

Inheritance and Chromosomal Assignment of Powdery Mildew Resistance Genes in Two Winter Wheat Germplasm Lines

G. Sрниć, J. P. Murphy,* J. H. Lyerly, S. Leath, and D. S. Marshall

ABSTRACT

Powdery mildew of wheat (*Triticum aestivum* L.), caused by *Blumeria graminis* DC f. sp. *tritici* Em. Marchal, occurs annually in eastern North America resulting in reduced grain yield and end-use quality in susceptible cultivars. The objectives of this study were to determine the inheritance, chromosomal location, and linkage with molecular markers of powdery mildew resistance genes in the two recently released germplasm lines NC96BGTA4 and NC99BGTAG11. Between 99 and 194 F_{2:3} progenies plus parents in two populations, 'Saluda' × NC96BGTA4 and Saluda × NC99BGTAG11, were evaluated in greenhouse and field nurseries for reaction to powdery mildew infection. Results indicated that the germplasm lines each contained a different, partially dominant, major resistance gene. The two segregating populations were subjected to amplified fragment length polymorphism (AFLP) and simple sequence repeat, or microsatellite (SSR) analyses. Both resistance genes were located on the long arm of chromosome 7A. The most likely locus order indicated that the resistance gene in NC96BGTA4 was flanked by the SSR loci *Xbarc292* and *Xwmc525*. The resistance gene in NC99BGTAG11 was most likely flanked by the AFLP markers *XE38M54-196* and *XE36M55-126*, and the SSR loci *Xgwm332* and *Xwmc525*. Both genes mapped to a chromosome arm that contains the powdery mildew resistance loci *Pm1* and *Pm9*. The resistance genes in the two germplasms are different from the *Pm1a* allele. Our mapping results suggested that the resistance genes were not alleles at the *Pm1* or *Pm9* loci, but further allelism tests are necessary to determine the relationships both between the two genes themselves and between the two genes and named *Pm* loci on chromosome 7AL.

POWDERY MILDEW of wheat is a cool temperature disease that significantly impacts grain yield and end-use quality in eastern North America. Grain yield reductions of 17 and 34% due to powdery mildew epidemics were recorded for North Carolina (Leath and Bowen, 1989) and Maryland (Johnson et al., 1979). The powdery mildew fungus can be observed on susceptible cultivars from the seedling stage through head emergence and yields are impacted through a decrease in numbers of mature tillers, kernels per head, and kernel weight (Leath and Bowen, 1989). Everts et al. (2001) reported that powdery mildew infection significantly reduced flour yield.

Host plant resistance is the most cost effective means for powdery mildew control. Monogenic resistance is

mainly via a hypersensitive foliar reaction involving major *Pm* genes in a gene-for-gene interaction (Bennett, 1984; Chen and Chelkowski, 1999; Hsam and Zeller, 2002). Thirty major gene loci have been identified to date in common wheat (McIntosh et al., 1998; Rong et al., 2000; Järve et al., 2000; Peusha et al., 2000; Zeller et al., 2002; Liu et al., 2002; Hsam et al., 2003; Singrun et al., 2003). Horizontal or quantitative resistance has been identified (Shaner, 1973; Griffey and Das, 1994; Chantret et al., 2001; Liu et al., 2001) and adult plants with this form of resistance exhibit a decrease in disease intensity compared with the fully susceptible lines.

Common sources of *Pm* genes are species within the primary, secondary, and tertiary gene pools of wheat (Hsam and Zeller, 2002). A program of interspecific hybridization between powdery mildew resistant diploid and tetraploid relatives and the soft red winter wheat cultivar Saluda (Starling et al., 1986) was initiated in 1986 by the small grains breeding and pathology projects at North Carolina State University. To date 11 germplasm lines have been released (Murphy et al., 1998, 1999a, 1999b, 2002; Navarro et al., 2000).

Selection of lines containing major *Pm* genes in breeding nurseries in the mid-Atlantic states is facilitated by annual powdery mildew epidemics, but selection of lines containing pyramids of *Pm* genes necessitates the use of molecular markers linked to resistance genes. There have been numerous demonstrations of the utility of molecular markers in wheat improvement, including the tagging of major *Pm* genes and quantitative trait loci (QTL) associated with horizontal resistance (reviewed by Huang and Roder, 2004). The multi-allelic *Pm1* locus on chromosome 7AL has been studied using restriction fragment length polymorphism (RFLP) (Ma et al., 1994; Hartl et al., 1995, 1999), randomly amplified polymorphic DNA (RAPD) (Hu et al., 1997), AFLP (Hartl et al., 1999), and SSR marker systems (Neu et al., 2002; Singrun et al., 2003).

Knowledge of the inheritance of resistance to powdery mildew and the linkage between the resistance genes and molecular markers in the recently released North Carolina germplasms would be advantageous for marker assisted selection in cultivar development. The objectives of this study were to determine the inheritance, chromosomal location, and linkage with molecular markers of genes for resistance to powdery mildew in two of these germplasm lines, NC96BGTA4 and NC99BGTAG11.

MATERIALS AND METHODS

Two germplasm lines resistant to powdery mildew, NC96BGTA4 (NCA4) and NC99BGTAG11 (NCAG11),

Abbreviations: AFLP, amplified fragment length polymorphism; SSR, simple sequence repeat, or microsatellite.

