Theor Appl Genet (2008) 116:797–806
DOI 10.1007/s00122-008-0713-7

ORIGINAL PAPER

Molecular mapping of genes for race-specific overall resistance to stripe rust in wheat cultivar Express

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Received: 25 August 2007 / Accepted: 8 January 2008 / Published online: 24 January 2008

Abstract 'Express', a hard red spring wheat cultivar that has been widely grown in the western United States, is used to differentiate races of *Puccinia striiformis* f. sp. *tritici*, the causal fungal pathogen of wheat stripe rust. To identify genes conferring race-specific, overall resistance to stripe rust, Express was crossed with 'Avocet S'. The parents and F_1, F_2, F_3 and F_5 populations were tested with races PST-1, PST-21, PST-43, and PST-45 of *P. striiformis* f. sp. *tritici* in the seedling stage under controlled greenhouse conditions. Two dominant genes for resistance to stripe rust were identified, one conferring resistance to PST-1 and PST-21, and the other conferring resistance to all four races. Linkage groups were constructed for the resistance genes using 146 F_5 lines to establish resistance gene analog and chromosome-specific simple sequence repeat marker polymorphisms. The gene for resistance to races PST-1 and PST-21 was mapped on the long arm of chromosome 1B, and that conferring resistance to all four races was mapped on the long arm of chromosome 5B. We temporarily designate the gene on 1BL as *YrExp1* and the gene on 5BL as *YrExp2*. Polymorphism of at least one of the two markers flanking *YrExp2* was detected in 91% of the 44 tested wheat genotypes, suggesting that they would be useful in marker-assisted selection for combining the gene with other resistance genes into many other wheat cultivars. Knowledge of these genes will be useful to understand recent virulence changes in the pathogen populations.

Communicated by J. W. Snape.

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Introduction

Stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is one of the most important diseases of wheat (*Triticum aestivum* L.) worldwide (Stubbs 1985; Chen et al. 1996; Chen 2005). The disease is preferably controlled through the release of resistant cultivars. However, rapid virulence changes in pathogen populations can render resistant cultivars susceptible. Isolates of the pathogen with the same virulence/avirulence pattern on a set of wheat genotypes are considered to be the same race or pathotype. Several sets of wheat genotypes are used to differentiate races of *P. striiformis* f. sp. *tritici* in different regions of the world, each set having been chosen to distinguish races meaningful for local wheat production and breeding programs. In the United States, a set of 20 wheat genotypes are used to differentiate races of *P. striiformis* f. sp. *tritici* (Chen et al. 2002; Chen 2005, 2007). Among them only 'Express' has unknown genes for resistance.

Express (PI 573003), a hard red spring wheat developed by Western Plant Breeders Inc., has been widely grown in

the western United States since its release in 1991 (http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=PI+573003). The cultivar was highly resistant to stripe rust until 1997, when stripe rust samples were collected from it in California. The stripe rust isolates could not be differentiated from previous races without considering the susceptible reaction on seedlings of Express. Therefore, Express was added to the differential set in order to distinguish races PST-58 and PST-59 from others (Line 2002).

The North American *P. striiformis* f. sp. *tritici* population has undergone dramatic changes in virulence structure since 2000 (Chen et al. 2002; Chen 2005, 2007). Pathogen races that had been identified before 2000 are considered "the old group" whereas races identified since 2000 and sharing virulences on Lemhi (*Yr21*), Lee (*Yr7*, *Yr22*, *Yr23*), Fielder (*Yr6*, *Yr20*), Express (*Yr*?), AVS/6*Yr8 (*Yr8*), AVS/6*Yr9 (*Yr9*), Clement (*Yr9*, *YrCle*) and Compair (*Yr8*, *Yr19*) are considered "the new group" of races. The new group has become predominant in North America and has caused widespread epidemics in the recent years (Chen 2005; Chen and Penman 2005, 2006). Virulence on seedlings of Express has been detected in most isolates of "new group" races. The identification of gene(s) for resistance in Express should lead to a better understanding of the virulence changes and development of better strategies for more effective control of stripe rust.

Although Express is susceptible to the recent predominant races in the seedling growth stage, it has remained moderately resistant in the field. Stripe rust damage on Express has been much less than on susceptible spring wheat cultivars such as Fielder, Zak and Jubilee (X. M. Chen, unpublished data). Stripe rust response data from commercial fields, experimental plots, and controlled greenhouse tests show that Express has non-race specific, high-temperature adult-plant (HTAP) resistance, as well as race-specific, overall (also called seedling) resistance to stripe rust (Chen 2005; Chen and Lin 2007). The mapping of quantitative trait loci (QTL) for HTAP resistance in Express will be published separately. Here, we report the genetics of genes conferring race-specific, overall resistance to stripe rust in Express and mapping them to chromosomal locations using resistance gene analog polymorphism (RGAP) (Chen et al. 1998; Shi et al. 2001) and simple sequence repeat (SSR) (Röder et al. 1998) markers.

