ABSTRACT

SRNIĆ, GORAN. Genetics of Resistance to Powdery Mildew in Several Wheat Germplasm Lines. (Under the direction of J. Paul Murphy.)

Powdery mildew (*Blumeria graminis* DC f. sp. *tritici*Em Marchal) of wheat (*Triticum aestivum* L.) is especially devastating in temperate, cool and moist regions. It increasingly affects wheat in drier and warmer areas as a consequence of changing crop practices such as irrigation, increased nitrogen fertilization, and the use of semi-dwarf cultivars. The highest impact on wheat yields have been observed in Europe, southwestern Asia, and in the eastern and western parts of the North America, including large areas of the southeastern USA. Resistant wheat cultivars remain the most cost efficient and effective means for powdery mildew control. Monogenic resistance is generally very efficient but tends to be a short time solution if variability in the pathogen population is great. A clearer understanding of the genetics of host resistance to wheat powdery mildew would be helpful in employing new resistance genes, especially for pyramidal complexes containing several resistance genes. The objectives of this study were to investigate the inheritance and efficiency of resistance to naturally occurring powdery mildew in the four wheat germplasm lines NC96BGTD1 (NCD1), NC96BGTA4 (NCA4), NC98BGTAB10 (NCAB10) and NC99BGTAG11 (NCAG11), and to identify AFLP markers linked to the resistance genes in NCD1 and NCAG11. Each germplasm was crossed to the susceptible parent Saluda, which was utilized as the recurrent parent in the development of each germplasm line and to each other. $F_{2:3}$ progenies of each of the four Saluda by germplasm line crosses and six germplasm by

germplasm crosses were tested in greenhouse and field studies. Resistance factors in all four germplasms segregated in a monogenic fashion. Five germplasm by germplasm populations (NCD1 x NCAG11, NCAG11 x NCAB10, NCA4 x NCAB10, NCD1 x NCAB10, and NCD1 x NCA4) segregated in a digenic fashion indicating independent segregation of their resistance loci. Resistance genes in NCA4 and NCAG11 co-segregated as if linked in repulsion phase. The recombination between these two genes was estimated to be 2%. The resistance genes in the four germplasms appear to be novel genes that were not previously used in commercial cultivars or breeding lines. A total of four AFLP markers were linked to resistance in NCAG11 (M-act/cct-196bp at 0.8 cM and M-acc/cga-126bp at 2.2 cM) and NCD1 (M-aca/cgt-182bp at 20.7 cM and Maag/cga-148bp at 36.9 cM). All four markers were also confirmed after two additional generations of self-pollination of resistant F_2 individuals from Saluda x NCD1 and Saluda x AG11 crosses. Pyramided markers from each parent were identified in $F_{2:4}$ wheat lines homogeneous for powdery mildew resistance from the cross between NCD1 and NCAG11. Testcrosses have been made to identify lines segregating for both resistance genes.

GENETICS OF RESISTANCE TO POWDERY MILDEW IN SEVERAL WHEAT GERMPLASM LINES

by

GORAN SRNIĆ

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

2003

APPROVED BY

Dr. Thomas G. Isleib	Dr. David S. Marshall
Dr. Steven Leath	Dr. J. Paul Murphy Chair of Advisory Committee

This dissertation is dedicated to my parents Mira and Slavko and to my sisters Jelena and Katarina, for their true, everlasting and unconditional love and support.

BIOGRAPHY

Goran Srnić was born November 16, 1969 in Cetinje, Yugoslavia. In 1989 Goran started General Agricultural Undergraduate studies at Agronomski fakultet, Čačak, Yugoslavia. In 1993 he came to the US and continued his undergraduate studies at the Department of Crop and Soil Sciences at Michigan State University. In 1996 he was awarded a Crop and Soil Science Undergraduate Scholarship. In 1996 he received a B.S. in Crop and Soil Sciences. In 1997 Goran started his M.S. degree in Crop and Soil Sciences at Michigan State University investigating genetics and inducible proteins of a Disease Lesion Mimic Trait in sugarbeet under the direction of Dr. Joseph W. Saunders. After completing this degree he moved to North Carolina State University and worked as a research technician with the Small Grains Breeding Program in the Crop Science Department from 1999-2000. In January 2001 he started a Ph.D. program at the Department of Crop Science under the direction of Dr. J. Paul Murphy.

ACKNOWLEDGEMENTS

I would like to express my deep appreciation to all of the people without whom this work would not be possible. I thank my thesis adviser Dr. J. Paul Murphy for all the time and dedication he has shown teaching me plant breeding. I am very thankful for all of his patience, support and guidance. I thank my committee members Dr. Thomas G. Isleib, Dr. Steven Leath, Dr. David Marshall, and Dr. David O'Malley for their support, ideas and valuable discussions during my studies. Special thanks to Rene Navarro for friendship, excellent technical support and for teaching me various field techniques. I thank Dr. James Holland for his help with the linkage analysis. Thanks to Amy Godwin and Lynda C. Whitcher for technical help with the field, greenhouse, and laboratory studies. I would like to thank all of the Plant Breeding Faculty and graduate students at Crop Science NCSU for their inputs and support.

My deep thanks to my family Mira, Slavko, Katarina and Jelena for their love and support. Special thanks to the families Radmilac and Srnić for their everlasting support. Many thanks to my uncle Branko Radmilac for being like a father to me. Thanks also to my fiance Wendy Pline for her love and for standing by me, and for all of her collegial support during all of these years.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

Chapter 1

Literature Review

Powdery mildew of wheat (*Triticum aestivum* L.) is a foliar disease caused by the obligate biotrophic fungus *Blumeria graminis* DC f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* DC f. sp. *Tritici* Marchal) of the order *Erysiphales*, which are filamentous Ascomycetes. The signs of this disease are well characterized by growth of the conidia and mycelia on leaf surfaces. Powdery mildew is capable of successfully infecting wheat at any growth stage from seedling to ripening of the seed (Large and Doling, 1962). The disease can cause loss in both grain yield and grain end-use quality. Breeding for resistance to powdery mildew begun over 80 years ago. Disease severity depends primarily on weather conditions, density of leaf canopies and susceptibility in cultivars (Lipps and Madden, 1989).

1. Disease distribution

Powdery mildew can occur wherever wheat is grown. It has been reported to be widespread through Africa, Asia, Australia, Europe and the Americas (Bennett, 1984). Disease severity on cereals has increased since the late 19th century with dramatic increase with increased levels of nitrogen fertilization which result in increased density and duration of green tissue necessary for development of the pathogen (Schafer, 1987; Wolfe, 1984). This pathogen is considered highly nomadic due to the annual nature of the host, a requirement for green tissue throughout most of the year and the high wind dispersal. Powdery mildew populations can move on favorable winds at a rate of 100 km/year. The spores can move throughout the canopy with a velocity of 1.2 cm/sec, even in still air (Limpert et al., 1999). Spore dispersal is likely in all directions but mainly depends on prevailing winds. The spores tend to move northward as the plants in southern regions are maturing, while dispersal from west to east and vice versa dispersal

is mainly directed by predominant winds. Increased distances, natural barriers such as mountains and host-free areas reduce the rate of dispersal. (Limpert et al., 1999).

Powdery mildew is considered a cool temperature disease, with optimum development at 15ºC to 25ºC and high relative humidity (Bennett, 1984; Leath and Bowen, 1989). Although it is especially devastating in temperate, cool and moist regions, it increasingly affects wheat in drier and warmer areas as a consequence of changing crop practices such as irrigation, increased nitrogen fertilization and the use of semi-dwarf cultivars (Bennett, 1984). The highest impacts on wheat yields have been observed in Europe, southwestern Asia, and in the eastern and western parts of the North America including large areas of the southeastern USA (Schafer, 1987). The disease can be observed throughout the growing season in many wheat fields in the southeastern US from one month after planting until late spring. Powdery mildew epidemics are inhibited when temperature increases above 28ºC.

2. Biology of powdery mildew

Following germination of an ascospore or conidium penetration of the wheat leaf and stem cuticule begins by softening it with enzymatic action. The cuticule is disrupted beneath the germ tube by appresoria (Jarvis et al., 2002). Infection is localized to epidermal cells by the primary germ tube of the fungus invaginating epidermal cell plasmalemma without penetrating the cells. A parasitic water relationship is established with the host cell which is needed for fungal development and subsequent infections. After establishing a successful infection the pathogen forms a penetration peg which penetrates through a papilla. The fungus obtains food parasitically from its host by single-celled haustoria which grow inside epidermal tissue (Götz and Boyle, 1998). Appressoria

attach the mycelium to the host surface and initiate the haustorial development. The first conidia and conidiophores develop about four days after successful primary infection (Schafer, 1987).

The fungus reproduces by both sexual and asexual mating system, with the possibility of both mating systems taking place on the same plant. An alternative host is not needed for completing the life cycle. The sexual or teleomorph stage is characterized by production of cleistothecia composed of three layers: atrosclerocortex, subcortex, and the dikaryotic inner layer of hymenium (Jarvis et al., 2002). These ascocarps of *B. graminis* are produced by generative mycelium at the end of the vegetative period. Ascocarps are fruiting bodies carrying asci within which the sexual diploid ascospores are produced. The fungus is heterothallic and needs two compatible biotypes to form the sexual stage (Schafer, 1987). The ascus is formed by union of the ascogonium or female sex cell and the antheridium or male sex cell (Agrios, 1997). Ascocarp formation occurs most frequently at the end of growing season when the amount of green host leaf tissue is reduced. Cleistothecia are the main oversummering and overwintering structures, but *B. graminis* can survive winters as mycelial mats on straw and volunteer plants. The role of cleistothecia is to assure survival under adverse conditions as well as sexual reproduction (Götz and Boyle, 1998; Jarvis et al., 2002). This promotes a high level of genotypic diversity which is of primary importance in pathogen evolution (McDonald and Linde, 2002; Wolfe and McDermott, 1994). The cleistothecium wall is permeable to water which causes an increase in internal pressure prior to spore discharge. Ascospores released in fall are the primary source of infection on winter wheat.

The asexual or anamorph stage is characterized by vegetative sporulation and the production of haploid asexual spores or conidia. They are single-celled, colorless, thin-walled and with a single nucleus. Conidia contain oil-droplets and grow in chains. They are variable in size, depending on humidity, age of host leaves and stage of season (Braun et al., 2002). The conidia are covered with a mucilaginous coating, except at the ends. Three distinct conidial stages can be recognized: 1) basal 'A' conidia are septate and not wider than a neck of a conidiophore, 2) 'B' conidia are elongated, but still completely attached in a chain, 3) 'C' conidia are mature and connected in the chain by papilla. Development of A conidia into B conidia into C conidia takes place all at the same time. There is a protoplasmic connection with neighboring conidium through a pore until the conidium is separated from the end of the chain. Cytoplasmic separation occurs at the time of release. Only C spores are released from the conidiophores under normal conditions. Conidia can be released either one by one from the chain, in chains or in clumps. The conidia are released daily, with a large peak at midday and sometimes a smaller peak after dark (Jarvis et al., 2002). Conidia germinate on the leaf surface to produce mycelium which has a white, powdery appearance. Initial infections produce chains of conidia which grow into thick mycelium. Germination of conidia is primarily dependent on availability of living leaf tissue, temperature and water. The primary mycelium is septate with thin walls. The hyphal cells have a single nucleus and vacuole (Braun et al., 2002).

Either sexual or asexual spores can penetrate the leaves leading to successful infections. Although free moisture is not required for spore germination and successful infection, optimum relative humidity is 100%. Powdery mildew has pathogenic activity

at temperatures ranging from 3ºC to 31ºC, with an optimum of 17ºC (Jarvis et al., 2002). Powdery mildew is more prevalent in shade than in dark areas.

3. Assessment and economy of the yield losses

On commercially grown wheat the disease signs and symptoms are initially observed on the older leaves and progress upward as the plants mature. Powdery mildew occurs annually in eastern North Carolina. Severe epidemics on susceptible cultivars are common. The fungus can be present on winter wheat continuously from fall until late spring (Bowen et al., 1991). Yield reductions of 34% in the cultivar Chancellor (CI 12333) in Maryland have been reported (Johnson et al., 1979), 16.7% in the cultivar Saluda and 3% in cultivars with effective levels of resistance such as Coker 983 in North Carolina (Leath and Bowen, 1989). When Saluda was grown in experimental plots in North Carolina the reductions of yield components in fungicide-untreated plots were large. The decrease in number of mature tillers was 9.6%, number of kernels per head 2.4%, 500 kernel weight 6.4% and yield 10.5% (Bowen and Leath, 1989). Saluda yields about 2,600 kg/ha under common management (Leath and Bowen, 1989) and a reduction of 17% is equivalent to $45 per hectare at today's prices.

Development of the whole plant is affected by the disease. The root system can be decreased up to 50% due to foliar infection (Jenkyn and Bainbridge, 1978). Early season infections can result in increased numbers of initial tillers but a reduced number of seed bearing tillers. The large number of tillers cannot be supported by the wheat plant and often do not survive. Initiated tillers that do not survive will deplete nutrients, primarily carbohydrates (Bowen et al., 1991; Everts and Leath, 1992). This can negatively impact yield because formation of a larger number of tillers reduces head size and number of

kernels per head. Leaf development is retarded and leaf senescence is accelerated which can severely reduce leaf area index (Jenkyn and Bainbridge, 1978).

Powdery mildew severity was negatively correlated with total leaf area in susceptible and slow-mildewing wheat cultivars Saluda, Knox 62 and HW3021 (Leath, 1987). Full season control of this disease resulted in lower disease severity, lower area under disease progress curve (AUDPC), and larger yields compared to other treatments (Leath, 1987). Yield reductions from 0 to 35% were reported in the US (Namuco et al., 1987), but may reach as high as 45% in the UK, New Zealand and India (Namuco et al., 1987; Chen and Chelkowski, 1999; Hsam and Zeller, 2002). The yield reduction seemed to be linearly related to disease severity of the whole plant in the cultivar Saluda and of the whole plant and of the flag leaf only in the cultivar Chancellor. Reductions in kernel weight and grain yield were observed in Saluda and Coker 983, even when the upper leaves had only small amounts of disease (Bowen et al., 1991). The predicted yield reductions for Saluda grown at Clayton and Plymouth, NC were 18% and 16% respectively when mildew severity was 10% at GS 10.3 (Large, 1954; Leath and Bowen, 1989). Powdery mildew also affects other characteristics of wheat besides yield. Flour quality may be decreased by altering the composition of flour protein resulting in losses of crop value as high as 50% (Johnson et al., 1979; Chen and Chelkowski, 1999). Isolines with the highest disease ratings were reported to have the lowest flour protein content (Johnson et al., 1979). Flour protein contents were highly negatively correlated with the amount of powdery mildew on the whole plant and on the flag leaf.

The assessment of disease severity in the field tends to be the most predictive of yield losses between head emergence and early stage of ripening. The correlation

between disease severity and yield loss was low prior to head emergence at GS 10 (sheat of last leaf grown out) (Lipps and Madden, 1989). The assessment can be done accurately at the stage GS 10.5 (all ears out of sheat) (Large and Doling, 1962), or GS 10.3 (half of heading process completed) (Leath and Bowen, 1989; Lipps and Madden, 1989). Assessment becomes inacurate with natural yellowing and senescence of the leaves after GS 11.1 (milky ripe). Several numerical methods were developed to estimate yield losses based on disease intensity. Large and Doling (1963) estimated that the percent loss of grain yield in the UK was twice the square root of the percent mildew assessment at GS 10.5. Linear regression of grain yield on disease severity estimate is commonly used (Large and Doling, 1963; Johnson et al., 1979; Leath and Bowen, 1989; Lipps and Madden, 1989). Yield loss can be predicted by regression of yield loss on AUDPC (Bowen et al., 1991). Correlation between disease intensity and yield loss can vary between cultivars (Jarvis et al., 2002). Highly susceptible cultivars had greater yield reduction per unit increase in disease severity, than moderately susceptible cultivars.

4. Disease control

A powdery mildew epidemic consists of a complex sequence of biological events taking place in a certain order in defined environments. To successfully implement disease control it is necessary to interrupt the disease cycle so that the time for completing a cycle is extended. The conditions for infections include phenology and susceptibility of the host and disease-conductive environment (Jarvis et al., 2002).

Disease control is based on avoiding environmental, soil and crop management factors that predispose the host to disease (Jarvis et al., 2002). Levels of powdery mildew infections may be reduced by altering cultural practices such as decreasing planting

density and crop rotation, application of fungicides and use of resistant cultivars. Management factors such as soil conditions and crop nutrition, row spacing and crop density, plant depth, seeding rate, sanitation and separation of crops and their interactions impact the intensity of powdery mildew infections (Broscious et al., 1985; Schafer, 1987; Jarvis et al., 2002). Of all macronutrients, nitrogen has the largest effect on host susceptibility. Disease intensity tends to increase linearly with spring applications of nitrogen (Broscious et al., 1985). Nitrogen applied in winter has little affect. Nitrogen added after flag leaf emergence will increase susceptibility, which cannot be associated with increase in growth rate but with change in the nutrient status of the plant (Jarvis et al., 2002). Increases in plant density population most often will lead to increase in disease severity under most management practices. Powdery mildew is a high-sugar pathogen and susceptibility will dramatically increase when the ratio of total carbohydrate to dry weight of the shoot exceeds 0.5 (Jarvis et al., 2002).

The atmospheric environment in the field can be little altered and altering management practices may not always be practical because of the potential negative effects. Altering management practices may reduce yield e.g. increasing or decreasing planting depth may decrease seed germination. The elimination of oversummering and overwintering inoculum is difficult in no-till systems. Chemical control, although costly and perhaps environmentally less desirable than host resistance, is available. Fungicide use is not always economically feasible and it is not always possible to accurately calculate economic thresholds (Leath and Bowen, 1989). Sulfur was the first fungicide used to control powdery mildew. It was followed by dithiocarbamates, benzimidazoles, strobilurines, quinolines and triazoles. Leath and Bowen (1989) suggested that in the

mid-1980's triadimenol seed treatment was the only economical treatment available to growers who anticipated low to moderate wheat yields. Only individual components of powdery mildew epidemiology have been modeled so far (Jarvis et al., 2002). They include meteorological factors such as wetness temperature, relative humidity, wind and winter frost. These components are commonly used to adjust fungicide spraying times.

5. Genetics of resistance to powdery mildew in wheat

Resistant wheat cultivars remain the most cost efficient and effective means for powdery mildew control. The pioneering work of Mains (1933, 1934), Powers and Sando (1957, 1960), Large and Doling (1962, 1963), initiated studies on resistance to wheat powdery mildew. The passive or preexisting defense mechanisms in plants include structural barriers such as waxy cuticle or reservoirs with antimicrobial compounds that prevent colonization of tissue by pathogens (Hutcheson, 1998). If the structural barriers are breached by a pathogen attack then primary, secondary and tertiary cellular defenses can be induced. Primary cellular defense responses are localized to the cells that are in contact with the pathogen and commonly result in programmed cell death (Dangl et al., 1996; Hutcheson, 1998). Secondary defense responses are induced in cells surrounding the initial infection site due to the response to signal molecules which are produced by the primary infection (Hutcheson, 1998). Defense response against pathogen attack is a very complex process, involving transcription of about 1000 genes simultaneously (Collinge et al., 2002). After pathogen recognition, an R protein activates signaling that coordinate the initial plant defense. Immediate downstream signaling molecules are functional kinases, phosphate cascades, transcription factors and reactive oxygen species (Hammond-Kosack and Jones, 1997). Among the most studied transcripts are those from

genes involved in oxidative bursts, signal transduction and enzymatic antimicrobial activity (Collinge et al., 2002). Defense responses in plants are frequently induced by different microorganisms. The most common responses are callose and lignin depositions, phytoalexins and salicylic acid synthesis, pathogenesis-related protein induction and hypersensitive response (Hammond-Kosack and Jones, 1997). Tertiary defense responses are associated with systemically acquired resistance that is induced throughout the whole plant (Dangl et al., 1996; Collinge et al., 2002; Hutcheson, 1998).

The first step to activate defense responses in the plant is the recognition of pathogen attack. Pathogen-produced proteins can function as eliciting stimuli that trigger the plant's primary responses. The primary function of most major resistance genes is to encode for receptors that bind specific pathogen-derived molecules according to the protein dimer model (Hutcheson, 1998), or gene-for-gene interaction (McDonald and Linde, 2002). According to this model, one or more pathogen proteins which are *Avr* gene products bind to a receptor domain of the plant's resistance (R) gene product and this stimulates signal transduction pathways to initiate defense responses (Dangl et al., 1996; Hutcheson, 1998; McDonald and Linde, 2002). The genetics of resistance can be roughly divided into three categories: monogenic (vertical), oligogenic, and quantitative (horizontal).

5.1. Monogenic, "vertical," "major-gene" resistance

Monogenic resistance is mainly via a hypersensitive foliar reaction directly involving single major R genes, designated as *Pm* (powdery mildew) genes, in a gene-for-gene interaction (Bennett, 1984; Chen and Chelkowski, 1999; Hsam and Zeller, 2002). This type of resistance can be complete or partial (Leath and Heun, 1990; Hsam

and Zeller, 2002; Paillard et al., 2000), and resistant plants may show from no to moderate signs and symptoms of infection, while susceptible plants show more severe signs and symptoms. Monogenic resistance is generally very efficient but tends to be a short term solution if variability in the pathogen population is great (Hsam and Zeller, 2002). Shifts in virulence are common ways for pathogens to overcome single resistance genes (Bennett, 1984). Out of more than 160 known genes conferring race-specific resistance against powdery mildew in wheat and barley (*Hordeum vulgare*) the *mlo* resistance gene in barley is the only exception conferring durable resistance to date (Limpert et al., 1999).

Mains (1933) demonstrated that there was more than one physiological form of *B. g. tritici*. A large number of virulent powdery mildew strains have been isolated which supports the inevitability of virulence shifts. There is strong evidence of dispersal of genes and genotypes of powdery mildew from the regional to the continental level and beyond (Limpert et al., 1999). Fixation of genes for virulence, avirulence and fungicide resistance cannot be expected due to a population size several orders of magnitude larger than plants and animals, a mixed reproduction system and high mutation rates (Limpert et al., 1999; McDonald and Linde, 2002). Sexual cycles produce many new allele combinations and asexual cycles can quickly increase the frequencies of selected genotypes. Selection is the main force that changes frequency of mutant alleles and genotypes. Directional selection is common when a major resistance gene becomes widely used over a large area. This will result in an increase in the frequency of the virulent mutant that has lost the elicitor (*Avr* gene mutated to *avr*, the virulence gene) and the resulting loss of effectiveness of the major gene (McDonald and Linde, 2002).

Virulence frequencies of the pathogenicity genes corresponding to *Pm* genes were found to cover a very wide range across different geographical regions for most of the tested R gene combinations (Chen and Chelkowski, 1999). In *B. g. hordei*, a close relative to *B. g. tritici*, Pedersen et al., (2002), described a very dynamic and evolutionarily potent genome based on distribution of 359 AFLP, RFLP, and SNP markers. The whole genome is organized in 34 linkage groups covering a genetic map of 2114 cM with a physical map estimated to be bigger than 35 Mb. Many known *Avr* genes were organized in several tightly linked clusters. They identified a single linkage group that contained 11 different *Avr* genes with distances from 2.4 cM to 46.3 cM.

As selection promotes a rapid increase in virulent races of the pathogens, new R genes that can match products of new *Avr* genes evolve in the host. It is currently believed that cross-over(s) and gene conversion are primary forces in generating R gene diversity (Hammond-Kosack and Jones, 1997). Common distributions of R loci throughout plant genomes are: 1) R allele as a single copy that is present in resistant and absent in susceptible line; 2) Single R locus with multiple alleles each giving specificity for different *Avr* recognition; 3) A complex R locus comprised of tandem arrays of closely linked R loci with multiple alleles with different specificities. An example is the complex *MlRE* locus comprised of 3 linked loci in wheat for resistance to powdery mildew (Chantret et al., 2000); 4) R genes for resistance to different pathogens are clustered into "major resistance complexes" (Hammond-Kosack and Jones, 1997). The *Pm6* gene, closely linked to the *Sr36* gene conferring resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in wheat is an example (Friebe et al., 1991). Clustering of R genes

is likely due to the initial duplication of the genomic segment within the ancestral gene (Hammond-Kosack and Jones, 1997).

5.1.1. Analysis of host-pathogen interactions

Analysis of host-pathogen interactions, also called gene postulation, is an efficient way of determining the presence or absence of specific *Pm* genes in wheat genotypes as well as virulence genes in mildew populations (Heun and Fischbeck, 1987; Chen and Chelkowski, 1999; Hsam and Zeller, 2002). A set of race-specific powdery mildew isolates is used to differentiate between host lines with different single resistance genes or specific gene combinations. Powers and Sando (1960) demonstrated that the gene-for-gene principle between wheat and its powdery mildew pathogen. They studied relationships between two independent genes for resistance to powdery mildew in wheat and two genes for pathogenicity in *E. graminis*. Briggle (1969) demonstrated that multiple alleles at the same *Pm* locus can be effective against different strains of *E. graminis*, and suggested that isolines with known *Pm* genes can be used to differentiate various strains of *E. graminis*. When hosts with a specific *Pm* gene are challenged with a powdery mildew isolate avirulent for that *Pm* gene, the incompatible infection types (resistance) are expressed. This can be used to identify which resistance genes are present by testing the lines with differential set of *E. graminis* isolates with known *Avr* genes (Hsam and Zeller, 2002). Virulent powdery mildew isolates are commonly identified by using sets of wheat genotypes that contain known defeated *Pm* genes and are known to be sensitive to powdery mildew infection (Namuco et al., 1987; Niewoehner and Leath, 1998; Hsam and Zeller, 2002). Alternatively, a tester set of known mildew strains can be

used to identify if known genes for powdery mildew resistance are incorporated in wheat plants (Heun and Fischbeck, 1987).

Leath and Heun (1990) were able to postulate the presence of resistance genes based on the gene-for-gene concept and pedigree analysis in a study of 22 soft red winter wheat cultivars inoculated with 27 powdery mildew isolates with known virulence genes. They found that some of the tested cultivars carried previously unknown genes. Near-isogenic lines of wheat, differing in *Pm* genes, have been used to test the efficacy of specific *Pm* genes (Briggle, 1969; Leath and Murphy, 1985). The detached seedling leaf technique is commonly used in the analysis of host-pathogen interactions (Hsam and Zeller, 2002). This method may have reduced efficacy in certain situations because plants with intermediate disease response show more severe infection on detached leaves than intact seedlings (Hsam and Zeller, 2002).

