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Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust

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Abstract The *Yr5* gene confers resistance to all races of the stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) of wheat in the United States. To develop molecular markers for *Yr5*, a BC_7:F_3 population was developed by backcrossing the *Yr5* donor '*Triticum spelta album*' (TSA) with the recurrent parent 'Avocet Susceptible' (AVS). Seedlings of the *Yr5* near-isogenic lines (AVS/6**Yr5*), AVS, TSA, and the BC_7:F_3 lines were tested with North American races of *P. striiformis* f. sp. *tritici* under controlled greenhouse conditions. The single gene was confirmed by a 1:2:1 segregation ratio for homozygous-resistant, heterozygous and homozygous-susceptible BC_7:F_3 lines. Genomic DNA was extracted from the parents (the *Yr5* near-isogenic line and AVS) and 202 BC_7:F_3 lines. The resistance gene-analog polymorphism (RGAP) technique was used to identify molecular markers. The parents and the homozygous-resistant and homozygous-susceptible BC_7:F_3 bulks were used to identify putative RGAP markers for *Yr5*. Association of the markers with *Yr5* was determined using segregation analysis with DNA from the individual BC_7:F_3 lines. Of 16 RGAP markers confirmed by segregation analysis with 109 BC_7:F_3 lines, and nine of the markers confirmed with an additional 93 BC_7:F_3 lines, three markers co-segregated with the resistance allele and three markers co-segregated with the susceptibility allele at the *Yr5* locus. The other four markers were tightly linked to the locus. Analysis of a set of Chinese Spring nulli-tetrasomic lines with three markers that co-segregated with, or were linked to, the susceptibility allele confirmed that the *Yr5* locus is on chromosome 2B. Of five RGAP markers that were cloned and sequenced, markers *Xwgp-17* and *Xwgp-18* that co-segregated with the *Yr5* locus were co-dominant and had 98% homology with each other in both DNA and translated amino-acid sequences. The two markers had 97% homology with a resistance gene-like sequence from *Aegilops ventricosa* and had significant homology with many known plant resistance genes, resistance gene analogs and expressed sequence tags (ESTs) from wheat and other plant species. The markers *Xwgp-17* and *Xwgp-18* also had significant homology with the NB-ARC domain that is in several genes for plant resistance to diseases, nematode cell death and human apoptotic signaling. These markers should be useful to clone *Yr5* and combine *Yr5* with other genes for durable and superior resistance for the control of stripe rust.

Keywords Molecular markers · *Puccinia striiformis* · Resistance gene-analog polymorphism · *Triticum aestivum* · Yellow rust

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Introduction

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*P. s. tritici*), is an important disease of wheat (*Triticum aestivum* L.) throughout the world. In the United States, the disease is most destructive in the Western United States and has become increasingly important in the south-central states (Line and Chen 1996; Chen et al. 2002). Growing resistant cultivars is the most-effective, economic and environmentally sound approach of controlling stripe rust (Line and Chen 1995). A series of resistance genes, *Yr1–Yr28*, and many other provisionally designated genes have been identified (Lupton and Macer 1962; Chen et al. 1998c; McIntosh et al. 1998) and incorporated into commercial cultivars (Allan and Purdy 1967; Allan et al. 1993; McIntosh et al. 1995). Stripe rust resistance mainly includes seedling re-

sistance, which can be detected at the seedling stage but is expressed in all growth stages and is race-specific, and high-temperature, adult-plant (HTAP) resistance, which is most effective in the adult-plant stage at high temperatures (Qayoum and Line 1985; Chen and Line 1995a, b). Cultivars with seedling resistance conferred by a single gene often become susceptible within a few years after their release because new virulent races of the pathogen appear and circumvent the resistance (Stubbs 1985; Line and Chen 1996; Chen et al. 2002). HTAP resistance is durable and non-specific (Milus and Line 1986a, b; Chen and Line 1995a, b), but it is difficult to incorporate into commercial cultivars because of its quantitative inheritance. It is even more difficult to combine both seedling resistance genes and genes for HTAP resistance. Molecular markers should accelerate the process of developing wheat cultivars with stronger and more durable resistance.

The gene *Yr5* was originally derived from *Triticum spelta album* (TSA), a hexaploid wheat. Using classic genetic analyses, Macer (1966) identified a single dominant gene in TSA and named it *Yr5*, which was also confirmed with several crosses tested with North American races of *P. s. tritici* (Chen and Line 1992b, c, 1993). The chromosomal location of *Yr5* was determined using cytogenetic approaches. Macer (1966) localized *Yr5* on chromosome 2B, and Law (1976) further mapped it on the long arm of chromosome 2B and 21-cM away from the centromere. *Yr5* confers resistance to almost all isolates of *P. s. tritici* in the world except for India and Australia (Nagarajan et al. 1986; Wellings and McIntosh 1990). It has been used in wheat breeding programs and transferred into some wheat cultivars (Kema 1992). The combination of *Yr5* with other seedling resistance genes and with HTAP resistance should provide durable and superior resistance. Molecular markers should be useful for transferring *Yr5* into commercial cultivars and combining it with other resistance genes. Markers directly associated with a resistance gene are always preferred to markers linked to the gene in marker-assisted selection. Recent advances in molecular characterization of plant resistance genes and molecular-marker techniques provide opportunities to develop direct markers for resistance genes.

