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ORIGINAL PAPER

Chromosomal location of *Pm35*, a novel *Aegilops tauschii* derived powdery mildew resistance gene introgressed into common wheat (*Triticum aestivum* L.)

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Abstract A single gene controlling powdery mildew resistance was identified in the North Carolina germplasm line NC96BGTD3 (NCD3) using genetic analysis of F_2 derived lines from a NCD3 X Saluda cross. Microsatellite markers linked to this *Pm* gene were identified and their most likely order was *Xcfd7*, 10.3 cM, X*gdm43*, 8.6 cM, *Xcfd26*, 11.9 cM, *Pm* gene. These markers and the *Pm* gene were assigned to chromosome 5DL by means of Chinese Spring Nullitetrasomic (Nulli5D-tetra5A) and ditelosomic (Dt5DL) lines. A detached leaf test showed a distinctive disease reaction to six pathogen isolates among the NCD3 *Pm* gene, *Pm2* (5DS) and *Pm34* (5DL). An allelism test showed independence between *Pm34* and the NCD3 *Pm* gene. Together, the tests provided strong evidence for the presence of a novel *Pm* gene in NCD3, and this gene was designated *Pm35.*

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Introduction

Wheat (*Triticum aestivum* L.) production in temperate areas is significantly affected by powdery mildew, a foliar disease caused by *Blumeria graminis* f. sp. *tritici*. Breeding for disease resistance has commonly relied on the use of major host resistance genes that originated in the primary, secondary or tertiary gene pools.

Interspecific gene transfer from species that share homologous genomes with cultivated wheat is easy to accomplish since normal chromosome pairing is expected (Hsam and Zeller 2002). *Aegilops tauschii* Coss. ($2n = 2X = 14$; genome DD), the donor of the D genome, is probably the most suitable among wheat relatives. Direct gene transfer can be accomplished using embryo rescue and adverse genetic interactions between the D genome of *A. tauschii* and the ABD genome of hexaploid wheat are uncommon (Gill and Raupp 1987).

Aegilops tauschii has been reported as a valuable source of powdery mildew resistance; numerous accessions have shown resistance and displayed diverse disease reactions (Cox et al. 1992; Lutz et al. 1994). *Pm19* and *Pm34* (Lutz et al. 1995; Miranda et al. 2006) were transferred from *A. tauschii* into cultivated wheat.

Molecular markers may be used in backcross breeding and near-isogenic line development to aid in the selection of the gene(s) of interest from the donor parent and to accelerate the recovery of the recurrent parent genotype (Zhou et al. 2005). Microsatellites are the preferred type of molecular markers for marker-assisted selection (MAS) in wheat

breeding because they are co-dominant, user friendly, highly polymorphic and provide good coverage of the wheat genome (Gupta et al. 1999; Langridge et al. 2001)

The small grains breeding program at North Carolina State University has released five powdery mildew resistance germplasm lines with different *A. tauschii* accessions serving as resistance donors and the soft red winter wheat cultivar 'Saluda' (Starling et al. 1986) serving as the recurrent parent (Murphy et al. 1998, 1999) In this study, we report the genetic characterization, microsatellite linkage mapping and allelism test performed for *Pm35*, the *A. tauschii*-derived powdery mildew resistance gene present in the germplasm line NC96BGTD3.

Materials and methods

The powdery mildew resistant germplasm line NC96BGTD3 (PI 603250) was crossed with the powdery mildew susceptible cultivar Saluda (PI 480474). NC96BGTD3, hereafter shortened to NCD3, is a homogeneously resistant BC_2F_5-derived line with the pedigree Saluda *3/TA2377 (Murphy et al. 1999). TA2377 is a powdery mildew resistant *A. tauschii* subsp. *strangulata* 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 NCD3/Saluda F_1 hybrid was selfed to produce F_2 seeds in the greenhouse. Two generations of F_2 derived lines were grown in the field without selection during the 2002–2003 and 2003–2004 seasons to produce $F_{2:4}$ seed for the 2005 greenhouse and field evaluations. NCD3 was also crossed to the North Carolina germplasm line NC97BGTD7 (NCD7) (PI 604033) that contains *Pm34* and the hybrid was advanced to the $F_{2:3}$ generation.

