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ORIGINAL PAPER

Yr45, a new wheat gene for stripe rust resistance on the long arm of chromosome 3D

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Abstract Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive diseases of wheat worldwide. Growing resistant cultivars is the most effective approach to control the disease, but only a few genes confer effective all-stage resistance against the current populations of the pathogen worldwide. It is urgent to identify new genes for diversifying sources of resistance genes and for pyramiding genes for different types of resistance in order to achieve high levels of durable resistance for sustainable control of stripe rust. The common spring wheat genotype 'PI 181434', originally from Afghanistan, was resistant in all greenhouse and field tests in our previous studies. To identify the resistance gene(s) PI 181434 was crossed with susceptible genotype 'Avocet Susceptible'. Adult plants of 103 F_2 progeny were tested in the field under the natural infection of *P. striiformis* f. sp. *tritici*. Seedlings of the parents, F_2 and F_3 were tested with races PST-100 and PST-127 of the pathogen under controlled greenhouse conditions. The genetic study showed that PI 181434 has a single dominant gene conferring all-stage resistance. Resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) techniques were used to identify molecular markers linked to the gene. A linkage map of 8 RGAP and 2 SSR markers was constructed for the gene using data from the 103 F_2 plants and their derived F_3 lines tested in the greenhouse. Amplification of the complete set of nulli-tetrasomic lines and selected ditelosomic lines of Chinese Spring with an RGAP marker and the two SSR markers mapped the gene on the long arm of chromosome 3D. Because it is the first gene for stripe rust resistance mapped on chromosome 3DL and different from all previously named *Yr* genes, the gene in PI 181434 was designated *Yr45*. Polymorphism rates of the two closest flanking markers, *Xwgp115* and *Xwgp118*, in 45 wheat genotypes were 73.3 and 82.2%, respectively. Single nucleotide polymorphisms (SNPs) were identified in the eight wheat genotypes sharing both flanking markers. The RGAP markers and potential SNP markers should be useful in incorporating the gene into wheat cultivars and in pyramiding it with other genes for durable resistance.

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Introduction

Wheat stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is a major disease of wheat (*Triticum aestivum* L.) worldwide (Stubbs 1985; Singh et al. 2000; Chen 2005, 2007). In the USA, the disease is most destructive in the western states and has become increasingly important in the south-central states in the past decade (Line and Chen 1996; Chen et al. 2002; Chen 2005, 2007). Growing resistant cultivars is

the most economic, effective and environment-friendly approach to control the disease (Line and Chen 1995; Chen 2005).

Stripe rust resistance mainly includes all-stage resistance (also called seedling resistance) and high-temperature adult-plant (HTAP) resistance (Qayoum and Line 1985; Chen and Line 1995a, b; Milus and Line 1986a, b; Chen 2005). All-stage resistance, which is often conferred by single genes, can be detected at the seedling stage, but is expressed in all growth stages and is usually race specific. A single-gene controlling all-stage resistance can generally provide complete resistance, but the resistance does not last very long due to the emergence of virulence in the pathogen population. On average, cultivars with all-stage resistance remain resistant for only 3 years in production (Chen 2005). In contrast, HTAP resistance is expressed in the later stages of plant growth when the weather becomes warm. This type of resistance is generally non-race specific, often quantitatively inherited, and durable (Line 2002; Chen 2005). Because HTAP resistance is usually partial, it may be inadequate if temperatures are too low for resistance to fully express. The best approach is to combine both HTAP and all-stage resistance genes to achieve complete and long-lasting protection of cultivars from stripe rust.

A number of stripe rust resistance genes have been identified and sorted into these two classes. To date, more than 70 stripe rust resistance genes with official and provisional designations have been reported in wheat (Chen 2005; McIntosh et al. 2007, 2009; Cheng and Chen 2010). The majority of these genes are race specific and virulences to them were detected in various parts of the world. Only a couple of genes, *Yr5* and *Yr15*, are effective against all races identified so far in the United States (Chen 2005, 2007; Chen et al., unpublished data). However, virulence to *Yr5* has been reported in India and Australia (Nagarajan et al. 1986; Wellings and McIntosh 1990). As cultivars with these genes are put into production, virulences to the genes are expected to emerge and spread. Therefore, it is urgent to identify new genes for effective resistance to stripe rust and to develop molecular markers for efficient incorporation and pyramiding of new genes into wheat cultivars.

