

Genetic Mapping of a *Triticum monococcum*-derived Powdery Mildew Resistance Gene in Common Wheat

L. M. Miranda,* L. Perugini, G. Srnić, G. Brown-Guedira, D. Marshall, S. Leath, and J. P. Murphy

ABSTRACT

Powdery mildew of wheat (*Triticum aestivum* L.) is a major fungal disease caused by *Blumeria graminis* DC f. sp. *tritici*. A microsatellite linkage map was developed for the *T. monococcum*-derived powdery mildew resistant gene present in the North Carolina germplasm line NCB-GT96A6 (NCA6). Genetic analysis of F₂-derived lines from the cross NCA6 × 'Saluda' indicated a single gene controlled powdery mildew resistance. Four microsatellite markers linked to the NCA6 *Pm* gene mapped to chromosome 7AL. The most likely order was *Xcfa2123*-0.9 cM-*Xbarc121*-1.7 cM resistance gene/*Xcfa2019*-3.0 cM-*Xgwm332*. A detached-leaf test indicated the disease reaction response of the NCA6 *Pm* gene was different from the five known alleles at the *Pm1* locus on 7AL. Deletion interval mapping showed a large physical to genetic distance ratio for these microsatellite marker loci. This may be due to suppressed recombination between the introgressed *T. monococcum* segment and the homologous region of the *T. aestivum* cultivar Saluda. Our results suggested that the NCA6 *Pm* gene is likely a novel source of resistance to powdery mildew but additional allelism studies are needed to establish the relationship between this locus and the other known *Pm* loci on 7AL.

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Abbreviations: LOD, decimal logarithm of odds; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA.

POWDERY MILDEW, caused by *Blumeria graminis* DC f. sp. *tritici* Em. Marchal, is a prevalent foliar disease of wheat (*Triticum aestivum* L.) in temperate or maritime climates. Breeding resistant cultivars is considered the most economic and environmentally safe approach to controlling this disease (Hsam and Zeller, 2002), but the use of single major genes that confer race specific disease resistance has proved ephemeral (Chen and Chelkowski, 1999). Nevertheless, major host resistance genes should provide a more durable disease resistance if deployed in combinations because directional selection pressure is disrupted (McDonald and Linde, 2002).

Several major genes can be deployed simultaneously using cultivar mixtures (Mundt, 2002), isolines with different resistance genes (Zhou et al., 2005) or by pyramiding different major genes into a single cultivar (Liu et al., 2000). These strategies can be applied more efficiently with the use of molecular markers that tag and identify disease resistance genes. Microsatellites offer several advantages among the different types of molecular markers: they are codominant, highly polymorphic, generally chromosome specific and more amenable to automatization than other marker systems (Gupta et al., 1999; Langridge et al., 2001).

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Diploid and tetraploid relatives of hexaploid wheat ($2n = 6x = 42$; genomes AABBDD) are commonly used sources of resistance to pests and diseases. The diploid wheat *T. monococcum* ($2n = 2x = 14$; genome A^mA^m) has proven to be a valuable source of disease resistance genes for leaf rust (*Puccinia triticina* Eriks) (Hussein et al., 1997; Anker et al., 2001) and stem rust (*Puccinia graminis* f.sp. *tritici*) (The, 1973; McIntosh et al., 1984).

Yao et al. (2007) reported the presence of two different powdery mildew resistance genes in the *T. monococcum* accessions TA2033 and M80. These genes, designated *Mlm2033* and *Mlm80*, were both mapped to chromosome 7AL and, based on their linkage maps, they are likely to be allelic or closely linked to *Pm1*.

Among the designated powdery mildew resistance genes only the *Pm1b* allele from the cultivar MocZlatka (Hsam et al., 1998) and *Pm25* from the North Carolina germplasm line NC96BGTA5 (Shi et al., 1998; Murphy et al., 1999) have been reported as being transferred from *T. monococcum* into common wheat. *Pm1* was mapped to chromosome 7AL by Sears and Briggie (1969) and microsatellite markers linked to this locus were identified by Singrün et al. (2003). Shi et al. (1998) mapped *Pm25* to chromosome 1AS and identified random amplified polymorphic DNA (RAPD) markers linked to this locus.

