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Molecular mapping of a gene for stripe rust resistance in spring wheat cultivar IDO377s

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Abstract Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat worldwide. The best strategy to control stripe rust is to grow resistant cultivars. One such cultivar resistant to most races in North America is 'IDO377s'. To study the genetics of its resistance this spring wheat cultivar was crossed with 'Avocet Susceptible' (AvS). Seedlings of the parents, F_2 plants, and F_3 lines were tested under controlled greenhouse conditions with races PST-43 and PST-45 of *P. striiformis* f. sp. *tritici*. IDO377s carries a single dominant gene for resistance. Resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) techniques were used to identify molecular markers linked to the resistance gene. A total of ten markers were identified, two of which flanked the locus at 4.4 and 5.5 cM. These flanking RGAP markers were located on chromosome 2B with nulli-tetrasomic lines of 'Chinese Spring'. Their presence in the ditelosomic 2BL line localized them to the long arm. The chromosomal location of the resistance gene was further confirmed with two 2BL-specific SSR markers and a sequence tagged site (STS) marker previously mapped to 2BL. Based on the chromosomal location, reactions to various races of the pathogen and tests of allelism, the IDO377s gene is different from all previously designated genes for stripe rust resistance, and is therefore designated *Yr43*. A total of 108 wheat breeding lines and cultivars with IDO377s or related cultivars in their parentage were assayed to assess the status of the closest flanking markers and to select lines carrying *Yr43*. The results showed that the flanking markers were reliable for assisting selection of breeding lines carrying the resistance gene. A linked stripe rust resistance gene, previously identified as *YrZak*, in cultivar Zak was designated *Yr44*.

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Introduction

Stripe rust (also known as yellow rust) is a disease of wheat, barley, rye and 59 additional grass species (Line 2002). Wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., has been reported in over 60 countries (Chen 2005). Although fungicides have proven effective for stripe rust control since their first use in North America in 1981 (Line 2002), the application of fungicides adds a significant extra cost to wheat production. The best strategy to control stripe rust is to grow resistant cultivars (Chen 2005).

Resistance to stripe rust can be separated into two categories: all-stage resistance (also called seedling resistance) and adult-plant resistance such as high temperature, adult-plant (HTAP) resistance. All-stage resistance is generally race-specific and can be detected at the seedling stage, but is also expressed at all growth stages (Chen 2005). Cultivars with all-stage resistance are almost always overcome by the pathogen soon after they have been commercialized, either as a consequence of selection of

previously rare variants or the occurrence of new virulences by mutation or somatic recombination (Wellings and McIntosh 1990; Line and Qayoum 1992; Chen 2005, 2007). In contrast, HTAP resistance is non-race specific, durable, and often quantitatively inherited (Qayoum and Line 1985; Chen and Line 1995a, b; Line 2002; Chen 2005). More than 70 stripe rust resistance genes, officially or provisionally designated *Yr* for yellow rust, have been identified in wheat (reviewed by Chen 2005). Molecular markers have been developed for many stripe rust resistance genes and quantitative trait loci (QTL) and have been used in marker-assisted selection for developing resistant cultivars (Chen 2005). New sources of resistance are required for developing resistant cultivars and are also very useful for characterizing the population dynamics of the stripe rust pathogen.

'IDO377s' (PI 591045), a hard white spring wheat cultivar (Souza et al. 1997), was released in 1994 by the southern Idaho breeding program. The cultivar has been grown in the Pacific Northwest and used in breeding programs. Greenhouse and field tests showed that IDO377s is resistant to most PST races (Chen et al. unpublished data). The cultivar was highly resistant to stripe rust until 2005 when susceptible reactions were first observed in experimental plots. However, it has never been severely affected and continues to be grown while the race(s) virulent on it remain at a low frequency (Chen, unpublished data).

Here, we demonstrate the presence of a single gene for stripe rust resistance in IDO377s, and identify molecular markers closely linked to it.

Materials and methods

Plant materials

IDO377s was crossed with Australian spring wheat selection 'Avocet Susceptible' (AvS). IDO377s confers a high level of resistance to most North American races of *P. striiformis* f. sp. *tritici*, whereas AvS is susceptible to most. About 120 F_2 seeds from a single F_1 plant were planted in the greenhouse to get 114 F_3 lines. The parents and F_1, F_2 and F_3 lines were used in seedling tests in the greenhouse to determine the inheritance of all-stage resistance to stripe rust. Leaves of each parent and the 114 F_3 lines were collected for DNA extraction. A total of 108 Pacific Northwest wheat breeding lines and cultivars with or without IDO377s in their pedigrees were used to validate flanking markers and determine their polymorphisms.

A complete set of 21 nulli-tetrasomic (N1AT1B, N1BT1A, N1DT1A, N2AT2D, N2BT2D, N2DT2A, N3AT3D, N3BT3D, N3DT3B, N4AT4D, N4BT4D, N4DT4B, N5AT5D, N5BT5A, N5DT5B, N6AT6D, N6BT6D, N6DT6A, N7AT7B, N7BT7A and N7DT7B) (Sears 1966) and relevant ditelosomic (Sears and Sears 1978) lines of 'Chinese Spring' (CS) wheat were used to localize RGAP markers to chromosome and chromosomal arms as previously described (Shi et al. 2001; Lin and Chen 2007, 2008; Sui et al. 2009).

