ABSTRACT

MIRANDA, LILIAN. Resistance to Powdery Mildew in Wheat Germplasm with Different Resistance Sources. (Under the direction of J. Paul Murphy).

Powdery mildew of wheat (*Triticum aestivum* L.) is an economically important fungal disease caused by *Blumeria graminis* f. sp. *tritici*. Breeding for disease resistance is regarded as the most efficient strategy to reduce yield losses caused by this disease. The use of monogenic disease resistance has been effective but ephemeral and novel sources of resistance are constantly needed. The objectives of this study were the genetic characterization, microsatellite linkage mapping and chromosomal assignment of powdery mildew resistance genes (*Pm* genes) present in the germplasm lines NC97BGTD7 (NCD7), NC96BGTD3 (NCD3), NC96BGTA5 (NCA5) and NC96BGTA6 (NCA6). NCD7 and NCD3 are *Aegilops tauschii*-derived lines and NCA5 and NCA6 are *Triticum monococcum* subsp. *aegilopoides*-derived lines. Mapping populations were developed by crossing each germplasm line to the susceptible cultivar Saluda. Greenhouse and field evaluations using F_2-derived families indicated monogenic segregation of disease resistance in all four germplasm lines. Microsatellite linkage maps were developed. The NCD7 and NCD3 *Pm* genes mapped to chromosome 5DL and were confirmed as two different loci in an allelism study conducted using an NCD3 X NCD7 population. The NCD7 and NCD3 *Pm* genes are novel *Pm* genes and were designated as *Pm34* and *Pm35* respectively.

The NCA5 *Pm* gene was previously identified as a novel *Pm* gene and was designated *Pm25*. The microsatellite linkage map developed in this study indicates that *Pm25* is tightly linked or might be an allele of the *Pm3* locus on chromosome 1AS.

The NCA6 *Pm* gene mapped to chromosome 7AL. *Pm1* is also on chromosome 7AL but comparison of the NCA6 linkage map developed in this study with published linkage maps for the *Pm1* locus suggested that the NCA6 *Pm* gene is proximal to the *Pm1* locus and is a novel *Pm* gene. An allelism study is suggested to confirm this hypothesis. Deletion mapping of the microsatellite markers linked to the NCA6 *Pm* gene showed a large physical to genetic distance ratio, indicating reduced recombination probably due to the alien *T. monococcum* introgression.

RESISTANCE TO POWDERY MILDEW IN WHEAT GERMPLASM WITH DIFFERENT RESISTANCE SOURCES

by

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A dissertation submitted to the Graduate Faculty of

North Carolina State University

in partial fulfillment of the

requirements for the Degree of

Doctor of Philosophy

CROP SCIENCE

Raleigh

2006

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BIOGRAPHY

Lilian Miranda was born in Lima, Peru. She did her undergraduate studies at the Universidad Nacional Agraria La Molina in Lima, receiving a bachelor degree in general Biology in 1993. In 1994 she joined the Plant Physiology Department at the International Potato Center (CIP). As a research assistant, she was involved in several research projects including non-symbiotic Nitrogen fixation, trypsin inhibitors and post-harvest quality traits in sweet potato and also, genotypic and stress induced variability in the glycoalkaloid content of potato tubers. While working at CIP, she also joined the graduate program in Plant Breeding at the Universidad Nacional Agraria La Molina. Seeking to continue her graduate education, Lilian traveled to the United States in September 2000. In the spring of 2003 she was accepted as a PhD student in the Crop Science Department at North Carolina State University. She worked in the wheat breeding program under the direction of Dr. J. Paul Murphy. After completion of her PhD degree, Lilian will continue working on wheat, now in a post-doctoral research position at the University of Georgia.

ACKNOWLEDGEMENTS

My deepest thanks to my advisor, Dr. J. Paul Murphy for giving me the opportunity to join his program without limiting my input to my thesis project but making me an active participant in the breeding program.

I am very thankful to Goran Srnić for generously sharing with me his knowledge and experience. Special thanks to Rene Navarro for his excellent technical support and his unconditional help. I also want to thank David Wooten for being such a wonderful co-worker and friend.

I thank my committee members, Dr. James Holland, Dr. David Marshall, Dr. Cavell Brownie and Dr. Steven Leath for their guidance. I am deeply grateful to Christina Cowger for her valuable inputs and her friendship. Many thanks to everybody in the Peanut Breeding and Genetics group for being the best collaborators.

My special thanks to all my friends in Raleigh for making my years in Graduate School a more pleasant experience.

I would like to acknowledge Jeannette Lyerly and Lynda Whitcher for their technical assistance.

Finally, all my gratitude to my family for their love and support during all these years.

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Literature review

Introduction

Powdery mildew, caused by *Blumeria graminis* DC f. sp. *tritici* Em. Marchal, is one of the most economically important fungal diseases of common wheat (*Triticum aestivum* L.) worldwide. In the southeastern United States, powdery mildew epidemics are commonly observed on winter wheat and yield losses attributed to this disease can be as high as 44% (Lipps and Madden 1989). The disease can be found continuously from one month after planting until late spring. Symptoms are first observed on the oldest leaves and tend to progress towards the flag leaf during the growing season (Bowen et al.1991). Since it is known that upper leaves contribute most to grain yield, early disease control (before flag leaf emergence) was not considered important, but Bowen et al. (1991) demonstrated that early season powdery mildew can affect yield by reducing the number of tillers that a plant produces or the number of kernels per head. The disease also affects milling and baking quality traits such as flour yield, flour protein, alkaline water retention capacity and kernel texture (Everts et al. 2001).

Powdery mildew biology

Blumeria graminis f.sp. *tritici* is an obligate parasite that belongs to the subdivision Ascomycotina in the order Erysiphales of higher fungi (Agrios 1997). Conidia, the asexual spores, germinate producing two germ tubes: the primary germ tube and the secondary germ tube. The primary germ tube emerges 0.5-2 h after deposition and it is thought to play a role in recognition and initial attachment to the host surface.

The secondary germ tube, also known as the appressorial germ tube, elongates to develop the appressoria, which are the structures that attach the mycelium to the host surface (Green et al. 2002).

The primary mycelium of powdery mildew is hyaline, septate and thin walled. Secondary mycelium is characterized by the development of thick-walled, sickle shaped mycelial bristles (Braun et al. 2002). Mycelial growth occurs only on the surface of plant tissue and nutrients are obtained from the plant by developing specialized feeding structures called haustoria. Haustoria are absorption structures that develop within the invaginated plasma membrane of the host epidermal cells (Green et al. 2002).

Under favorable environmental conditions, sexual spores or ascospores are produced within a sac-shaped structure known as the ascus. The asci of powdery mildew are produced inside a closed ascocarp known as cleisthothecium. Ascospores develop in groups of four or eight per ascus (Agrios 1997).

Powdery mildew infection

The infection process starts immediately after conidia land on the aerial parts of a host plant (Green et al. 2002). The optimal temperature for infection is around 15–20 °C, but infection can take place between 5-30°C. High humidity also favors spore germination but does not affect mycelium development (Jarvis et al. 2002). Under suitable environmental conditions, conidia germinate and this leads to the development of appressoria that attach the mycelium to the host surface and initiate the haustoria (Braun et al. 2002). The ramifying hyphae on the plant surface produce many generations of

haustoria until a new infection cycle starts with the development of wind-dispersed conidia on short conidiophores. Sporulating mildew colonies give the disease its characteristic powdery appearance (Green et al. 2002). Powdery mildew spores are short-lived, prefer high humidity and do not tolerate immersion in water. To compensate for these limitations, the fungi produce abundant conidia and have a short generation time (approximately one week), leading to large amounts of inoculum. In agricultural systems, where susceptible plants tend to be more crowded than in natural ecosystems, host plants are more readily available to provide abundant germinating surface for the spores (Bushnell 2002).

Chemical control of powdery mildew

Foliar spraying of fungicides and seed treatments with systemic fungicides have been shown to reduce crop losses due to powdery mildew infection (Hardwick et al., 1994; Da Luz and Bergstrom 1986; Christ and Frank 1989). Fungicides commonly used to control powdery mildew include ergosterol biosynthesis inhibitors (EBI) and strobilurins (Hollomon and Wheeler 2002).

EBI fungicides inhibit the ergosterol biosynthetic pathway and they include triadimenol, propiconazole and flutriafol, which belong to the triazole group and EBI fungicides of the morpholine type such as fenpropimorph, which have a different inhibition site than that of the triazoles (Svec et al. 1994). Strobilurins, also known as QoI inhibitors, act by inhibiting respiration by blocking electron transport and are generally

very effective at inhibiting germination of conidia but they do not have adequate eradicant action (Hollomon and Wheeler 2002).

Although chemical treatments can be effective to prevent yield losses, their use requires several considerations. The risk of a pathogen population developing fungicide resistance is higher for single site inhibitors because the resistance mechanism is monogenic (Fraaije et al 2002). When several genes are involved, fungicide resistance spreads more slowly, therefore, modeling studies suggest the use of fungicide mixtures with different modes of action (Hollomon and Wheeler 2002)

Fraajie et al. (2002) used allele-specific real-time PCR to monitor the dynamics of strobilurin-resistant A143 alleles in *B. graminis* f.sp. *tritici* populations under selection pressure of fungicides. After only three applications of azoxystrobin, the average allele frequency of A143 increased from 2.2 to 58 % but tebuconazole (EBI inhibitor fungicide) applications had no effect on strobilurin sensitivity. The study suggests that alternating fungicides with different cross-resistance groups should slow down fungicide resistance development in *B. graminis* f.sp. *tritici* populations, even if there is no fitness penalty associated with the strobilurin-insensitive allele.