The North Carolina germplasm lines NC96BGTA4 and NC96BGTA6 also have *T. monococcum* as their source of resistance to powdery mildew (Murphy et al., 1999). Srnić et al. (2005) characterized the powdery mildew resistance gene in NC96BGTA4 but due to its chromosomal location on 7AL, additional allelism studies are needed to differentiate this locus from the *Pm1* complex. In this study we report the genetic characterization and microsatellite linkage mapping of the powdery mildew resistance gene present in NC96BGTA6.

MATERIALS AND METHODS

The soft red winter wheat powdery mildew resistant germplasm line NC96BGTA6 (PI 599036) was crossed to the susceptible cultivar Saluda (PI 480474). NC96BGTA6 (NCA6) is a homogeneously resistant BC₂F₅-derived line with the pedigree Saluda *3/PI 427772 (Murphy et al., 1999). Saluda is a soft red winter wheat cultivar developed by the Virginia Polytechnic Institute and State University (Starling et al., 1986). It contains the major resistance gene *Pm3a* that has been defeated by the powdery mildew populations present in North Carolina (Leath and Heun, 1990). The powdery mildew resistance donor, PI 427772, is a *T. monococcum* L. subsp. *aegilopoides* accession collected in Iraq. The NCA6 × Saluda F₁ hybrid was selfed to produce F₂ seeds in the greenhouse. The F₂ spaced plants were grown in the field without selection and harvested on an individual plant basis to produce F_{2,3} lines used in the 2003 evaluations. The F_{2,3} lines were harvested by bulking 30 to 40 randomly selected heads from each line to produce F_{2,4} seed for the 2004 evaluations.

Greenhouse Disease Evaluations

The F₁ plants grown in the greenhouse were resistant to powdery mildew, indicating that powdery mildew resistance in NCA6 is a dominant trait. Ninety-nine F_{2,3} families from NCA6 × Saluda were evaluated for reaction to powdery mildew in 2003. Ten seeds per family were planted in two 10-cm pots, each of them with five seeds. Pots were randomly distributed on a greenhouse bench. One pot containing Saluda and one pot containing NCA6 were included at 10 pot intervals as susceptible and resistant controls. The potting mix consisted of Metro-Mix 200 (Scotts-Sierra Horticultural Products Co., Marysville, OH), 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 1000 W discharge lights.

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. The inoculum was maintained and propagated on Saluda plants under greenhouse conditions. Plants were inoculated 20 to 30 d after planting at Feekes growth stage 1.3 to 2 (Large, 1954) by shaking conidiospores from infected plants onto their leaves. Disease reactions were recorded 15 to 20 d 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 to 3 = resistant, increasing from (i) flecks with no necrosis to (ii) necrosis to (iii) chlorosis, while the amount of mycelium went from none to a detectable amount; 4 to 6 = intermediate reaction with chlorotic areas decreasing in amount while mycelium and conidia production increased from slight to moderate; 7 to 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 10 individual plants within each F₂-derived family with the Saluda and NCA6 reactions. Families for which all plants had a reaction similar to NCA6 were classified as homozygous resistant and as homozygous susceptible if all reactions were similar to Saluda. Families that had resistant and susceptible plants were included in the segregating class.

Field Disease Evaluations

One hundred twenty one F_{2,3} families from NCA6 × Saluda were planted at Kinston, in October 2002. All lines included in the greenhouse evaluations were included in the field evaluations. Forty to sixty seeds per family were planted in a 1.2-m row. Rows were spaced 30.5 cm apart. The NCA6 and Saluda rows were included at 40 plot intervals as controls. 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 ratings were recorded at the end of March, when plants were at Feekes Growth stage 9 to 10.1. Flag minus two leaves were rated using the scale of Leath and Heun (1990) previously described for the greenhouse test. Plots were visually screened to determine if all plants were within the NCA6 (resistant) or Saluda (susceptible) score ranges or if the plot had plants within both ranges (segregating).

A second field trial during the 2003–2004 season included only the 66 F_{2,4} families that were classified as either homozygous resistant or homozygous susceptible in the F_{2,3} generation.