Materials and methods

Plant materials

To study the genetics of overall resistance in Express, a cross was made between Express and 'Avocet S' (AVS), a line that is susceptible to most races of *P. striiformis* f. sp. *tritici*. To determine if Express and Alpowa have the same gene or genes for stripe rust resistance, Express was crossed with 'Alpowa', which carries *YrAlp* on the short arm of chromosome 1B (Lin and Chen 2007). Seeds from the parental plants were used for later phenotype and molecular marker studies. F_1, F_2 and F_3 populations of the two crosses were used to determine the mode of inheritance of overall resistance in Express. F_5 lines advanced from individual F_2 plants of the AVS/Express cross through single seed-descent were used for genetic analysis and molecular mapping of the resistance genes.

Wheat genotypes 'Compair' (*Yr8* and *Yr19*), 'Druchamp' (*Yr3a*, *YrDru, YrDru2*), 'Lemhi' (*Yr21*), 'Alpowa' (*YrAlp*) and AVS/6*Yr9 (*Yr9*) were used in molecular marker tests to determine if Express and these cultivars share a resistance gene(s) on chromosomes 1B or 5B; viz., *Yr21* in Lemhi, *YrAlp* in Alpowa, and *Yr9* in AVS/6*Yr9 on chromosome 1B and *Yr19* in Compair and *YrDru* in Druchamp on chromosome 5B (Chen et al. 1995; Shi et al. 2001; Chen 2005; Lin and Chen 2007). To determine polymorphism of the markers flanking one of the Express resistance genes, DNA of 44 spring and winter wheat cultivars and genotypes were tested with the markers flanking the resistance gene.

Evaluation of resistance to stripe rust

Seedling tests were conducted under controlled greenhouse conditions as described by Chen and Line (1992a, b). A total of 13 *P. striiformis* f. sp. *tritici* races (PST-1, PST-3, PST-7, PST-21, PST-23, PST-43, PST-45, PST-58, PST-59, PST-78, PST-98, PST-100, and PST-111) were initially chosen to confirm the response arrays of Express and AVS. Races that were avirulent on seedlings of Express but virulent on AVS were used to test F_1, F_2 and F_3 populations derived from AVS/Express and Alpowa/Express, and F_5 of AVS/Express. Seedlings of parents, and F_1, F_2, F_3 and F_5 populations were grown in controlled greenhouse conditions as previously described (Chen and Line 1992a, b). About 15 seeds of each parent, 3 seeds of F_1, 300 seeds for F_2, and 15 seeds for each of F_3 and F_5 line were planted in each pot, except for F_1s. A total of 146 F_3 and 146 F_5 lines were used for AVS/Express and 150 F_3 lines were used for Alpowa/Express.

Seedlings at the two-leaf stage were inoculated with selected races of *P. striiformis* f. sp. *tritici*, grown and evaluated in the seedling stage (Chen and Line 1992a, b). A set of stripe rust differential genotypes was included to confirm the identity of selected races. Infection type (IT) data were collected 18–21 days after inoculation based on the 0–9 scale of Line and Qayoum (1992).

To verify the genotypes of F_2 plants showing IT 4–6, the plants were trimmed, transplanted into bigger pots, and grown to obtain F_3 seeds. The F_3 seedlings were tested with the same races. The IT data of the progenies were used to determine genotypes of F_2 plants.

DNA extraction, PCR amplification, electrophoresis and gel visualization

Genomic DNA was isolated from more than 20 plants from two-leaf stage seedlings for each of the parents and F_5 lines using the CTAB method (Saghai-Maroof et al. 1984). The RGAP method (Chen et al. 1998; Shi et al. 2001) and the SSR procedure (Röder et al. 1998) were used to target genomic regions.

PCR reactions were performed in a GeneAmp® PCR System 9700 thermo-cycler. A 15 µl reaction mixture consisting of 30 ng of template DNA, 1.5 µl Mg-free 10X PCR buffer (Promega, Madison, WI, USA), 0.6 unit of Taq DNA polymerase (Promega), 5 mM of $MgCl_2$, 0.2 mM each of dCTP, dGTP, dTTP, and dATP (Sigma Chemical Co., St. Louis, MO, USA) and 30 ng of each primer synthesized by Operon Biotechnologies, Inc. (Huntsville, AL, USA). After 5 min of denaturation at 94°C, amplifications were programmed for 40 consecutive cycles, each consisting of 1 min at 94°C, 1 min at either 45, 50, 55, or 60°C (45°C for RGA primers, 50, 55 or 60°C for SSR primers depending on the individual primer pair), 2 min at 72°C and followed by a 7 min extension step at 72°C. After amplification, 6 µl of formamide loading buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.5% (W/V) xylene cyanol and 0.5% (W/V) bromophenol blue] was added to the PCR product. After 4 min denaturation at 94°C, about 7 µl of the PCR product and loading buffer mixture for each sample was loaded for electrophoresis in a 5% polyacrylamide gel as previously described (Chen et al. 1998). After electrophoresis, the gel was silver-stained according to the recommendation of the manufacturer (Promega).

Bulk segregant analysis

The initial resistant and susceptible bulks each consisted of equal amounts of DNA from ten lines based on the phenotypic data from the test with PST-21 that detected two resistance genes in Express. Because these bulks resulted in markers linked only to one of the two genes, additional 20 lines were added to each of the bulks to identify markers for the other gene. The new resistant bulk consisted of ten lines resistant to both races PST-21 and PST-43 that detected only one of the two genes and 20 lines resistant to PST-21 but susceptible to PST-43. The susceptible bulk consisted of all 20 lines susceptible to PST-21 and 10 lines resistant to PST-21, but susceptible to PST-43.