Genes conferring resistance to pathogens have been cloned from diverse species. The discovery of common motifs in cloned resistance genes, such as leucine-rich repeats (LRR), nucleotide-binding sites (NBS) and serine/threonine kinase domains, offers opportunities to isolate similar sequences in other plant species. Specific genomic DNA sequences amplified by degenerate primers based on these motifs are known as resistance gene analogs (RGAs). The RGA approach has been used to isolate plant resistance genes and develop molecular markers. Leister et al. (1996) obtained PCR products from potato that were homologous to known resistance genes and absolutely linked to the nematode resistance locus *Gro1* and the late blight resistance locus *R7*. Similarly, Kanazin et al. (1996) mapped several RGA loci that were near to known resistance genes in soybean. Yu et al. (1996) mapped RGAs to the vicinity of known soybean genes for resistance to potyviruses (*Rsv1* and *Rpv*), *Phytophtora* root rot (*Rps1*, *Rps2*, and *Rps3*) and powdery mildew (*rmd*). Feuillet et al. (1997) isolated a candidate gene *LrK10* for leaf rust resistance. However, in their reports, RGAs were detected using agarose-gel electrophoresis, which resulted in the appearance of the high heterogeneity of DNA fragments from single bands. Polymorphic markers were obtained using RGAs as probes in the time-consuming RFLP analysis.

Chen et al. (1998b) improved the RGA approach by using high-resolution electrophoresis and sensitive detection to separate PCR products amplified with primers based on conserved sequences of cloned plant resistance genes. Later, the technique was referred to as resistance gene-analog polymorphism (RGAP) (Chen et al. 1998a). The RGAP technique has been used successfully to develop molecular markers for genes conferring resistance to stripe rust of wheat, and stripe rust, leaf rust, scald, net blotch, barley yellow dwarf and scab of barley (Chen et al. 1998a, 1999; Toojinda et al. 2000; Shi et al. 2001). The objectives of the present study were to use the RGAP technique to identify molecular markers and construct a linkage map around *Yr5*, to clone RGAP markers for sequence comparison with genes in the GenBank and to demonstrate the efficiency of using the RGAP technique to develop direct or tightly linked markers for plant resistance genes.

Materials and methods

Plant materials and evaluation for stripe rust resistance

The wheat *Yr5* near-isogenic line (NIL) was developed at the Plant Breeding Institute, Sydney, Australia, by backcrossing the *Yr5* donor cultivar '*T. spelta album*' (TSA) with the recurrent parent 'Avocet Susceptible' (AVS). Three individual BC_6 plants of the *Yr5* NIL were backcrossed to AVS to develop the mapping population BC_7:F_3 lines. The wheat cultivar 'Chinese Spring' and the complete set of nulli-tetrasomic Chinese Spring lines (Sears 1966) were used to confirm the chromosomal location of *Yr5* and the markers.

Plants were grown and tested for stripe rust resistance under controlled greenhouse conditions using the methods described by Chen and Line (1992a). Seedlings of the *Yr5* NIL, AVS and TSA were tested with eight races (PST-17, PST-25, PST-29, PST-37, PST-43, PST-45, PST-58 and PST-59) of *P. s. tritici* (Line and Chen 1996; Chen et al. 2002), and 329 BC_7:F_3 lines were tested with PST-29 and PST-43. For the parents and the BC_7:F_3 population, 15 to 20 plants were tested for each line. For each plant, the infection type (IT) based on a 0–9 scale (Line and Qayoum 1991) was recorded 20 days after inoculation. Chi-square tests were used to analyze inheritance of stripe rust resistance.

DNA extraction, PCR amplification, electrophoresis and gel visualization

Three grams of fresh leaves from over 20 plants for each line were ground in liquid nitrogen. DNA was extracted from the leaf powder following the protocol described by Riede and Anderson (1996), 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 (Maniatis et al. 1982).

Table 1 Sequences of resistance gene analog (RGA) primers used to identify markers for *Yr5*

