring wheat cultivar Alpowa, a single major QTL for HTAP resistance was reported to explain about 64.2% of the total phenotypic variation for mean rAUDPC (Lin and Chen 2007). Similar results were reported in other wheat genotypes for adult-plant or HTAP resistance to stripe rust. Börner et al. (2000) reported *Yrns-B1* on chromosome 3BS determining non-race specific adult-plant resistance (presumably HTAP resistance) against stripe rust. Chen and Zhao (2007) reported a single QTL for HTAP resistance in the AVS/6*Yr8 line. However, multiple QTL were reported for durable resistance to stripe rust in several other reports. Mallard et al. (2005) identified six different QTL, which together explained 57–81% of the total phenotypic variation of stripe rust in wheat variety Camp Remy. In another study, 11 QTL were reported for durable resistance in the Japanese wheat variety Fukuho-komugi and these QTL together explained 41–52% of the total phenotypic variation (Suenaga et al. 2003). Bariana et al. (2001) identified four QTL for the durable resistance to stripe rust. Chen and Lin (2007) reported three QTL for HTAP resistance in hard red spring wheat cultivar Express. Thus, HTAP resistance to stripe rust can be controlled by a single or multiple QTL and different QTL can contribute different levels of resistance. These studies have identified numerous genes for HTAP resistance, which are available for breeding programs to develop cultivars with durable resistance.

The limited population size in this report might have underestimated QTL number, overestimated QTL effects, or failed to quantify QTL interactions (Melchinger et al.

1998; Schön et al. 2004). The number of lines used in many QTL experiments has been about 100 and such studies have identified QTL with large effect on the phenotype (Vales et al. 2005). Vales et al. (2005) reported effect of population size on estimation of QTL for stripe rust resistance in barley. They reported that in all cases, the number of QTL detected increased with population size. However, they also reported that QTL with large effects were detected even in small populations ($n = 94$), but QTL with small effects were detected only by increasing population size ($n = 409$). It was possible that QTL with small effects were not detected in this study, as indicated by the lack of RILs as resistant as Stephens at the Pullman. However, our objective was to identify QTL for HTAP resistance to stripe rust with relatively high effect so that they can be easily used in wheat breeding programs. The two QTL with relatively large effect, are more useful than possible undetected small effect QTL in breeding for stripe rust resistance.

Because the two QTL in Stephens are on chromosome 6B, they are likely different from all previously reported genes for durable resistance in wheat genotypes against stripe rust. Other genes for non-race specific adult-plant or HTAP resistance were mapped to different chromosomes, including *Yr16* (2DS), *Yr18* (7DS), *Yr29* (1BL), *Yr30* (3BS), *Yr39* (7BL), *Yrns-B1* (3BS) and three QTL in Express (1BL, 3BL, and 6AS) (McIntosh et al. 1995; Chen 2005; Chen and Lin 2007). Uauy et al. (2005) mapped *Yr36*, a HTAP resistance gene to chromosome 6BS, where *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* were also mapped in the present study. However, on the basis of an allelism test, it was determined that *Yr36* and these two QTL are different genes (Santra et al., unpublished). The SSR markers within *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* were mapped on 6BS based on physical mapping using ditelosomic and deletion lines of 6B. The SSR markers, flanking *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2*, were proximal and distal to the centromere, respectively, based on published maps of wheat genome (Qi et al. 2003; Somers et al. 2004; Song et al. 2005). Therefore, these two QTL on chromosome 6BS for HTAP resistance in Stephens are distinct from each other and they also are different from previously reported genes for resistance to stripe rust.

Since QTL for Stephens HTAP resistance are on different chromosomal regions from stripe rust resistance genes reported in other wheat genotypes, it is possible to pyramid these HTAP QTL and effective all-stage resistance genes into future wheat cultivars to achieve high-level and durable resistance. Molecular markers flanking the two QTL for HTAP resistance in Stephens can be used for this breeding objective. The best flanking markers for marker-assisted selection of *QYrst.wpg-6BS.1* were *Xbarc101* and *Xbarc136*. These two markers were presented in wheat cultivars with Stephens or Stephens-related genotypes in their pedigree and highly polymorphic in a set of 32 wheat genotypes tested in this study, which do not have Stephens-related genotype in their pedigree. However, in case of lack of polymorphism, other SSR markers (*Xbarc1169*, *Xbarc88*, *Xbarc198*, *Xgwm644* and *Xbarc211*) and RGAP marker *XRlrfNlrr8*, mapped within the region can be used. Although the flanking markers (*Xgwm132* and *Xgwm508*) for *QYrst.wgp-6BS.2* were less predictive for expected QTL allele due to longer genetic distances, they also had relatively high polymorphism among tested wheat genotypes. Therefore, they also can be used in marker-assisted selection although they may not be as effective as those for *QYrst.wgp-6BS.1*. In conclusion, the QTL for HTAP resistance and their closely linked molecular markers identified in this study should facilitate wider use of the durable type resistance in future cultivars for more effective and sustainable control of the devastating stripe rust disease.

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