Determination of chromosomal locations of the resistance genes

The nulli-tetrasomic lines of 'Chinese Spring' (CS) (Sears 1966) were used to locate RGAP markers on wheat chromosomes using the procedure as previously described (Shi et al. 2001; Lin and Chen 2007). SSR markers from the chromosomes identified with RGAP analysis of the CS nulli-tetrasomic lines were screened to map the resistance genes to chromosomal locations. A total of 15 SSR markers on chromosome 1B and 25 SSR markers on chromosome 5B were screened. The primer sequences of the SSR markers linked with the resistance genes are listed in Table 1.

Data analysis and linkage map construction

Chi-squared analysis was used to test the goodness of fit of observed segregations to specific ratios. The "chitest" procedure in the data analysis of Microsoft Office 2000 was used to calculate *P* values. Linkage maps were constructed using Mapmaker Macintosh version 2.0 (Lander et al. 1987). Two-point analysis with a logarithm of the odds (LOD) threshold of 3.0 or greater was used to determine linkage relationships among markers whereas multipoint analysis was used for determining the best locus order in linkage groups. Recombination values were converted to map distances using the Kosambi mapping function (Kosambi 1944).

Results

Genetics of resistance in Express to races of *P. striiformis* f. sp. *tritici*

The susceptible parent, AVS, was susceptible (IT 9) to all races, whereas Express was susceptible (IT 9) to races PST-58, 59, 78, 98, 100 and 111, but resistant (IT 2) to PST-1, 3, 7, 21, 23, 43 and PST-45 (Table 2). Alpowa was resistant only to PST-1 and PST-21. Based on these results, we chose PST-1, PST-21, PST-43 and PST-45 to evaluate the progenies of AVS/Express and Alpowa/Express to identify genes in Express and determine if Express and Alpowa have a common gene for resistance.

The reactions of F_1, F_2 and F_3 generations of cross AVS/Express to PST-1, PST-21, PST-43 and PST-45 are shown in Table 3. All three F_1 plants were resistant (IT 2) to PST-1. Among the 296 F_2 plants, 10 were recorded with IT 4 (three plants), 5 (four plants), and 6 (three plants). F_3 lines from the 10 plants were tested with PST-1. The progenies of the three plants with IT 4 were homozygous resistant, progenies of the four plant with IT 5 segregated, and

Table 1 Resistance gene analog (RGA) and simple sequence repeat (SSR) primers used to identify markers for stripe rust resistance genes in Express

Primer[a]	Sequence (5′–3′)	Gene	Domain	Reference
AS1	CAACGCTAGTGGCAATCC	*N*, *Rps2*	P-loop	Pahalawatta and Chen (2005a)
AS1-INV	CCTAACGGTGATCGCAAC	*N*, *Rps2*	P-loop	Lin and Chen (2007)
AS3-INV	CCIGAIGGIGAICGIG	*N*, *Rps2*	LRR	Yan et al. (2003)
Cre3LR-F	CACACACTCGTCAGTCTGCC	*Cre3*	LRR	Yan and Chen (2007)
NLRR-INV2	TCAGGCCGTGAAAAATAT	*N*	LRR	Lin and Chen (2007)
PtoFen-AS	TTGGCACAAAATTCTCATCAAGC	*Pto*	Fen	Pahalawatta and Chen (2005b)
Pto kin1	GCATTGGAACAAGGTGAA	*Pto*	Kinase	Chen et al. (1998)
Pto kin2	AGGGGGACCACCACGTAG	*Pto*	Kinase	Chen et al. (1998)
Pto kin3	TAGTTCGGACGTTTACAT	*Pto*	Kinase	Pahalawatta and Chen (2005a)
Pto kin2IN	GATGCACCACCAGGGGG	*Pto*	Kinase	Shi et al. (2001)
RLRR Rev	ACACTGGTCCATGAGGTT	*Rps2*	LRR	Chen et al. (1998)
S2	GGIGGIGTIGGIAAIACIAC	*N*, *Rps2*	P-loop	Leister et al. (1996)
Xa1NBS-F	GGCAATGGAGGGATAGG	*Xal*	NBS	Shi et al. (2001)
Xa1NBS-R	CTCTGTATACGAGTTGTC	*Xal*	NBS	Shi et al. (2001)
XLRR For	CCGTTGGACAGGAAGGAG	*Xa21*	LRR	Chen et al. (1998)
XLRR-INV1	TTGTCAGGCCAGATACCC	*Xa21*	LRR	Shi et al. (2001)
XLRR Rev	CCCATAGACCGGACTGTT	*Xa21*	LRR	Chen et al. (1998)
WMS268	L: AGGGGATATGTTGTCACTCCA R: TTATGTGATTGCGTACGTACCC	–	–	Somers et al. 2004
WMS499	L: ACTTGTATGCTCCATTGATTGG R: GGGGAGTGGAAACTGCATAA	–	–	Somers et al. (2004)
WMS604	L: TATATAGTTCAATATGACCCG R: ATCTTTTGAACCAAATGTG	–	–	Somers et al. (2004)
WMS639	L: CTCTCTCCATTCGGTTTTCC R: CATGCCCCCCTTTTCTG	–	–	Somers et al. (2004)
WMC118	L: AGAATTAGCCCTTGAGTTGGTC R: CTCCCATCGCTAAAGATGGTAT	–	–	Somers et al. (2004)
WMC631	L: TTGCTCGCCCACCTTCTACC R: GGAAACCATGCGCTTCACAC	–	–	Somers et al. (2004)