RGA primer	Sequence (5′–3′)	Gene	Domain[a]	Reference
S2	GGIGGIGTIGGIAAIACIAC	*N*, *Rps2*	P-loop	Leister et al. 1996
AS3	IAGIGCIAGIGGIAGICC	*N*, *Rps2*	LRR	Leister et al. 1996
RLRR Rev	ACACTGGTCCATGAGGTT	*Rps2*	LRR	Chen et al. 1998b
LM638	GGIGGIGTIGGIAAIACIAC	*L6*, *N*, *Rps2*	P-loop	Kanazin et al. 1996
Pto kin1IN	AAGTGGAACAAGGTTACG	*Pto*	Kinase	Shi et al. 2001
Pto kin2IN	GATGCACCACCAGGGGG	*Pto*	Kinase	Shi et al. 2001
XLRR Rev	CCCATAGACCGGACTGTT	*Xa21*	LRR	Chen et al. 1998b
Pto kin1	GCATTGGAACAAGGTGAA	*Pto*	Kinase	Chen et al. 1998b
Pto kin2	AGGGGGACCACCACGTAG	*Pto*	Kinase	Chen et al. 1998b
AS3-INV	CCIGAIGGIGAICGIG	*N*, *Rps2*	LRR	This study
CLRR-INV2	TCTTCAGCTATCTGC	*Cf9*	LRR	This study
Xa1LR-F	CTCACTCTCCTGAGAAAATTAC	*Xa1*	LRR	This study
Pto kin4	AGTGTCTTGTAGGGTATC	*Pto*	Kinase	Shi et al. 2001
Cre3LR-R	CAGGAGCCAAAAATACGTAAG	*Cre3*	LRR	This study
RLK-Rev	TCYGGYGCRATRTANCCNGGITGICC	*LrK10*	Kinase	Feuillet et al. 1997
Xa1NBS-F	GGCAATGGAGGGATAGG	*Xa1*	NBS	Shi et al. 2001
RLK-For	GAYGTNAARCCIGARAA	*LrK10*	Kinase	Feuillet et al. 1997
NLRR-INV1	TTGTCAGGCCAGATACCC	*N*	LRR	This study

[a] LRR, leucine-rich repeat; NBS, nucleotide-binding site

The primers used in this study were designed based on conserved motifs of cloned resistance genes (Table 1) and synthesized by Operon (Alameda, Calif.). The RGAP protocol, described by Chen et al. (1998b), with a modification to the reaction volume and the ingredient amount, was used. For each PCR reaction, the 15-µl volume contained 30 ng of template DNA, 0.2 mM each of dCTP, dGTP, dTTP and dATP (Promega, Madison, WI), 0.6 U of *Taq* DNA polymerase (Promega), 4 µM each of two primers, 1.5 µl of 10× PCR buffer (Promega, Mg-free), and 5 mM of $MgCl_2$ (Promega). Amplification was performed in DNA thermocyclers (Perkin Elmer and Ericomp, San Diego, Calif.) programmed for 5 min at 94 °C for initial denaturation and 45 cycles each consisting of 1 min at 94 °C, 1 min at 45 °C and 2 min at 72 °C, followed by a final 7-min extension at 72 °C. A 2.5-min ramp time was used between the 94 °C denaturation and the 45 °C annealing steps; for all other temperature transitions, the fastest possible ramp was employed.

After amplification, 6 µl of formamide loading buffer [98% formamide, 10 mM of EDTA (pH 8.0), 0.5% (w/v) bromophenol blue, and 0.5% (w/v) xylene cyanol] was added to the PCR product. A 4-µl mix of the PCR product and loading buffer was electrophoresed in a 1% agarose gel to check the success of the amplification.

Amplified DNA fragments were separated in a 5% denaturing polyacrylamide gel (398 mm × 338 mm × 0.4 mm), prepared as recommended by the manufacturer. A polymerized gel was prerun in 1× TBE buffer (90 mM of Tris-borate, 2 mM of EDTA, pH 8.0) for 40–60 min at 1,500 V, to bring the gel to 50 °C. After urea and the gel debris were removed from the well area, 6–8 µl of denatured PCR samples were loaded and the gel was run at 1,350 V for 2.5–3.5 h, depending on the approximate size of the bands of interest. The gel was silver-stained according to the manufacturer's recommendation (Promega). After the gel was dried at the room temperature, a photograph of the gel was produced using a silver-sequence automatic processor compatible film (Promega).

Genomic DNA samples from the *Yr5* NIL, AVS and two BC_7:F_3 DNA bulks, one of which was homozygous resistant (RR) and one homozygous susceptible (rr) based on BC_7:F_3 segregation data, were used for screening primers. Each bulk DNA contained equal amounts of DNA from 15 BC_7:F_3 homozygous lines. Polymorphic RGAP bands specific to the *Yr5* line and the resistant bulk, or to AVS and the susceptible bulk, were tested in a subpopulation with 20 individual BC_7:F_3 lines. Bands that matched the phenotypic disease data were tested further in a BC_7:F_3 population consisting of 109 progeny. Strong bands that were unique to the *Yr5* line were tested further with 93 additional BC_7:F_3 lines. The segregation data of RGAP markers and the disease data obtained from the BC_7:F_3 population were used to estimate the genetic linkage between the RGAP markers and the *Yr5* gene. The *Xwgp* series (Shi et al. 2001) was used to designate the RGAP markers.