As demonstrated by Hardwick et al. (1994), a late fungicide application (after ear emergence) does not have a positive effect on yield and several fungicide applications are generally more effective than any single spray treatment. Seed treatment with systemic fungicides such as triadimenol can prevent early establishment of the disease but it has been shown that this protection does not last the entire crop season and the seed treatment can produce delayed seed emergence and reduced plant growth (Da luz and Bergstrom

1986). Christ and Frank (1989) found that combining seed treatment with foliar sprays was more effective in reducing powdery mildew severity than either treatment alone.

Breeding for resistance to powdery mildew

Breeding of resistant cultivars is regarded as the most economically sound and environmentally safe approach for eliminating the use of fungicides and reducing crop losses caused by powdery mildew. The most common breeding strategy has been the use of major genes conferring hypersensitive types of resistance, but the effectiveness of this approach has commonly been ephemeral due to frequent changes in the pathogen population (Hsam and Zeller 2002). This type of resistance, also known as vertical, is effective only against certain races of the pathogen. Cultivars with race specific resistance genes exert a strong selection pressure against the pathogen populations and this results in a rapid build up of pathotypes with matching virulence genes (McDonald and Linde 2002).

In the southeastern United States, changes in virulence gene frequencies have been frequently observed over time and locations. In a study conducted in 1985, Leath and Murphy (1985) tested 11 near- isogenic lines of cultivar Chancellor (CI 12333), each with a different powdery mildew resistance gene, across different locations in North Carolina wheat fields. Virulence to all the genes tested: *Pm1*, *Pm2*, *Pm2+*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm4*, *Pm6*, *Pm7*, *Pm8* and a *Pm* gene from cultivar Michigan Amber (CI 11379) was found, but lower virulence frequencies were observed for *Pm1*, *Pm3b*, *Pm4*, *Pm7* and *Pm8*.

Persaud and Lipps (1995) tested 199 *B. graminis* f.sp. *tritici* isolates that were collected from 36 counties in Ohio during 1992 and 1993. Virulence to the genes *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm4a*, *Pm5*, *Pm6+2*, *Pm7* and *Pm8* was found in the isolates collected in 1992. Among the 1993 isolates, virulence to *Pm1* and *Pm17* was also detected at very low frequencies.

Niewoehner and Leath (1998) characterized 520 *B. graminis* f.sp. *tritici* isolates from 17 different states in the eastern United States. They found a decrease in the effectiveness of *Pm1*, *Pm3b*, *Pm4a* and *Pm8.* Among the *Pm* genes tested, *Pm12* and *Pm16* were the most effective genes for the region; very low virulence frequencies were detected only in Kentucky and North Carolina.

Another type of resistance is race non-specific or adult plant resistance (APR), which is effective only in adult plants but not in seedlings. APR is expressed quantitatively as a general decrease in infection growth and reproduction of the pathogen (Hsam and Zeller 2002). Quantitative resistance appears to be equally effective across different pathogen strains and is more sensitive to environmental conditions. As a consequence, selection pressure over the pathogen population is weaker, making this type of resistance more durable (McDonald and Linde 2002). Winter wheat cultivar Knox (CI 12798) and its derivatives are an example of the durability of this type of resistance. APR in these cultivars remained effective against powdery mildew infection during 20 years (Liu et al. 2001). Quantitative trait loci (QTLs) for adult plant resistance to powdery mildew have been identified by several authors (Keller et al. 1999; Chantret et al. 2000; Liu et al. 2001; Mingeot et al. 2002; Jakobson et al. 2005).

A more durable disease resistance is also possible using major genes if they are deployed using strategies that disrupt directional selection pressure. Simultaneous deployment of different *Pm* genes by using cultivar mixtures (Mundt 2002), isolines with different resistance genes (Zhou et al. 2005) or pyramiding different major genes into a single cultivar (Liu et al. 2000) increases the number of mutations that are needed in the pathogen population to overcome host resistance.

Manthey and Fehrmann (1993) tested the effect of wheat cultivar mixtures on powdery mildew, leaf rust (*Puccinia recondita*) and stripe rust (*Puccinia striiformis*) development. Infection levels were significantly reduced with the use of cultivar mixtures and the greatest reduction in disease development was observed for powdery mildew. According to Mundt (2002), powdery mildew should be an ideal target to control by using cultivar mixtures because of its relatively shallow dispersal gradient and the large number of pathogen generations per crop season.

Zhou et al (2005) developed near-isogenic lines (NILs) with powdery mildew resistance using molecular markers. Amplified fragment length polymorphisms (AFLPs) were used to assess the similarity of NILs to their recurrent parent and AFLPs and microsatellite markers linked to the *Pm* genes were used to select for powdery mildew resistance.

Three powdery mildew resistance gene combinations, *Pm2+Pm4a*, *Pm2+Pm21* and *Pm4a+Pm21* were pyramided into the Chinese elite wheat cultivar, 'Yang 158' by Liu et al. (2000). Selection of F_2 individuals with combined resistance was accomplished by screening the progeny with RFLP probes linked to these resistance genes.

Homozygous individuals carrying *Pm21* and *Pm2* were identified using co-dominant RFLP markers but the genotypic status of individuals carrying *Pm4a* could not be determined with the dominant marker used. Progeny tests with race specific isolates were needed to select for homozygous individuals carrying *Pm4a*.

Sources of *Pm* genes

Major genes for powdery mildew resistance (*Pm* genes) have been described at 33 gene loci in common wheat (Table1.1). The sources of these genes have been winter and spring wheat cultivars, land races and related species and genera (Huang and Röder 2004; Zhu et al. 2005).

The primary gene pool of cultivated hexaploid wheat (2n=6x=42, AABBDD) consists of hexaploid landraces and other closely related species such as *Triticum urartu*, (donor of the A genome), *Aegilops tauschii* (donor of the D genome), T. *turgidum* (durum wheat), *T. turgidum* ssp. *dicoccoides* (the immediate progenitor of cultivated durum and bread wheat), *T. monococcum* (A^mA^m) and *T. turgidum ssp. dicoccon* (AABB) that share only homologous genomes with common wheat (Jiang et al. 1994). Since chromosome pairing is homologous, hybrids are easily recovered within the primary gene pool, even when ploidy levels are different. Procedures to overcome crossing incompatibility between hexaploid wheat and its A and D genome donors are relatively simple and they include bridging crosses with tetraploid wheats and embryo rescue (Gill and Raupp 1987).

Polyploid *Triticum/Aegilops* species that have one homologous genome in common with *T. aestivum* such as *T. timopheevii* and *T. araraticum* are considered the secondary gene pool. This group also includes the diploid S genome species *A. speltoides* and *A. longissima* which are related to the B genome but have reduced chromosome pairing (Jiang et al. 1994). If the gene of interest is located in a homologous chromosome, gene transfer from the secondary gene pool is possible by homologous crossing over (Hsam and Zeller 2002) but if it is present in a homoeologous genome, special cytogenetic manipulations are required as in the case of gene transfer from the tertiary gene pool (Baum et al. 1992).

More distantly related species that share only homoeologous genomes with cultivated wheat are considered the tertiary gene pool. *Aegilops* species such as *Ae. caudate*, *Ae. ovata*, *Ae. umbellulata*, *Ae. triuncialis* and *Ae. variabilis* as well the less related ones *Secale cereale* and *Haynaldia villosa* can be included in this group (Feldman 2000; Hsam and Zeller 2002). Differences in crossability are commonly observed among wheat genotypes and they have a great impact on the success of wide hybridizations. The cultivar 'Chinese Spring' has at least four crossability genes (*Kr* genes) and is the most commonly used genotype in intergeneric hybridization (Jiang et al. 1994). Since F_1 intergeneric hybrids are generally sterile, production of amphiploids by chromosome doubling using colchicine is generally required to restore fertility (Mujeeb-Kazi and Asiedu 1990).

Gene transfer from the tertiary gene pool can also be achieved by producing alien chromosome addition and substitution lines. Addition lines are produced by interspecific

crossing followed by backcrossing to cultivated wheat and screening for monosomic additions. The procedure generates individual pairs of alien chromosomes added to the wheat genome (Islam and Shepherd 1990). Alien substitution lines are generated by replacing a pair of chromosomes by a pair from a foreign species. (Sears 1969). This can be done by identifying the homoeology of alien chromosomes in wheat-alien addition lines using genetic markers and crossing the addition line to the appropriate wheat monosomic (Jiang et al. 1994).

Wheat-alien chromosome addition and substitution lines can be used as bridge material to produce wheat-alien chromosome translocations (Jiang et al. 1994). These translocations can be induced by inactivating the *Ph1* gene. This gene, present on the long arm of chromosome 5B, prevents homoeologous pairing among non-homologous genomes (Feldman 2000). The *Ph1* gene can be removed by crossing a wheat line containing an alien chromosome to a line monosomic for 5B or the gene can be deleted by irradiation. Homoeologous pairing has the disadvantage of producing a very low seed set due to a high degree of multivalent pairing in the hybrid (Baum et al. 1992)

Wheat-alien translocations can also occur spontaneously or can be induced by ionizing radiation (Jiang et al. 1994). The ionizing radiation procedure consists of irradiating a monosomic addition line that has a full complement of wheat chromosomes and one alien chromosome with the desired gene (Morris and Sears 1967). Irradiation during meiosis is believed to induce intercalary translocations between the alien and the wheat chromosome (Baum et al. 1992)

Transfer and utilization of powdery mildew resistance from any of the germplasm pools available requires genetic studies to determine actual gene number, mode of inheritance and linkage and allelic relationships (Chung and Griffey 1995a; 1995b).