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were each crossed with the soft red winter wheat cultivar Saluda ($2n = 42$, genomes BBAADD). NCA4 and NCAG11 were homogeneous resistant BC₂F₅- and BC₂F₇-derived lines developed from interspecific hybridizations between diploid and tetraploid species and the recurrent parent Saluda. The donor species were *T. monococcum* subsp. *monococcum* ($2n = 14$, genomes AA) for NCA4, and *T. timopheevii* subsp. *armeniicum* ($2n = 28$, genomes GGAA) for NCAG11. Saluda contains the *Pm3a* gene that is ineffective against naturally occurring powdery mildew populations in North Carolina (Leath and Heun, 1990). F₁ plants from the two crosses (Saluda × NCA4 and Saluda × NCAG11) were grown in the greenhouse and self-pollinated. F₂ plants from the two populations were grown in the greenhouse at North Carolina State University, Raleigh, NC, during 2000 to produce F_{2,3} lines.

Greenhouse Evaluation of Inheritance of Resistance to Powdery Mildew

Ninety-nine F_{2,3} lines from each of the two populations were evaluated for reaction to powdery mildew in separate experiments during 2001 and 2002. 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. Two pots containing the relevant parental germplasm line and Saluda were included at 10-pot intervals as controls. Each control pot contained two plants. Seeds of parental germplasm lines and Saluda were derived from selfed progenies of the plants used in crosses to develop the two populations. The seeds were planted in a mixture of Metro-Mix 200 (Scotts-Sierra Horticultural Products Co., Marysville, OH), soil, and sand in a 50:40:10 ratio. Three grams of a slow release 14-14-14 (N-P-K) fertilizer was mixed with the potting medium in each pot. The temperature was maintained at 24°C (day) and 20°C (night). Plants were grown under a combination of plentiful natural light supplemented with artificial high intensity 1000-W discharge lights.

Plants were inoculated at Feekes growth stage 2 to 3 (Large, 1954) by gently shaking conidia from leaves of infected Saluda plants onto leaves of F_{2,3} lines and parental plants. The inoculum source was field grown Saluda plants dug at the Cunningham Research and Education Center, Kinston, NC, in April 2001. The inoculum was maintained through the year on Saluda plants in the greenhouse and under laboratory conditions on detached leaves according to the method of Leath and Heun (1990). Disease evaluations were conducted when all Saluda control plants showed abundant signs and symptoms of powdery mildew infection. The disease severity evaluation was on a scale from 0 to 9 as described by Leath and Heun (1990) where: 0 = immune, no visible signs of infection; 1 to 3 = resistant, increasing from (i) flecks, no necrosis to (ii) necrosis, to (iii) chlorosis, while the amount of mycelium went from none to a detectable amount; 4 to 6 = intermediate, chlorotic areas decreasing in amount while mycelium and conidia production increased from slight to moderate; 7 to 9 = susceptible, increasing amount, size, and density of mycelium and conidia to a fully compatible reaction. Disease reactions and intensities in F_{2,3} progeny similar to the parental germplasm line were classified as “resistant” and those similar to Saluda were classified as “susceptible.”

Field Evaluations of Inheritance of Resistance to Powdery Mildew

We planted 189 to 194 F_{2,3} lines from each of the two populations at Kinston, NC, in October 2001. All F_{2,3} lines evaluated

in the greenhouse tests were evaluated in the field tests. The experimental design was a randomized complete block with two replications. An experimental unit was a 1.2-m row sown with 20 to 60 seeds of each line. Rows were spaced 30.5 cm apart. Parental germplasm lines and Saluda were included as controls every 40 plots. In addition, each replication contained the cultivar Chancellor and 12 isolines with previously identified *Pm* genes backcrossed into Chancellor. The donor source and the major gene in each isolate were as follows: Axminster (*Pm1a*), Ulka (*Pm2*), Asosan (*Pm3a*), Chul (*Pm3b*), Sonora (*Pm3c*), Michigan Amber (*Pm3f*), Yuma (*Pm4*), Hope (*Pm5a*), Coker 747 (*Pm6*), Transec (*Pm7*), Federation/Kavkaz (*Pm8*), and Amigo (*Pm17*). Chancellor contains no *Pm* genes that are effective against wheat powdery mildew, but it contains *Pm10* and *Pm15* that are effective against wheatgrass powdery mildew (caused by *Blumeria graminis* DC f. sp. *agropyri*) (Briggles, 1969; Tosa and Tada, 1990). A 1.2-m border of Saluda surrounded the experiment. Irrigation, fertilization, and other agronomic practices, excluding fungicide application, followed standard management practices for North Carolina (Weisz, 2000). Homogeneous resistant and susceptible lines in both populations were harvested in June 2002. F_{2,4} seed of a random sample of the homogeneous resistant and susceptible lines, NCA4, NCAG11, Saluda, Chancellor, and the 12 isolines were reevaluated at Kinston, NC, in the 2002–2003 season using the same protocols.

Disease reaction evaluations were initiated at the end of March 2002 and 2003 when all Saluda plots showed uniform powdery mildew infection. Plants were between Feekes growth stage 9 and 10.1. Flag minus 2 leaves were evaluated using the modified 0 to 9 scale of Leath and Heun (1990). Disease reactions were recorded on 12 to 24 random plants in each row. A sample size of 11 is required to identify a single recessive plant with a $P = 0.95$ in a population segregating with a 3:1 ratio (Sedcole, 1977). Lines observed as segregating for resistance in one replication and homogenous for resistance in the other were classified as segregating.

Chi-square tests were conducted to evaluate the goodness of fit between observed and expected segregation ratios in the two populations (Snedecor and Cochran, 1956). The GLM procedure of the SAS software (SAS Institute, 1999) was utilized in the analysis of field data on parental and isogenic lines. The least significant difference (LSD) for comparison between germplasm and isogenic lines was computed as $LSD = t[MSE(g^{-1} + i^{-1})]^{1/2}$, where MSE was the estimated error mean square for testing the significance of variance among lines, g and i were the number of replicates per germplasm or isolate, respectively, and t was the t value at the $P = 0.05$ probability level for the number of degrees of freedom associated with MSE.

