Theor Appl Genet (2014) 127:935–945
DOI 10.1007/s00122-014-2269-z

ORIGINAL PAPER

Identification of *Yr59* conferring high-temperature adult-plant resistance to stripe rust in wheat germplasm PI 178759

X. L. Zhou · M. N. Wang · X. M. Chen · Y. Lu · Z. S. Kang · J. X. Jing

Received: 19 September 2013 / Accepted: 12 January 2014 / Published online: 1 February 2014

Abstract

Key message **This manuscript reports a new gene for non-race-specific resistance to stripe rust and molecular markers for incorporating it into wheat cultivars for control of the disease with durable resistance.**

Abstract Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive wheat diseases worldwide. The spring wheat germplasm 'PI 178759' originating from Iraq showed effective resistance to stripe rust in field evaluations over 8 years in Washington state, USA. To map the resistance gene(s), PI 178759 was crossed with 'Avocet Susceptible', and the parents and 176 $F_{2:3}$ lines were phenotyped in the fields under natural infection and in a greenhouse with selected races of *P. striiformis* f. sp. *tritici*. PI 178759 was identified to have high-temperature adult-plant (HTAP) resistance. Resistance gene analog polymorphism and simple sequence repeat techniques were used to identify molecular markers linked to the resistance gene and a chromosome region was mapped using a quantitative trait locus approach. One major gene was mapped to the long arm of chromosome 7B. Flanked by *Xwgp5175* and *Xbarc32* in a 2.1 cM region, the gene explained 31.8 and 54.7 % of the phenotypic variation in rAUDPC and IT, respectively. Based on genetic distances among markers and allelism tests, the HTAP resistance gene in PI 178759 is different from the previously reported *Yr39*, *Yr52*, *YrZH84*, and *YrC591*, also located on chromosome 7BL, and is therefore designated as *Yr59*. The gene and its flanking markers should be useful for developing wheat cultivars with durable resistance.

X. L. Zhou and M. N. Wang made equal contributions.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

Communicated by T. Miedaner.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2269-z) contains supplementary material, which is available to authorized users.

X. L. Zhou · Y. Lu · Z. S. Kang · J. X. Jing
State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China

X. L. Zhou · M. N. Wang · X. M. Chen · Y. Lu
Department of Plant Pathology, Washington State, University, Pullman, WA 99164-6430, USA

X. M. Chen (✉)
Wheat Genetics, Quality, Physiology and Disease Research Unit, US Department of Agriculture, Agricultural Research Service, Pullman, WA 99164-6430, USA
e-mail: xianming@wsu.edu

Introduction

Wheat is the most widely cultivated and important food crop in the world. It provides staple food for about 35 % of the human population (Huang and Röder 2004). Stripe rust, caused by the fungal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), is one of the most damaging diseases of wheat in many areas around the world especially in cooler and moister environments (Stubbs 1985). In the USA, the disease is most destructive in the western states, but has become increasingly important also in states east of the Rocky Mountains (Chen et al. 2010). Growing resistant cultivars is the most effective, economical and

environmentally safe approach to control stripe rust (Chen 2005).

Different types of resistance can be recognized, but two major types of resistance have been characterized and widely used in control of stripe rust: all-stage resistance (also known as seedling or overall resistance) and high-temperature adult-plant (HTAP) resistance (Chen 2005, 2013). All-stage resistance is generally race-specific and can be detected at the seedling stage, but is expressed at all growth stages. Cultivars with only all-stage resistance often become susceptible within a few years of release, either as a consequence of selection of previously rare races or development of new races (Wellings and McIntosh 1990; Line and Qayoum 1992; Chen 2005, 2007). In contrast, HTAP resistance is non-race-specific and has proven to be durable (Qayoum and Line 1985; Chen and Line 1995a, b; Line 2002; Chen 2005, 2013). However, HTAP resistance often does not provide adequate control as expression is partial and dependent on temperature and/or growth stage. Therefore, the best approach is to combine durable HTAP resistance with effective all-stage resistance to minimize the disadvantages of both types of resistance (Chen 2013).

More than 50 genes for stripe rust resistance have been formally named and many other genes or quantitative trait loci (QTL) have been tentatively designated (McIntosh et al. 2008; Chen 2013). The majority of genes conferring all-stage resistance are no longer effective, and many HTAP or adult-plant resistance QTL have small effects, making them more difficult to use than major genes in breeding programs. Genes with relatively large effects on HTAP resistance have also shown durability (Chen 2013; Chen et al. 2013). To diversify stripe rust resistance genes used in breeding programs, more genes need to be identified, especially those conferring high-level HTAP resistance.

PI 178759, originating from Iraq, is a spring wheat germplasm deposited in the USDA-ARS Small Grains Collection in 1949 (http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=PI+178759). The germplasm is susceptible to races PST-17, PST-37, PST-43, PST-45, PST-100, PST-114 and PST-127, which predominated in the US at different periods since 1970s (Line and Qayoum 1992; Chen 2005; Chen et al. 2010; Wan and Chen 2012), in low diurnal temperature seedling tests, but shows a high level of resistance in adult-plant tests at high temperatures and in field tests over several years (Wang et al. 2012). The contrast of greenhouse and field tests indicates that PI 178759 may have HTAP resistance. The objectives of this study were to characterize the type of stripe rust resistance, and to identify and map genes conferring HTAP resistance in PI 178759.

Materials and methods

Plant materials

PI 178759, a spring wheat germplasm originally provided by the USDA-ARS National Small Grains Collection, was crossed, as male parent, with susceptible spring wheat genotype 'Avocet Susceptible' (AvS). F_1, F_2, $F_{2:3}$ and late generation progenies were obtained as previously described (Wang et al. 2012).

To determine the chromosomal locations of resistance loci, Chinese Spring (CS) and its complete set of 21 nullitetrasomic lines and selected ditelosomic lines were used. To determine relationships of resistance genes in PI 178759 to those previously reported on the same chromosome, Alpowa with *Yr39* (Lin and Chen 2007), Zhou 8425B with *YrZH84* (Li et al. 2006) and PI 183527 with *Yr52* (Ren et al. 2012) were also used in the study. PI 178759 was crossed as male parent to these lines for tests of allelism.

