Theor Appl Genet (2006) 113:1497–1504
DOI 10.1007/s00122-006-0397-9

ORIGINAL PAPER

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

Received: 20 April 2006 / Accepted: 15 August 2006 / Published online: 5 September 2006
© Springer-Verlag 2006

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 F_2 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*.

Communicated by D. A. Lightfoot.

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

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.

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 BC_2F_6-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 F_1 hybrid was selfed to produce F_2 seeds in the greenhouse. F_2 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 isoline 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 (CACGACGTTGTAAAACGAC-) at the 5′ end for labeling purposes (Schulke 2000; Rampling et al. 2001).

The PCR reactions were conducted in a total volume of 10 μl containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM 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 ditelisomic 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 isoline 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 F_2-derived families			Total	$\chi^2_{(1:2:1)}$	*P*-value
	Resistant	Segregating	Susceptible			
Greenhouse						
$F_{2:3}$	22	47	32	101	2.47	0.29
$F_{2:4}$	23	33	24	80	2.48	0.29
Field						
F_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* isoline received a score of 4 and the *Pm1a* isoline 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 makers 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 nullitetrasomic 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 Nullitetrasomic 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.

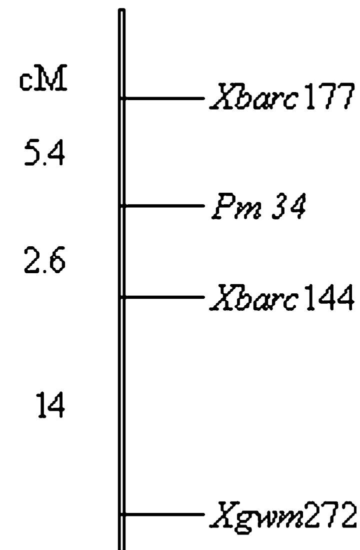

Fig. 1 Map position of *Pm34* on chromosome 5DL

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
Xbarc177	23	54	24	101	0.50	0.78
Xbarc144	27	51	23	101	0.33	0.85
Xgwm272	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

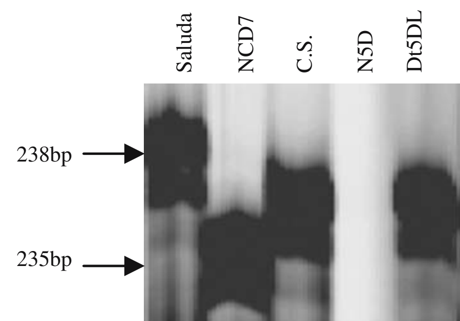

Fig. 2 Chromosomal localization of Microsatellite marker *Xbarc144* PCR products observed in NCD7, Saluda, Chinese Spring (*C.S.*) and Ditelosomic 5DL (*Dt5DL*) but no PCR products observed in Nullisomic 5D (*N5D-T5A*)

Discussion

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

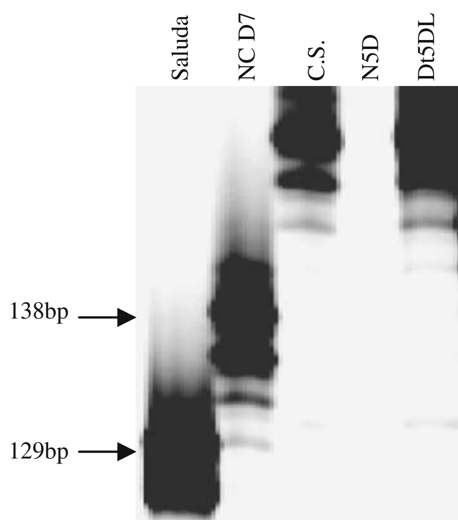

Fig. 3 Chromosomal localization of Microsatellite marker *Xbarc*177: PCR products observed in NCD7, Saluda, Chinese Spring (*C.S.*) and Ditelosomic 5DL (*Dt5DL*) but no PCR products observed in Nullisomic 5D (*N5D-T5A*)