Wheat genotypes 'AvSYr5NIL' (*Yr5*), 'Zak' (*YrZak*) and IDO377s, and F_2 plants from their possible crosses were used in studies of possible allelism.

Pathogen materials

A total of 15 *P. striiformis* f. sp. *tritici* races (Table 1), with various virulence gene combinations (Chen 2005; Chen and associates, unpublished), were chosen to test seedlings of IDO377s and AvS. PST-43 and PST-45, which were avirulent on IDO377s but virulent on AvS, were used to test seedlings of the F_1, F_2 and F_3 progenies. Urediniospores of each isolate representing a specific race were increased on susceptible genotypes and tested on the 20 wheat genotypes that are used to differentiate races of *P. striiformis* f. sp. *tritici* in the US (Chen et al. 2002; Chen 2005). This confirmed their purity before they were used for evaluation of stripe rust resistance of the parents and progeny of the AvS × IDO377s cross and the 108 wheat breeding lines and cultivars.

Evaluation of stripe rust response phenotype

Seeds of the two parents and F_1, F_2 and F_3 progeny were planted in plastic pots (5 × 5 × 5 cm) filled with a potting mixture of six peat moss:two perlite:three sand:three potting soil mix:four vermiculite, with lime, Osmocote 14-14-14, and ammonium nitrate added at 1.7, 3.3, and 2.2 g/l, respectively. About 15 seeds for each parent, 3 seeds for F_1, 230 seeds for F_2 and 15 seeds for each of the 114 F_3 lines were planted with about 15 seeds in each pot except for the F_1. Seedlings at the two-leaf stage (about 10 days after planting) grown in a rust-free greenhouse (diurnal temperature cycle gradually changing from 10°C at 2:00 a.m. to 25°C at 2:00 p.m. with the 16 h light/8 h dark cycle) were uniformly dusted with a mixture of urediniospores of the selected *P. striiformis* f. sp. *tritici* race with talc at a ratio of approximately 1:20. After inoculation, plants were placed in a dew chamber at 10°C for 24 h and then transferred to a growth chamber operating at 16 h light and 8 h dark with diurnal temperatures gradually changing from 4°C at 2:00 a.m. to 20°C at 2:00 p.m. (Chen and Line 1992a, b). A set of 20 wheat genotypes used to differentiate races of *P. striiformis* f. sp. *tritici* were also included in the tests to confirm the race identity. Infection type (IT) data were recorded 18-21 days after inoculation based on a 0–9 scale (Line and Qayoum 1992). Infection types 0–3, 4–6 and 7–9 were considered resistant, intermediate, and susceptible, respectively.

Table 1 Seedling infection types of IDO377s and Avocet Susceptible (AvS) to races of *Puccinia striiformis* f. sp. *tritici* tested under controlled greenhouse conditions

PST race[a]	Virulence formula	Infection type	
		AvS	IDO377s
PST-1	1, 2	9	2
PST-3	1, 3	9	2
PST-7	1, 3, 5	9	2
PST-17	1, 2, 3, 9, 11	9	2
PST-21	2	9	2
PST-23	1, 3, 6, 9, 10	9	2
PST-43	1, 3, 4, 5, 12, 14	9	2
PST-45	1, 3, 12, 13, 15	9	2
PST-59	1, 3, 11, 12, 16	9	2
PST-81	1, 14	9	2
PST-98	1, 3, 8, 10, 11, 12, 16, 17, 18, 19, 20	9	2
PST-100	1, 3, 8, 9, 10, 11, 12,16, 17, 18, 19, 20	9	2
PST-111	1, 3, 5, 8, 10, 11, 12, 16, 17, 18, 19, 20	9	2
PST-116	1, 3, 4, 5, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, 20	9	5
PST-127	1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20	9	9

[a] Races PST-1 to PST-116 were previously described by Line and Qayoum (1992), Chen et al. (2002), and Chen (2005, 2007). Race PST-127 is a new race that was collected from IDO377s in a field near Pullman, WA, and identified in 2007 during this study

Wheat lines AvSYr5NIL (*Yr5*), AvSYr7NIL (*Yr7*), and Zak (*Yr44*) were tested with seven races (PST-17, PST-43, PST-45, PST-59, PST-100, PST-116 and PST-127); the F_2 populations of the possible intercrosses AvSYr5NIL, Zak and IDO377s were tested with PST-43 or PST-45; and the 108 wheat breeding lines with or without IDO377s in their pedigree were tested with PST-43, PST-45 and PST-127 as described above.