PI 181434 is a spring wheat genotype originally from Afghanistan. It was resistant in germplasm screening nurseries planted in eastern and western Washington from 2004 to 2009 and in seedling tests in 2006 with five selected races covering all possible virulences up to that time under controlled greenhouse conditions. The objectives of this study were to identify the gene(s) conferring all-stage resistance in PI 181434 through genetic analysis and molecular mapping and to determine the usefulness of markers flanking the resistance gene.

Materials and methods

Plant materials

To study the genetics of all-stage resistance in PI 181434, the genotype was crossed with "Avocet Susceptible" (AvS). AvS is a spring wheat originally provided by Dr. Colin Wellings, Plant Breeding Institute, University of Sydney, Australia. It is susceptible to most races of *P. striiformis* f. sp. *tritici*. F_2 and $F_{2:3}$ populations were used in genetic analysis. F_2 plants tested in the field were used to map the resistance gene in PI 181434. Chinese Spring (CS) and its complete set of 21 nulli-tetrasomic lines and selected ditelosomic lines were used to determine the chromosomal location of the resistance gene. In addition to PI 181434 and AvS, 45 wheat cultivars and breeding lines, that do not have PI 181434 in their pedigree, were used to test for polymorphisms of molecular markers flanking the resistance gene.

Resistance evaluation in the greenhouse

Seedling tests were conducted under controlled greenhouse conditions as described by Chen and Line (1992a, b). Seven races (PST-17, PST-37, PST-43, PST-45, PST-78, PST-100 and PST-127) were used to test the responses of PI 181434 and AvS. These races collectively cover all possible virulences identified so far (Chen 2005, 2007; Chen et al., unpublished data). PST-100 has been the most predominant race in the USA since 2005 (Chen 2007) and PST-127 is the most widely virulent race with virulences to 17 of the 20 differential genotypes (Chen et al., unpublished data). Thus, PST-100 and PST-127 were used to test F_2 and $F_{2:3}$ populations derived from AvS/PI 181434.

Seedlings of parents and F_2 and $F_{2:3}$ populations were grown in the greenhouse. About 15 seeds of each parent, 120 F_2 seeds and 15 seeds for each of the 89 $F_{2:3}$ lines were planted in plastic pots (5 × 5 × 5 cm) filled with a potting mixture (6 L peat moss, 2 L perlite, 3 L sand, 3 L potting soil mix, 4 L vermiculite with lime, 1.5 cups Osmocote® and 2 L water).

Inoculation was conducted at the two-leaf stage as described by Chen and Line (1992a, b). Inoculated seedlings were kept in a dew chamber at 10°C for about 24 h without light, and then grown in a growth chamber operating at 16 h light and 8 h dark with diurnal temperatures gradually changing from 4°C at 2:00 am to 20°C at 2:00 pm. Infection type (IT) data were recorded 18–21 days after inoculation based on the 0–9 scale described by Line and Qayoum (1992).

Resistance evaluation in field

During the 2008 crop season, the parents and F_2 plants were grown in a field near Pullman, Washington, to evaluate

reactions to stripe rust under natural infection of *P. striiformis* f. sp. *tritici*. In April 2008, about 20 seeds of each parent were planted in 1 m rows and 150 F_2 seeds were space-planted in 1 m rows with 6 seeds in each row and 20 cm between rows. The plots were surrounded by susceptible spring wheat Lemhi as a spreader. After disease development, infection types were recorded at the soft dough stage based on a 0–9 scale similar to that described for seedling tests. $F_{2:3}$ seeds were harvested from the F_2 plants for testing $F_{2:3}$ lines in the greenhouse as described above.