Identification of powdery mildew resistance genes

Race specific isolates

The host-parasite relationship between wheat and powdery mildew follows the gene-for-gene hypothesis developed by Flor (1955). This means that for each resistance gene in the host there is a corresponding gene for avirulence in the pathogen. As a consequence, race specific powdery mildew isolates can be used to differentiate between lines with known resistance genes (Leath and Heun 1990). Resistance genes can be identified by comparing the resistant/susceptible reaction patterns after inoculating a standard set of powdery mildew differential isolates on detached leaf segments of lines or cultivars carrying different genes or gene combinations (Chen and Chelkowski 1999)

This has been the traditional approach but, with the increasing number of *Pm* genes, it is becoming less effective due to the lack of sufficient differential isolates. Combining several resistance genes in one cultivar (resistance gene pyramiding) also complicates the analysis because two or more *Pm* genes need to be identified simultaneously and the action of one gene could mask the effect of another (Langridge et al. 2001)

Cytogenetic analysis

Chromosomal location of resistance genes can be accomplished by crossing the phenotype of interest with a monosomic series in a cultivar with the contrasting phenotype (usually the 'Chinese Spring' series). In this procedure, each of the 21 different monosomics available in wheat is crossed as a female parent with the resistant line to ensure that most of the progeny will be monosomic. The F_1 plants are cytologically scored and if the resistance gene is recessive, direct phenotypic observation of the F_1 monosomics would suffice to determine the chromosomal location of the *Pm* gene. However, since dominant *Pm* alleles are most commonly observed, chromosomal assignment is generally determined by observing a deviation from the expected segregation ratio in the F_2 line carrying the resistance gene on the critical chromosome (Hsam and Zeller 2002). In the F_2 progeny of the non-critical crosses the expected genotypic ratio would be 1 homozygous resistant: 2 heterozygous: 1 homozygous susceptible and the observed phenotypic classes would have a 3 resistant: 1 susceptible distribution, but in the critical cross a reduced number of susceptible individuals should be observed (McIntosh 1987).

Molecular Markers

The most recent approach to identify resistance genes is the use of molecular markers. One marker or a set of closely linked molecular markers can be used to identify resistance loci (Chen and Chelkowski 1999). The large size of the wheat genome (16 x 10^9 bp) and its polyploid nature make mapping efforts more challenging. Southern

hybridization is more complex and has a higher rate of failure in wheat than in other cereals. The presence of three related genomes (A, B and D) increases the difficulty of marker analysis. The use of RFLP probes would commonly result in three sets of bands being observed, one for each genome (Langridge et al. 2001).

Microsatellites or simple sequence repeats (SSRs) are an alternative type of co-dominant marker more suitable for screening large populations than RFLPs. They are simple sequence repeats of only a few base pairs (1-6) that are commonly found in eukaryotic genomes (Gupta et al. 1999).

The use of microsatellite-based markers in plants was initially restricted due to their high development cost (Bryan et al. 1997). However, in recent years, several microsatellite linkage maps for hexaploid wheat have been developed by different research groups (Röder et al. 1998; Paillard et al. 2003; Gupta et al. 2000, 2002; Stephenson et al. 1998; Pestova et al. 2000; Sommers et al. 2004) and they show a much higher level of polymorphism and informativeness than any other maker system available for this crop (Röder et al. 1998)

Most of microsatellite markers are chromosome specific, thereby simplifying the assignment of linkage groups (Röder et al. 1998; Gupta et al. 1999). The genome specificity of microsatellite markers can also be used to infer the arm and sub-arm localization of disease resistance genes using Chinese Spring ditelosomic and deletion stocks (Endo and Gill 1996). Gene-flanking microsatellite markers can be assigned to chromosome arms and interval breakpoints by examining their presence or absence in ditelosomic and deletion lines (Plaschke et al. 1996; Sourdille et al. 2004).

Because they are not clustered but randomly distributed over different chromosomes and chromosomes arms in wheat, microsatellites should be useful for complete coverage of the wheat genome (Gupta et al. 1999). The level of polymorphism is not the same across the three genomes and higher levels of polymorphism seem to be positively correlated with the length of microsatellite repeats (Bryan et al. 1997). Generally, the D genome is more highly conserved between varieties and is therefore more difficult to map (Langridge et al. 2001) but microsatellite markers isolated from *Aegilops tauschii* have been used to improve the coverage of the D genome (Pestova et al. 2000)

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).

The *Pm* gene present in the cultivar 'Virest' was originally assigned to chromosome 1D and was named *Pm22*, but with the aid of molecular markers this gene was mapped on chromosome 7AL and its designation was changed to *Pm1e* (Singrün et al. 2003). *Xgwm344* is the closest microsatellite marker that has been reported linked to the *Pm1* locus (0.9cM) and is null for the alleles *Pm1a*, *Pm1b*, *Pm1d* and *Pm1e* (Stepien et al, 2004).

Bougot et al. (2002) found two microsatellite markers, *Xgdm33* and *Xpsp2999*, closely linked to the *Pm3g* locus. *Xpsp2999* produced the same amplification pattern in

lines with *Pm3a*, *Pm3b*, *Pm3e*, *Pm3f* and *Pm3g* alleles, therefore, it was concluded that this marker would be useful to detect *Pm3* alleles but cannot be used to differentiate among them. Huang et al. (2004) developed microsatellite linkage maps for *Pm3h*, *Pm3i* and *Pm3j*.

Ma et al. (2004) identified a microsatellite marker (*Xgwm356*) that mapped 4.8cM from *Pm4a*. This marker was co-dominant and showed the same amplification pattern in seven different pure lines carrying the *Pm4a* gene.

Huang et al. (2003) mapped *Pm5e* to the distal end of chromosome 7BL using microsatellite markers. The closest marker, *Xgwm1267*, was co-dominant and had a linkage distance of 6.6 cM to *Pm5e*. Only microsatellite markers located proximal to the *Pm5e* locus were found.

Pm16 was originally mapped to chromosome 4A using monosomic analysis (Reader and Miller 1991) but microsatellite linkage mapping conducted by Chen et al. (2005) indicated that *Pm16* is either located on 5BS or on a translocated 4A.5BS chromosome.

Huang et al. (2000) mapped *Pm24* close to the centromere on the short arm of chromosome 1D. Three microsatellite markers, *Xgwm337*, *Xgwm106* and *Xgwm458*, were found to be linked to *Pm*24, with linkage distances of 2.4 ± 1.2 cM, 19.2 ± 3.8 cM and 10.3 ± 2.5 cM respectively. *Pm24* segregated independently of gene *Pm22*, also located on chromosome 1D.

A genetic map for the region around *Pm27*, a *Triticum timopheevii* derived *Pm* gene, was developed by Järve et al. (2000), using RFLPs and SSR markers. Complete

linkage between *Pm27* and the microsatellite marker *Xpsp3131* was observed on the F_2 progeny.

Liu et al. (2002) introgressed a powdery mildew resistance gene from wild emmer (*T. dicoccoides)* into cultivated hexaploid wheat. Linkage of this locus with microsatellite marker *Xgwm159* helped determine its chromosome arm and sub-arm localization using 'Chinese Spring' nullitetrasomic and deletion lines. The *Xgwm159* locus was mapped to the 0.41-0.43 interval on chromosome 5BS. Since no other *Pm* gene had been reported to this region, the gene was designated *Pm30*.

Xie et al. (2003) found four polymorphic microsatellite markers, *Xpsp3029*, *Xpsp3071*, *Xpsp3152* and *Xgwm570*, linked to a powdery mildew resistance gene that had been transferred from an Israeli wild emmer (*T. dicoccoides*) into common wheat. This new gene mapped to chromosome 6AL and was designated *Pm31*.

Zhu et al. (2005) reported the identification of two powdery mildew resistance genes introduced from *T. carthlicum* using microsatellite markers. The two genes, *Pm*PS5A and *Pm*PS5B mapped to chromosomes 2AL and 2BL respectively. Based on the microsatellite linkage mapping information, *Pm*PS5A could be a member of the *Pm4* locus and *Pm*PS5B was proposed as a new *Pm* gene (*Pm33*).

Along with the selection of the more convenient marker system, bulked segregant analysis (BSA) can be an easier and more effective approach than full map construction for marker-assisted selection (Langridge et al. 2001). BSA for disease resistance is performed by making a DNA pool with resistant individuals and another pool with susceptible ones. These two pools are screened with molecular markers to identify

polymorphic bands. Polymorphisms observed between the two pools will likely be derived from regions of the genome that are associated with disease resistant (Michelmore et al. 1991)

Molecular markers linked to disease resistant loci will allow selection for resistant genotypes in the absence of the pathogen and can contribute to the development of pyramids of different powdery mildew resistance genes in one cultivar, providing a wider resistance spectrum (Langridge et al. 2001) Developing wheat cultivars with pyramids of resistance genes is a feasible breeding approach that involves conventional breeding procedures and can become more efficient with the aid of molecular markers.

Breeding for resistance to powdery mildew at North Carolina State University

In an effort to broaden the genetic base of resistance to powdery mildew, the small grain program and the plant pathology department at NC State University started a program to introgress powdery mildew resistance from diploid and tetraploid wheat relatives into the soft red winter wheat cultivar 'Saluda' (Starling et al. 1986) in 1987.

Interspecific hybridizations using *T. monococcum*, *T. turgidum*, *T. timopheevii* and *Ae. tauschii* as the resistance donors and the cultivar 'Saluda' as the recurrent parent have resulted in the release of 11 germplasm lines with resistance to powdery mildew (Table 1.2) (Murphy et al. 1998; 1999a; 1999b; 2002; Navarro et al. 2000)

Shi et al. (1998) reported a recombination frequency of 0.21 between the powdery mildew resistance gene present in the North Carolina germplasm line NC96BGTA5 and *Pm3a*. Based on this linkage information, the gene was mapped to chromosome 1AS and

was designated *Pm25*. Random amplified polymorphic DNA (RAPD) markers linked to *Pm25* were found, but they are not close enough to be useful for marker-assisted selection.