5.1.2. Cytogenetic and genetic analysis

These types of analysis can be used to determine the chromosomal location of certain *Pm* genes and the inheritance of resistance (Chen and Chelkowski, 1999; Hsam and Zeller, 2002). Cytogenetic methods are especially useful for identification of *Pm* genes introduced from wild species. The hexaploid genome of wheat is more tolerant of chromosome engineering than the diploid genomes. Homeologous chromosomes share many genes with the same function which allows compensation for each other in nullisomic and tetrasomic combinations and for alien chromosome interaction (Friebe et al., 1991). Chromosome banding methods such as C-banding can be used to identify all 21 chromosome pairs of common wheat (Friebe et al., 1991), as well as to detect wheat-alien species translocations (Jiang et al., 1994). Another cytogenetic method for detection

of wheat-alien translocations is *in situ* hybridization (Friebe et al., 1991; Jiang et al., 1994).

Monosomic analysis is commonly used to determine chromosomal locations of the resistance genes. Analyses using a monosomic series of the cultivar Chinese Spring was used to find chromosomes carrying different *Pm* genes (Friebe et al., 1991; Hsam et al., 1998; Sourdille et al., 1999). Each of the 21 monosomics can be used as a female parent in the cross with a resistant line. The F_1 individuals are cytologically evaluated and F_2 line segregation ratios can confirm the distorted segregation for the resistance if the genes are on the homologous chromosomes. Once the chromosomal locations have been determined, allelic crosses are made to lines with *Pm* genes known to be on that chromosome. Study of segregation patterns of combined genes reveals if they are alternate alleles at the same locus or different linked loci.

5.1.3. Molecular markers

Various molecular techniques have been used to characterize and manipulate resistance genes and to dissect different types of resistance. Molecular markers were used for mapping monogenic resistance, characterization of quantitative resistance in germplasms and marker-aided selection (Michelmore, 1995). Molecular identification of specific DNA sequences can be used to identify the presence or absence of *Pm* genes, their chromosomal location, the number of genes present in a cultivar and the way in which they are transmitted to progeny (Chen and Chelkowski, 1999). Molecular marker techniques commonly used for identification and confirmation of *Pm* genes to powdery mildew are: 1) Restriction Fragment Length Polymorphisms (**RFLP)**: *Pm1*, *Pm2*, *Pm3b*, *Pm4a* (Ma et al., 1994), *Pm1*, *Pm2*, and *Pm18* (Hartl et al., 1995), *Pm1c* (Hartl et al.,

1999), *Pm2* (Mohler and Jahoor, 1996), *Pm2*, *Pm4a*, *Pm21* (Liu et al, 2000), *Pm3a*, *Pm3b*, and *Pm3c* (Hartl et al., 1993), *Pm3g* (Sourdille et al., 1999), *Pm6* (Tao et al., 2000), *Pm12* (Jia et al.,1996), *Pm13* (Cenci et al., 1999), *Pm17* (Hsam et al., 2000), *Pm21* (Liu et al., 1999), *Pm26* (Rong et al., 2000), *Pm 27* (Järve et al., 2000), *Pm29* (Zeller et al., 2002); 2) Random Amplified Polymorphisms (**RAPD)**: *Pm1* (Hu et al., 1997), *Pm1*, *Pm2*, *Pm3*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm4a*, *Pm12* (Shi, 1997), *Pm13* (Cenci et al., 1999), *Pm18* (Hartl et al., 1995), *Pm21* (Qi et al., 1996; Liu et al., 1999), and *Pm25* (Shi et al., 1998); 3) Amplified Fragment Length Polymorphisms (**AFLP)**: *Pm1c*, and *Pm4a* (Hartl et al.,1999), *Pm17* (Hsam et al., 2000), *Pm24* (Huang et al., 2000a), *Pm29* (Zeller et al., 2002); 4) **Microsatellites**: *Pm24* (Huang et al., 2000a), *Pm27* (Järve et al., 2000), *Pm30* (Liu et al., 2002). 5) Sequence Tagged Site (**STS)**: *Pm1* (Hu et al., 1997), *Pm2* (Mohler and Jahoor, 1996), *Pm13* (Cenci et al., 1999), 6) Sequence Characterized Amplified Region (**SCAR)**: *Pm21* (Liu et al., 1999), 7) Differential Display Reverse Transcriptase (**DDTR)**: *Pm13* (Cenci et al., 1999). Various marker techniques have been used to identify and map not only *Pm* genes but other single genes (Chantret et al., 2000), ADPR genes (Eser, 1998), and/or QTL's (Mingeot et al., 2002), controlling resistance to powdery mildew in wheat.

The **AFLP** technique is based on selective PCR amplification of restriction fragments from total genomic DNA (Zabeau, 1993). This technique generates fingerprints of any genomic DNA regardless of the size or complexity of the genome (Vos et al., 1995). These restriction fragments can than be used to construct genetic maps of many species including wheat (Vos et al., 1995; Huang et al., 2000b). AFLP is currently one of the most efficient techniques for generating a large number of DNA

markers (Myburg et al., 2001) and is very suitable for analysis of large genomes such as in wheat. This technique has been successfully implemented to identify markers closely linked to *Pm* genes (Hartl et al., 1999) and generation of molecular maps in wheat (Huang et al., 2000b; Hazen et al., 2002).

5.1.4. Chromosomal locations of *Pm* genes

The presence of *Pm* resistant genes is crucial not only for monogenic resistance but defeated *Pm* genes often confer oligogenic and quantitative type resistance when combined together (Royer et al., 1984; Pedersen and Leath, 1988; Paillard et al., 2000). Over 43 alleles at about 30 loci (designated as *Pm1-Pm30*) have been identified as major genes for vertical resistance to powdery mildew in wheat (McIntosh et al., 1998; Chen and Chelkowski, 1999; Keller et al., 1999; Hsam and Zeller, 2002) (Table 1.1).

Analysis of host-pathogen interactions, chromosomal (cytogenetic) analysis and molecular marker techniques have been utilized for determining chromosomal locations of *Pm* genes, thus far. The locations of known *Pm* loci have been assigned as follows: 1A-*Pm3, Pm17, Pm25*; 1B-*Pm8, Pm28*; 1D-*Pm10*, *Pm22, Pm24* (only in Hsam and Zeller, 2002); 2A-*Pm4, Pm23*; 2B-*Pm6, Pm26*; 3B-3S (substituted)-*Pm13*, and 3B- and 3D-*Pm13*; 4A-*Pm7* (only in Bennet, 1984), *Pm16*; 4B-*Pm7*; 5B-*Pm30*; 5D-*Pm2*; 6A-*Pm21*; 6B-*Pm11, Pm12, Pm14, Pm20, Pm27*; 6D-*Pm24* (only in Chen and Chelkowski, 1999); 7A-*Pm1, Pm9, Pm18*; 7B- *Pm5*; 7D-*SuPm8*, *Pm15, Pm19, Pm29* (Bennett, 1984; Chen and Chelkowski, 1999; Hsam and Zeller, 2002). The chromosomes with the highest number of *Pm* loci are 6B and 7B with 5 known *Pm* loci, each. Chromosomes without known *Pm* genes are 2D, 3A, 4D, 5A, and possibly 6D (according to Hsam and Zeller, 2002). Loci that contain more than one resistance allele are *Pm*1 with 4 alleles, *Pm*3 with

9 alleles, *Pm*4 with 2 alleles, *Pm*5 with 4 alleles (Chen and Chelkowski, 1999; Hsam and Zeller, 2002). Hsam and Zeller (2002) stated that loci *Pm10*, *Pm11*, *Pm14*, and *Pm15* contain individual genes for resistance to *Erysiphe graminis* f.sp. *agropyri* and are not effective against *Blumeria graminis* f. sp. *tritici*.

5.1.5. Sources and distribution of resistance genes

Common sources of *Pm* genes are various species within the primary, secondary and tertiary gene pools. Common wheat is an allohexaploid species (2n=6x=42), with three distinct genomes (AABBDD). Many of the resistance genes were introduced from ancestral and other wild species related to common wheat such as *Triticum monococcum*, close relative of the A genome progenitor *Triticum uratu*, the B genome progenitor *Aegilops speltoides*, and the D genome progenitor *Ae. tauschii* (Hsam and Zeller, 2002; Jiang et al., 1994). Although Bennett (1984) reported that just a small number of *Pm* genes have been identified which originated in the cultivars of *T. aestivum*, Chen and Chelkowski (1999) and Hsam and Zeller (2002) reported a total of 22 resistance alleles at 10 loci in *T. aestivum* indicating that *Pm* genes may still be found in cultivated wheat. Mains (1933) identified the wild wheat relatives *T. monococcum* (AA genomes), *T. dicoccum* (AABB), and *T. timopheevi* (AAGG) as sources of resistance genes to powdery mildew as early as 1933. Screening of old wheat cultivars, land-races and related species for resistance to powdery mildew started in the 1930's (Hsam and Zeller, 2002). *Pm* genes were identified in many different, widely distributed wheat cultivars and landraces. *Pm5a* and *Pm5b*, followed by *Pm2*, *Pm6*, and *Pm8* are the most common in Europe and Mediterranean cultivars. *Pm3a* is commonly found in wheat cultivars grown in diverse geographical locations including the Balkans, Japan and the US. *Pm3c* was identified in

Germany, while *Pm3d* was found in several European countries and China. *Pm4a* has been used in commercial wheat cultivars in Germany and China. A number of commercially grown cultivars have been found to have *Pm* gene combinations (Heun and Fischbeck, 1987). The best known cultivars are Normandie with *Pm1*, *Pm2*, and *Pm9*, Maris Huntsman with *Pm2* and *Pm6*, Kronjuvel with *Pm4b* and *Pm8*, and 623/65 with *Pm4b* and *Pm8*.

Gene transfer from species within the primary gene pool of *Triticum* that have chromosomes homologous to wheat can be done directly by hybridization, homologous recombination and backcrossing. Powdery mildew resistant genes come from several different species within the primary gene pool, including: 1) diploid einkorn wheat (*T. monococcum*) (2n=2x=14, AA genome), the source of *Pm25* (Shi et al., 1998; Murphy et al., 1998); 2) diploid *Ae. tauschii* (2n=2x=14, DD genome) the source of powdery mildew resistance genes transferred into adapted germplasms (Cox et al., 1992; Murphy et al., 1998; 1999b), including *Pm2* and *Pm19* (Hsam and Zeller, 2002); 3) tetraploid *T. dicoccum* (2n=4x=28, AABB), a source of genes for resistance to powdery mildew (Bennett, 1984; Navarro et al., 2000; Hsam and Zeller, 2002) including *Pm4a* and *Pm5a* (Hsam and Zeller, 2002); 4) tetraploid *T. durum* (2n=4X=28, AABB), a somewhat less valuable source of resistance to powdery mildew (Mains, 1934; Hsam and Zeller, 2002), although it contributed *Pm3h* (Zeller and Hsam, 1998); 5) tetraploid wild emmer wheat (*T. dicoccoides*) (2n=4x=28, AABB), the progenitor of common tetraploid and hexaploid wheats (Liu et al., 2002) and the source of *Pm16*, *Pm26*, and *Pm30* (Rong et al., 2000; Liu et al., 2002; Hsam and Zeller, 2002). This species is highly regarded as a source for *Pm* and other resistance genes (Mains, 1934; Grechter-Amitai and Van Silfhout, 1984;

Hsam and Zeller, 2002); 6) tetraploid *T. carthlicum* (2n=4x=28, AABB genomes) was a donor of *Pm4b* (Hsam and Zeller, 2002).

Polyploid *Triticum* and *Aegilops* genotypes sharing at least one common genome with *T. aestivum* belong to the secondary gene pool. Gene transfer may be by direct hybridization (if genes are on the homologous chromosomes), or may require special cytogenetic techniques such as embryo rescue (Jiang et al., 1994) if genes are on homeologous chromosomes. A number of diploid and tetraploid species belong to this group, and some have been used as sources of resistance genes: 1) tetraploid cultivated *T. timopheevii* and it's wild form, *T. araraticum*, (2n=4x=28, AAGG), contributed *Pm6*, *Pm27* and at least one more *Pm* gene (Mains, 1934; Järve et al., 2000; Hsam and Zeller, 2002; Murphy et al., 2002); 2) *Ae. speltoides* (2n=2x=14, SS) was the donor of *Pm1d* and *Pm12* (Hsam and Zeller, 2002) and 3) *Ae. longissima* (2n=2x=14, SS), was the donor of *Pm13* (Cenci et al., 1999). *Ae. speltoides* and *Ae. longissima* are both diploid species with the S genome, which is closely related to the B genome of wheat and show colinearity with at least five chromosomes with the wheat D genome (Zhang et al., 2001; Hsam and Zeller, 2002).

Other species such as *Dasypyrum* (*Hylandia*)(2n=2x=14, VV), cultivated rye (*Secale cereale*) (2n=2x=14, RR), and some *Aegilops* species which do not share common genomes with wheat belong to the tertiary gene pool. With such donor parents a homologous recombination cannot be used for gene transfer. Genetic techniques such as induction of chromosome translocations by radiation or the induced mutation at the *Ph1* locus on chromosome 5BL or the lack of the 5B chromosomal pair can be used to facilitate gene transfer (Jiang et al., 1994). The products of these methods are wheat/alien

chromosome translocation, or recombination lines. Four *Pm* genes were transferred from rye into cultivated wheat (Hsam and Zeller, 2002). The 1RS chromosome arm from rye is the most widely incorporated alien chromatin in current wheat genomes (Hsam et al., 2000.). *Pm7* is present in the germplasm line Transec as a 4BS.4BL-5RL translocation. *Pm8* derived from rye cultivar Petkus (Ren et al., 1997) and *Pm17* are both located on the short arm of the 1R chromosome in rye. *Pm8* and *Pm17* segregated independently from each other in Amigo wheat which indicated two distinct translocations. *Pm8* is located in T1BL.1RS, and *Pm17* is located in T1AL.1RS wheat-rye translocation lines (Heun et al., 1990; Friebe et al., 1994). Ren et al., (1997) reported at least one major gene *SuPm8* controlling genetic suppression of the rye-derived gene. *Pm20* was transferred from the 6RL rye chromosome into common wheat.

Aegilops ovata (2n=4X=28, UUMM) was the donor of *Pm29* and the wild diploid *Hyanaldia vilosa* (2n=2x=14, VV) was the donor of *Pm21* (Zeller et al., 2002). Other species with potentially useful powdery mildew resistance genes are *Ae. caudata*, *Ae. markgrafii*, , *Ae. umbelluata*, *Ae. variabilis*, *Ae. triuncalis*, and *Ae. mutica*, as well as the perennial subspecies of *Triticae*, such as *Elymus*, *Leymus* and *Thinopyrum*, (Jiang et al., 1994; Eser, 1998; Hsam and Zeller, 2002).

5.2. Oligogenic and quantitative resistance

Distinction between oligogenic and quantitative (Hautea et al., 1987), or even qualitative and quantitative (Hsam and Zeller, 2002) resistance is not always possible. Qualitative resistance is considered to be race-specific with gene action controlled by individual *Pm* genes, while quantitative resistance is non-race specific with additive and possible some dominant gene effects. The main difference between oligogenic and

quantitative resistance is that the number of genes involved is smaller for oligogenic than quantitative resistance although it is possible that they can vary in degree of dominance for their gene effects. Complete resistance or immunity is usually expressed during all stages of the plant's life while some forms of partial resistance are only expressed as the plant develops (Kinane and Jones, 2000). Partial resistance, slow mildewing, horizontal resistance and field resistance and adult-plant resistance (APR) are common terms for resistance which does not confer immunity (Griffey and Das, 1994). These types of resistances are considered to be more durable than monogenic resistance (Chen and Chelkowski, 1999). They may be expressed as seedling, adult-plant, or whole plant resistance depending on the genetic background of the wheat plants (Bennett, 1984).

Oligogenic and quantitative resistances to powdery mildew are controlled by two to three genes with or without dominance effects (Das and Griffey, 1995; Chung and Griffey, 1995), or by larger number of polygenes with primarily cumulative effects (Hsam and Zeller, 2002). Both types of resistance are characterized by the degree of resistance, expressed in a form of reduction in the rate of disease development in the host, rather than by the presence or absence of disease. They are often described as partial resistance (Kinane and Jones, 2000). Quantitative field resistance refers to the degree of resistance in field conditions and Kmecel et al. (1995) suggested that it results from composite effects of environmentally determined resistance, acquired (induced) resistance, and horizontal resistance. Some components of partial resistance are latent period, pustule density and conidium production (Kinane and Jones, 2000). This type of resistance can be caused by an increase in incubation time and latent period, a decrease in infection frequency and infection period and a reduction in colony size and spore

production. The genetic component of partial resistance is believed to be controlled by many genes, each with a small effect. Breeding for this type of resistance is a labor-intensive and time-consuming process. An evaluation of partial resistance in the field may have a large experimental error due to environmental factors such as soil heterogeniety, biotic and abiotic stresses. Evaluations of components of resistance in controlled greenhouse environments will reduce experimental error but field tests are necessary to evaluate the true efficiency of the resistance. In a field study of 60 Chinese wheat cultivars, mean disease intensities ranged from close to 0 to over 90% (Yu et al., 2001). Analysis based on AUDPC found interaction effects of cultivar x location, cultivar x inoculation, location x inoculation, and cultivar x location x inoculation were all small compared to the main effects of cultivar and location (Yu et al., 2001). This study also demonstrated efficiency of partial resistance in the field.

The soft red winter wheat cultivars Knox and Massey have APR to powdery mildew. Both are completely susceptible to powdery mildew at the seedling stage but mature plants do not exhibit severe disease (Shaner, 1973; Griffey and Das, 1994). These cultivars exhibit a decrease in disease intensity as the leaves mature and age compared to fully susceptible lines. Another example of durable APR is the wheat landrace line k-15560 selected at the Derbent Experimental Breeding Station, Dagestan, Transcaucasia (Peusha et al., 2002). All three cultivars had continuously effective resistance in commercially grown conditions for at least 13 to 20 years. Knox and its progeny line Knox 62 were grown on more than 0.7 million ha annually from 1954-1969 (Shaner, 1973). No *Pm* resistance gene in wheat was reported as durable as the APR in three cultivars. Griffey and Das (1994) estimated the number of genes controlling APR in the

cultivars Knox and Massey to be two to three. Monosomic and hybridization studies of the wheat line k-15560 demonstrated that resistance at the seedling stage was controlled by a single dominant gene located on chromosome 7B. The resistance at the adult stage was controlled by two independent dominant genes (Peusha et al., 2002). A range of between one and seven QTLs governed APR in the wheat line RE714 (Mingeot et al., 2002). RE714 had two previously known resistance genes, *Pm4b* and *MlRE*. Two QTLs with large effects were located on the chromosome 5D and at the *MlRE* locus on chromosome 6A in two populations across all tested environments. Residual effect of *Pm4b* was significant in one genetic background (Mingeot et al., 2002). Chantret et al. (2000) found that the *MlRE* gene in RE714 was composed of three different loci, linked at 9.3 cM, and 9.4 cM.

Genetic effects controlling APR and quantitative resistance to powdery mildew in seven wheat winter and spring wheat lines were investigated in two separate studies. Das and Griffey (1995) conducted a diallel study of gene action for APR in four wheat lines. Additive-dominance and digenic epistasis models were sufficient to explain the variation in the expression of APR. Additive gene effects were predominant in all six crosses. Epistasis was significant in five crosses, dominance effects in four, additive x additive in three, additive x dominance in two and dominance x dominance in one cross. They suggested that selection for APR may be most effective in the advanced generations due to significance of non-additive gene effects. Hautea et al. (1987) studied inheritance of partial resistance to powdery mildew in four spring wheat cultivars. Additive gene effects were predominant in all four crosses. Additive x additive and additive x dominance effects were significant for one and two crosses, respectively. Dominance effects were

not significant in any of the crosses. Heritability estimates based at F_3/F_2 regression, were up to 32% indicated that selection may be insufficient when individual F_2 plants are used as selection units. Mass selection in F_2 and evaluation in F_4 was effective. Digenic epistatic components were significant in two crosses which might be due to interactions between alleles at homeologous loci. If this is the case the interacting alleles can be fixed in homozygous genotypes and used as a form of gene pyramiding of resistance genes.

Residual effects of several partially defeated *Pm* genes have been reported to have a significant role in resistance to powdery mildew (Nass et al., 1981, Bennet, 1984; Pedersen and Leath, 1988; Mingeot et al., 2002). Nas et al. (1981) suggested that use of combined *Pm* genes with residual resistance can be used in breeding programs for limiting epidemic development of powdery mildew. The ability of defeated *Pm* genes to show residual effects may depend on genetic background of the pathogen (Nass et al., 1981) and genetic background of the wheat line containing the *Pm* gene (Mingeot et al., 2002).

5.3. Gene pyramiding

Gene pyramiding, or the accumulation of multiple resistance genes into a single genotype, is one way to provide durable resistance to a range of pathogen races (Pedersen and Leath, 1988). Gene pyramiding is a method used to diversify host resistance if race-specific (*Pm*) genes are available (Hsam and Zeller, 2002). Various resistance genes can be pyramided together including major and minor genes, effective and defeated genes, race specific and race non-specific genes (Pedersen and Leath, 1988). Pyramiding of resistance genes is a long and expensive process similar to the development of a multiline. Success depends on the pyramiding of efficient alleles at multiple loci with the

primary criterion for a pyramid being its durability. In order to overcome pyramided genes the pathogen genotypes have to undergo multiple simultaneous changes, which are less likely than a mutation to overcome a single gene.

Durability of resistance is dependent on stabilizing selection. The selection pressure is smallest when there are compatible relationships for all R loci in the host to *Avr* loci in the pathogen. The host line that accumulates partial effects of many resistance genes will put some selection pressure on the pathogen. Low levels of disease are likely to develop over time. Such polygenic resistance should be long-term and controlled by additive gene action.

Some limitations and consequences of implementation of this system should be considered (Bennett, 1984; Pedersen and Leath, 1988; Hsam and Zeller, 2002). If the defeated genes have small effects, three or four genes may be needed to achieve a useful level of resistance. It may be difficult to assure that all resistance genes are in the homozygous state. The use of *Pm* genes already matched by the pathogen may result in only short-term resistance. Some combinations of pyramided *Pm* genes may become inefficient if challenged by complex pathogenicity. It is still unknown if this method could cause the development of the super race of powdery mildew. Such a race would exhibit a broad and complex virulence spectrum. A practical application of pyramiding *Pm* genes is in a progress. Liu et al. (2000) reported on marker-assisted selection of the three pairwise pyramids of *Pm2*, *Pm4a*, and *Pm21*. These gene combinations were introduced into an elite Chinese cultivar Yang 158 from wheat lines carrying *Pm2, Pm4a* and *Pm21* by six to eight backrosses. The isolines carrying different *Pm* genes were crossed pairwise and resulting F_2 populations were evaluated for presence of individual

Pm genes. Double-haploid lines from crosses between different lines carrying *Pm* genes were selected. Presence of the *Pm* genes in pyramids have been confirmed by specific tests of powdery mildew resistance and C-banding.

6. Efforts to breed for resistance to powdery mildew at North Carolina State University

In 1987 the small grains breeding and pathology programs at NC State University initiated a program of inter-specific hybridization, using powdery mildew resistant diploid and tetraploid relatives as donor parents and the soft red winter wheat cultivar Saluda as the recurrent parent. To date 11 BC_2F_5, BC_2F_6, and BC_2F_7 derived lines have been released with resistance genes incorporated from *T. monococcum, T. turgidum, T. timopheevii,* and *Ae. tauschii* (Murphy et al., 1998; 1999a; 2002; 1999b; Navarro et al., 2000). These 11 germplasm lines are being utilized in numerous wheat cultivar development programs in the Southern United States and Europe. Shi et al. (1998) studied the genetics of NC96BGTA5 and identified a single gene, *Pm25*, that confirmed powdery mildew resistance in this germplasm line. He identified three RAPD markers, distanced 11.3 cM, 15.5 cM, and 19.7 cM from the resistance locus. In order for these resistances to be combined in gene pyramids it is essential that we learn more about the genetics of resistance in these germplasm lines and their linkage to molecular markers.

7. Objectives

The objectives of this research are: 1) To determine the inheritance of resistance to powdery mildew and identify the number of resistance genes in the germplasm lines NC96BGTD1, NC96BGTA4, NC98BGTAB10, and NC99BGTAG11 and 2) To identify AFLP markers linked to the resistance genes which can be used to identifying plants with pyramided genes in segregating populations from crosses between the germplasm lines.

LITERATURE CITED:

Agrios, G. 1997. Plant Pathology. 4th. Edition. pp. 286-287. Academic Press, San Diego, CA.

Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathol. 33:279-300.

Bowen, K.L., and S. Leath. 1989. Yield reductions due to powdery mildew and leaf rust on winter wheat in North Carolina. Phytopathology. 79(3):371.

Bowen K.L., K.L. Everts, and S. Leath. 1991. Reduction in yield of winter wheat in North Carolina due to powdery mildew and leaf rust. Phytopathology. 81(5):503-511.

Braun, U, R.T.A. Cook, A.J. Inman, H.-D. Shin. 2002. The taxonomy of the powdery mildew fungi. p.13-55. *In* Bélanger, R.R., W.R. Bushnell, A.J. Dik and T.L.W. Carver (ed). The powdery mildews: A Comprehensive Treatise. The American Phytopathological Society, St. Paul, MN.

Briggle, L.W. 1969. Near-isogenic lines of wheat with genes for resistance to *Erysiphe graminis* f. sp. *tritici*. Crop Sci.9:70-72.

Broscious, S.C., F.A. James, and J.R. Frederick. 1985. Influence of winter wheat management practices on the severity of powdery mildew and septoria blotch in Pennsylvania. Phytopathology. 75(5):538-542.

Cenci, A., R. D'Ovidio, O.A. Tanzarella, C. Ceoloni, and E. Porceddu. 1999. Identification of molecular markers linked to *Pm13*, an *Aegilops longissima*gene

conferring resistance to powdery mildew in wheat. Theor. Appl. Genet.98: 448-454.

Chantret, N., P. Sourdille, M. Röder, M. Tavaud, M. Bernard, and D. Dousinault. 2000. Location and mapping of the powdery mildew resistance gene *MlRE* and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. Theor. Appl. Genet. 100:1217-1224.

Chen, Y., and J. Chelkowski. 1999. Genes for resistance to wheat powdery mildew. J. Appl. Genet. 40(4):317-334.