DNA extraction, PCR amplification, electrophoresis and gel visualization

At the early jointing stage (Zadoks GS 30), one or two leaf pieces for each F_2 plant and parents were collected for DNA extraction. Genomic DNA was isolated from the leaves using the CTAB method (Yan et al. 2003), dissolved in 1× TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0), and stored at −20°C. DNA was quantified using the mini-gel method and spectrophotometer. DNA concentration was adjusted to 30 ng/μL for use in PCR tests.

RGAP (Chen et al. 1998; Shi et al. 2001) and SSR methods (Röder et al. 1998) were used to identify markers linked to the resistance gene. PCR reactions were performed in a GeneAmp® PCR System 9700 thermo-cycler. For each PCR reaction, the 15 μL reaction mixture contained 30 ng of template DNA, 3.0 μL Mg-free 5× PCR buffer (Promega, Madison, WI, USA), 0.6 unit of Taq DNA polymerase (Promega), 5 mM of $MgCl_2$ (Promega), 0.25 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). PCR amplifications were conducted with 5 min of denaturation at 94°C, then amplifications were programmed for 45 cycles, each consisting of 1 min at 94°C, 1 min at either 45, 55 or 60°C (45°C for RGA primers, 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 products. After 4 min denaturation at 94°C, 4 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 for visualization (Chen et al. 1998).

Bulk segregant analysis

Based on phenotypic data, DNA bulks were constructed to screen the markers linked to the resistance gene locus. For DNA bulks, the ten most resistant and ten most susceptible F_2 plants, which were confirmed as homozygous resistant or homozygous susceptible by progeny testing the $F_{2:3}$ lines in the greenhouse, were chosen to construct the corresponding bulks by mixing equal amounts of DNA from each selected plant. A total of 528 RGA primer pairs were screened on the parents and bulks. Primer pairs generating bands specific to both PI 181434 and the resistant bulk, or AvS and the susceptible bulk, were used to genotype individual F_2 plants.

Chromosome localization of the resistance gene

A complete set of 21 nulli-tetrasomic lines of and the 3D ditelosomic lines of Chinese Spring were used to localize RGAP markers on wheat chromosomes using procedures previously described (Shi et al. 2001; Lin and Chen 2007, 2008, 2009; Sui et al. 2009; Cheng and Chen 2010). A total of 11 SSR markers specific to the long arm of chromosome 3D were screened to confirm the chromosome and further define the chromosomal region for the gene. Wheat SSR primers were synthesized according to the sequences published in the GrainGenes database (http://www.wheat.pw.usda.gov).

Statistical analysis and genetic mapping

Chi-squared (χ^2) tests were used to evaluate the goodness of fit of observed and expected segregation ratios for stripe rust reactions and molecular markers. The "chitest" procedure in the Excel data analysis of Microsoft Office 2007 was used to calculate *P* values. Linkage analysis was conducted using Mapmaker 3.0b (Lincoln et al. 1992). The Kosambi (Kosambi 1944) function was used to convert recombination values to map distance, and a LOD score of 3.0 was used as a threshold for the declaration of linkage.

Determination of sequence polymorphisms for flanking markers

Two flanking marker bands from PI 181434 and those with the same or similar sizes from eight spring wheat cultivars were sequenced to identify sequence polymorphism. The bands subjected to cloning and sequencing were excised from dried polyacrylamide gels after applying a drop of sterile water. The excised bands were soaked in 5 μL of H_2O for at least 1 h, and the solutions were used as the template DNA for re-amplification with the original RGA primers. The re-amplification products were tested in 2% agarose gels and the single band with the same size as the original band was cut and purified to get the target DNA using a TIANgel Midi Purification Kit (Tiangen Biotech Co. Ltd., Beijing). Three microliters of target DNA was

used for cloning into vector pGM-T (Tiangen Biotech Co. Ltd., Beijing) following the procedure recommended by the manufacturer. Plasmid DNA from ten single colonies derived from each cloning reaction was examined in a 2% agarose gel to determine the size of the inserted fragment. Positive clones with the expected insert size were sequenced using the universal primer T7 (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai) and an ABI 3130-XL DNA sequencer.