DNA extraction

Genomic DNA was extracted from 15 plants of each of the parents, F_3 lines of AvS/IDO377s and 108 breeding lines using the methods described by Riede and Anderson (1996). Three grams of fresh leaves were ground with a glass rod in liquid nitrogen and then mixed with 700 µl extraction buffer. After 30-min incubation at 65°C, 700 µl solution of 24:1 (v/v) chloroform/isoamyl alcohol was added, and the tube contents were vortexed thoroughly. Centrifugation was performed at 10,000 rpm for 12 min, and 600 µl of the upper phase solution was transferred to another 1.5-ml microcentrifuge tube. The DNA was precipitated with 1 ml of cold 95% ethanol (−20°C) and rinsed with 1 ml of 70% ethanol. The air-dried DNA was dissolved in 100 µl 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 (Maniatis et al. 1982) and spectrophotometer (NanoDrop ND-1000). The DNA stock solution was adjusted to 30 ng/µl with sterilized deionized and distilled water for use as the working solution for polymerase chain reaction (PCR).

Screening for RGAP markers using bulk segregant analysis

Based on the phenotypic data, aliquots of DNA from ten homozygous resistant and ten homozygous susceptible F_3 lines were combined into resistant and susceptible bulks, respectively. The resistance gene analog polymorphism (RGAP) method described previously (Chen et al. 1998; Shi et al. 2001) was used to screen for potential markers linked to the resistance locus. Resistance gene analog (RGA) primers were randomly paired and first screened on the parents and the two bulks and those that produced markers linked to the resistance locus are shown in Table 2. PCR were performed in a GeneAmp® PCR System 9700 thermo-cycler. A 15 µl reaction mixture consisting of 36 ng of template DNA, 1.5 µl Mg-free 10× PCR buffer (Promega, Madison, WI, USA), 0.6 unit of Taq DNA polymerase (Promega), 7.5 mM of $MgCl_2$, 3 mM each of dATP, dCTP, dGTP, and dTTP (Sigma Chemical Co., St. Louis, MO, USA), and 36 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 cycles, each consisting of 1 min at 94°C, 1 min at 45°C, 2 min at 72°C and followed by a 7 min extension step at 72°C. After PCR amplification, 6 µl of formamide loading buffer [98% formamide,

Table 2 Resistance gene analog (RGA) primers produced resistance gene analog polymorphism (RGAP) markers for mapping genes for resistance to races of *Puccinia striiformis* f. sp. *tritici* in IDO377s

RGA primers[a]	Sequence (5′–3′)	Gene	Domain	Reference
CLRR-INV2	TCTTCAGCTATCTGC	*Cf9*	LRR	Yan et al. (2003)
Cre3-k3	CTGCAGTAAGCAAAGCAACG	*Cre3*	Kinase	This study
Cre3LR-F	CACACACTCGTCAGTCTGCC	*Cre3*	LRR	Yan and Chen (2007)
NLRR Rev	TATAAAAAGTGCCGGACT	*N*	LRR	Chen et al. (1998)
NPLOOP	TCAATTAATGTTTGAGTTATTGTA	*N*	P-loop	This study
Pto kin1	GCATTGGAACAAGGTGAA	*Pto*	Kinase	Chen et al. (1998)
RLRR For	CGCAACCACTAGAGTAAC	*Rps2*	LRR	Chen et al. (1998)
RLRR Rev	ACACTGGTCCATGAGGTT	*Rps2*	LRR	Chen et al. (1998)
S1	GGTGGGGTTGGGAAGACAACG	*L6, N, Rps2*	NBS	This study
S2	GGIGGIGTIGGIAAIACIAC	*N, Rps2*	P-loop	Leister et al. (1996)
S2-INV	CAICAIAAIGGITGIGGIGG	*N, Rps2*	P-loop	Pahalawatta and Chen (2005)
XLRR For	CCGTTGGACAGGAAGGAG	*Xa21*	LRR	Chen et al. (1998)

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 followed by 4 min denaturation at 94°C for electrophoresis in 5% polyacrylamide gels. After electrophoresis, gels were silver-stained according to the recommendation of the manufacturer (Promega, Madison, WI) and dried overnight and digitally scanned. Primer pairs showing association with resistance in the bulk segregant analysis were used to genotype the F_3 population and those linked to the resistance locus were used to construct linkage maps. RGAP markers were designated using the *Xwgp* series (Shi et al. 2001).

Localization of RGAP markers to a wheat chromosome

To determine wheat chromosome and chromosomal arm, CS DNA was amplified with RGAP markers linked to the resistance gene. If the expected size of fragment was present in CS, the complete set of CS nulli-tetrasomic lines were tested with the markers. The chromosome absent in the nulli-tetrasomic line that did not show the fragment was determined to carry the RGAP marker locus. Once the chromosome was determined, the ditelosomic CS lines for that chromosome were tested with the marker to determine the chromosomal arm carrying the RGAP marker locus. Two RGAP markers were tested with the nulli-tetrasomic lines and ditelosomic lines to confirm the result.