Srnić et al. (2005) studied the inheritance of powdery mildew resistance in the North Carolina germplasm lines NC96BGTA4 and NC99BGTAG11 and determined that it was monogenically inherited in both lines. The two genes were mapped to the long arm of chromosome 7A using microsatellite markers but based on their disease reaction, it was concluded that they are either different alleles at the same locus or different genes.

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Table 1.1 Powdery mildew resistance loci, chromosomal location and source of resistance (Huang and Röder 2004; Zhu et al. 2005).

Locus	**Chromosome Location**	**Source**
Pm1a	7AL	*T. aestivum*
Pm1b	7AL	*T. aestivum*
Pm1c	7AL	*T. aestivum*
Pm1d	7AL	*T. spelta*
Pm1e	7AL	*T. aestivum*
Pm2	5DS	*Ae. tauschii*
Pm3a	1AS	*T. aestivum*
Pm3b	1AS	*T. aestivum*
Pm3c	1AS	*T. aestivum*
Pm3d	1AS	*T. aestivum*
Pm3e	1AS	*T. aestivum*
Pm3f	1AS	*T. aestivum*
Pm3g	1AS	*T. aestivum*
Pm3h	1AS	*T. durum*
Pm3i	1AS	*T. aestivum*
Pm4a	2AL	*T. dicoccum*
Pm4b	2AL	*T. carthlicum*
Pm5a	7BL	*T. dicoccum*
Pm5b	7BL	*T. aestivum*
Pm5c	7BL	*T. aestivum ssp. sphaerococcum*
Pm5d	7BL	*T. aestivum*
Pm5d	7BL	*T. aestivum*
Pm6	2B	*T. timopheevii*
Pm7	4BS.4BL-2RL	*Secale cereale*
Pm8	1BL.1RS	*S. cereale*
Pm9	7A	*T. aestivum*
Pm10	1D	*T. aestivum*
Pm11	6BS	*T. aestivum*
Pm12	6BS	*Ae. speltoides*

(**Table1.1** Continued)

Locus	**Chromosome Location**	**Source**
Pm13	3BL.3BS-3S	*Ae. longissima*
Pm14	6B	*T. aestivum*
Pm15	7DS	*T. aestivum*
Pm16	4A	*T. dicoccoides*
Pm17	1AL.1RS	*S. cereale*
Pm18	7A	*T. aestivum*
Pm19	7D	*Ae. squarrosa*
Pm20	6BS.6RL	*S. cereale*
Pm21	6VS.6AL	*Haynaldia villosa*
Pm22	1D	*T. aestivum*
Pm23	2AL	*T. aestivum*
Pm24	1D	*T. aestivum*
Pm25	1A	*T. monococcum*
Pm26	2BS	*T. dicoccoides*
Pm27	6B-6G	*T. timopheevii*
Pm28	1B	*T. aestivum*
Pm29	7DL	*A. ovata*
Pm30	5BS	*T. dicoccoides*
Pm30	6AL	*T. dicoccoides*
Pm32	T1BL.1SS	*Ae speltoides*
Pm33	2B	*T. carthlicum*

Table 1.2 Powdery mildew resistant germplasm lines released by North Carolina State University. (Murphy et al., 1998; 1999a; 1999b; 2002; Navarro et al., 2000)

Germplasm Line	Generation derived from	Source of Resistance
NC96BGTD1 (NC D1)	BC_2F_6	*Ae. tauschii subsp. tauschii*
NC96BGTD2 (NC D2)	BC_2F_6	*Ae. tauschii subsp. tauschii*
NC96BGTD3 (NC D3)	BC_2F_5	*Ae. tauschii subsp. strangulata*
NC96BGTA4 (NC A4)	BC_2F_6	*T. monococcum subsp. monococcum*
NC96BGTA5 (NC A5)	BC_2F_5	*T. monococcum subsp. aegilopoides*
NC96BGTA6 (NC A6)	BC_2F_5	*T. monococcum subsp. aegilopides*
NC97BGTD7 (NC D7)	BC_2F_6	*Ae. tauschii*
NC97BGTD8 (NC D8)	BC_2F_6	*Ae. tauschii*
NC97BGTAB9 (NC AB9)	BC_2F_6	*T. turgidum subsp. dicoccoides*
NC97BGTAB10 (NC AB10)	BC_2F_5	*T. turgidum subsp. dicoccoides*
NC99BGTAG11 (NCAG11)	BC_2F_7	*T. timopheevii subsp. armeniacum*

Chapter 2

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

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, resistance gene, 2.6cM, *Xbarc144-5D*, 14cM, *Xgwm272-5D*. These microsatellite markers were previously mapped to the long arm of the 5D chromosome and their positions were confirmed using Chinese Spring Nullitetrasomic Nulli5D-tetra5A and ditelosomic Dt5DL lines. *Pm2*, the only other known *Pm* gene on chromosome 5D has been mapped to the short arm and its specificity is different from that of *Pm34*.

Introduction

Powdery mildew of wheat (*Triticum aestivum* L.*)* is an economically important fungal disease caused by *Blumeria graminis* DC f. sp. *tritici* Em. Marchal. In areas with cool or maritime climates, such as the eastern United States, grain yield and end-use quality can be significantly affected by this disease (Everts et al. 2001; Lipps and Madden 1989). 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, 1999b, 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; Paillard et al. 2003; Gupta et al. 1999, 2002; Stephenson et al. 1998; Pestova et al. 2000; 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 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 10 entry 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 three grams of a slow release 14-14-14 (N-P-K) fertilizer per pot. The temperature was maintained between 20ºC and 24ºC and natural light was supplemented with artificial high intensity 1000 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 graminis* DC f. sp. *tritici* that were dug at the Cunningham Research and Education Center, Kinston, NC, 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 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 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 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 one $F_{2:3}$ lines were planted at Kinston, NC 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 to 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 minus 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://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).

PCR reactions were conducted in a total volume of 10 µl containing 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 200µM of each dNTP, 20nM of forward primer, 100nM of reverse primer, 100nM of M13 labeled primer (IRD700 or IRD800 label, LI-COR Biosciences), 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, 20mM 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 hours at 42W and 1500 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 $\geq$ 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 2.1. The χ^2 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: 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 2.1). 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: 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 4 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 2.1. 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). 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*/138bp and *Xbarc177*/129bp 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*/235bp and *Xgwm272*/144bp bands co-segregated with the NCD7 resistance gene and the *Xbarc144*/238bp and *Xgwm272*/127bp 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 2.2). The most likely order is illustrated in Figure 2.1. 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 (Figures 2.2, 2.3 and 2.4). 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.

Discussion

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

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 3.5 cM 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 NC99BGTAG11 (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.

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Table 2.1 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	X^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

Table 2.2 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	X^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

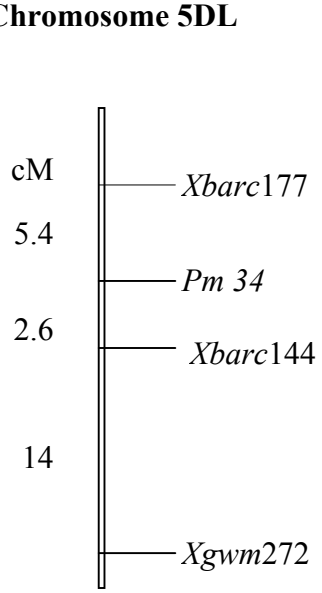

Figure 2.1 Map position of *Pm34* on chromosome 5DL

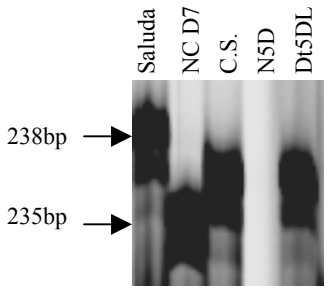

Figure 2.2 Chromosomal localization of Microsatellite marker *Xbarc144*

PCR products observed in NCD7, Saluda, Chinese Spring (C.S.) and Ditelosomic 5DL (Dt5DL) but no PCR products observed in Nullisomic 5D (N5D-T5A).

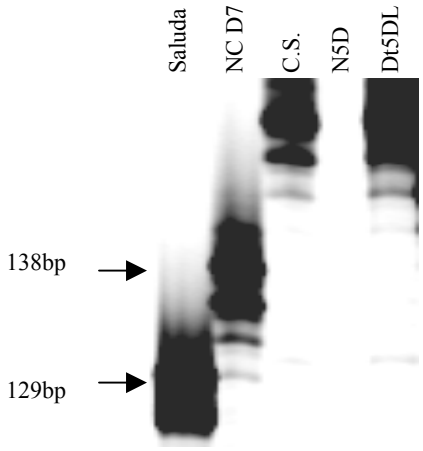

Figure 2.3 Chromosomal localization of Microsatellite marker *Xbarc*177

PCR products observed in NCD7, Saluda, Chinese Spring (C.S.) and Ditelosomic 5DL (Dt5DL) but no PCR products observed in Nullisomic 5D (N5D-T5A).

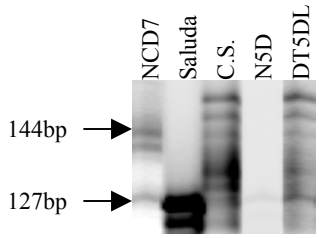

Figure 2.4 Chromosomal localization of Microsatellite marker *Xgwm272*

PCR products observed in NCD7, Saluda, Chinese Spring (C.S.) and Ditelosomic 5DL (Dt5DL) but no PCR products observed in Nullisomic 5D (N5D-T5A).