Results

Inheritance of stripe rust resistance in PI 181434

PI 181434 was resistant in seedling tests with tiny necrotic spots (IT 2) whereas AvS was susceptible to all seven races (Table 1) in each case with abundant sporulation with or without a chlorotic background (IT 8 or 9). PI 181434 showed IT 1–2 at the flowering to soft dough stages (Zadoks GS 50-70) in the Pullman and Mt. Vernon locations from 2004 to 2009. Therefore, PI 181434 has high-level all-stage resistance to the USA *P. striiformis* f. sp. *tritici* population.

As shown in Table 2, the F_2 segregation ratio indicated that resistance to both PST-100 and PST-127 was controlled by a single dominant gene. In the 2008 field test, the spaced planting of about 150 F_2 seeds produced 103 F_2 plants for recording stripe rust reactions, of which 77 were resistant (IT 1–2) and 26 susceptible (IT 8–9), a 3:1 ratio ($\chi^2 = 0.003$, $P = 0.95$) (Table 2). These results indicated that resistance in PI 181434 might be controlled by a single dominant gene.

Of the 103 F_2 plants, we harvested $F_{2:3}$ seeds from 89 plants. The 89 $F_{2:3}$ lines were tested with PST-100 and PST-127 under controlled greenhouse conditions (Table 2). The tests with both races produced identical results confirming segregation at a single locus. The 20 homozygous susceptible lines were derived from susceptible F_2 plants and the 21 resistant and 48 segregating lines were from resistant F_2 plants. The results indicated that a gene in PI 181434 conferred resistance to both PST-100 and PST-127, and that the phenotypic data were reliable for molecular mapping.

Molecular mapping of the stripe rust resistance gene in PI 181434

Seven RGA primer pairs that generated eight robust and repeatable polymorphic bands in bulked segregant analysis were selected to test the 103 F_2 plants. Seven of the RGAP markers were present in PI 181434 and one in AvS (Table 3). As an example, the presence of marker *Xwgp118* in the resistant parent and resistant bulk, absence in the susceptible parent and susceptible bulk, and segregation in the F_2 population are shown in Fig. 1a.

To determine the chromosome location, all eight RGAP markers were tested with CS and the parents and bulks. *Xwgp114*, present in CS as the same size band as in PI 181434, was used to test the CS nulli-tetrasomic lines. The band was present in all lines except nullisomic 3D tetrasomic 3B, indicating that the resistance gene was on chromosome 3D (Fig. 1b). When CS ditelosomic lines Dt3DL and Dt3DS were tested with the marker, only Dt3DL had the band, showing that the marker and linked resistance gene were located on the long arm of

Table 1 Infection types on wheat genotypes PI 181434 and Avocet S tested with races of *Puccinia striiformis* f. sp. *tritici* in the seedling and adult-plant stages

PST race	Virulence formula[a]	Infection type	
		PI 181434	Avocet S
PST-17	1, 2, 3, 9, 11	2	8
PST-37	1, 3, 6, 8, 9, 10, 11, 12	2	9
PST-43	1, 3, 4, 5, 12, 14	2	9
PST-45	1, 3, 12, 13, 15	2	9
PST-78	1, 3, 11, 12, 16, 17, 18, 19, 20	2	9
PST-100	1, 3, 8, 9, 10, 11, 12, 16, 17, 18, 19, 20	2	9
PST-127	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20	2	9
2008 field test		2	8

Numbers listed indicate the host lines to which those races were virulent

[a] Formulae were based on wheat genotypes used to differentiate races of *P. striiformis* f. sp. *tritici*: 1 = Lemhi, 2 = Chinese 166, 3 = Heines VII, 4 = Moro, 5 = Paha, 6 = Druchamp, 7 = Yr5AvSNIL, 8 = Produra, 9 = Yamhill, 10 = Stephens, 11 = Lee, 12 = Fielder, 13 = Tyes, 14 = Tres, 15 = Hyak, 16 = Express, 17 = Yr8AvSNIL, 18 = Yr9AvSNIL, 19 = Clement and 20 = Compair (Chen 2005)

