

**Validation, Saturation, and Marker-Assisted Selection of Quantitative
Trait Loci Conferring Adult Plant Resistance to Powdery Mildew in an
Elite Wheat Breeding Population**

Dominic Michael Tucker

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Crop and Soil Environmental Sciences

Dr. Carl A. Griffey, Co-Chair
Dr. M. A. Saghai Maroof, Co-Chair
Dr. Erik L. Stromberg
Dr. Carol A. Wilkinson

March 24, 2005
Blacksburg, VA

Keywords: *Blumeria graminis*, Microsatellite, Partial Resistance, *Triticum aestivum*

Validation, Saturation, and Marker-Assisted Selection of Quantitative Trait Loci
Conferring Adult Plant Resistance to Powdery Mildew in an Elite Wheat Breeding
Population

Dominic Michael Tucker

ABSTRACT

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most devastating diseases in wheat (*Triticum aestivum*) worldwide. Hypersensitive, race specific genes primarily have been deployed to control the disease, however recent efforts have shifted to breeding for more durable resistance, such as 'adult plant resistance' (APR). Molecular markers and quantitative trait loci (QTL) associated with APR to powdery mildew must first be validated and QTL effects evaluated in different genetic backgrounds and breeding populations to be useful in marker-assisted selection (MAS) programs. Eighteen simple sequence repeat (SSR) markers previously mapped near the three QTL in Massey for APR to powdery mildew were evaluated for association with APR for powdery mildew in a recombinant inbred line (RIL) breeding population derived from a cross between USG 3209 and a moderately susceptible cultivar Jaypee, wherein Massey is the resistance source for APR in USG 3209. Thirteen new SSR markers were added to the pre-existing genetic linkage maps near the three QTL associated with APR in the Becker by Massey (BM) population. Interval mapping analysis of mildew severity data collected in 2002 (F_{5:6}) and 2003 (F_{6:7}) field experiments with marker genotype data obtained in 2003 (F_{6:7}) confirmed the presence of three QTL for APR on chromosomes 1B, 2A, and 2B in the USG 3209 by Jaypee (UJ) population. The QTL on chromosomes 1B, 2A, and 2B explained 12% to 13%, 59% to 69%, and 22% to 48% of the phenotypic variance for powdery mildew severity in the UJ RIL populations, respectively, in the two field experiments. The efficiency of MAS was examined using powdery mildew data collected in 2002 and 2003 field experiments and also from a greenhouse experiment in 2004 (F_{7:8}), wherein adult plants of the 293 RILs were evaluated for disease severity using a composite of five different isolates of *B. graminis*. Selection of RILs possessing the QTL on chromosome 2A and to a lesser extent the one on chromosome 1B was effective in identifying powdery mildew resistance in both greenhouse and field

experiments, whereas the effect of the QTL on chromosome 2B was insignificant in the greenhouse. Overall, selecting RILs with QTL on chromosomes 2A and 2B was most successful in identifying highly resistant RILs compared to selecting RILs having other combinations of two or three QTL combinations. The RILs possessing both QTL on chromosomes 2A and 2B had mean mildew severities of 4.4% and 3.2% in 2002 and 2003 field experiments, respectively. Breeders implementing MAS programs for APR to powdery mildew via selection of RILs containing the two QTL on chromosomes 2A and 2B likely will obtain RILs having high levels of resistance in the field. However, combining all three QTL may ensure greater durability of APR, on the basis that resistance conferred by QTL on chromosome 2A and 1B are genetically stable across all environments in this study.

To my mother and father

ACKNOWLEDGEMENTS

Throughout the pursuit of my MS graduate studies and research, I have been extremely blessed by being exposed to individuals that were professional, inspirational, demanding, and visionary. Within each challenge faced, whether immense or minor, the highest standard of excellence was the expectation, for mediocrity would never be accepted. Therefore, it would be a great travesty to not give credit to the many notable individuals that have given me encouragement, direction, assistance, and support throughout this challenging endeavor.

To say the least, I am honored and fortunate to have been under the guidance of two major advisors throughout my thesis project. My advisors, Dr. Carl A. Griffey and Dr. Mohammad A. Saghai Maroof have my sincerest gratitude for their demand for excellence, modeled positive work ethic, and scientific vision. Furthermore, my committee members, Dr. Erik L. Stromberg and Dr. Carol A. Wilkinson are extended appreciation for their guidance in designing and preparing my MS thesis. Also, sincere acknowledgement is extended to Lynda Witcher, at North Carolina State University, for screening the USG 3209 by Jaypee populations and parental lines for race specific genes utilized in my research. In addition, a sincere gratitude is offered to the Virginia Crop Improvement Association (VCIA) for providing partial funding for this project.

Numerous members of the Small Grains, Breeding, and Genetics crew have provided invaluable assistance and comradery throughout this research. Members such as Thomas Pridgen, Wynse Brooks, Joe Paling, Wendy Rohrer, and Daryoosh Nabati have truly made my transition to Virginia Tech from Michigan State University a pleasant experience. Each generously provided not only technical assistance but also friendships within a family atmosphere. Patrick O'Boyle and Adrienne LaBranche provided much needed assistance in the set-up and inoculation of the greenhouse experiment. The following crew-members, Jason Kenner, Mark Vaughn, and Robyn Dustin of the Eastern Virginia Agricultural Experiment Station (Warsaw, VA) are highly accredited for their valuable support in maintaining, harvesting, and the data collecting process of the USG

3209 by Jaypee populations. Dr. Jafar Mammadov and Dr. Ruslan Biyashev provided immeasurable technical support in the lab, which has allowed me to develop the skills, expertise, and patience vital for the completion of my MS project. Also, recognition is extended to Jianli Chen whose office door was always open for technical advice and questions regarding lab portions of this study.

Several research facilities and personnel have either directly or indirectly assisted in this project. Dr. P. B. Cregan and Dr. Q. J. Song from USDA-ARS (Beltsville, MD) for providing sequence information of newly developed simple sequence repeats (*Xbarc*). Dr. M.D. Gale and Dr. Pauline Stephenson (John Innes Center, UK) for their assistance in obtaining simple sequence repeat primers (*Xpsp*) used in this study. Dr. Sixin Liu (University of Minnesota) for his initial work in locating the quantitative trait loci responsible for adult plant resistance to powdery mildew in the Becker by Massey population. In the event I inadvertently overlooked expressing appreciation to any individual or agency during the acknowledgement process, commendations are warmly extended. Also, words cannot describe nor articulate my sincere indebtedness and love toward my mother and father. Both have been pillars of strength, encouragement, stability, and support during my educational journey.

TABLE OF CONTENTS

	Pages
ABSTRACT	ii
ACKNOWLEDGEMENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
CHAPTER I	
Literature Review.....	1
Hypersensitive Resistance Genes	2
Adult Plant Resistance	4
Molecular Markers.....	5
Map Construction.....	10
Mapping Adult Plant Resistance.....	13
Marker-Assisted Selection.....	14
Literature Cited.....	17
CHAPTER II	
Validation and Saturation of Three Quantitative Trait Loci Conferring Adult Plant Resistance to Powdery Mildew with PCR-Based Markers in an Elite Wheat Breeding Population.....	29
Abstract.....	30
Introduction.....	31
Materials and Methods.....	33
Results.....	36
Discussion.....	39
Literature Cited.....	43

CHAPTER III

Potential Effectiveness of Marker-Assisted Selection for Three Quantitative Trait Loci Conferring Adult Plant Resistance to Powdery Mildew in an Elite Wheat Breeding Population.....	54
Abstract.....	55
Introduction.....	57
Material and Methods	59
Results.....	63
Discussion.....	68
Literature Cited.....	72
VITA	85

LIST OF TABLES

Pages

CHAPTER II

Table 1. Summary of publicly available wheat SSRs and chromosomal regions targeted to map additional markers near QTL associated with APR to powdery mildew in the F _{2:3} BM population.....	48
Table 2. One-way ANOVA of new SSR markers mapped in the F _{2:3} BM population applied to the UJ RIL breeding population that are associated with APR to powdery mildew.....	51
Table 3. Comparison of QTL effects on APR to powdery mildew using interval mapping analysis of original datasets of the F _{2:3} BM population (Liu et al., 2001) and the UJ population	52

CHAPTER III

Table 1. Seedling reactions of parental lines USG 3209 (APR possessing parent), Jaypee (moderately susceptible parent), Massey (APR source of USG 3209), and Becker (susceptible check with no known resistance genes) tested with <i>B. graminis</i> isolates used for adult-plant greenhouse screening tests.....	76
Table 2. Seedling reactions to a set of powdery mildew differentials containing genes <i>Pm1-Pm8</i> and <i>Pm17</i> screened with bulked <i>B. graminis</i> isolates used in the adult-plant greenhouse screening test	77
Table 3. One-way ANOVA to verify molecular markers associated with APR to powdery mildew in UJ RILs	81
Table 4. Average mildew severity values of RILs in the UJ population selected by single and flanking SSR markers for each QTL.....	82

LIST OF FIGURES

	Pages
CHAPTER II	
Figure 1 Alignment of the original mapping population, BM F _{2:3} , with the applied F _{6:7} UJ population	49
Figure 2. Distribution of BM F _{2:3} (1995) lines (Liu et al., 2001), and UJ F _{5:6} (2002) and F _{6:7} (2003) RILs for mean powdery mildew severity assessed under natural epidemics in the field at Warsaw, VA	50
Figure 3. Comparison of interval mapping analysis with flanking SSR markers for QTL associated with APR to powdery mildew located on chromosomes 2A, 2B, and 1B, respectively	53
CHAPTER III	
Figure 1 Linkage maps of wheat chromosomes 2A, 2B, and 1B developed from UJ RIL population	78
Figure 2. Histogram of mean powdery mildew disease severity in the field (2002 and 2003) and 2004 greenhouse screening of the 293 RILs derived from the cross of UJ.	79
Figure 3. Distribution of AUDPC values calculated for powdery mildew severity in the 2004 greenhouse screening	80
Figure 4. QTL for APR on chromosomes 2A, 1B, and 2B effects on AUDPC for RILs selected by MAS in the 2004 greenhouse experiment.....	83
Figure 5. Mean powdery mildew severity data from two field and one greenhouse experiments grouped by flanking markers for QTL on chromosomes 2A, 2B, and 1B simultaneously	84

CHAPTER I
Literature Review

Powdery mildew caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Ém. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the most common diseases of wheat (*Triticum aestivum* L.), prevalent throughout predominant wheat growing regions of the world including humid and semi-arid areas. Prior to the mid 19th century, powdery mildew was not considered a detrimental wheat disease, as it predominately occurred in areas having significant rainfall and maritime or semi-continental climates (Bennett, 1984). However, with the advent of semi-dwarf cultivars and extensive use of fertilizers, powdery mildew soon became one of the most economically important diseases of wheat (Hebert et al., 1948; Imani et al., 2002; Szunics et al., 2001).

Yield loss estimates attributed to powdery mildew vary greatly as yield loss and disease intensity vary from year to year depending on location, fertilization, weather conditions, and cultivars (Fried et al., 1979; Kingsland, 1982). Diseased seedlings produce fewer productive tillers compared to resistant plants or those protected with fungicides (Finney and Hall, 1972). Yield losses throughout the United States have ranged from 16% to 34% (Griffey et al., 1993; Johnson et al., 1979; Kingsland, 1982; Leath and Bowen, 1989), while yield losses as high as 30% have been reported in Hungary (Szunics et al., 2001).

Hypersensitive Resistance Genes

Powdery mildew has been successfully managed using resistant cultivars, crop rotation, delayed planting, occasionally foliar fungicide application, and seed treatments.

Utilization of resistance cultivars has provided the most economical and efficient control of the disease (Bennett, 1984). To date, 32 major gene, race-specific loci, designated Pm1-Pm32, and some other non-classified resistance genes have been reported to confer resistance to powdery mildew in wheat (Hsam et al., 2003; Huang and Röder, 2004; McIntosh et al., 1998; Singrun et al., 2003). Most race-specific major genes (*Pm* genes) are expressed by the two leaf stage and follow Flor's gene for gene model (Flor, 1956; Powers and Sando, 1960). Genes *Pm5* and *Pm6* are only fully expressed from the four to five leaf stage (Lesbock and Briggie, 1974).

Several *Pm* genes consist of complex loci having multiple alleles. The *Pm3* locus, located on the short arm of chromosome 1A (Briggle, 1969), has at least 10 different alleles (Zeller and Hsam, 1998). Gene *Pm4* has two alleles *Pm4a* and *Pm4b* that have been used in many breeding programs (Briggle, 1960; Jorgensen and Jensen, 1972). Six alleles have been reported at the *Pm5* locus (Huang et al., 2000; Huang et al., 2003a; Huang et al., 2003b).

A majority of these genes are dominant in action with only four recessive gene loci being reported including *Pm5* (Lesbock and Briggle, 1974), *Pm9* (McIntosh et al., 1998), *Pm26* (Rong et al., 2000), and the newly discovered gene *mIRD30* (Singrun et al., 2003).

Several of these *Pm* genes were identified in closely related species of *Triticum aestivum*. Genes *Pm4a* and *Pm5* originated from *T. dicoccum* (Briggle, 1966; Law and Wolfe, 1966) and *Pm4b* from *T. carthlicum* were introduced into hexaploid wheat (The et al., 1979). Genes *Pm6* and *Pm16* were derived from *T. timopheevii* and *T. dicoccoides*, respectively (Jorgensen and Jensen, 1972; Reader and Miller, 1991). Gene *Pm12* was transferred from *Aegilops speltoides* and *Pm19* from *Ae. Squarrosa* (Lutz et al., 1995; Miller et al., 1998). Four resistance genes, *Pm7*, *Pm8*, *Pm17*, and *Pm20* (Friebe et al., 1994; Heun et al., 1990a; Heun et al., 1990b) have been derived from rye (*Secale cereale*). Gene *Pm21* was introduced from *Haynaldia villosa* (Chen et al., 1995) and *Pm25* was introduced by *Triticum monococcum* (Shi et al., 1998).

The strategy of using predominantly race specific genes has not been highly effective due to lack of durability of these genes (Roberts and Calwell, 1970; Shaner, 1973). Many of the race specific genes thus far deployed in commercial cultivars have been overcome by rapidly changing and dynamic pathogen populations due to selection pressure placed on the pathogen population by these major genes (Menzies and MacNeill, 1986; Roberts and Calwell, 1970).

Pyramiding several race specific genes into single cultivars also has not provided long-term durable resistance. Virulence studies conducted in the Eastern United States and in portions of Europe have indicated that virulence spectra and complexity of isolates in a

given population is correlated with resistance genes possessed by cultivars grown in a particular area (Niewoehner and Leath, 1998; Szunics et al., 2001). Persaud and Lipp (1995) discovered that a majority of isolates collected in Ohio were complex and carry from four to eight virulence genes. A similar survey conducted by Imani et al. (2002) reported that 87% of the powdery mildew isolates in Morocco contained five or more virulence genes.

Adult Plant Resistance

Incomplete, non-race specific resistance retarding infection, growth, and reproduction of powdery mildew in adult plants has been termed ‘slow mildewing’ (Shaner, 1973) ‘adult-plant resistance’ (APR) (Gustafon and Shaner, 1982), or ‘partial resistance’ (Hautea et al., 1987). Partial resistance extends the latent period of the fungus and reduces its sporulation (Royer et al., 1984), therefore yields are not significantly reduced (Griffey et al., 1993).

Erosion of APR to powdery mildew has not been reported despite wide cultivation within the United States of APR possessing cultivars such as Knox (Roberts and Calwell, 1970) released in 1959, followed by Redcoat in 1969 (Rouse et al., 1980). Subsequently, Knox’s APR was deployed in cultivars Knox 62 (Patterson et al., 1978) and Massey (Starling et al., 1984), which have been widely utilized in breeding programs as a source of APR resistance.

Shaner and Finney (1975) first postulated that APR in cultivar Knox behaved genetically as a quantitative trait. Chae and Fishchbeck (1979) reported that 14 chromosomes were involved in the expression of APR in cultivar Diplomat utilizing monosomics of wheat varieties Chinese Spring and Caribo. Chromosomes of group 5 were of key importance along with, although with a lesser effect, chromosomes 7A and 4D.

Genetic studies conducted on APR in winter wheat cultivars Knox 62, Massey, Redcoat, and Houser indicated that two to three genes control APR with moderate to high heritabilities (Das and Griffey, 1994b; Griffey and Das, 1994). Additive effects were

predominant in the inheritance of the trait with some significance of dominance and digenic epistasis (Das and Griffey, 1995). Chantret et al. (1999) determined that the defeated, race-specific powdery mildew resistance gene, *MIRE*, produced a dominant residual effect that was a significant component of APR in winter wheat line RE714. The single race specific gene explained 27% to 30% of the total variation in a doubled haploid population.

Molecular Markers

More efficient methods of breeding and selection for APR have been sought because it is difficult to assess different levels of partial resistance in field research (Gustafon and Shaner, 1982) even with numerous replications in several different environments.

Molecular markers provide a viable and potential tool for breeding durable resistance in wheat. Utilizing molecular markers for indirect selection of a trait of interest, commonly referred to as marker-assisted selection or MAS, offer breeders a strategy for speeding up the breeding process.

Before the advent of molecular markers, breeders relied heavily on morphological markers for developing maps. Developing a genetic or physical map, required the occurrence of two or more alternate alleles of the same gene (normal and mutant), that were distinguishable. Few detailed genetic linkage maps were available and only for a few crops species, with a majority of markers being recessive morphological mutations that often have detrimental side effects (Melchinger, 1989). Tanksley (1983) first proposed use of two linked markers bracketing the target gene to improve marker-assisted selection.

Molecular markers offered the possibility of overcoming many of the problems associated with morphological markers by providing: 1) a virtually unlimited number of markers in any set of breeding material, 2) relatively large number of alleles, 3) markers that are not affected by environmental conditions, 4) genotyping of most molecular markers at an early developmental stage allowing for early screening, and 5) molecular markers that predominately demonstrate a co-dominant inheritance. To date, several

molecular markers have been utilized in wheat breeding: restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSRs or microsatellites), amplified length polymorphisms (AFLPs), sequence tagged sites (STS), and expressed sequence tags (ESTs).

