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Pm37, a new broadly effective powdery mildew resistance gene from *Triticum timopheevii*

L. D. Perugini · J. P. Murphy · D. Marshall · G. Brown-Guedira

Abstract Powdery mildew is an important foliar disease in wheat, especially in areas with a cool or maritime climate. A dominant powdery mildew resistance gene transferred to the hexaploid germplasm line NC99BGTAG11 from *T. timopheevii* subsp. *armeniacum* was mapped distally on the long arm of chromosome 7A. Differential reactions were observed between the resistance gene in NC99BGTAG11 and the alleles of the *Pm1* locus that is also located on chromosome arm 7AL. Observed segregation in $F_{2:3}$ lines from the cross NC99BGTAG11 × Axminster (*Pm1a*) demonstrate that germplasm line NC99BGTAG11 carries a novel powdery mildew resistance gene, which is now designated as *Pm37*. This new gene is highly effective against all powdery mildew isolates tested so far. Analyses of the population with molecular markers indicate that *Pm37* is located 16 cM proximal to the *Pm1* complex.

L. D. Perugini · J. P. Murphy
Department of Crop Science,
North Carolina State University, Raleigh, NC 27695, USA
e-mail: ldperugi@ncsu.edu

J. P. Murphy
e-mail: paul_murphy@ncsu.edu

D. Marshall
USDA-ARS Plant Science Research Unit,
Department of Plant Pathology,
North Carolina State University, Raleigh, NC 27695, USA
e-mail: david_marshall@ncsu.edu

G. Brown-Guedira (✉)
USDA-ARS Plant Science Research Unit,
Department of Crop Science,
North Carolina State University, Raleigh, NC 27695, USA
e-mail: gina.brown-guedira@ars.usda.gov

Simple sequence repeat (SSR) markers *Xgwm332* and *Xwmc790* were located 0.5 cM proximal and distal, respectively, to *Pm37*. In order to identify new markers in the region, wheat expressed sequence tags (ESTs) located in the distal 10% of 7AL that were orthologous to sequences from chromosome 6 of rice were targeted. The two new EST-derived STS markers were located distal to *Pm37* and one marker was closely linked to the *Pm1a* region. These new markers can be used in marker-assisted selection schemes to develop wheat cultivars with pyramids of powdery mildew resistance genes, including combinations of *Pm37* in coupling linkage with alleles of the *Pm1* locus.

Introduction

Powdery mildew, caused by *Blumeria graminis* (DC) Speer f. sp. *tritici* emend Marchal (*Bgt*), is an important foliar disease in wheat (*Triticum aestivum* L.), especially in areas with a cool or maritime climate. Powdery mildew impacts grain yield, significantly reduces flour yield and adversely affects other aspects of grain quality (Everts et al. 2001; Hsam and Zeller 2002). The use of resistant cultivars is an effective, economical, and environmentally safe approach that eliminates the use of fungicides and reduces production losses due to this disease.

The most common breeding strategy for resistance to powdery mildew in wheat has been the use of qualitative (race-specific) resistance conferring hypersensitive foliar reactions (Chen et al. 2005; Huang and Röder 2004). This type of resistance follows the gene-for-gene hypothesis described by Flor (1955), and because of the co-evolution of host and pathogen, race-specific resistance can be overcome by new races of the pathogen possessing corresponding

virulence genes. Leath and Murphy (1985) found that the 10 most widely used resistance genes had matching virulence genes in the Southeastern USA. In Europe, 10 powdery mildew resistance genes in wheat cultivars showed corresponding virulence genes in the pathogen (Clarkson 2000). Therefore, the search for and deployment of new powdery mildew resistance genes is necessary to provide wheat growers with resistant cultivars. Currently, 49 powdery mildew resistance genes mapped at 33 loci have been identified in wheat and its wild relatives (McIntosh et al. 2003, 2004, 2005).