Mapping the resistance gene to specific chromosomal region using SSR and STS markers

After the resistance gene in IDO377s was localized on chromosome 2BL, a total of 20 SSR markers specific to 2BL were tested for polymorphism with genomic DNA from the parents and F_3 lines to confirm chromosomal locations of the resistance gene and to identify more linked markers. The primer sequence information of SSR markers for 2BL tested in this study was obtained from the GrainGenes 2.0 website (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker). The protocol of Röder et al. (1998a, b) was followed for PCR amplification. The denatured polyacrylamide gel electrophoresis, silver-staining, and data collection were the same as described above for RGAP markers.

As the sequence tagged site (STS) marker Yr5STS7/8, which is tightly linked to the stripe rust resistance gene *Yr5*, is also on 2BL (Chen et al. 2003), the marker was tested with the mapping population of IDO377s. The PCR conditions, gel electrophoresis, and band scoring described by Chen et al. (2003) were followed.

Data analyses

Chi-squared tests were used to determine the goodness of fit of the observed numbers of plants or lines to the predicted segregation ratios of the F_2 and F_3 progenies to establish the number of stripe rust resistance genes, mode of inheritance, and relationships of genes for resistance to different races. A linkage map was constructed using the MAPMAKER program (Lander et al. 1987). Map distance in centi Morgans (cM) was calculated according to the Kosambi mapping function (Kosambi 1944). Chi-squared tests were also used to determine the goodness of fit to a single-locus model for each marker in the F_3 population.

Results

Phenotypic and genetic characterization of stripe rust resistance

The seedling IT data of IDO377s and AvS tested with the 15 *P. striiformis* f. sp. *tritici* races are shown in Table 1.

IDO377s was resistant to all races except PST-116 and PST-127, whereas AvS was susceptible to all. PST-116 produced IT 5 on IDO377s, an intermediate reaction with intermediate sporulation and necrosis. PST-127, originally collected from IDO377s in the field in 2007, produced IT 9, a highly susceptible reaction.

When tested with race PST-127 under both high (diurnal temperatures gradually changing from 10°C at 2:00 a.m. to 35°C at 2:00 p.m.) and low (diurnal temperatures gradually changing from 4°C at 2:00 a.m. to 20°C at 2:00 p.m.) temperatures, AvS and IDO377s were susceptible (IT 7–8) at both the seedling and adult-plant stages. These data indicated that IDO377s did not have HTAP resistance.

The numbers of resistant (ITs 0, 1 or 2) and susceptible (ITs 7, 8 or 9) plants in F_2 and the F_3 line classifications from tests with PST-43 and PST-45 are shown in Table 3. When tested with both races F_1 plants responded with IT 2 indicating dominance of resistance. The F_2 populations segregated three resistant: one susceptible ratios and the F_3 lines were distributed one homozygous resistant:two segregating:one homozygous susceptible confirming segregation at a single locus. Each of the F_3 lines had the same reaction to PST-43 and PST-45 and no recombinant lines were detected between reactions to these races, suggesting that the same gene conferred resistance to both PST-43 and PST-45.

RGAP markers linked the resistance locus

Of a total of 116 primer pairs from random combinations of 48 RGA primers screened in bulk segregant analysis, 38 primer pairs produced 40 amplicons that differentiated the AvS and the susceptible bulk (SB) from IDO377s and resistant bulk (RB). Eight primer pairs that generated strong and repeatable polymorphic bands were selected to test individual F_3 lines. The sequences of the 12 RGA primers producing the 8 polymorphic markers are shown in Table 2. As an example, Fig. 1a shows the banding pattern of F_3 lines screened with RGA primers RLRR For and NLRR Rev for marker *Xwgp110*. All eight RGAP markers were dominant and their designations, primers, sizes, presence or absence in IDO377s, AvS, and CS, and the *P* values for goodness of fit to a single locus are shown in Table 4. The relatively high *P* values indicated their reliability as single genes for constructing a linkage map.

Mapping the resistance gene to a wheat chromosome

To determine the chromosomal location of the resistance gene, eight RGAP markers that were present in IDO377s and RB but not in AvS and SB were tested with CS. RGAP markers *Xwgp103* and *Xwgp110* present in CS were used to test the 21 nulli-tetrasomic lines. The unique bands were detected in all lines except N2BT2D, as shown by marker *Xwgp103* in Fig. 1b, indicating that the RGAP markers and the linked resistance gene were located on wheat chromosome 2B. Both markers were present in CS ditelosomic 2BL, thus the resistance gene was located on the long arm. CS ditelosomic 2BS was not available for use as a control in this test.