Chung, Y.S., and C.A. Griffey. 1995. Powdery mildew resistance in wheat: I. Gene number and mode of inheritance. Crop Sci. 35:378-382.

Collinge, D.B., P.L. Gregersen, and H. Thordal-Christensen. 2002. The nature and role of defense response genes in cereals. p. 146-160. *In* Bélanger, R.R., W.R. Bushnell, A.J. Dik and T.L.W. Carver (ed). The powdery mildews: A comprehensive treatise. The American Phytopathological Society, St. Paul, MN.

Cox, T.S., W.J. Raupp, D.L. Wilson, B.S. Gill, S. Leath, W.W. Bockus, and L.E. Browder. 1992. Resistance to foliar diseases in collection of *Triticum tauschii* germ plasm. Plant Disease 76(10):1061-1064.

Dangl, J.L., Dietrich, R.A., and M.H. Richberg. 1996. Death don't have mercy: Cell death programs in plant-microbe interactions. Plant Cell 8:1793-1801.

Das, M.K., and C.A. Griffey. 1995. Gene action for adult-plant resistance to powdery mildew in wheat. Genome 38:277-282.

Eser, V. 1998. Characterization of powdery mildew resistant lines derived from crosses between *Triticum aestivum* and *Aegilops speltoides* and *Ae. mutica*. Euphytica 100:269-272.

Everts, K.L., and S. Leath. 1992. Effect of early season powdery mildew on development, survival, and yield contribution of tillers of winter wheat. Phytopathology 82(11):1273-1278.

Friebe, B., J. Jiang, W.J. Raupp, R.A. McIntosh, and B.S. Gill. 1991. Characterization of wheat-alien translocation conferring resistance to diseases and pests: current status. Euphytica: 91:59-87.

Friebe, B., M. Heun, N. Tuleen, F.J. Zeller, and B.S. Gill. 1994. Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci. 34:621-625.

Götz, M., and C. Boyle. 1998. Haustorial function during development of cleistothecia in *Blumeria graminis* f. sp. *tritici*. Plant Dis. 82(5):507-511.

Grechter-Amitai, Z.K. and C.H. Van Silfhout. 1984. Resistance to powdery mildew in wild emmer (Triticum dicoccoides Körn.). Euphytica 33:273-280.

Griffey, C.A., and M.K. Das. 1994. Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey winter wheats. Crop Sci. 34:641-646.

Hammond-Kosack, K.E., and J.D.G. Jones. 1997. Plant disease resistance genes. Annual Reviews in Plant Physiology and Plant Molecular Biology 48:575-607.

Hartl, L., H. Weiss, F.J. Zeller, and A. Jahoor. 1993. Use of RFLP markers for identification of alleles of the *Pm3* locus conferring powdery mildew resistance in wheat (*Triticum aestivum*, L.). Theor. Appl. Genet. 86:959-963.

Hartl, L., H. Weiss, U. Stephan, F.J. Zeller, and A. Jahoor. 1995. Molecular identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 90:601-606.

Hartl, L., V. Mohler, F.J. Zeller, S.L.K. Hsam and G. Schweizer. 1999. Identification of AFLP markers closely linked to powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L.). Genome 42:322-329.

Hautea, R.A., W.R. Coffman, M.E. Sorrells, and G.C. Bergstrom. 1987. Inheritance of partial resistance to powdery mildew in spring wheat. Theor. Appl. Genet. 73:609-615.

Hazen, S.P., P. Leroy, and R. Ward. 2002. AFLP in Triticum aetivum L.: Patterns of genetic diversity and genome distribution. Euphytica 125:89-102.

Heun, M., and G. Fischbeck. 1987. Identification of wheat powdery mildew resistant genes by analyzing host-pathogen interactions. Plant Breed. 98:124-129.

Heun, M., B. Friebe, and W. Bushuk. 1990. Chromosomal location of the powdery mildew resistance gene of Amigo wheat. Phytopathology 80:1129-1133.

Hsam, S.L.K., X.Q. Huang, F. Ernst, L. Hartl, and F.J. Zeller. 1998. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell). 5. Alleles at the *Pm1* locus. Theor. Appl. Genet. 96:1129-1134.

Hsam, S.L.K., V. Mohler, L. Hartl, G. Wenzel, and F.J. Zeller. 2000. Mapping of powdery mildew and leaf rust resistance genes on the wheat-rye translocated chromosome T1BL.1RS using molecular and biochemical markers. Plant Breed. 119:87-89.

Hsam, S.L.K., and F.J. Zeller 2002. Breeding for Powdery Mildew Resistance in common wheat (*Triticum aestivum* L.). p. 219-238. *In* Bélanger, R.R., W.R. Bushnell, A.J. Dik and T.L.W. Carver (ed). The powdery mildews: A comprehensive treatise. The American Phytopathological Society, St. Paul, MN.

Hu, X.Y., H.W. Ohm, and I. Dweikat. 1997. Identification of RAPD markers linked to the gene PM1 for resistance to powdery mildew in wheat. Theor. Appl. Genet. 94:832-840.

Huang, X.Q., S.L.K. Hsam, F.J. Zeller, G. Wenzel, and V. Mohler. 2000a. Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor. Appl. Genet. 101:407-414.

Huang, X., Zeller, F.J., S.L.K. Hsam, G. Wenzel, and V. Mohler. 2000b. Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. Genome 43:298-305.

Hucheson, S.W., 1998. Current concepts of active defense in plants. Annu. Rev. Phytopathol. 36:59-90.

Jarvis, W.R., W.G. Gubler, and G.G. Grove. 2002. Epidemiology of powdery mildew in agricultural pathosystems. p. 169-199. *In* Bélanger, R.R., W.R. Bushnell, A.J. Dik and T.L.W. Carver (ed). The powdery mildews: A comprehensive treatise. The American Phytopathological Society, St. Paul, MN.

Järve, K., H.O. Peusha, J. Tsymbalova, S. Tamm, K.M. Devos, and T.M. Eno. 2000. Chromosomal location of a *Triticum timopheevii*-derived powdery mildew resistance gene transferred to common wheat. Genome 43:377-381.

Jenkyn, J.F., and A. Bainbridge. 1978. Biology and pathology of cereal powdery mildews. p. 283-321. *In* Spencer, D.M. (ed). The Powdery Mildews. Academic Press, Inc. London, England.

Jia, J., K.M. Devos, S. Chao, T.E. Miller, S.M. Reader, and M.D. Gale. 1996. RFLP-based maps of group-6 chromosomes of wheat and their application in the tagging of *Pm12*, a powdery mildew resistance gene transferred from Aegilops speltoides to wheat. Theor. Appl. Genet 92:559-565.

Jiang, J., B. Friebe, and B.S. Gill. 1994. Recent advances in alien gene transfer in wheat. Euphytica 73: 199-212.

Johnson, J.W., P.S. Baenziger, W.T. Yamazaki, and R.T. Smith. 1979. Effects of powdery mildew on yield and quality of isogenic lines of 'Chancellor' wheat. Crop Sci. 19:349-352.

Keller M., B. Keller, G. Schachermayr, M., Winzeler, J.E. Schmid, P. Stamp, and M.M. Messmer. 1999. Quantitative trait loci for resistance against powdery mildew in segregating wheat x spelt population. Theor. Appl. Genet. 98:903-912.

Kinane, J.T., and P.W. Jones. 2000. Components of partial resistance to powdery mildew in wheat mutants. Europ. J. Plant Pathol. 106: 607-616.

Kmecel, A., F. Mauch, M. Winzeler, H. Winzeler, and R. Dudler. 1995. Quantitative field resistance to powdery mildew and defense reactions at the seedling stage: identification of a potential marker. Physiol. Mol. Plant Path. 47:185-199.

Large, E.C. 1954. Growth stages in cereals illustration of the Feekes scale. Plant Pathol. 3(4):128-129.

Large, E.C, and D.A. Doling. 1962. The measurement of cereal mildew and its effect on yield. Plant Pathol. 11:47-57.

Large, E.C, and D.A. Doling. 1963. Effects of mildew on yield of winter wheat. Plant Pathol. 12:128-130.

Leath, S., and J.P. Murphy. 1985. Virulence genes of the wheat powdery mildew fungus, *Erysiphe graminis* f. sp. *tritici* in North Carolina. Plant Dis. 69:905.

Leath, S. 1987. The effects of early-season infection of wheat by *Erysiphe graminis* f. sp. *tritici* on late-season leaf area and yield. Phytopathology 77(12):1741.

Leath, S., and K.L. Bowen. 1989. Effects of powdery mildew, triadimenol seed treatment, and triadimefon foliar sprays on yield of winter wheat in North Carolina. Phytopathology 79(2):152-155.

Leath, S., and M. Heun. 1990. Identification of powdery mildew resistance genes in cultivars of soft red winter wheat. Plant Dis. 74(10): 747-752.

Limpert, E., F. Godet, and K. Müller. 1999. Dispersal of cereal mildews across Europe. Agric. For. Meteorol. 97:293-308.

Lipps, P.E., and L.V. Madden. 1989. Assessment of methods of determining powdery mildew severity in relation to grain yield of winter wheat cultivars in Ohio. Phytopathology 79(4):462-470.

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

Liu Z., Q. Sun, and T. Yang. 1999. Development of SCAR markers linked to the Pm21 gene conferring resistance to powdery mildew in common wheat. Plant Breed. 118:215-219.

Liu, Z., Q. Sun, Z. Ni, E. Nevo, and T. Yang. 2002. Molecular characterization of a novel powdery mildew resistance gene Pm30 in wheat originating from wild emmer. Euphytica 123: 21-29.

Ma, Z.Q., M.E. Sorrells, and S.D. Tanksley. 1994. RFLP markers linked to powdery mildew resistance genes *Pm1, Pm2, Pm3,* and *Pm4* in wheat. Genome 37:871-875.

Mains, E.B. 1933. Host specialization of *Erysiphe graminis tritici*, Proc. Natl. Acad. Sci. USA 19:49-53.

Mains, E.B. 1934. Inheritance of resistance to powdery mildew, *Erysiphe graminis tritici*, in wheat. Phytopathology 24:1257-1261.

Mather, K. 1951. The measurement of linkage in heredity. John Wiley and Sons, Inc. New York.

McDonald, B.A., and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40: 349-379.

McIntosh, R.A., G.E. Hart, K.M. Devos, M. D. Gale, and W.J. Rogers. 1998. Catalogue of gene symbols for wheat. Proceedings of the 9th International Wheat Genetics Symposium. p.123-145.

Michelmore, R. 1995. Molecular approaches to manipulation of disease resistance genes. Annu. Rev. Phytopathol. 15:393-427.

Mingeot, D., N. Chantret, P.V. Baret, A. Dekeyser, N. Boukhatem, P. Sourdille, G. Doussinault, and J.M. Jacquemin. 2002. Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible backgrounds. Plant Breed. 121:133-140.

Mohler, V., and A. Jahoor. 1996. Allele-specific amplification of polymorphic sites for the detection of powdery mildew resistance loci in cereals. Theor. Appl. Genet. 93:1078-1082.

Murphy, J.P., S. Leath, D. Huynh, R.A. Navarro, and A. Shi. 1998. Registration of NC96BGTD1, NC96BGTD2, and NC96BGTD3 wheat germplasm resistant to powdery mildew. Crop Sci. 38:570.

Murphy, J.P., S. Leath, D Huynh, R.A. Navarro, and A. Shi. 1999a. Registration of NC96BGTA4, NC96BGTA5, and NC96BGTA6 wheat germplasm. Crop Sci. 39:883-884.

Murphy, J.P., S. Leath, D Huynh, R.A. Navarro, and A. Shi. 1999b. Registration of NC97BGTD7 and NC97BGTD8 wheat germplasms resistant to powdery mildew. Crop Sci. 39:884-885.

Murphy, J.P., R.A. Navarro, and S. Leath. 2002. Registration of NC99BGTAG11 Wheat germplasm resistant to powdery mildew. Crop Sci. 42:1382.

Myburg, A.A., D.L. Remington, D.M. O'Malley, R.R. Sederof, and R.W. Whetten. 2001. High-throughput AFLP analysis using infrared dye-labeled primers and an automated DNA sequencer. Biotechnology 30(2):348-357.

Namuco, L.O., W.R., Coffman, G.C. Bergstrom, and M.E. Sorrolles. 1987. Virulence spectrum of the *Erysiphe graminis* f.sp. *tritici* population in New York. Plant Dis. 71(6):539-541.

Nass, H.A., W.L. Pedersen, D.R. MacKenzie, and R.R. Nelson. 1981. Phytopathology 71:1315-1318.

Navarro, R.A., J.P. Murphy, and S. Leath. 2000. Registration of NC97BGTAB9, and NC97BGTAB10 wheat germplasm lines resistant to powdery mildew. Crop Sci. 40:1508-1509.

Niewoehner, A.S., and S. Leath. 1998. Virulence of *Blumeria graminis* f. sp. *tritici* on winter wheat in the Eastern United States. Plant Dis. 82(1):64-68.

Paillard, S., I. Goldringer, J. Enjalbert, G. Doussinault, C. de Vallavielle-Pope, and P. Brabant. 2000. Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. I- Is specific seedling resistance selected?. Theor. Appl. Genet. 101:449-456.

Pedersen, W.L., and S. Leath. 1988. Pyramiding major genes for resistance to maintain residual effects. Annu. Rev. Phytopathol. 26:369-378.

Pedersen, C., S.W. Rasmussen, and H. Giese. 2002. A genetic map of *Blumeria graminis*, based on functional genes, avirulence genes, and molecular markers. Fungal Genetics and Biology 35:235-246.

Peusha, H., T. Lebedeva, O. Prilinn, and T. Enno. 2002. Genetic analysis of durable powdery mildew resistance in a common wheat line. Hereditas 136:201-206.

Powers H.R., and W.J. Sando. 1957. Genetics of host-parasite relationship in powdery mildew of wheat. Phytopathology 47:453.

Powers, H.R., and W.J. Sando. 1960. Genetic control of the host-parasite relationship in wheat powdery mildew. Phytopathology 50:454-457.

Qi, L., M. Cao, P. Chen, W. Li, and D. Liu. 1996. Identification, mapping, and application of polymorphic DNA associated with resistance gene Pm21 of wheat. Genome 39:191-197.

Ren, S.X., R.A. McIntosh, and Z.J. Lu. 1997. Genetic suppression of the cereal rye-derived gene *Pm8* in wheat. Euphytica 93:353-360.

Rong, J.K., E. Millet, J. Manisterski, and M. Feldman. 2000. A new powdery mildew resistance gene: Introgression from wild emmer into common wheat and RFLP-based mapping. Euphytica. 115:121-126.

Royer, M.H., R.R. Nelson., D.R. Mac Kenzie, and D.A. Diehle. 1984. Partial resistance of near-isogenic lines compatible with *Erysiphe graminis* f. sp. *tritici*. Phytopathology 74:1001-1006.

Schafer, J.F. 1987. Wheat powdery mildew. p. 579-584. *In* Heyne, E.G. (ed). Wheat and wheat improvement, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. Phytopathology 63:867-872.

Shi, A. N. 1997. Genetic analysis of wheat powdery mildew resistance. Ph.D. Thesis. North Carolina State University.

Shi, A.N., S. Leath, and J.P. Murphy. 1998. A major gene for powdery mildew resistance transferred to common wheat from wild eikorn wheat. Phytopathology 88(2): 144-147.

Sourdille, P., P. Robe, M. Tixier, G. Doussinault, M. Pavione, and M. Bernard. 1999. Location of *Pm3g*, a powdery mildew resistance allele in wheat, by using a monosomic analysis and by identifying associated molecular markers. Euphytica 110:193-198.

Tao, W., D. Liu, J. Liu, Y. Feng, and P. Chen. 2000. Genetic mapping of the powdery mildew resistance gene *Pm6* in wheat by RFLP analysis. Theor. Appl. Genet. 100:564-568.

Vos, P., R. Hogers, M. Bleeker, M. Reijans., T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acid Res. 23(21): 4407-4414.

Wolfe, M.S. 1984. Trying to understand and control powdery mildew. Plant Pathol. 33:451-446.

Wolfe, M.S., and J.M. McDermott. 1994. Population genetics of plant pathogen interactions: The example of the *Erysiphe graminis-Hordeum vulgare* pathosystem. Annu. Rev. Phytopathol. 32:89-113.

Yu, D.Z., X.J. Yang, L.J. Yang, M.J. Jeger, and J.K.M. Brown. 2001. Assessment of partial resistance to powdery mildew in Chinese wheat varieties. 2001. Plant Breed. 120: 279-284.

Zabeau, M. 1993. European patent application. Bulletin 93/13.

Zeller, F.J., and S.L.K. Hsam. 1998. Progress in breeding for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). p. 178-180.*In* Slinkard, A. E. (ed). Proceedings of the 9th International Wheat Genetics Symposium. Vol.1. University of Saskatchewan, Saskatoon, Canada.

Zeller, F.J., L. Kong, L. Hartl, V. Mohler, and S.L.K. Hsam. 2002. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.) 7. Gene Pm29 in line Pova. Euphytica 123: 187-194.

Zhang, H., S.M. Reader, X. Liu, J.Z. Jia, M.D. Gale, and K.M. Devos. 2001. Comparative genetic analysis of the *Aegilops longissima* and *Ae. sharonensis* genomes with common wheat. Theor. Appl. Genet. 103:518-525.

Table 1.1 *Pm* gene designation, chromosomal location and species source of resistance. Modified from Chen and Chelkowski (1999), and Hsam and Zeller (2002).

Pm gene	Chromosome	Source
Pm1a	7AL	*T. aestivum*
Pm1b	7AL	*T. aestivum*
Pm1c	7AL	*T. aestivum*
Pm1d	7AL	*T. spelta*
Pm2	5DS	*A. 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*
Pm3j	1AS	*T. aestivum*
Pm4a	2AL	*T. dicoccum*
Pm4b	2AL	*T. carthlicum*
Pm5a	7BL	*T. dicoccum*
Pm5b	7BL	*T. aestivum*
Pm6	2BL	*T. timopheevii*
Pm7	4BL/2RL	*S. secale*
Pm8	1BL/1RS	*S. secale*
SuPm8	7D	*T. aestivum*
Pm9	7AL	*T. aestivum*
Pm10	1D	*T. aestivum*
Pm11	6BS	*T. aestivum*
Pm12	6BS	*Ae. speltoides*

Table 1.1 Continued

Pm gene	Chromosome	Source
Pm13	3S'1S	*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. tauschii*
Pm20	6BS/6RL	*S.cereale*
Pm21	6VS/6AL	*Hayanaldia vilosa*
Pm22	1D	*T. aestivum*
Pm23	2AL	*T. aestivum*
Pm24	6D	*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*

Chapter 2

Inheritance of Resistance to Powdery Mildew (*Blumeria graminis* DC f. sp. *tritici* Em. Marchal) in Four Soft Red Winter Wheat (*Triticum aestivum* L.) Germplasm Lines and Relationships Among the Resistance Genes

ABSTRACT

This study investigated the inheritance, efficiency and possible relationship with other sources of resistance to naturally occurring powdery mildew in the four wheat germplasm lines NC96BGTD1 (NCD1), NC96BGTA4 (NCA4), NC98BGTAB10 (NCAB10), and NC99BGTAG11 (NCAG11). Each germplasm was crossed to the susceptible parent Saluda, which was utilized as the recurrent parent in the development of each germplasm line, and to each other. $F_{2:3}$ progenies of each of the four Saluda by germplasm line crosses and six germplasm-by-germplasm crosses were tested in greenhouse and field studies. Between 33 and 204 $F_{2:3}$ progenies were developed for each population. Resistance factors in all four germplasms segregated in a monogenic fashion. Five germplasm x germplasm populations (NCD1 x NCAG11, NCAG11 x NCAB10, NCA4 x NCAB10, NCD1 x NCAB10, and NCD1 x NCA4) segregated in a digenic fashion, indicating independent segregation of their resistance loci. Resistance genes in NCA4 and NCAG11 co-segregated as if linked in repulsion phase. The recombination between these two genes was estimated to be 2%. NCAG11 was immune to powdery mildew infection, while NCD1 and NCA4 were highly resistant, and NCAB10 was moderately resistant. All four genes expressed dominant gene action and they can be combined together in pairwise combinations to provide more durable resistance to powdery mildew. The resistance genes in the four germplasms appear to be novel genes that were not previously used in commercial cultivars or breeding lines.

INTRODUCTION

Powdery mildew of wheat (*Triticum aestivum* L.) is a foliar disease caused by an obligate biotrophic fungus *Blumeria graminis* DC f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* DC f. sp. *Tritici* Marchal) of the order *Erysiphales*, which are filamentous Ascomycetes. Powdery mildew occurs annually in eastern North Carolina and severe epidemics on susceptible cultivars are common. The fungus can be present on winter wheat continuously from fall until late spring (Bowen et al., 1991). Yield reductions of 17% in the susceptible cultivar Saluda and 3% in cultivars with effective levels of resistance such as Coker 983 have been reported in North Carolina (Leath and Bowen, 1989). Johnson et al. (1979) reported yield reductions as high as 34% in the cultivar Chancellor in Maryland.

Resistant wheat cultivars remain the most cost efficient and effective means for powdery mildew control. The genetics of resistance can be roughly divided into three categories: monogenic (vertical), oligogenic, and quantitative (horizontal). Monogenic resistance is mainly via a hypersensitive foliar reaction directly involving single major R genes, designated as *Pm* (powdery mildew) genes, in a gene-for-gene interaction (Bennett, 1984; Chen and Chelkowski, 1999; Hsam and Zeller, 2002). This type of resistance can be complete or partial (Leath and Heun, 1990; Hsam and Zeller, 2002; Paillard et al., 2000). Resistant plants may show from no to moderate signs and symptoms of infection, while susceptible plants show more severe signs and symptoms. Monogenic resistance is generally very efficient but tends to be a short term solution if variability in the pathogen population is great (Hsam and Zeller, 2002). Shifts in

virulence are common ways for pathogens to overcome single resistance genes (Bennett, 1984).

Common sources of *Pm* genes are various species within the primary, secondary and tertiary gene pools. In 1987 the small grains breeding and pathology programs at NC State University initiated a program of inter-specific hybridization using powdery mildew resistant diploid and tetraploid relatives as donor parents and the soft red winter wheat cultivar Saluda (Starling et al., 1986) as the recurrent parent. To date 11 BC_2F_5, BC_2F_6, and BC_2F_7 derived lines have been released with resistance genes incorporated from *T. monococcum, T. turgidum, T. timopheevii,* and *Ae. tauschii* (Murphy et al, 1998, 1999a, 1999b, 2002; Navarro et al., 2000). These 11 germplasm lines are being utilized in numerous wheat cultivar development programs in the southern United States and Europe. Four of these germplasm lines, NC96BGTD1, NC96BGTA4, NC98BGTAB10, and NC99BGTAG11 were chosen for this study. Knowledge of inheritance, efficiency and relationship with other genes conferring resistance to powdery mildew in these germplasms is essential for incorporation of these genes into elite cultivars, either individually or in a form of gene pyramids.

The objectives of this study were to determine the inheritance and evaluate efficiency of resistance to powdery mildew in the germplasm lines NC96BGTD1, NC96BGTA4, NC98BGTAB10, and NC99BGTAG11.

MATERIALS AND METHODS

Four germplasm lines resistant to powdery mildew, NC96BGTD1 (NCD1), NC96BGTA4 (NCA4), NC97BGTAB10 (NCAB10) and NC99BGTAG11 (NCAG11), the soft red winter wheat cultivar Saluda and progenies of crosses between the five genotypes were used in this investigation (Table 2.1). The four germplasm lines were homogeneously resistant BC_2F_5- to BC_2F_7- derived lines from interspecific hybridizations between diploid and tetraploid species and Saluda. Saluda contains the *Pm3a* gene, an ineffective in the southeastern US (Leath and Heun, 1990). Each germplasm line was crossed to Saluda and to each of the other germplasm lines for a total of 10 populations. F_1 plants were grown in the greenhouse and were self-pollinated. F_2 plants from the four populations involving crosses between Saluda and the four germplasm lines were grown in the greenhouse during 2000 to produce $F_{2:3}$ lines. F_2 plants from the six crosses between the germplasm lines were planted at 30.5 cm spacing in October 2000 at the Lower Coastal Plain Tobacco Research Station in Kinston, NC. $F_{2:3}$ seeds were harvested in June 2001.

1. Greenhouse tests for resistance to powdery mildew

Ninety-seven to 100 $F_{2:3}$ lines from each of the four crosses between Saluda and the germplasm lines, and 33 to 204 $F_{2:3}$ lines from each of the six crosses between the germplasm lines were evaluated between March 2001 and November 2002 (Table 2.2). Ten seeds of each $F_{2:3}$ line were planted at five seeds per pot in two 10 cm pots. Two pots containing the parental germplasm line and Saluda were included at 10 pot intervals as controls. Each control pot contained two plants. Seeds of all parental germplasm lines

and Saluda were derived from selfed progenies of the original plants used in crosses to develop the 10 populations. The seeds were planted in a mixture of Metro-Mix 200:soil:sand in a 50:40:10 ratio with the addition of three grams Osmocote (14N:14P:14K) fertilizer. The temperature was maintained at 24°C/20°C (day/night). Plants were grown under a combination of plentiful natural light supplemented with artificial High Intensity Discharge 1000W lights for promoting plant growth. The experimental design was a completely randomized design with a single replication.

All populations were inoculated at Feekes growth stage 2 to 3 by gently shaking leaves of $F_{2:3}$ and control plants with leaves of infected Saluda plants. The inoculum source was field grown Saluda plants dug at Kinston, NC in April 2001, and April 2002. The inoculum was maintained through the year either in the greenhouse on susceptible Saluda plants or under laboratory conditions on detached leaves according to the method of Leath and Heun (1990). The first signs and symptoms of powdery mildew were evident 5 to 7 days after inoculation. The most prominent differences between resistant and susceptible reactions were evident 12 to 20 days after inoculation. Disease evaluations were conducted when all Saluda control plants showed intense signs and symptoms of powdery mildew infection. In some cases the assessments were repeated about a week after the first evaluation in order to assure the accuracy of the data. The disease severity evaluation was on a scale from 0 to 9 as described by Leath and Heun (1990) where: 0 = immune, no visible signs of infection; 1-3 = resistant, increasing from 1) flecks, no necrosis to 2) necrosis, to 3) chlorosis, while the amount of mycelium went from none to a detectable amount; 4-6 = intermediate, chlorotic areas decreasing in amount while mycelium and conidia production increased from slight to moderate; 7-9 =

susceptible, increasing amount, size and density of mycelium and conidia to a fully compatible reaction. Disease reactions and intensities in $F_{2:3}$ progeny similar to the parental germplasm line were classified as "resistant" and those similar to Saluda were classified as "susceptible."