The cultivated *Triticum timopheevii* subsp. *timopheevii* (Zhuk.) Zhuk. and its wild form *T. timopheevii* subsp. *armeniacum* (Jakubz.) van Slageran (A^{t}A^{t}GG, $2n = 28$) have been used as sources of pest resistance genes for wheat, including genes for resistance to powdery mildew. Resistance genes *Pm6* and *Pm27* were transferred to wheat chromosomes 2B and 6B from cultivated *timopheevii* wheat (Jarve et al. 2000; Jørgensen 1973). A powdery mildew resistance gene was transferred to the long arm of chromosome 7A (7AL) from the wild *T. timopheevii* subsp. *armeniacum* to germplasm line NC99BGTAG11 (Srnić et al. 2005). The *Pm1* locus is also located on chromosome 7AL and it possesses five dominant alleles, *Pm1a* to *Pm1e*. In addition, recessive genes *mlRD30* and *Pm9* have been reported to be linked to the *Pm1* locus (Schneider et al. 1991; Singrün et al. 2004). None of the alleles of the *Pm1* locus originate from tetraploid wheat relatives. The gene *Pm1b*, and two genes that are either new allelic variants of *Pm1* or closely linked to the *Pm1* locus (Yao et al. 2007) were introgressed from the diploid einkorn *Triticum monococcum* L. (A^{m}A^m, $2n = 14$), and *Pm1d* was introgressed from *Triticum aestivum* L. subsp. *spelta* (Hsam et al. 1998). Neu et al. (2002) suggested that *Pm1a* might have been derived from a wheat relative.

Molecular markers have been used for tagging and mapping powdery mildew resistance genes in wheat, including those on chromosome 7A. Srnić et al. (2005) reported simple sequence repeat (SSR) markers *Xgwm332* and *Xwmc525* as flanking the resistance gene in NC99BGTAG11 with genetic distances of 2.0 centimorgans (cM) proximal (towards the centromere) and 1.4 cM distal (towards the telomere), respectively. The RFLP markers *Xpsr148* and *Xpsr680* were reported to co-segregate with the powdery mildew resistance gene *Pm1* (Ma et al. 1994; Neu et al. 2002). The *Xpsr148* and *Xpsr680* markers were converted to STS markers named *Xmag1714* and *Xmag2185*, respectively (Yao et al. 2007).

The increasing availability of expressed sequence tag (EST) and genomic sequences from wheat is providing a potentially valuable source for marker enrichment. Currently, there are more than 580,000 wheat ESTs with 122,282 unique sequences (http://www.tigr.org/tigr-scripts/ tgi/T_index.cgi?species=wheat) deposited in public databases. This provides an excellent resource for mapping genes. A set of wheat deletion lines has been used to locate more than 8,300 unique ESTs into chromosome bin maps (Hossain et al. 2004; Lazo et al. 2004; Qi et al. 2004). The existing synteny between rice and wheat, as well as other cereals, can be exploited to tentatively position ESTs in silico based on orthology with sequences in the rice genome. Hossain et al. (2004) mapped 2148 EST loci to the three homeologous group 7 chromosomes of wheat, showed the distribution of mapped EST loci to the chromosome bins defined by the deletion stocks, and identified putative regions of conserved gene content between the wheat group 7 consensus chromosome and rice chromosome 6 and 8. In addition, several STS markers derived from wheat ESTs homologous to the coding DNA sequences of rice chromosome 6L were linked to two powdery mildew genes mapped on the distal region of 7AL (Yao et al. 2007).

The present study reports the genetic relationship between the resistance gene in NC99BGTAG11, now designated *Pm37*, and the *Pm1* locus, and the identification of additional molecular markers linked to these resistance genes.

Materials and methods

Plant material

Soft red winter wheat germplasm line NC99BGTAG11 (NCAG11 hereafter) (Reg. no. GP-729, PI 615588) is an F$_7$-derived line with the pedigree 'Saluda'*3/PI 427315 (Murphy et al. 2002). Saluda (PI 480474) is a soft red winter wheat developed and released by Virginia Polytechnic Institute and State University (Starling et al. 1986) and it possesses the powdery mildew resistance gene *Pm3a*. The *Pm3a* gene is not effective against naturally occurring powdery mildew populations in North Carolina (Leath and Heun 1990). PI 427315 is a winter growth habit accession of *T. timopheevii* subsp. *armeniacum* collected in Iraq. 'Axminster' (PI 228307) is a *T. aestivum* cultivar that possesses the powdery mildew resistance gene *Pm1a*. A population of 198 F$_{2:3}$ lines was developed from the cross NCAG11/Axminster to evaluate allelism of the *T. timopheevii*-derived resistance gene with *Pm1a*.