Restriction Fragment Length Polymorphisms

RFLPs were the first markers used for marker assisted selection in wheat. Several genetic maps were initially produced using this new technology in bread wheat *T. aestivum* (Anderson et al., 1992; Devos and Gale, 1993; Devos et al., 1992; Xie et al., 1993).

RFLP analysis begins with DNA isolation from suitable sets of plants. The DNA is then digested with a restriction enzyme that cuts DNA fragments at specific recognition sequences. These DNA fragments are then separated on an agarose gel using electrophoresis. Fragments are then transferred to a filter by Southern blotting where a radioactively labeled probe allows the detection of a specific DNA fragment. Individual fragments can then be visualized by autoradiography.

Hartl et al. (1993) followed by Ma et al. (1994) were the first to utilize RFLP markers to map race specific resistance genes to powdery mildew in wheat. Since then tightly linked markers have been identified for genes *Pm1*, *Pm2*, *Pm3*, *Pm4* (Ma et al., 1994), *Pm6* (Tao et al., 2000), *Pm8* (Hsam et al., 2000), *Pm12* (Jia et al., 1996), *Pm13* (Cenci et al., 1999), *Pm17* (Hsam et al., 2000), *Pm21* (Qi et al., 1996), *Pm24* (Huang et al., 1997b), and *Pm30* (Liu et al., 2002). However, RFLPs linked to race specific genes have had a limited impact on wheat breeding due to the nature of RFLP technology. RFLPs are time consuming and labor intensive as they cannot be automated and require large amounts of DNA. This technique is too expensive for rapid evaluations of large numbers of progenies commonly used in commercial breeding programs. Furthermore, levels of polymorphism revealed among adapted varieties is very low (Chao et al., 1989; Devos and Gale, 1992); therefore breeders may often only adopt the technology when transferring genes from wild relatives (Jia et al., 1996).

Polymerase Chain Reaction Markers

The *in vitro* polymerase chain reaction (PCR) has proven to be a revolutionary method for molecular biology since its inception in 1985. The PCR technique is rapid and inexpensive allowing many laboratories to adopt the new technology. PCR technology is based on use of oligonucleotide primers combined with thermal stable DNA polymerase, followed by varying levels of annealing and extension temperatures, that allows for exponential amplification of segments of DNA. PCR-based markers fall into two groups: those that use sequence arbitrary methods (RAPDs and AFLPs) and those which require sequence knowledge (SSRs, STS, and ESTs).

Random Fragment Amplified Polymorphisms DNA

Random amplified polymorphisms (RAPDs), developed by Williams et al. (1990), promised to overcome problems of RFLP technology. RAPDs are the result of amplification using a single, short, sequence arbitrary designed oligonucleotide. Amplification is carried out under low annealing temperature (35 to 37°C). Specific primers selected to carry out amplification are generally a 10 mer primer that can be transferred between species. DNA fragments of low complexity are visualized by ethidium bromide staining producing a dominant type marker.

RAPDs have been applied in wheat to tag powdery mildew resistance genes *Pm1* (Hu et al., 1997), *Pm8* (Iqbal and Rayburn, 1995), *Pm21* (Qi et al., 1996), and *Pm25* (Shi et al., 1998). However, similar to RFLPs, RAPDs had a limited impact on breeding for mildew resistance due to the low levels of polymorphism and reproducibility problems (Devos and Gale, 1992). Fortunately, RAPDs can be converted into a reliable type of PCR-based marker known as a SCAR (sequence characterized amplified regions) or sometimes referred to as sequence tagged site (STS) marker. Neu et al. (2002) were able to convert RAPD marker UBC638 (Hu et al., 1997) into a dominant SCAR marker, STS638, to select for *Lr20-Pm1* resistance locus in hexaploid wheat.

Amplified Fragment Length Polymorphisms

The amplified fragment length polymorphism (AFLP) technique is an arbitrary sequence, PCR method based on the digestion of DNA with one or more restriction enzymes (Vos et al., 1995). In this technique, DNA is first digested with one or more restriction endonucleases. An adapter of known sequence is ligated to the digested genomic DNA. The DNA fragments are then amplified using primers with sequence specificity for the adapter. The primer(s) also contain one or more bases on the 3' end which provide amplification selectivity by limiting numbers of perfect matches between the primer and adapter/DNA templates. Fragments are then visualized on acrylamide gels by labeling one of the primers. Observed patterns of AFLP are dominant in type and relatively complex, depending on primer pairs and endonucleases restriction.

The AFLP approach, which allows detection of high levels of polymorphic alleles coupled with high reproducibility, makes this technology attractive to plant breeders. Furthermore, Maughan et al. (1996) found AFLP markers to be inherited in a stable Mendelian fashion when assessing the variation of AFLPs in cultivated soybean (*Glycine max*) and wild soybean (*Glycine soja*) Zeller et al. (2002) closely linked three AFLP markers to gene *Pm29* in wheat line Pova derived from Poros wheat (*Aegilops ovata*) alien addition line. The AFLP technique compared to other PCR techniques, STS and SSR, is rather time consuming and labor intensive as DNA fragments must be digested followed by separate amplification steps of PCR. The large complex wheat genome also poses several problems that may result in detection of too many bands causing smearing of products.

Sequence Tagged Sites

A RFLP marker linked to a desired trait can be converted into a PCR, STS marker, based on the nucleotide sequence of the probe giving a polymorphic banding pattern (Olson et al., 1989). A STS marker is based on a pair of primers that are designed by sequencing a RFLP mapped with a low copy number sequence. These markers predominately produce a dominant banding pattern that can easily be assayed in marker-assisted selection for a trait of interest. Mohler et al. (2001) converted the RFLP probe, IAG95, into a dominant

STS marker developed for the selection of the *Pm8/Pm17* locus. The STS marker could differentiate between cultivars carrying gene *Pm8* from those with *Pm17*.

Simple Sequence Repeats

Simple sequence repeats (SSRs), otherwise known as microsatellites, are sequence dependent and have become a popular marker of choice for use in laboratories. The SSR technique is based on tandemly repeated DNA motifs found throughout an organism's genome. The repeat regions are generally di-, tri-, and tetra- repeated nucleotide DNA sequences. Each SSR must be cloned and sequenced before a useful marker can be generated. Small fragment genomic libraries are screened for clones containing the SSR sequence using oligonucleotide probe complementary to the repeat motif. Each positive clone from the library is then sequenced. Oligonucleotide primers complementary to the DNA sequence flanking both sides of the repeats are synthesized and used for PCR amplification. Radioactive labeling or fluorescent labeling of one deoxynucleotide followed by separation on a polyacrylamide or high agarose gel, allows amplified fragments to be visualized.

SSRs have become the marker system of choice in wheat due to high levels of polymorphism, their co-dominant inheritance, ease of distribution between laboratories, and in most cases chromosome specificity (Röder et al., 1995). Currently, over 450 microsatellite markers have been published and added to the wheat genetic map by various research groups (Gupta et al., 2002; Guyomarçh et al., 2002; Pestsova et al., 2000; Röder et al., 1998; Sourdille et al., 2003; Stephenson et al., 1998). Another 534 SSRs (Song et al., 2002) are unpublished with sequence information available to the general public at the GrainGenes database (<http://wheat.pw.usda.gov>). Somers et al. (2004), using all available markers, created a high density microsatellite consensus map for bread wheat with 1,235 microsatellite loci, covering 2,569 cM, giving an average interval distance of 2.2 cM.

Subsequently, SSRs have been applied to tag powdery mildew resistance genes in wheat. Bougot et al. (2002) were able to use the PSP2999 primer pair (Devos et al., 1995) to

distinguish different alleles at the *Pm3* locus for powdery mildew resistance in different genetic backgrounds. The same amplification pattern was observed among different lines with alleles *Pm3a*, *Pm3b*, *Pm3e*, *Pm3f*, and *Pm3g*. Lines carrying the *Pm3d* allele and susceptible allele at the *Pm3* locus, displayed a different banding pattern. Similarly, Huang et al. (2003a) mapped *Pm5e* to chromosome 7B and Xie et al. (2003) mapped the newly discovered gene, *MIG*, to chromosome 6 of wheat with tightly linked SSR markers.

Expressed Sequence Tagged Site-Simple Sequence Repeats

Traditional SSRs have minor problems that recent marker technology has attempted to overcome. Genomic SSRs were mostly derived from intergenic regions having no gene function and the procedure for developing markers was costly and tedious. Expressed-sequence tagged site simple sequence repeats (EST-SSRs) are derived from single-pass sequences of complimentary DNAs (cDNAs) from different species, which represent only the expressed sequences of the genome. EST-SSRs can be developed at little cost from an EST database and once developed can be transferred across a number of species (Gupta et al., 2003; Yu et al., 2004).

Gao et al. (2004) added a total of 101 EST-SSR markers covering 20 chromosomes to the existing wheat genetic map. Seventy-four of the 101 loci showed significant similarities to known genes. Similarly, La Rota and Sorrells (2003) and Yu et al. (2004) presented 5,425 and 400 EST-SSR sequences, respectively, on to the GrainGenes database (<http://wheat.pw.usda.gov>). Even though EST-SSRs are exceedingly easier to produce than genomic SSRs, their level of polymorphism thus far has been relatively low in wheat. Eujayl et al. (2002) found the level of polymorphism detected by EST-SSRs to be relatively low (25%) compared to genomic SSRs (53%) in wheat.

Map Construction

Genetic maps of molecular markers in plants have conventionally been constructed by segregation analysis of progeny derived from a F₁ cross of homozygous parents. The first genetic maps in plants and wheat were created from F₂ populations or backcross

generations. Several genes for powdery mildew resistance (Jia et al., 1996; Ma et al., 1994), and leaf rust resistance (*Puccinia recondita*) (Dedryver et al., 1996; Feuillet et al., 1995; Sacco et al., 1998), were mapped utilizing F₂ populations in wheat. However, performing marker analysis on all individual F₂ plants in many cases is very time consuming and tedious. Furthermore, obtaining accurate readings on an individual F₂ plant is often difficult. Therefore, other mapping strategies using recombinant inbred lines, doubled haploid lines, near isogenic lines, and bulk segregant analysis have been developed to map genes and quantitative trait loci (QTL).

Another method employed to map resistance genes is the use of near isogenic lines (NILs) developed by means of the backcross method. The donor parent carrying the gene of interest is repeatedly backcrossed to the recurrent parent, usually a cultivated line. Backcrossing is accompanied by selection for the resistance gene with recovery of the other properties of the recurrent parent. In theory, after five to six generations of backcrossing, isogenic lines will contain all portions of the genome of the recurrent parent and only a small segment of the target gene from the donor parent. Genetic markers that are polymorphic between the NIL and its recurrent parent are in theory linked to the target gene (Muehlbauer et al., 1988; Young et al., 1988).

Recently, Ma et al. (2004) were able to closely link a STS and a SSR marker to gene *Pm4a* using near isogenic line CI14123 (Briggle, 1969) developed by backcrossing durum wheat cultivar Yuma possessing *Pm4a* to the susceptible cultivar Chancellor for eight generations. Although NILs have been used with relative success in mapping powdery mildew and other disease resistance genes in wheat, development of such lines, especially in a self-pollinated crop, is a time consuming and tedious process.

Recombinant inbred lines (RILs) and doubled haploid (DH) populations provide a permanent mapping resource as seed can be continuously replenished from generation to generation allowing data to be continuously added in different environments. Therefore, RILs and doubled haploid populations are better suited for analysis of quantitative traits, compared to F₂ populations (Burr et al., 1988). Recombinant inbred lines are created

optimally by single seed descent from sibling F₂ plants through at least five or more generations of inbreeding. Doubled haploid lines are produced via interspecific and intergenetic crosses or androgenesis. Continuous selfing or haploid production leads to lines containing different combinations of linkage blocks from the original parents. Doubled haploid populations were utilized to locate and map mildew resistance gene *Pm3g* (Sourdille et al., 1999). RILs have been exploited to a greater extent in wheat. Durable resistance to leaf rust (*Puccinia recondita*) (Messmer et al., 2000), Fusarium head blight (*Fusarium graminearum*) (Guo et al., 2003; Shen et al., 2003), powdery mildew (Cenci et al., 1999), and single gene stripe rust resistance (Boukhatem et al., 2002) have been mapped with RILs.

Bulk segregant analysis (BSA) provides a rapid method for identifying markers linked to a gene of interest in a population (Michelmore et al., 1991). Using the BSA method, two bulks having distinct and often contrasting phenotypes for the trait of interest are generated from a segregating population from a single cross. Each pool, or bulk, contains individuals that are identical for a particular trait. All other genomic regions are considered arbitrary, other than those affecting the trait of interest. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools. In wheat there are only a few examples of where disease resistance genes have been tagged using the BSA method mainly due to the large genome size, low polymorphisms level, and existence of repeated sequences.

However, mapping and locating powdery mildew resistance in wheat by means of the BSA method has been relatively successful. Hartl et al. (1995) and Shi et al. (1998) created RAPD primers linked to *Pm18* and *Pm25*, respectively. Chantret et al. (2000) created a bulk consisting of nine resistant and nine susceptible individuals selected from 140 F₃ families from a cross of a powdery mildew resistant source, RE714, by a susceptible source, Hardi. As a result, the powdery mildew resistance gene, *MIRE*, and a QTL were mapped by microsatellite markers.

Mapping Adult Plant Resistance

Several QTL for APR to powdery mildew have been localized in different wheat populations using molecular markers. Keller et al. (1999) was first to map QTL associated with APR to powdery mildew in a population of 226 recombinant inbred lines derived from a cross of the spelt variety, Oberkulmer, by winter wheat cultivar, Forno. QTL analysis was based on a genetic map containing 176 RFLP probes and 9 SSRs. Eighteen total QTL were detected explaining 77% of the phenotypic variance. Two major QTL were consistently identified in all environments and years tested. QTL located on chromosomes 5A and 7B explained 22.9% and 31.8%, respectively. The 7B QTL was localized in the direct vicinity of gene *Pm5*.

Liu et al. (2001) detected three QTL in a Massey, a cultivar possessing APR, by Becker, susceptible cultivar, F_{2:3} population. The three QTL located on chromosome 1B, 2A, and 2B explained 17%, 29%, and 11% of the total variation, respectively. The major QTL located on chromosome 2A was located near the *Pm4* locus. In a multi-QTL model, the three QTL explained 50% of the variation for APR. Presence of the three QTL was confirmed in a set of 97 RILs over a three year period.

Similarly, Chantret et al. (2000) located and mapped one QTL and one hypersensitive gene (*MIRE*) with a residual effect on APR (Chantret et al., 1999) in winter wheat line RE714 using BSA. A total of 109 SSRs were surveyed in two bulks. *MIRE* was located on chromosome 6A and the QTL conferring APR was on chromosome 5D. The QTL explained 16.8% to 25.3% of the total variation in a F_{2:3} RE714 by Hardi population. A doubled haploid population was then created using the resistant source RE714 in two susceptible backgrounds, Hardi and Festin (Mingeot et al., 2002). A total of nine QTL were detected with the effects of residual genes *Pm4b* and *MIRE*. Two major QTL on chromosome 5D and the *MIRE* locus, located on 6A, were identified in all years and environments. Defeated hypersensitive gene *MIRE* and a 5D QTL explained 20.5% to 53.9% and 22.3% to 54.3% of the total variation, respectively. Effects of *Pm4b*, located on chromosome 2A, were only observed in two out of three locations tested in the RE714

by Festin population and explained 22.7% to 39.2% of the variation over a three year study.

Marker-Assisted Selection

Molecular markers tightly linked, co-segregating, or flanking a gene or QTL of interest provide plant breeders with a useful tool as they can: 1) identify resistance genes or QTL without tedious disease screening, 2) possibly shorten the time for cultivar development by identifying homozygous from heterozygous plants, and 3) allow the pyramiding of several disease resistance genes even when corresponding virulence spectra may overlap or may be non-existent.

Conventional assessment of quantitative trait resistance, particularly for APR to powdery mildew, relies heavily on repetitious ratings in several different environments to calculate an area under disease progress curve (AUDPC) (Das and Griffey, 1994a; Shaner and Finney, 1977). If mildew pressure is inadequate or nonexistent during a field season, disease assessments would not be accurate. Furthermore, a hypersensitive *Pm* gene conferring total resistance in the field would not allow varying levels of APR to be adequately assessed. Molecular markers linked to APR QTL for powdery mildew could assist breeders in selecting for APR in the presence of non-defeated *Pm* genes in early generations.

Molecular markers tightly linked to resistance genes can facilitate their pyramiding into a single elite cultivar, with the possibility of creating more durable or broad spectrum resistance. Liu et al. (2000) were able to successfully integrate three powdery mildew gene combinations *Pm2* + *Pm4a*, *Pm2* + *Pm21*, and *Pm4a* + *Pm21* into wheat cultivar Yang158. Double homozygote plants were selected from small F₂ populations with the assistance of RFLPs flanking the mildew resistance genes. As *Pm21* confers resistance to all known mildew isolates in China and Europe, conventional breeding strategies for pyramiding resistance would be difficult. Using similar pyramiding schemes in rice, four bacterial blight resistance genes *Xa-4*, *xa-5*, *xa-13*, and *Xa-21* (Huang et al., 1997a) and

three genes for blast resistance *pi1*, *Piz5*, and *Pita* (Hittalmani et al., 2000) were incorporated into single rice cultivars using PCR based markers and RFLPs.

Incorporating and pyramiding favorable QTL from wild relatives with the use of MAS involves the advanced backcross (AB)-QTL method first proposed by Tanksley and Nelson (1996). This method requires the development of NILs with a single QTL being integrated. Development of these NILs requires a controlled program of backcrossing, similar to the MAS program for a single gene, or only one QTL resistance allele. The targeted QTL, along with the non-targeted QTL, are monitored by a single marker or group of flanking markers as successive backcrosses occur to a recurrent parent. These NILs containing individual QTL and having a positive effect on the quantitative trait can then be backcrossed into a single line using markers to monitor each individual QTL.