Table 2 Segregation of stripe rust reactions in F_2 and $F_{2:3}$ progeny of Avocet S × PI 181434

Parent/progeny (race)	Observed no. of plants or lines			Expected ratio	χ^2	*P*
	Res	Seg	Sus			
Seedling testing in greenhouse						
PI 181434 (PST-100)	15	0	0			
Avocet S (PST-100)	0	0	14			
F_2 (PST-100)	58	–	23	3:1	0.50	0.48
$F^a_{2:3}$ (PST-100, PST-127)[b]	21	48	20	1:2:1	0.57	0.75
Adult-plant testing in field						
F_2	77	–	26	3:1	0.003	0.95

[a] The $F_{2:3}$ lines were derived from the F_2 plants in the field

[b] Races PST-100 and PST-127 were used separately to test the $F_{2:3}$ population and produced identical results

Table 3 Molecular markers linked to the gene for resistance to stripe rust in wheat genotype PI 181434, primer pairs, size and presence or absence in PI 181434, Avocet Susceptible (AvS) and Chinese Spring (CS), number of F_2 plants with or without the makers, and χ^2-tests to determine their single-locus inheritance

Marker[a]	Primer pair[b]	Size (bp)[c]	Presence (+) and absence (−) of the marker			No. of F_2 plants with or without the marker		χ^2	*P*
			PI 181434	AvS	CS	With	Without		
Xwgp111	Xa1NBS-F/Pto kin3	590	+	−	−	72	31	1.43	0.23
Xwgp112	Xa1NBS-F/Pto kin3	600	−	+	−	71	32	2.02	0.15
Xwgp113	Pto kin2/AS1-INV	720	+	−	−	69	34	3.52	0.06
Xwgp114	Pto kin 4/XLRR-INV2	680	+	−	+	73	30	0.94	0.33
Xwgp115	Pto kin4/NLRR-INV2	492	+	−	−	76	27	0.08	0.78
Xwgp116	XLRR Rev/LM637	220	+	−	−	76	27	0.08	0.78
Xwgp117	RLRR Rev/Pto kin2	760	+	−	−	71	32	2.02	0.15
Xwgp118	RLRR Rev/Pto kin 1IN	411	+	−	−	75	28	0.21	0.61
Xwmc656	WMC656 F/R	280/290	280	290	NT	84	19	2.36	0.12
Xbarc6	BARC6 F/R	360/365	360	365	NT	70	33	2.72	0.10

[a] *Xwmc656* and *Xbarc6* are SSR primers; the others are RGAP markers

[b] The sequences and characteristics of the RGA primers were previously described (Kanazin et al. 1996; Chen et al. 1998; Shi et al. 2001; Pahalawatta and Chen 2005; Lin and Chen 2007) and those of the SSR primers were described by Somers et al. (2004)

[c] The sizes of markers *Xwgp115* and *Xwgp118* were based on sequencing and those of the others were estimated using DNA size markers

chromosome 3D (Fig. 1c). As it is the first stripe rust resistance gene to be located on chromosome 3DL, the gene was designated as *Yr45*.

Of the 11 screened SSR markers specific to chromosome 3DL, markers *Xbarc6* and *Xwmc656* were polymorphic and linked to the resistance gene, confirming that the resistance gene was located on 3DL. Both SSR markers were co-dominant with the lower bands present in PI 181434. Presence in the resistant parent and bulk, absence in the susceptible parent and bulk and segregation of *Xbarc6* in the F_2 progeny are shown in Fig. 1d as an example. All markers, including eight RGAP markers and two SSR markers, segregated in 3:1 ratios for presence and absence (Table 3), indicating that these markers were single-locus markers.