The Wheat Genetics Resource Center at Kansas State University supplied the Chinese Spring (CS) wheat and chromosome 7A-related CS aneuploids used in the study. These included: nullisomic 7A-tetrasomic 7B (N7A-T7B), nullisomic 7A-tetrasomic 7D (N7A-T7D), nullisomic 7B-tetrasomic 7A (N7B-T7A), nullisomic 7D-tetrasomic 7A (N7D-T7A), and nullisomic 7D-tetrasomic 7B (N7D-T7B) (Sears 1966); ditelosomic lines 7AS (Dt7AS) and 7AL

(Dt7AL) (Sears and Sears 1979); and four CS deletion lines for the terminal region of the long arm of chromosome 7A. The deletion lines are designated by the chromosome arm carrying the deletion and the length of the terminal deletion, expressed as a fraction length (FL) of the whole arm. Deletion lines included were 7AL-16 (FL = 0.86), 7AL-2 (FL = 0.87), 7AL-20 (FL = 0.89), and 7AL-15 (FL = 0.99) (Endo and Gill 1996; Qi et al. 2003). Together, the nulli-somic–tetrasomic lines and the deletion lines were used for the chromosome and deletion bin mapping of EST-based STS and SSR markers linked to the powdery mildew resistance gene in NCAG11 (*Pm37*).

The soft winter (SW) wheat cultivars and breeding lines Ernie, Neuse, McCormick, Roane, Truman, Pioneer brand 2545, VA99W-200, Pioneer brand 2555, Superior, Patterson, Freedom, Patton, GA881130, and Batavia were analyzed with the SSR marker *Xgwm332* to determine the level of polymorphism for this marker in elite SRW germplasm.

Powdery mildew evaluations

Powdery mildew resistance was evaluated in the laboratory using the detached leaf technique. Primary leaf segments (1.5-cm) were floated on 0.5% water agar (w/v) amended with 50 mg l^{-1} benzimidazole in plastic plates. Lines possessing *Pm1a*, *Pm1b*, *Pm1c*, *Pm1d*, and *Pm1e*, NCAG11, Saluda, and the control Chancellor were inoculated with 14 different powdery mildew isolates collected from wheat fields in the eastern region of North Carolina. Plates were placed in a growth chamber maintained at 18°C, 85% relative humidity and with a photoperiod of 12 h. The disease severity evaluation was based on a scale from 0 to 9, in which 0–3 = resistant with (0) no visible signs of infection; (1) flecks with no necrosis, (2) necrosis, to (3) chlorosis; 4–6 = intermediate reaction with chlorotic areas decreasing in amount while mycelium and conidia production increased from slight to moderate; and 7–9 = susceptible with increasing amount, size and density of mycelium and conidia to a fully compatible reaction.

Twenty seeds each of 198 F$_{2:3}$ families from NCAG11/Axminster were grown at 18°C with a photoperiod of 12 h in a growth chamber and inoculated with the *Bgt* 'Yuma' isolate that was avirulent to both NCAG11 and Axminster, but virulent to Saluda (*Pm3a*). All plants were inoculated when they showed three fully developed leaves about 10–12 days after emergence. The resistant parents (NCAG11 and Axminster) were included as resistant controls and Saluda as the susceptible control. Evaluations were made when the susceptible controls showed distinct disease symptoms and the resistant parents showed no signs of disease. Susceptible and selected segregating families were re-screened with another avirulent *Bgt* isolate, Arapahoe, to confirm the

reactions. In these tests, the reaction of *Pm1a* (infection type, IT = 0) could not be distinguished from that of *Pm37* (IT = 0). The goodness-of-fit to the segregation ratio of 7 resistant:8 segregating:1 susceptible was tested using the χ^2 test.

An additional 20 seeds of each of 198 F$_{2:3}$ families from NCAG11/Axminster was grown at 18–20°C in a greenhouse where natural light was supplemented with artificial high intensity 1,000 W discharge lights to provide 12 h day:12 h night. Plants were inoculated with the *Bgt* 'E$_3$14' isolate that is virulent to *Pm1a*. Thirty plants of each of the parents and Saluda were included in the evaluation. All plants were inoculated at the three- to four-leaf stage about 15 days after emergence. The resistant parent (NCAG11) was considered as the resistant control and Axminster and Saluda were the susceptible controls. Evaluations were based on a 0–9 scale as described above, and data were obtained 15–20 days after inoculation when the susceptible controls showed distinct disease symptoms and the resistant parent showed no signs of disease.