[a] WMS268, WMS499, WMS604, WMS639, WMC118 and WMC631 are SSR primers and the remainder are RGA primers

progenies of the three plants with IT 6 were homozygous susceptible (IT 7–8). In the analysis in Table 3, seven plants were therefore included in the resistant class and three plants were pooled with the susceptible group. F_2 segregation fitted a 15:1 ratio ($P = 0.55$) for resistant and susceptible plants. The test with PST-21 produced similar results (Table 3). These results suggested that Express had two dominant genes for resistance to PST-1 and PST-21. These results were further confirmed by the segregation of F_3 lines into 7:8:1 ratio ($P = 0.52$) and of the F_5 lines in a 175:32:49 ratio ($P = 0.17$) for homozygous resistant, segregating and homozygous susceptible lines.

When inoculated with PST-43, F_1 plants had IT 2, and the F_2 population segregated three resistant: 1 susceptible ($P = 0.29$) without intermediate phenotypes. The segregation among 146 F_3 lines fitted a 1:2:1 ratio ($P = 0.18$) and the 146 F_5 lines segregated 7:2:7 ($P = 0.23$) for homozygous resistant, segregating and homozygous susceptible phenotypic classes. Re-grown plants were tested with PST-45. The results were the same as obtained in the earlier test, thus indicating that resistance to PST-43 and PST-45 was controlled by the same dominant gene.

All 45 F_3 lines resistant to PST-43 and PST-45 were also resistant to PST-1 and PST-21, and all 10 lines susceptible to PST-1 and PST-21 also were susceptible to PST-43 and PST-45. Further, all 74 F_5 lines resistant to PST-43 and PST-45 were also resistant to PST-1 or PST-21, and all 20 lines susceptible to PST-1 and PST-21 were susceptible to PST-43 and PST-45. These results indicated that the gene for resistance to PST-43 and PST-45 also conferred resistance to PST-1 and PST-21. We tentatively designated the gene conferring resistance only to PST-1 and PST-21 as *YrExp1* and the gene conferring resistance to all four races as *YrExp2*.

Table 2 Infection types of the parental genotypes produced by 13 races of *Puccinia striiformis* f. sp. *tritici*

PST race	Virulence formula[a]	Infection type produced on		
		Alpowa	AVS	Express
PST-1	1, 2	2	9	2
PST-3	1, 3	9	9	2
PST-7	1, 3, 5	9	9	2
PST-21	2	2	9	2
PST-23	1, 3, 6, 9, 10	9	9	2
PST-43	1, 3, 4, 5, 12, 14	9	9	2
PST-45	1, 3, 12, 13, 15	9	9	2
PST-58	1, 11, 12, 16	9	9	9
PST-59	1, 3, 11, 12, 16	9	9	9
PST-78	1, 3, 11, 12, 16–20	9	9	9
PST-98	1, 3, 8, 10–12, 16–20	9	9	9
PST-100	1, 3, 8–12,16–20	9	9	9
PST-111	1, 3, 5, 8, 10–12, 16–20	9	9	9

[a] Based on Chen 2005 (2005). *1* Lemhi, *2* Chinese 166, *3* Heines VII, *4* Moro, *5* Paha, *6* Druchamp, *7* the *Yr5* line, *8* Produra, *9* Yamhill, *10* Stephens, *11* Lee, *12* Fielder, *13* Tyee, *14* Tres, *15* Hyak, *16* Express, *17* AVS/6*Yr8, *18* AVS/6*Yr9, *19* Clement and *20* Compair

Identification and mapping of molecular markers associated with resistance

A total of 498 RGA primer pairs were screened for polymorphism between AVS and Express; 128 (25.7%) produced polymorphic bands. Polymorphic RGA primer pairs were tested in the bulk segregant analysis with F_5 lines to identify markers associated with resistance genes. A total of 16 RGAP markers were associated with resistance. Using Mapmaker software, the 16 markers were grouped into two linkage groups. Nine were linked to *YrExp1* and seven were linked to *YrExp2*. All were dominant, except, *Xwgp82* that was co-dominant. RGAP markers *Xwgp78* and *Xwgp82* are shown in Fig. 1 as examples.

RGAP markers *Xwgp74*, *Xwgp75, Xwgp 86* and *Xwgp87* were assayed on CS and its 21 nulli-tetrasomic lines to determine the chromosomes of the two linkage groups. Marker *Xwgp74* was present as an 800-bp band in AVS and CS, but not in Express. Markers *Xwgp75*, *Xwgp86* and *Xwgp87* were represented as bands of 810, 480 and 500 bp, respectively, in both Express and CS, but not in AVS. All nulli-tetrasomic lines, except N1BT1A, showed the 800- and 810-bp bands of *Xwgp74* and *Xwgp75*, indicating that *YrExp1* is located on chromosome 1B. The 480-bp band of *Xwgp86* and 500-bp band of *Xwgp87* were detected in all nulli-tetrasomic lines except N5BT5A, indicating that *YrExp2* was located on chromosome 5B.