Field Evaluation of Resistance to Powdery Mildew among Parental Lines

NCA4, NCAG11, and Saluda were planted in two replicate randomized complete block experiments between 20 October and 15 November in 2000, 2001, 2002, and 2003 at Kinston. Plots were 5.1 m² with seven rows spaced 18 cm apart. Irrigation, fertilization, and other agronomic practices, excluding fungicide application, followed standard management practices for North Carolina (Weisz, 2000). Powdery mildew severity was evaluated on a whole-plot basis once each spring between Feekes growth stage 8 and 10.1. Assessments were based on the extent and position of lesions in the canopy. A 0 to 9 scale was utilized where: 0 = no detectable lesions; 1 to 3 = lesions ranging from barely detectable to 1% coverage of leaf area in lower one-third of canopy; 4 to 6 = lesions ranging

from barely detectable to 5% coverage of leaf area in middle third of canopy; and 7 to 9 = lesions covering from 1% of the flag minus 1 leaf to 10% or more of flag leaf.

DNA Extraction

DNA was extracted from young leaf tissue of greenhouse-grown parental lines, 115 F₂ plants from the Saluda × NCA4 population and 127 F₂ plants from the Saluda × NCAG11 population. The F₂ plants were a random subset of those utilized to produce F_{2,3} lines for field evaluations of powdery mildew resistance, but included all F₂ plants utilized to produce F_{2,3} lines for greenhouse evaluations. In addition, DNA was extracted from one F₄ plant in each of 23 F_{2,4} lines homozygous for resistance or susceptibility in the Saluda × NCAG11 population and from 10 F₅ individuals in each of three F_{4,5} lines homozygous for resistance in the Saluda × NCA4 population. The DNeasy Plant Mini Kit (Qiagen, Valencia, CA) was used following the manufacturer's instructions, and DNA concentrations were adjusted to 200 ng per 12 μL of reaction mix.

Amplified Fragment Length Polymorphism Analysis

Restriction and ligation were performed according to the protocol supplied by Life Technologies (Gaithersburg, MD) with the Core Reagent Kit. Pre-amplifications were performed with *EcoRI* + A and *MseI* + C primers (Life Technologies). Polymerase chain reaction (PCR) conditions for pre-amplifications were: 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s (27 cycles). The selective amplifications were done with *EcoRI* + 3 and *MseI* + 3 primer combinations. Sequences of *EcoRI* primers (LI-COR, Lincoln, NE) were 5'-GACTGCGTACC AATTCNNN-3' and *MseI* primers (Sigma-Aldrich, Milwaukee, WI) were 5'-GATGAGTCCTGAGTAANNN-3'. The PCR conditions for selective amplifications were: 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s (12 cycles); 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s (22 cycles). The electrophoresis was conducted in LI-COR sequencers, Models 4000 and 4200L, on 8% polyacrylamide denaturing gels under 48°C, 42 W, 35 mA, and 1500 V for approximately 3 h. Electronic images of the gels were analyzed by AFLP Quantar 1.09 software (KeyGene Products, 2000).

Screening for polymorphisms between Saluda and NCA4 or NCAG11 utilized 88 primer combinations. Subsequently, the selected primer combinations were used to compare bulks containing DNA of 10 homozygous resistant F₂ individuals with bulks containing DNA of 10 homozygous susceptible F₂ individuals. The inheritance of polymorphisms identified through bulked segregant analyses was investigated utilizing 127 F₂ individuals in the Saluda × NCAG11 population. The AFLP marker nomenclature followed the protocol outlined at the GrainGenes website (<http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html>; verified 7 Mar. 2005).

Simple Sequence Repeat Analysis

Microsatellite primer sequences were obtained from Röder et al. (1998) and the GrainGenes database (<http://wheat.pw.usda.gov/>; verified 7 Mar. 2005). The SSR loci selected for screening for polymorphisms between Saluda and NCA4 or NCAG11 were evenly distributed across the A and B genomes. All primers were obtained from MWG Biotech (High Point, NC). Forward primers were modified to incorporate the M13 primer sequence (5'-CACGACGTTGTAAAACGAC-3') for the purpose of universal fluorescent labeling (Schuelke, 2000; Rampling et al., 2001). M13 primer was labeled with IRDye800 or IRDye700.

The PCR reactions contained 1× PCR buffer, 1.5 mM

MgCl₂, 0.2 mM dNTPs (Promega, Madison, WI), 0.15 pM forward primer, 0.75 pM reverse primer, 0.75 pM M13 labeled primer, 0.2 μL BSA (10 μg μL⁻¹; NEB, Beverly, MA), 0.75 U Taq polymerase (CLP, San Diego, CA), and 50 ng genomic DNA in a 10-μL total volume. Cycling was completed using the touchdown program as written by Rampling et al. (2001).

Reactions were diluted 1:1 with 95% formamide buffer [19 mL formamide (Fisher Scientific, Hampton, NH)], 1.0 mL 0.5 M EDTA (Fisher Scientific), pH 8.0, 0.16 g bromophenol blue (USB, Cleveland, OH), denatured for 3 min at 90°C, and placed immediately on ice. Samples were loaded onto 6.5% denaturing polyacrylamide gels and run on LI-COR sequencers for 2.5 h at 48°C, 42 W, 35 mA, and 1500 V. Gels were scored using AFLP Quantar 1.09 software (KeyGene Products, 2000).