Characterization of resistance

To determine whether PI 178759 has HTAP resistance, four-way tests (seedling and adult-plant tests at low and high temperatures) were conducted under controlled greenhouse conditions as previously described (Chen and Line 1995a; Chen 2013). *Pst* races PST-100, PST-114 and PST-127 were used in the four-way tests because the first has been the most widespread race throughout the USA since 2004, the second was the most predominant race in the US Pacific Northwest in 2006–2008 and the third became predominant after 2007 and has the broadest virulence range identified in the US (Chen et al. 2010; Wan and Chen 2012). For the purpose of comparing reactions, PI 183527, Alpowa and Zhou 8425B were included in these experiments and AvS was used as a susceptible check. Growing plants before and after inoculation, inoculation and recording infection type data were done using standard procedures (Chen and Line 1992a, b; 1995a). For seedling tests 7–10 plants were grown in small pots ($6 \times 6 \times 6$ cm^3) and for adult-plant tests three plants were grown in large pots (20 cm diameter × 15 cm height; three pots for each line arranged randomly). Seedling inoculations were done at the two-leaf stage and adult-plant inoculations were done at the booting stage. Inoculated plants were kept in a dew chamber at 10 °C for about 24 h without light, and then transferred to growth chambers with temperatures programmed for low- or high-temperature cycles. For the low-temperature cycle, temperature gradually changed from 4 °C at 2:00 a.m. to 20 °C at 2:00 p.m.; and for the high-temperature cycle, temperature changed between 10 and 30 °C (Chen and Line 1995a; Chen 2013). The light cycle

was the same for all tests, switching between a 16-h photoperiod and an 8-h dark period. Infection type (IT) data were recorded 18–21 days after inoculation based on the 0–9 scale described by Line and Qayoum (1992).

Genetic analysis of rust response

Greenhouse tests

Because PI 178759 was identified to have HTAP resistance, adult plants of the parents and progenies of AvS/PI178759 were tested with race PST-127 using the high-temperature cycle. Three F_1 plants and 181 F_2 plants, together with the parents, were tested at the same time. After the IT data were recorded, the F_2 plants were grown to maturity and harvested for $F_{2:3}$ seeds. Infection type was recorded for each plant, and ITs 0–3, 4–6 and 7–9 were considered resistant, intermediate and susceptible, respectively. $F_{2:3}$ lines were classified as homozygous resistant, segregating or homozygous susceptible groups.

Field tests

During the 2010 growing season, the $F_{2:3}$ lines and parents were planted at two locations, near Pullman (eastern Washington) and Mount Vernon (western Washington). The sites are about 500 km apart and separated by the Cascade Mountain Range. The two locations have different climatic conditions and often have different *Pst* races. The Mount Vernon location was planted on 15th April and the Pullman location on 25th April. Due to limited seed quantities, 176 lines were planted at the Pullman location and 94 at Mount Vernon. To ensure adequate disease evaluation, each site was planted using a randomized complete block design with three replications. About 50 seeds per line were planted in 1 m rows in each replication, with a 30 cm space between rows. The nurseries were surrounded by the susceptible spring wheat 'Lemhi' as a spreader. Standard practices for fertilization and weed control were used to manage the field nurseries. Infection types and disease severities (DS) were recorded at the booting, heading-flowering and soft dough stages when rust severities on AvS reached approximately 30, 60 and 95 %, respectively. Infection types were based on a 0–9 scale similar to that described for seedling tests. Data on disease severity were recorded across the range of 0–100 %. The DS data were used to calculate relative area under the disease progress curve (rAUDPC) as previously described (Chen and Line 1995a; Lin and Chen 2007). For genetic analysis, the $F_{2:3}$ lines were classified as resistant, segregating and susceptible based on IT data. Chi-squared tests were used to determine the goodness of fit of observed data with hypothesized genetic ratios. Both the rAUDPC and IT data were used in QTL mapping.

The SAS statistical package (SAS Institute, Cary, NC, USA) was used for analysis of variance (ANOVA) and was performed to estimate genetic, environmental and genotype × environment interaction effects. The ANOVA results were used to estimate the broad-sense heritability (h^2) of rAUDPC and IT. Broad-sense heritability (h^2) across the two locations was calculated using the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_e^2/re)$ where σ_g^2, σ_{ge}^2 and σ_e^2 were estimates of genotypic, genotype × environment interaction and error variances, respectively, and e and r were the numbers of environments and replicates per environment, respectively (Yang et al. 2005).

Genotyping and linkage map construction

DNA extraction, PCR amplification, electrophoresis and gel visualization

Genomic DNA was extracted from the leaves of each F_2 plant of AvS/PI178759 and parents using the CTAB method (Yan et al. 2003). The DNA was quantified using electrophoresis and spectrophotometry (NanoDrop ND-1000, Thermo Scientific, Wilmington, DE, USA) and concentration was adjusted to 30 ng/μl for use as a PCR template. The resistance gene analog polymorphism (RGAP) (Chen et al. 1998; Shi et al. 2001) and simple sequence repeat (SSR) (Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2005) techniques were used to identify markers linked to the resistance locus. Resistance gene analog (RGA) primers were used in random pairs as previously described (Yan et al. 2003; Lin and Chen 2007, 2009; Li et al. 2011; Ren et al. 2012). Primer sequences and chromosomal locations of SSR markers were obtained from the GrainGenes website (http://wheat.pw.usda.gov/). PCR amplification, electrophoresis and gel visualization were done following standard procedures and conditions (Yan et al. 2003; Lin and Chen 2007, 2009; Li et al. 2011).

Bulked segregant analysis

Resistant and susceptible bulks were made with equal amounts of DNA from ten homozygous resistant and ten homozygous susceptible F_2 plants, respectively. The IT and DS data of the ten resistant and the ten susceptible plants were the same as those of PI 178759 and AvS, respectively. Selection of these plants was also based on their homozygosity determined by $F_{2:3}$ data generated in the greenhouse and in field tests. The genomic DNA samples of parents and the two DNA bulks were used to screen the RGA and SSR primers.

Chromosomal location of the resistance gene

When an RGAP marker was associated with resistance in bulked segregant analysis, it was tested with CS and repeated with the parents and bulks. If the marker band was present in CS, the complete set of 21 nulli-tetrasomic lines (Sears 1966) was tested to identify the chromosome carrying the marker. Once a chromosome was identified, the corresponding ditelosomic and deletion lines were used to further locate the RGAP marker to a chromosomal region as previously described (Shi et al. 2001; Cheng and Chen 2010; Li et al. 2011). When a chromosome and chromosomal arm were determined, the SSR markers for the specific chromosome were screened to confirm the chromosomal location and further position the resistance locus to a specific chromosomal region.