Differential Disease Response to *Blumeria graminis* f. sp. *tritici* Isolates

A detached-leaf test was conducted to test for differences in disease reaction among NCA6 and the five *Pm1* alleles (*Pm1a-e*). The *Pm1* lines used were 'Axminster' (*Pm1a*), MocZlatka (*Pm1b*), M1N (*Pm1c*), *T. spelta* (*Pm1d*), and 'Virest' (*Pm1e*). Cultivars Chancellor and Saluda were used as susceptible controls. These lines were evaluated for their disease reaction using 14 *B. graminis* f. sp. *tritici* isolates originated from mildew samples collected in different locations in the eastern United States. The isolates were designated: A4/AG11-L, Yuma, Sturdy2K*Pm5*, 101a2, Asosan, Arapahoe, E314, Trego, W72-27, #8, 85063, Flat-7-11, 169-1b and AB9-10.

All isolates were maintained and propagated on 60- × 15-mm Petri dishes filled with 50 mg L⁻¹ benzimidazole-amended 6% agar containing 2.5-cm leaf segments of Chancellor that were obtained from 10- to 15-d-old seedlings. Plates were placed in a growth chamber and maintained at 18°C, 85% relative humidity and a photoperiod of 12 h.

Leaf segments from all the lines being tested were cultured on Petri dishes under the same conditions previously described and were inoculated using Chancellor leaf segments infected with each of the different isolates, such that each plate was inoculated with a single isolate. Three replicate plates were inoculated with each isolate. Disease reactions were recorded 7 to 8 d after inoculation, using the 0 to 9 scale previously described.

The disease reaction of NCA6 was also compared to the powdery mildew resistant germplasm line NC99BGTAG11 in a separate detached-leaf test conducted under the same conditions previously described but with four replicates and including only the following isolates: Yuma, Sturdy2K*Pm5*, 101a2, Asosan, Arapahoe, E314, Trego, W72-27, #8, 85063, Flat-7-11 and 169-1b.

Microsatellite Markers Analysis

Genomic DNA was extracted from leaf tissue samples of F₂ plants following the procedure described by Stein et al. (2001). Wheat microsatellite primers evenly distributed across the A genome were synthesized according to the sequences published in the GrainGenes database (<http://wheat.pw.usda.gov>), modifying all forward primers to include the M13 sequence (CAC-GACGTTGTAAAACGAC-) at the 5' end for labeling purposes (Schuelke 2000; Rampling et al., 2001). The polymerase chain reaction (PCR) protocols, separation of PCR products and fragment size calling were as described by Miranda et al. (2006).

Primer pairs that were polymorphic between resistant and susceptible parents were used for bulked segregant analysis (Michelmore et al., 1991). Resistant and susceptible bulks were made by pooling equal amounts of DNA from 10 lines phenotypically scored as homozygous resistant and 10 lines phenotypically scored as homozygous susceptible.

Chromosomal Assignment

Chromosomal location of the linked microsatellite markers was confirmed using Chinese Spring nullitetrasonics: Nullisomic7A-tetra7D (N7AT7D) and ditelosomics: ditelo 7AS (Dt7AS) and ditelo 7AL

(Dt7AL) lines (kindly provided by The Wheat Genetics Resource Centre, Kansas State University). Genomic DNA from NCA6, euploid Chinese Spring and the nullitetrasonomic and ditelosomic lines of the putatively assigned chromosome were used to perform PCR reactions with the microsatellite markers linked the powdery mildew resistance locus.

The chromosomal breakpoint interval was determined for the microsatellite markers flanking the NCA6 powdery mildew resistance gene using deletion lines 7AL1-0.39, 7AL10-0.49, 7AL17-0.71, 7AL21-0.74, 7AL16-0.86, and 7AL20-0.89 (this nomenclature describes the chromosome arm carrying the deletion—the arbitrary line number designation—percentage of the arm present). These deletion lines are all homozygous terminal deletions previously described by Endo and Gill (1996).

Data Analysis

Deviations of observed data from theoretically expected segregation ratios were tested using chi-squared (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 a decimal logarithm of odds (LOD) threshold score ≥ 3.0 .