Disease assessments

NCD3/Saluda

Greenhouse Ninety two $F_{2:4}$ lines from NCD3/Saluda were evaluated for reaction to powdery mildew under greenhouse conditions. An experimental unit was two 10-cm pots, each planted with five $F_{2:4}$ seeds of each line. The experimental design was a completely randomized design with a single replication. The methods of inoculation, greenhouse growing conditions and disease assessments were as described by Miranda et al. (2006). The inoculum source was a single-spored isolate with the following avirulence/virulence response: *Pm1a*, *1b*, *3b*, *4b*, *8*, *17*/*Pm2*, *3a*, *5*, *6*, *7*, *MA*.

Disease reactions were recorded 15–20 days after inoculation (Zadoks growth stage 26–29) (Zadoks et al. 1974) 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 (1) flecks with no necrosis, to (2) necrosis, to (3) chlorosis, while the amount of mycelium went from none to a detectable amount; 4 to 6 = intermediate reactions 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.

Field One hundred five $F_{2:4}$ lines were planted at Kinston, NC, in October 2004. The experimental design, field layout, inoculating conditions and assignment of phenotypic classes were as described by Miranda et al. (2006). Disease reactions were evaluated at the beginning of April 2005 when plants were at Zadoks growth stage 50–59 and all Saluda rows presented uniform powdery mildew infection. Flag minus 2 leaves were rated using the scale of Leath and Heun (1990).

A natural powdery mildew epidemic occurred during the field season. The following genes are effective for resistance to naturally occurring powdery mildew populations in North Carolina: *Pm1a*, *3d*, *3e*, *3g*, *8*, *12*, *16*, *17*, *25*, and *34* (C. Cowger, D. Marshall, L. Miranda, and J. P. Murphy, unpublished data). The following powdery mildew resistance genes are partially or completely defeated: *Pm2a*, *3a*, *3b*, *3c*, *3f*, *4a*, *4b*, *5a*, *6*, *7*, and *20*.

NCD3/NCD7 (Pm34)

Allelism test One hundred twenty eight $F_{2:3}$ families from the NCD3/NCD7 population were evaluated for their powdery mildew reaction under field conditions following the same procedures used for the NCD3 X Saluda cross.

Differential disease response to *Blumeria graminis* f. sp. *tritici* isolates.

A detached-leaf test was conducted to test for differences in disease reaction among NCD3, NCD7 (*Pm34*) and Ulka (*Pm2*). Cultivars Chancellor and Saluda were used as susceptible controls. The *B. graminis* f. sp. *tritici* isolates used were from mildew samples collected in different locations in the eastern United States. In North Carolina, field mildew populations have a low frequency of strains virulent to NCD3 and NCD7, respectively, and scattered pustules can be observed on each of these genotypes. The six isolates used were chosen for their varying interactions with cultivar Ulka and other known *Pm* genes. The six isolates were designated: C1-6, E2-5, H2-3, H2-8, J2-1 and J3-2. These single-ascosporic isolates were derived from cleistothecia on leaves of cultivar Coker 9663. The leaves were collected

in Kinston, NC (C1-6), Salisbury, NC (E2-5), Griffin, GA (H2-3 and H2-8), and Warsaw, VA (J2-1 and J3-2).

The isolates were maintained and propagated on 60 × 15 mm petri dishes filled with 50 mg/liter benzimidazole-amended 6% agar containing 2.5 cm leaf segments of cultivar Chancellor that were obtained from 10 to 15-day-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 7–8 days after inoculation, using the 0 to 9 scale previously described.

Microsatellite marker analysis

Genomic DNA was extracted from leaf tissue samples of $F_{2:4}$ 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 (CACGACGT TGTAAAACGAC-) at the 5′ end for labeling purposes (Schulke 2000; Rampling et al. 2001). PCR protocols, separation of PCR products and fragment size designations were as described by Miranda et al. (2006).

Primer pairs that were polymorphic between NCD3 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.