Linkage map construction

Single locus segregation of each marker was determined using Chi-squared tests. Markers with normal single locus segregation ratios were included in linkage map construction using software QTL IciMapping V3.2 (Li et al. 2007, 2008; Wang 2009; http://www.isbreeding.net/download_software_ICIM.aspx). The Kosambi function (Kosambi 1944) was used to convert recombination values to map distances in centiMorgans (cM). A logarithm of odds (LOD) threshold of 3.0 was used for grouping, and the algorithm "nnTwo Opt" was used for ordering. Linkage groups were assigned to chromosomes and compared with previously published wheat consensus maps (Somers et al. 2004; http://wheat.pw.usda.gov).

QTL analysis

Quantitative trait locus mapping was conducted using rAUDPC and IT data for each $F_{2:3}$ line and the parents at each location. Stripe rust resistance QTL was detected in the population using QTL IciMapping V3.2 (Wang 2009; http://www.isbreeding.net/download_software_ICIM.aspx). Specifically, inclusive composite interval mapping (ICIM) analysis was performed on IT and rAUDPC scores of $F_{2:3}$ lines at each field site. In the first step of stepwise regression, the significance level was set at 0.01 and the threshold LOD score was set at 2.5 based on a 1,000 permutation test for declaring significant QTL for all methods. QTL effects were estimated as proportions of the phenotypic variance (R^2).

Relationships between resistance genes on chromosome 7BL

Because stripe rust resistance genes in wheat cultivars PI 183527 (*Yr52*), Alpowa (*Yr39*) and Zhou 8425B (*YrZH84*) were previously mapped on the long arm of chromosome 7B, they were compared with PI 178759 using races PST-100 and PST-127 in the greenhouse. These cultivars were also crossed with PI 178759, and the F_2 populations were used in allelism tests to determine the genetic relationships of the resistance genes. The PI 178759 crosses with Alpowa and PI 183527 were tested in a field near Pullman during the 2011 growing season. Because Zhou 8425B is a winter wheat cultivar, the F_2 population from the cross with PI 178759 was vernalized for 4 weeks before transplanting to the field, and the F_2 population of its cross with PI 183527 was vernalized and tested with race PST-127 in the greenhouse at the adult-plant stage under the high-temperature testing cycle. Critical $F_{2:3}$ lines of these crosses were tested in the field in the 2012 crop season to confirm the phenotypes of the F_2 plants which had susceptible reactions. Tests of the F_2 populations were repeated in 2013. The frequency (p) of susceptible plants in each F_2 population was used to estimate the genetic distance between the genes.

Polymorphisms of molecular markers flanking *YrPI178759*

To determine the applicability of molecular markers closely linked to *YrPI178759*, primers for two SSR markers (*Xwmc557* and *Xbarc32*) flanking the locus were used to amplify DNA samples of 73 wheat cultivars, breeding lines and genetic stocks maintained in our laboratory. PCR amplification and product detection were the same as described above. Samples producing the same peak as PI 178759 were recorded as the presence of the marker in coupling with the resistance allele (presence) and those producing the same peak as AvS, a peak different from both PI 178759 and AvS, or no peak were recorded as absence of the marker for the resistance allele.

Results

HTAP resistance in PI 178759

Four-way tests with PST-100, PST-114 and PST-127 under the controlled conditions in the greenhouse clearly showed that PI 178759 has HTAP resistance to the tested races of the wheat stripe rust pathogen; however, the resistance phenotype was influenced more by temperature than by plant stage (Table 1; Supplementary Fig. 1). In all tests, the susceptible check, AvS, had IT 8 or 9 with abundant uredinia. PI 178759 had IT 8 with abundant uredinia in the seedling tests with the three races under the low-temperature cycle, and produced IT 7 or 8 with reduced uredinia in the adult-plant tests under the low-temperature cycle. In contrast, it had a resistant reaction under the high-temperature cycle at both the seedling (IT 2–3) and adult-plant (IT 2) stages.

However, under the high-temperature cycle, the necrotic patches produced on the seedlings were much larger, showing a high DS (30–60 %) with a limited number of uredinia, whereas the adult-plant leaves had a limited number of narrow necrotic stripes without uredinia, showing a low DS (2–10 %). The results indicated that HTAP resistance in PI 178759 could be detected in the seedling stage under high temperatures, but was expressed at a much higher level on adult plants tested at higher temperatures.

Table 1 Infection types produced on seedlings and adult plants of five wheat varieties inoculated with three *Puccinia striiformis* f. sp. *tritici* races under controlled temperature conditions

Cultivar	Race	Infection type			
		Seedling		Adult plant	
		LT[a]	HT[b]	LT[a]	HT[b]
AvS	PST-100	9	9	8	8 (>70 %)[d]
	PST-114	9	9	8	8 (>70 %)
	PST-127	9	9	8	8 (>70 %)
PI 178759	PST-100	8	2–3[c]	7	2 (5 %)
	PST-114	8	2–3[c]	7	2 (5 %)
	PST-127	8	2–3[c]	8	2 (10 %)
PI 183527	PST-100	8	8	8	2 (5 %)
	PST-114	8	8	8	2 (10 %)
	PST-127	8	8	8	2 (20 %)
Alpowa	PST-100	9	8	8	3 (30 %)
	PST-114	9	8	8	3–4 (30 %)
	PST-127	9	8	8	3–4 (30 %)
Zhou 8425B	PST-100	9	8	8	2 (20 %)
	PST-114	8	–	–	2 (20 %)
	PST-127	9	7	8	2 (20 %)

[a] LT, low-temperature diurnal cycle gradually changing from 4 °C at 2 a.m. to 20 °C at 2 p.m

[b] HT, high-temperature diurnal cycle gradually changing from 10 °C at 2 a.m. to 30 °C at 2 p.m

[c] Although the seedlings tested under the HT profile had a low infection type, similar to the adult plants tested under the HT, the seedlings developed more severe necrotic patches

[d] Mean severity (percentage of leaf areas with necrotic or chlorotic symptoms and/or sporulation)

Inheritance of resistance

When adult plants were tested with race PST-127 under high-temperature conditions in the greenhouse, all plants of AvS were susceptible (IT 8) and all plants of PI 178759 were highly resistant (IT 2). The three F_1 plants from AvS/PI 178759 were resistant (ITs 3–4), producing mostly necrotic stripes with a few small uredinia (Table 2). The data indicated that HTAP resistance in PI 178759 was partially dominant. The F_2 population segregated in a 3 resistant (ITs 2–4):1 susceptible (ITs 7–9) ratio and segregation of the $F_{2:3}$ lines fitted a 1 resistant:2 segregating:1 susceptible ratio (Table 2). These results indicated that the HTAP resistance to PST-127 in PI 178759 was controlled by a single gene. Five F_2 plants scored susceptible failed to produce seed.