RESULTS

Greenhouse Evaluation

The disease reaction of NCA6 was immune to resistant, with scores ranging from 0 to 2. Saluda exhibited a susceptible reaction with scores ranging from 7 to 8. A χ^2 test value of 0.76 ($P = 0.68$) was obtained when testing for a 1:2:1 phenotypic ratio in the F₂ generation, indicating monogenically inherited disease resistance (Table 1).

Field Evaluations

2003 evaluation: The disease reaction of NCA6 was immune to resistant, with scores ranging from 0 to 2. Saluda exhibited a susceptible reaction with scores ranging from 7 to 9. The observed segregation of powdery mildew resistance in the NCA6 × Saluda population also fitted a 1:2:1 ratio ($P = 0.51$) (Table 1).

2004 evaluation: Sixty-six F_{2:4} lines from the NCA6 × Saluda population that were categorized either as homozygous susceptible or homozygous resistant were included in the 2004 experiment. There was complete agreement in phenotypic classes for all lines in both years.

Table 1. Segregation ratios for powdery mildew reaction of F₂-derived families from the NCA6 × 'Saluda' cross evaluated in the greenhouse and field.

Generation	Number of F ₂ families			Total	X ² (1:2:1)	P value
	Resistant	Segregating	Susceptible			
F _{2:3}	Greenhouse			99	0.76	0.68
	26	52	21			
F _{2:3}	Field (2003)			121	1.35	0.51
	35	55	31			

Differential Disease Response to *Blumeria graminis* f. sp. *tritici* Isolates

The disease reaction responses observed in the detached-leaf test indicated the NCA6 *Pm* gene was different from the five designated *Pm1* alleles (Table 2). Unlike NCA6, NC99BGTAG11 was resistant to isolates Sturdy2K*Pm*5, Asosan, and Arapahoe (data not shown).

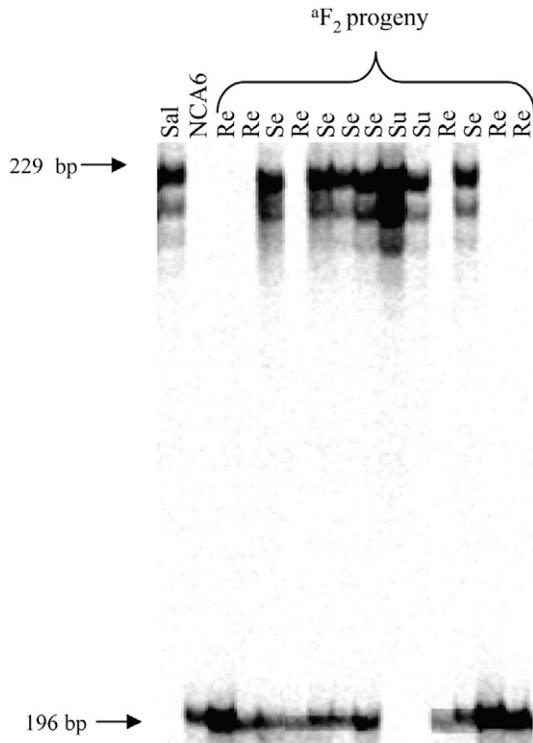


Figure 1. Genotyping of the NCA6 × Saluda F₂ population using microsatellite marker *Xbarc121*. ^aF₂ progeny labeled according to the disease reaction of their F₂-derived family: Re, resistant; Se, segregating; Su, susceptible.

Table 2. Differential reactions of NCA6, 'Axminster' (*Pm1a*), 'MocZlatka' (*Pm1b*), M1N (*Pm1c*), *T. spelta* (*Pm1d*), and 'Virest' (*Pm1e*) to 14 isolates of *Blumeria graminis* f. sp. *tritici* evaluated in a detached-leaf test.