Castro et al. (2003a) pyramided one QTL for barley (*Hordeum vulgare*) stripe rust (*Puccinia striiformis*) resistance obtained from experimental line 'D1-72', carrying a resistance QTL on chromosome 5, and two QTL on chromosomes 4 and 7 from cultivar 'Orca'. The three QTL were then backcrossed into stripe rust susceptible line 'Harrington' using flanking RFLPs. Together, the three QTL explained 94% of the genotypic variance in a new genetic background for adult plant resistance to stripe rust (Castro et al., 2003b).

However, MAS, particularly for QTL, has had a limited impact on plant breeding to date. Numerous studies and simulations have attempted to increase and maximize the efficiency of marker-assisted selection for quantitative traits, yet have yielded little progress (van Berloo and Stam, 1998; van Berloo and Stam, 1999; Yousef and Juvik, 2001). Often markers linked to genes or QTL in one population are monomorphic in a population containing the same resistance source; therefore making selection in the new population not possible.

A significant problem encountered when mapping quantitative traits is the effect of genotype by environmental (GE) interaction. QTL mapped in one environment often do

not have the same contribution in other environments or they may have opposite genetic effects on a quantitative trait (Melchinger et al., 1998). Yan et al. (1999) mapped eight quantitative traits in rice and found significant GE interaction involved in all traits observed. QTL identified in one environment explaining a significant portion of the variance were often non-existent in other environments. Other QTL were only controlled by the GE interaction without main genetic effects.

One of the most promising contributions in mapping QTL offered to the plant breeder was the ability to concurrently assay 100 to 200 plants to accurately map QTL locations and contributions. Early published studies with replicated trials employed 100 to 200 progeny, requiring excessive labor and costs for phenotyping and genotyping large populations with RFLPs. However, studies have shown that assaying small numbers of progeny (less than 200) leads to over estimation of QTL effects and underestimation of QTL numbers (Lande and Thompson, 1990; Melchinger et al., 1998). Knapp and Bridges (1990) demonstrated that for any quantitative trait, QTL mapping is improved by increasing the number of lines contained in the population and evaluating the population once, rather than evaluating small populations in several environments.

As large numbers of SSR markers are publicly available in all crop species and marker screening can be easily automated, new QTL mapping projects consist of large populations, tested in several environments, and genetic backgrounds. Breeders attempting to apply QTL mapped in small populations must first verify QTL effects in new populations as estimates may be biased in the mapping population (Langridge and Chalmers, 1998). Additional markers should be added to original mapping populations to increase the level of polymorphic markers in different genetic backgrounds and decrease distances between the QTL and marker loci. Once additional markers are added and QTL effects are validated in a different genetic background, a MAS selection program can be initiated.

Literature Cited

- Anderson, J.A., Y. Ogihara, M.E. Sorrells, and S.D. Tanksley. 1992. Development of a chromosomal arm map for wheat based on RFLP markers. *Theor. Appl. Genet.* 83:1035-1043.
- Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programs. *Plant Path.* 33:279-300.
- Bougot, Y., J. Lemoine, M.T. Pavoine, D. Barloy, and G. Doussinault. 2002. Identification of a microsatellite marker associated with *Pm3* resistance alleles to powdery mildew in wheat. *Plant Breed.* 121:325-329.
- Boukhatem, N., P.V. Baret, D. Mingeot, and J.M. Jacquemin. 2002. Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. *Theor. Appl. Genet.* 104:111-118.
- Briggle, L.W. 1960. Sources of resistance to powdery mildew from tetraploid *Triticum* species. *Agron. Abs.* 65:45.
- Briggle, L.W. 1966. Three loci in wheat involving resistance to *Erysiphe graminis* f. sp. *tritici*. *Crop Sci.* 9:70-72.
- Briggle, L.W. 1969. Near isogenic lines of wheat with genes for resistance to *Erysiphe graminis* f. sp. *tritici*. *Crop Sci.* 9:70-72.
- Burr, B., F.A. Burr, K.H. Thompson, M.C. Albertson, and C.W. Stuber. 1988. Gene mapping with recombinant inbreds in maize. *Genetics* 118:519-525.
- Castro, A.J., X. Chen, P.M. Hayes, and M. Johnston. 2003b. Pyramiding quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on resistance at the seedling stage. *Crop Sci.* 43:651-659.
- Castro, A.J., X. Chen, A. Corey, T. Filichkina, P.M. Hayes, C. Mundt, K. Richardson, S. Sandoval Islas, and H. Vivar. 2003a. Pyramiding and validation of quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: Effects on adult plant resistance. *Crop Sci.* 43:2234-2239.
- 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.
- Chae, Y.A., and G.W. Fishchbeck. 1979. Genetic analysis of powdery mildew resistance in wheat cultivar "Diplomat". *Z. Plazenzuchtg* 83:272-280.

Chantret, N., M.T. Pavoine, and G. Doussinault. 1999. The race-specific resistance gene to powdery mildew, *MIRE*, has a residual effect on adult plant resistance of winter wheat line RE714. *Phytopathology* 89:533-539.

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

Chao, S., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner, and M.D. Gale. 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theor. Appl. Genet.* 78:495-504.

Chen, P.D., L.L. Qi, B. Zhou, S.Z. Zhang, and D.J. Liu. 1995. Development and molecular cytogenetic analyses of wheat-Haynaldia villosa 6VS, 6AL translocation lines specifying resistance to powdery mildew. *Theor. Appl. Genet.* 91:1125-1128.

Das, M.K., and C.A. Griffey. 1994a. Diallel analysis of adult-plant resistance to powdery mildew in wheat. *Crop Sci.* 34:948-952.

Das, M.K., and C.A. Griffey. 1994b. Heritability and number of genes governing adult-plant resistance to powdery mildew in Houser and Redcoat winter wheats. *Phytopathology* 84:406-409.

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

Dedryver, F., M.F. Jubier, J. Thouvenin, and H. Goyeau. 1996. Molecular markers linked to the leaf rust resistance gene *Lr24* in different wheat cultivars. *Genome* 39:830-835.

Devos, K.M., and M.D. Gale. 1992. The use of random amplified polymorphic DNA markers in wheat. *Theor. Appl. Genet.* 84:567-572.

Devos, K.M., and M.D. Gale. 1993. Extended genetic maps of the homoeologous group 3 chromosomes of wheat, rye and barley. *Theor. Appl. Genet.* 85:649-652.

Devos, K.M., M.D. Atkinson, C.N. Chinoy, C.J. Liu, and M.D. Gale. 1992. RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theor. Appl. Genet.* 83:931-939.

Devos, K.M., G.J. Bryan, A.J. Collins, P. Stephenson, and M.D. Gale. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. *Theor. Appl. Genet.* 90:247-252.

- Eujayl, I., M.E. Sorrells, M. Baum, P. Wolters, and W. Powell. 2002. Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. *Theor. Appl. Genet.* 104:399-407.
- Feuillet, C., M. Messmer, G. Schacehermayr, and B. Keller. 1995. Genetic and physical characterization of the *LRI* leaf rust resistance locus in wheat (*Triticum aestivum* L.). *Mol. Gen. Genet.* 248:553-562.
- Finney, J.R., and D.W. Hall. 1972. The effect on an Autumn attack of mildew (*Erysiphe graminis*) on growth and development of winter barley. *Plant Path.* 73-76.
- Flor, H.H. 1956. The complementary genetic systems in flax and flax rust. *Adv. Genet.* 8:29-54.
- 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.
- Fried, P.M., D.R. MacKenzie, and R.R. Nelson. 1979. Disease progress curves of *Erysiphe graminis* f. sp. *tritici* on Chancellor wheat and four multilines. *Phytopathology* 95:151-166.
- Gao, L.F., R.L. Jing, N.X. Huo, Y. Li, X.P. Li, R.H. Zhou, X.P. Chang, J.F. Tang, Z.Y. Ma, and J.Z. Jia. 2004. One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. *Theor. Appl. Genet.* 108:1392-1400.
- 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.
- Griffey, C.A., M.K. Das, and E.L. Stromberg. 1993. Effectiveness of adult plant resistance in reducing loss to powdery mildew in winter wheat. *Plant Dis.* 77:618-622.
- Guo, P.G., G.H. Bai, and G.E. Shaner. 2003. AFLP and STS tagging of a major QTL for *Fusarium* head blight resistance in wheat. *Theor. Appl. Genet.* 106:1011-1017.
- Gupta, P.K., S. Rustgi, S. Sharma, R. Singh, N. Kumar, and H.S. Balyan. 2003. Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. *Mol. Genet. Gen.* 270:315-323.
- Gupta, P.K., H.S. Balyan, K.J. Edwards, P. Isaac, V. Korzun, M. Röder, M.F. Gautier, P. Joudrier, A.R. Schlatter, and J. Dubcovsky. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theor. Appl. Genet.* 105:413-422.

- Gustafon, G.D., and G. Shaner. 1982. Influence of plant age on the expression of slow-mildewing resistance in wheat. *Phytopathology* 72:746-749.
- Guyomar ch, H., P. Sourdille, K.J. Edwards, and M. Bernard. 2002. Studies of the transferability of microsatellites derived from *Triticum tauschii* to hexaploid wheat and to diploid related species using amplification, hybridization and sequence comparisons. *Theor. Appl. Genet.* 105:736-744.
- Hartl, L., H. Weiss, F.J. Zeller, and A. Jahoor. 1993. Use of RFLP markers for the 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.
- Hautea, R.A., W.R. Coffman, M.E. Sorrells, and G.C. Berstrom. 1987. Inheritance of partial resistance to powdery mildew in spring wheat. *Theor. Appl. Genet.* 79:609-615.
- Hebert, T.T., W.H. Rankin, and G.K. Middleton. 1948. Interaction of nitrogen fertilization and powdery mildew on yield of wheat. *Phytopathology* 38:569-570.
- Heun, M., B. Friebe, and W. Bushuk. 1990a. Chromosomal location of powdery mildew resistance gene of Amigo wheat. *Phytopathology* 80:1129-1133.
- Heun, M., B. Friebe, and W. Bushuk. 1990b. Introgression of powdery mildew resistance from rye into wheat. *Phytopathology* 80:242-245.
- Hittalmani, S., A. Parco, T.V. Mew, R.S. Zeigler, and N. Huang. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theor. Appl. Genet.* 100:1121-1128.
- Hsam, S.L.K., I.F. Lapochkina, and F.J. Zeller. 2003. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). Gene *Pm32* in a wheat-*Aegilops speltoides* translocation line. *Euphytica* 133:367-370.
- 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 molecular and biochemical markers. *Plant Breed.* 119:87-89.
- 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, N., E.R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, N. Kumaravadivel, J. Bennett, and G.S. Khush. 1997a. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* 95:313-320.
- Huang, X.-Q., and M.S. Röder. 2004. Molecular mapping of powdery mildew resistance genes in wheat: a review. *Euphytica* 137:203-223.
- Huang, X.Q., S.L.K. Hsam, and F.J. Zeller. 1997b. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 4. Gene *Pm24* in Chinese landrace Chiyacao. *Theor. Appl. Genet.* 95:950-953.
- Huang, X.Q., L.K. Hsam, and F.J. Zeller. 2000. Chromosomal location of two novel genes for resistance to powdery mildew in Chinese landraces (*Triticum aestivum* L. em Thell.). *J. Genet. Breed.* 54:311-317.
- Huang, X.Q., H. Coster, M.W. Ganal, and M.S. Röder. 2003b. Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 106:1379-1389.
- Huang, X.Q., L.X. Wang, M.X. Xu, and M.S. Röder. 2003a. Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 106:858-865.
- Imani, Y., A. Ouassou, and C.A. Griffey. 2002. Virulence of *Blumeria graminis* f. sp. *tritici* populations in Morocco. *Plant Dis.* 86:383-388.
- Iqbal, M.J., and A.L. Rayburn. 1995. Identification of the 1RS rye chromosomal segment in wheat by RAPD analysis. *Theor. Appl. Genet.* 91:1048-1053.
- Jia, J., K.M. Devos, S. Chao, T.E. Miller, S.M. Reader, and M.D. Gale. 1996. RFLP-based maps of the homoeologous 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.
- Johnson, J.W., P.S. Bänziger, 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.
- Jorgensen, J.H., and C.J. Jensen. 1972. Gene for resistance to powdery mildew in derivatives of *Triticum timopheevii* and *Triticum carthlicum*. *Euphytica* 21:121-128.

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 a segregating wheat x spelt population. *Theor. Appl. Genet.* 98:903-912.

Kingsland, G.C. 1982. Triadimefon for control of powdery mildew of wheat. *Plant Dis.* 66:139-141.

Knapp, S.J., and W.C. Bridges. 1990. Using molecular markers to estimate quantitative trait locus parameters power and genetic variances for unreplicated and replicated progeny. *Genetics* 126:769-777.

La Rota, M., and M.E. Sorrells. "Development of microsatellite markers that map across related species" In *GrainGenes* <http://wheat.pw.usda.gov/ggpages/ITMI/EST-SSR/LaRota/>. (22 Nov. 2003).

Lande, R., and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* 124:743-756.

Langridge, P., and K. Chalmers. 1998. Techniques for marker development, p. 107-117, In A. E. Slinkard, ed. *Proceeding of 9th Int'l Wheat Genetics Symposium*. University Extension Press, Saskatoon, Canada.

Law, C.N., and M.S. Wolfe. 1966. Location for genetic factors for mildew resistance and ear emergence on chromosome 7B of wheat. *Can. J. Genet. Cytol.* 8:462-470.

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* 79:152-155.

Lesbock, K.L., and L.W. Briggles. 1974. Gene *Pm5* for resistance to *Erysiphe graminis* f. sp. *tritici* in Hope wheat. *Crop Sci.* 14:561-563.

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, S., C.A. Griffey, and M.A. Saghai Maroof. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. *Crop Sci.* 41:1268-1275.

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.

Lutz, J., L.K. Hsam, and L.E. Zeller. 1995. Chromosomal location of powdery mildew resistance genes in *Triticum aestivum* L. (common wheat). 2 Genes *Pm2* and *Pm19* from *Aegilops squarrosa* L. *Heredity* 74:152-156.

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.

Ma, Z.Q., J.B. Wei, and S.H. Cheng. 2004. PCR-based markers for the powdery mildew resistance gene *Pm4a* in wheat. *Theor. Appl. Genet.* 109:140-145.

Maughan, P.J., M.A. Saghai Maroof, G.R. Buss, and G.M. Huestis. 1996. Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theor. Appl. Genet.* 93:392-401.

McIntosh, R.A., G.E. Hart, K.M. Devos, and M.D. Gale. 1998. Catalogue of gene symbols for wheat, *In* A. E. Slinkard, ed. *Proceeding 9th International Wheat Genetics Symposium*, Vol. 5. University Extension Press, University of Saskatchewan, Saskatoon Canada.

Melchinger, A.E. 1989. Use of molecular markers in breeding for oligogenic resistance. *Plant Breed.* 104:1-19.

Melchinger, A.E., H.F. Utz, and C.C. Schon. 1998. Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. *Genetics* 149:383-403.

Menzies, J.G., and MacNeill. 1986. Asexual recombination in *Erysiphe graminis* f. sp. *tritici*. *Can. J. Plant Path.* 8:400-404.

Messmer, M.M., R. Seyfarth, M. Keller, G. Schachermayr, M. Winzeler, S. Zanetti, C. Feuillet, and B. Keller. 2000. Genetic analysis of durable leaf rust resistance in winter wheat. *Theor. Appl. Genet.* 100:419-431.

Michelmore, R.W., I. Paran, and R.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.

- Miller, T.E., S.M. Reader, C.C. Ainsworth, and R.W. Summers. 1998. The introduction of a major gene for resistance to powdery mildew of wheat, *Erysiphe graminis* f. sp. *tritici*, from *Aegilops splotoides* into wheat, *Triticum aestivum*., p. 179-183, In M. L. Jorna and L. A. J. Sloodmaker, eds. Cereal production related to integrated cereal production, Pudoc, Netherlands.
- 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 genetic backgrounds. *Plant Breed.* 121:133-140.
- Mohler, V., S.L.K. Hsam, F.J. Zeller, and G. Wenzel. 2001. An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the *Pm8/Pm17* locus of common wheat. *Plant Breed.* 120:448-450.
- Muehlbauer, G.J., J.E. Specht, M.A. Thomas Compton, P.E. Staswick, and R.L. Bernard. 1988. Near-isogenic lines--a potential resource in the integration of conventional and molecular marker linkage maps. *Crop Sci.* 28:729-735.
- Neu, C., N. Stein, and B. Keller. 2002. Genetic mapping of the *Lr20-Pm1* resistance locus reveals suppressed recombination on chromosome arm 7AL in hexaploid wheat. *Genome* 45:737-744.
- Niewoehner, A.S., and S. Leath. 1998. Virulence of *Blumeria graminis* f.sp. *tritici* on winter wheat in the Eastern United States. *Plant Dis.* 82:64-68.
- Olson, M., L. Hood, C. Cantor, and D. Botstein. 1989. A common language for physical mapping of the human genome. *Science* 245:1434-1435.
- Patterson, F.L., J.F. Schafer, and R.L. Gallun. 1978. Registration of Knox 62 wheat. *Crop Sci.* 18:527.
- Persaud, R.R., and P.E. Lipps. 1995. Virulence genes and virulence gene frequencies of *Blumeria graminis* f. sp. *tritici* in Ohio. *Plant Dis.* 79:494-499.
- Pestsova, E., M.W. Ganai, and M.S. Röder. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome* 43:689-697.
- 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.L., M.S. Cao, P.D. Chen, W.L. Li, and D.J. Liu. 1996. Identification, mapping, and application of polymorphic DNA associated with resistance gene *Pm21* of wheat. *Genome* 39:191-197.