A total of 188 SSR loci were examined for polymorphism between the parents in both populations. Subsequently, the selected primer combinations were used for bulked segregant analysis of DNA samples prepared as described from resistant and susceptible individuals for each population. Forty-eight primer combinations were tested in the Saluda × NCA4 population, and 50 primer combinations were tested in the Saluda × NCAG11 population. The inheritance of polymorphisms identified through bulked segregant analyses was investigated utilizing 115 F₂ individuals in the Saluda × NCA4 population and 105 F₂ individuals in the Saluda × NCAG11 population.

Linkage Analysis

Linkage relationships between marker loci and resistance genes were determined by Mapmaker/Exp (Version 3.0 b) (Lincoln et al., 1993). Map distances were calculated using the Kosambi function to correct for crossover interference in estimation of recombination fractions. The decimal logarithm of odds (LOD) ratio was set to 3.0. Loci were ordered on the chromosome using the "sequence" and "compare" commands.

RESULTS AND DISCUSSION

Powdery mildew development was excellent in all greenhouse and field evaluations. Readily identifiable differences in disease reactions between resistant and susceptible parents were observed in both environments (Table 1). There was good correlation in classification of F_{2,3} progenies into discrete genotypic classes between the two field replications and between field and greenhouse tests. Agreement between the two field replications was 98% in both crosses. Agreement between the field and greenhouse evaluations was 86% for the

Table 1. Means, standard deviations, and ranges of powdery mildew severity scores for parents of two wheat populations evaluated in greenhouse and field tests in 2001–2002.

Cross no.	Parents	Mean	Range
Greenhouse			
1	Saluda	7.1 ± 0.24	7.0–8.0
	NCA4	4.7 ± 0.41	4.0–5.0
2	Saluda	7.5 ± 0.51	7.0–8.0
	NCAG11	0.0	–
Field test			
1	Saluda	7.7	7.0–9.0
	NCA4	0.0	–
	LSD _{0.05}	0.45	
2	Saluda	7.1	6.0–8.0
	NCAG11	0.0	–
	LSD _{0.05}	0.36	

Saluda × NCA4 population and 99% for the Saluda × NCAG11 population. Powdery mildew resistance in both Saluda × germplasm line populations segregated as a partially dominant monogenic trait in greenhouse and field evaluations (Table 2).

Cross 1 (Saluda × NCA4)

Greenhouse

Saluda had a mean disease rating of 7.1 ± 0.24 with a range of 7.0 to 8.0 (Table 1). NCA4 had a mean disease rating of 4.7 ± 0.41 with a range of 4.0 to 5.0. Eighteen $F_{2,3}$ lines were homozygous resistant with a mean of 4.8 ± 0.26 and a range of 4.0 to 5.0 (Table 2). Fifty-two lines were segregating. Twenty-nine lines were homozygous susceptible with a mean of 6.9 ± 0.16 and a range of 6.4 to 7.0. The chi-square test value for the expected 1:2:1 ratio was 2.70 ($P = 0.26$), indicating that resistance evaluated in the greenhouse segregated as a monogenic trait.

Field

Saluda had a mean disease rating of 7.7 ± 0.67 and a range of 7.0 to 9.0 (Table 1). NCA4 was immune with a mean of 0.0. Forty-one $F_{2,3}$ lines were homozygous resistant (Table 2). One-hundred-eight $F_{2,3}$ lines were segregating. Forty-five $F_{2,3}$ lines were homozygous susceptible, with a mean of 7.1 ± 0.64 and a range of 6.0 to 8.0. The chi-square test value for the expected 1:2:1 ratio was 2.66 ($P = 0.26$), indicating that resistance evaluated in the field segregated as a monogenic trait.

NCA4 and the resistant progenies had notably different disease reactions in the greenhouse versus the field environment, while Saluda and the susceptible progenies had similar reactions in both environments. In the greenhouse, NCA4 and the resistant progenies exhibited an intermediate response with chlorosis and some mycelium production. NCA4 and the resistant progenies had an immune reaction in the field in 2002. The different disease ratings in the two environments may have reflected differences due to stage of plant development (Shaner, 1973; Griffey and Das, 1994). It is also possible that the powdery mildew population in the greenhouse had a more complex pathogenicity profile, such as multiple *avr* genes, so the resistance gene in NCA4 was very effective against some powdery mildew isolates and less effective against others. Although differences in level of resistance expression were observed

in the two environments, both data sets indicated that NCA4 contained a single major resistance gene.

Cross 2 (Saluda × NCAG11)

Greenhouse

Saluda had a mean disease rating of 7.5 ± 0.51 and a range of 7.0 to 8.0 (Table 1). NCAG11 was immune. Twenty-six $F_{2,3}$ lines were homozygous resistant with a mean of 0.0 (Table 2). Forty-one $F_{2,3}$ lines were segregating. Thirty-two lines were homozygous susceptible. The chi-square test value for the expected 1:2:1 ratio was 3.65 ($P = 0.16$), indicating that resistance evaluated in the greenhouse segregated as a monogenic trait.

Field

Saluda had a mean disease rating of 7.1 ± 0.57 and a range of 6.0 to 8.0 (Table 1). NCAG11 was immune. Fifty-two $F_{2,3}$ lines were homozygous resistant with a mean of 0.0 (Table 2). Eighty-one $F_{2,3}$ lines were segregating. Fifty-six $F_{2,3}$ lines were homozygous susceptible with a mean of 7.2 ± 0.53 and a range of 5.0 to 8.0. The chi-square test value for the expected 1:2:1 ratio was 4.03 ($P = 0.13$), indicating that resistance evaluated in the field segregated as a monogenic trait.