Cloning and sequencing

The bands subjected to cloning and sequence analysis were excised from a dried polyacrylamide gel after applying a drop of sterile water. An excised band was soaked in 2 µl of H_2O for at least 1 hour, and the solution was used as the template DNA for re-amplification with the original RGA primers. The re-amplification product that generated a single band with the same size as the original band in a 1% agarose gel was selected. Four microliters of the re-amplification mixture were used for cloning into vector TOPO TA pCR2.1 (Invitrogen, Carlsbad, Calif.) following the procedures as the manufacturer recommended. Plasmid DNA from ten single colonies derived from each cloning reaction was examined in a 1% agarose gel to determine the size of the inserted fragment. To obtain more-accurate sequences for major markers, two or more clones with the expected insert size were sequenced using the ABI 377 sequencer (Applied Biosystems, Foster City, Calif.).

Data analyses

Chi-square tests were used to analyze the segregating population for stripe rust resistance and the RGAP markers. Linkage analyses and map construction of RGAP markers and the resistance locus were performed with the computer program Mapmaker, version 3.0 (Lander et al. 1987). A LOD score of 3.0 or greater and Kosambi's (1944) mapping function were used to establish the linkage. DNA and peptide-sequence similarities between the cloned RGAP markers and other genes or sequences were determined by searches through the GenBank database, using the computer program BLAST (basic local alignment search tool; Altschul et al. 1997) at the website http://www.ncbi.nlm.nih.gov/BLAST/.

Results

Genetic and phenotypic analyses

In the tests with eight races (PST-17, PST-25, PST-29, PST-37, PST-43, PST-45, PST-58 and PST-59) of *P. s. tritici*, the *Yr5* line and *T. spelta album* (TSA) were resis-

Table 2 Resistance gene-analog polymorphism (RGAP) markers for *Yr5*, primers used to identify the marker and size of the markers

RGAP marker	Primer pair	Size (bp)[a]
Xwgp-17	S2/AS3	546
Xwgp-18	S2/AS3	540
Xwgp-19	RLRR Rev/LM638	480
Xwgp-20	RLRR Rev/LM638	475
Xwgp-21	Pto kin1IN/Pto kin2IN	248
Xwgp-22	Cre3LR-R/Pto kin2	221
Xwgp-23	Cre3LR-R/Pto kin2	210
Xwgp-24	Xa1LR-F/Pto kin4	310
Xwgp-25	XLRR Rev/Pto kin1	490
Xwgp-26	Pto kin2/AS3-INV	260
Xwgp-27	CLRR-INV2/Pto kin1	608
Xwgp-28	RLRR Rev/Pto kin4	340
Xwgp-29	RLK-Rev/Xa1NBS-F	180
Xwgp-30	LM638/S2	390
Xwgp-31	Pto kin2/RLK-For	360
Xwgp-32	Pto kin2/NLRR-INV1	210

[a] Sizes of *Xwgp-17*, *Xwgp-18*, *Xwgp-21*, *Xwgp-22* and *Xwgp-27* were based on sequence data. All others were estimated based on the 1-kb-plus marker

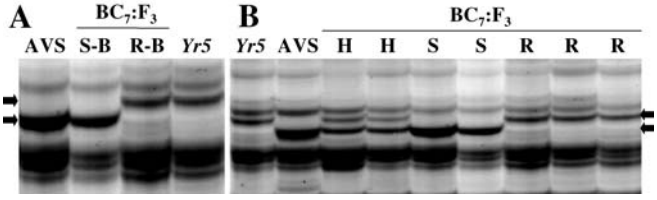

Fig. 1A, B Silver-stained denaturing polyacrylamide gels showing the resistance gene-analog polymorphism (RGAP) markers *Xwgp-17* (*upper arrows*, 546 bp) and *Xwgp-18* (*lower arrows*, 540 bp) amplified with primers S2 (5′-GGIGGIGTIGGIAAIACIAC-3′) and AS3 (5′-IAGIGCIAGIGGIAGICC-3′). Both completely co-segregated with *Yr5* in a population consisting of 202 BC_7:F_3 lines. **A** The bulk segregant analysis with the recurrent parent 'Avocet Susceptible' (AVS), the susceptible bulk (S-B), the resistant bulk (R-B) and the resistant parent, the *Yr5* near-isogenic line (*Yr5*). **B** The co-segregating analysis with the resistant parent (*Yr5*), the susceptible parent (AVS) and individual BC_7:F_3 lines. *R* stands for homozygous-resistant, *H* for heterozygous, and *S* for homozygous-susceptible lines determined by seedling tests with North American races PST-29 and PST-43 of *P. striiformis* f. sp. *tritici*

tant with either infection type (IT) 0 (no visible symptom) or IT 1 (tiny necrotic or chlorotic flecks). Avocet Susceptible (AVS) was susceptible with IT 9 (heavy sporulation without necrosis and chlorosis). Of all 329 BC_7:F_3 lines tested with races PST-29 and PST-43, 87 were homozygous-resistant, 157 were segregating, and 85 were homozygous-susceptible, which fit a 1:2:1 ratio (P = 0.7). Within each of the heterozygous BC_7:F_3 line, segregation of resistant and susceptible plants fit a 3:1 ratio. Therefore, the *Yr5* BC_6 NIL that was used as the resistant parent had a single resistance gene, *Yr5*.