Reader, S.M., and T.E. Miller. 1991. The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer wheat. *Euphytica* 53:57-60.

Roberts, J.J., and R.M. Calwell. 1970. General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in "Knox" wheat (abstract). *Phytopathology* 60:1310.

Röder, M.S., J. Plaschke, S.U. König, A. Börner, M.E. Sorrells, S.D. Tanksley, and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen. Genet.* 246:327-333.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W. Ganal. 1998. A microsatellite map of wheat. *Genetics* 149:2007-2023.

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 map mapping. *Euphytica* 115:121-126.

Rouse, D.I., R.R. Nelson, D.R. MacKenzie, and C.R. Armitage. 1980. Components of rate-reducing resistance in seedlings of four wheat cultivars and parasitic fitness in six isolates of *Erysiphe graminis* f. sp. *tritici*. *Phytopathology* 70:1097-1100.

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

Sacco, F., E.Y. Suarez, and T. Naranjo. 1998. Mapping of the leaf rust resistance gene *Lr3* on chromosome 6B of Sinvalocho MA wheat. *Genome* 41:686-690.

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

Shaner, G., and R.E. Finney. 1975. Inheritance of slow mildewing resistance in wheat. *Proc. Am. Phytopath. Soc.* 2:49.

Shaner, G., and R.E. Finney. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.

- Shen, X., M. Zhou, W. Lu, and H. Ohm. 2003. Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. *Theor. Appl. Genet.* 106:1041-1047.
- Shi, A.N., S. Leath, and J.P. Murphy. 1998. A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. *Phytopathology* 88:144-147.
- Singrun, C., S.L.K. Hsam, L. Hartl, F.J. Zeller, and V. Mohler. 2003. Powdery mildew resistance gene *Pm22* in cultivar Virest is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L. em Thell.). *Theor. Appl. Genet.* 106:1420-1424.
- Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:1105-1114.
- Song, Q.J., E.W. Fickus, and P.B. Cregan. 2002. Characterization of trinucleotide SSR motifs in wheat. *Theor. Appl. Genet.* 104:286-293.
- Sourdille, P., P. Robe, M.H. Tixier, G. Doussinault, M.T. Pavoine, 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.
- Sourdille, P., T. Cadalen, H. Guyomar ch, J.W. Snape, M.R. Perretant, G. Charmet, C. Boeuf, S. Bernard, and M. Bernard. 2003. An update of the Courtot x Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. *Theor. Appl. Genet.* 106:530-538.
- Starling, T.M., C.W. Roane, and H.M. Camper, Jr. 1984. Registration of Massey wheat. *Crop Sci.* 24:1000.
- Stephenson, P., G. Bryan, J. Kirby, A. Collins, K. Devos, C. Busso, and M. Gale. 1998. Fifty new microsatellite loci for the wheat genetic map. *Theor. Appl. Genet.* 97:946-949.
- Szunics, L., G. Vida, Z. Bedo, and M. Svec. 2001. Dynamics of changes in the races and virulence of wheat powdery mildew in Hungary between 1971 and 1999. *Euphytica* 119:143-147.
- Tanksley, S.D. 1983. Molecular markers in plant breeding. *Plant Mol. Bio. Rep.* 1:3-8.
- Tanksley, S.D., and J.C. Nelson. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTL from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* 92:191-203.

- Tao, W., D. 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.
- The, T.T., R.A. McIntosh, and F.G.A. Bennett. 1979. Cytogenetical studies in wheat. IX. Monosomic analyses, telocentric mapping, and linkage relationships of genes *Sr21*, *Pm4* and *Mle*. *Australian Journal of Biological Sciences* 32:115-125.
- van Berloo, R., and P. Stam. 1998. Marker-assisted selection in autogamous RIL populations: a simulation study. *Theor. Appl. Genet.* 96:147-154.
- van Berloo, R., and P. Stam. 1999. Comparison between marker-assisted selection and phenotypical selection in a set of *Arabidopsis thaliana* recombinant inbred lines. *Theor. Appl. Genet.* 98:113-118.
- 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. *Nucleic Acids Res.* 23:4407-4414.
- Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.
- Xie, C., Q. Sun, Z. Ni, T. Yang, E. Nevo, and T. Fahima. 2003. Chromosomal location of a *Triticum dicoccoides*-derived powdery mildew resistance gene in common wheat by using microsatellite markers. *Theor. Appl. Genet.* 106:341-345.
- Xie, D.X., K.M. Devos, G. Moore, and M.D. Gale. 1993. RFLP-based genetic maps of the homoeologous group 5 chromosomes of bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 87:70-74.
- Yan, J., J. Zhu, C. He, M. Benmoussa, and P. Wu. 1999. Molecular marker-assisted dissection of genotype x environment interaction for plant type traits in rice (*Oryza sativa* L.). *Crop Sci.* 39:538-544.
- Young, N.D., D. Zamir, M.W. Ganai, and S.D. Tanksley. 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. *Genetics* 120:579-585.
- Yousef, G.G., and J.A. Juvik. 2001. Comparison of phenotypic and marker-assisted selection for quantitative traits in sweet corn. *Crop Sci.* 41:645-655.
- Yu, J.K., M. La Rota, R.V. Kantety, and M.E. Sorrells. 2004. EST derived SSR markers for comparative mapping in wheat and rice. *Mol. Genet. Genom.* 271:742-751.

Zeller, F.J., and L.K. Hsam. 1998. Progress in breeding for resistance to powdery mildew in common wheat (*Triticum aestivum*, L.), p. 178-181, *In* A. E. Slinkard, ed. Proc. 9th Int. Wheat Genet. Symp. Univ. Press, Saskatoon.

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* 123:187-19

CHAPTER II

Validation and Saturation of Three Quantitative Trait Loci Conferring Adult Plant Resistance to Powdery Mildew with PCR-Based Markers in an Elite Wheat Breeding Population

Abstract

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most devastating diseases in wheat (*Triticum aestivum*) worldwide. Hypersensitive, race specific genes primarily have been deployed to control the disease, however recent efforts have shifted to breeding for more durable resistance, such as ‘adult plant resistance’ (APR). Molecular markers and quantitative trait loci (QTL) associated with APR to powdery mildew must first be validated and QTL effects evaluated in different genetic backgrounds and breeding populations to be useful in marker-assisted selection (MAS) programs. Previously, three QTL for APR resistance to powdery mildew in cultivar Massey were mapped in a Becker by Massey (BM) F_{2:3} population and further confirmed over a three year period in a set of recombinant inbred lines (RILs). Eighteen simple sequence repeat (SSR) markers previously mapped near the three QTL in Massey for APR to powdery mildew were examined for association with APR to powdery mildew in a RIL breeding population derived from a cross between USG 3209 and a moderately susceptible cultivar Jaypee, wherein Massey is the resistance source for APR in USG 3209. Thirteen new SSR markers were added to the pre-existing BM F_{2:3} linkage maps near the QTL for APR on chromosomes 1B, 2A, and 2B. Genetic linkage maps comprised of 21 SSRs on chromosomes 1B, 2A, and 2B were constructed in the USG 3209 by Jaypee (UJ) F_{6:7} population. Interval mapping analysis of mildew severity data collected in 2002 (F_{5:6}) and 2003 (F_{6:7}) field experiments with marker genotypic data obtained in 2003 (F_{6:7}) confirmed the presence of three QTL for APR on chromosomes 1B, 2A, and 2B in the UJ population. The QTL on chromosomes 1B, 2A, and 2B explained 12% to 13%, 59% to 69%, and 22% to 48% of the phenotypic variance for powdery mildew severity in the UJ RIL populations, respectively, in two field experiments. The current study verifies that the elite wheat cultivar USG 3209 possesses the same QTL for APR as its parent Massey and validates that SSRs from the original BM F_{2:3} mapping population can be used successfully for MAS in the UJ elite breeding population.

Introduction

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the most devastating diseases of common wheat (*Triticum aestivum*). Yield losses ranging from 16% to 34% have been reported in the United States (Griffey et al., 1993; Johnson et al., 1979; Kingsland, 1982; Leath and Bowen, 1989). Utilization of resistance cultivars has provided the most economical and efficient control of the disease (Bennett, 1984). To date, 32 major gene, race-specific loci, designated *Pm1-Pm32*, as well as other non-characterized resistance genes have been reported to confer resistance to powdery mildew in wheat (Hsam et al., 2003; Huang and Röder, 2004; McIntosh et al., 1998; Singrun et al., 2003). These genes confer complete resistance to the pathogen throughout the life cycle of the host. However, this resistance is ephemeral and generally has been overcome by rapidly changing, dynamic powdery mildew populations (Roberts and Calwell, 1970; Shaner, 1973).

Some wheat cultivars exhibit resistance that delays infection and reduces growth of the pathogen, rather than conferring immunity or complete resistance. This form of resistance has been termed 'slow mildewing' (Roberts and Calwell, 1970), 'adult plant resistance' (APR) (Gustafon and Shaner, 1982), or 'partial resistance' (Hautea et al., 1987). APR is more durable than that conferred by race-specific major genes, but it is difficult to assess in field conditions due to its quantitative nature (Gustafon and Shaner, 1982). Quantitative trait loci (QTL) linked to APR for powdery mildew in wheat cultivars Forno (Keller et al., 1999), Massey (Liu et al., 2001), and RE714 (Chantret et al., 2000; Chantret et al., 2001; Mingeot et al., 2002) have been mapped using simple sequence repeats (SSRs) and restriction fragment length polymorphisms (RFLPs).

SSRs have become the marker system of choice in wheat because they are highly polymorphic, genome specific, and current coverage of genetic maps are sufficient for densely saturating QTL regions (Röder et al., 1995; Röder et al., 1998; Somers et al., 2004). Use of SSRs, known to be associated with QTL for APR, could potentially provide an approach for implementing routine marker-assisted selection (MAS) of resistant plants in segregating breeding populations. However, use of molecular markers,

particularly for quantitative traits, has had limited practical impact in plant breeding programs. While many QTL have been validated in mapping populations often in several different environments and years, MAS for QTL has not been widely deployed. Inefficiency in MAS has been due to overestimation of QTL effects in small mapping populations (Beavis, 1998), varying genetic effects of QTL in different backgrounds, and lack of polymorphic markers for effective application of MAS in new populations or backgrounds.

Successful application of molecular markers for MAS in plant breeding depends on the availability of markers that have been validated in different genetic backgrounds and well characterized and verified QTL effects (Fasoula et al., 2003; Langridge and Chalmers, 1998; Li et al., 2001). Several studies have reported QTL with major effects for APR for powdery mildew that were not consistent across environments (Keller et al., 1999; Mingeot et al., 2002). Validation of QTL for APR to powdery mildew in additional breeding populations will assist breeders in developing effective MAS programs.

Massey, a derivative of Knox 62 (Shaner, 1973), has retained effective powdery mildew resistance since its release in 1981 (Starling et al., 1984). Previous molecular and conventional genetic studies reported that APR in Massey was conferred by three QTL with moderate to high heritabilities (Griffey and Das, 1994; Liu et al., 2001). Three QTL were mapped to wheat chromosomes 1B, 2A, and 2B in a Becker by Massey (BM) F_{2:3} population using SSR and RFLP markers (Liu et al., 2001). The three QTL explained 17%, 29%, and 11% of the phenotypic variance, respectively. Presence and contribution of the three QTL to APR were validated in a set of recombinant inbred lines (RILs) derived from the BM cross over a three year period.

Objectives of this current study were to validate and map SSRs previously identified near QTL responsible for APR in cultivar Massey in the breeding population of USG 3209 by Jaypee (UJ), and to identify and map additional SSRs in the three QTL regions. The UJ breeding population was selected for this study because the APR of USG 3209 putatively was derived from Massey and USG 3209 is a modern, widely grown commercial cultivar.

Effects of the three QTL on APR to powdery mildew, assuming that USG 3209 contains all three QTL from Massey, in a different genetic background will be evaluated to determine the feasibility and effectiveness for implementing a MAS breeding program for APR.

Materials and Methods

Plant Materials

Previously mapped and newly added SSR markers in BM F_{2:3} population were assayed in the RIL population of UJ. USG 3209 (PI617055) was derived from the cross Saluda/4/Massey*2/3/Massey*3/Balkan//Saluda. Seedling tests conducted at North Carolina State University and the SSR marker Xpsp2999 (Bougot et al., 2002) confirmed that USG 3209 possesses *Pm3a* (from Saluda). USG 3209 also contains *Pm8* (from Balkan), and other unidentified genes for seedling mildew resistance. Jaypee (PI592760) was derived from a cross between Arthur 6 and AR39-3 (Doublecrop//Forlani/Garibaldo) and is moderately susceptible to *B. graminis* populations prevalent in the Mid-Atlantic region.

The UJ RIL population was initiated by crossing USG 3209 and Jaypee during the 1997 spring greenhouse crossing season. The population was planted yearly and advanced to the F₄ generation using a modified bulk breeding method, which is implemented in our cultivar development program. The F₂ and F₃ generations were grown in 20.8 m² blocks at Blacksburg and Warsaw, VA from which spikes, selected at both locations solely on the basis of desirable plant type, were harvested, threshed in bulk and used as a source of seed in subsequent generations. In the F₄ generation, 293 spikes were selected arbitrarily, harvested, and threshed separately to develop RILs and were planted in 1.4 m rows at Warsaw, VA during fall 2001. Subsequent generations (F₅-F₈) were advanced yearly by selecting a single spike from the head row and planting it in Warsaw, VA the following growing season.

Genetic Materials

DNA from 180 previously developed F_{2:3} lines, derived from a cross of BM (Liu et al., 2001) was used in mapping SSR markers to saturate QTL regions responsible for APR. Massey (Citr17953) has long exhibited durable powdery mildew resistance and is known to possess APR. Becker (PI494524) does not possess any known genes for powdery mildew resistance and is highly susceptible to powdery mildew populations in the Mid-Atlantic region.

Plant tissue was collected in the field from each F_{6:7} UJ RIL row. A 5 cm leaf section was cut from 10 to 15 plants within the 1.4 m row and bulked. DNA was extracted as described by Saghai Maroof et al. (1984). DNA integrity was determined by running undigested DNA in 0.8% agarose gel followed by staining with ethidium bromide (EtBr) and visualizing under ultraviolet (UV) light. DNA concentration was measured with a DyNA Quanta2000 Fluorometer (Hoefer Scientific, San Francisco, CA).

Microsatellite Analysis and Mapping in Becker by Massey F_{2:3} Population and USG 3209 by Jaypee F_{6:7} RILs

Sixty-eight new SSR markers publicly available on the GrainGenes website (<http://wheat.pw.usda.gov>) or in published papers (Table 1), located near QTL contributing to APR powdery mildew in Massey were assayed for polymorphism using the parental lines Becker and Massey.

Thirty-one SSRs located near the three QTL responsible for APR in the BM population (Liu et al., 2001) were assayed for polymorphism between USG 3209 and Jaypee. Two DNA bulks were created by selecting RILs which exhibited homogeneous disease reaction in the F_{5:6} field experiment. The bulks contained equal amounts of DNA from eight homozygous resistant and eight homozygous susceptible F_{5:6} RILs, respectively. DNA from the two bulks, APR resistance source Massey, and parental lines USG 3209 and Jaypee was used to survey SSR markers for putative association with APR to powdery mildew in the UJ population. Markers exhibiting a similar pattern of polymorphism between the resistant parents (Massey and USG3209), the susceptible

parent (Jaypee), and the two respective bulks were considered as putative resistance-related markers.

PCR reactions were performed in a total volume of 10 ul in a Perkin Elmer 9700 (Perkin Elmer, Norwalk, CN) thermal cycler. After the initial denaturing step, 4 min at 94°C, followed by 32 or 40 cycles (depending on the primer pair) of 0.5 min at 95°C, 1 min at either 50°C, 55°C, or 60°C (depending on the primer pair), 1 min at 73°C, followed by a final extension step for 5 min at 73°C. The reaction mixture and polyacrylamide gel electrophoresis were performed as described by Saghai Maroof et al. (1994).

Powdery Mildew Assessment and Statistical Analysis

To determine whether new SSRs mapped near QTL for APR in Massey were associated with powdery mildew resistance in the BM population, disease severity ratings (James, 1971) assessed in 1995 at Warsaw (Liu et al., 2001) were analyzed for association with genotypic data. Disease ratings likewise were assessed for the F_{5:6} (2002) and F_{6:7} (2003) UJ RILs for mildew severity under natural epidemics at Warsaw using the James scale assessments. Average powdery mildew severity was scored on F-2 (two leaves below the flag leaf) and F-1 leaves (one leaf below the flag leaf). A score of 0% was given to plants without any mildew lesions while a score of 50% was given when plants reached maximum disease coverage on the F-2 and F-1 leaves.

Statistical analysis was performed using SPSS statistical software version 12.0 (SPSS, 1998). A one-way ANOVA was used to determine the significance ($P \leq 0.05$) between markers and APR to powdery mildew. Log₁₀ was used to only transform the mildew severity data in the F_{2:3} BM population prior to analysis due to a skewed distribution of the data.

Map Construction

MAPMAKER 3.0b (Lander et al., 1987) was used in linkage group map construction in both MB and UJ populations. Initial grouping of markers in both populations was performed using the “group” command at a LOD score of 3.0 with a maximum Haldane

distance of 50 cM. The “order” and “compare” commands were used to determine the most probable marker order in the populations. Markers not meeting the threshold were placed in intervals using the “try” command.

Genetic distances and marker order calculated by MAPMAKER were imported into QTL Cartographer version 2.0 (Basten et al., 1994) to create linkage group figures. Interval mapping (Lander and Botstein, 1989) was used to search for QTL in UJ population and compared to QTL resulting from the BM population using QTL Cartographer 2.0 software. Significant thresholds for QTL were determined using a 1000 permutation test included in the software (Doerge and Churchill, 1996). A mean LOD threshold of 3.0 was selected for all traits to declare a putative QTL significance corresponding to a genome wide α error of 0.05. Estimates of LOD peaks and variance explained were obtained from the Zmapqtl program run with model 3 in QTL Cartographer 2.0. Field disease assessments for both BM and UJ populations were analyzed for QTL effects.