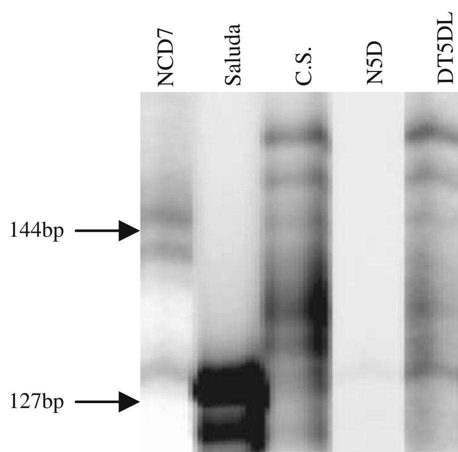

Fig. 4 Chromosomal localization of Microsatellite marker *Xgwm272*: PCR products observed in NCD7, Saluda, Chinese Spring (*C.S.*) and Ditelosomic 5DL (*Dt5DL*) but no PCR products observed in Nullisomic 5D (*N5D-T5A*)

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 identical 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 Isoline 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 *Pm*2 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, *Xbarc*144-*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. tauschi* 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.

Acknowledgments The authors thank Rene Navarro and David Wooten for their help with field work, Jeannette Lyerly for her technical assistance and Lynda Whitcher for providing the powdery mildew isolate. We gratefully acknowledge Prof. Bob McIntosh for some excellent additions to the final draft of this manuscript.

References

Anderson JA, Ogihara Y, Sorrells ME, Tanksley SD (1992) Development of a chromosome arm map for wheat based on RFLP markers. Theor Appl Genet 83:1035–1043

Bougot Y, Lemoine J, Pavoine MT, Barloy D, Doussinault G (2002) Identification of a microsatellite associated with *Pm3* resistance alleles to powdery mildew in wheat. Plant Breed 121:325–329

Chen XM, Luo YH, Xia XC, Xia LQ, Chen X, Ren ZL, He ZH, Jia JZ (2005) Chromosomal location of powdery mildew resistance gene *Pm16* in wheat using SSR marker analysis. Plant Breed 124:225–228

Everts K, Leath S, Finney PL (2001) Impact of powdery mildew and leaf rust on milling and baking quality of soft red winter wheat. Plant Dis 85:423–429

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390

Gupta PK, Baylan HS, Edwards KJ, Isaac P, Korzun V, Röder MS, Gautier MF, Joudrier P, Schlatter AR, Dubcovscy J, De la Pena RC, Khairalla M, Penner G, Hayden MJ, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P (2002) Genetic mapping of 66 microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V (2000) Molecular mapping of wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor Appl Genet 101:407–414

Huang XQ, Wang LX, Xu MX, Röder MS (2003) Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). Theor Appl Genet 106:858–865

Huang XQ, Hsam SLK, Mohler V, Röder MS, Zeller F (2004) Genetic mapping of three alleles at the *Pm3* locus conferring powdery mildew resistance in common wheat (*Triticum aestivum* L.). Genome 47:1130–1136

Huang XQ, Röder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica 137:203–223

Hsam SLK, Zeller FJ (2002) Breeding for powdery mildew resistance in common wheat (Triticum aestivum L.) In: Berlanger RR, Bushnell WR, Dik AJ, Carver DL (eds) The powdery mildews: a comprehensive treatise. American Phytopathological Society MN, pp 219–238

Järve K, Peusha HO, Tsybalova J, Tamm S, Devos KM, Enno TM (2000) Chromosomal location of a *Triticum timopheevi* derived powdery mildew resistance gene transferred to common wheat. Genome 43:377–381

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

Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ (2001) Trends in genetics and genome analyses in wheat: a review. Aust J Agric Res 52:1043–1077

Large EC (1954) Growth stages in cereals. Illustrations of the Feeke's scale. Plant Pathol 3:129

Leath S, Heun M (1990) Identification of powdery mildew resistance genes in cultivars of soft red winter wheat. Plant Dis 74:747–752

Lincoln SE, Daly MJ, Lander ES (1993) Constructing linkage maps with MAPMAKER/Exp Version 3.0. A tutorial reference manual, 3rd edn. Whitehead Institute for Medical Res., Cambridge, MA

Lipps PE, Madden V (1989) Assesment of methods of determining powdery mildew severity in relation to grain yield of winter wheat cultivars in Ohio. Phytopathology 79:462–470

Liu J, Liu D, Tao W, Li W, Wang S, Chen P, Cheng S, Gao D (2000) Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. Plant Breed 119:21–24

Liu ZY, Sun QX, Ni ZF, Nevo E, Yang TM (2002) Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer. Euphytica 123:21–29