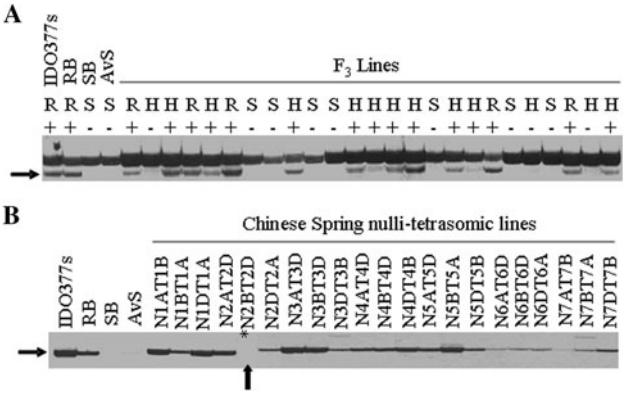

Fig. 1 Silver-stained denaturing polyacrylamide gels showing **a** the resistance gene analog polymorphism (RGAP) marker *Xwgp110* (*arrow*, 1,000 bp) amplified with primers RLRR For and NLRR Rev in bulk segregant analysis of F_3 progeny. *RB* resistant bulk and *SB* susceptible bulk. For the stripe rust reaction phenotype, *R* homozygous resistant, *S* homozygous susceptible, and *H* heterozygous. For the marker type, + indicates presence of the marker and – indicates absence of the marker. **b** Segregation patterns of RGAP marker *Xwgp103* (*arrow*, 680 bp) amplified with primers RLRR For and S2 and its amplification in 21 nulli-tetrasomic lines

Table 3 AvS/IDO377 F_2 plants and F_3 lines segregation for seedling resistance to races PST-43 and PST-45 of *Puccinia striiformis* f. sp. *tritici*

Race	Generation	Observed number of F_2 plants or F_3 lines			Expected ratio	χ^2	*P*
		Resistance	Segregating	Susceptible			
PST-43	F_2	226	–	74	3:1	0.02	0.99
	F_3	31	56	27	1:2:1	0.32	0.85
PST-45	F_2	223	–	77	3:1	0.07	0.93
	F_3	31	56	27	1:2:1	0.32	0.85

Table 4 Primer pairs, probability (*P*) values for 3:1 segregation ratios among F_3 progeny, and sizes of markers used for mapping the *Yr43* gene conferring resistance to *Puccinia striiformis* f. sp. *tritici* and their presence or absence in the resistant parent IDO377s, susceptible parent Avocet S (AvS) and Chinese Spring (CS)

Marker	Primer pair	No. of F_3 lines		*P* for 3:1	Size (bp)[a]	Marker present (+) in		
		Marker present	Marker absent			IDO377s	AvS	CS
RGAP								
Xwgp103	RLRR For/S2	86	30	0.83	680	+	–	+
Xwgp104	RLRR Rev/S2-INV	89	27	0.67	580	+	–	–
Xwgp105	NPLOOP/S2-INV	90	26	0.52	680	+	–	–
Xwgp106	RLRR For/CLRR-INV2	86	30	0.83	550	+	–	+
Xwgp107	Pto kin1/S1	92	24	0.28	800	+	–	–
Xwgp108	Cre3-k3/Cre3LR-F	85	31	0.67	480	+	–	+
Xwgp109	XLRR For/Cre3LR-F	91	25	0.39	650	+	–	–
Xwgp110	RLRR For/NLRR Rev	88	28	0.83	1000	+	–	+
SSR								
Xgwm501	GWM501	89	27	0.67	166	+	–	–
Xgwm501	GWM501	89	27	0.67	172	–	+	+
Xbarc139	BARC139	88	28	0.83	156	+	–	–
STS								
Yr5STS 7/8	Yr5STS7/8	86	30	0.83	480	+	–	+

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

Confirmation of the chromosomal location of the resistance gene with SSR and STS markers

To identify additional markers and to further confirm the chromosomal location of the resistance gene, 20 SSR markers covering the long arm of chromosome 2B were screened among the two parents and two bulks. Two markers, *Xgwm501* amplified by the GWM501 primers (F: 5′-GGCTATCTCTGGCGCTAAAA-3′ and R: 5′-TCCACAAACAAGTAGCGCC-3′) (Röder et al. 1998a) and *Xbarc139* amplified by the BARC139 primers (F: 5′-AGAAGCTCCCCTAAACTGAG-3′ and R: 5′-CGACGCTGATGAATGAAT-3′) (Liu and Anderson 2003), produced bands specific to both IDO377s and resistant bulk or both AvS and susceptible bulk. *Xgwm501* was a co-dominant marker producing a 166-bp band in IDO377s and a 172-bp band in AvS. *Xbarc139* was a dominant marker (156 bp) present in IDO377s. Linkage analysis using the two markers with the 114 F_3 lines indicated that they were linked to the resistance gene. The known associations of the two SSR markers with the long-arm of chromosome 2B further confirmed the location of the resistance gene.

Because *Yr5* was previously mapped to chromosome 2BL (Chen et al. 2003), an *Yr5* specific sequence tagged site (STS) marker, *Yr5STS7/8*, amplified by primers Yr5STS7 (5′-GTGTACAATTCACCTAGAG-3′) and Yr5STS8 (5′-GCAAGTTTTCTCCCTAT-3′) (Chen et al. 2003), was assayed on the F_3 lines of the AvS/IDO377s cross (Table 4). This co-dominant marker produced polymorphic bands that were specific to both resistant parent and resistant bulk or both susceptible parent and the susceptible bulk. Linkage analysis using the *Yr5STS7/8* marker with the 114 F_3 lines indicated that *YrIDO377s* was linked to *Yr5*, confirming the location of *YrIDO377s* on chromosome 2BL.