Results

Mapping in Becker by Massey and USG 320 by Jaypee Populations

Of the 68 new SSRs assessed for polymorphism in the BM population, 22 (32%) were polymorphic between Becker and Massey. Thirteen of the 22 (59%) SSRs cosegregated with markers known to be linked to QTL for APR to powdery mildew in Massey and were added to the preexisting genetic map (Liu et al., 2001). A majority of the new markers added were mapped near QTL on chromosomes 2A and 2B (Fig. 1). Newly added SSR marker Xbar15 on chromosome 2A, located 20 cM away from the QTL, provided the second closest SSR marker on one side of the QTL. The nine markers near the QTL on chromosome 2B were all located in close proximity to the QTL region. Specifically, two closely linked markers Xgwm191 and Xbar200 mapped to one side of the QTL where previously only RFLP markers were located. Marker Xbar80 was the only SSR marker mapped to the linkage group of chromosome 1B; however the newly mapped marker, 10 cM away from the QTL, provided the necessary flanking PCR based marker on one side of the QTL for efficient MAS.

Thirteen of the eighteen (72%) SSR markers previously mapped in the BM population were polymorphic in the UJ population. Eleven of these thirteen (84%) SSR markers were putatively linked to APR QTL on the basis of BSA results. These markers were used to genotype the UJ F_{6:7} RILs and linkage groups were constructed by MAPMAKER 3.0b (Fig. 1). Marker order was consistent between the two populations. Only slight rearrangement of marker order was observed on the linkage group of chromosome 2B for marker intervals Xgwm501-Xgwm47 and Xgwm191-Xbar200. Marker order on linkage groups 2A and 1B was entirely conserved between the two populations.

Of ten SSRs mapped near the QTL on chromosome 2A in the original BM mapping population, five (50%) were polymorphic and mapped to the linkage group on chromosome 2A in the UJ population. Similarly, only four of the original eight (50%) SSRs mapped near the QTL on chromosome 2B in the BM population were polymorphic and mapped to the UJ 2B linkage group. Two SSR markers mapped to chromosome 1B in the original BM mapping population were added to the UJ map. Another two SSRs newly mapped by this study were also added to the linkage group. A severe distortion in marker distances in the UJ population was observed in mapping the 1B linkage group compared to the BM population. Marker interval Xgwm259 to Xpsp3100 in the UJ population spanned a region of 67 cM, while the interval in the BM population only measured 11.2 cM. This distortion may have resulted from the presence of the 1BL.1RS translocation in USG 3209.

A large gap (80 cM) between markers Xgwm304 and Xbar353B on chromosome 2A resulted partially from six SSRs that were not polymorphic or not linked to the linkage group of chromosome 2A in the UJ population. A large gap (33 cM) also exists between markers Xgwm304 and Xgwm312 where the QTL on chromosome 2A is located in the BM population. Somers et al. (2004) also reported large gaps in the SSR consensus map in this region of chromosome 2A.

Three new markers not mapped in BM population were added to the UJ map. Marker XSTS1231 mapped to chromosome 2B, is a sequence tagged site (STS) marker designed

from the RFLP probe Xbcd1231 (Ma et al., 2004) which was mapped in the BM population (Liu et al., 2001). Simple sequence repeat markers Xbar353b and Xbar174 were not linked to linkage group of chromosomes 2A and 1B, respectively, in the BM population. Mapped locations of the two SSRs in the UJ population are in agreement with published maps (<http://wheat.pw.usda.gov>).

Linear Regression of Powdery Mildew Severity Ratings

Field assessments of powdery mildew severity in both MB and UJ populations were skewed toward resistance (Fig. 2). Association of newly mapped markers with APR to powdery mildew in the BM population and UJ RILs was assessed via a one-way ANOVA (Table 2). Results indicate that all newly mapped markers on chromosomes 1B, 2A, and 2B mapped in the BM F_{2:3} and UJ RILs had significant effects ($P \leq 0.05$) on mildew severity. In agreement with Liu et al. (2001), newly mapped markers located on chromosome 2A had the largest effect on powdery mildew severity in the original BM mapping population while markers mapped to chromosome 2B in the UJ RILs were highly associated with mildew severity data.

Interval Mapping

Interval mapping was conducted using QTL Cartographer 2.0 to compare QTL effects in BM and UJ populations. A total of 21 and 43 markers on three linkage groups located on chromosomes 1B, 2A, and 2B were analyzed in the UJ and MB populations, respectively.

The QTL mapped on chromosomes 2A and 2B in the UJ populations explained significantly more of the total variation for mildew severity compared to original estimates reported in the BM populations both years of field disease assessments (Table 3). The interval mapping method located one major QTL between markers Xgwm304 and Xbar353b on chromosome 2A which was consistent with the location for APR in Massey (Fig. 3). Association of the QTL on chromosome 2A with field data in the F_{6:7} UJ population had the highest LOD value (20.6) and explained 69% of phenotypic variation. To a lesser extent the QTL was associated with field severity data in the F_{5:6} RIL population with a LOD of 10.7 explaining 59% of phenotypic variation.

Similar to the BM population, interval mapping located two major peaks on chromosome 2B in the UJ population. The major peak identified in the analysis was in the marker interval of Xgwm501 and Xgwm191 in both UJ RIL populations. QTL analysis involving markers and field severity data collected in the F_{5:6} UJ population displayed the largest LOD peak at 25.2 explaining 48% of the phenotypic variation. The phenotypic effect of the QTL on chromosome 2B in the F_{6:7} RILs was reduced to 22% (LOD = 12.6).

Location of the QTL on chromosome 1B in the UJ populations was shifted toward the end of the 1B chromosome compared to the same QTL in the BM populations (Fig. 3). Quantitative trait loci effects were consistent for both RIL populations with LOD peaks of approximately 6.0 explaining 13% of the phenotypic variance in the F_{5:6} and F_{6:7} generations.

Discussion

In this study, 18 SSRs associated with three QTL for APR to powdery mildew in Massey were successfully mapped in an elite breeding population, UJ, where Massey is the resistance source of APR in the parental line USG 3209. Marker order was remarkably conserved between the maps resulting from the two populations. Presence of the three QTL in USG 3209 was confirmed in analysis of marker and powdery mildew severity data assessed in the UJ F_{5:6} and F_{6:7} RIL populations in two field experiments. An additional 13 SSRs also were added to the pre-existing BM genetic map. The newly mapped SSR markers in the BM population replaced the closely linked, RFLP markers on one side of the QTL on chromosomes 1B and 2B, allowing efficient selection for the QTL for APR to powdery mildew by utilizing PCR-based markers on both sides of the QTL.

QTL regions in the original mapping populations should be saturated with PCR-based flanking markers to facilitate MAS, in different genetic backgrounds. Zhou et al. (2003), reported that polymorphism of SSR markers linked to the 3BS QTL for fusarium head blight (*Fusarium graminearum*) resistance in wheat cultivar Ning7840 varied in 132 different wheat genetic backgrounds. Three out of six SSR markers, mapped near 3BS

QTL, were polymorphic in less than 50% of genetic backgrounds tested. In the current study, none of the three QTL regions in the UJ population could be flanked by SSRs mapped in the original mapping population even though SSRs are generally accepted as being highly polymorphic and genome specific (Röder et al., 1995). Additional markers had to be added in the current study to the pre-existing BM genetic map in order to flank all three QTL for APR to powdery mildew in the UJ breeding population. Therefore, applying only existing markers mapped in the original mapping population would have resulted in markers mapping only to one side of the QTL in the breeding population, significantly decreasing the efficiency of MAS (Tanksley, 1983).

An additional requirement for successful MAS is validation of QTL effects, interaction, and stability in different genetic backgrounds and environments. Mingeot et al. (2002) mapped QTL responsible for APR to powdery mildew in RE714 in two susceptible backgrounds and detected a total of nine QTL in both populations; however only two QTL were consistently expressed in both genetic backgrounds. In the current study, the three QTL reported in the original mapping study (Liu et al., 2001) were consistently expressed in UJ population and mapped in the same marker intervals.

The QTL effects in the UJ populations differed from the original mapping population of BM. The QTL on chromosome 2A explained significantly more of the phenotypic variance in the UJ population; however it is likely that an overestimation of the QTL effect occurred due to the large gap between Xgwm304 and Xbar353b where the QTL resides. Therefore, as additional SSRs become available they should be added to this region to increase the efficiency of MAS. The QTL on chromosome 2B in the USG 3209 genetic background explained significantly more of the phenotypic variation for APR while the QTL on chromosome 1B explained less when compared to the values reported in the original mapping population (Liu et al., 2001). Castro et al. (2003) evaluated three QTL for APR to barley (*Hordeum vulgare*) stripe rust (*Puccinia striiformis*) in different genetic backgrounds and reported that one QTL with a small genetic effect in the mapping population explained significantly more of the variation in a different genetic background. Therefore, it is likely that QTL effect will vary in different genetic

backgrounds due to both positive and negative epistasis among genes affecting host resistance and susceptibility.

An interaction that must be considered is between QTL associated with APR to powdery mildew in UJ population with defeated hypersensitive *Pm* resistance genes. Several studies have reported a contribution to APR from defeated race specific genes *Pm4a* (Martin and Ellingboe, 1976; Nass et al., 1981), *Pm4b* (Mingeot et al., 2002), *Pm5* (Keller et al., 1999), and *MIRE* (Chantret et al., 2000; Mingeot et al., 2002) in different populations. USG 3209 possess race specific genes *Pm3a*, *Pm8*, and other unidentified hypersensitive genes, while Jaypee is moderately susceptible to the prevalent mildew populations found in the Mid-Atlantic region. The defeated *Pm3a* gene likely contributed little additional resistance as the SSR marker Xpsp3100 was not associated with mildew resistance and Saluda (*Pm3a* source of USG 3209) is highly susceptible to prevalent mildew populations found in Virginia. Cultivars carrying *Pm8* are not as susceptible as those cultivars carrying only *Pm3a*, although virulence to *Pm8* exists in Virginia. The original mapping population developed by Liu et al. (2001) consisted of a susceptible parent, Becker that contains no known hypersensitive genes, and Massey, an APR possessing cultivar that is susceptible to the prevalent mildew populations found in the Mid-Atlantic region at the seedling stage and contains no known hypersensitive resistance genes. Therefore, such an interaction with the three QTL for APR to powdery mildew in the original mapping population of BM was less likely.

The QTL on chromosome 1B in the UJ populations was shifted toward the end of the chromosome and marker distances were distorted when compared to the BM genetic map. This distortion and shift in the location of the QTL may be due to reduced transmission of the rye (*Secale cereale*) derived 1RS chromosomal segment, carrying the resistance gene *Pm8*, possessed by USG 3209 (Hsam and Zeller, 1997). Rayburn and Mornhinweg (1988) and Ren et al. (1997) reported reduced transmission of the 1RS chromosomal segment in crosses, therefore segregation data for markers near the QTL on chromosome 1B associated with APR to powdery mildew may have been affected.

In summary, three previously mapped QTL for APR to powdery mildew in cultivar Massey were validated in the UJ RIL populations, where Massey is the source of APR in USG 3209. The SSR markers mapped in BM populations were used to develop a genetic linkage map comprised of the same chromosomal regions in the UJ population. However, without mapping of additional markers, the QTL regions responsible for APR in USG 3209 would have lacked flanking markers on both sides of the QTL. Therefore, simply applying the SSRs mapped in the original BM mapping population for MAS in the UJ breeding population would not likely be as effective without flanking markers for the three QTL. While genetic effects varied between original mapping and breeding populations, SSRs mapped near the three QTL responsible for APR to powdery mildew can be transferred and selected in other populations where Massey or a derivative of Massey are the parental source of APR. As additional SSRs become publicly available, they should be added to the existing BM genetic map to increase the density of polymorphic markers available for MAS in other breeding populations where Massey is the ancestral resistant source of APR to powdery mildew.

Literature Cited

Basten, C.J., Z.-B. Zeng, and B.S. Weir. 1994. Zmap-a QTL Cartographer, p. 65-66, *In* C. Smith, et al., eds. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software, Vol. 22. Published by the Organizing Committee, 5th World Congress on Genetics Applied to Livestock Production, Guelph, Ontario, Canada.

Beavis, W.D. 1998. QTL analyses: Power, precision, and accuracy, p. 145-161, *In* A. H. Patterson, ed. Molecular dissection of complex traits. CRC Press, Boca Raton, FL.

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

Bougot, Y., J. Lemoine, M.T. Pavoine, D. Barloy, and G. Doussinault. 2002. Identification of a microsatellite marker associated with *Pm3* resistance alleles to powdery mildew in wheat. *Plant Breed.* 121:325-329.

Bryan, G.J., A.J. Collins, P. Stephenson, A. Orry, J.B. Smith, and M.D. Gale. 1997. Isolation and characterization of microsatellites from hexaploid bread wheat. *Theor. Appl. Genet.* 94:557-563.

Castro, A.J., X. Chen, A. Corey, T. Filichkina, P.M. Hayes, C. Mundt, K. Richardson, S. Sandoval Islas, and H. Vivar. 2003. Pyramiding and validation of quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: Effects on adult plant resistance. *Crop Sci.* 43:2234-2239.

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

Chantret, N., D. Mingeot, P. Sourdille, M. Bernard, J.M. Jacquemin, and G. Doussinault. 2001. A major QTL for powdery mildew resistance is stable over time and at two developmental stages in winter wheat. *Theor. Appl. Genet.* 103:962-971.

Doerge, R.W., and G.A. Churchill. 1996. Permutation tests for multiple loci affecting a quantitative character. *Genetics* 142:285-294.

Fasoula, V.A., D.K. Harris, M.A. Baily, D.V. Phillips, and H.R. Boerma. 2003. Identification, mapping, and confirmation of a soybean gene for bud blight resistance. *Crop Sci.* 43:1754-1759.

- 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.
- Griffey, C.A., M.K. Das, and E.L. Stromberg. 1993. Effectiveness of adult plant resistance in reducing loss to powdery mildew in winter wheat. *Plant Dis.* 77:618-622.
- Gupta, P.K., S. Rustgi, S. Sharma, R. Singh, N. Kumar, and H.S. Balyan. 2003. Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. *Mol. Genet. Gen.* 270:315-323.
- Gustafon, G.D., and G. Shaner. 1982. Influence of plant age on the expression of slow-mildewing resistance in wheat. *Phytopathology* 72:746-749.
- Guyomar ch, H., P. Sourdille, K.J. Edwards, and M. Bernard. 2002. Studies of the transferability of microsatellites derived from *Triticum tauschii* to hexaploid wheat and to diploid related species using amplification, hybridization and sequence comparisons. *Theor. Appl. Genet.* 105:736-744.
- 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.
- Hsam, S.L.K., and F.J. Zeller. 1997. Evidence of allelism between genes *Pm8* and *Pm17* and chromosomal location of powdery mildew and leaf rust resistance genes in the common wheat cultivar 'Amigo'. *Plant Breed.* 116:119-122.
- Hsam, S.L.K., I.F. Lapochkina, and F.J. Zeller. 2003. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). Gene *Pm32* in a wheat-*Aegilops speltoides* translocation line. *Euphytica* 133:367-370.
- Huang, X.-Q., and M.S. R der. 2004. Molecular mapping of powdery mildew resistance genes in wheat: a review. *Euphytica* 137:203-223.
- James, C. 1971. A manual of assessment keys for plant diseases. Can. Dep. Agric. Publ. No. 1458. American Phytopathological Society, St. Paul, MN.
- Johnson, J.W., P.S. B enziger, 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 a segregating wheat x spelt population. *Theor. Appl. Genet.* 98:903-912.

Kingsland, G.C. 1982. Triadimefon for control of powdery mildew of wheat. *Plant Dis.* 66:139-141.

Lander, E.S., and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.

Langridge, P., and K. Chalmers. 1998. Techniques for marker development, p. 107-117, *In* A. E. Slinkard, ed. *Proceeding of 9th Int'l Wheat Genetics Symposium*. University Extension Press, Saskatoon, Canada.

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* 79:152-155.

Li, Z., L. Jakkula, R.S. Hussey, J.P. Tamulonis, and H.R. Boerma. 2001. SSR mapping and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode. *Theor. Appl. Genet.* 103:1167-1173.

Liu, S., C.A. Griffey, and M.A. Saghai Maroof. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. *Crop Sci.* 41:1268-1275.

Ma, Z.Q., J.B. Wei, and S.H. Cheng. 2004. PCR-based markers for the powdery mildew resistance gene *Pm4a* in wheat. *Theor. Appl. Genet.* 109:140-145.

Martin, T., and A. Ellingboe. 1976. Differences between compatible parasite/host genotypes involving the *Pm4* locus of wheat and the corresponding genes in *Erysiphe graminis* f. sp. *tritici*. *Genetics* 66.

McIntosh, R.A., G.E. Hart, K.M. Devos, and M.D. Gale. 1998. Catalogue of gene symbols for wheat, *In* A. E. Slinkard, ed. *Proceeding 9th International Wheat Genetics Symposium*, Vol. 5. University Extension Press, University of Saskatchewan, Saskatoon Canada.

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 genetic backgrounds. *Plant Breed.* 121:133-140.