In the field tests, AvS was highly susceptible (ITs 8–9) with about 30, 60, and 95 % DS at the boot, heading-flowering and soft dough stages, respectively, at both the Pullman and Mount Vernon locations. PI 178759 was highly resistant (IT 2) with necrotic stripes and no uredinia at all three scoring dates. The necrotic stripes were much shorter and the severity values (DS 2–5 %) were much lower than in the greenhouse. Both the tests of 176 $F_{2:3}$ lines at Pullman and 94 lines at Mount Vernon fitted a 1 resistant:2 segregating:1 susceptible ratio. Because all 43 HS $F_{2:3}$ lines identified in the adult-plant test in the greenhouse with race PST-127 were from susceptible F_2 plants tested in the field near Pullman, and they also showed a homozygous susceptible reaction at both Pullman and Mount Vernon locations under natural infections, the same gene apparently conferred the HTAP resistance observed in all tests. The resistance allele was temporarily designated as *YrPI178759*.

Table 2 Adult plant responses of the parents and hybrid generations of cross AvS/PI 178759 to race PST-127 when tested under the high-temperature profile in the greenhouse

Generation	No. of plants or lines[a]			Expected ratio (Res:Seg:Sus)	χ^2	*P*
	Resistant (ITs 1–4)	Segregating (ITs 1–9)	Susceptible (ITs 7–9)			
AvS			All	–	–	–
PI 178759	All			–	–	–
F_1	3	–	0	–	–	–
F_2	133	–	48	3:1[b]	0.15	0.64
$F_{2:3}$	40	93	43	1:2:1	0.67	0.71

[a] For the F_2 generation, plants with infection types (ITs) 1–4 were resistant and those of ITs 7–9 susceptible. For the F_3 generation, lines with ITs 1–4 were homozygous resistant and those with ITs 7–9 homozygous susceptible; segregating lines produced ITs 1–9

[b] For resistant:susceptible

When the DS values of the three sets of data were used to calculate rAUDPC using the mean values of AvS as 100 %, the rAUDPC values of PI 178759 were 2.3 % at Pullman and 4.6 % at Mount Vernon. When the mean IT and rAUDPC values were used to show the frequencies of different resistance levels, both data sets indicated a continuous variation with one peak (Fig. 1a, b). The IT data between the two locations were strongly correlated ($r = 0.80$, $P < 0.01$), and the rAUDPC data were also significantly correlated ($r = 0.62$, $P < 0.01$), indicating the same gene for resistance at the two locations. The field results indicated that the resistance in PI 178759 was mostly quantitatively inherited. Broad-sense heritability (h^2) was estimated at 75.5 % for the IT data and 76.3 % for the rAUDPC data across both locations based on the ANOVA. In general, the results of the quantitative analyses were consistent with the qualitative analysis.

Mapping resistance QTL

Screening of 280 RGA primer pairs in bulked segregant analysis resulted in 82 bands produced by 55 primer pairs being associated with resistance. After repeating tests and further screening on a subset of the mapping population, nine strong and repeatable RGAP markers were selected to test the entire F_2 population which was used to produce the $F_{2:3}$ generation for phenotyping. *Xwgp5271* was co-dominant and the remaining eight markers were dominant and all segregated as single loci, five (*Xwgp2132*, *Xwgp4042*, *Xwgp5175*, *Xwgp5258* and *Xwgp4546*) present in PI 178759 and three (*Xwgp5668*, *Xwgp1315* and *Xwgp538*) in AvS (Table 3).

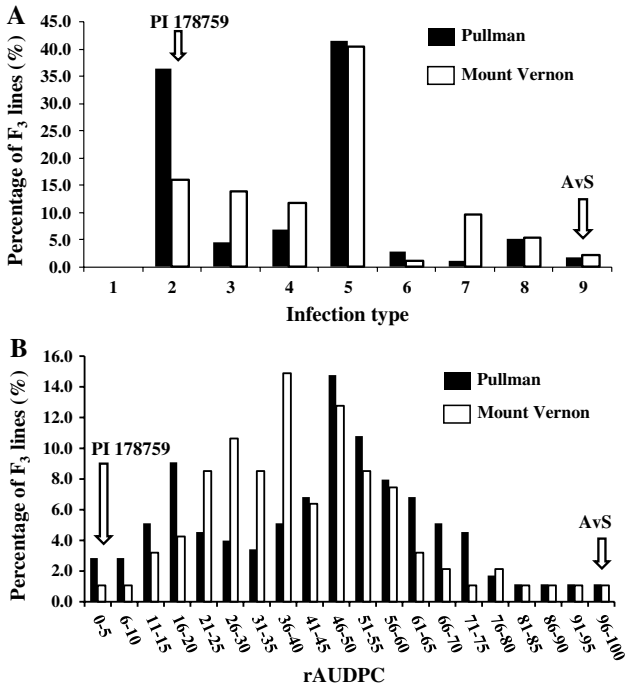

Fig. 1 Distribution of infection type (IT) (**a**) and relative area under the disease progress curve (rAUDPC) (**b**). Data from the AvS × PI 178759 mapping population averaged across the three sets of data at different adult growth stages

Seven RGAP markers (*Xwgp2132*, *Xwgp4546*, *Xwgp1315*, *Xwgp5175*, *Xwgp5258*, *Xwgp5271* and *Xwgp5668*) were also present in CS (Table 3). Tests of the 21 nulli-tetrasomic CS lines with four (*Xwgp2132*, *Xwgp1315*, *Xwgp5175* and *Xwgp5258*) of the markers revealed that they were on chromosome 7B (Supplementary Fig. 2). The markers were tested on the CS 7B ditelosomic lines, and only Dt7BL had the bands further localizing the markers to the long arm of chromosome 7B.

A total of 23 SSR primer pairs on chromosome 7BL were screened for polymorphism using bulked segregant analysis. Ten markers, *Xgpw1144*, *Xcfa2040*, *Xbarc32*, *Xbarc182*, *Xgwm577*, *Xmag1708*, *Xwmc166*, *Xwmc557*, *Xwmc581* and *Xgpw2338*, were used to genotype the F_2 mapping population. For example, *Xcfa2040* is shown in Fig. 2. Each of the ten markers segregated as single loci, of which three (*Xgpw1144*, *Xcfa2040* and *Xbarc32*) were co-dominant and the others dominant (Table 3). Except *Xgpw2338* in AvS the target bands of the dominant markers were present in PI 178759. These markers showed significant association with stripe rust resistance in the ANOVA tests (Supplementary Table 1). Tests of the CS 7B ditelosomic lines with four (*Xcfa2040*, *Xbarc182*, *Xwmc557* and *Xgpw2338*) of the SSR markers further confirm the markers and the linked resistance locus to 7BL.

