



***Pm34*: a new powdery mildew resistance gene transferred from *Aegilops tauschii* Coss. to common wheat (*Triticum aestivum* L.)**

L. M. Miranda · J. P. Murphy · D. Marshall · S. Leath

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Abstract Powdery mildew is a major fungal disease in wheat growing areas worldwide. A novel source of resistance to wheat powdery mildew present in the germplasm line NC97BGTD7 was genetically characterized as a monogenic trait in greenhouse and field trials using F₂ derived lines from a NC97BGTD7 X Saluda cross. Microsatellite markers were used to map and tag this resistance gene, now designated *Pm34*. Three co-dominant microsatellite markers linked to *Pm34* were identified and their most likely order was established as: *Xbarc177-5D*, 5.4cM, *Pm34*, 2.6cM, *Xbarc144-5D*, 14cM, *Xgwm272-5D*. These microsatellite markers were previously mapped to the long arm of the 5D chromosome and their positions were confirmed using

Chinese Spring nullitetrasomic Nulli5D-tetra5A and ditelosomic Dt5DL lines. *Pm2*, the only other known *Pm* gene on chromosome 5D, has been mapped to the short arm and its specificity is different from that of *Pm34*.

Introduction

Powdery mildew of wheat (*Triticum aestivum* L.) is an economically important fungal disease caused by *Blumeria graminis* DC f. sp. *tritici* Em. Marchal. In areas with cool or maritime climates, such as the eastern US, grain yield and end-use quality can be significantly affected by this disease (Lipps and Madden 1989; Everts et al. 2001). Major host resistance genes have been identified at 33 loci (Huang and Röder 2004; Zhu et al. 2005). Five of these loci (*Pm1*, *Pm3*, *Pm4*, *Pm5*, and *Pm8*) have more than one allele conferring resistance, making a total of 49 named *Pm* resistance alleles.

Wild and cultivated relatives of hexaploid wheat ($2n = 6x = 42$; genomes AABBDD) are frequently used as sources of resistance to powdery mildew and approximately one half of the named genes originated outside the cultivated gene pool (Hsam and Zeller 2002). The small grains breeding program at North Carolina State University released 11 powdery mildew resistant germplasms developed through interspecific hybridization and backcrossing (Murphy et al. 1998, 1999a, b, 2002; Navarro et al. 2000). Diploid and tetraploid wheat relatives were utilized as the resistance donors and the soft red winter wheat cultivar Saluda (Starling et al. 1986) as the recurrent parent.

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L. M. Miranda · J. P. Murphy (✉)
Department of Crop Science,
North Carolina State University, Box 7629,
Raleigh, NC 27695, USA
e-mail: paul_murphy@ncsu.edu

D. Marshall
United States Department of Agriculture—Agricultural
Research Service, North Carolina State University,
Box 7619, Raleigh, NC 27695, USA
e-mail: david_marshall@ncsu.edu

S. Leath
Department of Plant Pathology,
North Carolina State University, Box 7619,
Raleigh, NC 27695, USA
e-mail: steven_leath@ncsu.edu

Molecular markers tightly linked to disease resistance genes allow selection for resistance in the absence of the pathogen and facilitate combining more than one effective disease resistance gene to a single pathogen (resistance gene pyramiding) (Langridge et al. 2001). Pyramiding several major genes into a single cultivar should provide a more durable disease resistance than deployment of single major genes individually because the pathogen population is less likely to undergo multiple simultaneous changes corresponding to each resistance gene (McDonald and Linde 2002). In the absence of molecular markers, race-specific pathogen isolates have been used to differentiate among major genes, but virulent isolates are not always available for newly discovered genes (Hsam and Zeller 2002). Molecular markers provide an alternative methodology. For example, Liu et al. (2000) utilized restriction fragment length polymorphisms (RFLPs) to incorporate three different powdery mildew resistance gene combinations into the wheat cultivar Yang158.