The 8 RGAP and 2 SSR markers were used to construct a linkage map for *Yr45*; the map spanned 55.3 cM (Fig. 2). SSR markers *Xbarc6* and *Xwmc656* were 11.7 and 12.6 cM, respectively, from the resistance locus and located on the same side. Two flanking RGAP markers, *Xwgp115* and *Xwgp118*, were 5.8 and 4.8 cM from the gene, respectively, potentially more useful for marker-assisted selection.

Polymorphism of flanking markers for *Yr45* in other wheat genotypes

To determine the usefulness of the flanking markers, *Xwgp115* and *Xwgp118*, in marker-assisted selection, 30 spring wheat and 15 winter wheat genotypes were tested for polymorphism (Table 4). Of the 45 genotypes, 33 (73%) did not have the *Xwgp115* band and 37 (82%) did not have the *Xwgp118* band. Three spring cultivars (Alturas, Nick and Otis) and one winter cultivar (Paha) had only the *Xwgp115* band. For such cultivars, *Xwgp118* could be used. However, *Xwgp115* and *Xwgp118* were present in

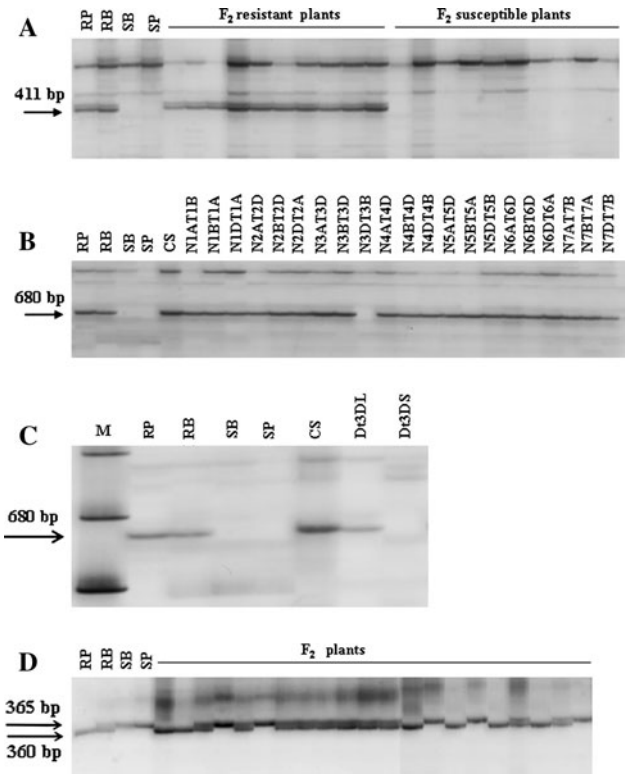

Fig. 1 Silver-stained polyacrylamide gels showing resistance gene analog polymorphism (RGAP) marker *Xwgp118* (**a**), the presence and absence of RGAP marker *Xwgp114* in the 21 Chinese Spring nulli-tetrasomic lines (**b**) and ditelosomic lines (**c**), which mapped the resistance gene in PI 181434 to wheat chromosome 3D and 3DL, respectively, and simple sequence repeat (SSR) marker *Xbarc6* (**d**). *RP* resistant parent (PI 181434), *RB* resistant bulk, *SB* susceptible bulk of F_2 plants, *SP* susceptible parent (Avocet Susceptible), *CS* Chinese Spring. *Dt3DL* ditelosomic 3DL, *Dt3DS* ditelosomic 3DS and *M* molecule size marker

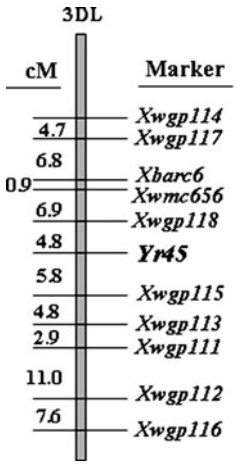

Fig. 2 A linkage map for *Yr45* on chromosome 3DL based on 103 F_2 plants from AvS/PI181434. All markers were RGAP markers except *Xwmc656* and *Xbarc6* were SSR markers. All RGAP markers were present in PI 181434 except for *Xwgp112* that was present in AvS

eight spring genotypes (Hank, Jeff Pronto, Jefferson, Jerome, Lolo, Tara 2002, UI Cataldo and Weikea). Thus, these RGAP markers could not be directly used in marker-assisted selection for *Yr45* in crosses of these genotypes with PI 181434 as donor due to lack of polymorphism of the markers in these cultivars.