Isolates	Genotypes [†]								
	NCA6	'Axminster' (<i>Pm1a</i>)	'MocZlatka' (<i>Pm1b</i>)	M1N (<i>Pm1c</i>)	<i>T. spelta</i> (<i>Pm1d</i>)	'Virest' (<i>Pm1e</i>)	'Saluda' [‡]	'Chancellor' [‡]	
A4/AG11L	S	R	R	R	R	R	S	S	
Yuma	I	R	R	R	R	R	S	S	
Sturdy2K <i>Pm</i> 5	S	R	I	I	R	R	S	S	
101a2	R	S	R	I	S	S	S	S	
Asosan	S	R	R	R	R	R	S	S	
Arapahoe	S	R	R	I	R	R	S	S	
E314	I	S	I	R	S	S	S	S	
Trego	I	R	R	R	R	R	S	S	
W72-27	R	S	S	R	S	S	S	S	
#8	R	S	R	R	R	S	S	S	
85063	R	S	R	I	S	S	S	S	
Flat-7-11	I	R	R	I	R	R	S	S	
169-1b	R	R	R	I	R	R	S	S	
AB9-10	S	R	R	I	R	R	S	S	

[†]R = resistant = 0–3; I = intermediate = 4–6; S = susceptible = 7–9.

[‡]Saluda = recurrent parent in NCA6; Chancellor = susceptible check.

Microsatellite Markers Analysis

Fifteen of the 56 A genome primer pairs included in the parental screening were polymorphic between NCA6 and Saluda. These primer pairs were used to perform bulk segregant analysis. The primer pair BARC121 generated polymorphic fragments between the contrasting bulks and was tested in the F₂ progeny (Fig. 1). A 196-bp fragment observed in NCA6 was associated with the resistant allele and a 229-bp band observed in Saluda was associated with the susceptibility allele. This microsatellite marker had been mapped to chromosome 7AL; therefore, 34 additional 7AL microsatellite markers were tested on the population. *Xcfa2123*, *Xcfa2019*, and *Xgwm332* were linked to the NCA6 powdery mildew resistance. The *Xcfa2019* marker was dominant and linked in coupling to the NCA6 *Pm* gene. The *Xcfa2019*/196bp band segregated with the resistant allele and was absent in all susceptible F₂ individuals. *Xbarc121*, *Xcfa2123*, and *Xgwm332* were codominant and fitted expected 1:2:1 monogenic ratios (Table 3). The most likely gene order was *Xcfa2123*-0.9 cM-*Xbarc121*-1.7 cM-NCA6 powdery mildew resistance allele-0 cM-*Xcfa2019*-3.0 cM-*Xgwm332*. Figures 2 and 3 compare the linkage map for the NCA6 *Pm* gene with other genetic maps for *Pm* loci on chromosome 7AL. Six other orders had LOD scores that differed by ≤3 from this most likely order; in four of them *Xcfa2123* and *Xgwm332* flanked the NCA6 *Pm* gene. The most likely order of the region between *Xcfa2123* and *Xbarc121* is inverted compared to the physical map (Fig. 4), but two other orders with LOD scores of 3.4 from the most likely order placed *Xbarc121* proximal to *Xcfa2123*.

The microsatellite marker *Xgwm334* was tested on NCA6, Saluda, and PI 427772, the *T. monococcum* donor of resistance to powdery mildew in NCA6. This marker is