The nine RGAP and ten SSR markers were placed in one linkage group using the software QTL IciMapping V3.2 (Supplementary Table 1). Using both rAUDPC and averaged IT data from the Pullman and Mount Vernon sites, one QTL, corresponding to *YrPI178759*, was mapped to chromosome 7BL by inclusive composite interval mapping (ICIM) (Fig. 3). The QTL was located in a 2.1 cM interval flanked by SSR marker *Xbarc32* and RGAP marker *Xwgp5175* (Fig. 3). This QTL explained 40.1 % of the phenotypic variation in rAUDPC at Pullman and 33.7 % at Mount Vernon. The corresponding variances explained on the basis of IT were 51.4 and 49.2 %, respectively (Table 4). Although the QTL was detected by IT and rAUDPC in both locations, variations between the two locations were found in the levels of association for the flanking markers and their explained portions of the phenotypic variations. *YrPI178759* was detected at LOD values ranging from 7.3 to 33.7 depending on IT or rAUDPC and location (Table 4). The QTL explained 29.2 % (rAUDPC at Mount Vernon) to 66.7 % (IT at Pullman) of the total variations in stripe rust response based on ICIM mapping (Table 4; Fig. 3), likely due to the different weather conditions at the two locations as the resistance gene is sensitive to temperature.

Table 3 Molecular and genetic data for the cross AvS/PI 178759

Marker	Primer pair	Size (bp)[a]	Presence (+) and absence (−)			No. of F_2 plants		Test for 3:1	
			PI 178759	AvS	CS	RPA	SPA	χ^2	*P*
RGAP									
Xwgp2132	S1/NLRR-INV2	425	+	−	+	131	50	0.53	0.41
Xwgp4042	wlrk-S/Cre3-k3	215	+	−	−	131	50	0.53	0.41
Xwgp4546	Xa1NBS-F/Xa1NBS-R	285	+	−	+	129	52	1.15	0.25
Xwgp1315	S1/NLRR-INV2	423	−	+	+	48	133	0.53	0.41
Xwgp538	RLRR For/AS3-INV	225	−	+	−	52	129	1.15	0.25
Xwgp5175	rga51/rga75	450	+	−	+	133	48	0.22	0.64
Xwgp5258	rga52/rga58	650	+	−	+	135	46	0.01	0.90
Xwgp5271	rga52/rga71	300/310	300	310	310	132[d]	49	1.24	0.26
Xwgp5668	rga56/rga68	430	−	+	+	43	138	0.15	0.70
SSR									
Xgpw1144	GPW1144	140/147	140	147	NT	140[b]	41	0.41	0.47
Xgwm577	GWM577	150	+	−	NT	137	44	0.01	0.83
Xmag1708	MAG1708	680	+	−	NT	132	49	0.31	0.52
Xcfa2040	CFA2040	228/247	228	247	241	134[d]	47	0.05	0.76
Xbarc182	BARC182	75	+	−	+	132	49	0.31	0.52
Xwmc557	WMC557	315	+	−	+	135	46	0.002	0.90
Xbarc32	BARC32	165/175	165	175	175	133[b]	48	0.002	0.90
Xwmc581	WMC581	310	+	−	NT	49	132	0.31	0.52
Xgpw2338	GPW2338	70	−	+	NT	46	135	0.002	0.90
Xwmc166	WMC166	305	+	−	NT	46	135	0.002	0.90

RPA resistant parent (PI 178759) allele, *SPA* susceptible parent (AvS) allele, *NT* not tested

[a] Sizes of all markers were estimated based on 1 kb plus DNA ladder

[b] The lines with the homozygous resistant parental allele and heterozygous alleles were combined

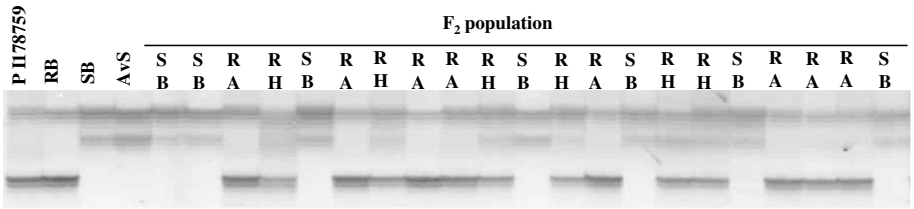

Fig. 2 Polyacrylamide gel showing SSR marker *Xcfa2040* that was polymorphic among PI 178759, AvS, resistant (RB) and susceptible (SB) bulks and among F_2 progeny of the AvS/PI 178759 cross

To physically map *Yr178759* to a specific chromosomal region, CS deletion lines Del7BL-3, Del7BL-7, Del7BL-9, Del7BL-10 and Del7BL-14, each carrying a different deletion of chromosome 7B, were amplified with RGAP marker *Xwgp5258* and SSR markers *Xbarc32*, *Xbarc182*, *Xwmc557* and *Xcfa2040*. The CS alleles of these marker loci were not present in all tested deletion lines, including 7BL-3 which contains the entire short arm and the largest fragment (0.86) of the long arm among the deletion lines. Therefore, *Yr178759* is physically located in the distal region (bin 7BL-0.86–1.00) of the long arm of chromosome 7BL.

Relationships of *YrPI178759* to other stripe rust resistance genes on 7BL

In order to determine the relationships of *Yr178759* with *Yr52*, *Yr39* and *YrZH84* reported on chromosome 7BL, PI 178759 (*YrPI178759*), PI 183527 (*Yr52*), Alpowa (*Yr39*) and Zhou 8425B (*YrZH84*) were tested with races PST-100, PST-114 and PST-127 and the flanking markers, with the exception that Zhou 8425B was not tested at high temperatures in the seedling stage or at low temperatures at the adult stage (Table 1). In the low-temperature tests, all four varieties were susceptible (ITs 7–9) to the three races at both the seedling and adult-plant stages. In the high-temperature seedling tests, the four varieties displayed different infection types: PI 178759 having a resistant reaction (IT 2–3) and PI 183527, Alpowa and Zhou 8425B having susceptible reactions (IT 7–8). In tests on adult plants under the high-temperature profile, the four varieties were all resistant (IT 2–4), but the actual responses were different. PI 178759 and PI 183527 had IT 2 (5–15 % severity) with short necrotic stripes without uredinia; Zhou 8425B had IT 2 (20 % severity);