Construction of a linkage group for the resistance gene

A linkage group containing the resistance gene in IDO377s (Fig. 2) was constructed with eight RGAP markers, two SSR markers, and one STS. The *Xgwm501* SSR marker mapped another stripe rust resistance gene, *YrZak* in spring wheat cultivar ‘Zak’, to chromosome 2BL (Sui et al. 2009). The *Yr5STS7/8* STS marker also placed *Yr5* on the linkage group. The two closest flanking RGAP markers, *Xwgp103* and *Xwgp110*, were linked to the resistance gene in IDO377s with genetic distances of 4.4 and 5.5 cM, respectively. The closest SSR marker, *Xgwm501*, was 16.0 cM proximal to the resistance gene. This marker indicated that *YrZak* is 12.0 cM away from the resistance locus in IDO377s. The *Yr5STS7/8* STS marker had a genetic distance of 38.6 cM from the resistance locus in IDO377s. These data indicated that *YrIDO377s*, *YrZak* and *Yr5* are at different loci.

Comparison of *YrIDO377s* to other *Yr* genes on 2BL using various races and allelic tests

Yr genes reported on chromosome 2BL include *Yr5*, *Yr7*, *YrQz* and *YrZak* (Deng et al. 2004; Chen 2005; Sui et al.

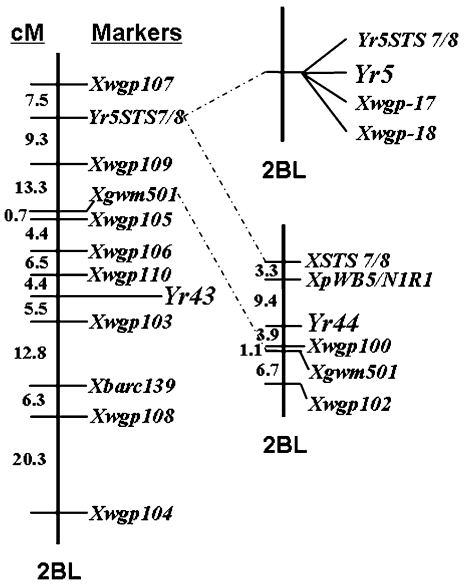

Fig. 2 Linkage map for the resistance gene *Yr43* constructed with 8 RGAP markers (*Xwgp103* to *Xwgp110*), 2 SSR markers (*Xgwm501* and *Xbarc139*) and 1 STS marker (*Yr5STS7/8*). The markers linked to the resistance gene *Yr43* are shown on the *right*. The linkage on the long arm of chromosome 2B was determined by two RGAP markers *Xwgp103* and *Xwgp110* using 21 nulli-tetrasomic Chinese Spring lines. Genetic map of *Yr5* was from Yan et al. (2003) and Chen et al. (2003) and genetic map of *Yr44* (*YrZak*) was from Sui et al. (2009)

2009). To compare the specificities of *YrIDO377s* and others, IDO377s, AvSYr5NIL (*Yr5*), AvSYr7NIL (*Yr7*) and Zak (*YrZak*) were tested with seven races (PST-17, PST-43, PST-45, PST-59, PST-100, PST-116 and PST-127). The reactions shown in Table 5 strongly suggested that *YrIDO377s*, *Yr5* and *YrZak* were different genes.

Tests on F_2 populations of 300 seedlings from intercrosses among AvSYr5NIL, Zak and IDO377s using PST-43 showed that the resistance genes in these wheat genotypes involved different loci (Table 6). In Zak/IDO377s there was one plant with IT 8 (susceptible); two plants with IT 3 were considered resistant, but different from the infection type produced by either Zak or IDO377s. Six plants were susceptible in Zak/AvSYr5NIL and 10 were susceptible in AvSYr5NIL/IDO377s. In each cross the frequencies of resistant and susceptible plants deviated from 15:1 ratios expected for independent segregation of dominant alleles at two loci ($P < 0.05$). Assuming linkage, the frequency of susceptible plants in repulsion phase crosses such as these in this study is $p^2/4$ where p is the recombination value. Thus the recombination values were estimated at 0.115, 0.283 and 0.365 for the intervals *YrZak-YrIDO377s*, *Yr5-YrZak* and *Yr5-YrIDO377s*, respectively. On conversion to map distances using the formula $d = -1/2\ \mathrm{Ln}(1 - 2p)$ the values become 13.1, 41.5 and 65.5 cM, respectively. Although these estimated distances exceed those indicated on the molecular maps in Fig. 2, the gene order is consistent with the molecular mapping results. The gene in IDO377s is thus considered to be at a new locus and was named *Yr43*. At the same time *YrZak* is also unique and it is designated *Yr44*.