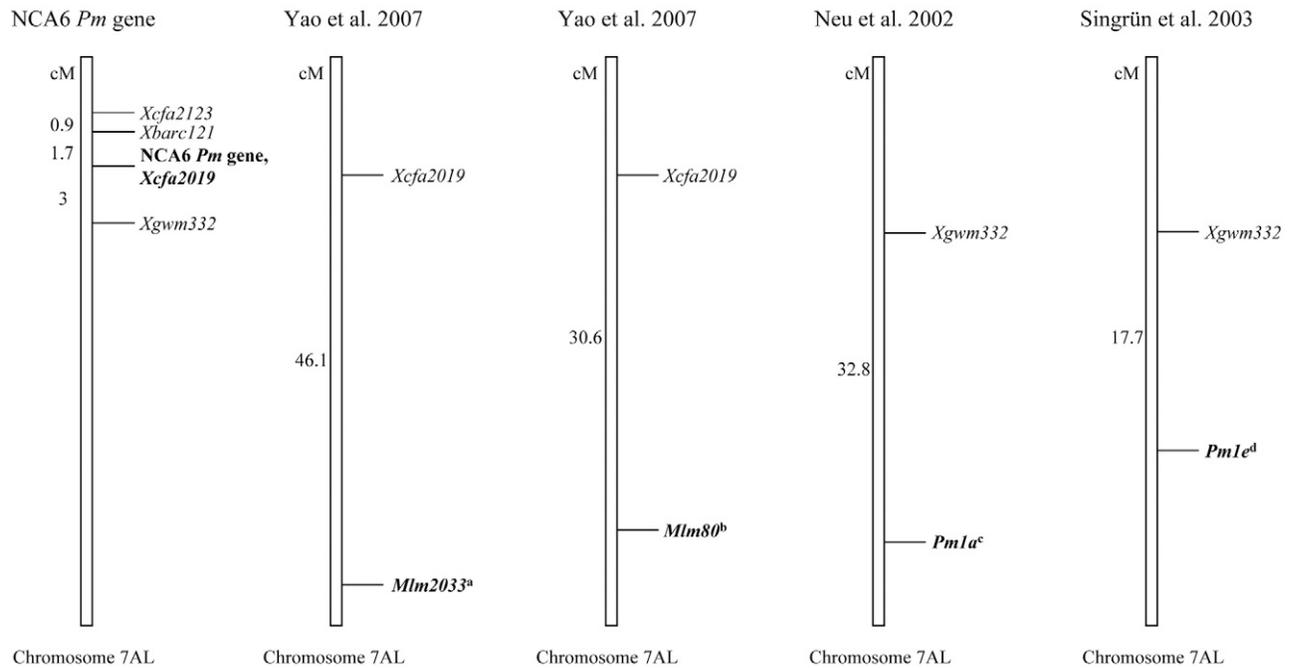


Figure 2. Comparative view of the NCA6 linkage map with other previously published genetic maps for *Pm* loci on chromosome 7AL. ^aMap position of *Mlm2033* according to Yao et al. (2007), ^bmap position of *Mlm80* according to Yao et al. (2007), ^cmap position of *Pm1a* according to Neu et al. (2002), ^dmap position of *Pm1e* according to Singrün et al. (2003).

tightly linked in repulsion to the *Pm1* locus (Singrün et al., 2003). The GWM344 primer pair amplified a 142-bp monomorphic fragment in Saluda and NCA6. A different 127-bp fragment was present in accession PI 427772, indicating that this locus in NCA6 traced to the recurrent parent.

Chromosomal Assignment

Marker loci linked to NCA6 were present in euploid Chinese Spring and ditelosomic 7AL but were absent in Nulli7A-Tetra7D and ditelosomic 7AS. Mapping distances among the microsatellite markers linked to the NCA6 powdery mildew resistance gene were not in good agree-

ment with published SSR linkage maps, therefore sub-arm localization of the microsatellite markers was performed using 7AL deletion lines. *Xbarc121* was located within the 0.49 to 0.71 interval, *Xcfa2123* was in the 0.74 to 0.86 interval and *Xgwm332* and *Xcfa2019* mapped to the distal 0.11 end of 7AL (Fig. 4).

DISCUSSION

Powdery mildew resistance in NCA6 was inherited as a monogenic trait and was mapped to chromosome 7AL. The *Pm1* (Sears and Briggles 1969) and *Pm9* (Schneider et al., 1991) loci have been mapped to the long arm of chromosome 7A,