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, NCD3 and Saluda were used to perform PCR reactions with the microsatellite markers putatively linked to the NCD3 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.

In the allelism test conducted in the field using the NCD3 X NCD7 population, the disease reaction of the NCD3 resistance gene could not be distinguished from that of *Pm34*. F_2 lines heterozygous at both resistance loci would have a segregation ratio of 15:1 (resistant:susceptible) in their F_3 progeny and such a low frequency of susceptible individuals within a family would have been hard to detect in a field experiment. Therefore, to allow for more accurate phenotyping, the resistant and segregating families were grouped in a single phenotypic class, and the susceptible families in another. The phenotypic classes were defined as: resistant/segregating (Re/Se) = homozygous for *Pm34* and/or NCD3 *Pm* gene, heterozygous at both *Pm* loci or heterozygous at one locus and homozygous recessive at the other locus; susceptible (Su) = homozygous recessive at both loci.

Results

Greenhouse evaluations

NCD3 and Saluda reactions ranged from 2 to 4 and from 6 to 8, respectively. The observed number of F_2-derived families per phenotypic class fitted the expected 1:2:1 ratio ($\chi^2_{1:2:1} = 0.59$, $P = 0.74$) for monogenic resistance (Table 1).

Field evaluations

NCD3 and NCD7 exhibited resistant disease reactions, with scores ranging from 1 to 3. A slightly higher frequency of mildew pustules was observed on NCD3 than on NCD7. Saluda exhibited an intermediate or susceptible reaction with scores ranging from 5 to 7. The observed segregation for resistance to powdery mildew fitted a 1:2:1 ratio ($\chi^2_{1:2:1} = 1.45$, $P = 0.48$), confirming a monogenic disease resistance (Table 1). Three lines that were classified as resistant and one that was classified as susceptible in the greenhouse test were re-classified as heterozygous. The field phenotypic data was used for the linkage mapping analysis.

Table 1 Segregation ratios for powdery mildew reaction of F_2 derived families from the NCD3/Saluda cross

Generation	Number of F_2-derived families			Total	$\chi^2_{1:2:1}$	*P* value
	Resistant	Segregating	Susceptible			
	Greenhouse					
$F_{2:4}$	26	45	21	92	0.59	0.74
	Field					
$F_{2:4}$	27	57	21	105	1.45	0.48

Allelism test

The chi-squared test did not show a significant deviation from the expected 15:1 ratio for two dominant genes segregating independently ($P = 0.27$, Table 2).

Differential disease response to *Blumeria graminis* f. sp. *tritici* isolates

NCD3, NCD7 and Ulka exhibited distinctive disease response patterns from one another when exposed to the six different *B. graminis* f. sp. *tritici* isolates (Table 3). Ulka was clearly distinguished from both NCD3 and NCD7 by isolates H2-3 and J2-3. Isolates E2-5 and H2-3 produced distinguishing responses in NCD3 and NCD7 (Fig. 1); while these cases both involved intermediate phenotypes, the differences were marked and consistent among the replicate plates.

Microsatellite marker analysis

Among the microsatellite markers tested in the bulked segregant analysis, *Xcfd26* was polymorphic between the contrasting bulks. When tested in the F_2 progeny, a 231 bp fragment was associated with the NCD3 allele and a 261 bp band was associated with the susceptibility allele. This microsatellite locus was mapped to chromosome 5DL (Somers et al. 2004). An additional 43 primer pairs mapping to this chromosome arm were tested. These included the microsatellite markers *Xbarc177-5DL*, *Xbarc144-5DL* and *Xgwm272-5DL* linked to *Pm34* (Miranda et al. 2006). Microsatellite markers *Xcfd7* and *Xgdm43* were also linked to the NCD3 powdery mildew resistance gene. The $Xcfd7_{251}$ and $Xgdm43_{126}$ bands were associated with the NCD3 allele, and the $Xcfd7_{240}$ and $Xgdm43_{150}$ bands were associated with the Saluda allele. The segregation ratios for the three microsatellite markers linked to the NCD3 *Pm* locus fitted the expected 1:2:1 ratio for co-dominant markers (Table 4). A microsatellite linkage map depicting the most likely order is shown in Fig. 2. No other locus had a LOD within 3 of this most likely order. Microsatellite markers distal from *Xcfd26* were not polymorphic between NCD3 and Saluda; thus, no distal markers could be added to the linkage map. A comparative view of the *Pm* loci on