Molecular marker analyses

Leaf tissue was harvested in bulk from 20 young plants each of the 198 F$_{2:3}$ lines from the NCAG11/Axminster population, the parents, CS wheat, and the CS aneuploids and deletion lines, and stored at −80°C. Frozen leaf tissue was ground in a GenoGrinder (Spex, Metuchen, NJ) and genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Genomic DNA was amplified with SSR markers and EST-STS markers. Polymerase chain reaction (PCR) amplifications were performed in 12-μl reactions with 1.2 μl of 10× PCR buffer (containing 1.5 mM magnesium chloride), 0.97 μl of dNTPs (2.5 mM each dNTP), 0.5 μl of each forward and reverse primers (10 pmol/μl) and 40–60 ng of DNA in an Eppendorf Mastercycler® Gradient (Brinkmann Instruments, Inc., NY, USA). Forward SSR primers were 5′ labeled with fluorescent dyes (6-FAM and VIC). After initial denaturation at 95°C for 3 min, 40 amplification cycles were performed with 94°C for 45 s, 54–62°C (marker dependent) for 45 s and 72°C for 1 min, and a final extension at 72°C for 10 min. Sizing of the SSR fragments was resolved in an ABI3130 DNA analyzer (Applied Biosystems, Foster, CA) following manufacture's instructions, and results were analyzed in GeneMarker v1.5 (SoftGenetics LLC, State College, PA). PCR products of EST-STS markers were resolved in 2.3% high resolution agarose (Gene Pure HiRes Agarose, ISC BioExpress) gels with 0.5× TBE buffer and visualized by ethidium bromide staining. After the fragment size of the PCR products from EST-STS markers were verified in agarose gels, the PCR products were denatured and separated on

380 × 500 × 0.4 mm single stranded conformation polymorphism (SSCP) gels using a mutation detection enhancement (MDE) gel solution (Martins-Lopes et al. 2001). The gel mix was made in an 80-ml total volume containing a final concentration of 0.5× gel solution (Cambrex Bio-science Rockland, Rockland, ME) and 0.6× TBE buffer, and polymerized by the addition of 0.16 ml of 20% ammonium persulphate and 24 μl of tetramethylethylenediamine (TEMED). Fragments were electrophoresed for 16 h at a constant power of 4 W at room temperature; and then, silver stained as described by Bassam et al. (1991). The STS marker *XstsBE406627* was subsequently assayed using a fluorescent labeled primer and resolved in the ABI3130 sequencer. Genomic DNA of 14 SRW wheat lines was amplified with SSR marker *Xgwm332* and electrophoresis was conducted at 110 W for 2.0 h on a 6% denaturing polyacrylamide gel (19 acrylamide:1Bis), 8 M urea and 1× TBE. Amplified fragments were visualized by silver-staining.

SSR markers of the terminal region of 7AL were evaluated for polymorphism between NCAG11 and Axminster. The physical locations of two wheat SSR markers (*Xgwm332* and *Xwmc525*) that were previously reported to flank the powdery mildew resistance gene in NCAG11 (Srnić et al. 2005) and other SSR markers previously mapped in the terminal region of 7AL, including *Xcfa2257*, *Xcfa2293*, *Xwmc790*, *Xgwm63*, *Xwmc633*, *Xcfa2019*, *Xgwm554*, *Xgwm346*, *Xwmc273*, *Xgwm344*, and *Xcfa2040*, were determined by evaluating the markers on the CS aneuploid and deletion line stocks. In addition, two STS markers, *Xmag2185* and *Xmag1714*, developed from RFLP markers (*Xpsr680* and *Xpsr148*, respectively) and one STS marker, *Xmag1759* developed from a wheat EST (Yao et al. 2007) were evaluated. The SSR and STS data indicated the physical location of the resistance gene in NCAG11 and were used for subsequent identification of wheat ESTs likely linked to the resistance gene. The terminal region of chromosome 7AL of wheat is syntenic to the distal region of chromosome 6L of rice (La Rota and Sorrells 2004) and was targeted for marker enrichment. Fifty-seven unique wheat EST sequences that mapped distal to the 7AL-18 (FL = 0.90) deletion breakpoint (http://wheat.pw.usda.gov/

cgi-bin/westsql/map_locus.cgi) were used to search the rice genome database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) using the BLASTn program (Altschul et al. 1997). Sequences in the target region of the rice genome were also used as queries in BLASTn searches of the wheat EST database (http://tigrblast.tigr.org/tgi/) to identify additional unmapped wheat ESTs that were potentially linked to *Pm37*. Wheat EST sequences with high levels of identity (E values < e−15) to sequences from chromosome 6L of rice were used to design primers for EST-derived STS markers. Primer design was conducted with the software Primer3 (Rozen and Skaletsky 2000), and amplicons of 200–500 base pairs (bp) were targeted. All markers were mapped physically using the CS deletion lines. Polymorphic markers that physically mapped in the target region were evaluated on 198 lines of the NCAG11/Axminster $F_{2:3}$ mapping population. Primer sequences for the two polymorphic STS markers mapped in this study are listed in Table 1.