Identification of Molecular Markers Linked to the Resistance Gene in NCA4

Fifty-five AFLP primer combinations generated 167 polymorphisms between Saluda and NCA4, but none were considered viable markers for the resistance gene in NCA4 following bulked segregant analysis. The NCA4 × Saluda population was not subjected to further AFLP investigation.

Polymorphisms between Saluda and NCA4 were detected using bulked segregate analysis at the *Xwmc525*, *Xbarc292*, and *Xgwm4* SSR loci (Fig. 1). *Xwmc525* was the only locus displaying codominant gene action and the stutter observed was consistent with the description of this locus in GrainGenes. A 211 bp fragment was observed at the *Xwmc525* locus in NCA4 and a 230 bp fragment was observed in Saluda. The large allele separation at this locus allowed for consistent scoring of this locus despite the stutter. A 219 bp fragment was observed in Saluda at *Xbarc292*. A 253 bp fragment was observed in Saluda at *Xgwm4*. The F_2 segregation pattern at the *Xwmc525* locus followed the expected

Table 2. Observed and expected ratios among $F_{2,3}$ lines in greenhouse and field evaluations of two wheat crosses segregating for powdery mildew resistance.

Cross no.	Cross	Number of $F_{2,3}$ lines				Expected ratio†	χ^2	P
		Resistant	Segregating	Susceptible	Total			
Greenhouse								
1	Saluda × NCA4	18	52	29	99	1R:2Se:1S	2.70	0.26
2	Saluda × NCAG11	26	41	32	99	1R:2Se:1S	3.65	0.16
Field test								
1	Saluda × NCA4	41	108	45	194	1R:2Se:1S	2.66	0.26
2	Saluda × NCAG11	52	81	56	189	1R:2Se:1S	4.03	0.13

† R, resistant; Se, segregating; S, susceptible.

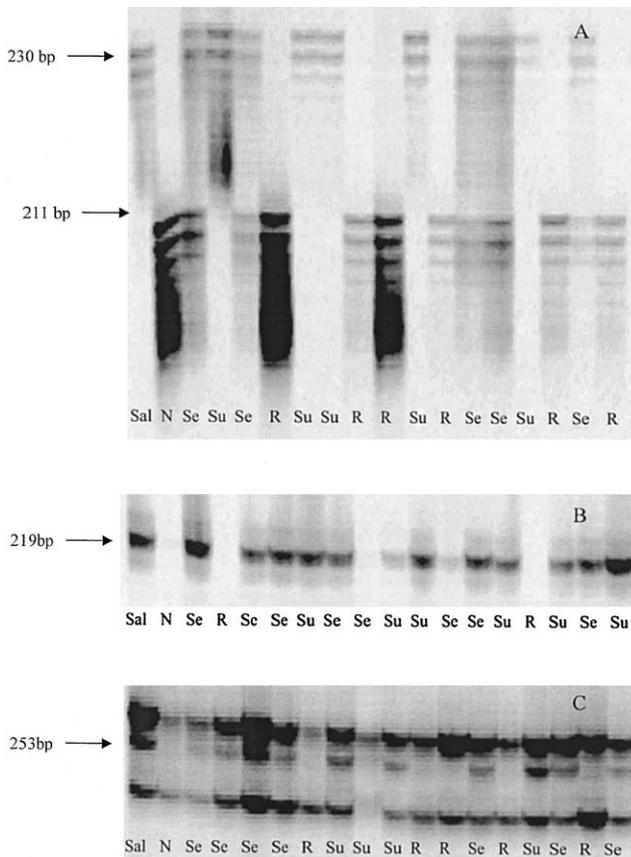


Fig. 1. Gel images for simple sequence repeat (SSR) markers in the Saluda \times NCA4 population. (A) *Xwmc525* with fragment sizes of 230 bp (Saluda) and 211 bp (NCA4). (B) *Xbarc292* with fragment sizes 219 bp (Saluda) and null (NCA4). (C) *Xgwm4* with fragment sizes 253 bp (Saluda) and null (NCA4). Lanes 1 and 2 are Saluda (Sal) and NCA4 (N). Lanes 3 to 18 are F_2 plants labeled according to the disease reaction of their F_{23} progeny as follows: R, resistant; Se, segregating; and Su, susceptible.

1:2:1 ratio of a codominant marker while the segregation patterns at the *Xgwm4* and *Xbarc292* loci followed the expected 3:1 ratio of a single dominant marker (Table 3). The inheritance of powdery mildew resistance in the F_{23} lines derived from the random F_2 plants that underwent SSR analyses followed the expected 1:2:1 ratio (data not shown).

Table 3. Observed and expected segregation ratios for amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers among F_2 individuals in the Saluda \times NCA4 and Saluda \times NCA11 wheat populations.

	AA [†]	H [‡]	BB [§]	+	-	Total	Expected ratio	χ^2	P
Saluda \times NCA4									
SSR									
<i>Xwmc525</i>	18	54	23			95	1:2:1	0.32	0.85
<i>Xbarc292</i>				82	22	104	3:1	0.63	0.43
<i>Xgwm4</i>				89	26	115	3:1	0.24	0.63
Saluda \times NCA11									
AFLP									
<i>XE36M55-126</i>				87	40	127	3:1	2.52	0.11
<i>XE38M54-196</i>				84	37	121	3:1	1.72	0.19
SSR									
<i>Xgwm332</i>	28	45	29			102	1:2:1	1.27	0.49
<i>Xwmc525</i>	29	43	33			105	1:2:1	3.72	0.15

[†] Homozygous for germplasm SSR allele.