Chapter 3

Genetic mapping of two *Triticum monococcum*-derived powdery mildew resistance genes

Abstract

Microsatellite linkage maps were developed for two different *Triticum monococcum*-derived powdery mildew resistant genes present in the North Carolina germplasm lines NCBGT96A6 and NCBGT96A5. Genetic analysis of F_2 derived lines from a NCBGT96A6 X Saluda cross indicated a single dominant gene controlling powdery mildew resistance. The NCBGT96A6 *Pm* gene was mapped to chromosome 7AL and the most likely order was *Cfa2123*, 0.9cM, *Xbarc121*, 1.7cM, NCBGT96A6 powdery mildew resistance gene, 0cM, *Cfa2019*, 3.0cM, *Xgwm332.* The linkage map developed for *Pm25*, present in the germplasm line NCBGT96A5, confirmed its previous assignment to chromosome 1AS. The most likely order was: *Xgwm905*, 7.7cM, *Xgdm33*, 6.9cM, *Pm25, 68.0cM, Xgwm1148.*

Introduction

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 of resistant cultivars is considered the most economic and environmentally safe approach to prevent yield losses caused by this disease (Hsam and Zeller 2002). The use of single major genes that confer race specific disease resistance has not provided a long lasting protection against this disease (Chen and Chelkowski 1999) but major host resistance genes should provide a more durable disease resistance when they are deployed in gene 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 to tag and identify disease resistance genes. Microsatellites offer several advantages among the different types of molecular markers: they are co-dominant, highly polymorphic, generally chromosome specific and more amenable to automatization than RFLPs (Gupta et al. 1999; Langridge et al. 2001).

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). 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 Briggle (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 germplasm lines NC96BGTA4 and NC96BGTA6 developed by the small grains breeding program at North Carolina State University (Murphy et al. 1999) also have *T. monococcum* as their source of resistance to powdery mildew. 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 linkage mapping of the powdery mildew resistance gene present in NC96BGTA6, and the identification of microsatellite markers linked to *Pm25*.

Materials and Methods

The soft red winter wheat powdery mildew resistant germplasm lines NC96BGTA6 (PI 599036) and NC96BGTA5 (PI 599035) were each crossed to the susceptible cultivar Saluda (PI 480474). NC96BGTA6 (NCA6) and NC96BGTA5 (NCA5) are homogeneously resistant BC_2F_5 -derived lines with the pedigrees Saluda *3 / PI 427772 and Saluda *3 / PI 427662 respectively (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 *Pm*3a that has been defeated by the powdery mildew populations present in North Carolina (Leath and Heun, 1990). The powdery mildew resistance donors, PI 427772 and PI 427662, are two different *T. monococcum* L. subsp. *aegilopoides* accessions collected in Iraq. The NCA6 X Saluda and NCA5 X Saluda F_1 hybrids were selfed to produce F_2 seeds in the greenhouse. The F_1 plants grown in the greenhouse were resistant to powdery mildew, indicating that the resistance in both germplasm lines is a dominant trait. F_2 spaced plants were grown in the field without selection and harvested per individual plant to produce $F_{2:3}$ lines that were used in the 2003 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 2004 evaluations.

Disease assessments

Greenhouse

Ninety nine $F_{2:3}$ lines from the NCA6 X Saluda population and 84 $F_{2:3}$ lines from the NCA5 X Saluda population were evaluated for their reaction to powdery mildew in

two independent greenhouse trials during 2003. An experimental unit was two 10-cm pots, each planted with five seeds of each line. The experimental design was a completely randomized design with a single replication. One pot containing Saluda and one pot containing the parental germplasm were included at 10 entry 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 three grams of a slow release 14-14-14 (N-P-K) fertilizer per pot. The temperature was maintained between 20ºC and 24ºC and natural light was supplemented with artificial high intensity 1000 W discharge lights.

The source of inoculum was powdery mildew infected Saluda plants collected at the Cunningham Research and Education Center, Kinston, NC. The inoculum was maintained and propagated on Saluda plants under greenhouse conditions. Plants were inoculated 20-30 days after planting at Feekes growth stage 1.3-2 (Large, 1954) by shaking conidia from infected plants onto their leaves. 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 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 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 all individual plants within each F_2 derived family with Saluda and the corresponding resistant germplasm line parent. Lines for which all plants had a reaction similar to the germplasm line 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.

Field

One hundred twenty one $F_{2:3}$ lines from the NCA6 X Saluda cross and 96 lines from the NCA5 X Saluda cross were planted at Kinston, NC in October 2002. All lines included in the greenhouse evaluations were included in the field evaluations. The experimental design was a completely randomized design with a single replication. An experimental unit was a 1.2-m row planted with 40 to 60 seeds per line. Rows were spaced 30.5-cm apart. Parental germplasm lines and Saluda rows were included at forty 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.

Uniform disease spread was verified in all Saluda rows in the NCA6 X Saluda experiment. Poor canopy development due to spindle streak mosaic virus resulted in less than uniform disease spread among the susceptible checks within the NCA5 X Saluda population. This population was not evaluated in the 2002-03 season. Disease ratings

were taken at the end of March, when plants were at Feekes Growth stage 9 - 10.1. Flag minus 2 leaves were rated using the scale of Leath and Heun (1990) previously described for the greenhouse test. Lines were classified as homozygous resistant or homozygous susceptible when only one phenotypic class was observed. The $F_{2:3}$ lines from the NCA5 X Saluda population were advanced one generation and phenotyped in the $F_{2:4}$ generation during the 2003-04 season following the procedures previously described. A second field trial of the NCA6 X Saluda population during the 2003-04 season included only the $F_{2:4}$ lines that were classified as either homozygous resistant or homozygous susceptible in the $F_{2:3}$ generation.

Microsatellite markers analysis

Genomic DNA was extracted from leaf tissue samples of F_2 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 (CACGACGTTGTAAAACGAC-) at the 5' end for labeling purposes (Schulke 2000; Rampling et al. 2001). Microsatellite primers GWM905 and GWM1148 were kindly provided by Dr. M. Röder, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany).

PCR reactions were conducted in a total volume of 10 µl containing 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 200µM of each dNTP, 20nM of forward primer, 100nM of reverse primer, 100nM of M13 labeled primer (IRD700 or IRD800

label, LI-COR Biosciences), 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, 20mM 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 hours at 42W and 1500 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 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 ten lines phenotypically scored as resistant and ten lines phenotypically scored as susceptible respectively.

Chromosomal assignment.

Chromosomal location of the linked microsatellite markers was confirmed using Chinese Spring nullitetrasomics: Nulli1A-tetra1D (N1ATA) and Nulli7A-tetra7D (N7AT7D) and ditelisomics: ditelo 1AS (Dt1AS), ditelo 1AL (Dt1AL), ditelo 7AS (Dt7AS) and ditelo 7AL (Dt7AL) lines (kindly provided by The Wheat Genetics Resource Center, Kansas State University). Genomic DNA from each germplasm line,

their resistance donor, euploid Chinese Spring and the nullitetrasomic and ditelosomic lines of the putatively assigned chromosome were used to perform PCR reactions with the microsatellite markers linked to each powdery mildew resistance gene.

The chromosomal breakpoint interval was also 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 chromosome arm carrying the deletion-number of the line-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-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 $\geq$ 3.0.

Results

NCA6 X Saluda

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, indicating monogenically inherited disease resistance (Table 3.1).

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 x Saluda population also fitted a 1:2:1 ratio (P=0.51) (Table 3.1).

2004 evaluation: Sixty six $F_{2:4}$ lines from the NCA6 x 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.

Microsatellite markers analysis

Fifteen out 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. A 196 bp fragment observed in NCA6 was also present in the resistant bulk and a 229bp band was observed in Saluda and the susceptible bulk. Genotyping of the F_2 progeny is shown in figure 3.1. This microsatellite marker has been mapped to chromosome 7AL, therefore, 34 additional 7AL microsatellite markers were tested on the population. *Cfa2123*, *Cfa2019* and *Xgwm332* were found linked to the NCA6 powdery mildew resistance. The *Cfa2019* marker was dominant and linked in coupling to the NCA6 *Pm* gene. The *Cfa2019*/196bp band segregated with the resistant allele and was absent in all susceptible individuals of the F_2 progeny. *Xbarc121*, *Cfa2123* and *Xgwm332* were co-dominant markers and fitted the expected 1:2:1 monogenic ratio (Table 3). The most likely order was *Cfa2123*, 0.9cM, *Xbarc121*, 1.7cM, NCA6 powdery mildew resistance allele, 0cM, *Cfa2019*, 3.0cM, *Xgwm332* (Fig. 3.2). Six other orders had a LOD scores that differ by ≤ 3 from this most likely order and in four of them *Cfa2123* and *Xgwm332* were flanking the NCA6 *Pm* gene. The most likely order of the region between *Cfa2123* and *Xbarc121* is inverted compared to the physical map (Fig. 3.3), but two other orders with LOD scores of 3.4 from the most likely order placed *Xbarc121* proximal to *Cfa2123.*

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

Chromosomal assignment - 7AL.

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 agreement with published SSR linkage maps, therefore sub-arm localization of the microsatellite markers was performed using 7AL deletion lines. *Xbarc121* was located within the 0.49-0.71 interval, *Cfa2123* was in the 0.74-0.86 interval and *Xgwm332* and *Cfa2019* mapped to the distal 0.11 end of 7AL (Fig. 3.3)

NCA5 X Saluda

Greenhouse evaluation.

NCA5 showed disease reaction scores ranging from 0 to 3 and Saluda scores ranged from 6 to 8. The observed number of F_2-derived lines per phenotypic class followed the expected 1:2:1 monogenic inheritance ratio (P=0.41) (Table 3.2).