The microsatellite or simple sequence repeat (SSRs) linkage maps developed for wheat provide the extensive genome coverage that is required for marker-assisted breeding strategies (Röder et al. 1998; Stephenson et al. 1998; Gupta et al. 1999, 2002; Pestova et al. 2000; Paillard et al. 2003; Sommers et al. 2004). Linked microsatellite markers have already been found for *Pm1e* (Singrün et al. 2003), *Pm3g* (Bougot et al. 2002), *Pm3h*, *Pm3i*, *Pm3j* (Huang et al. 2004), *Pm4a* (Ma et al. 2004), *Pm5e* (Huang et al. 2003), *Pm16* (Chen et al. 2005), *Pm24* (Huang et al. 2000), *Pm27* (Järve et al. 2000), *Pm30* (Liu et al. 2002), *Pm31* (Xie et al. 2003) and *Pm33* (Zhu et al. 2005). A new *Pm* gene transferred to common wheat from *Triticum urartu* Tum. was mapped to chromosome 7AL and was temporarily designated *PmU* (Qiu et al. 2005). In addition, Srnić et al. (2005) mapped two powdery mildew resistance genes in the North Carolina germplasm lines NC96BGTA4 and NC99BGTAG11 to the long arm of chromosome 7A.

In this study we report the use of microsatellite markers to identify and map a new powdery mildew resistance gene, *Pm34*, derived from *Aegilops tauschii* Coss. ($2n = 2x = 14$; genome DD) that is present in the North Carolina germplasm line NC97BGTD7.

Materials and methods

The powdery mildew resistant germplasm line NC97BGTD7 (PI 604033) was crossed with the powdery mildew susceptible cultivar Saluda (PI 480474). NC97BGTD7, hereafter shortened to NCD7, is a

homogeneously resistant BC₂F₆-derived line with the pedigree Saluda *3/TA2492 (Murphy et al. 1999b). TA2492 is a powdery mildew resistant *Ae. tauschii* Coss. accession. Saluda contains the major resistance gene *Pm3a*, but this gene has been defeated by the powdery mildew populations present in North Carolina (Leath and Heun 1990). The NCD7 X Saluda F₁ hybrid was selfed to produce F₂ seeds in the greenhouse. F₂ spaced plants were grown in the field without selection during 2002–2003 to produce the F_{2,3} lines that were used in the 2004 evaluations. F_{2,3} lines were harvested by bulking 30–40 randomly selected heads from each line to produce F_{2,4} seed for the 2005 evaluations.

Disease assessments

Greenhouse

F_{2,3} generation One hundred and one F_{2,3} lines were evaluated for reaction to powdery mildew in 2004. An experimental unit was two 10-cm pots, each planted with five F_{2,3} seeds of each line. The experimental design was a completely randomized design with a single replication. One pot containing Saluda and one pot containing NCD7 were included at ten entry intervals as susceptible and resistant controls. The potting mix consisted of Metro-Mix 200 (Scotts-Sierra Horticultural Products Co., Marysville, OH, USA), soil and sand (50:40:10) ratio, supplemented with 3 g of a slow release 14-14-14 (N-P-K) fertilizer per pot. The temperature was maintained between 20 and 24°C and natural light was supplemented with artificial high intensity 1,000 W discharge lights.

Plants were inoculated 20–30 days after planting at Feekes growth stage 1.3–2 (Large 1954) by shaking conidia from infected plants onto their leaves. The inoculum source was field grown Saluda plants infected with *Blumeria gramininis* DC f. sp. *tritici* that were dug at the Cunningham Research and Education Center, Kinston, NC, USA during the winter of 2003–2004. The inoculum was maintained and propagated on Saluda plants under greenhouse conditions. Disease reactions were recorded 15–20 days after inoculation (Feekes growth stage 3–4) following the rating scale developed by Leath and Heun (1990). In this scale: 0 = immune, no visible signs of infection; 1–3 = resistant, increasing from (1) flecks with no necrosis, to (2) necrosis, to (3) chlorosis, while the amount of mycelium went from none to a detectable amount; 4–6 = intermediate reaction with chlorotic areas decreasing in amount while mycelium and conidia production increased from slight to moderate; 7–9 = susceptible