Table 2 Segregation ratios for powdery mildew reaction of F_2 derived families in the allelism test of the NCD3 *Pm* gene (*Pm35*) and *Pm34* (F_2 generation)

Population	Number of F_2 individuals		χ^2 (15:1)[a]	*P* value
	Resistant	Susceptible		
NCD3 X NCD7	123	5	1.2	0.27

[a] (15:1 = resistant:susceptible)

Table 3 Differential reactions of NCD3 (*Pm35*), NCD7 (*Pm34*) and Ulka (*Pm2*) to six isolates of *Blumeria graminis* f. sp. *tritici* in a detached-leaf test

Line	*Blumeria graminis* f. sp. *tritici* isolate					
	C1-6	E2-5	H2-3	H2-8	J2-1	J2-3
NCD3	I[a]	S	I	S	I	S
NCD7	I	I	R	S	I	S
Ulka	R[b]	R	S	S	S	R
Saluda	S[c]	S	S	S	S	S
Chancellor	S	S	S	S	S	S

[a] I = intermediate = 4–6

[b] R = resistant = 0–3

[c] S = susceptible = 7–9

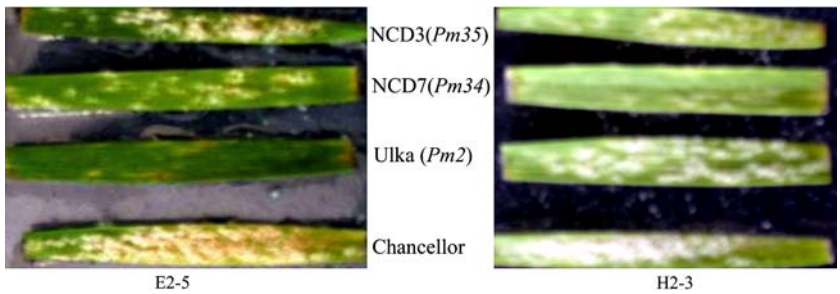

Fig. 1 Virulence test for NCD3 (*Pm35*), NCD7 (*Pm34*), Ulka (*Pm2*) and Chancellor using *B. graminis* f.sp. *tritici* isolates E2-5 and H2-3

chromosome 5D with respect to the wheat consensus map (Somers et al. 2004) was constructed based on the *Pm2* (Qiu et al. 2006) and *Pm34* (Miranda et al. 2006) linkage maps and our present findings (Fig. 2).

Chromosomal assignment

All three microsatellite markers linked to the NCD3 *Pm* gene mapped to 5DL. PCR products were observed in euploid Chinese Spring and Dt5DL, but not in N5DT5A.

Discussion

The NCD3 *Pm* gene was assigned to chromosome 5D using a microsatellite linkage map developed in this study. Two other *Pm* genes, *Pm2* and *Pm34*, have been located on chromosome 5D. The disease responses of the NCD3 *Pm* gene were shown to be different from those of *Pm2* and *Pm34*, indicating that the NCD3 *Pm* gene is a novel *A. tauschii*-derived powdery mildew resistance specificity.

The physical mapping of the microsatellite markers linked to the NCD3 *Pm* gene confirmed the presence of this locus in the long arm of 5D. *Pm2* was physically mapped to chromosome 5DS by McIntosh and Baker (1970), and further evidence of the chromosomal location of this locus has been provided by the microsatellite linkage map recently developed by Qiu et al. (2006). Therefore, we concluded that the NCD3 *Pm* gene is different from *Pm2*. Also, *Pm34* was previously mapped to 5DL (Miranda et al. 2006) but the chi-square test showed no evidence of linkage between the NCD3 *Pm* gene and *Pm34*.