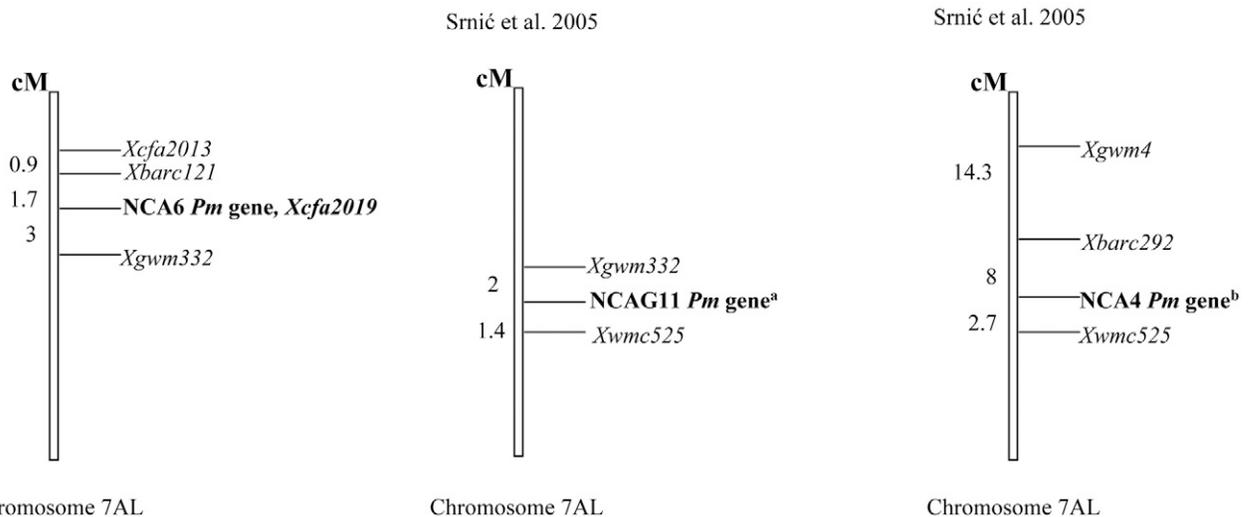


Figure 3. Comparative view of the NCA6 linkage map with the genetic maps for two other major powdery mildew resistance genes mapped to chromosome 7AL in North Carolina germplasm lines. ^aMap position of the NCAG11 *Pm* gene according to Sрниć et al. (2005), ^bmap position of the NCA4 *Pm* gene according to Sрниć et al. (2005).

Table 3. Segregation ratios for microsatellite (SSR) markers among F_2 individuals in the NCA6 \times 'Saluda' population.

Marker	Observed segregation ratio	Expected [†]	χ^2	<i>P</i> value
<i>Xbarc121</i>	36:55:30	1:2:1 A:H:B	1.26	0.53
<i>Xcfa2019</i>	90:31	3:1 D:B	0.02	0.87
<i>Xcfa2123</i>	34:56:31	1:2:1 A:H:B	0.82	0.66
<i>Xgwm332</i>	35:61:25	1:2:1 A:H:B	1.66	0.44

[†]A = homozygous for the NCA6 allele; H = heterozygous; B = homozygous for the Saluda allele; D = not homozygous for the Saluda allele.

separated by 8.5 cM (Schneider et al., 1991). Only one resistance allele has been reported for *Pm9*, and it has a recessive mode of action. A dominant mode of action for the NCA6 powdery mildew resistance gene was observed in NCA6 \times Saluda. NCA6 had a distinctive disease reaction response when compared to all the *Pm1* alleles in the detached-leaf test, indicating that this is a unique specificity.

The mapping distances among the microsatellite markers linked to the NCA6 *Pm* gene in this experiment were considerably less than the distances previously reported on microsatellite linkage maps (<http://wheat.pw.usda.gov/>). The microsatellite linkage map for the NCA6 *Pm* gene constituted an interval of <6 cM (Fig. 2) and a physical distance of at least 20% (Fig. 4) of the long arm of chromosome 7A. This low genetic to physical distance ratio could be explained by the presence of a relatively large segment introgressed from *T. monococcum* with suppressed recombination with *T. aestivum*. Lack of recombination could make locating the NCA6 powdery mildew resistance gene within a smaller physical interval a difficult task.

The order of the microsatellite markers *Xcfa2123* and *Xbarc121* was also in disagreement with the physical mapping, but discrepancies between genetic and physical maps have been previously reported (Sourdille et al., 2004),