[‡] Heterozygous.

[§] Homozygous for Saluda SSR allele.

|| +, AFLP or SSR fragment present; -, AFLP or SSR fragment absent.

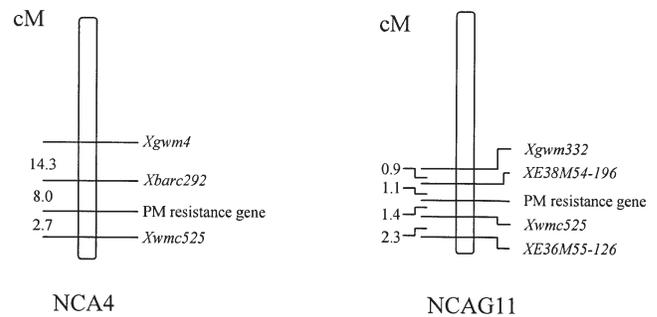


Fig. 2. Map positions of the powdery mildew resistance genes on wheat chromosome 7AL in the germplasm lines NCA4 and NCA11. PM, powdery mildew.

The most likely order of loci in the linkage group was *Xgwm4*, 14.3 cM, *Xbarc292*, 8.0 cM, powdery mildew resistance gene, 2.7 cM, *Xwmc525* (Fig. 2). The second most likely order of loci, which differed by a LOD score of -2.1 , was *Xgwm4*, 14.6 cM, *Xbarc292*, 10.1 cM, *Xwmc525*, 2.9 cM, powdery mildew resistance gene. Ma et al. (1994) and Neu et al. (2002) reported that *Pm1a* cosegregated with the restriction fragment length polymorphism (RFLP) locus *Xcdo347* on chromosome 7A. Shi et al. (2003) located the *Xbarc292* locus 24.4 cM proximal to *Xcdo347* on chromosome 7AL. The *Xwmc525* locus is located on 7AL distal to *Xgwm4* (Somers et al., 2004). The locus order we observed for *Xgwm4*, *Xbarc292*, and *Xwmc525* was consistent with the previous studies and indicated that the major resistance gene in NCA4 was located on chromosome 7AL proximal to the *Pm1* locus.

Forty F_{24} lines derived from 20 homozygous resistant and 20 homozygous susceptible F_{23} lines in the Saluda \times NCA4 population were evaluated for resistance to powdery mildew in the field in 2003. Nineteen of the 20 F_{24} lines derived from resistant F_{23} lines were categorized as resistant and one was categorized as segregating. There was complete agreement in the categorization of the 20 F_{24} progenies and the parental F_{23} lines with respect to the susceptible class. Twenty-nine F_5 plants in three homozygous resistant F_{25} lines plus the parents were evaluated for the presence of the SSR alleles at the *Xgwm4*, *Xbarc292*, and *Xwmc525* loci. All 29 indi-