Microsatellite markers *Xbarc177* and *Xgwm272* flanked *Pm34* at proximal and distal positions, respectively (Miranda et al. 2006), but these markers were not polymorphic in the NCD3/Saluda population. However, the wheat composite map (http://www.wheat.pw.usda.gov) indicates a distance of 45 cM between *Xbarc177* and *Xcfd26*, and the consensus map of Somers et al. (2004) has a distance of 69 cM between *Xgwm272* and *Xcfd26*. These distances are in agreement with our findings that *Pm34* and the present resistance gene are genetically independent.

Thus, the experiments conducted in the present study provide strong evidence for a novel powdery mildew resis-

Table 4 Segregation ratios for microsatellite (SSR) markers among F_2 individuals in the NCD3/Saluda population

SSR marker	AA[a]	H[b]	BB[c]	Total	χ^2 (1:2:1)	*P* value
Xcfd26	25	56	24	105	0.49	0.78
Xcfd7	25	53	27	105	0.10	0.95
Xgdm43	22	60	22	104	2.72	0.26

[a] AA = Homozygous for the NCD3 allele

[b] H = Heterozygous

[c] BB = Homozygous for the Saluda allele

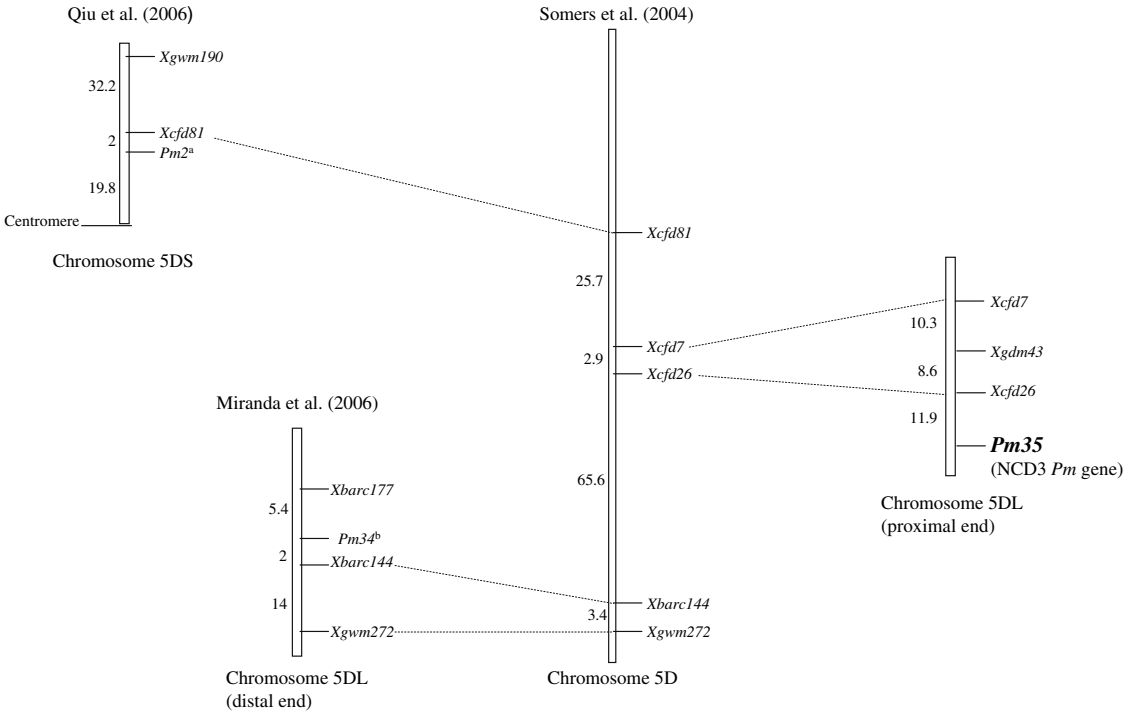

Fig. 2 Comparative view of the *Pm2*, *Pm34* and *Pm35* (NCD3 *Pm* gene) linkage maps with respect to the 5D consensus map by Somers et al (2004)

tance gene in NCD3. We propose to designate this gene as *Pm35*.

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