Field evaluation

NCA5 disease scores ranged from 0 to 3 and Saluda scores ranged from 6 to 8. Only 4 lines received a phenotypic classification that disagreed with the greenhouse evaluation. Three susceptible and one resistant line were re-classified as segregating. These differences can be attributed to sampling error. The phenotypic classification of $F_{2:4}$ lines from the NCA5 X Saluda population confirmed the expected 1:2:1 monogenic ratio (Table 3.2).

Microsatellite markers analysis

Among the microsatellite markers included in the bulk segregant analysis, *Xgdm33* was polymorphic between the contrasting bulks. A 148bp band was observed in NCA5 and the resistant bulk and was absent in Saluda. Two additional monomorphic bands of 130 and 119 bp were also observed. Genotyping of the F_2 progeny confirmed the linkage of this marker to the powdery mildew resistance (Fig. 3.4). The GDM33 microsatellite primer pair was originally isolated from *Aegilops tauschii* (Pestova et al. 2000) and mapped to chromosome 1D, but was reported to amplify homoeologous loci on 1A and 1B (Sommers et al 2004). Because *Pm25* was reported linked to the *Pm3* locus located on chromosome 1A (Shi et al.1998), 39 additional primers mapped to chromosome 1AS were tested in the population. Microsatellite markers *Xgwm905* and *Xgwm1148* were linked to *Pm25*. *Xgwm1148* and *Xgwm905* segregated as co-dominant markers. Marker bands *Xgwm1148*/191bp and *Xgwm905*/253 were associated with *Pm25* and fragments *Xgwm1148*/180bp and *Xgwm905*/276bp were associated with the susceptible allele. Segregation of *Xgdm33* fitted a 3:1 ratio and segregation of *Xgwm1148* and *Xgwm905* fitted the expected 1:2:1 ratio of co-dominant markers (Table 3.4). The most likely order was: *Xgwm905*, 7.7cM, *Xgdm33*, 6.9cM, *Pm25, 68.0cM, Xgwm1148* (Fig. 5). Five other locus orders had LOD scores that differ by ≤ 3 from the most likely order and two of them had *Xgwm905* and *Xgdm33* flanking *Pm25*. *Xgwm1148* remained proximal to *Pm25* in all five other most likely orders.

Chromosomal assignment- 1AS.

The microsatellite primer pairs linked to NCA5 amplified PCR products of the expected size on euploid and ditelosomic 1AS Chinese Spring but not on Nulli1A - Tetra1D and ditelosomic 1AL.

Discussion

The NCA6 and NCA5 loci for powdery mildew resistance were both introgressed from *T. monococcum* but their chromosomal locations indicated they are different genes. The disease response of these germplasm lines to the powdery mildew populations in the two field experiments was immune or highly resistant. Greenhouse and field experiments confirmed that both resistances are inherited as a monogenic trait.

Two other known *Pm* loci have been mapped to the long arm of chromosome 7AL: *Pm1* (Sears and Briggle 1969) and *Pm*9 (Schneider et al.1991). Only one 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 the F_1 generation of the NCA6 X Saluda cross. The *Pm1* and *Pm9* loci are separated by a mapping distance of 8.5cM (Schneider et al. 1991). Five alleles have been reported for the *Pm1* locus (Huang and Röder, 2004).

The mapping distances among the microsatellite markers linked to the NCA6 powdery mildew resistance gene in this experiment were considerably shorter 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 less than 6cM (Fig.1) and a physical distance of at least 20% (Fig.3) of the whole long arm of chromosome 7A. This low genetic/physical distance ratio can be explained by the presence of a relatively large segment introgressed from *T. monococcum* that suppresses

recombination. This could make locating the NCA6 powdery mildew resistance gene within a smaller physical interval a difficult task.

Neu et al. (2002) also reported suppressed recombination, without physical proximity, between *Lr20* and *Pm1*, probably due to an alien introgression. A similar situation may have occurred in our experiment where *T. monococcum* regions flanking the NCA6 *Pm* gene are reducing recombination despite the absence of a tight physical linkage. The absence of recombination between the microsatellite marker *Cfa2019* and the NCA6 *Pm* gene and the lack of PCR products in Saluda and the susceptible progeny, can be explained by the fact that CFA2019 is a *T. monococcum*- derived primer pair (Sourdille et al. 2001). Nevertheless, this marker amplified a fragment in Chinese Spring and its physical location was determined.

The order of the microsatellite markers *Cfa2123* 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) particularly in proximal regions and when genetic distances were very short. These two markers were separated by a distance of only 0.9 cM and were the most proximal loci in the NCA6 linkage map.

Xgwm332 has been mapped 17.7cM proximal to *Pm1e* (Singrün et al. 2003) and 32.8 proximal to *Pm1a* (Neu et al. 2002). Since we mapped Xgwm332 3cM distal to the NCA6 *Pm* gene, the distance between these two *Pm* loci should be about 20cM or even greater, considering that recombination is likely to be reduced by the alien *T. monococcum* segment.

Xgwm344 is the closest microsatellite marker reported linked to the *Pm1* locus (0.9cM) 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 can be traced back to the recurrent parent 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 source of powdery mildew resistance in NC96BGTA4 is the *T. monococcum* accession PI 221414 and the donor of the NC99BGTAG11 *Pm* gene is the *T. timopheevii* subsp. *armeniacum* accession PI 427315 (Murphy et al. 1999, 2002). The linkage maps for NC96BGTA4 and NC99BGTAG11 (Srnić et al. 2005) suggested that both are distal to the NCA6 *Pm* gene.

All the previous cross-references to published linkage maps for *Pm* genes on chromosome 7AL indicate that the NCA6 powdery mildew resistance gene is likely to be a novel *Pm* gene but additional allelism studies are needed to differentiate between this locus and *Pm1*.

The presence of *Pm25* on chromosome 1AS was previously inferred due to its linkage with the *Pm3* locus (Shi et al. 1998) and was confirmed with the linked microsatellite markers in this study. The proximity and co-dominant nature of some of these markers makes them more suitable for marker-assisted selection than the linked RAPD markers previously found (Shi et al. 1997). The disease reaction of *Pm25* against *Blumeria graminis* differential isolates produced a virulence profile that was different

from *Pm3a*, *Pm3b*, *Pm3c*, *Pm3e* and *Pm3f* (R. Parks and C. Cowger, United States Department of Agriculture-Agricultural Research Service, Raleigh, North Carolina, personal communication).

Shi et al. (1998) reported a recombination frequency of 0.21 between *Pm25* and *Pm3a* but comparison of the microsatellite linkage map for *Pm25* with microsatellite linkage maps for *Pm3* alleles (Bougot et al. 2002; Huang et al. 2004) suggested a closer linkage to the *Pm3* locus.

The *Pm* genes present in NCA6 and NCA5 provided effective powdery mildew resistance in all field and greenhouse trials, are non-allelic and could be easily incorporated into breeding programs through marker-assisted selection.

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Table 3.1 Segregation ratios for powdery mildew reaction of F_2 derived families from the NCA6 X Saluda cross

Generation	Number of F_2 families			Total	X^2(1:2:1)	P value
	Resistant	Segregating	Susceptible			
		Greenhouse				
$F_{2:3}$	26	52	21	99	0.76	0.68
		Field				
$F_{2:3}$	35	55	31	121	1.35	0.51

Table 3.2 Segregation ratios for powdery mildew reaction of F_2 derived families from the NCA5 X Saluda cross

Generation	Number of F_2 families			Total	X^2(1:2:1)	P value
	Resistant	Segregating	Susceptible			
		Greenhouse				
$F_{2:3}$	26	37	21	84	1.79	0.41
		Field				
$F_{2:4}$	29	47	20	96	1.73	0.42

Table 3.3 Segregation ratios for Microsatellite (SSR) markers among F_2 individuals in the NCA6 X Saluda population

SSR marker	Observed segregation ratio	Expected	X^2	P value
Xbarc121	36:55:30	1:2:1 [a]A: [b]H : [c]B	1.26	0.53
Cfa2019	90:31	3:1 [d]D:B	0.02	0.87
Cfa2123	34:56:31	1:2:1 A: H : B	0.82	0.66
Xgwm332	35:61:25	1:2:1 A:H : B	1.66	0.44

[a]A=Homozygous for the NCA6 allele

[b]H= Heterozygous

[c]B= Homozygous for the Saluda allele

[d]D= not homozygous for the Saluda allele

Table 3.4 Segregation ratios for Microsatellite (SSR) markers among F_2 individuals in the NCA5 X Saluda population.