Chi-squared (χ^2) tests were used to test for deviations of observed data from theoretically expected segregation ratios in the allelism tests and in the mapping population. Genetic maps were constructed using the program JoinMap®4 (van Ooijen 2006) and recombination frequencies were converted to cM using the Kosambi mapping function (Kosambi 1944) to estimate genetic distances with a minimum LOD of 3.0.

Results

Differential powdery mildew responses

Differential reactions were observed on lines possessing *Pm1a*, *Pm1b*, *Pm1c*, *Pm1d*, and *Pm1e*, NCAG11, Saluda, and Chancellor inoculated with 14 *Bgt* isolates (Table 2). All the *Pm1* alleles could be differentiated from each other and from the gene in NCAG11, indicating that the powdery mildew resistance in NCAG11 was different from the five designated *Pm1* alleles. NCAG11 was highly resistant to all isolates. The Chancellor control was fully susceptible to all isolates. The cultivar Saluda used as the recurrent parent in the development of NCAG11 has the *Pm3a* resistance gene

Table 1 The ESTs from which STS markers were developed, primer sequences, and annealing temperature of two wheat EST-derived STS markers linked to *Pm37* in deletion bin 7AL20 (FL = 0.89)-7AL15 (FL = 0.99)

Wheat EST[a]	STS marker designation	Primer sequence	Annealing temperature (°C)
BE406627	STSBE406627	F 5′-GTCTGCGAAAAGAACGAAA-3′	58
		R 5′-CTTCTCTAGCGCTCCATGCT-3′	
BE445653	STS BE445653	F 5′-GCGTGGTATCCCATATACCG-3′	54
		R 5′-CTTTTGAACCGGATCTGCTC-3′	

[a] Designations of ESTs (GenBank) as of July 2007

Table 2 Reactions of five genotypes possessing *Pm1* alleles, NCAG11, Saluda, and the susceptible control Chancellor after inoculation with 14 isolates of *Blumeria graminis* f.sp. *tritici* (*Bgt*)

Cultivar/line[a]	*Pm* gene	*Bgt* isolates													
		ZoneLP	Yuma	Sturdy 2KPm5	101a2	Asosan	Arapahoe	E₃14	Trego	W72-27	#8	85063	Flat 7–11	169-1b	AB9-10
Axminster	*Pm1a*	R[b]	R	R	S	R	R	S	R	S	S	S	R	R	R
MocZlatka	*Pm1b*	I	R	R	R	R	R	S	R	S	R	I	R	R	R
M1N	*Pm1c*	R	I	I	I	R	S	R	S	R	R	S	S	S	S
T. spelta var. duhamelianum	*Pm1d*	R	R	R	S	R	R	S	R	S	I	S	R	R	R
Virest	*Pm1e*	R	R	R	S	R	R	S	R	S	S	S	I	R	R
NCAG11	*Pm37*	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Saluda	*Pm3a*	S	S	S	R	S	S	S	S	R	R	R	S	R	S
Chancellor	–	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[a] Axminster, Sears and Briggle (1969); MocZlatka, M1N, and *T. spelta*, Hsam et al. (1998); Virest, Singrün et al. (2003); NCAG11, Murphy et al. (2002); Saluda, Starling et al. (1986); Chancellor, Briggle (1969)

[b] R = resistant; I = intermediate; S = susceptible

and gave intermediate or resistant reactions with five isolates. MocZlatka (*Pm1b*) was fully susceptible to only two *Bgt* isolates and intermediate to two others. In contrast, lines possessing other *Pm1* alleles were fully susceptible to four or more *Bgt* isolates.

Allelism test

Eight of the *Bgt* isolates were avirulent to the genes in Axminster and NCAG11 and virulent to *Pm3a* that is present in Saluda (Table 2). The *Bgt* 'Yuma' and *Bgt* 'Arapahoe' isolates were selected to evaluate the segregating population from the cross NCAG11/Axminster to determine if the *T. timopheevii*-derived gene in NCAG11 was an allele of the *Pm1* locus. All of the 198 F$_{2:3}$ lines showed clear reactions when inoculated with the *Bgt* 'Yuma' isolate. The reaction of susceptible and some segregating families were confirmed in a separate test using the *Bgt* 'Arapahoe' isolate. Three families were fully susceptible when inoculated with both isolates, indicating that a new powdery mildew locus is present in NCAG11 that is not an allele of *Pm1*. However, the observed ratio of 153 resistant lines:42 segregating lines:3 susceptible lines significantly differed from the 7 resistant:8 segregating:1 susceptible ratio ($\chi^2 = 90.78$, df = 2, $P < 0.0001$) expected for two independently segregating dominant genes. Thus, the new resistance gene in NCAG11 is linked to the *Pm1* locus. The *T. timopheevii*-derived resistance gene in accession NCAG11 is designated *Pm37*.