Because the eight spring wheat genotypes are susceptible to PST-127 (data not shown), they cannot have *Yr45*. Comparison of the sequences of the bands from the eight genotypes with the *Xwgp115* and *Xwgp118* sequences revealed polymorphisms among the genotypes and PI 181434 (Fig. 3). Along the 492 bp of *Xwgp115*, single nucleotide polymorphisms (SNP) were found at 13 sites (2.64% polymorphism) and along the 411 bp of *Xwgp118*, there were 11 SNPs (2.68% polymorphism). In the case of *Xwgp118*, Hank, Tara 2002 and Waikea had the same sequence as PI 181434, and at least one SNP was found in other genotypes. There were at least two SNPs for each of the eight genotypes compared to the *Xwgp115* sequence in PI 181434. These results confirmed that the eight genotypes were unlikely to have *Yr45* and that it could be incorporated into such cultivars using SNPs in marker-assisted selection.

Discussion

We identified an effective stripe rust resistance gene in wheat genotype PI 181434 and mapped it to the long arm of chromosome 3D. To date, there are more than 40 permanently designated *Yr* genes and many others with temporary designations (Chen 2005; McIntosh et al. 2007; Sui et al. 2009; Cheng and Chen 2010). Because no previously named *Yr* gene was located to chromosome 3DL, the gene in PI 181434 was designated *Yr45*. This gene should be useful in developing cultivars with effective resistance to stripe rust.

The combined RGAP, nulli-tetrasomic lines and chromosome-specific SSR marker approach to genetic analysis is efficient for identifying and mapping genes to particular chromosomal regions. Chen and associates have used this approach to map several genes and QTLs for resistance to stripe rust in wheat (Shi et al. 2001; Yan et al. 2003; Lin and Chen 2007; Cheng and Chen 2010) and in barley (Toojinda et al. 2000; Castro et al. 2002; Yan and Chen 2006, 2007, 2008). In the present study, we first identified the RGAP marker *Xwgp114* linked to the resistance gene and present in CS. This marker was located in CS using CS nulli-tretrasomic and ditelosomic lines, thus locating *Yr45* on chromosome 3DL. *Yr45* was then located to a 10.6-cM region flanked by two RGAP markers identifiable by length polymorphisms or by SNPs. Although virulence has not been identified in the USA, the *Yr45* resistance gene should be considered race specific. The donor of the gene could be tested in the region of its origin in central Asia and other countries to determine if virulence is present. Even for countries or regions, such as the USA, where virulence to

Table 4 Polymorphism of RGAP markers *Xwgp115* and *Xwgp118* in wheat lines not possessing *Yr45*