Value of the flanking markers in marker-assisted selection

A total of 108 cultivars and breeding lines with IDO377s or related genotypes in their pedigrees were tested with races PST-43, PST-45, and PST-127 and assayed for the two flanking markers *Xwgp103* and *Xwgp110* (Supplemental table 1). Ninety lines (83.3%) were resistant to PST-43 and PST-45, but susceptible to PST-127. Because the IT responses and response patterns were very similar to those of IDO377s, these genotypes were postulated to carry *Yr43*. The remaining 18 lines (16.7%) were susceptible and therefore, cannot carry the gene. Among the potential carriers 76 lines (84.4%) had both *Xwgp103* and *Xwgp110*; 4 lines (4.4%) had only *Xwgp110*; 9 lines had only *Xwgp103*; and only 1 line (1.1%) carried neither marker. Of the 18 susceptible lines 13 (72.2%) had neither marker; 2 (11.1%) had *Xwgp110*; 3 (16.7%) had *Xwgp103*; and none both. When the two groups were combined, 89 lines (82.4%) had marker genotypes that predicted the reaction phenotypes, 1 line (0.9%) had a marker type not matching the reaction phenotype, and 18 lines (16.7%) had one of the markers. These results indicated that the flanking markers were quite reliable, but not perfect, in predicting the presence of *Yr43*.

Table 5 Infection types on wheat genotypes with *Yr* genes on long arm of chromosome 2B produced by races of *Puccinia striiformis* f. sp. *tritici*

Yr gene	Wheat genotype	Infection types produced by race						
		PST-17	PST-43	PST-45	PST-59	PST-100	PST-116	PST-127
Yr5	AvS + Yr5	2	2	2	2	2	2	2
Yr7	AvS + Yr7	8	8	8	8	8	8	8
Yr43	IDO377s	2	2	2	2	2	6	8
Yr44	Zak	2	2	2	2	8	8	8

Table 6 Segregation of resistant and susceptible plants in the F_2 populations of IDO377s (*Yr43*)/AvSYr5NIL (*Yr5*), AvSYr5NIL/Zak (*Yr44*) and IDO377s/Zak tested with race PST-43 of *Puccinia striiformis* f. sp. *tritici*, recombination value and map distance between genes in each of the crosses

Cross		Observed F_2 plants		Recombination[a] value	Map distance[b] (cM)
Female parent	Male parent	Resistant	Susceptible		
IDO377s	Zak	299[c]	1	0.115	13.1
AvSYr5NIL	Zak	294	6	0.283	41.5
IDO377s	AvSYr5NIL	290	10	0.365	65.5

[a] Recombination value (p) was calculated using the formula $f = p^2/4$, where f is the frequency of susceptible plants in a F_2 population

[b] Map distance was calculated using the formula $d = -1/2$ Ln $(1 - 2p)$, where p is the recombination value

[c] Two of the 299 plants had infection type (IT) 3, which was different from IT 2 on the remaining 297 plants and both parents

Discussion

Genetic analysis of stripe rust resistance in IDO377s indicated that a single gene conferred resistance to races PST-43 and PST-45, and presumably many other races. However, it was fully susceptible to PST-127. Using the CS nulli-tetrasomic lines and chromosome specific SSR markers, we mapped the gene to the long arm of chromosome 2B. Through comparison of its reaction to various races of *P. striiformis* f. sp. *tritici*, presence and absence of molecular markers for some other genes on 2BL, and genetic analysis of segregations of IDO377s with wheat lines carrying these genes, we determined that the IDO377s resistance gene has not been previously reported and named it *Yr43*.

The molecular markers identified in this study were linked to *Yr43* and other resistance genes on chromosome 2BL. From combined linkage groups constructed from data for three separate crosses (AvS/Zak, AvS/AvSYr5NIL and AvS/IDO377s), *Yr43* was estimated to be 12.0 cM proximal to *Yr44* (*YrZak*) and 39.3 cM proximal to *Yr5*. In the intercrosses (AvSYr5NIL/Zak, AvSYr5NIL/IDO377s and Zak/IDO377s), the occurrence of susceptible F_2 segregations indicated that the genes were not allelic. The gene order indicated by the intercross F_2 data was consistent with that predicted from the molecular maps (Fig. 2), but the respective predicted linkage values were somewhat higher. *YrQz* also has been reported on 2BL (Deng et al. 2004). Because the *YrQz* line was not available, it was not possible to make crosses with the *Yr* gene lines used in this study. However, we could estimate its relative distances to *Yr43* and *Yr44* based on SSR markers. As previously discussed (Sui et al. 2009), *YrQz* is at a locus about 28.3 cM from *Yr44* based on a common SSR marker, *Xgwm388*, and other reference markers (Deng et al. 2004; Sui et al. 2009). The map distance between the *Yr43* and *YrQz* loci is estimated to be at least 7 cM. Other genes located in chromosome 2BL include *Yr7* in Lee and *YrSp* in Spaldings Prolific. Both genes are allelic with *Yr5* (Zhang et al. 2009). Therefore, *Yr43* is different from these genes.