Fifteen SSR markers on 1B and 25 on 5B were screened for polymorphism to confirm the chromosome locations. Two polymorphic SSR markers on the long arm of chromosome 1B and four SSR markers on the long arm of chromosome 5B were found to be associated with the RGAP linkage groups.

All dominant markers, including 15 RGAP and one SSR markers, segregated in 9:7 ratios for presence and absence, and six co-dominant markers (one RGAP and five SSR markers) segregated in 7:2:7 ratios for presence of the Express band, both bands, and the AVS band in each case (Table 4). The results indicated that these markers were single copy and should be reliable for constructing the linkage maps.

The RGAP and SSR markers were used to construct linkage groups for the resistance genes (Fig. 2). *YrExp1* mapped proximal to *Xwmc631* outside of the *Xwmc631* - *Xgwm268* interval. *YrExp2* fell in the *Xgwm604* - *Xgwm639* interval. According to Röder et al. (1998) and Somers et al. (2004), markers *Xgwm268* and *Xwmc631* are on the long arm of 1B and *Xgwm604* and *Xgwm639* are on the long arm of 5B; thus *YrExp1* and *YrExp2* should also be on chromosome 1BL and 5BL, respectively.

Relationships of the resistance genes in Express to other genes on chromosomes 1B and 5B

Wheat genotypes Lemhi, AVS/6*Yr9 and Alpowa were reported to have genes *Yr21* on 1B (Pahalawatta and Chen 2005a), *Yr9* on 1RS (1RS/1BL) (Shi et al. 2001) and *YrAlp* on 1BS (Lin and Chen 2007), respectively. In the present study, resistance gene *YrExp1* was mapped on 1BL. In order to determine the relationships of *YrExp1* with *Yr21*, *Yr9* and *YrAlp*, we tested Express, Lemhi, AVS/6*Yr9 and Alpowa with markers flanking *YrExp1* (*Xwmc631* and *Xwgp78*), *Yr9* (*Xwgp1* and *Xwgp15*), *YrAlp* (*Xwgp47* and *Xwgp48*) and markers closely linked to *Yr21* (*M1* and *M2*). The flanking markers for *Yr9* and *YrAlp*, and markers closely linked with *Yr21* were not detected in Express, indicating that *YrExp1* was different from these genes. Eight susceptible plants were observed in the F_2 population of the Alpowa/Express cross, which segregated in a 63R:1S ratio, further confirming that *YrExp1* was different from *YrAlp*.

Genes *Yr19* in Compair (Chen et al. 1995) and *YrDru* in Druchamp (Chen et al. 1996) confering race-specific, overall resistance were reported on chromosome 5B. When the wheat genotypes were amplified with the primers for the *YrExp2* flanking markers (*Xwgp81* and *Xwgp82*), they did not have the marker bands, indicating that *YrExp2* was different from *Yr19* and *YrDru*.

Polymorphism of the *YrExp2* flanking markers in wheat cultivars

A total of 44 wheat cultivars were tested for polymorphism of the RGAP markers (*Xwgp81* and *Xwgp82*)

Table 3 Observed numbers of plants or lines in reaction groups for parents and F_1, F_2, F_3 and F_5 populations of crosses AVS/Express and Alpowa/Express when tested with different races of *Puccinia striiformis* f. sp. *tritici*, expected ratios, numbers of genes and probability values for chi-squared tests of goodness of fit

Cross[a]	Test race	Parent or population	Observed number of plants or lines[b]			Expected ratio Res:Seg:Sus	No. of genes	P[c]
			IT 2–3 (Res.)	IT 4–6 (Seg.)	IT 8–9 (Sus.)			
0AVS/Exp	PST-1	AVS	0	0	13	–		–
		Express	12	0	0	–		–
		F_1	3	0	0	–		–
		F_2	268	10[d]	18	15:1	2	0.55
		F_3	57	79	10	7:8:1	2	0.52
		F_5	110	16	20	175:32:49	2	0.17
	PST-21	AVS	0	0	13	–		–
		Express	12	0	0	–		–
		F_1	3	0	0	–		–
		F_2	271	7[d]	18	15:1	2	0.55
		F_3	57	79	10	7:8:1	2	0.52
		F_5	110	16	20	175:32:49	2	0.17
	PST-43	AVS	0	0	14	–		–
		Express	15	0	0	–		–
		F_1	3	0	0	–		–
		F_2	217	0	83	3:1	1	0.29
		F_3	45	63	38	1:2:1	1	0.18
		F_5	74	15	57	7:2:7	1	0.23
	PST-45	AVS	0	0	14	–		–
		Express	15	0	0	–		–
		F_1	3	0	0	–		–
		F_2	217	0	83	3:1	1	0.29
		F_3	45	63	38	1:2:1	1	0.18
		F_5	74	15	57	7:2:7	1	0.23
Alp/Exp	PST-1	Alpowa	15	0	0	–		–
		Express	13	0	0	–		–
		F_1	3	0	0	–		–
		F_2	294	0	8	63:1	3	0.13
		F_3	95	55	0	37:26:1	3	0.16
	PST-21	Alpowa	15	0	0	–		–
		Express	13	0	0	–		–
		F_1	3	0	0	–		–
		F_2	294	0	8	63:1	3	0.13
		F_3	95	55	0	37:26:1	3	0.16
	PST-43	Alpowa	0	0	14	–		–
		Express	15	0	0	–		–
		F_1	3	0	0	–		–
		F_2	219	0	80	3:1	1	0.48
		F_3	43	79	28	1:2:1	1	0.18
	PST-45	Alpowa	0	0	15	–		–
		Express	15	0	0	–		–
		F_1	3	0	0	–		–
		F_2	216	0	80	3:1	1	0.42
		F_3	43	79	28	1:2:1	1	0.18