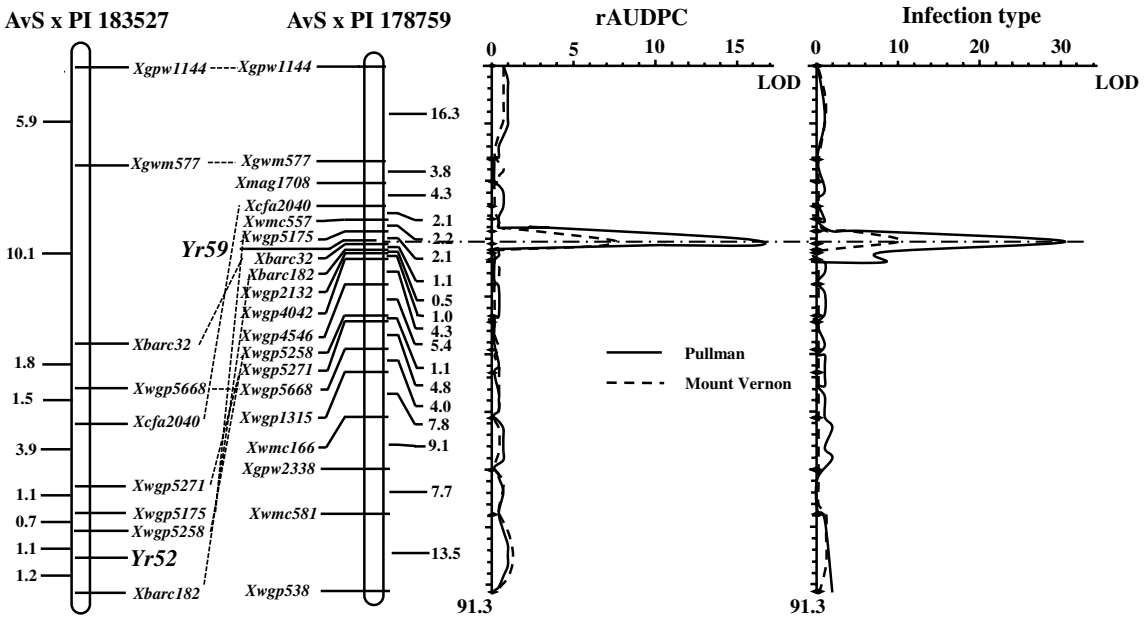

Fig. 3 Map of *Yr59* on chromosome 7BL identified by inclusive composite interval mapping (ICIM) using phenotypic and marker data from AvS/PI 178759. The map of *Yr52* on 7BL constructed with the AvS × PI 183527 (Ren et al. 2012) was used to compare markers linked to *Yr52* with those linked to *Yr59*. Map distances are in cM

Table 4 Summary of stripe rust resistance QTL by composite interval mapping based on relative area under the disease progress curve (rAUDPC) and infection type data for AvS/PI 178759 $F_{2:3}$ lines tested at two locations

Marker interval	Location	rAUDPC			Infection type		
		LOD	PVE (%)	Total R^2 (%)	LOD	PVE (%)	R^2 (%)
Xwgp5175–Xbarc32	Pullman	16.6	40.1	34.3	33.7	51.4	66.7
	Mt. Vernon	7.3	33.7	29.2	11.8	49.2	42.7
	Combined			31.8			54.7

PVE phenotypic variance explained by the QTL

and Alpowa had IT 3–4 (20–30 % severity) with longer necrotic stripes and some uredinia. The phenotypic data confirmed that all four varieties have HTAP resistance and that the resistances were possibly controlled by different genes.

PI 183527 (*Yr52*), Zhou 8425B (*YrZH84*) and Alpowa (*Yr39*), together with PI 178759 and AvS, were tested with SSR markers *Xcfa2040*, *Xwmc557*, *Xbarc32*, *Xbarc182* and *Xgpw2338* and RGAP markers *Xwgp5175*, *Xwgp5258* and *Xwgp1315* linked to the *YrPI178759* locus (Fig. 3). The presence and absence of the different alleles of these markers are shown in Table 5. PI 178759 differed from Zhou 8425B at *Xwmc557* and *Xwgp5175* and from Alpowa at *Xwgp5175* and *Xbarc32*, indicating that *YrPI178759* is different from *YrZH84* and *Yr39*. However, PI 178759 and PI 183527 could not be differentiated by the haplotypes of the eight markers.

Allelism tests were conducted to further determine the genetic relationships among *Yr* genes *Yr178759*, *Yr52*, *Yr39* and *YrZH84*. The adult plants of the F_2 populations from the intercrosses, together with the parents, were tested in a field near Pullman under natural infection of *Pst* in 2011. The resistant parental varieties had ITs 2 or 3 as observed in the greenhouse tests described above. F_1 plants had ITs 1–3. The total number of F_2 plants tested and number of susceptible plants identified in each cross are shown in Table 6. In the tests of critical $F_{2:3}$ lines obtained from the individual F_2 plants in the field near Pullman in 2012, all lines from susceptible F_2 plants were homozygous susceptible. The genetic distances between these genes are shown in Table 6; the linkage order is *Yr39*, 41.1 cM; *Yr52*, 5.4 cM; *YrPI178759*, 6.0 cM; *YrZH84*. The results show that *YrPI178759* is different from previously reported *Yr* genes on chromosome 7BL, and therefore, the gene was designated *Yr59*.