with increasing amount, size and density of mycelium and conidia to a fully compatible reaction. Phenotypic classes were assigned by comparing the disease reactions of the ten individual plants within each F_2 -derived line with the Saluda and NCD7 reactions. Lines for which all plants had a reaction similar to NCD7 were classified as homozygous resistant and as homozygous susceptible if all reactions were similar to Saluda. Lines that had resistant and susceptible plants were included in the segregating class.

$F_{2,4}$ generation Eighty $F_{2,4}$ lines were evaluated for reaction to powdery mildew in 2005 following the protocol described above for 2004. These lines were a random sub-set of the 101 $F_{2,3}$ lines evaluated in 2004. A single spore culture isolate with the following avirulence/virulence response to known *Pm* genes was utilized: *Pm1a*, *1b*, *3b*, *4b*, *8*, *17/Pm2*, *3a*, *5*, *6*, *7*, *MA* (Dr. D. Marshall, personal communication).

Field

$F_{2,3}$ generation One hundred and one $F_{2,3}$ lines were planted at Kinston, NC, USA in October 2003. The experimental design was a randomized complete block with two replications. An experimental unit was a 1.2-m row planted with 40–60 seeds per line. Rows were spaced 30.5-cm apart. NCD7 and Saluda rows were included at forty plot intervals as controls. In addition, one of the replications contained 12 isolines of the susceptible cultivar Chancellor each containing a previously identified *Pm* gene. The donor source and major gene in each Chancellor isolate were: Axminster (*Pm1a*), Ulka (*Pm2*), Chul (*Pm3b*), Sonora (*Pm3c*), Michigan Amber (*Pm3f*), Yuma (*Pm4a*), Hope (*Pm5a*), Coker 747 (*Pm6*), Transec (*Pm7*) and Federation*4/Kavkaz (*Pm8*). Irrigation, fertilization, and other agronomic practices followed standard management practices for North Carolina (Weisz 2000). The experiment was surrounded by a 1.2-m Saluda border to promote homogeneous disease spread. Disease reactions were evaluated at the beginning of April when plants were at Feekes Growth stage 10.1–10.5 and all Saluda rows presented uniform powdery mildew infection. Flag –2 leaves were rated using the scale of Leath and Heun (1990) previously described for the greenhouse test. The results from the two replications were combined to assign the phenotypic classes. Lines were classified as homozygous resistant or homozygous susceptible when only one phenotypic class was observed in both replications and segregating when both resistant and susceptible plants were identified in the family.

$F_{2,4}$ generation All lines classified as either homozygous resistant or homozygous susceptible in the $F_{2,3}$ generation and the 12 Chancellor isolines were included in a second evaluation in 2005, using the same protocols described above for 2004.

Microsatellite markers analysis

Genomic DNA was extracted from leaf tissue samples of $F_{2,3}$ plants following the procedure described by Stein et al. (2001). Leaf samples from the ten plants per line grown in the greenhouse experiment were bulked to perform the DNA extractions.

Wheat microsatellite primers evenly distributed across the D genome were synthesized according to the sequences published in the GrainGenes database (<http://www.wheat.pw.usda.gov>), with all forward primers modified to include the M13 sequence (CACGACGTTGTAACGAC-) at the 5' end for labeling purposes (Schulke 2000; Rampling et al. 2001).