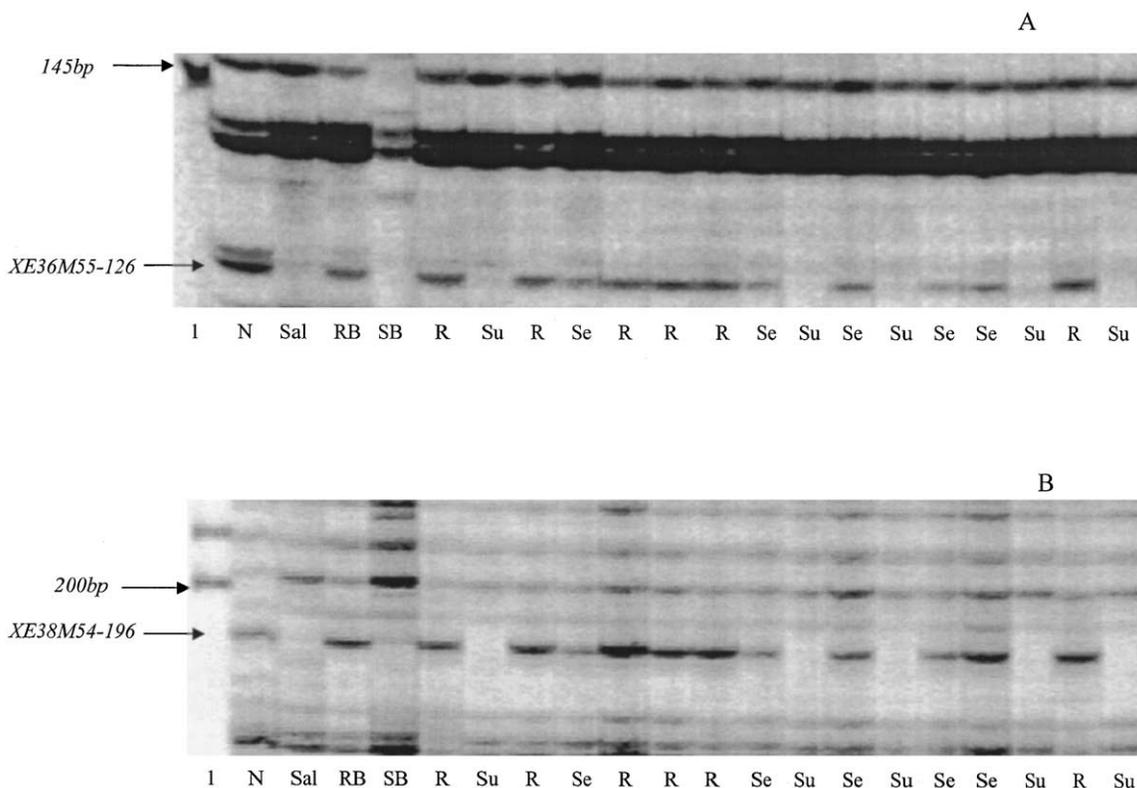


Fig. 3. Gel images for amplified fragment length polymorphism (AFLP) markers in the Saluda \times NCAG11 population. (A) *XE36M55-126* with a fragment size of 126 bp (NCAG11) and null (Saluda). (B) *XE38M54-196* with a fragment size of 196 bp (NCAG11) and null (Saluda). Lane 1 is the molecular weight standards. Lanes 2 and 3 are NCAG11 (N) and Saluda (Sal). Lanes 4 and 5 are the resistant (RB) and susceptible bulks (SB). Lanes 6 to 21 are F_2 plants labeled according to the disease reaction of their F_{23} progeny as follows: R, resistant; Se, segregating; and Su, susceptible.

viduals contained the 211 bp fragment from NCA4 at the *Xwmc525* locus. None contained the 219 bp fragment from Saluda at the *Xbarc292* locus. One plant contained the 253 bp fragment from Saluda at the *Xgwm4* locus. These results were consistent with the preferred locus order and estimated distances between the powdery mildew resistance locus and the flanking SSR loci.

Identification of Molecular Markers Linked to the Resistance Gene in NCAG11

Fifty-two AFLP primer combinations generated 138 polymorphisms between Saluda and NCAG11 and two AFLPs, *XE36M55-126* and *XE38M54-196*, were selected for further study based on bulked segregant analysis (Fig. 3). Both fragments were present in NCAG11 and absent in Saluda. The F_2 segregation patterns for both markers followed the expected 3:1 ratio of a single dominant gene (Table 3). The inheritance of powdery mildew resistance in the F_{23} lines derived from the F_2 plants involved in SSR and AFLP analyses followed the expected 1:2:1 ratio (data not shown). The two primer combinations used to generate the AFLPs of interest in this study were not among those used to construct published AFLP maps of wheat (Hazen et al., 2002; Huang et al., 2000). Thus the chromosome location of the resistance gene in NCAG11 was not identified by AFLP analysis.

Polymorphisms between Saluda and NCAG11 were observed at the *Xgwm332* and *Xwmc525* SSR loci (Fig. 4). Both loci displayed codominant gene action. A 210 bp fragment was observed at the *Xgwm332* locus in NCAG11 while a 212 bp fragment was observed in Saluda. A 258 bp fragment was observed at the *Xgwm525* locus in NCAG11 while a 228 bp fragment was observed in Saluda. The stutter observed at this locus in the NCA4 \times Saluda population was observed in this population also. Nevertheless the large allele size separation allowed for consistent scoring. The F_2 segregation pattern at both loci followed the expected 1:2:1 ratio of a single codominant marker (Table 3).

The most likely order of loci in the linkage group was *Xgwm332*, 0.9 cM, *XE38M54-196*, 1.1 cM, powdery mildew resistance gene, 1.4 cM, *Xwmc525*, 2.3 cM, *XE36M55-126* (Fig. 2). Twelve other locus orders were within a LOD score of -3.0 of the most likely order reflecting the close linkage between the loci and the relatively small population size evaluated. Nevertheless, 11 of these 13 likely orders had the two SSR loci flanking the powdery mildew resistance gene. The sixth most likely order was the first to suggest that the SSR loci did not flank the resistance gene. This order differed from the most likely order by a LOD score of -1.67 . The *Xgwm332* locus mapped 17.7 cM (Singrun et al., 2003) and 23.3 cM (Singrun et al., 2004) proximal to the *Pm1* locus on chromosome 7AL in a population from the cross of

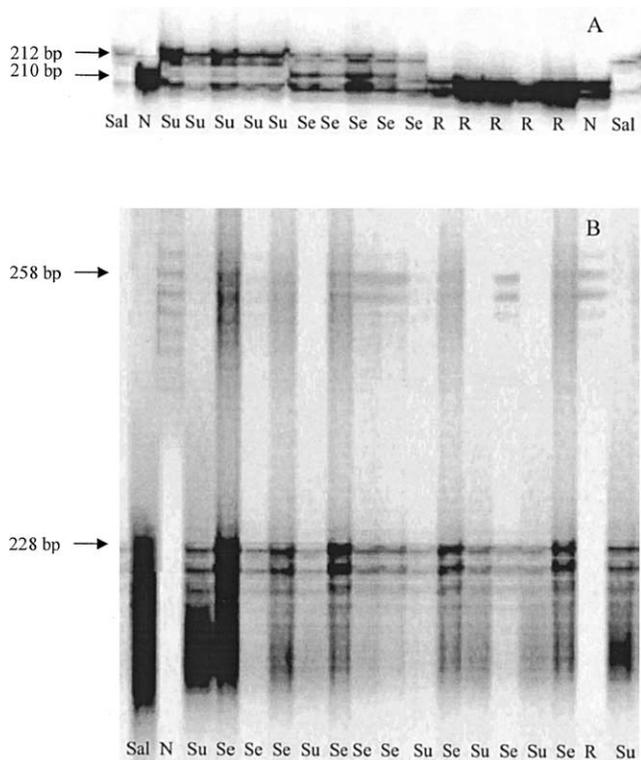


Fig. 4. Gel images for simple sequence repeat (SSR) markers in the Saluda \times NCAG11 population. (A) *Xgwm332* with fragment sizes of 212 bp (Saluda) and 210 bp (NCAG11). Lanes 1 and 19 are Saluda (Sal). Lanes 2 and 18 are NCAG11 (N). Lanes 3 to 17 are $F_{2,3}$ plants labeled according to the disease reaction of their $F_{2,3}$ progeny as follows: R, resistant; Se, segregating; and Su, susceptible. (B) *Xwmc525* with fragment sizes of 228 bp (Saluda) and 258 bp (NCAG11). Lanes 1 and 2 are Saluda and NCAG11. Lanes 3 to 18 are $F_{2,3}$ plants labeled according to the disease reaction of their $F_{2,3}$ progeny.