Mapping the *Pm37* powdery mildew resistance gene

Five *Bgt* isolates were virulent to *Pm1a*, but only *Bgt* 'E$_3$14' was virulent on Saluda (Table 2); and therefore, was selected to evaluate the F$_{2:3}$ lines from the cross NCAG11/Axminster in order to map *Pm37*. The observed ratio of 44 resistant:101 segregating:48 susceptible did not significantly differ from the 1:2:1 ratio ($\chi^2 = 1.94$, df = 2, $P = 0.38$) expected for a single dominant gene for powdery mildew resistance, indicating that resistance to the *Bgt* 'E$_3$14' isolate was conferred by a single resistance gene.

Seven SSR markers *Xgwm332*, *Xwmc790*, *Xcfa2019*, *Xwmc346*, *Xwmc525*, *Xwmc273*, and *Xcfa2040* were polymorphic between Axminster, NCAG11 and the recurrent parent Saluda (Table 3). Several other SSR markers that mapped on the distal region of 7AL, such as *Xcfa2257*, *Xcfa2293*, *Xgwm63*, *Xwmc633*, and *Xgwm344*, were not polymorphic between NCAG11 and Axminster, but did show polymorphism between NCAG11 and Saluda (data not shown), indicating that the distal portion of 7AL in NCAG11 was derived from the donor parent. In this study, the powdery mildew resistance gene *Pm37* was flanked by SSR loci *Xgwm332* and *Xwmc790* with genetic distances of 0.5 cM proximal and distal, respectively (Fig. 1). This indicates that there were two recombination events in the population of 198 F$_2$ families, one on each side of the gene. Both SSR markers *Xgwm332* and *Xwmc790* were co-dominant. The 193-bp allele amplified in NCAG11 by the SSR marker *Xgwm332* was not present in the 14 soft red winter cultivars and breeding lines tested (Fig. 2). The STS markers *Xmag2185* and *Xmag1714* were developed from RFLP markers *Xpsr680* and *Xpsr148*, respectively (Yao et al. 2007), and reported to co-segregate with *Pm1* (Neu et al. 2002). The STS markers mapped more than 16 cM distal to *Pm37* (Fig. 1). The STS markers were dominant and linked in repulsion to the *Pm1a* allele.

Table 3 Fragments of SSR and EST-based STS markers when amplified from parents of the mapping population and Saluda

Genotype	Marker								
	GWM332	WMC790	CFA2019	WMC346	WMC525	CFA2040	WMC273	STSBE406627	STSBE445653
NCAG11	193	76	231	200	243	237	268	431	720
Axminster	195	100	Null	203	255	310	250	Null	Null
Saluda	195	100	237	198	214	304	244	435	725

Numbers in columns are sizes in base pairs
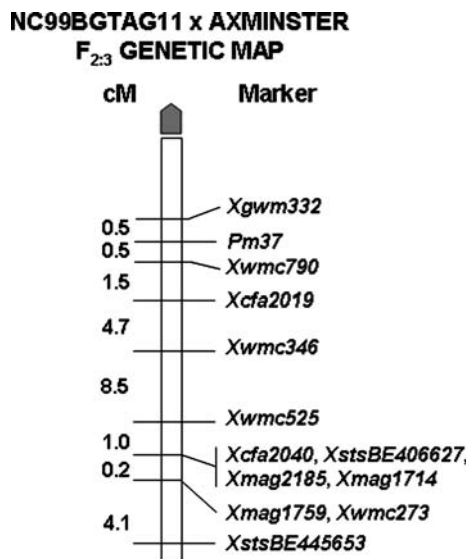
Fig. 1 Genetic map of the wheat chromosome 7AL region. The gray arrow indicates the direction of the centromere

Physical mapping and marker enrichment

The SSR markers *Xgwm332*, *Xwmc790*, *Xwmc525*, and *Xcfa2040* were located on chromosome arm 7AL in other maps of *T. aestivum* (Röder et al. 1998, Somers et al. 2004; Sourdille et al. 2004). In our study, the order of these SSR

loci agreed well with the established SSR maps of chromosome arm 7AL. Our analysis of the Chinese Spring aneuploid stocks and deletion lines confirmed the presence of these markers on 7AL and their location in the terminal 11% of the chromosome arm between the deletion breakpoints 7AL-20 (FL = 0.89) and 7AL-15 (FL = 0.99).