[a] *AVS* 'Avocet S'*, *Exp* 'Express' and *Alp* 'Alpowa'

[b] *Res* resistant, *Seg* segregating, and *Sus* susceptible. For parents, F_1 and F_2, the values are numbers of plants. For F_3 and F_5, the values are numbers of lines. There were no plants or lines with IT 0, 1, and 7. The segregating lines included plants with IT 2–3 and 7–8. The F_2 ratios are for resistant and susceptible plants, and the F_3 and F_5 ratios are for homozygous resistant, segregating and homozygous susceptible lines

[c] *P* probability of chi-squared test for goodness of fit

[d] For the chi-squared test, seven plants were placed in the resistant category and three were included in the susceptible group on the basis of progeny test results (see text)

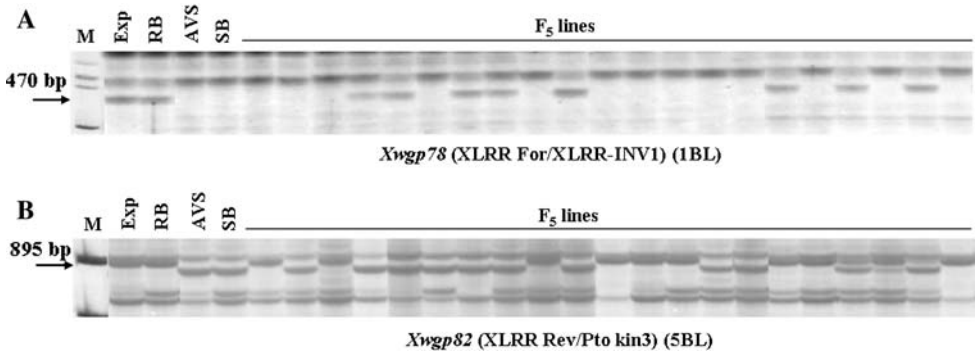

Fig. 1 Polyacrylamide gels showing resistance gene analog polymorphism (*RGAP*) markers *Xwgp78* (**a**) and *Xwgp82* (**b**) in linkage groups 1BL and 5BL, respectively. *M* is 100-bp DNA ladder. *Exp* 'Express'. *AVS* 'Avocet S'. *RB* resistant bulk and *SB* susceptible bulk of selected F_5 lines from AVS/Express

Table 4 Resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) markers linked to overall stripe rust resistance loci and their primer pairs, size, presence (+) and absence (−) in Express, Avocet Susceptible (AVS) and Chinese Spring (CS), and probability (*P*) values for goodness of fit to 9:7 ratios for dominant markers and 7:2:7 ratios for co-dominant markers in the F_5 population of the AVS/Express cross

Marker[a]	Primer pair	Size[b]	Presence (+) or absence (−) of markers in			
		(bp)	Express	AVS	CS	*P*
Xwgp72	Ptokin3/S2	470	+	−	NT[c]	0.42
Xwgp73	Pto kin3/AS1	800	+	−	NT	0.42
Xwgp74	Pto kin2/AS3-INV	800	−	+	+	0.75
Xwgp75	Pto kin2/AS1	810	+	−	+	0.75
Xwgp76	XLRR For/S2	960	+	−	NT	0.42
Xwgp77	XLRR For/AS1	820	+	−	NT	0.63
Xwgp78	XLRR For/XLRR-INV1	470	+	−	NT	0.23
Xwgp79	Pto kin3/XalNBS For	900	+	−	NT	0.63
Xwgp80	XLRR For/PtoFen-AS	720	+	−	NT	0.88
Xwgp81	Pto kin2/RLRR Rev	855	+	−	NT	0.18
Xwgp82	XLRR Rev/Pto kin3	895/880	895	880	NT	0.32
Xwgp83	Pto kin2IN/RLRR Rev	480	+	−	NT	0.23
Xwgp84	NLRR-INV2/XLRR For	485	+	−	NT	0.06
Xwgp85	NLRR-INV2/XLRR For	480	−	+	+	0.60
Xwgp86	Pto kin1/RLRR Rev	550	+	−	NT	0.23
Xwgp87	Pto kin2/Cre3LR For	500	+	−	+	0.23
Xwmc631	WMC631	220/180	220	180	NT	0.52
Xgwm268	WMS268	420/480	420	480	NT	0.53
Xgwm499	WMS499	210/230	210	230	NT	0.49
Xgwm604	WMS604	360/330	360	330	NT	0.16
Xgwm639	WMS639	200/220	200	220	NT	0.11
Xwmc118	WMC118	400	+	−	NT	0.23

[a] All *Xwgp* markers were RGAP markers and *Xgwm* were SSR markers

[b] Fragment size was visually estimated based on the 100-bp DNA ladder

[c] *NT* not tested

flanking *YrExp2* (Table 5). Besides Express, both markers were present in four cultivars or breeding lines (Expresso, Blanca Grande, Jeff Pronto and Buck Proto), suggesting that these cultivars may have *YrExp2*. Six cultivars (Zak, Eden, Lolo, IDO377s, Cashup and Druchamp) had only *Xwgp81* whereas Macon and Waikea had only *Xwgp82*. Thirty-two (73%) had neither marker, suggesting these markers would be useful in marker-assisted selection for combining *YrExp2* with other stripe rust resistance genes.