Stripe rust is a major problem in wheat production in the U.S. (Chen 2005, 2007). Because new races of PST can overcome race-specific resistance in cultivars, more resistance genes are needed for breeding programs. The race-specific, all-stage resistance that is conferred mostly by single dominant genes can provide high level resistance, but is easily overcome by new races of the pathogen (Line and Qayoum 1992; Chen 2005, 2007). The spring cultivar IDO377s used in this study was highly resistant to all races in the Pacific Northwest before 2005, but became moderately resistant to PST-116 and highly susceptible to PST-127, which were detected after 2005 (Chen and associates, unpublished data). Although HTAP resistance is durable and non-race specific, incorporating HTAP resistance into commercial cultivars is more difficult because it is partial, often controlled by multiple genes, and affected by plant growth stage and temperature (Line and Chen 1995, Chen 2005). Therefore, a preferred strategy for breeding resistant cultivars is to combine all-stage resistance with HTAP resistance.

In spite of stripe rust resistance being overcome after 2005, IDO377s continues to be grown and used in breeding programs because of many other desirable traits. The markers identified in this study can efficiently be used to detect *Yr43* in US northwestern wheat breeding lines and cultivars. The flanking markers *Xwgp103* and *Xwgp110* identified in the study showed high reliability for tagging *Yr43* by testing 108 cultivars and breeding lines. The spring wheat cultivars Lolo (PI 614840) and Otis (PI 634866), the former released by the southern Idaho breeding program in 2000 (Souza et al. 2003), and the latter released by the Washington State University spring wheat breeding program in 2005 (Kidwell et al. 2006) were included in the study because they were developed using IDO377s as a parent. Both the rust reactions and molecular data showed that Lolo has *Yr43* and Otis does not. These results agreed with multiple-year field and greenhouse testing data for stripe rust resistance (Chen, unpublished data). Interestingly, the mismatch by *Xwgp110* to the race reaction phenotype was only in 6 of 108 lines (5.6%) and that by

Xwgp103 was 11 of 108 lines (10.2%). When these mismatch rates were treated as frequencies of recombination between the gene and the markers, the data correlated well with the genetic distances, 4.4 and 5.5 cM, between *Yr43* and *Xwgp110* and between the *Yr43* and *Xwgp103*, respectively. The results demonstrated the usefulness of association mapping, an increasingly used approach that does not require crosses (Cockram et al. 2008).

The RGAP technique used in the study permitted rapid identification of resistance-gene linked markers in bulk segregant analysis. Many resistance genes in wheat and barley have been identified since the RGAP technique was adopted in 1998 (Shi et al. 2001; Yan et al. 2003; Yan and Chen 2007, 2008, Lin and Chen 2007, 2008). New RGA primers can be developed to improve the efficiency and power of the strategy by utilizing the recent report of nucleotide binding site leucine rich repeat genes in other plant species (Tan et al. 2007).

The origin of *Yr43* is not clear because cultivar IDO377s has a complex pedigree, Chova/59Ab10293-5 = Gallo/Yecora Selection/3/Aurora//Kalyansona/Bluebird/4/Norin 10/Brevor//Baart/Onas (http://www.laughlintrading.com/extended%20variety%20descriptions/377s.htm). However, we can postulate the possible sources based on stripe rust responses of the genotypes in the pedigree from our data of germplasm evaluation (http://www.ars-grin.gov/npgs/acc/acc_queries.html). Several of the wheat genotypes in the pedigree can be determined not to be the donor of *Yr43*. Kalyansona (PI 376839), which has *Yr2* (Singh and Johns 1988), was susceptible to races PST-17, 37, 45 and 78. Bluebird, which is also known as Yecora Rojo (CItr17414), was resistant to PST-37 and 45, but susceptible to PST-43 and 100. Norin 10 (PI 156641) was susceptible to PST-17, 20, 27, 29, 37 and 45 and intermediate to PST-43. Baart (PI 45401) was resistant to PST-17 and 27 but susceptible to PST-20 and 29. Onas (PI 46796) was resistant to PST-17 but susceptible to PST-20, 27 and 29. Brevor (CItr 12385) has not been tested in the seedling stage with individual races, but it was resistant to moderately susceptible in the field tests for the last 10 years (Chen, unpublished data). Therefore, these genotypes could not be the source of *Yr43* as IDO377s was resistant to these races and completely resistant in the field up to 2005. Gallo (PI583710) was a possible donor of *Yr43* as it was resistant to PST-43 and 45. The genotype should be tested with more races and the markers for *Yr43* to prove the hypothesis.

In conclusion, this study identified a new gene in wheat for resistance to stripe rust. Because the *Yr43* resistance is race-specific, it should be used in combination with other genes or QTL for either all-stage resistance or durable HTAP resistance. The flanking markers, *Xwgp103* and *Xwgp110*, should be useful for combining *Yr43* with other genes for durable resistance. Because these RGAP markers need to be run in polyacrylamide gels, which are relatively time-consuming and complicated, more user-friendly STS markers can be developed from the flanking markers for use of agarose gel electrophoresis to tag the resistance gene in breeding lines.

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