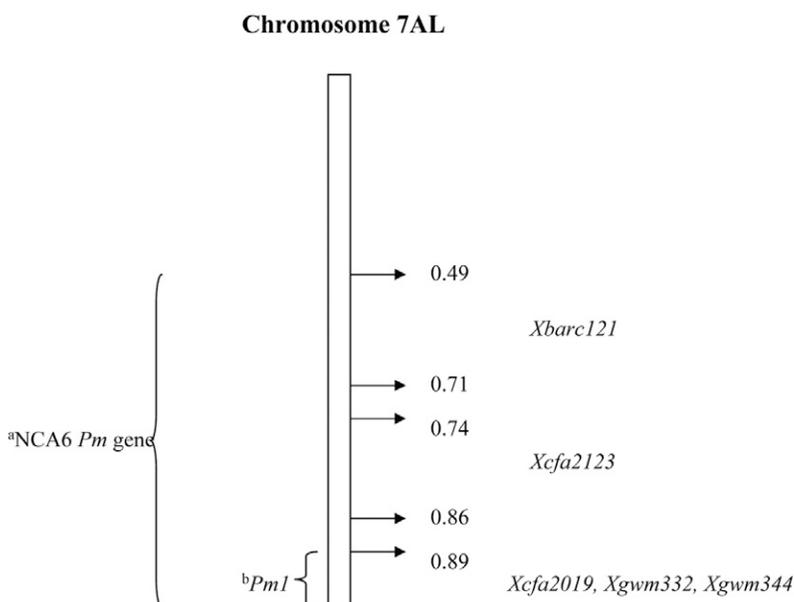


Figure 4. Chromosomal breakpoint intervals for microsatellite markers linked to the NCA6 *Pm* gene. ^aNCA6 *Pm* gene interval, ^b*Pm1* interval.

particularly in proximal regions and when there is little recombination. These two markers were separated by a distance of only 0.9 cM and were the most proximal loci in the NCA6 linkage map. Despite the unresolved order between *Xcfa2123* and *Xbarc121*, both the physical and the genetic map indicate that these two marker loci are proximal to the NCA6 *Pm* gene and *Xgwm332* is distal.

Marker *Xgwm332* was mapped 17.7 cM proximal to *Pm1e* (Singrün et al., 2003) and 32.8 cM proximal to *Pm1a* (Neu et al., 2002). Because we mapped *Xgwm332* 3 cM distal to the NCA6 *Pm* gene, the distance between this *Pm* locus and *Pm1* should be at least 20 cM (Fig. 2). Also, considering that recombination is likely to be reduced by the alien *T. monococcum* segment, additional backcross generations to the recurrent parent Saluda could increase the genetic distance between the NCA6 *Pm* locus and *Pm1*.

Marker *Xgwm344* is the closest reported microsatellite linked to *Pm1* (0.9 cM) and is null for *Pm1a*, *Pm1b*, *Pm1d* and *Pm1e* (Stepien et al., 2004). This marker locus was monomorphic between NCA6 and Saluda but polymorphic between NCA6 and *T. monococcum* accession PI 427772, indicating that the allele at this locus in NCA6 is derived from Saluda.

The powdery mildew resistance genes in the North Carolina germplasm lines NC96BGTA4 and NC99BGTAG11 are also located on chromosome 7AL (Srnić et al., 2005). The disease reaction response of NCA6 differs from both NC96BGTA4 (Murphy et al., 1999) and NC99BGTAG11 (our results). The source of powdery mildew resistance in NC96BGTA4 was the *T. monococcum* accession PI 221414 and the donor of the NC99BGTAG11 *Pm* gene was the *T. timopheevii* subsp. *armeniicum* accession PI 427315 (Murphy et al., 1999, 2002). The linkage maps for NC96BGTA4 and NC99BGTAG11 (Srnić et al., 2005) suggested that the resistance genes in these germplasms are distal to the NCA6 *Pm* gene (Fig. 3).

The 7AL linkage group also includes the *T. monococcum Pm* genes *Mlm2033* and *Mlm80* (Yao et al., 2007). The linkage maps developed for these two loci indicated they are allelic or tightly linked to *Pm1*, thereby distal to the NCA6 *Pm* gene. Microsatellite marker *Xcfa2019* co-segregated with the NCA6 *Pm* gene and was mapped 46.1 cM proximal to *Mlm2033* and 30.6 cM proximal to *Mlm80* (Fig. 2).

All the previous cross-references to published linkage maps for *Pm* genes on chromosome 7AL and the distinctive virulence profile of NCA6 indicate that this germplasm line has novel powdery mildew resistance specificity and is likely to be a novel *Pm* locus in hexaploid wheat.

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