2. Field tests for resistance to powdery mildew

One hundred-eighty-five to 194 $F_{2:3}$ lines from each of the four crosses between Saluda and the germplasm lines, and 146 $F_{2:3}$ lines from the cross NCD1 x NCAG11 were planted at Kinston in October 2001 (Table 2.5). The $F_{2:3}$ lines in each population included the 97 to 100 evaluated in greenhouse tests. The experimental design was a randomized complete block with two replications. Thirty to sixty seeds of each line were planted in 1.2 m rows spaced 30.5 cm apart. Parental germplasm lines and Saluda were included as controls every forty plots. Included in each replication were 12 isolines with previously identified *Pm* genes backcrossed into susceptible cultivar Chancellor, Aximinister (*Pm1*), Ulka (*Pm2*), Asosan (*Pm3a*), Chul (*Pm3b*), Sonora (*Pm3c*), Michigan Amber (*Pm3f*), Yuma (*Pm4*), Hope (*Pm5*), Coker 747 (*Pm6*), Transec (*Pm7*), Federation/Kavkaz (*Pm8*), Amigo (*Pm17*) and Chancellor itself with no known *Pm* genes. A 1.2 m border of Saluda surrounded the experiment. Irrigation, fertilization and other agronomic practices were applied as needed and followed standard management practices for North Carolina (Weisz, 2000).

Disease reaction evaluations were initiated at the end of March 2002 when all Saluda rows showed uniform powdery mildew infection. Plants were between growth stage 9 and 10.1. Flag leaf minus 2 or flag leaf minus 3 were evaluated using the modified scale of Leath and Heun (1990). The same leaf was evaluated within a

population, but changed between populations because of differences in overall levels of disease. Scores were recorded on 12 random plants in homogenous susceptible or homogenous resistant rows, and 24 individual plants in rows segregating for disease reactions. All parental controls and isolines containing different *Pm* genes were evaluated three times (March 29, April 12, and April 18) in 2002, and (March 14, April 14, and April 29) in 2003. Homogenous resistant and susceptible $F_{2:3}$ lines were harvested in June 2002 and $F_{2:4}$ seed were planted in October, 2003 at Kinston in a completely random design, with a single replication. Plot design, management and evaluation of resistance reactions in 2003 followed the same protocol as in 2002.

Lines classified as segregating in one replication or environment and homogenous for resistance or susceptibility in another were considered as segregating in all ten populations derived from Saluda x germplasm and germplasm x germplasm crosses. Lines showing full resistance or susceptibility in one replication or environment versus those segregating in the other was likely due to inadequate sample size. According to Sedcole's (1977) Method I in order to identify a single susceptible or resistant plant with $P = 0.95$ from a population that will segregate 3:1 and 15:1 a minimum sample size of 11 and 47 individual plants is needed, respectively to identify the smaller category. This sample size was achieved in the field but not in the greenhouse studies.

Chi-square tests were conducted to test the goodness of fit between observed and expected segregation ratios (Snedecor and Cochran, 1956). Yates correction factor was applied for tests with one degree of freedom. In the NCA4 x NCAG11 cross three phenotypes were expressed in segregating $F_{2:3}$ lines: immune, partially resistant and susceptible. Seven combinations were considered for linkage analysis: all immune (“A”

class); immune and partially resistant ("B" class); immune and susceptible ("C" class); immune, partially resistant and susceptible ("D" class); all partially resistant ("E" class); partially resistant and susceptible ("F" class); and all susceptible ("G" class). Linkage analyses were calculated using the probability formula $P = [{}^{1}/_{2}r*(1-r)]^{(B+C+F)} * [{}^{1}/_{2}+r*(1-r)]^{D} * [{}^{1}/_{4}*(1-r)^{2}]^{E} * ({}^{1}/_{4}r^{2})^{G}$, where r is recombination frequency and A, B, C, D, E, F and G were different classes of segregating resistance phenotypes (Mather, 1951).

RESULTS AND DISCUSSION

Resistance in all Saluda x germplasm line populations segregated as a monogenic trait in greenhouse and field studies (Table 2.2). Resistance in four out of six germplasm x germplasm populations segregated as a digenic trait in greenhouse studies, indicating independence among resistance loci in NCD1 and NCAG11, NCAG11 and NCAB10, NCA4 and NCAB10, and NCD1 and NCAB10 combinations (Table 2.2). The NCD1 x NCA4 cross contained only 33 $F_{2:3}$ lines, an inadequate number to test for digenic inheritance, but the inheritance pattern was different from monogenic and consistent with digenic inheritance. Segregation of resistance in NCA4 x NCAG11 indicated that resistance genes in NCA4 and NCAG11 are located on the same chromosome. The segregation pattern between $F_{2:3}$ lines from germplasm x germplasm crosses indicated all four resistance alleles were dominant to susceptibility.

1. Analysis of $F_{2:3}$ lines from individual Saluda x germplasm crosses in greenhouse and field tests

Cross 1 (Saluda x NCD1). The 20 Saluda control pots were classified as susceptible in greenhouse evaluations with a mean disease rating of 7.0±0.00 (Table 2.3). The 20 NCD1 control pots were classified as resistant with a mean of 4.6±0.47 and a pot range of 4.0-5.0. A total of 24 $F_{2:3}$ lines were classified as uniformly resistant, with a mean of 4.6±0.28 and a range of 4.0-5.0. Fifty-two lines were segregating with a mean of 5.9±0.47 and a range of 4.8-6.8. Twenty-four lines were classified as susceptible with a mean of 6.9±0.31 and a range of 6.4-7.0. The chi-square test value for the expected ratio

of 1:2:1 (R:Se:S) was $\chi^2_{1:2:1(R:Se:S)}$= 0.16 ($P \leq 0.92$), indicating that resistance in this population evaluated in the greenhouse segregated as a monogenic trait (Table 2.2).

The eight Saluda control rows were classified as susceptible in field evaluations with a mean of 6.6±0.52 and a range of 6.0-7.0 (Table 2.4). All 8 NCD1 control rows were resistant with a mean of 1.4±0.53 and a range of 1.0-2.0. A total of 48 $F_{2:3}$ lines were resistant with a mean of 1.7±1.16 and a range of 0.0-4.0. One hundred-four $F_{2:3}$ lines were segregating with a mean of 4.1±0.64 and a range of 2.5-5.5. Forty-one $F_{2:3}$ lines were classified as susceptible with a mean of 6.3±0.67 and a range of 5.0-7.0. The chi-square test value for the expected ratio of 1:2:1 (R:Se:S) was $\chi^2_{1:2:1(R:Se:S)}$= 1.67 ($P \leq 0.43$), indicating that resistance in this population evaluated in the field segregated as a monogenic trait (Table 2.5).

Of the 193 $F_{2:3}$ lines evaluated, 177 were placed in the same categories in both replications in the field. Eleven lines were classified as susceptible in one replication and segregating in the other. Four were classified as segregating in one replication and resistant in the other. Only one was classified as susceptible in one replication and resistant in the other and was considered as segregating for the further analysis.

Of the 100 $F_{2:3}$ lines evaluated in the greenhouse and field evaluations 81 were placed in the same categories in both environments. Two were classified as susceptible in the field and segregating in the greenhouse. Seven were classified as segregating in the field and susceptible in the greenhouse. Two were classified as segregating in the field and resistant in the greenhouse. One was classified as resistant in the field and susceptible in the greenhouse and was considered as segregating for the further analysis. Seven were classified as resistant in the field and segregating in the greenhouse. Differences in

agreement between the two field replications and between field and greenhouse were likely due to sample size. Nevertheless, differences in growth stage of plants, environmental conditions and possibly some shifts in virulence of powdery mildew populations may have contributed to differences in different observations.

Cross 2 (Saluda x NCA4). The 20 Saluda control pots were classified as susceptible in greenhouse evaluations with a mean disease rating of 7.1±0.24 and a pot range of 7.0-8.0 (Table 2.3). The 20 NCA4 control pots were classified as resistant with a mean of 4.7±0.41 and a range of 4.0-5.0. A total of 18 $F_{2:3}$ lines were classified as uniformly resistant with a mean of 4.8±0.26 and a pot range of 4.0-5.0. Fifty-two lines were segregating, with a mean of 6.2±0.35 and a range of 5.2-6.8. Twenty-nine lines were classified as susceptible with a mean of 6.9±0.16 and a range of 6.4-7.0. The chi-square test value for the expected ratio of 1:2:1 (R:Se:S) was $\chi^2_{1:2:1(R:Se:S)}$= 2.70 ($P \leq 0.69$), indicating that resistance in this population evaluated in the greenhouse segregated as a monogenic trait (Table 2.2).

The 10 Saluda control rows were classified as susceptible in field evaluations with a mean of 7.7±0.67 and a range of 7.0-9.0 (Table 2.4). All 10 NCA4 control rows were immune with a mean of 0.0±0.00. Forty-one $F_{2:3}$ lines were immune with a mean of 0.0±0.00. One hundred-eight $F_{2:3}$ lines were segregating with a mean of 3.4±0.25 and a range of 2.5-4.0. Forty-five $F_{2:3}$ lines were classified as susceptible, with a mean of 7.1±0.64 and a range of 6.0-8.0. The chi-square test value for the expected ratio of 1:2:1 (R:Se:S) was $\chi_{1:2:1(R:Se:S)}$= 2.66 ($P \leq 0.26$), indicating that resistance in this population evaluated in the field segregated as a monogenic trait (Table 2.5).

Of the 194 $F_{2:3}$ lines evaluated, 191 were placed in the same categories in both replications in the field. Two were classified as susceptible in one replication and segregating in the other. One was classified as segregating in one replication and resistant in the other. Of the 99 $F_{2:3}$ lines evaluated in the greenhouse and the field, 85 were placed in the same categories in both environments. Five were classified as susceptible in the field and segregating in the greenhouse. Seven were classified as segregating in the field and susceptible in the greenhouse. Two were classified as segregating in the field and resistant in the greenhouse.

Cross 3 (Saluda x NCAB10). The 20 Saluda control pots were classified as susceptible in greenhouse evaluations with a mean disease rating of 6.5±0.57 and a pot range of 6.0-8.0. (Table 2.3). The 20 NCAB10 control pots were classified as resistant with a mean of 3.5±0.38 and a range of 3.0-4.0. A total of 21 $F_{2:3}$ lines were classified as uniformly resistant with a mean of 3.3±0.16 and a range of 3.0-3.7. Fifty-nine $F_{2:3}$ lines were segregating with a mean of 4.4±0.60 and a range of 3.2-5.7. Seventeen $F_{2:3}$ lines were classified as susceptible with a mean of 6.7±0.29 and a range of 6.0-7.0. The chi-square test value for the expected ratio of 1:2:1 (R:Se:S) was $\chi^2_{1:2:1(R:Se:S)}$= 4.88 ($P \leq 0.09$), indicating that resistance in this population evaluated in the greenhouse segregated as a monogenic trait (Table 2.2).

The 10 Saluda control rows were classified as susceptible in field evaluations with a mean of 7.1±0.57 and a range of 6.0-8.0 (Table 2.4). All 10 NCAB10 control rows were partially resistant with a mean of 4.7±0.48 and a range of 4.0-5.0. A total of 14 $F_{2:3}$ lines were partially resistant with a mean of 4.7±0.55 and a range of 3.0-5.0. One hundred forty-two were segregating with a mean of 5.5±0.43 and a range of 4.0-6.5. Twenty-nine

lines were classified as susceptible with a mean of 6.7±0.54 and a range of 6.0-8.0. The chi-square test value for the expected ratio of 1:2:1 (R:Se:S) was $\chi^2_{1:2:1(R:Se:S)}$= 55.41 (P<0.001), indicating that resistance in this population evaluated in the field did not segregate as a monogenic trait (Table 2.5).

Of the 185 $F_{2:3}$ lines evaluated 124 were placed in the same categories in both replications in the field. Thirty-six were classified as susceptible in one replication and segregating in the other. Twenty-two were classified as segregating in one replication and resistant in the other. Three were classified as susceptible in one replication and resistant in the other and were considered as segregating for the further analysis. Of the 97 $F_{2:3}$ lines evaluated in the greenhouse and field evaluations, 68 were placed in the same categories in both environments. Five were classified as susceptible in the field and segregating in the greenhouse. Five were classified as segregating in the field and susceptible in the greenhouse. Sixteen were classified as segregating in the field and resistant in the greenhouse. Three were classified as resistant in the field and segregating in the greenhouse.

This was the only instance where segregation of NCAB10 resistance deviated from a monogenic pattern. In the greenhouse tests as well as in crosses with other resistant germplasms (Cross 6, 7 and 8), resistance in this germplasm segregated as a monogenic trait (Table 2.2). It is likely that the deviation from monogenic segregation was due to altered expressivity of the resistance gene in this specific environment. It is also possible that the powdery mildew population had complex pathogenicity factors, such as multiple *avr* genes, so the resistance gene in NCAB10 was very efficient against some powdery mildew isolates and less efficient against others. Powdery mildew

inoculum used for greenhouse inoculations was collected at Kinston in April 2001, and field evaluations were conducted at the end of March 2002. Due to virulence shifts, a large number of virulent strains can be produced and fixation of *avr* genes in a population is very unlikely (Limpert et al., 1999; McDonald and Linde, 2002). All plants grown at the greenhouse were inoculated at Feekes growth stages 2 to 3 and evaluated at stages 3 to 4. Plants grown in the field were evaluated at growth stages 9 to 10.1. It has been well documented that certain types of resistance to powdery mildew in Knox and Massey wheats were efficient only in mature plants, and completely susceptible at the seedling stage (Shaner, 1973; Griffey and Das, 1994).

Cross 4 (Saluda x NCAG11). The 20 Saluda control pots were classified as susceptible in greenhouse evaluations with a range of 6.0-7.0. The 20 NCAG11 pots were immune with a mean of 0.0. A total of 26 $F_{2:3}$ lines were classified as immune with a mean of 0.0 (Table 2.3). Forty-one $F_{2:3}$ lines were segregating for resistance. Thirty-two lines were classified as susceptible. The chi-square test value for the expected ratio of 1:2:1 (R:Se:S) was $\chi^2_{1:2:1(R:Se:S)}$= 3.65 ($P \leq 0.16$), indicating that resistance in this population evaluated in the greenhouse segregated as a monogenic trait (Table 2.2).

The 10 Saluda control rows were classified as susceptible in field evaluations with a mean of 7.1±0.57 and a range of 6.0-8.0 (Table 2.4). All 10 NCAG11 control rows were immune with mean of 0.0. A total of 52 $F_{2:3}$ lines were immune with a mean of 0.0. Eighty-one $F_{2:3}$ lines were segregating with a mean of 3.4±0.21 and a range of 3.0-3.5. Fifty-six $F_{2:3}$ lines were classified as susceptible with a mean of 7.2±0.53 and a range of 5.0-8.0. The chi-square test value for the expected ratio of 1:2:1 (R:Se:S) was

$\chi^2_{1:2:1(R:Se:S)}$= 4.03 ($P \leq 0.13$), indicating that resistance in this population evaluated in the field segregated as a monogenic trait (Table 2.5).

Of the 189 $F_{2:3}$ lines evaluated, 188 were placed in the same categories in both replications. One was classified as susceptible in one replication and segregating in the other. Of the 99 $F_{2:3}$ lines evaluated in the greenhouse and field evaluations, 94 were placed in the same categories in both environments. Three were classified as segregating in the field and susceptible in the greenhouse. One was classified as segregating in the field and resistant in the greenhouse. One was classified as resistant in the field and segregating in the greenhouse.

2. Analysis of $F_{2:3}$ lines from individual germplasm x germplasm crosses in greenhouse and field tests

Cross 5 (NCD1 x NCAG11). The 29 Saluda control pots were classified as susceptible in the greenhouse evaluation with a mean disease rating of 6.0±0.51 and a range of 5.0-7.0 (Table 2.3). The 29 NCD1 control pots were classified as resistant with a mean of 2.9±1.17 and a range of 0.5-5.0. The 29 NCAG11 control pots were classified as immune with a mean of 0.0. A total of 70 $F_{2:3}$ lines were classified as resistant with a mean of 0.2±0.37 and a range of 0.0-2.1. Sixty-nine lines were segregating with a mean of 2.1±1.41 and a range of 0.5-5.6. Seven lines were classified as susceptible with a mean of 6.3±0.25 and a range of 5.9-6.6. Resistant and segregating classes were pooled because ten individual plants per line was not an adequate sample size to distinguish between resistant and different classes of segregating lines. The chi-square test value for the expected ratio of 15:1 (R+Se:S) was $\chi^2_{15:1(R+Se:S)}$= 0.31 ($P \leq 0.58$), indicating that

resistance in this population evaluated in the greenhouse segregated as a digenic trait (Table 2.2).

The eight Saluda control rows were classified as susceptible in field evaluations with a mean of 7.4±0.52 and a range of 7.0-8.0 (Table 2.4). All eight NCD1 control rows were classified as resistant with a mean of 1.4±1.06 and a range of 0.0-3.0. All eight NCAG11 control rows were classified as immune with a mean of 0.0. A total of 54 $F_{2:3}$ lines were resistant with a mean of 0.3±0.89 and a range of 0.0-4.0. Eighty-two were segregating with a mean of 3.4±0.39 and a range of 2.5-5.0. Ten $F_{2:3}$ lines were classified as susceptible with a mean of 7.2± 0.40 and a range of 7.0-8.0. The chi-square test value for the expected ratio of 7:8:1 (R:Se:S) was $\chi^2_{7:8:1(R:Se:S)}$= 2.72 ($P \leq 0.26$), consistent with digenic segregation (Table 2.5).

Of the 146 $F_{2:3}$ lines evaluated, 139 were placed in the same categories in both replications. Seven were classified as segregating in one replication and resistant in the other. Of the 146 $F_{2:3}$ lines evaluated in the greenhouse and field evaluations, 123 were placed in the same categories in both environments. Three were classified as susceptible in the field and segregating in the greenhouse. Eighteen were classified as segregating in the field and resistant in the greenhouse. Two were classified as resistant in the field and segregating in the greenhouse. The lower percent agreement between greenhouse and field studies was likely due to inadequate sample size within individual lines to clearly identify all lines segregating for resistance. A larger sample size is needed to identify lines segregating for digenic, than for monogenic inheritance pattern.

Cross 6 (NCAG11 x NCAB10). The 39 Saluda control pots were classified as susceptible in greenhouse evaluations with a mean disease rating of 6.2±0.68 and a range

of 5.0-7.0 (Table2.3). The 39 NCAG11 control pots were classified as immune with a mean of 0.0. The 39 NCAB10 control pots were classified as resistant with a mean of 1.4±1.38 and a range of 0.0-5.0. A total of 118 $F_{2:3}$ lines were classified as resistant with a mean of 0.1±0.32 and a range of 0.0-1.9. Seventy-six $F_{2:3}$ lines were segregating for resistance with a mean of 2.2±1.28 and a range of 0.5-5.9. Ten lines were classified as susceptible with a mean of 6.4±0.44 and a range of 5.7-6.9. The chi-square test value for the expected ratio of 15:1 (R+Se:S) was $\chi^2_{15:1(R+Se:S)}$= 0.42 ($P \leq 0.52$), indicating that resistance in this population segregated as a digenic trait (Table 2.2).

Cross 7 (NCA4 x NCAB10). The 23 Saluda control pots were classified as susceptible in greenhouse evaluations with a mean disease rating of 6.0±0.48 and a range of 5.0-7.0 (Table 2.3). The 23 NCA4 control pots were classified as resistant with a mean of 2.2±0.91 and a range of 1.0-3.5. The 23 NCAB10 control pots were classified as resistant with a mean of 2.7±0.65 and a range of 1.5-4.0. A total of 50 $F_{2:3}$ lines were classified as resistant with a mean of 1.4±1.21 and a range of 0.0-3.9. Sixty-three lines were segregating with a mean of 3.5±0.96 and a range of 1.0-5.3. Eight lines were classified as susceptible with a mean of 6.1±0.47 and a range of 5.4-6.9. The chi-square test value for the expected ratio of 15:1 (R+Se:S) was $\chi^2_{15:1(R+Se:S)}$= 5.51×10^{-4} ($P \leq 0.98$), indicating that resistance in this population evaluated in the greenhouse segregated as a digenic trait (Table 2.2).

Cross 8 (NCD1 x NCAB10). The 24 Saluda control pots were classified as susceptible with a mean disease rating of 6.7±0.44 and a range of 6.0-7.5 (Table 2.3). The 24 NCD1 control pots were classified as resistant with a mean of 4.2±0.67 and a range of 3.0-5.0. The 24 NCAB10 control pots were classified as resistant with a mean of

4.2±0.49 and a range of 3.5-5.0. A total of 68 $F_{2:3}$ lines were classified as resistant with a mean of 2.9±0.95 and a range of 0.8-4.8. Forty-three $F_{2:3}$ lines were segregating with a mean of 4.4±0.96 and a range of 1.8-5.9. Eleven $F_{2:3}$ lines were classified as susceptible with a mean of 6.2±0.26 and a range of 6.0-7.7. The chi-square test value for the expected ratio of 15:1 (R+Se:S) was $\chi^2_{15:1(R+Se:S)}$= 1.16 ($P \leq 0.28$), indicating that resistance in this population evaluated in the greenhouse segregated as a digenic trait (Table 2.2).

Cross 9 (NCD1 x NCA4). The seven Saluda control pots were classified as susceptible in greenhouse evaluations with a mean disease rating of 6.9±0.24 and a range of 6.5-7.0 (Table 2.3). The seven NCD1 control pots were classified as resistant with a mean of 3.8±0.49 and range of 3.0-4.5. The seven NCA4 control pots were classified as resistant with a mean of 1.7±0.49 and a range of 1.0-2.5. A total of 17 $F_{2:3}$ lines were classified as resistant with a mean of 1.3±0.93 and a range of 0.0-3.1. Fifteen lines were segregating for with a mean of 4.0±1.05 and a range of 2.4-6.2. One line was classified as susceptible with a mean of 7.0. The chi-square test value for the expected ratio of 15:1 (R+Se:S) was $\chi^2_{15:1(R+Se:S)}$= 0.16 ($P \leq 0.69$), indicating that resistance in this population evaluated in the greenhouse segregated as a digenic trait (Table 2.2).

Cross 10 (NCA4 x NCAG11). The 39 Saluda control pots were classified as susceptible with a mean disease rating of 7.1±0.36 and a range of 6.5-8.0 (Table 2.3). The 39 NCA4 control pots were classified as resistant with a mean of 3.2±0.78 and a range of 2.0-5.0. The 39 NCAG11 control pots were immune with a mean of 0.0. A total of 192 $F_{2:3}$ lines were classified as resistant with a mean of 1.0±1.00 and a range of 0.0-4.0. Three $F_{2:3}$ lines were segregating with a mean of 2.7±0.86 and a range of 1.9-3.6. There

were no susceptible lines. The chi-square test value for the expected ratio of 15:1 (R+Se:S) was $\chi^2_{15:1(R+Se:S)}$= 11.96 ($P \leq 5.45 \times 10^{-4}$), indicating that resistance in this population evaluated in the greenhouse did not segregate as a digenic trait (Table 2.2). One hundred-ninety-two $F_{2:3}$ lines were classified as resistant (immune, partially resistant, or both) indicating linkage, but not allelism between the resistance genes in these two germplasm lines. The 195 $F_{2:3}$ lines were classified based on their progeny phenotype. Seven resistance classes were observed: all immune (A= 52), immune+partially resistant (B= 97), immune+susceptible (C= 0), immune+partially resistant+susceptible (D= 3), all partially resistant (E= 43), partially resistant+susceptible (F= 0), and all susceptible (G= 0). Average individual plant values for each of the categories were 0.0 for immune, 2.21±0.94 and a range of 1.0-5.0 for partially resistant and 6.0 for susceptible. The two-gene model was insufficient due to the small observed frequencies of classes C and F. The model to fit these data was one with three linked genes for resistance to powdery mildew. The expected frequencies of classes B, C and F were the same, when tested for different values of r. The relative probability for r= 0.02 was the closest to zero:

$$P = [{}^1/_2 r*(1-r)]^{(B+C+F)}*[{}^1/_2+r*(1-r)]^D*[{}^1/_4*(1-r)^2]^E*({}^1/_4 r^2)^G$$

$$P = [{}^1/_2 r*(1-0.02)]^{(97+0+0)}*[{}^1/_2+0.02*(1-0.02)]^3*[{}^1/_4*(1-0.02)^2]^{43}*({}^1/_4 0.02^2)^0$$

$$P = 1.03*10^{-125}.$$

An explanation for these results may be that resistance genes from NCA4 and NCAG11 are linked to each other, but also linked to *Pm3a* from Saluda. *Pm3a* in Saluda comes from Asosan, and is located on the chromosome 1AS (Hsam and Zeller, 2002), so it is likely that other two resistance genes are located on this chromosome as well.

Resistance in NCA4 was incorporated from diploid *T. monococcum* (AA genomes) (Murphy et al., 1999a) and in NCAG11 from tetraploid *T. timopheevii* (AAGG genomes) (Murphy et al., 2002). Because the resistance gene in NCA4 is located on the A genome, it is also very likely that the resistance gene in NCAG11 is also located on the A genome and not on the G genome. *Pm3a* resistance is not effective in North Carolina, but there may be some pleiotropic effects that make it detectable as partial resistance in this specific cross.

3. Comparison of resistant and susceptible $F_{2:3}$ lines and their $F_{2:4}$ progenies from Saluda x germplasm lines and NCD1 x NCAG11 crosses in the field

Based on both greenhouse and field evaluations the consistently homogeneously resistant and susceptible $F_{2:3}$ lines from the four Saluda x germplasm line and the NCD1 x NCAG11 crosses were harvested and $F_{2:4}$ progenies were evaluated in Kinston in 2003 (Table 2.6).

Cross 1 (Saluda x NCD1). A single control row of Saluda was homogeneously susceptible with a mean of 6.0 (standard deviations and ranges were not calculated for sample sizes less than 3 and 2, respectively). A single control row of NCD1 was homogeneously resistant with a mean of 1.0. Twenty $F_{2:4}$ lines were homogenous for resistance with a mean of 1.9±0.72 and a range of 1.0-3.0. Twenty $F_{2:4}$ lines were homogenous for susceptibility with a mean of 6.9±0.60 and a range of 6.0-8.0. There was a complete agreement in the categorization of the selfed $F_{2:3}$ lines and their $F_{2:4}$ progenies.