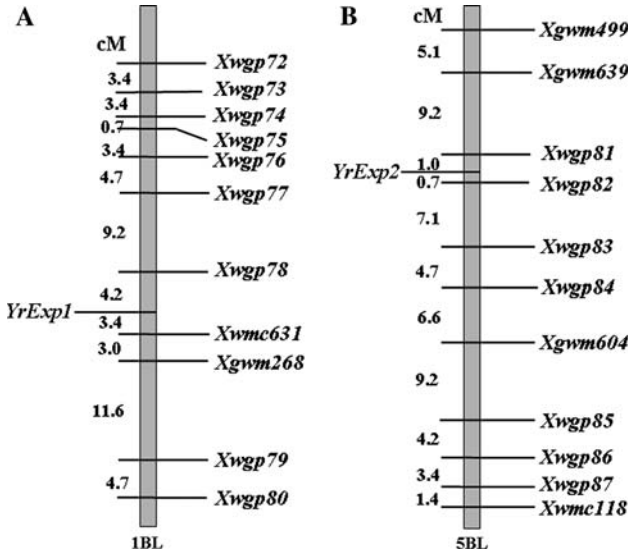

Fig. 2 Linkage maps for *YrExp1* on the long arm of chromosome 1B (**a**) and *YrExp2* on the long arm of chromosome 5B (**b**) based on the AVS/Express mapping population of 146 F_5 lines. All resistant gene analog polymorphism (*RGAP*) markers were present in Express except for *Xwgp74-1B* and *Xwgp86-5B* present in AVS. The linkage groups on 1BL and 5BL were determined by analyzing the nulli-tetrasomic Chinese Spring lines with markers *Xwgp74* and *Xwgp75* on 1B and *Xwgp86* and *Xwgp87* on 5B and confirmed with SSR markers *Xgwm268* and *Xwmc631* on 1BL and *Xgwm499*, *Xgwm604*, *Xgwm639*, and *Xwmc118* on 5BL

Discussion

Among the 20 wheat genotypes that are used to differentiate races of *P. striiformis* f. sp. *tritici* in the United States, Express was the only one without identified resistance genes (Chen 2005). In this study, we determined two genes conferring race-specific, overall stripe rust resistance in Express. Using 13 selected races, we confirmed that Express was resistant to previously identified races when added to the differential set (Line 2002; Chen 2005) and showed that Express is a valuable differential because it has genes different from those in the other 19 differential genotypes (Chen 2005).

Express was developed from the cross Veery/BH1146 (http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=PI+573003). Because 'Veery' has resistance gene *Yr9* in the 1B/1R wheat/rye chromosome translocation (Merker 1982), Express was thought to have *Yr9*. However, races PST-58 and PST-59 that are virulent on Express are not virulent on genotypes with only *Yr9*, even though most US *Yr9*-virulent races detected since 2000 are virulent on Express (Line 2002; Chen 2005). Results from the present study further confirmed that Express does not carry *Yr9*.

Other stripe rust resistance genes on chromosome 1B include *Yr10*, *Yr15*, *Yr21*, *Yr24*, *YrH52* and *YrAlp* in

Table 5 Presence (+) and absence (−) of RGAP markers flanking *YrExp2* in wheat cultivars and genetic stocks

Cultivar/genetic stock		Wheat habit	*YrExp2*	
Name	ID number		*Xwgp81*	*Xwgp82*
Avocet S	WG00001	Spring	−	−
Express	PI 573003	Spring	+	+
Expresso	Expresso	Spring	+	+
Blanca Grande	PI 631481	Spring	+	+
Buck Pronto	T0001052	Spring	+	+
Jeff/Pronto	Jeff/Buck	Spring	+	+
Eden	PI 630983	Spring	+	−
IDO377s	PI 591045	Spring	+	−
Lolo	PI 614840	Spring	+	−
Zak	PI 607839	Spring	+	−
Macon	PI 617072	Spring	−	+
Waikea	BZ 998447	Spring	−	+
Alpowa	PI 566596	Spring	−	−
Alturas	PI 620631	Spring	−	−
Compair	PI 325842	Spring	−	−
Edwall	PI 477919	Spring	−	−
Fielder	CI 017268	Spring	−	−
Hank	PI 613585	Spring	−	−
Jefferson	PI 603040	Spring	−	−
Jerome	IDO00566	Spring	−	−
Lemhi	CI 011415	Spring	−	−
Louise	PI 634865	Spring	−	−
Nick	BZ 698031	Spring	−	−
Otis	PI 634866	Spring	−	−
Produra	CI 017406	Spring	−	−
Scarlet	PI 601814	Spring	−	−
Solano	DA900229	Spring	−	−
Tara 2002	PI 617073	Spring	−	−
UI Cataldo	PI 642361	Spring	−	−
Wakanz	PI 506352	Spring	−	−
Wawawai	PI 574538	Spring	−	−
Cashup	PI 601237	Winter	+	−
Druchamp	CI 013723	Winter	+	−
Barbee	CI 017417	Winter	−	−
Chinese 166	CI 011765	Winter	−	−
Crew	CI 017951	Winter	−	−
Hill 81	CI 017954	Winter	−	−
Hiller	PI 587026	Winter	−	−
Jacmar	PI 608016	Winter	−	−
Moro	CI 013740	Winter	−	−
Omar	CI 013072	Winter	−	−
Paha	CI 014485	Winter	−	−
Rely	PI 542401	Winter	−	−
Riebesel 47–51	PI 295999	Winter	−	−
Rohde	PI 582529	Winter	−	−
Tres	CI 017917	Winter	−	−