SSR marker	Observed segregation ratio	Expected	X^2	P value
Xgwm905	30:43:23	1:2:1 [a]A: [b]H : [c]B	2.06	0.36
Xgwm1148	29:40:27	1:2:1 A: H : B	2.75	0.25
Xgdm33	72:24	3:1 [d]D: B	0	1

[a]A= Homozygous for the NCA5 allele

[b]H= Heterozygous

[c]B= Homozygous for the Saluda allele

[d]D=not homozygous for the Saluda allele

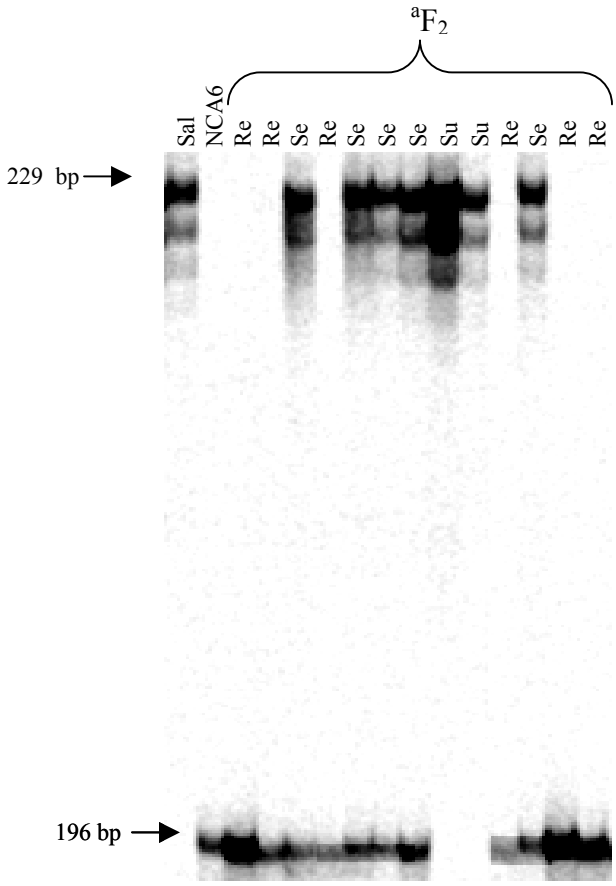

Figure 3.1 Genotyping of the NCA6 X Saluda F_2 population using microsatellite marker *Xbarc121*.

[a]F_2 progeny labeled according to the disease reaction of their F_2 derived family: Re, resistant; Se, segregating and Su, Susceptible

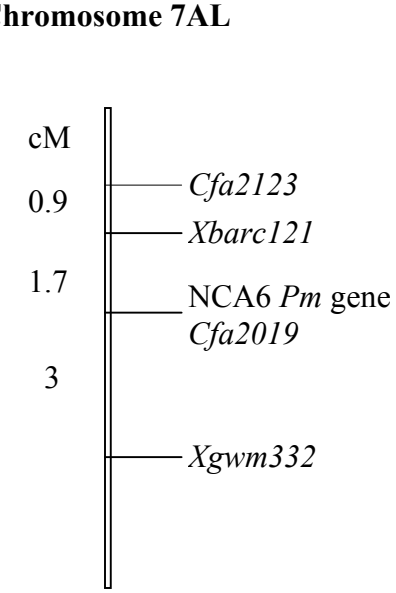

Figure 3.2 Map position of the NCA6 *Pm* gene on chromosome 7AL

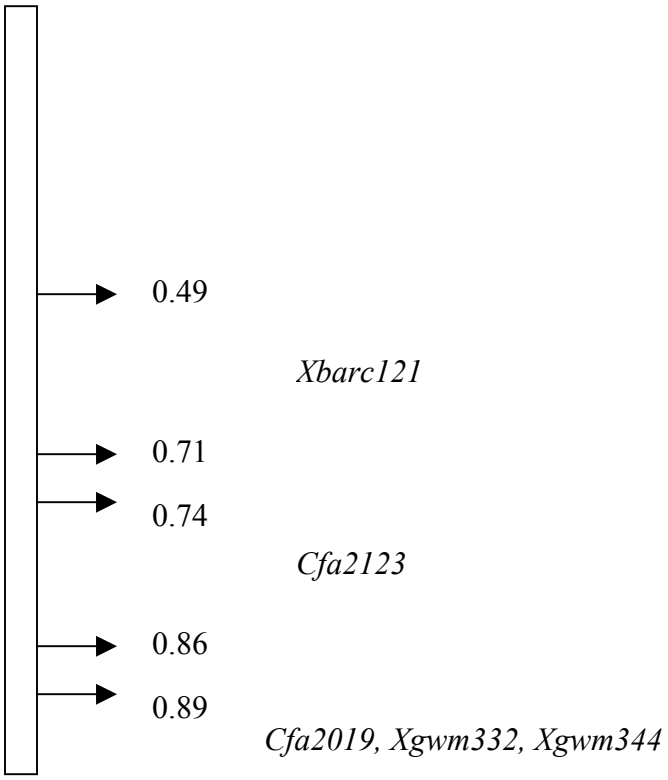

Figure 3.3 Chromosomal breakpoint intervals for microsatellite markers linked to the NCA6 *Pm* gene

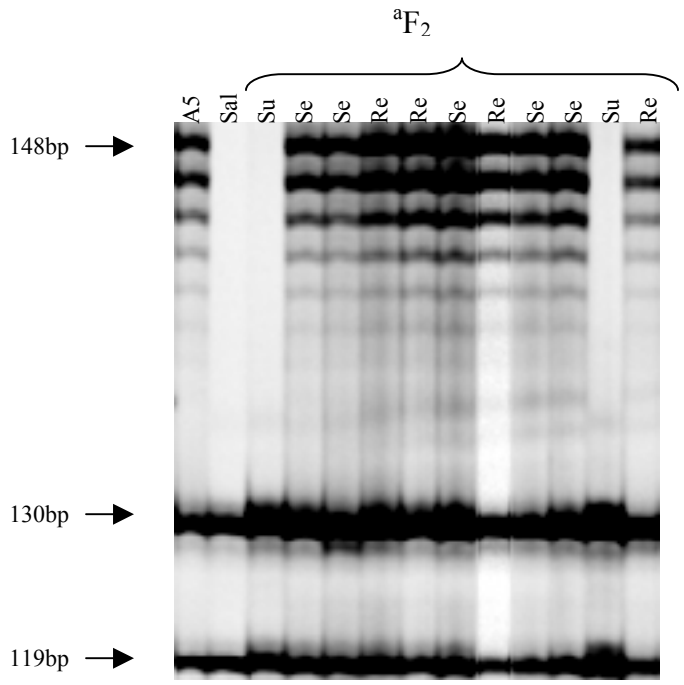

Figure 3.4 Genotyping of the NCA5 X Saluda F_2 population using microsatellite marker *Xgdm33*.

[a]F2 progeny labeled according to the disease reaction of their F_2 derived family: Re, resistant; Se, segregating and Su, Susceptible

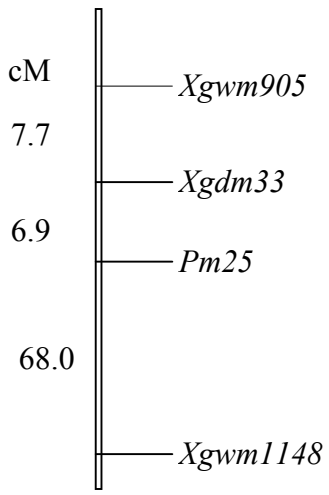

Figure 3.5 Map position of *Pm25* on chromosome 1AS

Chapter 4

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

Abstract

A single gene controlling powdery mildew resistance was identified in the North Carolina germplasm line NC96BGTD3 using genetic analysis of F_2 derived lines from a NC96BGTD3 X Saluda cross. Microsatellite markers linked to this *Pm* gene were identified and their most likely order was *Cfd7,* 8.2cM, X*gdm43*, 6.7cM, *Cfd26*, 13.4cM, *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 among the NC96BGTD3 *Pm* gene, *Pm2* (5DS) and *Pm34* (5DL) and the allelism test conducted showed recombination between *Pm34* and the NC96BGTD3 *Pm* gene. All the tests conducted provided strong evidence of the presence of a novel *Pm* gene in NC96BGTD3 and this gene should be designated *Pm35.*

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 easier 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 *Ae. tauschii* and the D 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 screened showed resistance and had diverse virulence profiles (Cox et al. 1992; Lutz et al. 1994). *Pm2*, *Pm19* and *Pm34* (McIntosh and Baker 1970; Lutz et al. 1995; Miranda et al. 2006) were transferred from *Ae. tauschii* into cultivated wheat.

Molecular markers are 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 *Ae. 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 *Ae. 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 *Ae. 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 X 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 X Saluda

Greenhouse

Ninety three $F_{2:4}$ lines from the NCD3 X Saluda cross 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. One pot containing Saluda and

one pot containing NCD3 were included at 10 entry 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 three grams of a slow release 14-14-14 (N-P-K) fertilizer per pot. The temperature was maintained between 20ºC and 24ºC and natural light was supplemented with artificial high intensity 1000 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 a single spore culture isolate with the following avirulence / virulence response to known *Pm* genes: *Pm1a*, *1b*, *3b*, *4b*, *8*, *17*/*Pm2*, *3a*, *5*, *6*, *7*, *MA*.

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 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 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 ten individual plants within each F_2-derived line with the Saluda and NCD3 reactions. Lines for which all plants had a reaction similar to NCD3 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.

Field

One hundred six $F_{2:4}$ lines were planted at Kinston, NC in October 2004. The experimental design was a randomized complete block with two replications. An experimental unit was a 1.2-m row planted with 40 to 60 seeds per line. Rows were spaced 30.5-cm apart. NCD3 and Saluda rows were included at forty 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 reactions were evaluated at the beginning of April 2005 when plants were at Feekes Growth stage 10.1-10.5 and all Saluda rows presented uniform powdery mildew infection. Flag minus 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.

NCD3 x NCD7 (*Pm34*)

Allelism test.

One hundred twenty eight $F_{2:3}$ families from the NCD3 X NCD7 cross were evaluated for their powdery mildew reaction under field conditions using the same procedures previously described 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. The seven isolates used were designated: C1-6, C4-6, 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 and C4-6), 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 x 15 mm petri-dishes filled with 50mg/liter benzimidazole amended 6% agar containing 2.5cm leaf segments of cultivar Chancellor that were obtained from 10-15 days old seedlings. Plates were placed in a growth chamber and maintained at 18ºC, 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. Disease reactions were recorded 7-8 days after inoculation using the same 0 to 9 scale previously described.

Microsatellite markers 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://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).