The PCR reactions were conducted in a total volume of 10 μ l containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 200 μ M of each dNTP, 20 nM of forward primer, 100 nM of reverse primer, 100 nM of M13 labeled primer (IRD700 or IRD800 label, LI-COR Biosciences, San Diego, CA, USA), 0.75U *Taq* DNA polymerase and 50 ng of genomic DNA. Amplifications were performed using a touchdown PCR protocol with the following conditions: 94°C for 4 min, 15 cycles of 94°C for 30 s, 65°C for 30 s (–1°C per cycle) and 72°C for 1 min, followed by 25 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 45 s and a final extension step at 72°C for 3 min. PCR products were mixed 1:1 with loading buffer (95% formamide, 20 mM EDTA and 0.08% bromo-phenol blue) denatured at 95°C for 3 min and loaded on 6.5% polyacrylamide gels (KB Plus gel matrix, LI-COR Biosciences) that were run in LI-COR sequencers (Model 4300) for 2.5 h at 42 W and 1,500 V. Gel images were scored using AFLP Quantar 1.09 software and 19 bp from the M13 tail were subtracted from all band sizes obtained.

Primer pairs that were polymorphic between NCD7 and Saluda were used for bulked segregant analysis (Michelmore et al. 1991). Resistant and susceptible bulks were made by pooling equal amounts of DNA from ten lines phenotypically scored as resistant and ten lines phenotypically scored as susceptible, respectively.

Chromosomal assignment

Chromosomal locations of the linked microsatellite markers were confirmed using Chinese Spring Nullisomic5D-tetra5A (N5DT5A) and ditelomic 5DL

(Dt5DL) lines (kindly provided by the Wheat Genetics Resource Center, Kansas State University). Genomic DNA from N5DT5A, Dt5DL, euploid Chinese Spring, NCD7 and Saluda were used to perform PCR reactions with the microsatellite markers putatively linked to the NCD7 gene. All PCR reactions included DNA of the N5DT5A and Dt5DL lines amplified with a primer pair that maps to the A genome as positive controls.

Data analysis

Deviations of observed data from theoretically expected segregation ratios were tested using chi-square (χ^2) tests for goodness-of-fit. Linkage analysis was performed using MAPMAKER/Exp Version 3.0b (Lincoln et al. 1993). Map distances were determined using the Kosambi mapping function (Kosambi 1944) and loci were ordered using the ‘sequence’ and ‘compare’ commands, with an LOD threshold score ≥ 3.0 and a maximum distance allowed between markers set to 50.0.

Results

Greenhouse evaluations

*F*_{2,3} generation

In 2004 the powdery mildew seedling disease reaction of NCD7 was resistant to intermediate with scores ranging from 2 to 5. Saluda exhibited an intermediate to susceptible reaction with scores ranging from 6 to 8. The phenotypic classification of the 101 *F*_{2,3} lines is shown in Table 1. The chi-square test value was in good agreement with the expected 1:2:1 ratio of a monogenic trait ($\chi^2_{1:2:1} = 2.47$, $P = 0.29$).

*F*_{2,4} generation

The NCD7 and Saluda scores in 2005 ranged from 1 to 3 and from 7 to 9, respectively. The observed phenotypic classes also fitted the 1:2:1 ratio ($\chi^2_{1:2:1} = 2.48$, $P = 0.29$) confirming a single gene controlled resistance

(Table 1). The single spore culture isolate used as the inoculum source elicited less powdery mildew symptoms in the resistant genotypes allowing a more clear distinction between resistant and susceptible seedlings. Seven among the 80 *F*₂-derived lines received a different phenotypic classification in 2005. Three resistant and two susceptible lines were re-classified as segregating and two segregating lines were re-classified as resistant. These changes were confirmed with the phenotypic data obtained in the field trials.

Field evaluations

*F*_{2,3} generation

The NCD7 exhibited a resistant disease reaction with scores ranging from 2 to 3. Saluda exhibited an intermediate reaction with scores ranging from 5 to 6. Thirty *F*_{2,3} lines were classified as resistant, 43 as segregating and 28 as susceptible. The $\chi^2_{1:2:1}$ test value was 2.31, indicating a good fit to the expected 1:2:1 ratio ($P = 0.31$). The Chancellor isolines with genes *Pm3b*, *Pm3f*, *Pm5a*, and *Pm6* received the highest score (6); *Pm2*, *Pm4*, and *Pm7* received a score of 5; *Pm3c*, and *Pm8* received a score of 4; *Pm1a* had a disease score of 2.