RGAP makers associated with *Yr5*

Of the 379 primer pairs from 48 individual RGA primers that were screened in bulk segregant analyses, 78 pairs (21%) produced 89 polymorphic bands that differentiated the *Yr5* line and the resistant bulk from AVS and the susceptible bulks. Thirteen primer pairs that produced 16 stronger, repeatable polymorphic bands were selected for co-segregation analysis (Table 1). Segregations of the 16 RGAP markers were studied using 109 BC_7:F_3 lines. The 16 markers segregated in a 3:1 ratio for the presence and absence of each marker in the BC_7:F_3 lines with P values of 0.09 to 0.37. The RGAP markers *Xwgp-17* and *Xwgp-18* were co-dominant, and so were *Xwgp-19* and *Xwgp-20*. Genetic relationships of the RGAP markers with *Yr5* were determined by linkage analysis. The fragment sizes and primer pairs of the 16 RGAP markers are shown in Table 2. Figure 1 shows co-dominant markers *Xwgp*-17 (546 bp) and *Xwgp*-18 (540 bp) that completely co-segregated with the *Yr5* locus. Linkage analyses of the 16 RGAP markers with the 109 BC_7:F_3 lines showed that five markers were coincident with the resistant allele and four markers were coincident with the susceptibility allele at the *Yr5*

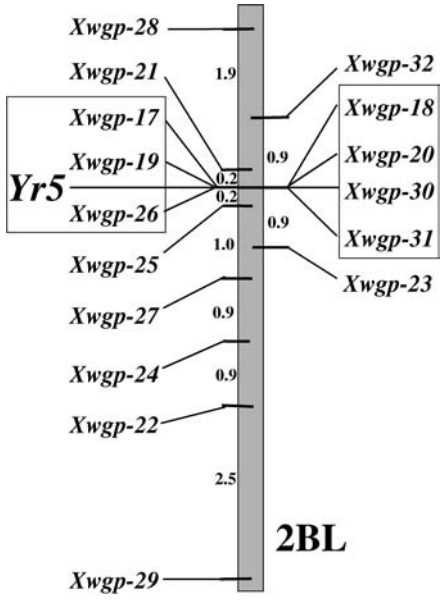

Fig. 2 Linkage map constructed with resistance gene-analog polymorphism (RGAP) markers using the MAPMAKER, version 3.0. The markers co-segregating with, or linked to, the resistance allele at the *Yr5* locus are shown on the left, and the markers co-segregating with, or linked to, the susceptibility allele at the *Yr5* locus are shown on the right. Note: the six RGAP markers (*Xwgp-17*, *Xwgp-18*, *Xwgp-19*, *Xwgp-20*, *Xwgp-26* and *Xwgp-31*) completely co-segregated with the *Yr5* locus in a population of 202 BC_7:F_3 lines, and *Xwgp-30* completely co-segregated with the *Yr5* locus in a population of 109 BC_7:F_3 lines. The linkage on chromosome 2B was confirmed by analyzing the nulli-tetrasomic Chinese Spring lines with *Xwgp-18*, *Xwgp-20* and *Xwgp-23*

locus. The other five markers in coupling and two markers in repulsion were linked to *Yr5* with a genetic distance ranging from 0.5 to 7.3 cM. Of the nine markers that were tested with 93 additional BC_7:F_3 lines, three markers in coupling and three in repulsion co-segregated with *Yr5*, and three markers were closely

Fig. 3 The alignment of amino-acid sequences of the RGAP markers *Xwgp-17* and *Xwgp-18* for wheat resistance gene *Yr5* with a resistance gene-like fragment (AJ249949) from *A. ventricosa* and seven selected plant resistance genes. Residues identical to those of *Xwgp-17* are highlighted with *gray backgrounds* and conserved residues are highlighted with *open boxes*. The GenBank accession numbers of the genes are the following: *Mi*, T06267; *Rpp13*, T51185; *Bs2*, AF202179; *Rpm1*, AB028231; *Rx*, AJ011801; *Prf*, T07589 and *I2*, AF118127

linked in coupling to *Yr5*. Figure 2 shows the linkage map around *Yr5* using the combined 109 and 202 BC_7:F_3 lines.

When the genomic DNA of TSA was amplified with the primer pairs for three *Yr5*-coupling markers (*Xwgp-17*, *Xwgp 19* and *Xwgp-22*), the markers were all detected. The results demonstrated that these markers were from TSA, the original *Yr5* donor.

In the tests of the 21 nulli-tetrasomic lines and the disomic line of Chinese Spring with three markers (*Xwgp-18*, *Xwgp-20* and *Xwgp-23*) that were coincident or closely linked to the susceptibility allele at the *Yr5* locus, the unique bands were detected in all lines except the N2BT2D line. These results show that these markers are on chromosome 2B and confirm the previous report that *Yr5* is on chromosome 2B, which was based on monosomic analysis (Law 1976).