Wheat ESTs located in the terminal region of 7AL were targeted in an effort to obtain additional markers linked to *Pm37*. Out of 57 wheat ESTs previously mapped distal to the deletion breakpoint 7AL-18 (FL = 0.90) (Hossain et al. 2004), 22 had a significant homology to sequences on the terminal region of rice chromosome 6L (from 24.90 to 31.10 Mb), 23 had no obvious orthologous sequences in rice, and the remaining 12 had significant hits elsewhere in the rice genome. Twenty-two wheat ESTs, whose only significant orthologous rice sequences were in the terminal region of chromosome 6 were selected for primer design.

Six of the selected EST-based primer pairs amplified fragments that were located in the distal 10% of chromosome 7AL, consistent with the previous assignment of the ESTs based on RFLP analysis (Hossain et al. 2004). The other 16 EST-based primer pairs failed to amplify fragments that could be deletion mapped using SSCP analysis. Of the six markers that were deletion mapped, two STS markers, *XstsBE406627* and *XstsBE445653* (Table 1), were polymorphic between NCAG11 and Axminster and were evaluated on the mapping population. Marker
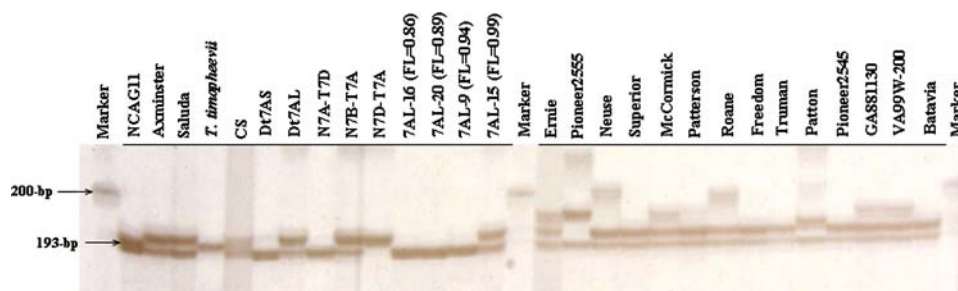

Fig. 2 A denaturing polyacrylamide gel pattern of the deletion mapping and screening of 14 soft red wheat cultivars (SRW) of the locus *Xgwm332*. Amplified fragments are observed in CS, Dt7AL, N7B-T7D, N7D-T7B, and deletion line 7AL-15, but not in Dt7AS, N7A-T7D, and deletion lines 7AL-16, 7AL-20, and 7AL-9. Polymorphism of the A-genome fragment was observed between NCAG11 and Axminster. The 193-bp allele in NCAG11 was not present in the 14 SRW wheat lines surveyed. Size standard HyperLadder IV (Bioline, Randolph, MA), with a 200-bp marker fragment indicated as Marker

XstsBE406627 co-segregated with markers *Xmag2185* and *Xmag1714* and was located 16.2 cM distal to *Pm37* (Fig. 1). Marker *XstsBE445653* was located 20.4 cM, distal to *Pm37*.

Discussion

Our differential tests, combined with allelism and molecular marker analyses, indicated that a new dominant powdery mildew resistance gene designated *Pm37* was transferred from *T. timopheevii* subsp. *armeniacum* to germplasm line NCAG11. *Pm37* is proximal to the *Pm1* locus. This new gene is highly effective in the field in southeastern U.S. and confers resistance to more than 60 different isolates of *Blumeria graminis* f. sp. *tritici* (data not shown).

The *Pm37* gene is the first powdery mildew resistance gene transferred from *T. timopheevii* to the A genome of common wheat. Two other powdery mildew resistance genes, *Pm6* and *Pm27*, were transferred from cultivated *T. timopheevii*, but these are located on chromosomes 2BL and 6B, respectively (Jørgensen 1973; Jarve et al. 2000).