Alpowa (Chen 2005; Lin and Chen 2007). *Yr10*, *Yr15*, *Yr24*, *Yr26* and *YrH52* are located on 1BS whereas *YrExp1* is on 1BL. *Yr29* confers non-race specific, adult-plant resistance (William et al. 2003) whereas *YrExp1* is a gene for race-specific, overall resistance. In wheat genotype Lemhi, gene *Yr21* confers resistance only to PST-21 (Chen et al. 1995; Chen 2005), and a closely linked gene, *RpsLem*, confers resistance to some races of *P. striiformis* f. sp. *hordei* (the barley stripe rust pathogen) (Pahalawatta and Chen 2005a). The RGAP markers linked to these genes were absent in Express and markers flanking *YrExp1* were absent in Lemhi (data not shown). *YrExp1* confers resistance to PST-1, to which *Yr21* is not effective, therefore, *YrExp1* and *Yr21* should be different. *YrAlp* in Alpowa confers resistance only to races PST-1 and PST-21 (Lin and Chen 2007); however, this gene is located on 1BS whereas *YrExp1* is on 1BL. The genetic analysis of Alpowa/Express and the reciprocal marker tests also indicated that they are located at different loci.

Two genes, *Yr19* in Compair and *YrDru* in Druchamp, for race-specific resistance to stripe rust were reported on chromosome 5B (Chen et al. 1995, 1996). Several races, including PST-23 used in this study, are virulent on Druchamp but not on Express (Chen 2005). These observations indicate that *YrExp2* on chromosome 5BL is different from *YrDru*. The relationship between *YrExp2* and *Yr19* is less clear because Compair also has *Yr8* and 27 races virulent on Express also are virulent on Compair (Chen 2005). However, 17 races, including PST-58 and PST-59 used in this study, are virulent on Express, but avirulent on Compair. Five races (PST-68, PST-87, PST-104 and PST-106) are virulent on Express and the *Yr8* single gene line, but avirulent on Compair. Further, race PST-121, detected in 2005, is virulent on Compair and avirulent on Express (Chen 2007). Moreover, the *YrExp2* flanking markers were not present in Compair and indicated that *YrExp2* and *Yr19* are different. Based on chromosomal locations, race reactions, and presence/absence of molecular markers, the two genes in Express are different from previously reported genes. Further studies are needed to determine the genetic distances between *YrExp1* and those genes on 1BL and between *YrExp2* and those on 5BL.

Since *YrExp2* confers resistance to more races than *YrExp1* (the latter conferring resistance only to the widely avirulent races PST-1 and PST-21), *YrExp2* is more useful than *YrExp1* for developing resistant cultivars. As *YrExp2* confers race-specific overall resistance and is not effective against the current US. *P. striiformis* f. sp. *tritici* population, it should be used in combination with other effective genes, especially non-race specific HTAP resistance genes.

One of the major obstacles of marker-assisted selection is lack of polymorphism at marker loci. To determine how useful markers flanking *YrExp2* in a wide range of wheat genetic backgrounds, we tested 23 spring and 21 winter wheat genotypes. Among the spring wheat genotypes, Expresso, Blanca Grande, Buck Pronto and Jeff/Pronto had both flanking marker bands, indicating that these genotypes may have *YrExp2*. The presence of the markers in Expresso was expected because it was developed from Express. However, the presence of *YrExp2* in the other three spring wheat cultivars could not be determined because of lack of their pedigree information. In a seedling test of spring wheat germplasms with six races (PST-17, PST-37, PST-43, PST-45, PST-100, and PST-116), all four cultivars and Express were resistant to PST-43 and PST-45 (data not shown), to which *YrExp2* is effective as shown in the present study. While the results suggest that these cultivars may have *YrExp2*, data are not conclusive because some of them were also resistant to races that were virulent on Express, indicating the presence of additional or different resistance genes. The absence of both tightly linked markers in 32 (73%) and one of the markers in eight (18%) of surveyed wheat genotypes indicated potential usefulness of these markers in combining *YrExp2* with other genes in wheat cultivars.

Acknowledgments This research was supported by the US Department of Agriculture, Agricultural Research Service, Vogel Foundation, and Washington Wheat Commission. PPNS no. 0466, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences Research Center, project no. 13Z-3061-3824 and 13C-3061-3923, Washington State University, Pullman, WA 99164-6430, USA. We are grateful to Dr. Robert A. McIntosh and Dr. Jayaveeramuthu Nirmala for their critical review of the manuscript.

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