Table 5 Presence and absence of molecular markers in wheat varieties with stripe rust resistance genes on the long arm of chromosome 7B

Variety	*Yr* gene	Marker band (bp)							
		Xcfa2040	*Xwmc557*	*Xwgp5175*	*Xbarc32*	*Xbarc182*	*Xwgp5258*	*Xwgp1315*	*Xgpw2338*
PI 178759	*Yr59*	228	315	450	165	75	650	–	–
PI 183527	*Yr52*	228	315	450	165	75	650	–	–
Zhou 8425B	*YrZH84*	228	–	–	165	75	650	–	–
Alpowa	*Yr39*	228	315	–	175	75	650	–	–
AvS	(Susc. check)	247	–	–	175	–	–	423	70

– no band for this marker

Table 6 Allelism tests for estimating the genetic distances between stripe rust resistance genes *Yr39*, *Yr52*, *YrZH84* and *Yr59* on wheat chromosome 7BL

Genes involved	Cross	No. of F_2 plants						Mean distance (cM)
		2011			2013			
		Total	Susc.	Distance (cM)	Total	Susc.	Distance (cM)	
Yr39/Yr52	Alpowa/PI183527	179	3	28.6	184	4	33.9	31.2 ± 3.7
Yr39/Yr59	Alpowa/PI178759	165	2	23.6	141	6	58.6	41.1 ± 24.7
Yr52/Yr59	PI183527/PI178759	726	2	10.7	780	0	0	5.4 ± 7.6
Yr52/YrZH84	PI183527/Zhou8425B	243	1	13.1	200	1	11.2	12.2 ± 1.3
YrZH84/Yr59	Zhou8425B/PI178759	295	1	11.9	352	0	0	6.0 ± 8.4

[a] Map distances were calculated from recombination values using the Kosambi function $d = 0.25 \ln [(1 + 2p)/(1 - 2p)]$, where p is the recombination frequency

Polymorphisms of markers flanking *Yr59*

When 73 wheat genotypes were tested with *Xwmc557* (Supplementary Table 2), 28 did not have the 315 bp product present in PI 178759, indicating a 60.3 % polymorphism rate. When these genotypes tested with *Xbarc32*, only one (Buck Pronto) amplified a fragment with the same size as that from PI 178759, indicating a 98.6 % polymorphism rate. As Buck Pronto did not have marker *Xwmc557*, the variety is unlikely to have *Yr59*. Therefore, using both *Xwmc557* and *Xbarc32* wheat lines with or without *Yr59* could be distinguished.

Discussion

In this study, we characterized the stripe rust resistance in wheat germplasm PI 178759 as HTAP resistance and mapped a gene with major effect to the long arm of chromosome 7B. Unlike many other previously reported HTAP resistance genes which confer a low to moderate level (ITs 3–7) of resistance, *YrPI178759*, named here as *Yr59*, confers a high level of HTAP resistance (IT 2) with short necrotic stripes and no sporulation on adult plants under field and high-temperature greenhouse conditions.

PI 178759 was susceptible to all tested *Pst* races in standardized low temperature seedling tests. Greenhouse and field tests over several years suggest that the resistance is race non-specific and, therefore, likely to be durable. The relatively high level of resistance controlled by a single gene or major QTL allows for relatively easy use in breeding programs.

Yr59 was mapped to the long arm of chromosome 7B and determined to be different from *Yr39* (Lin and Chen 2007), *Yr52* (Ren et al. 2012) and *YrZH84* (Li et al. 2006) previously reported on chromosome 7BL. Although *Yr39* is also a HTAP resistance gene, it should be different from *Yr59* based on the estimated genetic distance (41.1 ± 24.7 cM). Both PI 178759 (*Yr59*) and PI 183527 (*Yr52*) had similar reactions to stripe rust in field tests and each has a single major gene for similar levels of HTAP resistance. The *Yr52* resistance in PI 183527 is a more typical HTAP type and only adult plants under the high-temperature profile showed a high level of resistance and seedlings at both low and high temperatures were highly susceptible (Ren et al. 2012). In contrast, HTAP resistance in PI 178759 is more temperature sensitive. The molecular linkage maps of *Yr59* and *Yr52* share nine markers (5 SSR and 4 RGAP) on the long arm of chromosome 7B (Fig. 3). However, the shared markers were not in the same order in the two mapping populations, AvS × PI 178759

and AvS × PI 183527, indicating a chromosomal rearrangement between the two resistant genotypes. *Yr59* is delimited by markers *Xbarc32* and *Xwgp5175* in a 2.1 cM interval, whereas *Yr52* is in a 3.3 cM interval flanked by markers *Xbarc182* and *Xwgp5258*. The markers surrounding *Yr59* and *Yr52* were assigned to the distal 0.86–1.0 bin on chromosome 7BL. Linkage between the resistance genes was estimated to be 5.4 ± 7.6 cM. Both genes were also at loci different from *Yr39* and *YrZH84*.

Stripe rust resistance in Zhou 8425B, a Chinese winter wheat line with genes *Yr9* and *YrZH84*, was studied by Li et al. (2006). In seedling tests under a 12/17 °C night/day temperature cycle, the line gave variable but resistant reactions (ITs 0–2 on a 0–4 scale) to a collection of 25 *Pst* isolates from different countries, and was susceptible (ITs 3 and 3^+ and 4) to two isolates from Chile and the Netherland isolates (Li et al. 2006). When *Yr9* was effective, Zhou 8425B displayed IT 0; when *Yr9* is not effective (e.g. tests with races CYR29 and CYR32), the line produced ITs 1^+ and 2 (necrotic patches with low to moderate sporulation), equivalent to ITs 4–5 of the 0–9 scale used in the present study. The temperature range used by Li et al. (2006) is in the middle of our low-temperature (4–20 °C) and high-temperature (10–30 °C) cycles. Under their conditions, HTAP resistance in Zhou 8425B detected in the present study is likely to be similar to the reaction they described, but lower than reaction we observed under our high-temperature regime. In the present study, Zhou 8425B and PI 178759 differed by two marker loci among eight tested markers closely linked to both *YrZH84* and *Yr59*. The marker data indicated that the two genes are closely linked, but at different loci, and hence in agreement with the allelism test.

YrC591, conferring all-stage resistance, was also reported on chromosome 7BL (Li et al. 2009). Because seed of C591 was not available, it was not possible to conduct an allelism test of *YrC591* and *Yr59*. However, we estimated the genetic distance between the two genes based on common SSR markers used in their study and the present work. As previously discussed (Li et al. 2006, 2009), the 7B SSR map was obtained from the GrainGenes 2.0 website (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker). Four SSR markers, *Xcfa2040*, *Xbarc32*, *Xwmc166* and *Xgwm577* identified in the present study, were also used in the map of *YrC591* (Li et al. 2009). Except for *Xgwm577* that was distally located from both *Yr59* and *YrC591*, the common markers had similar orders and genetic distances in both studies. Based on the distances of the common markers, *YrC591* is about 12.3 cM distal to *Yr59*.

Yr59 is effective against all tested North American *Pst* races and its HTAP resistance suggests a likelihood of durability. PI 178759 is an old Iraqi landrace and has obviously undesirable agronomic traits. We selected resistant line F_4-158 from the F_4 population of AvS × PI 178759 and registered it as PI 660061 (Wang et al. 2012). The new germplasm line with *Yr59* and improved agronomic traits should be more useful than the original donor in breeding programs. The molecular markers identified in this study should be useful for marker-assisted selection for incorporating the useful stripe rust resistance gene into commercial wheat cultivars. Currently, we are selecting lines with *Yr59* combined with *Yr39*, *Yr52* and *YrZH84* from the crosses used in the allelism tests. The stacked two-gene lines will provide more durable and higher levels of resistance to stripe rust.