Cross 2 (Saluda x NCA4). A single control row of Saluda was homogeneously susceptible with a mean of 7.0. A single control row of NCA4 was homogeneously resistant with a mean of 2.0. Nineteen $F_{2:4}$ lines were homogenous for resistance with a

mean of 0.5±0.53 and a range of 0.0-1.0. A single $F_{2:4}$ line was segregating for resistance with a mean of 2.5. Twenty $F_{2:4}$ lines were homogenous for susceptibility with a mean of 6.7±0.47 and a range of 6.0-7.0. Of the 40 $F_{2:3}$ lines homogenous for resistance 39 of their $F_{2:4}$ progenies were placed in the same categories. One $F_{2:4}$ line derived from a resistant $F_{2:3}$ line was classified as segregating.

Cross 3 (Saluda x NCAB10). A total of 39 rows were evaluated (Table 2.6). A single control row of Saluda was homogeneously susceptible with a mean of 7.0. A single control row of NCAB10 was homogeneously resistant with a mean of 2.0. Thirteen $F_{2:4}$ lines were homogenous for resistance with a mean of 3.5±0.66 and a range of 2.0-4.0. Twenty-four $F_{2:4}$ lines were homogenous for susceptibility with a mean of 6.5±0.88 and a range of 5.0-8.0. Of the 37 $F_{2:3}$ lines homogenous for resistance 31 of their $F_{2:4}$ progenies were placed in the same categories. Six $F_{2:4}$ lines derived from resistant $F_{2:3}$ lines were classified as susceptible, indicating that expression of resistance in these lines was very dependent on the all factors of the disease triangle as discussed in the Chapter 1.

Cross 4 (Saluda x NCAG11). A single control row of Saluda was homogeneously susceptible with a mean of 7.0. A single NCAG11 control row was homogeneously immune with a mean of 0.0. Eighteen $F_{2:4}$ lines were homogenous for immunity with a mean of 0.0. Twenty $F_{2:4}$ lines were homogenous for susceptibility with a mean of 6.7±0.57 and a range of 6.0-8.0. This was a complete agreement in the categorization of the selfed $F_{2:3}$ lines and their $F_{2:4}$ progenies.

Cross 5 (NCD1 x NCAG11). A single control row of Saluda was homogeneously susceptible with a mean of 7.0. A single control row of NCD1 was homogeneously resistant with a mean of 1.0. A single control row of NCAG11 was homogeneously

immune with mean of 0.0. Thirty $F_{2:4}$ lines were homogenous for resistance with a mean of 0.3±0.55 and a range of 0.0-2.0 A single $F_{2:4}$ line was segregating for resistance with a mean of 3.0. Ten $F_{2:4}$ lines were homogeneous for susceptibility with a mean of 6.4±0.52 and a range of 6.0-7.0. Of the 41 $F_{2:3}$ lines homogenous for resistance 40 of their $F_{2:4}$ progenies were placed in the same categories. One $F_{2:4}$ line derived from a resistant $F_{2:3}$ line was classified as segregating.

4. Comparison of resistance genes in NCD1, NCA4, NCAB10 and NCAG11 with known *Pm* genes

NCAG11 exhibited immunity to powdery mildew in all greenhouse and field experiments (Table 2.3, 2.4, 2.6). NCA4 and NCD1 had very low levels of infection in both field and greenhouse tests. Resistance levels in NCA4 and NCD1 were almost the same in the greenhouse (Table 2.3). In the field in 2002, NCA4 was more resistant than NCD1 (Table 2.4). In the field in 2003, NCD1 was slightly more resistant than NCA4 (Table 2.6). NCAB10 was the least resistant of the germplasms (Table 2.3, 2.4, 2.6), but also can be considered as a very useful source of resistance for breeding purpose. Resistance from this germplasm was efficient in greenhouse and field tests.

The importance of environmental factors in the expression of disease resistance increases as the level of resistance decreases. This reduces accuracy in identifying resistance reactions. This also suggests that selection for resistance is the most efficient for complete resistance, and decreases as levels of resistance decreases. Lipps and Madden (1989) recommended evaluations be conducted only after head emergence so that the correlation between disease intensity and yield losses are greatest.

Correspondingly, powdery mildew infections were the most evenly distributed across the plots at this stage in field nurseries in 2002 and 2003.

Agreement in scoring between greenhouse and field evaluations and between replication one and two in field evaluation were high. The agreement between different replications in the field in 2002 ranged from 67% for Saluda x NCAB10, to 99% for Saluda x NCAG11. The agreement between both replications in the field 2002 and the greenhouse environments ranged from 70% for Saluda x NCAB10 to 95% for Saluda x NCAG11.

The agreement in scoring between selected $F_{2:3}$ lines homogenous for resistance and susceptibility in the field during 2002 and their $F_{2:4}$ progenies in 2003 were also high, ranging from 84% for Saluda x NCAB10 to 100% for Saluda x NCD1 and Saluda x NCAG11. The agreement was the highest for the crosses using the most resistant parents NCAG11 and NCD1, followed by NCA4, and the lowest for NCAB10. The NCD1 x NCAG11 cross was consistent with this pattern. The highest agreement between reactions to powdery mildew was between selected $F_{2:3}$ lines in the field and their $F_{2:4}$ progenies.

The percentage agreement between greenhouse and field results ranged from 65% for Saluda x NCAB10 to 95% for Saluda x NCAG11. This wide range may be due to several factors such as differences in growth stages of plants at evaluation time, different environments, and some alteration of virulence in greenhouse versus field conditions. These results indicated that selection for resistance to powdery mildew in the greenhouse may still be efficient, although not as efficient as in field tests.

Parental lines NCD1, NCA4 and NCAB10 in the greenhouse, NCAG11 and NCA4 in the field nursery in 2002, and NCA4 and NCAB10 in the field nursery in 2003

had similar levels of resistance (Table 2.3, 2.4, 2.6). However, their levels of resistance were different from the susceptible parent Saluda, most of the 12 isolines carrying different known *Pm* genes (*Pm1a*, *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3f*, *Pm4a*, *Pm5a*, *Pm6*, *Pm7*, *Pm8*, *Pm17*), and Chancellor without any known *Pm* genes (Tables 2.7, 2.8). Only three out of twelve isolines scored 0 to 4. Amigo (*Pm17*) was immune in the field in both years. Aximinister (*Pm1*) was partially resistant, with a disease score of 2 to 4 in 2002 and 4 in 2003. Federation/Kavkaz (*Pm8*) was moderate to fully susceptible in 2002, with scores of 5 to 8, and fully to partially resistant in 2003, with scores 0 to 1 (Tables 2.7, 2.8).

Pm10, *Pm11*, *Pm14* and *Pm15* are genes conferring resistance to *E. graminis* f. sp. *agropyri* and not effective against *E. graminis* f. sp.*tritici* (Hsam and Zeller, 2002), so they can be excluded as identical with any powdery mildew resistance genes in the four germplasms evaluated. *Pm13*, *Pm20*, *Pm21*, and *Pm29* from *A. longissima*, *S. secale*, *H. villosa* from *A. ovata*, respectively were introgressed from species that are not closely related to any of the species that were sources of resistance genes in the four germplasms. It is very unlikely that any of four genes for resistance to powdery mildew in the four germplasms are identical to the four *Pm* genes from these species.

NCD1. Compared to all 12 isolines evaluated in both 2002 and 2003 NCD1 exhibited resistance levels similar to Federation/Kavkaz (*Pm8*) in 2003 only (Table 2.8). Federation/Kavkaz (*Pm8*) was moderate to fully susceptible in 2002, with scores of 5 to 8 (Table 2.7), and fully to partially resistant in 2003, with scores of 0 to 1 (Table 2.8), while NCD1 was partially resistant in 2002, with scores of 0-3 (Table 2.4) and a score of 1 in 2003 (Table 2.6). It can be concluded that the resistance gene in NCD1 was different

from the 12 resistance genes present in the isolines. These results confirmed the conclusions of Murphy et al. (1998) that the resistance gene in NCD1 was different from *Pm2*, *Pm3a*, *Pm3b*, *Pm4a*, *Pm6* and *Ma*. It is very likely that the resistance gene from NCD1 is located on a chromosome of the D genome, due to homologous recombination or chromosome substitution rather than on a chromosome belonging to the A or B genomes, due to homeologous recombination or chromosome substitution. *Pm1b, Pm1c, Pm1d* (7AL), *Pm3d*, *Pm3e, Pm3g, Pm3h, Pm3j* (1AS), *Pm4b* (2AL), *Pm5b*, *Pm5c* (7BL), *Pm9* (7AL), *Pm12* (6BS-6SS.6SL), *Pm16* (4A), *Pm18* (7A), *Pm23* (2AL), *Pm25*, *Pm26* (2BS), *Pm27* (6B-6G), *Pm28* (1B), and *Pm30* (5BS) are unlikely to be identical to the resistance gene from NCD1. None of these genes is located on the D genome and they are unlikely to be identical to the resistance gene in NCD1. *Pm19*, *Pm22* and *Pm24* are located on chromosomes 7D, 1D and 6D, respectively. *Pm22* and *Pm24* originated from *T. aestivum* and not from *Ae. tauschii*. *Pm19* located on chromosome 7D, was introgressed from *Ae. tauschii*. Hsam and Zeller (2002) report that *Pm19* is not very effective in the adult stage. Resistance in NCD1 was very effective against powdery mildew in greenhouse and field tests in all tested growth stages, suggesting that it is different from *Pm19*.

NCA4. Compared with all 12 isolines evaluated in both 2002 and 2003 NCA4 exhibited resistance levels similar to Amigo (*Pm17*) in year 2002 only (Table 2.7). Amigo (*Pm17*) was immune in both years, with a score of 0 (Table 2.7, 2.8). NCA4 was immune in 2002 (Table 2.4), and partially resistant in 2003, with a score of 2 (Table 2.6). It can be concluded that the resistance gene in NCA4 was different from the 12 resistance genes present in the isolines. These results confirmed the conclusions of Murphy et al.

(1999a) that the resistance gene in NCA4 was different from *Pm2*, *Pm3a*, *Pm3b*, *Pm4a*, *Pm6* and *Ma*. It is very likely that the resistance gene from NCA4 is located on a chromosome of the A genome, due to homologous recombination or chromosome substitution rather than on a chromosome belonging to the B or D genomes resulting from homeologous recombination or chromosome substitution. *Pm5b*, *Pm5c*, *Pm5d* (7BL), *Pm12* (6BS), *Pm19* (7DL), *Pm22* (1D), *Pm24* (6D), *Pm26* (2BS), *Pm27* (6B-6G), *Pm28* (1B) and *Pm30* (5BS) are unlikely to be identical to the resistance gene from NCA4. None of these genes is located on the A genome and they are unlikely to be identical to the resistance gene in NCA4. *Pm1b* (7AL) from *T. aestivum*, *Pm1c* (7AL) from *T. aestivum*, *Pm1d* (7AL) from *T. spelta*, *Pm3d* (1AS) from *T. aestivum*, *Pm3e* (1AS) from *T. aestivum*, *Pm3g* (1AS) from *T. aestivum*, *Pm3h* (1AS) from *T. durum*, *Pm3j* (1AS) from *T. aestivum*, *Pm4b* (2AL) from *T. carthilicum*, *Pm9* (7AL) from *T. aestivum*, *Pm16* (4A) from *T. dicoccoides*, *Pm18* (7A) from *T. aestivum* and *Pm23* (2AL) from *T. aestivum* did not originate from *T. monococcum*, and it is likely they are different from the resistance gene in NCA4. *Pm25* (in the NC96BGTA5 germplasm) located on chromosome 1A was introgressed from *T. monococcum* (Murphy et al., 1999a). The donor parent for the NCA4 germplasm was PI 221414 a *T. monococcum* L. subsp. *monococcum* accession from Yugoslavia. The donor parent for NC96BGTA5 was PI 427662, a *T. monococcum* L. subsp. *aegilopoides* accession from Iraq. Given different subspecies and geographical distance where the two were collected it is likely that the two donor accessions carry different resistance genes.

NCAB10. Compared with the 12 isolines evaluated in both 2002 and 2003 NCAB10 exhibited resistance levels somewhat similar to Aximinister (*Pm1a*) in 2002

only (Table 2.7). Aximinister (*Pm1a*) was partially resistant in 2002 with scores of 2 to 4 (Table 2.7) and 4 in 2003 (Table 2.8). NCAB10 was partially resistant in 2002, with scores of 4 to 5 (Table 2.4) and 2 in 2003 (Table 2.6). It can be concluded that the resistance gene in NCAB10 was different from the 12 resistance genes present in the isolines. According to Navarro et al. (2000), the resistance gene in NCAB10 is different from *Pm1a*, *Pm1b*, *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm4a*, *Pm4b*, *Pm5*, *Pm6*, *Pm7*, *Pm8*, *Pm9*, *Pm10*, *Pm12*, *Pm13*, *Pm14*, *Pm15*, *Pm16*, *Pm17*, *Pm19*, *Pm20*, *Pm21*, *Pm22*, *Pm24*, and *Ma*. It is likely that the resistance gene in NCAB10 is located on a chromosome of the A or B genomes, resulting from homologous recombination or chromosome substitution rather than on a chromosome belonging D genome, resulting from homeologous recombination or chromosome substitution. *Pm1c* (7AL) from *T. aestivum*, *Pm1d* (7AL) from *T. spelta*, *Pm3d* (1AS) from *T. aestivum*, *Pm3e* (1AS) from *T. aestivum*, *Pm3g* (1AS) from *T. aestivum*, *Pm3j* (1AS) from *T. aestivum*, *Pm5b* (7BL) from *T. aestivum*, *Pm5c* (7BL) from *T. aestivum*, *Pm5d* (7BL) from *T. aestivum*, *Pm12* (6BS) from *Ae. speltoides*, *Pm18* (7A) from *T. aestivum*, *Pm23* (2AL) from *T. aestivum*, *Pm25* (1A) from *T. monococcum*, *Pm27* (6B-6G) from *T. timopheevii* and *Pm28* (1B) from *T. aestivum* did not originate from *T. turgidum*, and it is likely that they differ from the resistance gene in NCAB10. The only three *Pm* genes with possible relationships with resistance gene in NCAB10 are *Pm3h* (Zeller and Hsam, 1998), *Pm26* (Rong et al., 2000), and *Pm30* (Liu et al., 2002), all introgressed into common wheat from tetraploid durum wheat.

NCAG11. Compared with the 12 isolines in both 2002 and 2003 NCAG11 exhibited similar resistance levels with Amigo (*Pm17*) in both years. Amigo (*Pm17*) was immune in 2002 and 2003 with scores of 0 (Table 2.7, 2.8). NCAG11 was also immune

in 2002 and 2003 with scores of 0 (Table 2.4, 2.6). Powdery mildew resistance genes in NCAG11 and *Pm17* are likely to be different, because *Pm17* in Amigo was introgressed from rye on the 1AL/1RS translocation (Hsam and Zeller, 2002), while resistance in NCAG11 was incorporated from tetraploid *T. timopheevii* (AAGG genomes) (Murphy et al., 2002). It is likely that resistance gene in NCAG11 was different from the 12 resistance genes present in the isolines. According to Murphy et al. (2002) the resistance gene in NCAG11 is different from *Pm1a*, *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3f*, *Pm4a*, *Pm4b*, *Pm5*, *Pm6*, *Pm7*, *Pm8*, *Pm9*, *Pm16*, *Pm17*, *Pm19* and *Pm20*. It is likely that the resistance gene in NCAG11 is located on a chromosome of the A genome, resulting from homologous recombination or chromosome substitution rather than on a chromosome belonging to the B or D genomes, resulting from homeologous recombination or chromosome substitution. *Pm4b* (2AL), *Pm5b* (7BL), *Pm5c* (7BL), *Pm5d* (7BL), *Pm12* (6BS), *Pm19* (7D), *Pm22* (1D), *Pm24* (6D), *Pm26* (2BS), *Pm28* (1B) and *Pm30* (5BS) are unlikely to be identical to the resistance gene in NCAG11.

Pm1b (7AL) from *T. aestivum*, *Pm1c* (7AL) from *T. aestivum*, *Pm1d* (7AL) from *T. spelta*, *Pm3d* (1AS) from *T. aestivum*, *Pm3e* (1AS) from *T. aestivum*, *Pm3g* (1AS) from *T. aestivum*, *Pm3h* (1AS) from *T. durum*, *Pm3j* (1AS) from *T. aestivum*, *Pm9* (7AL) from *T. aestivum*, *Pm16* (4A) from *T. dicoccoides*, Pm18 (7A) from T. aestivum, *Pm23* (2AL) from *T. aestivum*, Pm25 (1A) from *T. monococcum* did not originate from *T. timopheevii* and it is likely they are different from the resistance gene in NCAG11. The *Pm* gene whose possible relationship with resistance gene in NCAG11 is the least known is *Pm27* (Järve et al., 2000), introgressed into common wheat from *T. timopheevii*. This

gene is located at chromosome 6B-6G, while the resistance gene from AG11 is most-likely located on a chromosome of the A genome.

In conclusion, four different single genes all with dominant gene action control resistance to powdery mildew in the germplasm lines NCD1, NCA4, NCAB10 and NCAG11. All the genes were efficient against powdery mildew in North Carolina in field and greenhouse tests in March 2001-May 2003. Selection for resistance was efficient in segregating populations derived from susceptible x resistant parent. Genes for resistance to powdery mildew from these four germplasms can be successfully combined together, in all pairwise combinations to generate lines with increased durability of resistance (Pedersen and Leath, 1988).

LITERATURE CITED:

Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: A review of its use in agriculture and breeding programmes. Plant Pathol. 33:279-300.

Bowen, K.L., K.L. Everts, and S. Leath. 1991. Reduction in yield of winter wheat in North Carolina due to powdery mildew and leaf rust. Phytopathology. 81(5):503-511.

Chen, Y., and J. Chelkowski. 1999. Genes for resistance to wheat powdery mildew. J. Appl. Genet. 40(4):317-334.

Griffey, C.A., and M.K. Das. 1994. Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey winter wheats. Crop Sci.34:641-646.

Hsam, S.L.K., and F.J. Zeller 2002. Breeding for Powdery Mildew Resistance in common wheat (*Triticum aestivum* L.). p. 219-238. *In* Bélanger, R.R., W.R. Bushnell, A.J. Dik and T.L.W. Carver (ed). The powdery mildews: A Comprehensive Treatise. The American Phytopathological Society, St. Paul, MN.

Järve, K., H.O. Peusha, J. Tsymbalova, S. Tamm, K.M. Devos, and T.M. Eno. 2000. Chromosomal location of a *Triticum timopheevii*-derived powdery mildew resistance gene transferred to common wheat. Genome 43:377-381.

Johnson, J.W., P.S. Baenziger, W.T. Yamazaki, and R.T. Smith. 1979. Effects of powdery mildew on yield and quality of isogenic lines of 'Chancellor' wheat. Crop Sci. 19:349-352.

Leath, S., and K.L. Bowen. 1989. Effects of powdery mildew, triadimenol seed treatment, and triadimefon foliar sprays on yield of winter wheat in North Carolina. Phytopathology 79(2):152-155.

Leath, S., and M. Heun. 1990. Identification of powdery mildew resistance genes in cultivars of soft red winter wheat. Plant Dis. 74(10): 747-752.

Limpert, E., F. Godet, and K. Müller. 1999. Dispersal of cereal mildews across Europe. Agric. For. Meteorol. 97:293-308.

Lipps, P.E., and L.V. Madden. 1989. Assessment of methods of determining powdery mildew severity in relation to grain yield of winter wheat cultivars in Ohio. Phytopathology 79(4):462-470.

Liu, Z., Q. Sun, Z. Ni, E. Nevo, and T. Yang. 2002. Molecular characterization of a novel powdery mildew resistance gene Pm30 in wheat originating from wild emmer. Euphytica 123: 21-29.

Mather, K. 1951. The measurement of linkage in heredity. John Wiley and Sons, Inc. New York.

McDonald, B.A., and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Reviews of Phytopathology 40: 349-379.

Murphy, J.P., S. Leath, D. Huynh, R.A. Navarro, and A. Shi. 1998. Registration of NC96BGTD1, NC96BGTD2, and NC96BGTD3 wheat germplasm resistant to powdery mildew. Crop Sci. 38:570.

Murphy, J.P., S. Leath, D Huynh, R.A. Navarro, and A. Shi. 1999a. Registration of NC96BGTA4, NC96BGTA5, and NC96BGTA6 wheat germplasm. Crop Sci. 39:883-884.

Murphy, J.P., S. Leath, D Huynh, R.A. Navarro, and A. Shi. 1999b. Registration of NC97BGTD7 and NC97BGTD8 wheat germplasms resistant to powdery mildew. Crop Sci. 39:884-885.

Murphy, J.P., R.A. Navarro, and S. Leath. 2002. Registration of NC99BGTAG11 wheat germplasm resistant to powdery mildew. Crop Sci. 42:1382.

Navarro, R.A., J.P. Murphy, and S. Leath. 2000. Registration of NC97BGTAB9, and NC97BGTAB10 wheat germplasm lines resistant to powdery mildew. Crop Sci. 40:1508-1509.

Paillard, S., I. Goldringer, J. Enjalbert, G. Doussinault, C. de Vallavielle-Pope, and P. Brabant. 2000. Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. I- Is specific seedling resistance selected?. Theor. Appl. Genet. 101:449-456.

Pedersen, W.L., and S. Leath. 1988. Pyramiding major genes for resistance to maintain residual effects. Annu. Rev. Phytopathol. 26:369-378.

Rong, J.K., E. Millet, J. Manisterski, and M. Feldman. 2000. A new powdery mildew resistance gene: Introgression from wild emmer into common wheat and RFLP-based mapping. Euphytica. 115:121-126.

Sedcole, J.R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. Phytopathology 63:867-872.

Snedecor, G.W., and W.G. Cochran. 1956. Statistical methods applied to experiments in agriculture and biology. The Iowa State College Press, Ames, IA.

Starling, T.M., C.W. Roane, and H.M. Camper, Jr. 1986. Registration of 'Saluda' wheat. Crop Sci. 26:200.

Zeller, F.J., and S.L.K. Hsam. 1998. Progress in breeding for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). *In* Slinkard, A. E. (ed). Proceedings of the 9th International Wheat Genetics Symposium. University of Saskatchewan, Saskatoon, Canada. Vol.1 pp. 178-180.

Weisz, R. 2000. Small grain production guide 2000-01. North Carolina Cooperative Extension Service.

Table 2.1 Powdery mildew resistant germplasms, their pedigrees and sources of resistant genes.

Germplasm Line (Reference)	Pedigree [Working ID]	Donor Specie (Genomes)
NC96BGTD1 (Murphy et al., 1998.)	'Saluda'*3/TA2570 (BC_2F_6) [NCD1]	*A. tauschii* subsp. t*auschii* (DD)
NC96BGTA4 (Murphy et al., 1999.)	'Saluda'*3/PI22141 (BC_2F_6) [NCA4]	*T. monococcum* subsp. *monococcum* (AA)
NC97BGTAB10 (Navarro et al., 2000.)	'Saluda'*3//'Ward'/ PI471746 (BC_2F_5) [NCAB10]	*T. turgidum* subsp. *dicoccoides* (AABB)
NC99BGTAG11 (Murphy et al. 2002.)	'Saluda'*3/PI427315 (BC_2F_7) [NCAG11]	*T. timopheevii* subsp. *armeniacum* (AAGG)

Table 2.2 Observed and expected ratios among $F_{2:3}$ lines in greenhouse evaluations of 10 wheat crosses segregating for powdery mildew resistance.

No.	Cross	Number of $F_{2:3}$ lines				Expected Ratio	χ^2	*P*
		Resistant	Segregating	Susceptible	Total			
1.	Saluda x NCD1	24	52	24	100	1R[†]:2Se:1S	0.16	0.92
2.	Saluda x NCA4	18	52	29	99	1R:2Se:1S	2.70	0.69
3.	Saluda x NCAB10	21	59	17	97	1R:2Se:1S	4.88	0.09
4.	Saluda x NCAG11	26	41	32	99	1R:2Se:1S	3.65	0.16
5.	NCD1 x NCAG11	70	69	7	146	15(R+Se):1S	0.31[‡]	0.58
6.	NCAG11 x NCAB10	118	76	10	204	15(R+Se):1S	0.42[‡]	0.52
7.	NCA4 x NCAB10	50	63	8	121	15(R+Se):1S	5.51×10^{-4}[‡]	0.98
8.	NCD1 x NCAB10	68	43	11	122	15(R+Se):1S	1.16[‡]	0.28
9.	NCD1 x NCA4	17	15	1	33	15(R+Se):1S	0.16[‡]	0.69
10.	NCA4 x NCAG11	192	3	0	195	15(R+Se):1S	11.96[‡]	5.45×10^{-4}

[†]R, resistant; Se, segregating; S, susceptible.
[‡]Chi-square tests calculated with Yates correction factor for one degree of freedom.

Table 2.3 Mean disease scores in greenhouse evaluations for the four germplasm lines, Saluda, and $F_{2:3}$ progenies in 10 segregating populations.

Cross		N	Mean	Range
1.	Saluda x NCD1 $F_{2:3}$			
	Saluda	20	7.0± 0.00	-
	NCD1	20	4.6±0.47	4.0-5.0
	Resistant	24	4.6±0.28	4.0-5.0
	Segregating	52	5.9±0.47	4.8-6.8
	Susceptible	24	6.9±0.17	6.4-7.0
2.	Saluda x NCA4 $F_{2:3}$			
	Saluda	20	7.1±0.24	7.0-8.0
	NCA4	20	4.7±0.41	4.0-5.0
	Resistant	18	4.8±0.26	4.0-5.0
	Segregating	52	6.2±0.35	5.2-6.8
	Susceptible	29	6.9±0.16	6.4-7.0
3.	Saluda x NCAB10 $F_{2:3}$			
	Saluda	20	6.5±0.57	6.0-8.0
	NCAB10	20	3.5±0.38	3.0-4.0
	Resistant	21	3.3±0.16	3.0-3.7
	Segregating	59	4.4±0.60	3.2-5.7
	Susceptible	17	6.7±0.29	6.0-7.0
4.	Saluda x NCAG11 $F_{2:3}$			
	Saluda	20	-	6.0-7.0
	NCAG11	20	0.0±0.00	-
	Resistant	26	0.0±0.00	-
	Segregating	41	-	-
	Susceptible	32	-	-
5.	NCD1 x NCAG11 $F_{2:3}$			
	Saluda	29	6.0.±0.51	5.0-7.0
	NCD1	29	2.9±1.17	0.5-5.0
	NCAG11	29	0.0±0.00	-
	Resistant	70	0.2±0.37	0.0-2.1
	Segregating	69	2.1±1.41	0.5-5.6
	Susceptible	7	6.3±0.25	5.9-6.6

Table 2.3 Continued.