Sequence analyses of the *Yr5* markers

Five RGAP markers were cloned and sequenced. The sequences of each *Xwgp-17* and *Xwgp-18* were obtained from three different clones. *Xwgp-17* had 546 bp and *Xwgp-18* had 540 bp, and they shared 98% homology in both DNA and theoretical protein sequences. The marker *Xwgp-18* had a 6-bp deletion and eight base pair substitutions (four transitions and four transversions) compared with *Xwgp-17*. The results suggested that *Xwgp-17* and *Xwgp-18* were alleles. In the BLAST search of the GenBank database, *Xwgp-17* produced 79, 744 and 501 homology hits, and *Xwgp-18* produced 60, 666 and 59 homology hits in DNA, protein and expressed sequence tag (EST) searches, respectively. Of the 744 accessions with significant homology to *Xwgp-17*, 719 were plant resistance genes, putative resistance genes or resistance gene analogs (RGAs). *Xwgp-17* had homology in amino-acid sequence with genes *Rpp13* (58%) *Rpm1* (55%) and *Rpp8* (53%) in *Arabidopsis thaliana*; *Mla6* (54%) and *Mla1* (54%) in barley; *BS2* (55%) in pepper; *Rx* (56%) and *Rx2* (54%) in potato; *Pib* (53%) in rice; *Mi* (60%), *Prf* (56%), *Sw-5* (56%), *I2* (58%), *I2c-1* (55%), *I2C-2* (54%) and *I2C-5* (57%) in tomato; and RLK (a leaf rust resistance kinase, thus putative for the wheat *Lr10* gene for leaf rust resistance) (53%) in wheat. *Xwgp-17* has significant homology with *Yr10* (49%), the only cloned gene for resistance to stripe rust (André Laroche, personal communication). Amino-acid sequence homology of *Xwgp-17* with *Rps2*, *Rps4*, *Rpp1* and *Rpp5* in *A. thaliana*; *L*, *L6*, *M*, *N1-B*, *N1-C*, *N1-D*, *N2-A*, *N2-B*, *N2-C*, *N2-D*, *Nbi-C*, *Nbi-D*, *Ngc-A*, *Ngc-C*, *Ngc-D*, *Nho-C* and *P2* in Flax; *Rp1-D*, *Rp1-dp2*, *Rp1-dp3*, *Rp1-dp7*, *Rp1-dp8*, *Rp1-kp1* and *Rp1-kp-3* in Maize; *Pi-ta* and *Xa1* in rice; and *N* and *Cf9* in tobacco ranged from 41 to 49%.

Xwgp-17 had significant homology with RGA or putative resistance genes from 27 plant species including wheat and wheat-related species. The highest homology (97%) of *Xwgp-17* and *Xwgp-18* in both DNA and amino-acid sequences were obtained with a resistance gene-like sequence (AJ249949) from *Aegilops ventricosa*, a close relative of wheat. The amino-acid sequence alignment of *Xwgp-17* and *Xwgp-18* with eight selected plant-resistant genes and resistance-like sequences are shown in Fig. 3.

The remaining 25 accessions that had significant homology with *Xwgp-17* included a myosin-heavy chain homolog in *A. thaliana* (S71195), a pollen-signaling protein with adenylyl cyclase activity in maize (AJ307886), a Chain A ATP sulfurylase in yeast (gi 14488754), a cytidylate kinase in *Sulfolobus solfatericus*, an *Apaf-1* like-gene in zebrafish (AF251502) and 20 accessions related to *Apaf-1* for the apoptotic protease activating factor 1 in humans (e.g. AJ243009, XP_39639.1 and NP_177430.1).

The BLAST search for conserved domains with *Xwgp-17* and *Xwgp-18* revealed that they are within a NB-ARC (nucleotide binding – *Apaf-1* in humans for apoptotic signaling, resistance genes in plants and *Ced-4* in nematode for cell death) domain, a novel signaling-motif shared by plant resistance gene products and regulators of cell death in animals (van der Biezen and Jones 1998). The consensus NB-ARC domain (pfam00931) has 308 amino acids. The theoretical translated sequence of *Xwgp-17* matched the residues between the 50th to 221st amino-acid positions with 73 identities (40%) and 108 positive (59%) residues. The alignment of amino-acid sequences of *Xwgp-17* and the consensus NB-ARC domain is shown in Fig. 4.

The EST that had the highest homology with *Xwgp-17* and *Xwgp-18* was a wheat etiolated seedling root cDNA clone (BE405507). *Xwgp-17* and the wheat EST had two significant homologous regions; 52 of 58 (89%) identities (Score = 67.9 bits, E = 2e-09) and 38 of 45 (84%) identities. These results suggested that *Xwgp-17* might be part of a functional gene.