Lutz J, Hsam SLK, Limpert E, Zeller FJ (1994) Powdery mildew resistance genes in *Aegilops tauschii* Coss. and synthetic hexaploid wheats. Genet Resour Crop Evol 41:151–158

Ma ZQ, Sorrels ME, Tanksley SD (1994) RFLP markers linked to powdery mildew resistance genes *Pm1*, *Pm2*, *Pm3* and *Pm4* in wheat. Genome 37:871–875

Ma ZQ, Wei JB, Chen SH (2004) PCR based markers for the powdery mildew resistance gene *Pm4a* in wheat. Theor Appl Genet 109:140–145

McIntosh RA, Baker EP (1970) Cytogenetic studies in wheat IV Chromosomal location and linkage studies involving the *Pm2* locus for powdery mildew resistance. Euphytica 19:71–77

McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential and durable resistance. Annu Rev Phytopathol 40:349–379

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating population. Proc Natl Acad Sci USA 88:9828–9832

Murphy JP, Leath S, Huynh D, Navarro RA, Shi A (1998) Registration of NC96BGTD1, NC96BGTD2 and NC96BGTD3 wheat germplasm resistant to powdery mildew. Crop Sci 38:570–571

Murphy JP, Leath S, Huynh D, Navarro RA, Shi A (1999a) Registration of NC96BGTA4, NC96BGTA5 and NC96BGTA6 wheat germplasm. Crop Sci 39:883–884

Murphy JP, Leath S, Huynh D, Navarro RA, Shi A (1999b) Registration of NC97BGTD7 and NC97BGTD8 wheat germplasms resistant to powdery mildew. Crop Sci 39:884–885

Murphy JP, Navarro RA, Leath S (2002) Registration of NC99BGTAG11 wheat germplasm resistant to powdery mildew. Crop Sci 42:1382

Navarro RA, Murphy JP, Leath S, Shi A (2000) Registration of NC97BGTAB9 and NC97BGTAB10 wheat germplasm lines resistant to powdery mildew. Crop Sci 40:1508–1509

Niewoehner AS, Leath S (1998) Virulence of *Blumeria graminis* f. sp. *tritici* on winter wheat in the eastern United States. Plant Dis 82:64–68

Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An integrative genetic linkage map of wheat. Theor Appl Genet 107:1235–1242

Pestova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Plaschke JB, Börner A, Wendehake K, Ganal MW, Röder MS (1996) The use of aneuploids for the chromosomal assignment of microsatellite loci. Euphytica 89:33–40

Qiu YC, Zhou RH, Kong XY, Zhang SS, Jia JZ (2005) Microsatellite mapping of a *Triticum urartu* Tum. derived powdery mildew resistance gene transferred to common wheat (*Triticum aestivum* L.). Theor Appl Genet 111:1524–1531

Rampling LR, Harker N, Shariflou MR, Morell MK (2001) Detection and analysis systems for microsatellite markers in wheat. Aust J Agric Res 52:1131–1141

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Schuelke M (2000) An economic method for fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234

Singrün CH, Hsam SL, Zeller FJ, Mohler V (2003) Powdery mildew resistance gene *Pm22* is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L). Theor Appl Genet 106:1420–1424

Sommers DJ, Isaac P, Edward K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Srnić G, Murphy JP, Lyerly JH, Leath S, Marshall DS (2005) Inheritance and chromosomal assignment of powdery mildew resistance genes in two winter wheat germplasm lines. Crop Sci 45:1578–1586

Starling TM, Roane CW, Camper HM (1986) Registration of Saluda wheat. Crop Sci 26:200

Stein N, Herren G, Keller B (2001) A new DNA extraction method for high throughput marker analysis in a large genome species such as *Triticum aestivum* L. Plant Breed 120:354–356

Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale M (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 100:564–568

Weisz R (2000) Small grain production guide 2000–2001. North Carolina Coop. Ext. Serv., Raleigh, NC

Xie CJ, Sun QX, Ni ZF, Yang ZM, Nevo E, Fahima T (2003) Chromosomal location of a *Triticum dicoccoides*-derived powdery mildew resistance gene in common wheat by using microsatellite markers. Theor Appl Genet 106:341–345

Zhu ZD, Zhou RH, Kong XY, Dong YC, Jia JZ (2005) Microsatellite markers linked to two genes conferring resistance to powdery mildew in common wheat introgressed from *Triticum carthlicum* accession PS5. Genome 48:585–590