Cross		N	Mean	Range
6.	NCAG11 x NCAB10 $F_{2:3}$			
	Saluda	39	6.2±0.68	5.0-7.0
	NCAG11	39	0.0±0.00	-
	NCAB10	39	1.4±1.38	0.0-5.0
	Resistant	118	0.1±0.32	0.0-1.9
	Segregating	76	2.2±1.28	0.5-5.9
	Susceptible	10	6.4±0.44	5.7-6.9
7.	NCA4 x NCAB10 $F_{2:3}$			
	Saluda	23	6.0±0.48	5.0-7.0
	NCA4	23	2.2±0.91	1.0-3.5
	NCAB10	23	2.7±0.65	1.5-4.0
	Resistant	50	1.4±1.21	0.0-3.9
	Segregating	63	3.5±0.96	1.0-5.3
	Susceptible	8	6.1±0.47	5.4-6.9
8.	NCD1 x NCAB10 $F_{2:3}$			
	Saluda	24	6.7±0.44	6.0-7.5
	NCD1	24	4.2±0.67	3.0-5.0
	NCAB10	24	4.2±0.49	3.5-5.0
	Resistant	68	2.9±0.95	0.8-4.8
	Segregating	43	4.4±0.96	1.8-5.9
	Susceptible	11	6.2±0.26	6.0-6.7
9.	NCD1 x NCA4 $F_{2:3}$			
	Saluda	7	6.9±0.24	6.5-7.0
	NCD1	7	3.8±0.49	3.0-4.5
	NCA4	7	1.7±0.49	1.0-2.5
	Resistant	17	1.3±0.93	0.0-3.1
	Segregating	15	4.0±1.05	2.4-6.2
	Susceptible	1	7.0±0.00	-
10.	NCA4 x NCAG11 $F_{2:3}$			
	Saluda	39	7.1±0.36	6.5-8.0
	NCA4	39	3.2±0.78	2.0-5.0
	NCAG11	39	0.0±0.00	-
	Resistant	192	1.0±1.00	0.0-4.0
	Segregating	3	2.7±0.86	1.9-3.6
	Susceptible	0	-	-

Table 2.4 Mean disease scores in Kinston 2002 of the four germplasm lines, Saluda, and $F_{2:3}$ progenies in five segregating populations.

Cross		N	Mean	Range for lines
1.	Saluda x NCD1 $F_{2:3}$			
	Saluda	8	6.6±0.52	6.0-7.0
	NCD1	8	1.4±0.53	1.0-2.0
	Resistant	48	1.7±1.16	0.0-4.0
	Segregating	104	4.1±0.64	2.5-5.5
	Susceptible	41	6.3±0.67	5.0-7.0
2.	Saluda x NCA4 $F_{2:3}$			
	Saluda	10	7.7±0.67	7.0-9.0
	NCA4	10	0.0±0.00	-
	Resistant	41	0.0±0.00	-
	Segregating	108	3.4±0.25	2.5-4.0
	Susceptible	45	7.1±0.64	6.0-8.0
3.	Saluda x NCAB10 $F_{2:3}$			
	Saluda	10	7.1±0.57	6.0-8.0
	NCAB10	10	4.7±0.48	4.0-5.0
	Resistant	14	4.7±0.55	3.0-5.0
	Segregating	142	5.5±0.43	4.0-6.5
	Susceptible	29	6.7±0.54	6.0-8.0
4.	Saluda x NCAG11 $F_{2:3}$			
	Saluda	10	7.1±0.57	6.0-8.0
	NCAG11	10	0.0±0.00	-
	Resistant	52	0.0±0.00	-
	Segregating	81	3.4±0.21	3.0-3.5
	Susceptible	56	7.2±0.53	5.0-8.0
5.	NCD1 x NCAG11 $F_{2:3}$			
	Saluda	8	7.4±0.52	7.0-8.0
	NCD1	8	1.4±1.06	0.0-3.0
	NCAG11	8	0.0±0.00	-
	Resistant	54	0.3±0.89	0.0-4.0
	Segregating	82	3.4±0.39	2.5-5.0
	Susceptible	10	7.2±0.40	7.0-8.0

Table 2.5 Observed and expected ratios among $F_{2:3}$ lines in Kinston, 2002 of five crosses segregating for powdery mildew resistance.

No.	Cross	Number of $F_{2:3}$ lines				Expected Ratio	χ^2	*P*
		Resistant	Segregating	Susceptible	Total			
1.	Saluda x NCD1	48	104	41	193	1R[†]:2Se:1S	1.67	0.43
2	Saluda x NCA4	41	108	45	194	1R:2Se:1S	2.66	0.26
3.	Saluda x NCAB10	14	142	29	185	1R:2Se:1S	55.41	9.28×10^{-13}
4.	Saluda x NCAG11	52	81	56	189	1R:2Se:1S	4.03	0.13
5.	NCD1 x NCAG11	54	82	10	146	7R:8Se:1S	2.72	0.26

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

Table 2.6 Mean disease scores in Kinston, 2003 of the four germplasm lines, Saluda, and $F_{2:4}$ progenies from resistant and susceptible $F_{2:3}$ lines from Saluda x germplasm and NCD1 x NCAG11crosses.

Cross		N	Mean	Range
1.	Saluda x NCD1 $F_{2:4}$			
	Saluda	1	6.0	-
	NCD1	1	1.0	-
	Resistant	20	1.9±0.72	1.0-3.0
	Segregating	0	-	-
	Susceptible	20	6.9±0.60	6.0-8.0
2.	Saluda x NCA4 $F_{2:4}$			
	Saluda	1	7.0	-
	NCA4	1	2.0	-
	Resistant	19	0.5±0.53	0.0-1.0
	Segregating	1	2.5	-
	Susceptible	20	6.7±0.47	6.0-7.0
3.	Saluda x NCAB10 $F_{2:4}$			
	Saluda	1	7.0	-
	NCAB10	1	2.0	-
	Resistant	13	3.5±0.66	2.0-4.0
	Segregating	0	-	-
	Susceptible	24	6.5±0.88	5.0-8.0
4.	Saluda x NCAG11 $F_{2:4}$			
	Saluda	1	7.0	-
	NCAG11	1	0.0	-
	Resistant	18	0.0±0.00	-
	Segregating	0	-	-
	Susceptible	20	6.7±0.57	6.0-8.0
5.	NCD1 x NCAG11 $F_{2:4}$			
	Saluda	1	7.0	-
	NCD1	1	1.0	-
	NCAG11	1	0.0	-
	Resistant	30	0.3±0.55	0.0-2.0
	Segregating	1	3.0	-
	Susceptible	10	6.4±0.52	6.0-7.0

Table 2.7 Mean powdery mildew disease scores in Kinston 2002 for isolines with different *Pm* genes.

	Isoline	N	Times evaluated	Mean	Range for lines
1.	Aximinister (*Pm1*)	2	3	3.5±0.84	2.0-4.0
2.	Ulka (*Pm2*)	2	3	7.5±0.55	7.0-8.0
3.	Asosan (*Pm 3a*)	2	3	7.2±0.75	6.0-8.0
4.	Chul (*Pm3b*)	2	3	8.0±0.00	-
5.	Sonora (*Pm3c*)	2	3	7.0±1.41	6.0-8.0
6.	MI Amber (*Pm3f*)	2	3	7.2±0.50	7.0-8.0
7.	Yuma (*Pm4*)	2	3	7.0±0.00	-
8.	Hope (*Pm5*)	2	3	7.7±0.52	7.0-8.0
9.	Coker 747 (*Pm6*)	2	3	7.0±0.00	-
10.	Transec (*Pm7*)	2	3	7.8±0.41	7.0-8.0
11.	Federation/Kavkaz (*Pm8*)	2	3	6.7±1.51	5.0-8.0
12.	Amigo (*Pm17*)	2	3	0.0±0.00	-
13.	Chancellor	2	3	7.5±0.55	7.0-8.0

Table 2.8 Mean powdery mildew disease scores in Kinston 2003 for isolines with different *Pm* genes.

	Isoline	N	Times evaluated	Mean	Range for lines
1.	Aximinister (*Pm1*)	1	3	4.0±0.00	-
2.	Ulka (*Pm2*)	1	3	7.3±0.58	7.0-8.0
3.	Asosan (*Pm 3a*)	1	3	8.0±0.00	-
4.	Chul (*Pm3b*)	1	3	8.0±0.00	-
5.	Sonora (*Pm3c*)	1	3	8.0	-
6.	MI Amber (*Pm3f*)	1	3	8.0±0.00	-
7.	Yuma (*Pm4*)	1	3	7.0±0.00	-
8.	Hope (*Pm5*)	1	3	8.0±0.00	-
9.	Coker 747 (*Pm6*)	1	3	7.7±0.58	7.0-8.0
10.	Transec (*Pm7*)	1	3	8.0±0.00	-
11.	Federation/Kavkaz (*Pm8*)	1	3	0.5±0.71	0.0-1.0
12.	Amigo (*Pm17*)	1	3	0.0±0.00	-
13.	Chancellor	1	3	-	-

Chapter 3

Identification of AFLP Markers Linked to Genes Conferring Resistance to Powdery Mildew (*Blumeria graminis* DC f. sp. *tritici* Em. Marchal) in Soft Red Winter Wheat (*Triticum aestivum* L.) Germplasm Lines and Their Application in Gene Pyramiding

ABSTRACT

Powdery mildew (*Blumeria graminis* DC f. sp. *tritici* Em. Marchal) of wheat (*Triticum aestivum* L.) is especially devastating in temperate, cool and moist regions. It increasingly affects wheat in drier and warmer areas as a consequence of changing crop practices such as irrigation and increased nitrogen fertilization and the use of semi-dwarf cultivars. Resistant wheat cultivars remain the most cost efficient and effective means for powdery mildew control. Monogenic resistance is generally very efficient but tends to be a short time solution if variability in the pathogen population is great. Gene pyramiding or the accumulation of multiple resistance genes into a single genotype is one way to provide durable resistance to a range of pathogen races. The development of pyramidal complexes of powdery mildew resistance genes in wheat requires detailed knowledge of the genetics of resistance sources and the identification of molecular markers tightly linked to the resistance alleles. The objectives of this research were to 1) identify AFLP markers linked to resistance genes in the germplasm lines NC96BGTD1 (NCD1) and NC99BGTAG11 (AG11) by bulked segregant analysis, 2) estimate linkages between identified markers and the resistance genes in segregating F_2 populations from Saluda x NCD1 and Saluda x NCAG11 crosses, 3) study inheritance of the identified markers in $F_{2:4}$ resistant and susceptible lines from Saluda x NCD1 and Saluda x NCAG11 crosses, and 4) identify pyramided resistance genes in $F_{2:4}$ lines from the cross between NCD1 and NCAG11. A total of four AFLP markers were linked to resistance in NCAG11 (M-act/cct-196bp at 0.8 cM and M-acc/cga-126bp at 2.2 cM) and NCD1 (M-aca/cgt-182bp at 20.7 cM and Maag/cga-148bp at 36.9 cM). All four markers were inherited after two

generations of self-pollination of resistant and susceptible F_2 individuals from Saluda x NCD1 and Saluda x AG11 crosses. Pyramided markers from each parent were identified in $F_{2:4}$ wheat lines homogeneous for powdery mildew resistance from the cross between NCD1 and NCAG11. Testcrosses have been made to identify lines segregating for both resistance genes.

INTRODUCTION

Powdery mildew of wheat (*Triticum aestivum* L.) is a foliar disease caused by an obligate biothropic fungus *Blumeria graminis* DC f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* DC f. sp. *Tritici* Marchal)of the order *Erysiphales*, which are filamentous Ascomycetes. Powdery mildew occurs annually in Eastern North Carolina and severe epidemics on susceptible cultivars are common. The fungus can be present on winter wheat continuously from fall until late spring (Bowen et al., 1991). Yield reductions of 17% in the susceptible cultivar Saluda and 3 % in cultivars with effective levels of resistance such as Coker 983 have been reported in North Carolina (Leath and Bowen, 1989). Johnson et al. (1979) reported yield reductions as high as 34% in the cultivar Chancellor in Maryland.

Molecular identification of specific DNA sequences can be used to identify the presence or absence of *Pm* genes, their chromosomal location, the number of genes present in a cultivar and the way in which they are transmitted to progeny (Chen and Chelkowski, 1999). Molecular marker techniques used for identification and confirmation of *Pm* genes to powdery mildew were: 1) Restriction Fragment Length Polymorphisms (**RFLP)**, 2) Random Amplified Polymorphic DNA (**RAPD)**, 3) Amplified Fragment Length Polymorphisms (**AFLP)**, 4) **Microsatellites**, 5) Sequence Tagged Site (**STS)**, 6) Sequence Characterized Amplified Region (**SCAR)**, 7) Differential Display Reverse Transcriptase (**DDTR)**.

AFLP is currently one of the most efficient techniques for generating a large number of DNA markers (Myburg et al., 2001) and is very suitable for analysis of large

genomes such as in wheat. This technique has been successfully implemented to identify markers closely linked to *Pm* genes (Hartl et al., 1999) and generation of molecular maps in wheat (Huang et al., 2000b; Hazen et al., 2002).

Gene pyramiding or the accumulation of multiple resistance genes into a single genotype is one way to provide durable resistance to a range of pathogen races (Pedersen and Leath, 1988). Various resistance genes can be pyramided together including major and minor genes, effective and defeated genes, race specific and race non-specific genes (Pedersen and Leath, 1988). Success depends on the pyramiding of efficient alleles at multiple loci with the primary criterion for a pyramid being its durability.

Liu et al. (2000) reported on marker-assisted selection of the three pairwise pyramids of *Pm2*, *Pm4a*, and *Pm21*. These gene combinations were introduced into an elite Chinese cultivar Yang 158 from wheat lines carrying *Pm4a*, *Pm2* and *Pm21* by six to eight backrosses. The isolines carrying different *Pm* genes were crossed pairwise and resulting F_2 populations were evaluated for presence of individual *Pm* genes. Double-haploid lines from crosses between different lines carrying *Pm* genes were selected.

Small grains breeding and pathology programs at NC State University have released 11 germplasm lines with powdery mildew resistance genes to date. These genes were incorporated from *T. monococcum, T. turgidum, T. timopheevii,* and *Ae. tauschii* into the soft red winter wheat cultivar Saluda and released as BC_2F_5, BC_2F_6, and BC_2F_7 derived lines (Murphy et al., 1998, 1999a; 1999b, 2000; Navarro et al., 2000). Genetics of resistance in four germplasm lines: NC96BGTD1 (NCD1), NC96BGTA4 (NCA4), NC98BGTAB10 (NCAB10) and NC99BGTAG11 (NCAG11) were studied as described in Chapter 2. Each germplasm carried a different single gene for resistance to powdery

mildew. All four genes were successfully combined in all pairwise combinations to generate lines with more than one gene for resistance to powdery mildew.

The objectives of this research were to: 1) identify AFLP markers linked to resistance genes in NCD1 and NCAG11 and 2) identify $F_{2:4}$ lines with pyramided resistance genes from the cross between NCD1 and NCAG11.

MATERIALS AND METHODS

1. Plant material

Two germplasm lines resistant to powdery mildew, NC96BGTD1 (NCD1) (Murphy et al., 1998), and NC99BGTAG11 (NCAG11) (Murphy et al., 2002), the soft red winter wheat cultivar Saluda (Starling et al., 1986) and progenies of crosses between the three genotypes were used in this investigation. NCD1 was selected as a BC_2F_6-derived line from interspecific hybridizations between *A. tauschii* and the soft red winter wheat cultivar Saluda, and NCAG11 was selected as a BC_2F_7- derived line from interspecific hybridization between *T. timopheevi* and Saluda. Both germplasm lines were crossed to Saluda and to each other for a total of three populations, Saluda x NCD1, Saluda x NCAG11 and NCD1 x NCAG11. Plant material utilized was described in Chapter 2. Greenhouse and field tests for resistance to powdery mildew were described in Chapter 2. Resistance in Saluda x NCD1 and Saluda x NCAG11 segregated as a monogenic trait in greenhouse and field studies. Resistance in the NCD1 x NCAG11 population segregated as a digenic trait in greenhouse and field studies.

2. PCR analysis

DNA was isolated from 1) 94 F_2 plants from the cross of Saluda x NCD1 and 128 F_2 plants from the cross of Saluda x NCAG11 plants grown in the greenhouse. 2) Twenty-two $F_{2:4}$ plants from the cross of Saluda x NCD1, 22 $F_{2:4}$ plants from the cross of Saluda x NCAG11 grown in the field during January and February 2003. 3) Twenty-nine $F_{2:4}$ lines, with three plants per line, from the cross between NCD1 x NCAG11 grown in the field during February 2003. 4) Selfed progenies of all parents involved in the crosses.

DNA isolated from selfed progenies of the three parental lines was used in screening for polymorphisms between parents, in F_2 bulked segregant analysis and as controls in $F_{2:4}$ segregation analyses. For Saluda x NCD1 F_2 only, DNA was extracted with the 2X CTAB system according to Santos (2000). For all other populations and parents the DNA was extracted with DNeasy Plant Mini Kit (Qiagen, Inc. Valencia, CA). The DNA concentration was adjusted to 200 ng per 12.5 µl of reaction mixture.

DNA from 10 homozygous resistant and 10 homozygous susceptible individuals identified in greenhouse and field tests were pooled to generate resistant and susceptible bulks for the Saluda x NCD1 and Saluda x NCAG11 F_2 and $F_{2:4}$ populations (Michelmore et al., 1991). Restriction and ligation were according to the protocol supplied by Life Technologies (Gaithersberg, MD) with the Core Reagent Kit. Preamplifications were performed with *EcoRI*+A, *MseI*+C (Life Technologies). PCR conditions for preamplifications were: 94°C 30s, 60°C 30s, 72°C 60s (27 cycles). The preamplification products were diluted to 1:50 and stored at -4°C short-term, or -35°C for long-term storage. Selective amplifications were done with *EcoRI*+3 and *MseI*+3 primer combinations. Sequences of *EcorI* primers (LI-COR, Inc. Lincoln, NE) were 5'-GACTGCGTACCAATTCNNN-3' and MseI primers (Sigma-Aldrich, Inc. Milwaukee, WI) were 5'-GATGAGTCCTGAGTAANNN-3'. PCR for selective amplifications were: 94°C 30s, 65°C 30s-0.7°C/cycle, 72°C 60s (12 cycles); 94°C 30s, 56°C 30s, 72°C 60s (22 cycles). Five microliters of formamide loading dye were added to each 10 µl of the products of selective amplification. The mixture was denatured at 95°C for 3 minutes and immediately chilled on ice for at least 5 minutes, and centrifuged prior to loading. Between 0.6 µl and 0.8 µl of the final reaction mixture were loaded to 2.5 mm, 8%

polyacrylamide gels. The electrophoresis was done in LI-COR sequencers, Models 4000, and 4200L (LI-COR, Inc. Lincoln, NE), under 48°C, 42W, 35mA, 1500V for ~3hr (Myburg and Remington, 2000).

A total of 88 EcorI/MseI primer combinations were tested for polymorphisms between NCD1 and NCAG11 powdery mildew resistant germplasms versus the susceptible parent Saluda (Table 3.1). Primer combinations producing polymorphisms between Saluda and at least one of the germplasms were used to conduct bulked segregant analysis (BSA) of F_2 individuals from Saluda x NCD1 and Saluda x NCAG11 populations based on phenotypic data collected in the greenhouse and field studies. Primer combinations showing polymorphisms between a resistant germplasm and Saluda, and resistant versus susceptible bulks, were tested for F_2 segregation. $F_{2:3}$ progenies of Saluda x NCD1 population, derived from a total of 94 F_2 individuals with corresponding marker data and $F_{2:3}$ progenies of Saluda x NCAG11 derived from a total 128 F_2 individuals with corresponding marker data were tested for segregation of resistance in the field at the Lower Coastal Plain Tobacco Research Station in Kinston, NC during 2002 as described in Chapter 2. Bands reproduced within 2 bp in subsequent analyses were considered as the same. Once the linkages between markers and genes for powdery mildew resistance were detected, 10 resistant and 11 lines from the Saluda x NCD1 cross and 11 resistant and 12 susceptible lines from the Saluda x NCAG11 cross were developed to confirm the inheritance of the marker phenotypes from the F_2 generation to the $F_{2:4}$ generation. Markers identified as linked to the resistance genes in NCD1 and NCAG11 were used to test $F_{2:4}$ lines homozygous for resistance from the cross NCD1 x NCAG11 for the presence of all four markers.

Electronic images of the gels were analyzed by AFLP Quantar 1.0 software (KeyGene Products B.V., The Netherlands). The scored bands were evaluated as dominantly inherited and a value for each was assigned as present ('+'), or absent ('-'). The molecular weight (MW) of the polymorphic bands was evaluated, based on MW size standards, ranging from 50bp to 700bp. Bands in the range of 75bp to 450bp were considered for the majority of gels.

Chi-square analyses were used to test for expected 3(+):1(-) segregation of markers and for expected 1(R):2(Se):1(S) segregation of resistance in segregating lines. Linkages between marker loci and resistance genes were determided by Mapmaker/Exp (Version 3.0 b) (Lincoln et al., 1993). The marker and phenotypic data were coded as follow: marker present = 'D' (not homozygous for recessive allele), marker absent= 'B' (homozygous for recessive allele), an F_2 individual homozygous resistant = 'A', heterozygous= 'H' and homozygous susceptible= 'B', and missing data point= '-'. The data was entered into the Mapmaker/Exp program in ASCII format. Kosambi's mapping function, which assumes a moderate amount of interference between crossovers was set by the 'cent kos' command. The decimal logarithm of odds (“LOD”) ratio was set by the 'group 3' command to 3.0 according to Risch (1992). The order of loci in a linkage group was tested by the 'compare' command and the best order was determined based on the best maximum likelihood estimates. The recombination values between loci were obtained by the 'map' command.

RESULTS AND DISCUSSION

1. Screening primer combinations and bulked segregant analysis (BSA)

NCD1. Fifty *EcoRI/MseI* primer combinations generated a total of 101 polymorphisms between Saluda and NCD1 (Table 3.2). Sixty-two polymorphisms were in coupling phase where the band was present in NCD1 and absent in Saluda. Thirteen primer combinations agc/cct, agc/ccg, act/cgt, act/cct, act/ccg, act/ccc, act/cca, act/cta, aca/cgt, aag/cta, aag/cgt, aag/cga and aag/cct were retested in F_2 BSA. Two primer combinations produced polymorphic bands (aca/cgt-182bp and aag/cga-148bp) between Saluda and NCD1 and susceptible versus resistant bulks (Table 3.3) (Figure 3.1, 3.2).

NCAG11. Fifty-two *EcoRI/MseI* primer combinations generated a total of 138 polymorphisms between Saluda and NCAG11 (Table 3.2). Eighty-eight polymorphisms were in coupling phase where the marker was present in NCAG11 and absent in Saluda. Twelve primer combinations agc/ccg, act/cgg, act/cga, act/cct, act/ccg, act/cat, acc/cga, aca/cgt, aca/cta, aca/cgt, aag/cgc and aag/ccg were retested in F_2 BSA. Six primer combinations produced polymorphic bands (act/cga-231bp, acc/cga-126bp (Figure 3.3), act/cct-196bp (Figure 3.4), act/ccg-164bp, aag/cgc-111bp and aag/ccg-297bp) between Saluda and NCAG11 and susceptible versus resistant bulks (Table 3.4). A single acc/cga-126bp band was not identified as polymorphic between Saluda and NCAG11 in primer screening, but was identified in bulked segregant analysis as present in NCAG11 and the resistant bulk and absent in Saluda and the susceptible bulk.

Primer screening was used as a tool for identifying *EcoRI/MseI* primer combinations that produced clearly identified band patterns between Saluda and NCD1

and NCAG11. According to Zabeau (1993) five to 200 bands per lane are desirable for an AFLP gel profile, and such a profile would be considered as accurately scoreable. Lanes with greater than 200 bands tended to produce a smear and it was difficult to distinguish between bands in close proximity to each other. Primer screening was a good way to test for primer combinations that will produce 'readable' gel profiles, but also to initially identify polymorphic bands between resistant and susceptible parents.

Bulked segregant analysis was an efficient way of narrowing down candidate markers linked to either side of the resistance locus in NCD1 and NCAG11. This method is widely used to allow fast mapping of single genes for resistance using segregating populations (Michelmore, 1995). It is especially valuable for identification of marker bands linked within 15 cM (Michelmore et al., 1991), although in the present case the method was successful in identifying bands even more distant to a resistant locus. This method has been successfully used in wheat for identification of major resistance genes such as Hessian fly (*Mayetiola destructor*) resistance gene *vH6* (Stuart et al., 1998), powdery mildew resistance genes *Pm25* (Shi et al., 1998), *Pm 1* (Hu et al., 1997), *Pm1* and *Pm2* (Hartl et al., 1995), *Pm1c* (Hartl et al., 1999), *Pm24* (Huang et al., 2000a), *Pm29* (Zeller et al., 2002), and *MIRE* (Chantret et al., 2000), and a major QTL controlling scab (*Fusarium graminearum*) resistance in wheat (Bai et al., 1999).

2. Identification of markers linked to the resistance genes in segregating F_2 populations from Saluda x NCD1 and Saluda x NCAG11 crosses

Saluda x NCD1. A total of 87 F_2 individuals were evaluated for the segregation of the aca/cgt-182bp band (Figure 3.1.C). Sixty-four individuals had the marker band present ('+') and 23 had the marker absent ('-'). The chi-square test value for the expected

ratio of 3(+):1(-) was $\chi^2_{3:1(+):(-)}$= 0.03 ($P \leq 0.85$), indicating that the marker allele followed independent monogenic segregation (Table 3.5).

A total of 83 individuals were evaluated for segregation of the aag/cga-148bp band (Figure 3.2.C). Forty-four individuals had the marker band present and 39 had the marker absent. The chi-square test value for the expected ratio of 3(+):1(-) was $\chi^2_{3:1(+):(-)}$= 20.24 ($P \leq 6.81 \times 10^{-6}$), indicating that the marker allele did not follow independent monogenic segregation (Table 3.5). The order of loci in the linkage group was aag/cga-148bp-->16.2 cM<--aca/cgt-182bp-->20.7 cM<--allele for resistance (Table 3.7). The log-likelihood value of -80.77, corresponding to the relative log-likelihood of 0.00 indicated this was the best order.