- Nass, H., D. Pedersen, D. MacKenzie, and R. Nelson. 1981. The residual effects of some 'defeated' powdery mildew resistance genes in isolines of chancellor winter wheat. *Phytopathology* 71:1315-1318.
- Rayburn, A.L., and D.W. Mornhinweg. 1988. Inheritance of a 1BL/1RS wheat-rye translocated chromosome in wheat. *Crop Sci.* 28:709-711.
- 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.
- Roberts, J.J., and R.M. Calwell. 1970. General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in "Knox" wheat (abstract). *Phytopathology* 60:1310.
- Röder, M.S., J. Plaschke, S.U. König, A. Börner, M.E. Sorrells, S.D. Tanksley, and M.W. Galal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen. Genet.* 246:327-333.
- Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W. Galal. 1998. A microsatellite map of wheat. *Genetics* 149:2007-2023.
- Saghai Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. (USA)* 81:8014-8018.
- Saghai Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang, and R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. *Proc. Natl. Acad. Sci. (USA)* 91:5466-5470.
- Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. *Phytopathology* 63:867-872.
- Singrun, C., S.L.K. Hsam, L. Hartl, F.J. Zeller, and V. Mohler. 2003. Powdery mildew resistance gene *Pm22* in cultivar Virest is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L. em Thell.). *Theor. Appl. Genet.* 106:1420-1424.
- Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:1105-1114.
- Song, Q.J., E.W. Fickus, and P.B. Cregan. 2002. Characterization of trinucleotide SSR motifs in wheat. *Theor. Appl. Genet.* 104:286-293.

Sourdille, P., T. Cadalen, H. Guyomar ch, J.W. Snape, M.R. Perretant, G. Charmet, C. Boeuf, S. Bernard, and M. Bernard. 2003. An update of the Courtot x Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. *Theor. Appl. Genet.* 106:530-538.

SPSS Inc. 1998. SPSS Base 12.0 for Windows User's Guide. SPSS Inc., Chicago, IL.

Starling, T.M., C.W. Roane, and H.M. Camper, Jr. 1984. Registration of Massey wheat. *Crop Sci.* 24:1000.

Stephenson, P., G. Bryan, J. Kirby, A. Collins, K. Devos, C. Busso, and M. Gale. 1998. Fifty new microsatellite loci for the wheat genetic map. *Theor. Appl. Genet.* 97:946-949.

Tanksley, S.D. 1983. Molecular markers in plant breeding. *Plant Mol. Bio. Rep.* 1:3-8.

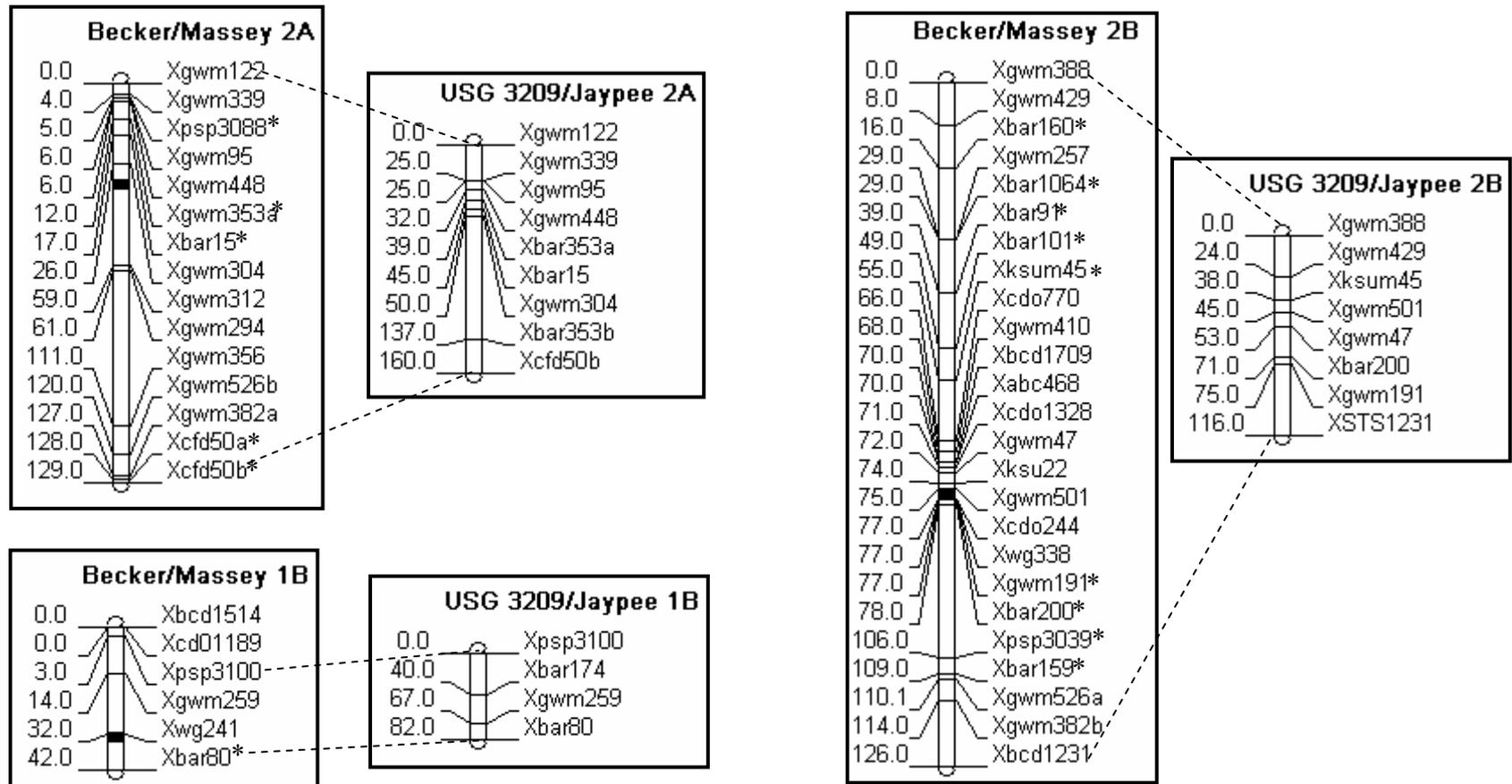
Yu, J.K., M. La Rota, R.V. Kantety, and M.E. Sorrells. 2004. EST derived SSR markers for comparative mapping in wheat and rice. *Mol. Genet. Genom.* 271:742-751.

Zhou, W.C., F.L. Kolb, G.H. Bai, L.L. Domier, L.K. Boze, and N.J. Smith. 2003. Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. *Plant Breed.* 122:40-46.

Table 1. Summary of publicly available wheat SSRs and chromosomal regions targeted to map additional markers near QTL associated with APR to powdery mildew in the F_{2:3} BM population.

SSR Code	Num. of Primer Pairs Screened	Chromosomes Mapped			Source
		1B	2A	2B	
Xwmc	3	1	1	1	Gupta et al. (2003), http://wheat.pw.usda.gov
Xgwm	23	3	14	6	Röder et al. (1998)
Xbar	28	11	9	8	Song et al.(2002), http://wheat.pw.usda.gov
Xcfd	2	0	1	1	Sourdille et al. (2003), http://wheat.pw.usda.gov
Xcfa	3	2	1	0	Guyomaréh et al. (2002), http://wheat.pw.usda.gov
Xksum	4	0	1	3	Yu et al. (2004), http://wheat.pw.usda.gov
Xpsp	5	1	3	1	Bryan et al. (1997), Stephenson et al. (1998)

Fig. 1 Alignment of the original mapping population, BM F_{2,3}, with the applied F_{6,7} UJ population. Black blocked areas on the linkage groups represent previously mapped QTL locations on chromosome 2A, 2B, and 1B. Markers highlighted with "*" indicate that they were added to the pre-existing BM map in this study.



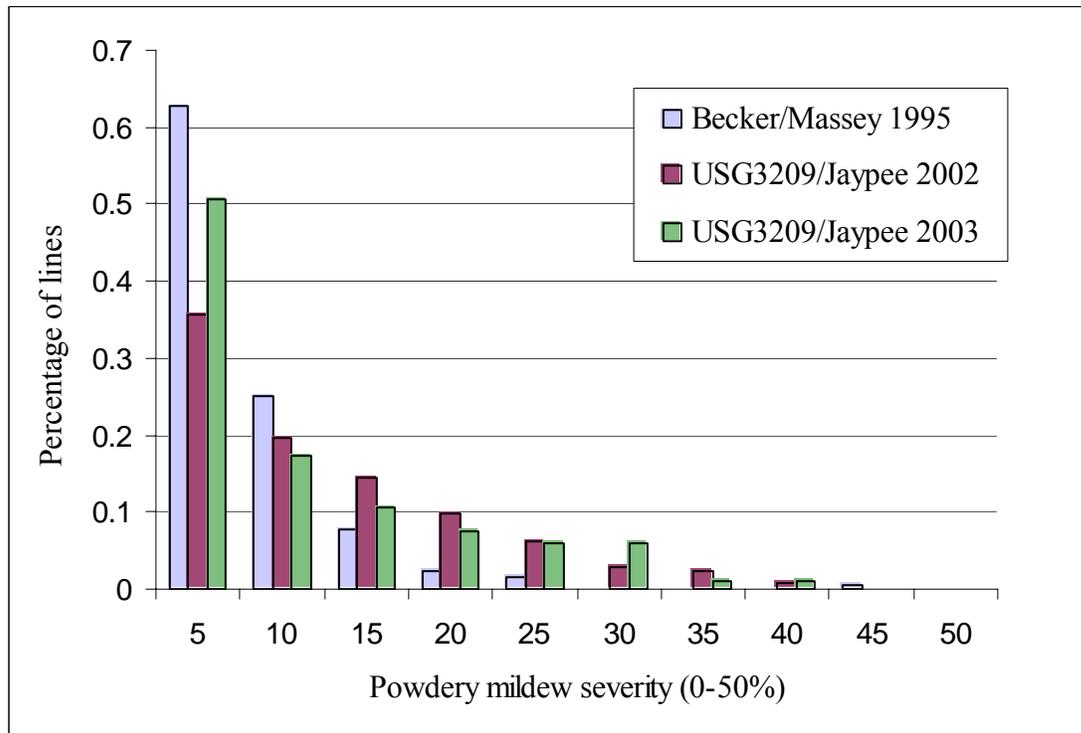


Fig. 2. Distribution of BM F_{2:3} (1995) lines (Liu et al., 2001), and UJ F_{5:6} (2002) and F_{6:7} (2003) RILs for mean powdery mildew severity assessed under natural epidemics in the field at Warsaw, VA.

Table 2. One-way ANOVA of new SSR markers mapped in the F_{2:3} BM population applied to the UJ RIL breeding population that are associated with APR to powdery mildew.

		Becker by Massey F _{2:3}			USG 3209 by Jaypee RILs ^d			
Marker	Chrom.	df of error ^a	P	R ^{2b}	Gen ^c	df of error	P	R ²
Xbar15	2A	171	≤0.001	8.8	F _{5:6}	248	≤0.001	8.3
					F _{6:7}	272	≤0.001	7.6
Xbar353a	2A	175	≤0.001	10.2	F _{5:6}	268	≤0.001	7.3
					F _{6:7}	290	≤0.001	4.7
Xbar200	2B	175	≤0.001	7.3	F _{5:6}	248	≤0.001	17.1
					F _{6:7}	271	≤0.001	7.2
Xbar80	1B	117	=0.092	2.4	F _{5:6}	258	≤0.001	9.8
					F _{6:7}	274	≤0.001	8.2

^a = Degrees freedom of error

^b = Regression of correlation

^c = Generation of UJ population

^d = Recombinant inbred lines

Table 3. Comparison of QTL effects on APR to powdery mildew using interval mapping analysis of original datasets of the F_{2:3} BM population (Liu et al., 2001) and the UJ population.

Loc ^a	Becker by Massey Populations			USG 3209 by Jaypee Populations		
	Population	LOD	R ^{2b}	Population	LOD	R ²
2A	F _{2:3}	9.2	0.29	F _{5:6}	10.7	0.59
2A	RILs ^c	4.7	0.26	F _{6:7}	20.6	0.69
2B	F _{2:3}	4.3	0.11	F _{5:6}	25.2	0.48
2B	RILs	2.9	0.15	F _{6:7}	12.6	0.22
1B	F _{2:3}	5.3	0.17	F _{5:6}	6.1	0.13
1B	RILs	2.5	0.15	F _{6:7}	6.2	0.13

^a = Location of quantitative trait loci

^b = Phenotypic variation explained

^c = Recombinant inbred lines

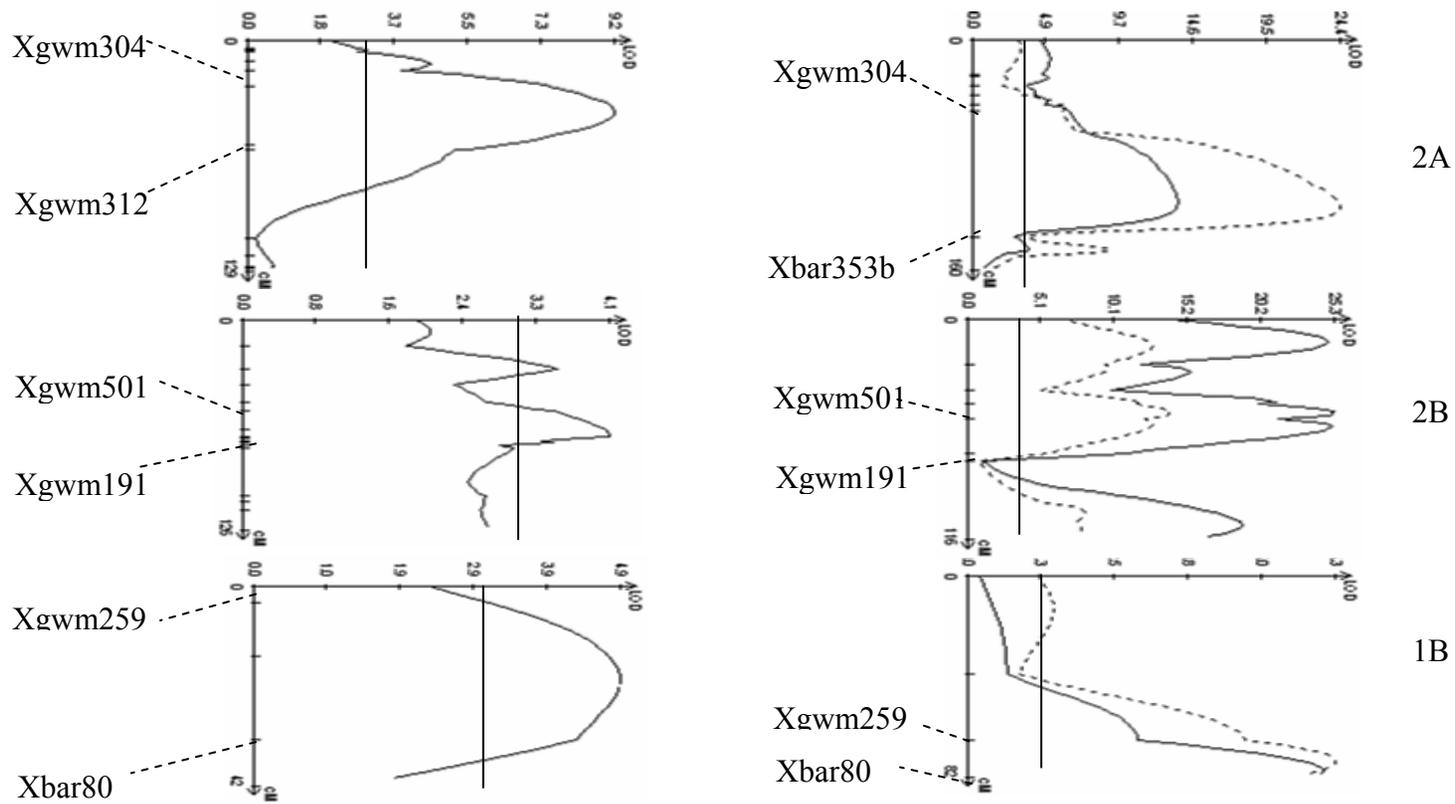


Fig 3. Comparison of interval mapping analysis with flanking SSR markers for QTL associated with APR to powdery mildew located on chromosomes 2A, 2B, and 1B, respectively. Three QTL from the original BM $F_{2:3}$ mapping population are presented in the bottom hand column and those for UJ population are presented in the top column. The solid line in graphs of the UJ population represents interval mapping of mildew data taken in the $F_{5:6}$ while the dashed line is for mildew data taken in the $F_{6:7}$ generation. The vertical lines at LOD 3.0 represents the minimum LOD required for significance.

CHAPTER III

Potential Effectiveness of Marker-Assisted Selection for Three Quantitative Trait Loci Conferring Adult Plant Resistance to Powdery Mildew in an Elite Wheat Breeding Population

Abstract

Adult plant resistance (APR) in wheat (*Triticum aestivum* L.) to powdery mildew caused by *Blumeria graminis* f. sp. *tritici*, has provided effective and durable resistance compared to that conferred by conventional short-lived race specific genes. Three quantitative trait loci (QTL) associated with APR to powdery mildew were mapped in a previous study in a cross between wheat cultivars Massey (APR) and Becker (susceptible). Confirmation of QTL effects in the original Becker by Massey (BM) mapping population must be demonstrated in other genetic backgrounds or breeding populations to ascertain their effectiveness in marker-assisted selection (MAS) programs. A 293 recombinant inbred line (RIL) breeding population derived from the cross of USG 3209 by Jaypee (UJ) was used to verify inheritance of Massey's QTL for APR to powdery mildew in USG 3209, and to validate the QTL effects and potential use in MAS. Powdery mildew severity of the 293 UJ RILs was evaluated in 2002 (F_{5:6}) and 2003 (F_{6:7}) under natural disease pressure in the field. Adult plants of the 293 RILs were also evaluated for disease severity in a 2004 (F_{7:8}) greenhouse experiment using a composite of five different isolates of *B. graminis*. The QTL on chromosome 2B, selected on the basis of flanking markers, had the largest impact on powdery mildew resistance in the field. Lines containing the allele from USG 3209 had mean mildew severities of 5.8% while RILs containing the Jaypee allele had mean mildew severities of 14.4% to 17.7% in the 2002 and 2003 field experiments. Selection of RILs possessing the QTL on chromosome 2A and to a lesser extent the one on chromosome 1B was effective in identifying powdery mildew resistance in both greenhouse and field experiments, whereas the effect of the QTL on chromosome 2B was insignificant in the greenhouse. Overall, selecting RILs with QTL on chromosomes 2A and 2B was most successful in identifying highly resistant RILs compared to selecting RILs having other combinations of two or three QTL combinations. The RILs possessing both QTL on chromosomes 2A and 2B had mean mildew severities of 4.4% and 3.2% in 2002 and 2003 field experiments, respectively. Breeders implementing MAS programs for APR to powdery mildew via selection of RILs containing the two QTL on chromosomes 2A and 2B likely will obtain RILs having high levels of resistance in the field. However, combining all

three QTL may ensure greater durability of APR, on the basis that resistance conferred by QTL on chromosome 2A and 1B are genetically stable across all environments in this study.