Genotype		Habit	Presence (+) and absence (−) of marker	
Name	ID number		*Xwgp115*	*Xwgp118*
Alpowa	PI 566596	Spring	−	−
Avocet S	WG00001	Spring	−	−
Blanc Grande	PI 631481	Spring	−	−
Buck Pronto	T0001052	Spring	−	−
Compair	PI 325842	Spring	−	−
Edwall	PI 477919	Spring	−	−
Express	PI 573003	Spring	−	−
Expresso	Expresso	Spring	−	−
Eden	PI 630983	Spring	−	−
Fielder	CI 017268	Spring	−	−
Lemhi	CI 011415	Spring	−	−
Louise	PI 634865	Spring	−	−
Macon	PI 617072	Spring	−	−
Produra	CI 017406	Spring	−	−
Scarlet	PI 601814	Spring	−	−
Solano	DA 900229	Spring	−	−
Wakanz	PI 506352	Spring	−	−
Wawawai	PI 574538	Spring	−	−
Zak	PI 607839	Spring	−	−
Alturas	PI 620631	Spring	+	−
Nick	BZ 698031	Spring	+	−
Otis	PI 634866	Spring	+	−
Hank	PI 613585	Spring	+	+
Jeff Pronto	Jeff/Buck	Spring	+	+
Jefferson	PI 603040	Spring	+	+
Jerome	IDO00566	Spring	+	+
Lolo	PI 614840	Spring	+	+
Tara 2002	PI 617073	Spring	+	+
UI Cataldo	PI 642361	Spring	+	+
Weikea	BZ 998447	Spring	+	+
Barbee	CI 017417	Winter	−	−
Cashup	PI 601237	Winter	−	−
Chinese 166	CI 011765	Winter	−	−
Crew	CI 017951	Winter	−	−
Druchamp	CI 013723	Winter	−	−
Hill 81	CI 017954	Winter	−	−
Hiller	PI 587026	Winter	−	−
Jacamar	PI 608016	Winter	−	−
Moro	CI 013740	Winter	−	−
Omar	CI 013072	Winter	−	−
Rely	PI 542401	Winter	−	−
Riebesel 47–51	PI 295999	Winter	−	−
Rohde	PI 582529	Winter	−	−
Tres	CI 017917	Winter	−	−
Paha	CI 014485	Winter	+	−

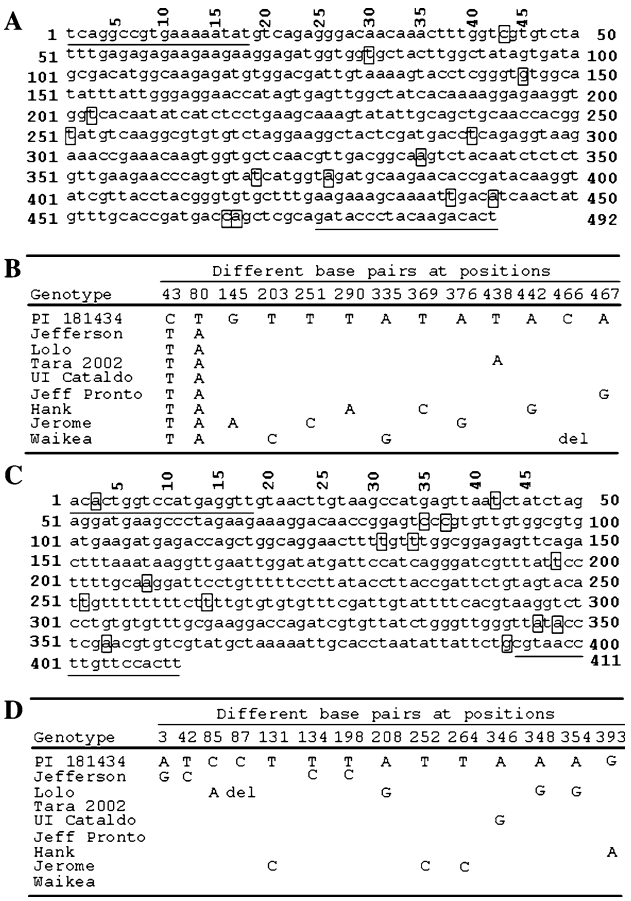

Fig. 3 DNA sequences and sequence polymorphisms of RGAP markers *Xwgp115* (**a** and **b**) and *Xwgp118* (**c** and **d**) flanking *Yr45* in wheat genotype PI 181434 and the same sized fragments in eight other spring wheat cultivars. The nucleotides that were different in some of the eight cultivars are *marked with a box*. The NLRR IN2 primer for *Xwgp115* (**a**) and the RLRR Rev primer for *Xwgp118* (**c**) are *underlined* from the 5′ ends of the sequences, while the Pto kin4 primer for *Xwgp115* (**a**) and the Pto kin1IN primer for *Xwgp118* (**c**) are *underlined* from the 3′ends, respectively. *del* Deletion

Yr45 has not been found, the gene should be used in combination with other genes for effective all-stage resistance or non-race specific HTAP resistance for high level and durable resistance.

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