Saluda x NCAG11. A total of 127 F_2 individuals were evaluated for segregation of the acc/cga-126bp band (Figure 3.3.B). Eighty-seven individuals had the marker band present ('+') while 40 individuals had the band absent ('-'). The chi-square test value for the expected ratio of 3(+):1(-) was $\chi^2_{3:1(+):(-)}$= 2.52 ($P \leq 0.11$), indicating that the marker allele followed independent monogenic segregation (Table 3.6).

A total of 121 F_2 individuals were evaluated for segregation of the act/cct-196bp band (Figure 3.4.C). Eighty-four individuals had the band present and 37 had the band absent. The chi-square test value for the expected ratio of 3(+):1(-) was $\chi^2_{3:1(+):(-)}$= 1.72 ($P \leq 0.19$), indicating that the marker allele followed independent monogenic segregation (Table 3.6).

The order of loci in the linkage group was act/cct-196bp-->0.8 cM<--allele for resistance-->2.2 cM<--acc/cga-126bp (Table 3.8). The log-likelihood value of -68.08, corresponding to the relative log-likelihood of 0.00 indicated the best order.

Tightly linked markers such as the two AFLPs flanking the powdery mildew resistance gene at 0.8cM and 2.2cM in NCAG11 are especially valuable. If both markers are present then the probability that the resistant gene is present is *P*= 1-r1*r2= 1- 0.008 * 0.022= >0.99. AFLP marker band aca/cgt-182bp is 20.7 cM apart from the resistance gene in NCD1 and very valuable, but the 20.7% recombination rate must be considered, whenever the presence of this marker alone is used to determine the presence of the resistance gene. Another AFLP marker band linked to aca/cgt-182bp at 16.2 cM and the resistance gene in NCD1 at 36.9cM is the aag/cga-148bp marker. This marker may be of lesser practical value due to the large distance from the resistance gene, and the distorted segregation ratio. However, the aca/cgt-182bp marker can still be used to generate data that will support selection.

All four markers were compared to the existing AFLP maps from the Opata 85 x W7984 cross (Hazen et al., 2002) and nulli-tetrasomic and several ditelosomic lines of 'Chinese Spring' (Huang et al., 2000b) wheat. Out of 10 *EcoRI/MseI* primer combinations tested by Hazen et al. (2002) and 15 *EcoRI/MseI* primer combinations assigned to individual chromosomes by Huang et al. (2000b), no primer combinations were the same as the four identified as AFLP markers linked to the powdery mildew resistance genes in NCD1 and NCAG11. Knowledge of the chromosomal location of the resistance genes from NCD1 and NCAG11 would be helpful to understand their possible relationship and proximity to mapped resistance genes.

3. Confirmation of markers linked to the resistance genes in $F_{2:4}$ lines derived from Saluda x NCD1 and Saluda x NCAG11 crosses

Saluda x NCD1. A total of 21 $F_{2:4}$ lines derived from 10 homozygous resistant and 11 homozygous susceptible $F_{2:3}$ lines were evaluated for resistance to powdery mildew in the field (Table 3.9). The results confirmed a complete agreement in the categorization of the selfed $F_{2:3}$ lines and their $F_{2:4}$ progenies. One individual per $F_{2:4}$ line, Saluda, NCD1, the resistant and susceptible $F_{2:4}$ bulks were tested for the presence of aca/cgt-182bp (Figure 3.1.D) and aag/cga-148bp (Figure 3.2.D) marker bands.

NCD1 and the resistant bulk showed the aca/cgt-182bp marker band, while Saluda and the susceptible bulk lacked it. The band was present in nine $F_{2:4}$ individuals and absent in 12 (Figure 3.1.D). Of the 21 $F_{2:4}$ individuals evaluated for resistance and the presence of the marker band, eight resistant individuals had this band present while two resistant individuals had this band absent (Table 3.9). The band was absent in all 11 susceptible individuals.

NCD1 and the resistant bulk showed the aag/cga-148bp marker band, while Saluda and the susceptible bulk lacked it. The band was present in 10 $F_{2:4}$ individuals and absent in 11 (Figure 3.2.D). Of the 21 $F_{2:4}$ individuals evaluated for resistance and presence of the marker band, nine resistant individuals had this band present and one resistant individual had this band absent (Table 3.9). Out of 11 susceptible individuals, 10 had the marker band absent and one had this band present.

Saluda x AG11. A total of 23 $F_{2:4}$ lines derived from 11 resistant and 12 susceptible $F_{2:3}$ lines were evaluated for resistance to powdery mildew in the field (Table 3.10). The results confirmed a complete agreement in the categorization of the selfed $F_{2:3}$ lines and their $F_{2:4}$ progenies. One individual per $F_{2:4}$ line, Saluda, NCAG11, resistant and

susceptible bulks were tested for the presence of acc/cga-126bp (Figure 3.3.C) marker bands and act/cct-196bp (Figure 3.4.D).

NCAG11 and resistant bulk showed the acc/cga-126bp marker band, while Saluda and the susceptible bulk lacked it. The band was present in 11 $F_{2:4}$ individuals and absent in 9 (Figure 3.3.C). This was a complete agreement between marker band and resistance phenotypes (Table 3.10).

NCAG11 and the resistant bulk showed the act/cct-196bp marker band, while Saluda and the susceptible bulk lacked it. The band was present in 11 $F_{2:4}$ individuals and absent in 12 (Figure 3.4.D). Of the 23 $F_{2:4}$ individuals evaluated for resistance and presence of the marker band, all 11 resistant individuals had the marker present while all 12 susceptible individuals had this band absent (Table 3.10). This was a complete agreement between marker band and resistance phenotypes.

The confirmation of marker alleles in subsequent generations was important to demonstrate that marker alleles were inherited from generation to generation. Only heritable markers can be successfully applied to investigate the inheritance of linked genes. In this study, all four markers linked to powdery mildew resistance genes in NCD1 and NCAG11 persisted in the population after two generations of selfing. If the markers are not 100% linked to the genes of interest it is important to combine marker-aided with phenotypic selection in subsequent generations where a resistant locus may be in a heterozygous or homozygous recessive state.

4. Identification of $F_{2:4}$ lines homozygous for the marker loci, derived from NCD1 x NCAG11 cross

A total of 22 $F_{2:4}$ progenies derived from 19 resistant and three susceptible $F_{2:3}$ lines, were evaluated for resistance to powdery mildew in the field. The 19 resistant lines were homogenously resistant in the $F_{2:3}$ and $F_{2:4}$ generations. The three susceptible lines were homogenously susceptible in the $F_{2:3}$ and $F_{2:4}$ generations.

NCD1, NCAG11, Saluda and 19 resistant and three susceptible $F_{2:4}$ lines, were evaluated for the aag/cga-148bp and aca/cgt-182bp bands, linked to the resistance gene in NCD1 (Table 3.11). NCD1 had both the aag/cga-148bp and aca/cgt-182bp bands, while Saluda and NCAG11 lacked these bands (Figure 3.5). Out of 19 resistant $F_{2:4}$ lines, 14 lines had both bands present, one had only the aag/cga-148bp, two had only the aca/cgt-182bp band and two resistant lines had both markers absent. The three susceptible lines did not have either of the two bands present.

NCAG11, NCD1, Saluda and the same 19 resistant and three susceptible $F_{2:4}$ lines, were evaluated for acc/cga-126bp and act/cct-196bp bands linked to the resistance gene in NCAG11 (Table 3.11). NCAG11 had present both the acc/cga-126bp and act/cct-196bp bands, while Saluda and NCD1 lacked both markers. Out of 19 resistant $F_{2:4}$ lines, 13 lines had both bands present, one had only the act/cct-196bp band and five resistant lines had both bands absent. The three susceptible lines had neither of the bands present.

Out of 19 resistant $F_{2:4}$ lines, eight lines had all four bands present. The remaining 11 resistant lines had between zero and three of the bands present. The three susceptible lines did not have any of the four markers present. Resistant lines with all four bands present were test-crossed to the susceptible parent Saluda to develop $F_{2:3}$ segregating lines to identify resistant lines homozygous for resistance genes from NCD1 and NCAG11.

The application of molecular markers linked to resistance genes can be a great aid in identifying lines that carry multiple genes for powdery mildew resistance (Liu et al., 2000). The application of marker-aided selection is most valuable when phenotypic selection may be inefficient to identify all genes controlling the phenotype, as in the case of pyramided powdery mildew resistance genes. When dominant markers are used to identify lines homozygous for both resistance genes, additional test-crosses are needed. This means that distinction between some of the loci heterozygous and homozygous dominant for the resistance alleles was not possible. The identification of $F_{2:3}$ lines as resistant, segregating and susceptible was crucial in order to assure that only homogenously resistant lines from NCD1 x NCAG11 cross were selected. The markers not completely linked to the resistance genes, produce progenies where the presence of the markers means presence of the resistance genes in 1-(No. recombinant genotypes/No. total genotypes). The genotypes that need to be identified by test-crossing will include AABB, AABb, AaBB, AaBB, which cannot be identified by dominant AFLP markers and others derived by recombination between marker and resistance alleles.

Wide use of a small number of currently effective single genes for powdery mildew increases risks of resistance breakdowns due to virulence shifts (Liu et al., 2000). Pyramiding genes for powdery mildew resistance is one of the primary ways of increasing durability of resistance to a range of pathogenic races (Pedersen and Leath, 1988). The durability of resistance is dependent on stabilizing selection. If a single resistant genotype is composed of numerous resistance genes with partial effects there will be a little selection pressure on the pathogen, and only small levels of disease should develop over time. The application of molecular markers is a major tool in pyramiding

resistance genes and identification of genotypes carrying multiple resistance genes in segregating populations. A combination of marker-aided and phenotypic selection appears to be the most efficient way of manipulating alleles at resistance loci.

The line that accumulates partial effects of many resistance genes will put a little selection pressure on the pathogen. Such polygenic resistance should be long-term and exhibit additive gene action. Some limitations and consequences of implementation of this system should be considered (Bennett, 1984; Pedersen and Leath, 1988; Hsam and Zeller, 2002). If the defeated genes continue to exert small effects on disease development, three or four of them may be needed to achieve a useful level of resistance. It also may be difficult to assure that all resistance genes are in the homozygous state. The use of *Pm* genes already matched by the pathogen may result in only short-term resistance. Some combinations of pyramided *Pm* genes may become inefficient if challenged by complex pathogenicity. It is still unknown if this method could cause the development of a "super race" of powdery mildew. Such a race would exhibit broad and complex virulence spectrum. A practical application of pyramiding *Pm* genes is in progress.

LITERATURE CITED:

Bai, G., F.L. Kolb, G.Shaner, and L.L. Doimer. 1999. Amplified fragment length polymorphism marker linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathol. 89(4):343-347.

Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathol. 33:279-300.

Bowen, K.L., K.L. Everts, and S. Leath. 1991. Reduction in yield of winter wheat in North Carolina due to powdery mildew and leaf rust. Phytopathology. 81(5):503-511.

Chantret, N., P. Sourdille, M. Röder, M. Tavaud, M. Bernard, and D. Dousinault. 2000. Location and mapping of the powdery mildew resistance gene *MlRE* and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. Theor. Appl. Genet. 100:1217-1224.

Chen, Y., and J. Chelkowski. 1999. Genes for resistance to wheat powdery mildew. J. Appl. Genet. 40(4):317-334.

Hartl, L., H. Weiss, U. Stephan, F.J. Zeller, and A. Jahoor. 1995. Molecular identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 90:601-606.

Hartl, L., V. Mohler, F.J. Zeller, S.L.K. Hsam and G. Schweizer. 1999. Identification of AFLP markers closely linked to powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat (*Triticum aestivum* L.). Genome 42:322-329.

Hazen, S.P., P. Leroy, and R. Ward. 2002. AFLP in Triticum aetivum L.: patterns of genetic diversity and genome distribution. Euphytica 125:89-102.

Hsam, S.L.K., and F.J. Zeller 2002. Breeding for Powdery Mildew Resistance in common wheat (*Triticum aestivum* L.). *In* Bélanger, R.R., W.R. Bushnell, A.J. Dik and T.L.W. Carver (ed). The powdery mildews: A Comprehensive Treatise. The American Phytopathological Society, St. Paul. pp. 219-238.

Huang, X.Q., S.L.K. Hsam, F.J. Zeller, G. Wenzel, and V. Mohler. 2000a. Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor. Appl. Genet. 101:407-414.

Huang, X., Zeller, F.J., S.L.K. Hsam, G. Wnezel, and V. Mohler. 2000b. Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. Genome 43:298-305.

Hu, X.Y., H.W. Ohm, and I. Dweikat. 1997. Identification of RAPD markers linked to the gene PM1 for resistance to powdery mildew in wheat. Theor. Appl. Genet. 94:832-840.

Johnson, J.W., P.S. Baenziger, W.T. Yamazaki, and R.T. Smith. 1979. Effects of powdery mildew on yield and quality of isogenic lines of 'Chancellor' wheat. Crop Sci. 19:349-352.

Leath, S., and K.L. Bowen. 1989. Effects of powdery mildew, triadimenol seed treatment, and triadimefon foliar sprays on yield of winter wheat in North Carolina. Phytopathology 79(2):152-155.

Lincoln, S. E., M. J. Daly, and E. Lander. 1993. Constructing Linkage Maps with Mapmaker/Exp Version 3.0; A Tutorial and Reference Manual. 3rd Edition. A Whitehead Institute for Medical Research. Cambridge, MA.

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

Michelmore, R.W., I. Paran, and V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Michelmore, R. 1995. Molecular approaches to manipulation of disease resistance genes. Annu. Rev. Phytopathol. 15:393-427.

Murphy, J.P., S. Leath, D. Huynh, R.A. Navarro, and A. Shi. 1998. Registration of NC96BGTD1, NC96BGTD2, and NC96BGTD3 wheat germplasm resistant to powdery mildew. Crop Sci. 38:570.

Murphy, J.P., S. Leath, D. Huynh, R.A. Navarro, and A. Shi. 1999a. Registration of NC96BGTA4, NC96BGTA5, and NC96BGTA6 wheat germplasm. Crop Sci. 39:883-884.

Murphy, J.P., S. Leath, D. Huynh, R.A. Navarro, and A. Shi. 1999b. Registration of NC97BGTD7 and NC97BGTD8 wheat germplasms resistant to powdery mildew. Crop Sci. 39:884-885.

Murphy, J.P., R.A. Navarro, and S. Leath. 2002. Registration of NC99BGTAG11 Wheat germplasm resistant to powdery mildew. Crop Sci. 42:1382.

Myburg, A.A., and D.L. Remington. 2000. Protocol for high-throughput AFLP analysis using *LI-COR IR*^{2} automated sequencers. North Carolina State University.

Myburg, A.A., D.L. Remington, D.M. O'Malley, R.R. Sederof, and R.W. Whetten. 2001. High-throughput AFLP analysis using infrared dye-labeled primers and an automated DNA sequencer. Biotechnology 30(2):348-357.

Navarro, R.A., J.P. Murphy, and S. Leath. 2000. Registration of NC97BGTAB9, and NC97BGTAB10 wheat germplasm lines resistant to powdery mildew. Crop Sci. 40:1508-1509.

Pedersen, W.L., and S. Leath. 1988. Pyramiding major genes for resistance to maintain residual effects. Annu. Rev. Phytopathol. 26:369-378.

Risch, N. 1992. Genetic linkage: Interpreting lod scores. Science 255:803-804.

Santos, A. G. 2000. Quantitative trait loci associated with components of winterhardiness in cultivated oats. Ph.D. Dissertation. North Carolina State University.

Shi, A.N., S. Leath, J.P. Murphy. 1998. A major gene for powdery mildew resistance transferred to common wheat from wild eikorn wheat. Phytopathology 88(2):144-147.

Starling, T.M., C.W. Roane, and H.M. Camper, Jr. 1986. Registration of 'Saluda' wheat. Crop Sci. 26:200.

Stuart, J.J., S.J. Schulte, P.S. Hall, and K.M. Mayer. 1998. Genetic mapping of Hessian fly avirulence gene *vH6* using bulked segregant analysis. Genome 41:702-708.

Zabeau, M. 1993. European patent application. Bulletin 93/13.

Zeller, F.J., L. Kong, L. Hartl, V. Mohler, and S.L.K. Hsam. 2002. Chromosomal location of genes for resistance to powdery mildew in common wheat (Triticum aestivum L. em Thell.) 7. Gene Pm29 in line Pova. Euphytica 123:187-194.

Table 3.1 *EcoRI/MseI* primer combinations used to screen for polymorphisms between Saluda versus NCD1 and NCAG11.

	E-AAG	E-ACA	E-ACC	E-ACT	E-AGC	E-AGG
M-CAA	AAG/CAA	ACA/CAA	ACC/CAA	ACT/CAA	AGC/CAA	AGG/CAA
M-CAC	AAG/CAC	ACA/CAC	ACC/CAC	ACT/CAC	AGC/CAC	AGG/CAC
M-CAG	AAG/CAG	ACA/CAG	ACC/CAG	ACT/CAG	AGC/CAG	AGG/CAG
M-CAT	AAG/CAT	-	ACC/CAT	ACT/CAT	AGC/CAT	AGG/CAT
M-CCA	AAG/CCA	-	ACC/CCA	ACT/CCA	AGC/CCA	AGG/CCA
M-CCC	AAG/CCC	-	ACC/CCC	ACT/CCC	AGC/CCC	AGG/CCC
M-CCG	AAG/CCG	-	ACC/CCG	ACT/CCG	AGC/CCG	AGG/CCG
M-CCT	AAG/CCT	-	ACC/CCT	ACT/CCT	AGC/CCT	AGG/CCT
M-CGA	AAG/CGA	-	ACC/CGA	ACT/CGA	AGC/CGA	AGG/CGA
M-CGC	AAG/CGC	-	ACC/CGC	ACT/CGC	AGC/CGC	AGG/CGC
M-CGG	AAG/CGG	-	ACC/CGG	ACT/CGG	AGC/CGG	AGG/CGG
M-CGT	AAG/CGT	ACA/CGT	ACC/CGT	ACT/CGT	AGC/CGT	AGG/CGT
M-CTA	AAG/CTA	ACA/CTA	ACC/CTA	ACT/CTA	AGC/CTA	AGG/CTA

Table 3.1 Continued.

	E-AAG	E-ACA	E-ACC	E-ACT	E-AGC	E-AGG
M-CTC	AAG/CTC	ACA/CTC	ACC/CTC	ACT/CTC	AGC/CTC	AGG/CTC
M-CTG	AAG/CTG	ACA/CTG	ACC/CTG	ACT/CTG	AGC/CTG	AGG/CTG
M-CTT	AAG/CTT	ACA/CTT	ACC/CTT	ACT/CTT	AGC/CTT	AGG/CTT

Table 3.2 *EcoRI/MseI* primer combinations and polymorphisms between Saluda versus NCD1 and NCAG11.

No.	Primer combination	bp	Saluda	NCD1	NCAG11
1	AGG/CTT	460.7	-	+	
		300.3	-	+	+
2	AGG/CTC	131.2	-	+	+
3	AGG/CTA	399.1	+		-
		143.6	+		-
		89.6	-		+
4	AGG/CGT	271.1	-		+
		252.0	-		+
		233.5	-		+
		214.7	-		+
		195.9	-		+
		176.3	-		+
5	AGG/CGG	111.8	-		+
6	AGG/CCG	277.5	-	+	
7	AGG/CAG	171.0	-		+
		160.2	-	+	+
		114.4	-	+	+
8	AGG/CAC	204.3	+		-
		166.8	+		-
9	AGG/CAA	169.0	-	+	
10	AGC/CTT	347.5	-	+	
11	AGC/CTG	242.9	-	+	
		200.4	+	-	
12	AGC/CTC	164.1	-		+
13	AGC/CTA	160.8	-	+	
14	AGC/CGT	215.8	-	+	
		137.5	+		-
15	AGC/CGG	260.5	-		+
		209.6	+		-
16	AGC/CGC	197.3	-		+
		186.0	-	+	
		95.6	+	-	-
17	AGC/CCT	195.5	+	-	-
		195.1	+	-	-
		148.8	+		-
		136.3	-	+	
		123.6	+	-	
		123.3	+	-	
18	AGC/CCG	137.5	-		+
		129.8	+	-	
		129.3	-		+

Table 3.2 Continued.

No.	Primer combination	bp	Saluda	NCD1	NCAG11
19	AGC/CCC	257.4	-	+	
		221.3	-	+	+
		191.3	+		-
20	AGC/CCA	238.9	-		+
		157.4	-	+	
21	AGC/CAT	122.5	+	-	-
22	AGC/CAG	296.2	-		+
		264.9	+		-
		140.7	+		-
		105.4	+		-
		93.3	+		-
23	AGC/CAC	244.4	-		+
		190.0	-	+	
		83.4	-	+	
24	ACT/CGT	196.5	-		+
		97.7	+		-
		97.0	+		-
		82.6	+	-	-
		81.7	+	-	
25	ACT/CGG	491.6	-		+
		478.1	-		+
		444.7	+		-
		259.2	+	-	
		248.0	-		+
26	ACT/CGC	292.5	-		+
		126.6	-		+
		104.2	-		+
		78.8	+		-
27	ACT/CGA	495.0	-	+	+
		408.8	-		+
		364.3	+	-	
		363.4	+	-	
		289.9	-	+	+
		289.2	-	+	+
		270.9	+		-
		249.8	-		+
		247.8	-		+
		231.6	-		+
		212.5	-		+
		113.1	-	+	+
28	ACT/CCT	357.7	+		-

Table 3.2 Continued.

No.	Primer combination	bp	Saluda	NCD1	NCAG11
		323.8	-	+	
		307.7	-	+	+
		245.8	+		-
		196.5	-		+
		179.8	+		-
		137.7	-	+	
29	ACT/CCG	514.4	-		+
		316.4	-		+
		188.9	+		-
		188.7	+		-
		175.5	+		-
		175.4	+		-
		166.0	-		+
		166.0	-		+
30	ACT/CCC	387.2	-		+
		387.0	-		+
		349.7	-	+	+
		253.3	-	+	
		165.0	+		-
		130.7	-		+
		130.5	-		+
31	ACT/CCA	310.5	-	+	
		168.3	+	-	
		144.7	-	+	
32	ACT/CAT	357.0	-		+
		330.3	-		+
		278.9	-	+	+
		150.3	-	+	
33	ACT/CAG	189.2	-	+	
		147.5	+	-	
34	ACT/CAA	159.8	-	+	
35	ACC/CTT	359.4	+		-
36	ACC/CTG	212.8	-		+
37	ACC/CTA	280.5	-	+	+
38	ACC/CGT	500.0	+		-
		394.6	-	+	+
		317.8	-		+
		158.3	+		-
		155.1	-		+
		128.8	+		-
		108.5	-		+

Table 3.2 Continued.

No.	Primer combination	bp	Saluda	NCD1	NCAG11
		64.5	-		+
39	ACC/CGG	205.4	+		-
40	ACC/CGA	516.6	-		+
		424.1	-		+
		208.4	-	+	+
		111.0	-		+
		53.6	-		+
41	ACC/CCT	416.9	-	+	+
42	ACC/CCG	403.1	+		-
		213.2	+		-
		143.6	-		+
		139.6	+		-
		137.6	-		+
43	ACC/CCC	204.6	-		+
		173.1	-		+
		131.4	-	+	+
		119.5	-	+	+
44	ACC/CAT	287.6	+	-	
		201.1	+	-	
45	ACC/CAG	528.4	-	+	+
		148.8	+	-	
46	ACC/CAC	190.1	-	+	
47	ACC/CAA	468.4	-	+	
		402.4	-	+	
		337.5	+		-
48	ACA/CTG	124.1	-	+	
		97.3	-		+
49	ACA/CTC	351.2	-	+	
		205.9	+	-	
		140.2	+	-	
50	ACA/CTA	291.8	-		+
51	ACA/CGT	546.6	-		+
		263.5	+		-
		186.4	+	-	
		183.2	-	+	
		159.4	-	+	+
52	ACA/CGG	411.7	-	+	
		319.3	-		+
		299.7	-		+
53	ACA/CGC	230.7	-		+
		213.0	+	-	

Table 3.2 Continued.

No.	Primer combination	bp	Saluda	NCD1	NCAG11
		191.7	+	-	-
		172.2	+	-	
		167.4	+	-	
		128.7	-		+
		112.7	+	-	-
54	AAG/CTT	307.5	+		-
55	AAG/CTG	81.6	+	-	-
56	AAG/CTC	525.1	-	+	+
		450.5	+	-	
57	AAG/CTA	503.0	+	-	
		431.4	-	+	
		413.0	+	-	
		333.6	+	-	
		293.3	-	+	
		282.1	-	+	
		276.9	+	-	
		122.1	-	+	+
58	AAG/CGT	237.7	-	+	
		212.9	+	-	-
59	AAG/CGG	485.5	+		-
		307.4	+	-	-
		376.6	-	+	+
		274.8	+	-	
		238.1	+	-	-
		112.4	-		+
60	AAG/CGA	495.0	-	+	
		147.3	-	+	
61	AAG/CCT	412.4	+	-	-
		181.2	+	-	
62	AAG/CCG	365.7	-		+
		295.5	-	+	+
		143.8	-		+
63	AAG/CCA	245.7	-	+	
		225.2	-	+	
		179.2	+	-	
64	AAG/CAC	164.6	-	+	
65	AAG/CAA	405.4	-	+	+
		219.3	+		-
	No of primer combinations		65	50	52
	Total No. of polymorphisms		202	101	138
	Coupling polymorphisms		125	62	88

Table 3.3 Identification of markers linked to the resistance gene in NCD1 by bulked segregant analysis.

No. Polym.	No. Primers	Primer combination-bp	NCD1	R†-Bulk	S-Bulk	Saluda
1	1	ACA/CGT-182	+	+	-	-
2	2	AAG/CGA-148	+	+	-	-

† R= resistant, S= susceptible.

Table 3.4 Identification of markers linked to the resistance gene in NCAG11 by bulked segregant analysis.