The sequences of other markers had similarity with some functional plant genes. For instance, *Xwgp-21*, which was about 0.5-cM away from *Yr5*, had a sequence similarity to an EST (BG590490) from potato inoculated with *Phytophthora infestans*. The predicted peptide of *Xwgp-22*, which was about 0.9-cM away from *Yr5*, had homology with 246 accessions related to a gypsy type retrotransposon (e.g. AP002486), a GAG-POL precursor (e.g. AB030283), a polyprotein (e.g. AF111709) and reverse transcriptase (e.g. AF112702) in plant and animal species. *Xwgp-27*, which was 0.8-cM away from *Yr5*, had homology with the ribulosebisphosphate carboxylase/oxygenase activase gene (gi167094) and ESTs from *Pseudomonas*-resistant tomato (AI774612), *Medicago truncatula* leaves infected with *Collectotrichum trifolii* (BF520995) and in vitro expressed *Cladosporium fulvum* cDNA similar to an elongation factor 1-alpha mRNA sequence (BE188008).

Fig. 4 Amino-acid sequence alignment of the RGAP marker *Xwgp-17* for wheat stripe rust resistance gene *Yr5* with the consensus NB-ARC domain (pfam00931), a novel signaling-motif shared by plant resistance gene products and regulators of cell death in animals (van der Biezen and Jones 1998). The two peptides share 73 of 182 (40%) identical residues highlighted with *gray backgrounds* and 108 of 182 (59%) conserved residues highlighted with *open boxes*

Discussion

The resistance gene-analog polymorphism (RGAP) technique, which was first used by Chen et al. (1998b) to detect polymorphisms in wheat, rice and barley, has proved to be highly efficient for identifying molecular markers for disease resistance genes. The technique uses high-resolution electrophoresis and sensitive detection of DNA fragments amplified using primers based on conserved domains of plant resistance genes. In the previous studies, several RGAP markers were coincident with resistance to different diseases (Chen et al. 1998a, 1999; Toojinda et al. 2000). Using BC_7:F_2 and BC_7:F_3 populations, Shi et al. (2001) identified 16 RGAP markers for the *Yr9* gene resistance to wheat stripe rust. Four of the markers co-segregated with *Yr9*. Using the RGAP markers, they successfully determined the presence or absence of the *Yr9* gene in cultivars that have been postulated to have *Yr9*. In this study, 89 putative RGAP markers were identified for *Yr5* using 379 RGA primer pairs in bulk segregant analyses. Associations of 16 of the markers with *Yr5* were confirmed with the BC_7:F_3 populations. Six of the markers co-segregated with the *Yr5* locus, and 11 markers were tightly linked to the locus. These results demonstrate that the RGAP technique is efficient for identifying direct or tightly linked markers for disease resistance genes.

Direct markers that are completely associated with resistance genes are more effective than linked markers in marker-assisted selection. Conserved domains shared by genes from diverse plant species for resistance to various pathogens (Michelmore 1996; Baker et al. 1997; Hammond-Kosack and Jones 1997) provide the possibility of identifying direct markers. In this study, we report that *Xwgp-17* and *Xwgp-18* had high homology with many plant resistance genes. These two DNA fragments are almost identical (97% homology) to a cloned resistance-gene-like fragment from *Ae. ventricosa*, a wild wheat

species that was the donor of the *Yr17* gene for resistance to stripe rust, the *Lr37* gene for resistance to leaf rust and the *Sr38* gene for resistance to stem rust (McIntosh et al. 1998), and the *CreX* gene for resistance to cereal cyst nematode (Seah et al. 2000). Moreover, *Xwgp-17* and *Xwgp-18* are part of the NB-ARC domain. The NB-ARC have been found in several plant resistance gene products, including *I2C-1* and *Prf* in tomato, *Rpm1* and *Rps2* in *A. thaliana*, and *L6* in flax for resistance to *Fusarium* wilt and *Pseudomonas* spot of tomato, *Pseudomonas* spot of *Arabidopsis* and flax rust, respectively (van der Biezen and Jones 1998). This study demonstrates that the RGAP technique can produce markers not only co-segregating with a resistance gene but are also highly homologous to other plant resistance genes. The markers identified in this study should be useful in cloning *Yr5* and studying molecular plant-pathogen interactions, because they co-segregate with the gene, have a resistance-gene like sequence structure and may be part of the sequences of the resistance and susceptibility alleles at the *Yr5* locus.

Isolates of *P. s. tritici* with virulence to *Yr5* are extremely rare (Stubbs 1985; McIntosh et al. 1995), suggesting that *Yr5* is an excellent gene in breeding for stripe rust resistance. Because the *Yr5* resistance is race-specific, the gene should be used in combination with other effective genes and/or with non-race-specific high-temperature, adult-plant resistance. The direct or tightly linked markers developed in this study should be useful for combining *Yr5* with other genes to obtain superior and durable resistance.