Several powdery mildew resistance genes have been mapped on chromosome 7A of wheat. Among these, *Pm1*, *Pm9*, *mlRD30*, and two dominant powdery mildew resistance genes introgressed from *T. monoccocum* (Schneider et al. 1991; Sears and Briggle 1969; Singrün et al. 2004; Yao et al. 2007) are located on the long arm. Our allelism data indicates that *Pm37* is not an allele at the *Pm1* locus. The map location of the resistance genes introgressed from *T. monoccocum* by Yao et al. (2007) suggests that they are alleles at the *Pm1* locus. The resistance genes *Pm9* and *mlRD30* were reported to be recessive. The cultivar Normandie carries the recessive powdery mildew resistance gene *Pm9*, which is 8.5 cM, distal to *Pm1a* (Schneider et al. 1991; Singrün 2002). The recessive powdery mildew resistance gene *mlRD30* was located 1.8 cM distal to SSR marker *Xgwm344* (Singrün et al. 2004). We were not able to map *Xgwm344* as it was not polymorphic between NCAG11 and Axminster. However, marker *Xgwm344* was located 16 cM distal to *Xgwm332* (Singrün et al. 2003), and was reported to co-segregate or to map distal to *Xmag2185* in four different populations (Yao et al. 2007). In our study, the STS marker *Xmag2185* was 16.2 cM distal to *Pm37*.

In our allelism tests, the reaction of *Pm1a* could not be distinguished from that of *Pm37* using isolates *Bgt* 'Yuma' and *Bgt* 'Arapahoe'. The number of lines segregating for resistance was less than expected if *Pm37* and *Pm1a* are 16 cM apart. Given that the population size for each $F_{2:3}$ line was only 20 plants and that the resistance genes are linked, we likely have overestimated the number of homozygous resistant lines. This hindered our ability to estimate linkage between *Pm37* and *Pm1a* based on the phenotypic data. However, the identification of three homozygous susceptible $F_{2:3}$ lines in our population did not differ significantly from the 1.26 expected given a genetic distance of 16 cM between *Pm37* and the *Pm1* locus ($\chi^2 = 2.4$, df = 1, $P < 0.12$).

The powdery mildew resistance allele *Pm1a* was reported to co-segregate with RFLP marker *Xcdo347* (Ma et al. 1994; Neu et al. 2002). The *Xcdo347* marker also co-segregated with RFLP marker, *Xpsr680*, which was converted to STS marker *Xmag2185* (Yao et al. 2007) that was mapped in this study. These RFLP and STS markers were mapped in different populations at 32.8 cM and more than 30 cM from *Xgwm332* and *Xcfa2019*, respectively (Neu et al. 2002; Yao et al. 2007). Comparison of these marker analyses with our analysis suggests that *Pm37* is proximal to the other powdery mildew resistance loci on the long arm of chromosome 7A, *Pm1*, *Pm9* and *mlRD30*.

Srnić et al. (2005) reported *Xgwm332* and *Xwmc525* as flanking the resistance gene in NCAG11 with genetic distances of 2.0 cM proximal and 1.4 cM distal, respectively. Although our map positions agree, slightly less recombination was observed between *Pm37* and *Xgwm332* while more recombination was observed between *Pm37* and *Xwmc525* in our population. This may be due to the origin of the chromosome segments in the NCAG11/Axminister cross since it has been suggested that *Pm1a* was derived from an alien introgression (Neu et al. 2002). The distal region of 7AL in NCAG11 was transferred from *T. timopheevii* subsp. *armeniacum*. Neu et al. (2002) reported that complete linkage of *Xcdo347*, *Xc607*, *Xpsr121*, *Xpsr148*, and *Xpsr680* with *Pm1a* was caused by suppressed recombination in hexaploid wheat rather than physical linkage. Although we did not locate any STS marker derived from wheat ESTs in the *Pm37* region, we identified a new marker in the *Pm1* region that originated from a wheat EST with homology to the syntenic region in rice that might be useful for fine mapping the *Pm1* locus.

Introgression of disease resistance genes from related species into wheat has become crucial in developing resistant genotypes. The resistance gene *Pm37* introgressed from *T. timopheevii* subsp. *armeniacum* has provided full resistance to all powdery mildew isolates tested in this study, and so far, no virulence to *Pm37* has been found. It is now also possible to combine several *Pm* resistance genes into a single improved wheat genotype for more durable powdery mildew resistance. Co-dominant SSR markers *Xgwm332* (proximal) and *Xwmc790* (distal) are closely linked to *Pm37* and could be used in marker-assisted selection to develop powdery mildew-resistant lines having this gene in combination with other resistance genes. Using the markers linked to *Pm37* and *Pm1a*, we identified recombinant plants having the genes in coupling; such genotypes can be used to develop cultivars with additional resistance genes.

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