*F*_{2,4} generation

The 2005 field evaluation included the 58 *F*_{2,4} lines that were categorized either as homozygous susceptible or homozygous resistant in the 2004 experiment. Disease levels were higher than in 2004 and Saluda exhibited an intermediate to fully susceptible reaction with scores ranging from 6 to 8. NCD7 scores remained between 2 and 3. Three resistant and four susceptible lines were re-classified as segregating. Phenotypic classes of the *F*₂-derived lines based on the two-year field data are shown in Table 1. These values were a good fit to the expected 1:2:1 ratio ($\chi^2_{1:2:1} = 0.19$, $P = 0.91$). This phenotypic classification agreed with the second greenhouse ratings and was used for the molecular marker analysis.

The Chancellor isolate containing *Pm6* received the highest disease score (7), followed by *Pm3b* (6).

Table 1 Segregation ratios for powdery mildew reaction of *F*₂ derived families from the NCD7 X Saluda cross

Generation	Number of <i>F</i> ₂ -derived families			Total	$\chi^2_{(1:2:1)}$	<i>P</i> -value
	Resistant	Segregating	Susceptible			
Greenhouse						
<i>F</i> _{2,3}	22	47	32	101	2.47	0.29
<i>F</i> _{2,4}	23	33	24	80	2.48	0.29
Field						
<i>F</i> ₂ -derived	27	50	24	101	0.19	0.91

Isolines containing *Pm2*, *Pm3c*, *Pm3f*, *Pm4a*, *Pm5a*, and *Pm8* received a score of 5; the *Pm7* isolate received a score of 4 and the *Pm1a* isolate again showed the lowest disease score (3).

Microsatellite markers

Twenty-three of the 67 microsatellite markers chosen for the initial primer screening were polymorphic between NCD7 and Saluda. These primer pairs were included in the bulk segregant analysis and one of them, BARC177, showed polymorphism between the bulks. The polymorphic fragments *Xbarc177*/138 bp and *Xbarc177*/129 bp observed in NCD7 and Saluda, respectively, were also present in the resistant and susceptible bulks. Genotyping of the F_2 progeny confirmed the linkage of this marker to the powdery mildew resistance.

Since this microsatellite marker was previously mapped to the long arm of chromosome 5D, an additional 28 primer pairs on the same chromosome arm were tested. Two additional microsatellite markers, *Xbarc144* and *Xgwm272*, linked to the NCD7 powdery mildew resistance were identified. The *Xbarc144*/235 bp and *Xgwm272*/144 bp bands co-segregated with the NCD7 resistance gene and the *Xbarc144*/238 bp and *Xgwm272*/127 bp bands co-segregated with the susceptible allele from Saluda. The three microsatellite markers were co-dominant and segregated in the expected 1:2:1 ratio (Table 2). The most likely order is shown in Fig. 1. No other marker locus order was within a LOD score of 3.0 from this most likely order.

Chromosomal assignment

Pm34 was putatively assigned to the long arm of chromosome 5D, based on the reported chromosomal locations of the three linked microsatellite markers (Sommers et al. 2004). However, since microsatellite markers are not always chromosome specific (Plashke et al. 1996), the locations of the three linked microsatellite loci were confirmed using the CS nullitetrasonic N5DT5A and ditelosomic Dt5DL lines. The three microsatellite primer pairs amplified products of the expected size in Chinese Spring and the Ditelosomic 5DL lines but no PCR products were observed in the Nullitetrasonic N5DT5A line for any of the three primer pairs (Figs. 2, 3, 4). The absence of PCR products in the N5DT5A and their presence in the Dt5DL confirmed the assignment of the three microsatellite markers to the long arm of chromosome 5D.