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References

Allan RE, Purdy LH (1967) Single gene control of stripe rust resistance in wheat. Plant Dis Rep 51:1041–1043

Allan RE, Peterson CJ, Line RF, Rubenthaler GL, Morris CF (1993) Registration of 'Rely' wheat multiline. Crop Sci 33:213–214

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BALAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Baker B, Zambryski P, Stazkawicz B, Dinesh-Kumar SP (1997) Signaling in plant-microbe interactions. Science 276:726–733

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

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

Chen XM, Line RF (1992c) Genes for resistance to stripe rust in 'Tres' wheat. Crop Sci 32:692–696

Chen XM, Line RF (1993) Inheritance of stripe rust (yellow rust) resistance in the wheat cultivar Carstens V. Euphytica 71:107–113

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

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

Chen XM, Hayes PM, Toojinda T, Vivar H, Kudrna D, Kleinhofs A, Leung H, Line RF (1998a) Genetic mapping of genes for stripe rust resistance in barley using resistance gene analog polymorphism and AFLP markers. Phytopathology 88:S16

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

Chen XM, Line RF, Shi ZX, Leung H (1998c) Genetics of wheat resistance to stripe rust. In: Proc 9th Int Wheat Genet Symposium, 2–7, August, 1998, Saskatoon, Saskatchewan, Canada. Slinkard AE (ed) University Extension Press, University of Saskatchewan, Saskatoon, Vol. 3, pp 237–239

Chen XM, Line RF, Hayes PM, Toojinda T, Vivar H, Kleinhofs A, Kudrna D (1999) Mapping barley genes for resistance to stripe rust, leaf rust and scab, using resistance gene analog polymorphism and restriction fragment length polymorphism. Phytopathology 89:S15

Chen XM, Moore M, Milus EA, Long D, Marshall D, Line RF, Jackson L (2002) Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. Plant Dis 86:39–46

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like-kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J 11:45–52

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Mol Biol 48:575–607

Kanazin V, Marek LF, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93:11,746–11,750

Kema GHJ (1992) Resistance in spelt wheat to yellow rust, I. Formal analysis and variation for gliadin patterns. Euphytica 63:207–217

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

Lander ES, Green P, Abrahamson J, Barlow A, Daly JM, Lincoln SE, Newberg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Law CN (1976) Genetic control of yellow rust resistance in *T. spelta album*. Plant Breeding Institute, Cambridge, Annual Report 1975, pp 108–109

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plant. Nature Genet 14:421–429

Line RF, Chen XM (1995) Success in breeding for and managing durable resistance to wheat rusts. Plant Dis 79:1254–1255

Line RF, Chen XM (1996) Wheat and barley stripe rust in North America. In: Proc 9th European and Mediterranean Cereal Rusts and Powdery Mildews Conf, 2–6 September 1996, Lunteren, The Netherlands, pp 101–104

Line RF, Qayoum A (1991) Virulence, aggressiveness, evolution, and distribution of races (of the causes of stripe rust of wheat) in North America. 1968–87. Tech Bull US Dept Agric No. 1788

Lupton FGH, Macer RCF (1962) Inheritance of resistance to yellow rust (*Puccinia glumarum* Erikss & Henn) in seven varieties of wheat. Trans Br Mycol Soc 45:21–45

Macer RCF (1966) The formal and monosomic genetic analysis of stripe rust (*Puccinia striiformis*) resistance in wheat. In: Proc 2nd Int Wheat Genet Symposium, Mackey J (ed) Lund, Sweden 1963. Hereditas Suppl 2:127–142

Maniatis TA, Frisch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO, Australia

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symposium, 2–7 August 1998, Saskatoon, Saskatchewan, Canada. University Extension Press, University of Saskatchewan, Saskatoon. vol. 5, pp 1–235

Michelmore R (1996) Flood warning – resistance genes unleashed. Nature Genet 14:376–378

Milus EA, Line RF (1986a) Number of genes controlling high-temperature, adult-plant resistance to stripe rust in wheat. Phytopathology 76:93–96

Milus EA, Line RF (1986b) Gene action for inheritance of durable, high-temperature, adult-plant resistance to stripe rust in wheat. Phytopathology 76:435–441

Nagarajan S, Nayar SK, Bahadur P (1986) Race 13 (67 S8) virulent on *Triticum spelta* var. *album* in India. Plant Dis 70:173

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

Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci 36:905–909

Seah S, Spielmeyer W, Jahier J, Sivasithamparam K, Lagudah ES (2000) Resistance gene analogs within an introgressed chromosomal segment derived from *Triticum ventricosum* that confers resistance to nematode and rust pathogens in wheat. Mol Plant-Microbe Interact 13:334–341

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

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

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

Toojinda T, Broers L, Chen XM, Hayes PM, Kleinhofs A, Korte J, Kudrna D, Leung H, Line RF, Powell W, Vivar H (2000) Mapping qualitative and quantitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor Appl Genet 101:580–589

van der Biezen EA, Jones JDG (1998) The NB-ARC domain: a novel signaling motif shared by plant resistance gene products and regulators of cell death in animals. Curr Biol 8:226–227

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

Yu YG, Buss GR, Maroof MA (1996) Isolation of a superfamily of candidate disease resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93:11,751–11,756