Introduction

Powdery mildew caused by the pathogen *Blumeria graminis* f. sp. *tritici*, is a major disease of winter wheat (*Triticum aestivum* L.) in areas with maritime or semi-continental climates (Bennett, 1984). Race specific major genes, designated as *Pm* genes, were first deployed in wheat cultivars to control powdery mildew. However, wide spread cultivation of such resistant cultivars consistently has led to evolution of mildew populations with matching virulence alleles. Breeders therefore have shifted their efforts to breeding for more durable resistance. Incomplete, non-race specific resistance retarding growth and reproduction of powdery mildew in adult plants has been termed 'slow mildewing' (Shaner, 1973), 'adult-plant resistance' (APR) (Gustafon and Shaner, 1982), or 'partial resistance' (Hautea et al., 1987). Partial resistance extends the latent period and reduces sporulation of the fungus (Royer et al., 1984), thereby reducing disease development and losses in grain yield (Griffey et al., 1993). Genetic studies conducted on APR in winter wheat cultivars Knox 62, Massey, Redcoat, and Houser indicate that APR is controlled quantitatively by two to three genes with moderate to high heritability (Das and Griffey, 1994; Griffey and Das, 1994). Resistance to powdery mildew in APR cultivars such as Knox (Roberts and Calwell, 1970), Redcoat (Rouse et al., 1980), Knox 62 (Patterson et al., 1978), and Massey (Starling et al., 1984) has remained effective for over 20 yr.

It is difficult to assess differing types and levels of APR in field experiments (Gustafon and Shaner, 1982), which require numerous replications in several diverse environments. Classical breeding methods also greatly limit efficient incorporation, selection, and pyramiding of diverse genes governing APR to powdery mildew. Application of molecular markers and marker-assisted selection (MAS) provides a potential tool for breeding durable resistance in wheat. Quantitative trait loci (QTL) responsible for APR have been mapped in segregating populations using restriction fragment length polymorphism (RFLP) and PCR-based simple sequence repeat (SSR) markers. Recently, SSRs or microsatellites have been employed frequently due to their highly polymorphic nature, chromosome specificity, and distribution throughout the genome (Röder et al., 1995).

Keller et al. (1999) mapped 18 QTL associated with APR to powdery mildew in a recombinant inbred line (RIL) population derived from a cross between APR wheat cultivar Forno and spelt cultivar Oberkulmer. Liu et al. (2001) identified three QTL in cultivar Massey governing powdery mildew resistance using a F_{2:3} Becker by Massey (BM) population. The three QTL were further confirmed in a RIL population over a three year period. The QTL located on chromosomes 1B, 2A, and 2B explained 17%, 29%, and 11% of the total variation, respectively. The major QTL located on chromosome 2A was located near the *Pm4* locus. The QTL controlling APR to powdery mildew in wheat line RE714 have been mapped extensively using doubled haploid populations, F_{2:3} populations (Chantret et al., 2001), and F₃ bulk populations (Chantret et al., 2000), including two susceptible backgrounds (Mingeot et al., 2002) and different environments including greenhouse and field conditions. Two major QTL, one on chromosome 5D and the *MIRE* locus on chromosome 6A, were consistent in all studies. The defeated major gene, *Pm4b*, also contributed a residual effect in RE714 in some genetic backgrounds.

Although, several studies have mapped QTL responsible for disease resistance, including APR to powdery mildew in wheat, relatively few studies have attempted to apply the mapped QTL to MAS. The 3BS QTL for fusarium head blight (*Fusarium graminearum*) resistance in wheat (Liu and Anderson, 2003; Zhou et al., 2003) and three QTL for stripe rust (*Puccinia striiformis*) resistance at the seedling (Castro et al., 2003b) and adult plant stage (Castro et al., 2003a) in barley (*Hordeum vulgare*) have successfully been utilized in MAS selection studies. However, initial promises of MAS for quantitative traits have been tempered by overestimation of QTL effects in small original mapping populations (Beavis, 1998) and lack of polymorphic markers in different genetic backgrounds. Therefore, polymorphic SSRs which are linked to the QTL of interest must be examined for their effects and be validated in different genetic backgrounds (Langridge and Chalmers, 1998).

Development of elite cultivars using SSR markers for selection of QTL governing APR in breeding populations derived directly from Massey is hindered because Massey is a

not a desirable parent due to low yield potential, tall plant height, weak straw strength, susceptibility to leaf rust, and poor combining ability. In addition lack of knowledge as to whether markers mapped near the QTL in Massey are polymorphic and selectable in elite breeding populations containing parents that are derivatives of Massey restricts implementation of effective MAS programs. The objective of the current study was to validate whether SSR markers mapped near QTL in Massey for APR to powdery mildew can be applied to MAS in a RIL breeding population comprised of a resistant parent (USG 3209), derived from Massey, and a moderately susceptible parent, Jaypee.

Material and Methods

Plant Materials

USG 3209 (PI617055) winter wheat was derived from the cross Saluda/4/Massey*2/3/Massey*3 /Balkan//Saluda. Massey is known to possess APR (Griffey and Das, 1994) and seedling tests conducted at North Carolina State University confirmed that USG 3209 possesses *Pm3a* (from Saluda), *Pm8* (from Balkan), and other unidentified genes for seedling mildew resistance. While seedlings of Massey are susceptible to most races of powdery mildew, seedlings of USG 3209 are resistant to some races and susceptible to others. Jaypee (PI59760), a winter wheat cultivar developed at the University of Arkansas (Bacon et al., 1998), is moderately susceptible to prevalent populations of powdery mildew found in Virginia.

A cross between USG 3209 and Jaypee, hereafter referred to as UJ, was made in a greenhouse during the spring of 1997. The F₁ and subsequent generations were planted in the field yearly and advanced to the F₄ generation using the same modified bulk breeding method implemented in the cultivar development program. The F₂ and F₃ generations were grown in 20.8 m² blocks at Blacksburg and Warsaw, VA from which spikes, selected at both locations solely on the basis of desirable plant type, were harvested, threshed in bulk and used as a source of seed in subsequent generations. In the F₄ generation, 293 spikes were selected arbitrarily, harvested, and threshed separately to develop RILs. These 293 RILs were planted in 1.4 m rows in the field at Warsaw, VA. Parents USG 3209 and Jaypee, and check cultivars Becker and Massey, were planted

approximately at 25 row intervals throughout the nursery. Spreader strips, 1.2 m wide by 24 m long, of the mildew susceptible cultivar Becker were planted adjacent to test plots to ensure presence of ample mildew inoculum. At maturity, a random head selection from each row was harvested, threshed, and planted during each subsequent year.

Fungal Material: Field

APR in the field was assessed under natural disease epidemics at Warsaw, Virginia in 2002 and 2003. Virulence for race specific genes *Pm1* through *Pm8* has been identified in the *Blumeria graminis* populations of the mid-Atlantic region (Niewoehner and Leath, 1998).

Fungal Material: Greenhouse

Five *B. graminis* f. sp. *tritici* isolates (E3-14, 101a2, #8, #9, and 127) maintained via culture in Petri dishes on approximately 15 to 20, 1.5 cm leaf sections of wheat per isolate were obtained from North Carolina State University and used in the APR greenhouse screening experiment conducted in fall, 2004. Leaf sections obtained had visible conidia growing on the green leaf sections. Collectively, the isolates had known virulence for resistance genes *Pm1*, *Pm3a*, *Pm4a*, *Pm4b*, *Pm6*, and *Pm8*. Seedling tests conducted at North Carolina State University also confirmed that one or both parents were susceptible to one or more of the isolates used in the greenhouse experiment (Table 1).

The five isolates were cultured and increased separately in the greenhouse (12 hrs light, $890 \text{ } \mu\text{mol s}^{-1} \text{ m}^{-2}$) from single leaf discs by rubbing each sporulating leaf section over the upper leaf blade of densely planted (approximately 50-80 seeds per pot) 10 day old Becker seedlings planted in small clay pots (8.9 cm diameter, 8.3 cm in depth). Pots were fertilized as needed with 20-20-20 (N-P₂O₅-K₂O) water-soluble fertilizer. Five weeks after initial inoculations, isolates were bulked together by shaking conidia from plant in two clay pots per isolate containing plants over 4 flats (24 pots per flat) of differential cultivars (Table 2) with known resistance genes and parental lines. The mildew composite was subsequently increased on eight flats of known susceptible

cultivars following the same procedure. Seedling reaction of a set of host differential lines known to contain genes *Pm1-Pm8* and *Pm17* and parental lines was rated 12 d after inoculation (Table 2) before inoculating the UJ F_{7:8} RILs.

Disease Assessments: Adult Plant Resistance Field Screening

Powdery mildew severity was assessed on parents, checks, and 293 RILs in 2002 (F_{5:6}) and 2003 (F_{6:7}) at Warsaw, VA under natural mildew epidemics. Disease severity was assessed twice each year utilizing the James assessment key (James, 1971) on the basis of 0-50% leaf area coverage where 50% represents maximum leaf coverage. The first disease rating was assessed as the average coverage of F-2 leaves (2nd leaf below the flag leaf) of five to ten plants per row. The second rating was assessed as average coverage of F-1 leaves (1st leaf below the flag) of five to ten plants per row. Disease severity of the F-1 and F-2 leaves was then averaged to derive mean mildew severity of each line.

Disease Assessments: Adult Plant Resistance Greenhouse Screening

Individual seeds, of parents, check cultivars Becker and Massey, and each of the UJ F_{7:8} RILs were planted in small square plastic pots (7.0 cm wide, 5.8 cm in depth) and germinated in the greenhouse at $22 \pm 5^\circ\text{C}$ (12 hrs light, $890 \text{ umol s}^{-1} \text{ m}^{-2}$) in plastic flats (32 pots per flat). Three replications comprised of single plants of the 293 UJ RILs, parents, and checks were planted. Seed of each line was derived from a single spikelet, harvested from the parents, checks, and F_{6:7} RILs in the field from the previous year. Ten days after planting, the seedlings were transferred into a powdery mildew free CMP 3000 vernalization chamber (Conviron, Winnipeg, Manitoba) for 87 d at $5 \pm 0.1^\circ\text{C}$ (12 hrs light, $92 \text{ umol s}^{-1} \text{ m}^{-2}$). Thereafter, the vernalized seedlings were removed and placed in the greenhouse for 5 d to acclimate and subsequently transplanted into plastic pots (15.3 cm diameter, 15.3 depth) with a soil and peat mixture (3:1 peat/soil). Vernalized seedlings were grown in the greenhouse for 18 days at $13 \pm 5^\circ\text{C}$ (12 hrs of daylight, $750 \text{ umol s}^{-1} \text{ m}^{-2}$) without artificial light exposure to promote tillering. Plants were arranged on the greenhouse bench in a completely randomized block design, staked upright using 260 cm canes and wire ties 10-15 days after transplanting. Replications 1 and 2 were contained in a single greenhouse room and replication 3 was placed in an adjacent room.

Plants were inoculated when approximately 50% of the plants in the experiment were booting at Zadoks' growth stage 45 (Zadoks et al., 1974). Entire replications were inoculated under 23 ± 5 °C day temperatures (18 hrs of daylight, $1060 \text{ umol s}^{-1} \text{ m}^{-2}$) and 18 ± 5 °C night temperatures by shaking conidia from inoculated susceptible lines over plants on greenhouse benches. Plants were inoculated three times at two day intervals. Resistant checks USG 3209 and Massey, and susceptible checks Becker and Jaypee were interspersed throughout each replication approximately every 25 pots. Average powdery mildew severity on several F-2 (2nd leaf below the flag) leaves of each plant was assessed 7 d after inoculation and subsequently at 7 d intervals using the James Disease Assessment Key (James, 1971). Three readings were conducted on each replication and data averaged to derive the mean mildew severity of each line. Area under the disease progress curve (AUDPC) was calculated for each plant from the weekly mildew severity readings following the formula: $\sum_i [(x_i + x_{i+1})/2] t_i$, where x_i is the phenotypic value on date i , and t_i the number of days between dates i and $i+1$ (Bjarko and Line, 1988). Three individual AUDPC values from each replication were then averaged to derive an AUDPC for each RIL.

Simple Sequence Repeat Analysis

Plant tissue was collected in the field from parents and each F_{6,7} RIL. A 5 cm leaf section was cut from approximately 10 to 15 plants in each individual head row and bulked for DNA extraction. DNA was extracted as described by Saghai Maroof et al. (1984). DNA integrity was determined by running undigested DNA in 0.8% agarose gel followed by staining in ethidium bromide (EtBr) and visualizing under ultraviolet (UV) light. DNA concentration was measured with a DyNA Quanta2000 Fluorometer (Hoefer Scientific, San Francisco, CA). PCR reactions were performed in a total volume of 10 ul in PE 9700 thermal cycler (Perkin Elmer, Norwalk, CN). After the initial denaturing step, for 4 min at 94°C, followed by 32 or 40 cycles (depending on the primer pair) of 0.5 min at 95°C, 1 min at either 50°C, 55°C, or 60°C (depending on the primer pair), 1 min at 73°C, followed by a final extension step for 5 min at 73°C. Protocols for reaction mixtures and

polyacrylamide gel electrophoresis were conducted as described by Saghai Maroof et al. (1994) and Liu et al. (2001).

Molecular Marker and QTL Analysis

Presence of three QTL for APR to powdery mildew was confirmed in the UJ population. Markers from the developed UJ F_{6:7} genetic linkage maps of chromosomes 1B, 2A, and 2B were used in this study to test their ability to select for APR via MAS. Nine SSR markers on chromosome 2A, 7 SSR markers and 1 sequence tagged site (STS) marker on 2B, and 4 SSR markers on 1B were used to genotype the 293 F_{6:7} UJ RILs (Fig. 1). Chromosomal maps for the APR QTL region on 1B, 2A, and 2B were previously created by QTL Cartographer version 2.0 (Basten et al., 1994). Statistical analysis was performed using SPSS statistical software version 12.0 (SPSS, 1998). A one-way ANOVA was used to determine the significance ($P \leq 0.05$) between markers and APR to powdery mildew. A pairwise *t* test at $P = 0.05$ was used to compare mean mildew severity values of different genotype groups.

Results

Analysis of Phenotypic Data: Field Experiments

Powdery mildew severity of the parents ranged from 0% to 5% for USG 3209 and 5% to 15% for Jaypee over two years in field experiments. The average powdery mildew severity of the RILs was 10.8% and 8.9% in 2002 and 2003, respectively. Massey averaged 10 to 15% mildew severity and susceptible check Becker averaged 38% to 45% mildew severity. Disease pressure in 2003 was significantly lower than in 2002 due to poor seedling emergence and winter injury, which resulted in thin stands. Nevertheless, field severity data over the two year experiments had a coefficient of correlation of $r = 0.68$ ($P \leq 0.001$). Distribution of disease data over two years displayed continuous variation and was significantly skewed toward lower disease severity (Fig. 2).

Analysis of Phenotypic Data: Greenhouse Experiment

Greenhouse disease severity was significantly higher than that observed in the field as in 2002 when powdery mildew reached normal epidemic proportions (Fig. 2). Average disease severity of the RILs was 12.4% with mildew severity of the parents ranging 0% to 5% for USG 3209 and 15% to 30% for Jaypee. Mildew severity of Massey ranged from 15% to 22% and that of susceptible check Becker averaged 40% to 50%. Mildew severity for replications 1 and 2 was highly correlated with correlation coefficients of $r = 0.81$ ($P \leq 0.001$). Replications 1 versus 3 and 2 versus 3 had coefficients of correlations of $r = 0.72$ ($P \leq 0.001$) and $r = 0.73$ ($P \leq 0.001$), respectively. AUDPC values followed similar trends as disease severity (Fig. 3.) with Jaypee having a 19-fold higher AUDPC value than USG 3209.

Single Marker Regression

Based on the $F_{6,7}$ RIL map for the UJ population (Fig. 1), 9 SSR markers on chromosome 2A, 7 SSR and 1 STS (XSTS1231) markers on chromosome 2B, and 4 SSR markers on chromosome 1B were analyzed for correlation with powdery mildew resistance in field and greenhouse experiments. One-way ANOVA for each marker was conducted to determine its effect on APR to powdery mildew. Results indicated that all markers mapped near the QTL responsible for APR in Massey had significant ($P \leq 0.05$) effects on disease severity in RILs evaluated in 2002 and 2003 field experiments (Table 3). Markers located on chromosome 2B had the largest effect followed by markers on chromosome 2A in the two year field study. Marker Xgwm501, located on chromosome 2B, explained the most variation for APR in 2002 (29.6%) and 2003 (16.6%) field experiments. Markers on all chromosomes, except Xgwm259 and Xbar353b, explained less of the total variation for powdery mildew in 2003 than in 2002.

All markers evaluated in the 2004 greenhouse experiment, except Xbar353b, displayed a significant decrease in the total variation in powdery mildew resistance explained (Table 3). Markers on 2B explained 11-fold less of the total variation for mildew severity in the greenhouse than in 2002 field experiment. Markers on chromosome 2A displayed the largest effect on APR to powdery mildew in the greenhouse.