Chromosome 5DL

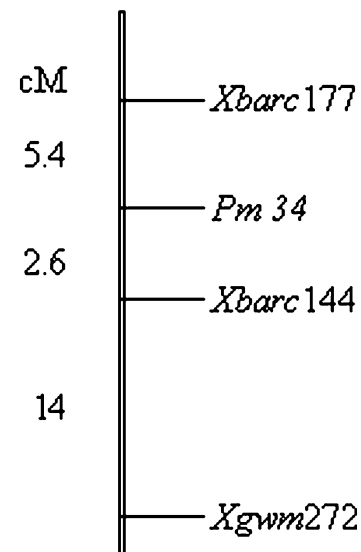


Fig. 1 Map position of *Pm34* on chromosome 5DL

Table 2 Segregation ratios for Microsatellite (SSR) markers among F_2 individuals in the NCD7 X Saluda population

SSR marker	AA ^a	H ^b	BB ^c	Total	$\chi^2_{(1:2:1)}$	P-value
<i>Xbarc177</i>	23	54	24	101	0.50	0.78
<i>Xbarc144</i>	27	51	23	101	0.33	0.85
<i>Xgwm272</i>	23	56	22	101	1.22	0.54

^a AA homozygous for the NCD7 allele

^b H heterozygous

^c BB homozygous for the Saluda allele

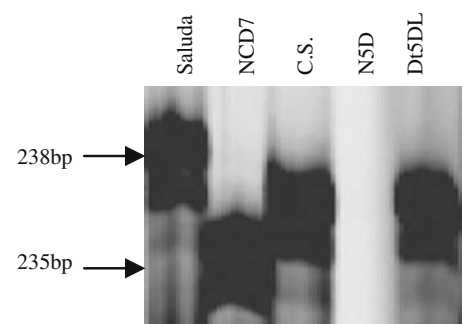


Fig. 2 Chromosomal localization of Microsatellite marker *Xbarc144* PCR products observed in NCD7, Saluda, Chinese Spring (C.S.) and Ditelosomic 5DL (Dt5DL) but no PCR products observed in Nullitetrasonic 5D (N5D-T5A)

Discussion

The NCD7 powdery mildew resistance introgressed from *Ae. tauschii* was confirmed in greenhouse and field experiments to be a monogenic trait. Good

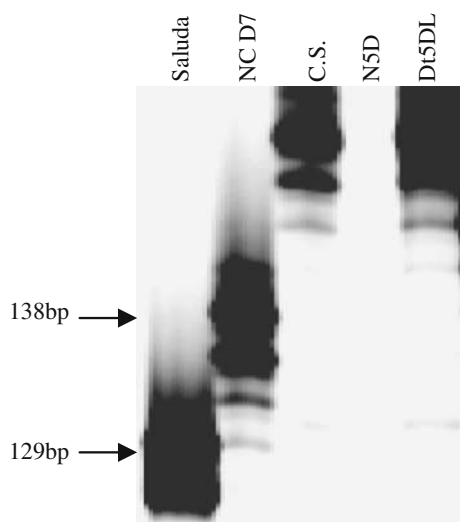


Fig. 3 Chromosomal localization of Microsatellite marker *Xbarc177*: PCR products observed in NCD7, Saluda, Chinese Spring (C.S.) and Ditelosomic 5DL (*Dt5DL*) but no PCR products observed in Nullisomic 5D (*N5D-T5A*)

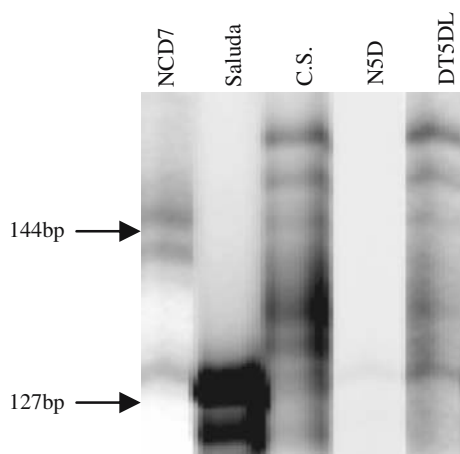


Fig. 4 Chromosomal localization of Microsatellite marker *Xgwm272*: PCR products observed in NCD7, Saluda, Chinese Spring (C.S.) and Ditelosomic 5DL (*Dt5DL*) but no PCR products observed in Nullisomic 5D (*N5D-T5A*)

overall agreement between the greenhouse and field data was observed and the results from the first year greenhouse and field ratings were confirmed with a second greenhouse test using a single spore culture isolate of known virulence profile and a field test with $F_{2:4}$ putatively homozygous susceptible and homozygous resistant lines.