PCR reactions were conducted in a total volume of 10 µl containing 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 200µM of each dNTP, 20nM of forward primer, 100nM of reverse primer, 100nM of M13 labeled primer (IRD700 or IRD800 label, LI-COR Biosciences), 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, 20mM 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 hours at 42W and 1500 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 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 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, 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 $\geq$ 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*, therefore, the phenotypic classes were defined as: resistant = homozygous for *Pm34* and/or NCD3 *Pm* gene; segregating = heterozygous at both *Pm* loci or heterozygous at one locus and homozygous recessive at the other locus and susceptible = homozygous recessive at both loci. To estimate the recombination between these two loci, the expected frequencies of these classes, assuming linkage in repulsion phase, were expressed as functions of the recombination frequency (r): frequency of resistant $F_{2:4}$ families = ½(1- r/2), frequency of segregating families = ½ and frequency of susceptible families = $r^2/4$. This estimated value of recombination frequency was converted to mapping units using the Kosambi mapping function.

Results

Greenhouse evaluations.

NCD3 and Saluda scores 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 ($\chi^2_{1:2:1}$=1.4, P=0.5) of a monogenic resistance (Table 4.1).

Field evaluations.

NCD3 exhibited a resistant disease reaction with scores ranging from 1 to 3. 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 ($\chi^2_{1:2:1}$=1.83, P=0.4) confirming a monogenic disease resistance (Table 4.1). 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.

Allelism test

The observed number of $F_{2:3}$ families per phenotypic class in the NCD3 X NCD7 population is shown in table 3. The χ^2 value for the expected 7:8:1 (resistant: segregating: susceptible) was 14.06, indicating that the two *Pm* genes are linked (P=0.001, Table 4.2). A mapping distance of 33cM was estimated based on a maximum likelihood estimated recombination frequency of 0.29. However, the observed phenotypic classes for the $F_{2:3}$ families indicated a distortion in the phenotypic classification. For two loci

undistinguishable phenotypically and linked in repulsion phase, the frequency of segregating $F_{2:3}$ families is independent of the recombination frequency and should be equal to 0.5. Since a significant deviation from this expected number of segregating families was observed, the recombination frequency (r) was also estimated using the number of susceptible families as the only informative phenotypic class (number of susceptible families =r/4) and combining the resistant and segregating families in one single class, as could also be done in the case of an allelism test with F_2 progeny. The chi-square test did not show a significant deviation from the expected 15:1 ratio for two dominant genes segregating independently (P=0.27, Table 4.3) and the estimated recombination frequency (0.40) was equivalent to a distance of 53.6cM when converted to Kosambi mapping units.

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 seven different *B. graminis* f. sp. *tritici* isolates tested (Table 4.4).

Microsatellite markers analysis.

Among the microsatellite markers tested in the bulked segregant analysis, C*fd26* was polymorphic between the contrasting bulks. When tested in the F_2 progeny, a 231bp fragment was associated with the NCD3 allele and a 261bp band was associated with the susceptible allele. This microsatellite locus was mapped to chromosome 5DL (Sommers

et al. 2004). therefore, additional 43 primer pairs mapped 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 *Cfd7* and *Xgdm43* were also linked to the NCD3 powdery mildew resistance. *Cfd7*/251bp and *Xgdm43*/ 126bp bands were associated with the NCD3 allele and *Cfd7*/240bp and *Xgdm43*/ 150bp bands were associated with the Saluda allele. The segregation ratios for the three microsatellite markers linked to the NCD3 *Pm* gene fitted the expected 1:2:1 ratio for co-dominant markers (Table 4.5). A microsatellite linkage map depicting the most likely order is shown in figure 4.1. No other locus order had a LOD score within 3 of this most likely order. Microsatellite markers linked to *Pm34* were not polymorphic between NCD3 and Saluda.

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 the microsatellite linkage map developed in this study. Two other known *Ae. tauschii*-derived *Pm* genes, *Pm2* and *Pm34,* have been mapped for chromosome 5D:. The disease reaction response of the NCD3 *Pm* gene was shown to be different from *Pm2* and *Pm34,* indicating that the NCD3 *Pm* gene is at least a novel *Ae. tauschii*-derived powdery mildew resistance allele.

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. Because *Pm2* was mapped to the short arm of chromosome 5D (McIntosh and Baker 1970) we concluded that the NCD3 *Pm* gene is different from *Pm2*. Also *Pm34* was previously mapped to 5DL (Miranda et al. 2006) but the allelism test in this study showed recombination between the NCD3 *Pm* gene and *Pm34.* Therefore the NCD3 *Pm* gene and *Pm34* are non-allelic.

The phenotypic classification of $F_{2:3}$ lines in the NCD3 X NCD7 population probably overestimated the number of resistant families because F_2 lines heterozygous at both resistant 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 was hard to detect in the field experiment. Since the susceptible families could be accurately distinguished phenotypically, the data was also analyzed using the phenotypic classification in the F_2 generation and a larger distance (53.6cM) between the two loci was estimated. Despite these discrepancies, the susceptible $F_{2:3}$ families in the NCD3 X NCD7 progeny were derived from homozygous susceptible F_2 plants that could only be present if there was recombination between the two loci.

Microsatellite markers *Xbarc177* and *Xgwm272* were mapped flanking *Pm34* at proximal and distal positions respectively (Miranda et al. 2006) but these markers could not be mapped in the NCD3 X Saluda population because they were not polymorphic between the parents. However, the wheat composite map (http://wheat.pw.usda.gov) reported a distance of 45cM between *Xbarc177* and *cfd26* and the consensus map (Sommers et al 2004) reported a distance of 60cM between *Xgwm272* and *Cfd26*. These distances are in good agreement with our findings.

All the experiments conducted in this study provide strong evidence of the presence of a novel powdery mildew resistance gene in NCD3 and we propose to designate this gene as *Pm35*.

Field trials with different powdery mildew resistant germplasm lines released by the small grains breeding program at North Carolina State University have been conducted annually since 1999. NCD3 has been shown to have superior resistance to spindle streak mosaic virus in comparison to its recurrent parent Saluda, in addition to its powdery mildew resistance. Multiple disease resistance present in *Ae. tauschii* accessions was also reported by Lutz et al. (1994) who found resistance to other foliar diseases such as leaf rust (*Puccinia triticina* Eriks), stem rust (*Puccinia graminis* f.sp. *tritici* and tan spot (*Pyrenophora tritici-repentis*), among powdery mildew resistant accessions. Resistance to spindle streak mosaic virus makes this germplasm line even more valuable as a parent to be included in wheat breeding programs.

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Table 4.1 Segregation ratios for powdery mildew reaction of F_2 derived families from the NCD3 X Saluda cross

Generation	Number of F_2-derived families			Total	X^2(1:2:1)	P value
	Resistant	Segregating	Susceptible			
		Greenhouse				
$F_{2:4}$	26	46	21	93	1.40	0.50
		Field				
$F_{2:4}$	26	59	21	106	1.83	0.40

Table 4.2 Segregation ratios for powdery mildew reaction of F_2 derived families in the allelism test of the NCD3 *Pm* gene and *Pm34* ($F_{2:3}$ generation)

Population	Observed number of $F_{2:3}$ families			X^2 (7:8:1)[a]	P value	%Recombination ± SD
	Resistant	Segregating	Susceptible			
NCD3 X NCD7	77	46	5	14.06	0.001	29 ± 1.4

[a](7:8:1= resistant:segregating:susceptible)

Table 4.3 Segregation ratios for powdery mildew reaction of F_2 derived families in the allelism test of the NCD3 *Pm* gene and *Pm34* (F_2 generation)

Population	Number of F_2 individuals		X^2 (15:1)[a]	P value	%Recombination ± SD
	Resistant	Susceptible			
NCD3 X NCD7	123	5	1.2	0.27	40± 1.8

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

Table 4.4 Differential reaction of NC3, NCD7 (*Pm34*) and Ulka (*Pm2*) after inoculation with seven isolates of *Blumeria graminis* f. sp. *tritici* in a detached-leaf test

Line	*Blumeria graminis* f. sp. *tritici* Isolates						
	H2-3	C1-6	H2-8	C4-6	E2-5	J2-1	J2-3
NCD3	I[a]	I	S	S	S	I	S
NCD7	R[b]	I	S	S	I	I	S
Ulka	S[c]	R	S	S	I	S	R
Saluda	S	S	S	S	S	S	S
Chancellor	S	S	S	S	S	S	S

[a]I = intermediate = 4-6

[b]R = resistant = 0-3

[c]S = susceptible= 7-9

Table 4.5 Segregation ratios for Microsatellite (SSR) markers among F_2 individuals in the NCD3 X Saluda population

SSR marker	AA[a]	H[b]	BB[c]	Total	X^2 (1:2:1)	P value
Cfd26	26	56	23	105	0.64	0.73
Cfd7	26	53	25	104	0.06	0.97
Xgdm43	22	58	20	100	2.64	0.27

[a]AA=Homozygous for the NCD3 allele

[b]H= Heterozygous

[c]BB= Homozygous for the Saluda allele

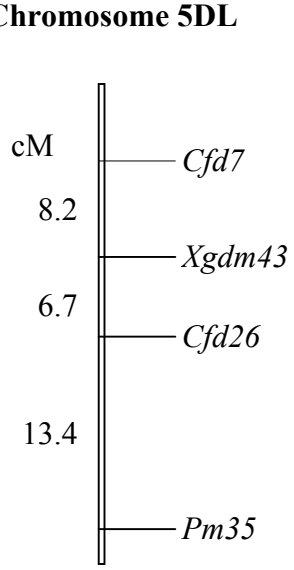

Figure 4.1 Map position of *Pm35* on chromosome 5DL