Acknowledgments This research was supported by the US Department of Agriculture, Agricultural Research Service (Project No. 5348-22000-015-00D), National Key Basic Research Program of China (2013CB127700), Washington Wheat Commission (Project No. 13C-3061-3923) and Vogel Foundation (Project No. 13Z-3061- 3824). We thank Ms. Lu Hou for technical assistance and Dr. Robert McIntosh for critical review of the manuscript. A scholarship to Xinli Zhou from the China Scholarship Council was greatly appreciated. The research is also part of a Northwest A&F University Plant Pathology "111" Project (B07049).

Conflict of interest None.

Ethical standard 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.

References

Chen XM (2005) Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can J Plant Pathol 27:314–337

Chen XM (2007) Challenges and solutions for stripe rust control in the United States. Aust J Agric Res 58:648–655

Chen XM (2013) Review article: high-temperature adult-plant resistance, key for sustainable control of stripe rust. Am J Plant Sci 4:608–627

Chen XM, Line RF (1992a) Inheritance of stripe rust resistance in wheat cultivars used to differentiate races of *Puccinia striiformis* in North America. Phytopathology 82:633–637

Chen XM, Line RF (1992b) Identification of stripe rust resistance genes in wheat genotypes used to differentiate North American races of *Puccinia striiformis*. Phytopathology 82:1428–1434

Chen XM, Line RF (1995a) Gene action in wheat cultivars for durable high-temperature adult-plant resistance and interactions with race-specific, seedling resistance to stripe rust caused by *Puccinia striiformis*. Phytopathology 85:567–572

Chen XM, Line RF (1995b) Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant resistance and race-specific resistance to *Puccinia striiformis*. Phytopathology 85:573–578

Chen XM, Line RF, Leung H (1998) Genome scanning for resistance gene analogs in rice, barley and wheat by high-resolution electrophoresis. Theor Appl Genet 97:345–355

Chen XM, Penman L, Wan AM, Cheng P (2010) Virulence races of *Puccinia striiformis* f. sp. *tritici* in 2006 and 2007 and

development of wheat stripe rust and distributions, dynamics, and evolutionary relationships of races from 2000 to 2007 in the United States. Can J Plant Pathol 32:315–333

Chen XM, Coram T, Huang XL, Wang MN, Dolezal A (2013) Understanding molecular mechanisms of durable and non-durable resistance to stripe rust in wheat using a transcriptomics approach. Curr Genom 14:111–126

Cheng P, Chen XM (2010) Molecular mapping of a gene for stripe rust resistance in spring wheat cultivar IDO377s. Theor Appl Genet 121:195–204

Huang XQ, Röder MS (2004) Molecular mapping of powdery resistance genes in wheat: a review. Euphytica 137:203–223

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Li ZF, Zheng TC, He ZH, Li GQ, Xu SC, Li XP, Yang GY, Singh RP, Xia XC (2006) Molecular tagging of stripe rust resistance gene *YrZH84* in Chinese wheat line Zhou 8425B. Theor Appl Genet 112:1098–1103

Li HH, Ye GY, Wang JK (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Li HH, Ribaut JM, Li Z, Wang JK (2008) Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor Appl Genet 116:243–260

Li Y, Niu YC, Chen XM (2009) Mapping a stripe rust resistance gene *YrC591* in wheat variety C591 with SSR and AFLP markers. Theor Appl Genet 118:339–346

Li Q, Chen XM, Wang MN, Jing JX (2011) *Yr45*, a new wheat gene for stripe rust resistance mapped on the long arm of chromosome 3D. Theor Appl Genet 122:189–197

Lin F, Chen XM (2007) Genetic and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor Appl Genet 114:1277–1287

Lin F, Chen XM (2009) Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar express. Theor Appl Genet 118:631–642

Line RF (2002) Stripe rust of wheat and barley in North America: a retrospective historical review. Annu Rev Phytopathol 40:75–118

Line RF, Qayoum A (1992) Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968–87. US Dep Agric Tech Bull 1788:44

McIntosh R, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Somers D, Appels R, Devos K (2008) Catalogue of gene symbols for wheat. In: Proceedings of 11th international wheat genetic symposium, University of Sydney Press, Australia. http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/

Qayoum A, Line RF (1985) High-temperature, adult-plant resistance to stripe rust of wheat. Phytopathology 75:1121–1125

Ren RS, Wang MN, Chen XM, Zhang ZJ (2012) Characterization and molecular mapping of a new gene for high-temperature adult-plant resistance to stripe rust in spring wheat germplasm PI 183527. Theor Appl Genet 125:847–857

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:207–333

Sears ER (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulations and plant genetics. Oliver and Boyd, Edinburgh, pp 29–45

Shi ZX, Chen XM, Line RF, Leung H, Wellings CR (2001) Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. Genome 44:509–516

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Sourdille P, Gandon B, Chiquet V, Nicot N, Somers D, Murigneux A, Bernard M (2005) Wheat genoplante SSR mapping data release: a new set of markers and comprehensive genetic and physical mapping data. http://wheat.pw.usda.gov/GG2/index.shtml. Accessed 26 Nov 2012

Stubbs RW (1985) Stripe rust. In: Roelfs AP, Bushnell WR (eds) The cereal rusts, vol II. Academic Press, NY, pp 61–101

Wan AM, Chen XM (2012) Virulence, frequency, and distribution of races of *Puccinia striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* identified in the United States in 2008 and 2009. Plant Dis 96:67–74

Wang JK (2009) Inclusive composite interval mapping of quantitative trait genes. Acta Agron Sinica 35:239–245

Wang MN, Chen XM, Xu LS, Cheng P, Bockelman H (2012) Registration of 70 common spring wheat germplasm lines resistant to stripe rust. J Plant Regist 6:104–110

Wellings CR, McIntosh RA (1990) *Puccinia striiformis* f. sp. *tritici* in Australasia: pathogenic changes during the first 10 years. Plant Pathol 39:316–325

Yan GP, Chen XM, Line RF, Wellings CR (2003) Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. Theor Appl Genet 106:636–643

Yang J, Bai G, Shaner GE (2005) Novel quantitative trait loci (QTL) for Fusarium head blight resistance in wheat cultivar Chokwang. Theor Appl Genet 111:1571–1579