The potential of *Ae. tauschii* as a source of powdery mildew resistance genes had been previously reported. Lutz et al. (1994) screened 400 *Ae. tauschii* accessions using nine powdery mildew isolates and found 276 that showed complete or isolate specific resistance patterns. Forty accessions had a disease response pattern identi-

cal to *Pm2*, which is also derived from *Ae. tauschii*. The remaining 236 accessions had disease specificities that were different from *Pm2*. TA2492, the donor of powdery mildew resistance to NCD7, was not included in their screening.

The *Pm2* is the only named *Pm* gene mapped to chromosome 5D. The differences observed in field disease reactions between NCD7 and the Chancellor Iso-line with *Pm2* indicated that the NCD7 gene is a different gene or at least a different specificity. The NCD7 powdery mildew resistance gene was effective against the powdery mildew population present in Kinston, North Carolina but virulence to *Pm2* had been previously reported in North Carolina (Niewoehner and Leath 1998) In addition, the isolate used for the greenhouse test in 2005 was virulent to *Pm2* but avirulent to NCD7.

McIntosh and Baker (1970) assigned *Pm2* to chromosome 5D using monosomic analysis and suggested the short arm as the most likely location of this gene, based on telocentric mapping evidence. Further, evidence from this same study was provided by the absence of linkage between *Pm2* and *Lr1* (located on 5DL) and also by the observation that chimaeras resulting from the loss of one chromosome arm in a line homozygous resistant for both genes did not show identical patterns of resistance or susceptibility to both pathogens. Ma et al. (1994) reported the RFLP marker *Xbcd1871-5D* linked to the *Pm2* locus with a distance of 3.5cM and confirmed the location of probe BCD1871 on chromosome 5DS using filters with aneuploid DNA of Chinese Spring (Anderson et al. 1992).

The three microsatellite markers linked to the NCD7 *Pm* gene have all been mapped to the distal half of 5DL (Röder et al. 1998; Sommers et al. 2004) and this was verified using the Chinese Spring nullitetrasomic (N5DT5A) and ditelosomic (Dt5DL) lines. Although a Ditelosomic 5DS line was not available, the presence of PCR products of the same size in euploid Chinese Spring and the CS Dt5DL and their absence in CS N5DT5A confirmed the location of these markers on 5DL.

The order of the microsatellite loci linked to the NCD7 gene was in good agreement with previous microsatellite linkage maps for chromosome arm 5DL (<http://wheat.pw.usda.gov/>). The close linkage and co-dominant nature of these markers should facilitate the incorporation of *Pm34* in cultivar development programs. The recombination frequencies between the two flanking markers, *Xbarc144-5D* and *Xbarc177-5D*, and *Pm34* were 2.6 and 5.4%, respectively. These recombination frequencies give a 99.98% probability of recovering the trait when performing selection

based on the markers alone. NCD7 can also be used in crosses with the previously characterized North Carolina germplasm lines NC96BGTA4 and NC99BGTA G11 (Srnić et al. 2005) to develop powdery mildew resistant germplasm lines with pyramids of effective *Pm* genes. NCD7 was selected for maturity and plant type similar to Saluda (Murphy et al. 1999b) and the high degree of homology between the D genomes of *Ae. tauschii* and *T. aestivum* (Pestova et al. 2000) suggest greater recombination and less linkage drag should occur in this case than when other wheat relatives are used for introgression of useful traits.

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