No. Polym.	No. Primers	Primer combination-bp	NCAG11	R†-Bulk	S-Bulk	Saluda
1	1	ACT/CGA-231	+	+	-	-
2	2	ACT/CCT-196	+	+	-	-
3	3	ACT/CCG-164	+	+	-	-
4	4	ACC/CGA-126	+	+	-	-
5	5	AAG/CGC-111	+	+	-	-
6	6	AAG/CCG-297	+	+	-	-

† R= resistant, S= susceptible.

Table 3.5 Chi-square tests for independent segregation of resistance of $F_{2:3}$ lines, and independent segregation of AFLP markers in F_2 segregating population, from the Saluda x NCD1 cross.

Phenotype	R†	S	Su	+	-	Total	$\chi^{2‡}$	*P*
Resistance	27	45	22			94	0.70	0.70
M-aca/cgt-182bp				64	23	87	0.03	0.85
M-aag/cga-148bp				44	39	83	20.24	6.81×10^{-6}

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

‡ Chi-square test for resistance 1R:2Se:1S and for marker 3(+):1(-). Yates correction factor was used for 3:1 ratios.

Table 3.6 Chi-square tests for independent segregation of resistance of $F_{2:3}$ lines, and independent segregation of AFLP markers in F_2 segregating population, from the Saluda x NCAG11 cross.

Phenotype	R†	Se	Su	+	-	Total	$\chi^{2‡}$	*P*
Resistance	33	56	39			128	2.56	0.28
M-acc/cga-126bp				87	40	127	2.52	0.11
M-act/cct-196bp				84	37	121	1.72	0.19

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

‡ Chi-square test for resistance 1R:2Se:1S and for marker 3(+):1(-). Yates correction factor was used for 3:1 ratios.

Table 3.7 Linkage group of resistant locus and two AFLP marker loci in NCD1.

Linkage Group	Distance [cM]	Log-Likelihood	Relative Log-Likelihood
M-aag/cga-148bp	16.2	-80.77	0.0
M-aca/cgt-182bp	20.7		
Resistance locus	-		

Table 3.8 Linkage group of resistant locus and two AFLP marker loci in NCAG11.

Linkage Group	Distance [cM]	Log-Likelihood	Relative Log-Likelihood
M-act/cct-196bp	0.8	-68.08	0.0
Resistance locus	2.2		
M-acc/cga-126bp	-		

Table 3.9 Conformation of AFLP markers linked to the resistance gene in NCD1, evaluated in $F_{2:4}$ resistant and susceptible lines from Saluda x NCD1 cross.

	Resistance reaction	M-aca/cgt-182bp	M-aag/cga-148bp
NCD1	R[†]	+	+
Saluda	S	-	-
R bulk	R	+	+
S bulk	S	-	-
$F_{2:4}$ lines			
-1	R	+	+
-2	S	-	-
-3	R	+	+
-4	S	-	-
-5	S	-	-
-6	R	-	-
-7	R	+	+
-8	R	+	+
-9	S	-	-
-10	S	-	-
-11	S	-	+
-12	S	-	-
-13	S	-	-
-14	R	+	+
-15	R	+	+
-16	R	+	+
-17	S	-	-
-18	R	+	+
-19	S	-	-
-20	R	+	+
-21	S	-	-
Total			
R or +	10	9	10
S or -	11	12	11
Sum	21	21	21
R/+		8	9
R/-		2	1
S/-		11	10
S/+		0	1
Sum		21	21

[†] R= resistant, S= susceptible, (+)= marker present, (-)= marker absent.

Table 3.10 Conformation of AFLP markers linked to the resistance gene in NCAG11, evaluated in $F_{2:4}$ resistant and susceptible lines from Saluda x NCAG11 cross.

	Resistance reaction	M-act/cct-196bp	M-acc/cga-126bp
NCAG11	R[†]	+	+
Saluda	S	-	-
R bulk	R	+	+
S bulk	S	-	-
$F_{2:4}$ lines			
-1	R	+	+
-2	R	+	+
-3	S	-	.
-4	S	-	-
-5	R	+	+
-6	S	-	.
-7	S	-	-
-8	S	-	-
-9	R	+	+
-10	S	-	-
-11	S	-	-
-12	S	-	-
-13	R	+	+
-14	R	+	+
-15	S	-	-
-16	R	+	+
-17	S	-	-
-18	S	-	-
-19	R	+	+
-20	R	+	+
-21	S	-	.
-22	R	+	+
-23	R	+	+
Total			
R or +	11	11	11
S or -	12	12	9
Sum	23	23	20
R/+		11	11
R/-		0	0
S/-		12	9
S/+		0	0
Sum		23	20

[†] R= resistant, S= susceptible, (+)= marker present, (-)= marker absent.

Table 3.11 Identification of $F_{2:4}$ lines from NCD1 x NCAG11 cross, homogenous for presence of four AFLP markers linked to resistance genes in NCD1 and NCAG11.

	Resistance reaction	M-aag/cga-148bp	M-aca-cgt-182bp	M-acc/cga-126bp	M-act/cct-196bp
NCD1	R[†]	+	+	-	-
NCAG11	R	-	-	+	+
Saluda	S	-	-	-	-
$F_{2:4}$ lines					
-1	R	+	+	+	+
-2	R	+	+	+	+
-3	R	+	+	-	-
-4	R	+	+	-	-
-5	R	+	+	-	-
-6	R	+	+	+	+
-7	R	-	-	+	+
-8	R	+	+	-	-
-9	R	+	+	+	+
-10	R	-	-	+	+
-11	R	+	+	+	+
-12	R	-	+	+	+
-13	R	+	+	-	-
-14	R	-	+	+	+
-15	R	+	+	+	+
-16	R	+	-	+	+
-17	R	+	+	+	+
-18	R	+	+	-	+
-19	R	+	+	+	+
-20	S	-	-	-	-
-21	S	-	-	-	-
-22	S	-	-	-	-
Total for $F_{2:4}$ lines					
R or +	19	15	16	13	14
S or -	3	7	6	9	8
Sum	22	22	22	22	22
R/+		15	16	13	14
R/-		4	3	6	5
S/-		3	3	3	3
S/+		0	0	0	0
Sum		22	22	22	22

[†] R= resistant, S= susceptible, (+)= marker present, (-)= marker absent.

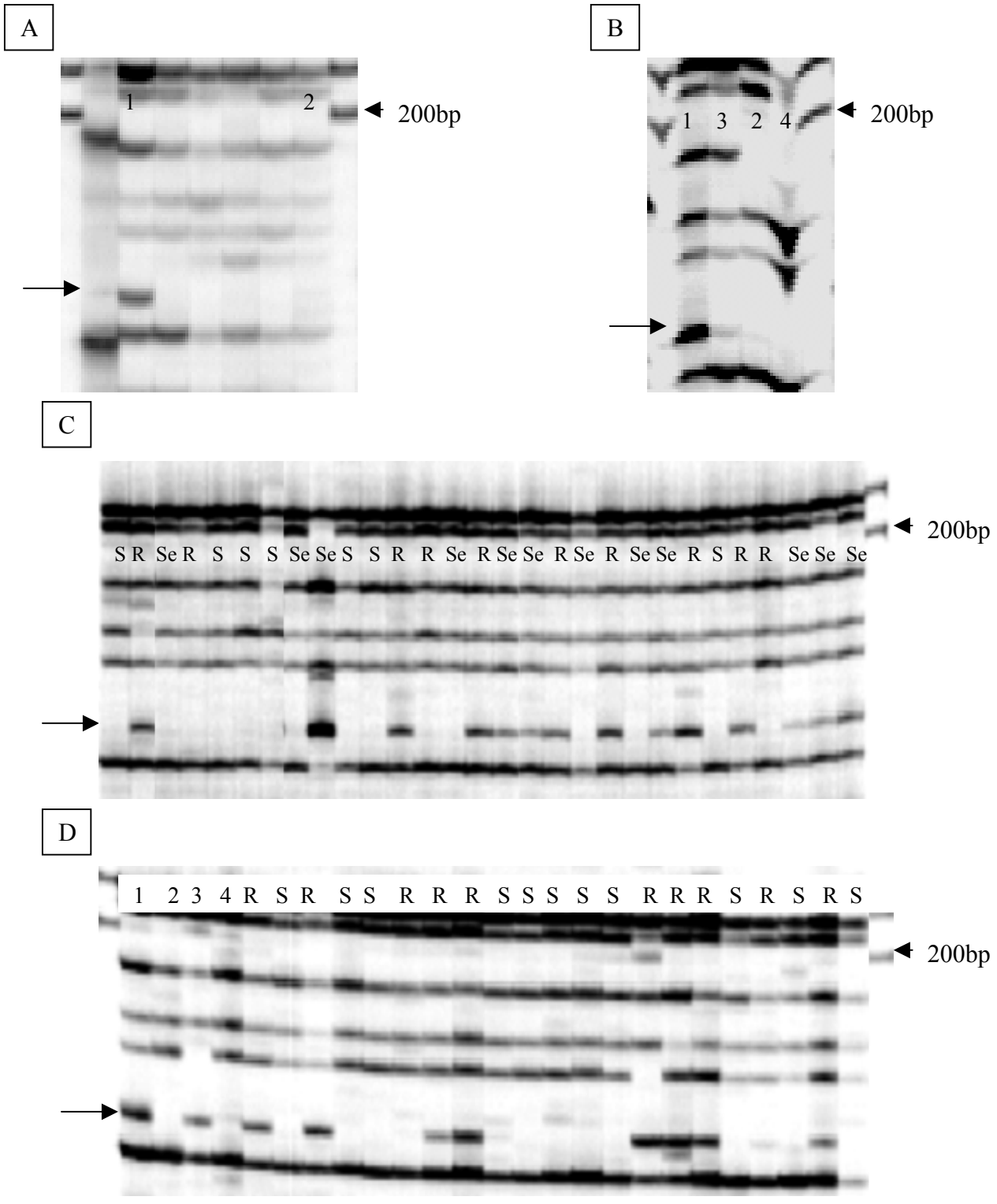

Figure 3.1 Gels illustrating the linkage between AFLP marker aca/cgt-182bp and resistance gene in NCD1. A= primer screen, B= F_2 bulked segregant analysis, C= F_2 segregating population, D= $F_{2:4}$ resistant and susceptible lines. 1= NCD1, 2= Saluda, 3= resistant bulk, 4= susceptible bulk, R= resistant, Se= segregating, S= susceptible.

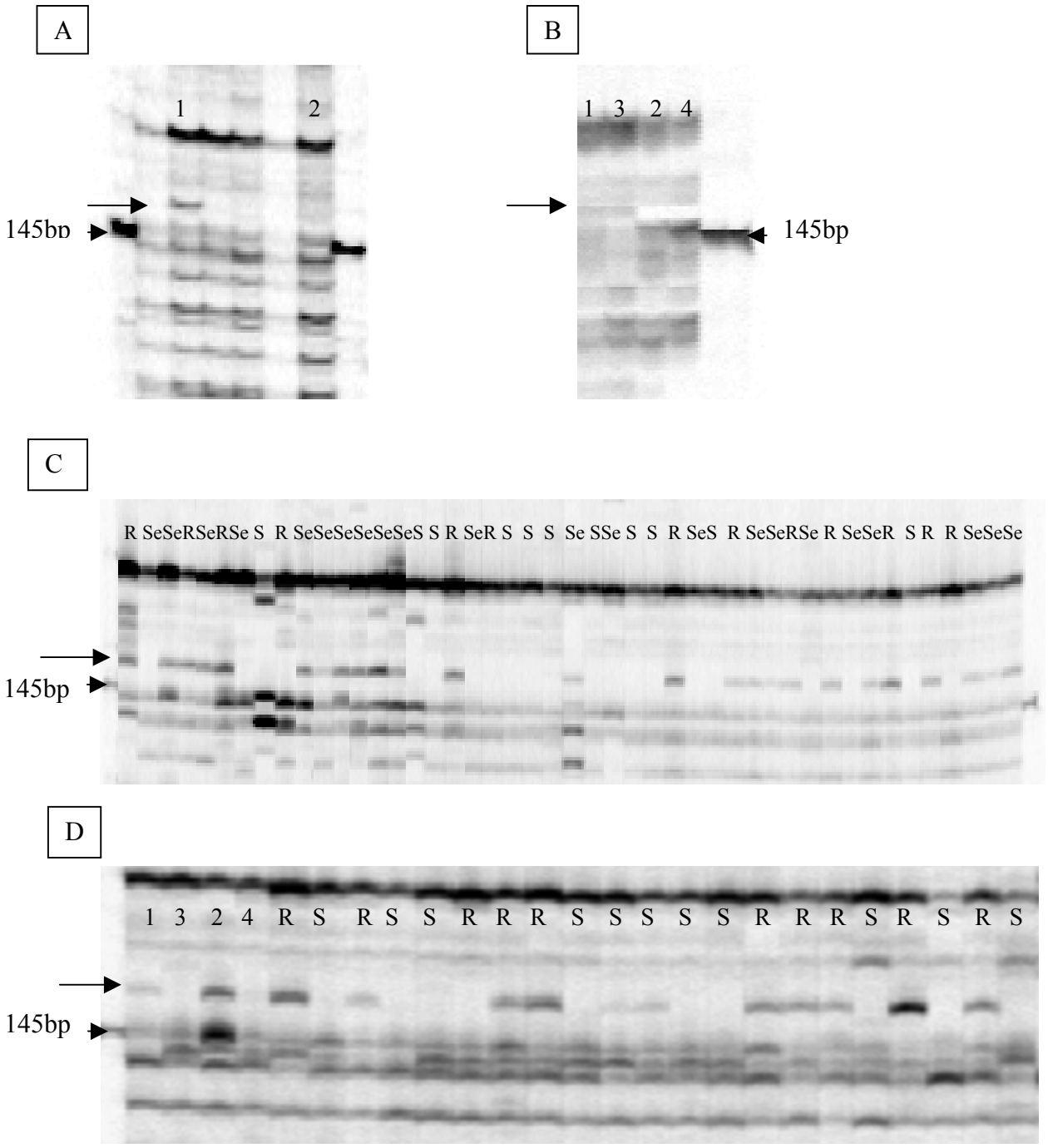

Figure 3.2 Gels illustrating the linkage between AFLP marker aag/cga-148bp and resistance gene in NCD1. A= primer screen, B= F_2 bulked segregant analysis, C= F_2 segregating population, D= $F_{2:4}$ resistant and susceptible lines. 1= NCD1, 2= Saluda, 3= resistant bulk, 4= susceptible bulk, R= resistant, Se= segregating, S= susceptible.

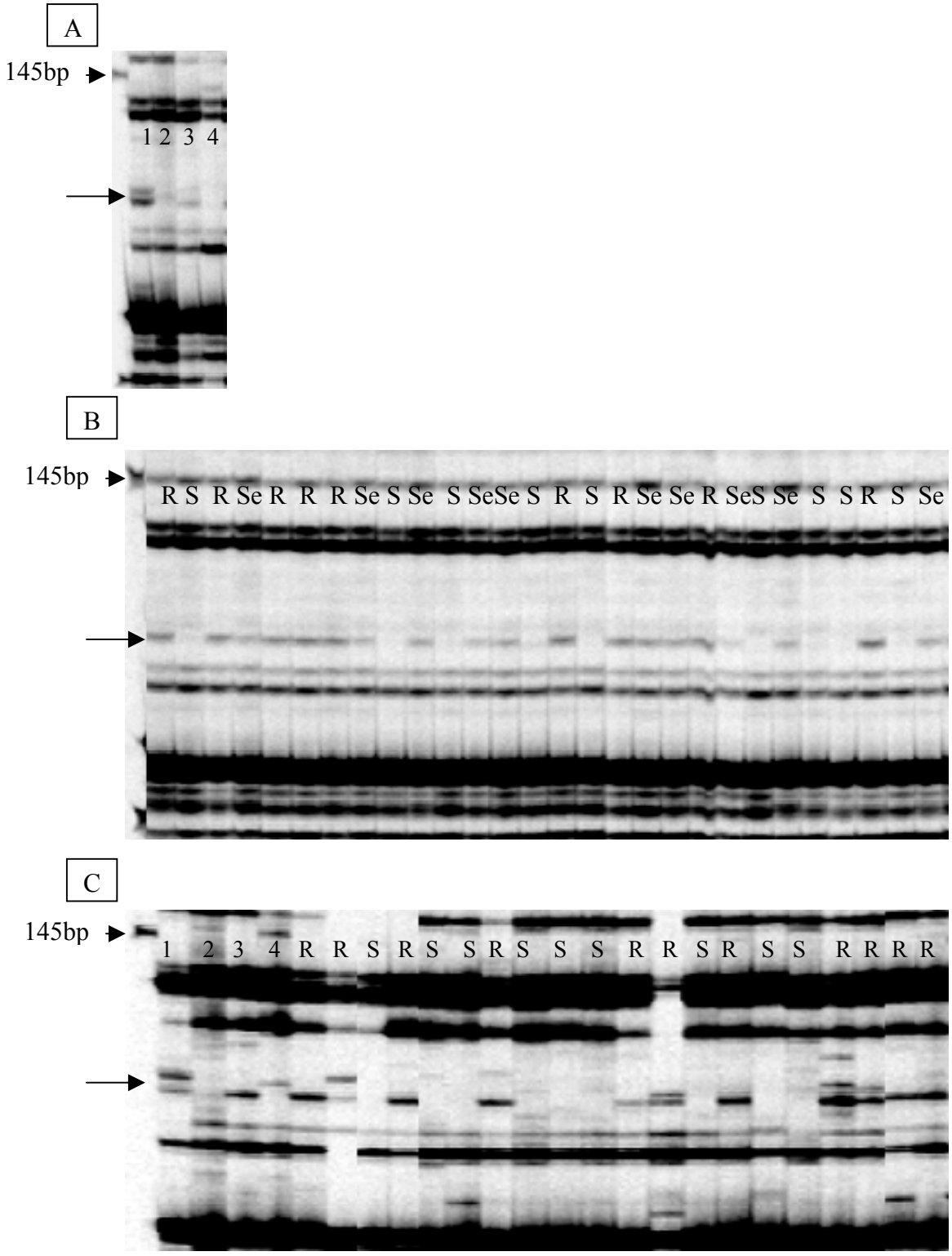

Figure 3.3 Gels illustrating the linkage between AFLP marker acc/cga-126bp and resistance gene in NCAG11. A= F_2 bulked segregant analysis, B= F_2 segregating population, C= $F_{2:4}$ resistant and susceptible lines. 1= NCAG11, 2= Saluda, 3= resistant bulk, 4= susceptible bulk, R= resistant, Se= segregating, S= susceptible.

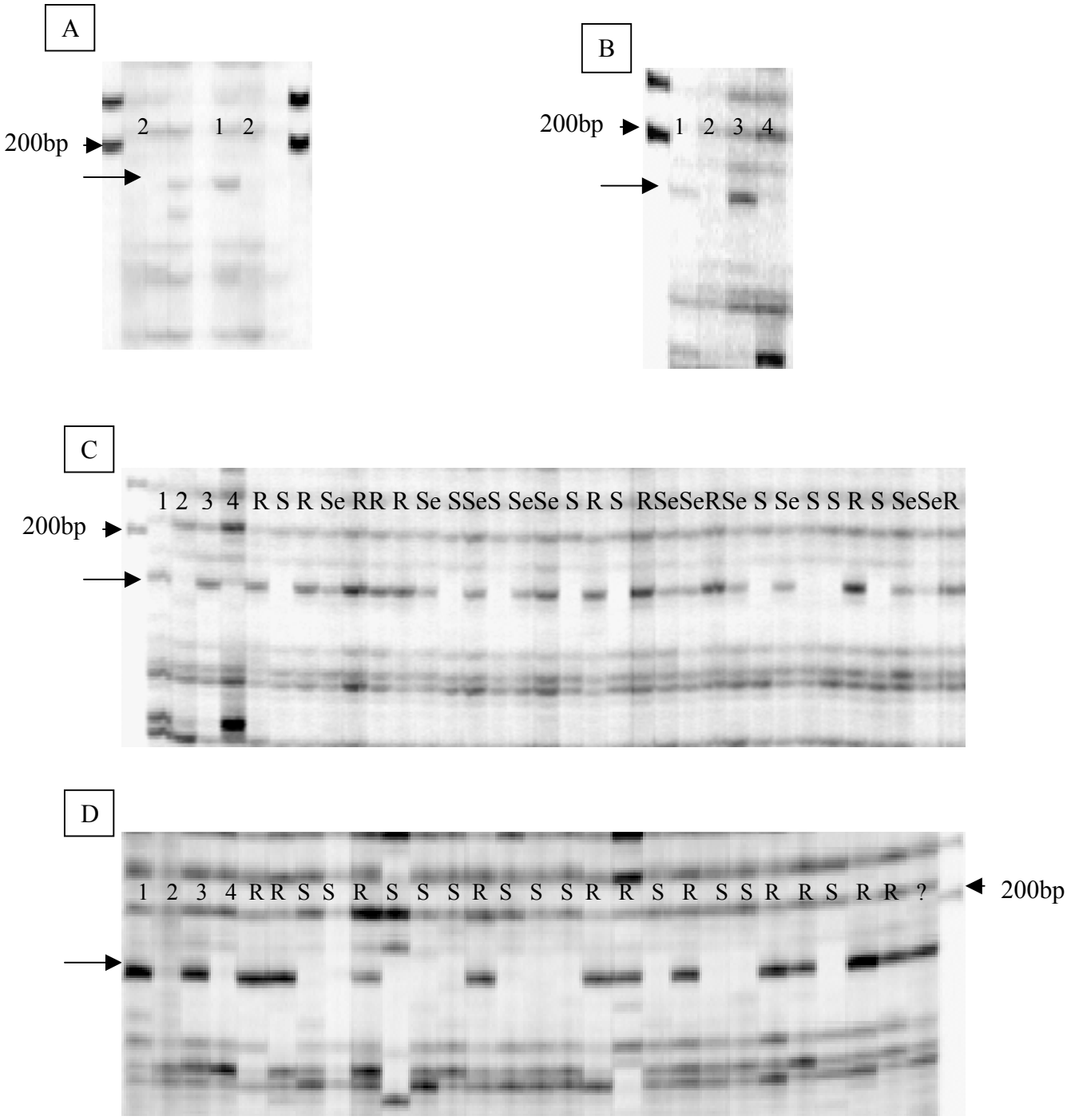

Figure 3.4 Gels illustrating the linkage between AFLP marker act/cct-196bp and resistance gene in NCAG11. A= primer screen, B= F_2 bulked segregant analysis, C= F_2 segregating population, D= $F_{2:4}$ resistant and susceptible lines. 1= NCD1, 2= Saluda, 3= resistant bulk, 4= susceptible bulk, R= resistant, Se= segregating, S= susceptible.

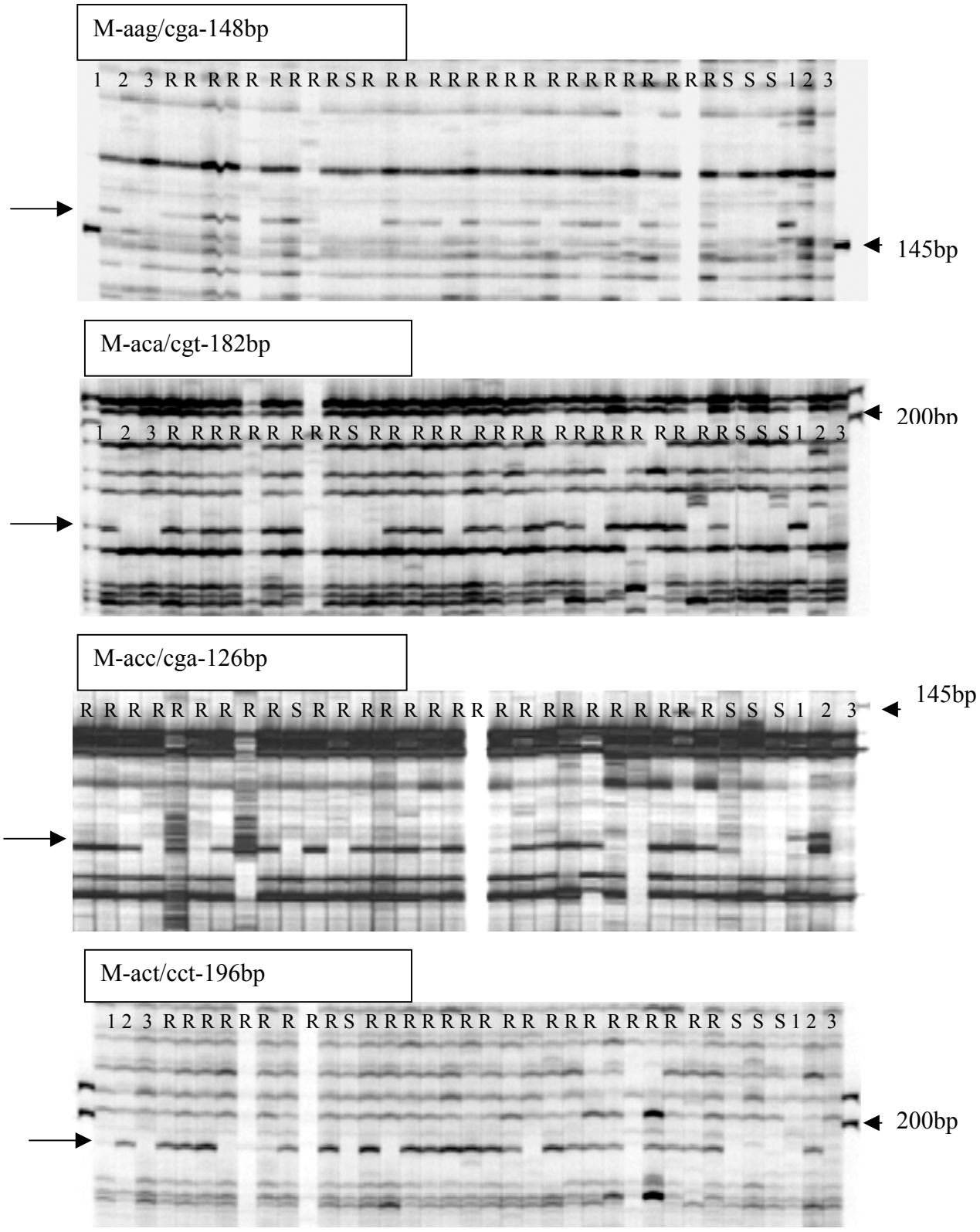

Figure 3.5 Band pattern of 31 $F_{2:4}$ lines in the NCD1 x NCAG11 population homogenous for presence of the four AFLP markers linked to resistance genes in NCD1 and NCAG11. 1= NCD1, 2= NCAG11, 3= Saluda, R= resistant, S= susceptible.