Chinese Spring \times 'Virest'. Virest contains the *Pm1e* allele. Neu et al. (2002) mapped the *Xgwm332* locus 32.8 cM proximal to the *Pm1* locus in a population from the cross of Chancellor \times 'Axminster'/8*Chancellor. Axminster contains the *Pm1a* allele. Our data suggested that the major resistance gene in NCAG11 was located on chromosome 7AL proximal to the *Pm1* locus.

Twenty-three $F_{2,4}$ lines derived from 11 homozygous resistant and 12 homozygous susceptible $F_{2,3}$ lines in the Saluda \times NCAG11 population were evaluated for resistance to powdery mildew in the field in 2003. There was complete agreement in the categorization of $F_{2,4}$ progenies and the parental $F_{2,3}$ lines with respect to powdery mildew reaction. One individual per $F_{2,4}$ line and the parents were evaluated for the presence of the AFLP bands *XE36M55-126* and *XE38M54-196* and SSR alleles at the *Xgwm332* and *Xwmc525* loci. There was complete agreement between expected and observed AFLP and SSR allele presence in the resistant and susceptible progeny reflecting the tight linkage among the loci.

The resistance genes in NCA4 and NCAG11 are both located on chromosome 7AL. It is not surprising that both resistance genes were introgressed into the A genome given the homology between the A genomes of

Table 4. Mean powdery mildew scores for four wheat germplasm lines and the Chancellor isoline series containing known *Pm* genes evaluated at Kinston, NC, in 2002 and 2003.

Germplasm/isoline	Disease rating (0–9)
NCA4	0.2
NCAG11	0.0
Chancellor	7.5
<i>Pm1a</i>	3.3
<i>Pm2</i>	7.3
<i>Pm3a</i>	7.0
<i>Pm3b</i>	8.0
<i>Pm3c</i>	8.0
<i>Pm3f</i>	8.0
<i>Pm4</i>	7.0
<i>Pm5a</i>	8.0
<i>Pm6</i>	7.3
<i>Pm7</i>	7.7
<i>Pm8</i>	4.7
<i>Pm17</i>	0.0
LSD _{0.05} between two NC germplasms	0.5
LSD _{0.05} between two <i>Pm</i> genes	1.4
LSD _{0.05} between NC germplasms and <i>Pm</i> genes	1.0

the donor species *T. monococcum* subsp. *monococcum* and *T. timopheevii* subsp. *armeniicum* with the A genome of common hexaploid wheat (Feldman, 2001). The disease scores of the two germplasms in greenhouse evaluations indicated that they contain either different genes or different alleles at a single locus (Table 1). This difference was supported by four years of evaluation in 5.1-m² plots at Kinston where significantly different disease severity means for Saluda (6.3), NCA4 (3.3), and NCAG11 (0.25) (LSD_{0.05} = 0.56) were recorded. Two years of field evaluations in single 1.2-m rows indicated that the resistance genes in NCA4 and NCAG11 are different from *Pm1a* (Table 4). The *Pm17* isoline exhibited a disease score similar to NCA4 and NCAG11, but this resistance gene was introgressed from rye (*Secale cereale* L.) and is located on the 1AL.1RS wheat-rye translocation segment (Heun et al., 1990). Murphy et al. (2002) reported that the resistance gene in NCAG11 was different from both *Pm1a* and *Pm9*. The *Pm1* and *Pm9* loci are located on chromosome 7AL separated by a map distance of 8.5 cM (Schneider et al., 1991; McIntosh et al., 1998). Five alleles (*1a-1e*) have been identified at the *Pm1* locus (Hsam et al., 1998; Singrun et al., 2003). The single allele conferring powdery mildew resistance at *Pm9* has a recessive mode of action. Singrun et al. (2004) identified an additional recessive allele tightly linked and distal to the *Pm1* locus. Given the chromosomal location of the resistance genes in NCA4 and NCAG11 they could be alleles of the *Pm1* or *Pm9* loci, or alleles of a new locus, or loci, in a cluster of closely linked resistance genes (Chantret et al., 2000). A mean map distance of 24.6 cM between the *Xgwm332* and *Pm1* loci has been reported (Singrun et al., 2003, 2004; Neu et al., 2002) and Shi et al. (2003) reported a distance of 24.4 cM between *Pm1* and *Xbarc292*. These results suggested that the resistance genes in NCA4 and NCAG11 are not alleles at the *Pm1* locus. Nevertheless, flanking diploid and tetraploid germplasm introgressed with the resistance genes may suppress recombination between *Pm1* and *Xgwm332* in NCAG11 and *Pm1* and *Xbarc292* in NCA4. Tests for allelism and further

identification of linked markers are necessary to resolve these different scenarios.

The powdery mildew resistance conferred by these two germplasm lines should have utility in cultivar development programs. The germplasms exhibited immune to intermediate levels of resistance in greenhouse and field environments when exposed to natural inoculum in North Carolina. Both have a plant type and maturity similar to Saluda. The tightly linked and flanking markers reported in this manuscript should aid in the incorporation of these resistant genes into future cultivars.

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