Marker-Assisted Selection for the 2A, 2B, and 1B QTL in the USG 3209 by Jaypee RILs

Mean powdery mildew severity values of the UJ RILs were derived from the average of two disease severity assessments from two separate years in the field and one greenhouse experiment. To determine the effect of QTL located by interval mapping on chromosomes 2A, 2B, and 1B on powdery mildew severity, RILs were genotyped with flanking markers. Two genotypes, one homozygous for the USG 3209 alleles (MM) and one homozygous for the Jaypee alleles (mm), were identified for each marker. ‘M’ refers to the marker allele associated with the resistant parent USG 3209 and ‘m’ refers to the allele associated with the susceptible parent Jaypee. RILs which produced a heterozygous banding pattern in the UJ population were excluded from the analysis. Average mildew severity of the RILs selected on the basis of genotypes for individual and flanking markers near 2A, 2B, and 1B QTL is presented in Table 4. Significant differences in powdery mildew severity was observed among RILs selected for individual markers for all three QTL when field data were used in comparisons. The RILs possessing the USG 3209 marker allele consistently displayed lower mildew severity than lines containing the Jaypee marker allele. Markers near the QTL on chromosome 2B, Xgwm501 and Xgwm191, had the largest impact on powdery mildew severity when selecting for powdery mildew severity with RILs possessing the USG 3209 allele having a mean severity of 6.3% to 6.7%, while RILs possessing the Jaypee allele had a mean severity of between 11.1% to 17.0%.

In agreement with simple linear regression, markers on chromosome 2B (Xgwm501 and Xgwm191) had only minor effects on the mean mildew severity in the greenhouse experiment. In contrast, selection of USG 3209 marker alleles for Xgwm304 and Xbar353b on chromosome 2A and to a lesser extent markers on chromosome 1B (Xgwm259 and Xbar80) had a significant effect on mildew severity in the greenhouse experiment.

The effect of selecting QTL for APR to powdery mildew using flanking markers was also examined. Microsatellite markers Xgwm304 and Xbar353b; Xgwm501 and Xgwm191;

and Xgwm259 and Xbar80 flanking the QTL on 2A, 2B, and 1B, respectively, were genotyped in the RILs (Table 4). Selecting markers flanking the QTL on chromosome 2B had the largest impact on mean mildew severity, with markers possessing USG 3209 alleles ($M_3M_3M_4M_4$) having a mean severity of 5.8% while lines carrying the Jaypee alleles ($m_3m_3m_4m_4$) had mean severities of approximately 14% to 17% in field experiments. Markers flanking the QTL on chromosome 1B were least efficient in selecting for powdery mildew resistance in field experiments. The RILs possessing USG 3209 alleles at the 1B QTL had mean severities of 7.1% and 6.0% while those lacking the QTL ($m_5m_5m_6m_6$) had mean mildew severities of 12.7% and 11.8% in 2002 and 2003, respectively.

Markers Xgwm304 and Xbar353b were the only group of flanking markers highly effective in selection for powdery mildew resistance in the greenhouse experiment. RILs possessing the QTL on chromosome 2A ($M_1M_1M_2M_2$) displayed a slow development of the disease while RILs not having the QTL on chromosome 2A ($m_1m_1m_2m_2$) showed a linear increase in disease severity in the weekly disease assessments in the greenhouse experiment (Fig. 4). Overall, RILs containing USG 3209 alleles ($M_1M_1M_2M_2$) had a mean mildew severity of 10.0% while lines having Jaypee alleles ($m_1m_1m_2m_2$) had a mean severity of 19.7% in the greenhouse experiment. RILs selected on the basis of the QTL on chromosome 1B also had a slower development of disease in the greenhouse, although less prominent than the effect of the QTL on chromosome 2A. Weekly mean disease severity assessments were 7.3%, 11.2%, and 12.3% for RILs containing the QTL on chromosome 1B ($M_5M_5M_6M_6$) while RILs lacking the QTL on chromosome 1B ($m_5m_5m_6m_6$) were 10.6%, 15.6%, and 18.9% for the 7, 14, and 21 d disease assessment increments, respectively.

Combining Multiple QTL for APR to Powdery Mildew via MAS

RILs having combinations of two or more APR QTL based on flanking markers were evaluated for mildew severity. If the sets of flanking markers had USG 3209 alleles (e.g. $M_1M_1M_2M_2$ and $M_3M_3M_4M_4$), the RIL was considered to possess multiple APR QTL. RILs containing Jaypee alleles for corresponding sets of flanking markers (e.g.

$m_1m_1m_2m_2$ and $m_3m_3m_4m_4$) were considered as lacking the QTL for APR to powdery mildew. All RILs selected with multiple QTL had lower mean mildew severity than lines selected for only a single QTL. Thirty-two RILs were identified as carrying all three QTL (U1U2U3) for APR from USG 3209 (Fig. 5). Lines containing all three of these APR QTL had mean mildew severities of 5.1%, 4.1%, and 11.4% in 2002, 2003, and 2004 experiments, respectively. Ten recombinant inbred lines lacking all three QTL for APR from USG 3209 had mean mildew severities of 19.1%, 21.3%, and 20.0% in 2002, 2003, and 2004 disease assessment experiments.

Selection of RILs containing the combination of APR QTL (U1U2) located on chromosome 2A and 2B was most effective in applying MAS for selecting mildew resistance in both field and greenhouse experiments (Fig. 4). Fifty-seven of 293 RILs were identified as having QTL (U1U2) on chromosomes 2A and 2B for APR using flanking markers Xgwm304-Xbar353b and Xgwm501-Xgwm191. Mean mildew severities of RILs having these two QTL (U1U2) were 4.3%, 3.2%, and 9.8% in the 2002, 2003, and 2004 disease assessment experiments (Fig. 5). Twelve RILs lacking the APR QTL on chromosomes 2A and 2B (J1J2) and possessing Jaypee alleles at both sets of flanking markers, had mean mildew severities of 19.1%, 22.0%, and 21.0% in 2002, 2003, and 2004 experiments, respectively.

Selecting the QTL of chromosome 1B in combination with other QTL for APR contributed relatively little additional field powdery mildew resistance compared to simply selecting lines for single QTL on chromosomes 2A and 2B or selecting RILs having a combination of both QTL chromosomes 2A and 2B via MAS. Fifty lines were identified as having the two QTL of chromosomes 2A and 1B (U1U3) with mean mildew severities of 6.5% and 5.2% in 2002 and 2003, respectively. Similarly, selecting lines containing APR QTL on chromosomes 2B and 1B (U2U3) gave mean mildew severities of 5.3% and 5.1% in 2002 and 2003 field experiments, respectively. However, selection of the two QTL on chromosomes 2A and 1B (U1U3) was most effective in selecting for mildew resistance in the greenhouse experiment as RILs with the combination of these

QTL had a mean mildew severity of 9.8% while RILs lacking the two QTL pyramid (J1J3) had a mean mildew severity of 21.4%.

Discussion

QTL Detection and Application in Different Genetic Backgrounds

Previous studies identified three QTL associated with APR to powdery mildew in cultivar Massey located on wheat chromosomes 2A, 1B, and 2B (Liu et al., 2001). The QTL explained 29%, 17%, and 15% of the phenotypic variance, respectively. In the current study, application of microsatellite markers, mapped near the QTL responsible for APR in Massey, were tested in a RIL breeding population derived from a cross between cultivars Jaypee and USG 3209 with the latter being a derivative of Massey. Results of the study demonstrate that SSR markers mapped in the original mapping population of BM can be applied in MAS for APR to powdery mildew in populations where more desirable genotypes derived from Massey are used as the APR donor parent. USG 3209 contains all three APR QTL from Massey and markers mapped near the QTL can be used effectively to select for powdery mildew resistance in breeding populations.

The QTL mapped on chromosome 2A in the Massey population as well as in the USG 3209 population is located in the vicinity of the *Pm4* locus. However, seedling tests have confirmed that Massey and USG 3209 do not possess *Pm4a* or *Pm4b*. The defeated gene *Pm4a* has been shown to have residual effects on APR to powdery mildew (Martin and Ellingboe, 1976; Nass et al., 1981). Mingeot et al. (2002) mapping QTL involved with APR in winter wheat line RE714 in two different susceptible backgrounds, found that gene *Pm4b* had a residual effect in only one population and explained 22% to 40% of the phenotypic variation for mildew resistance in field studies. Similarly, the QTL location on chromosome 2B in the original mapping and USG 3209 populations is in the vicinity of the mapped location of *Pm6* locus (Tao et al., 2000). The *Pm6* gene has not been documented as having residual effects on APR to powdery mildew and seedling test confirm that USG 3209 and Massey do not possess *Pm6*.

The QTL on chromosome 2B evaluated in the UJ RIL populations explained more of the phenotypic variation in field studies than the same QTL detected in the Massey by Becker population. Castro et al. (2003a) also reported differences in phenotypic variance for barley stripe rust explained by QTL in the mapping population and derived lines containing the QTL. A bias could exist in the estimation of QTL effects in the different populations or the QTL may have a modified genetic effect due to epistasis in different backgrounds.

While the QTL on chromosome 2B had a significant effect on mildew resistance in the field experiments, the QTL had only minor effects on mildew resistance in the greenhouse experiment. Conversely, the APR QTL on chromosome 2A and to a lesser extent the QTL on chromosome 1B were stable over all environments, inclusive of both field and greenhouse. Chantret et al. (2001) compared QTL for APR to powdery mildew in field experiments to those identified in adult plants evaluated for reaction to four individual isolates in a greenhouse experiment. In field studies, three QTL were identified in doubled haploid and $F_{2:3}$ populations, however in greenhouse studies only one QTL had a significant effect for all four isolates tested. Chantret et al. (2001) concluded that the QTL detected in all environments was a race non-specific adult plant resistance factor, and that major genes (race specific factors) are involved in expression of APR (Borner et al., 2000). However, a gene cluster theory was also considered as a possibility. Results of the current study are in agreement with those of Chantret et al. (2001) in that greenhouse conditions were not highly predictive of APR expression of QTL involved under field conditions. However in both studies, greenhouse assessments of APR did provide useful information regarding QTL having major effects on powdery mildew resistance and those having stable and consistent expression over environments.

Combining QTL for APR to Powdery Mildew

Marker-assisted selection on the basis of haplotypes of markers flanking QTL was effective in identifying RILs having APR expression similar to the resistant parent USG 3209. Liu et al. (2001) reported that selection for the QTL on chromosome 2A with the single SSR marker Xgwm304 in the 97 BM RIL population was highly effective in

selecting for resistance. The RILs with the Massey allele at the Xgwm304 marker locus had a mean mildew severity of 5.4% while lines carrying the Becker allele had a mean mildew severity of 12.8%. Similarly in the USG 3209 population selection for a single QTL was effective in identifying RILs having high levels of APR resistance, particularly for the QTL on chromosome 2B, and selection for two QTL further increased resistance.

Combining QTL on chromosomes 2A and 2B and the three QTL combination was most effective in selecting for powdery mildew resistance in the field. Lack of significant increase in mildew resistance in the field when all three QTL for APR were selected likely resulted from the large proportion of phenotypic variance contributed by QTL on chromosomes 2A and 2B versus the rather small amount of variation (13%) explained by the QTL on chromosome 1B. Selection for the three QTL in the BM RIL population was most effective with RILs containing all three QTL for APR having a mean mildew severity of 3.4% while RILs with Becker alleles at all three loci had a mildew severity of 22.3% (Liu et al., 2001). Nevertheless, results from the current study are in agreement with Liu et al. (2001) in that the QTL on chromosome 1B contributes little to overall mildew resistance. Selection for the single Massey QTL on chromosome 1B with marker Xpsp3100 gave RILs with a mean powdery mildew severity of 6.5% while RILs carrying the Becker allele had a mean mildew severity of 10.9%. Furthermore, the two QTL combinations of chromosome 1B with 2A and 1B with 2B in the BM RIL populations were less effective in selecting for mildew resistance than RILs carrying only the QTL on chromosome 2A (Liu et al., 2001).

In summary, this study demonstrates that microsatellite markers identified in original mapping populations can be applied to MAS in breeding populations comprised of parental lines derived from the original resistance source. Microsatellite markers for three QTL for APR to powdery mildew identified in a Becker by Massey mapping population (Liu et al., 2001) were efficiently used for MAS selection in a UJ RIL breeding population. Although the QTL effects observed in the original mapping population and the applied breeding population varied in magnitude, the current study demonstrated that selection for two QTL (located on chromosome 2A and 2B) were

effective in identifying RILs having high levels of APR to powdery mildew in the UJ breeding population. However, combining all three QTL may ensure greater durability of APR, on the basis that resistance conferred by QTL on chromosome 2A and 1B are genetically stable across all environments tested in this study.

Literature Cited

- Bacon, R.K., J.T. Kelly, and G.A. Milus. 1998. Registration of 'Jaypee' wheat. *Crop Sci.* 38:1723.
- Basten, C.J., Z.-B. Zeng, and B.S. Weir. 1994. Zmap-a QTL cartographer, p. 65-66, *In* C. Smith, et al., eds. *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software*, Vol. 22. Published by the Organizing Committee, 5th World Congress on Genetics Applied to Livestock Production, Guelph, Ontario, Canada.
- Beavis, W.D. 1998. QTL analyses: Power, precision, and accuracy, p. 145-161, *In* A. H. Patterson, ed. *Molecular dissection of complex traits*. CRC Press, Boca Raton, FL.
- Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programs. *Plant Path.* 33:279-300.
- Bjarko, M.E., and R.F. Line. 1988. Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. *Phytopathology* 78:457-461.
- Borner, A., M.S. Röder, O. Unger, and A. Meinel. 2000. The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. *Theor. Appl. Genet.* 100:1095-1099.
- Castro, A.J., X. Chen, P.M. Hayes, and M. Johnston. 2003b. Pyramiding quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on resistance at the seedling stage. *Crop Sci.* 43:651-659.
- Castro, A.J., X. Chen, A. Corey, T. Filichkina, P.M. Hayes, C. Mundt, K. Richardson, S. Sandoval Islas, and H. Vivar. 2003a. Pyramiding and validation of quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: Effects on adult plant resistance. *Crop Sci.* 43:2234-2239.
- Chantret, N., P. Sourdille, M. Röder, M. Tavaud, M. Bernard, and G. Doussinault. 2000. Location and mapping of the powdery mildew resistance gene *MIRE* and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. *Theor. Appl. Genet.* 100:1217-1224.
- Chantret, N., D. Mingeot, P. Sourdille, M. Bernard, J.M. Jacquemin, and G. Doussinault. 2001. A major QTL for powdery mildew resistance is stable over time and at two developmental stages in winter wheat. *Theor. Appl. Genet.* 103:962-971.

- Das, M.K., and C.A. Griffey. 1994. Heritability and number of genes governing adult-plant resistance to powdery mildew in Houser and Redcoat winter wheats. *Phytopathology* 84:406-409.
- 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.
- Griffey, C.A., M.K. Das, and E.L. Stromberg. 1993. Effectiveness of adult plant resistance in reducing loss to powdery mildew in winter wheat. *Plant Dis.* 77:618-622.
- Gustafon, G.D., and G. Shaner. 1982. Influence of plant age on the expression of slow-mildewing resistance in wheat. *Phytopathology* 72:746-749.
- Hautea, R.A., W.R. Coffman, M.E. Sorrells, and G.C. Berstrom. 1987. Inheritance of partial resistance to powdery mildew in spring wheat. *Theor. Appl. Genet.* 79:609-615.
- James, C. 1971. A manual of assessment keys for plant diseases. Can. Dep. Agric. Publ. No. 1458. American Phytopathological Society, St. Paul, MN.
- 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 a segregating wheat x spelt population. *Theor. Appl. Genet.* 98:903-912.
- Langridge, P., and K. Chalmers. 1998. Techniques for marker development, p. 107-117, *In* A. E. Slinkard, ed. *Proceeding of 9th Int'l Wheat Genetics Symposium*. University Extension Press, Saskatoon, Canada.
- Liu, S., and J.A. Anderson. 2003. Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. *Crop Sci.* 43:760-766.
- Liu, S., C.A. Griffey, and M.A. Saghai Maroof. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. *Crop Sci.* 41:1268-1275.
- Martin, T., and A. Ellingboe. 1976. Differences between compatible parasite/host genotypes involving the *Pm4* locus of wheat and the corresponding genes in *Erysiphe graminis* f. sp. *tritici*. *Genetics* 66.
- 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 genetic backgrounds. *Plant Breed.* 121:133-140.

- Nass, H., D. Pedersen, D. MacKenzie, and R. Nelson. 1981. The residual effects of some 'defeated' powdery mildew resistance genes in isolines of chancellor winter wheat. *Phytopathology* 71:1315-1318.
- Niewoehner, A.S., and S. Leath. 1998. Virulence of *Blumeria graminis* f.sp. *tritici* on winter wheat in the Eastern United States. *Plant Dis.* 82:64-68.
- Patterson, F.L., J.F. Schafer, and R.L. Gallun. 1978. Registration of Knox 62 wheat. *Crop Sci.* 18:527.
- Roberts, J.J., and R.M. Calwell. 1970. General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in "Knox" wheat (abstract). *Phytopathology* 60:1310.
- Röder, M.S., J. Plaschke, S.U. König, A. Börner, M.E. Sorrells, S.D. Tanksley, and M.W. Ganai. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen. Genet.* 246:327-333.
- Rouse, D.I., R.R. Nelson, D.R. MacKenzie, and C.R. Armitage. 1980. Components of rate-reducing resistance in seedlings of four wheat cultivars and parasitic fitness in six isolates of *Erysiphe graminis* f. sp. *tritici*. *Phytopathology* 70:1097-1100.
- Royer, M.H., R.R. Nelson, D.R. MacKenzie, and D.A. Diehle. 1984. Partial Resistance of near-isogenic wheat lines compatible with *Erysiphe graminis* f. sp. *tritici*. *Phytopathology* 74:1001-1006.
- Saghai Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. (USA)* 81:8014-8018.
- Saghai Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang, and R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. *Proc. Natl. Acad. Sci. (USA)* 91:5466-5470.
- Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. *Phytopathology* 63:867-872.
- SPSS Inc. 1998. SPSS Base 12.0 for Windows User's Guide. SPSS Inc., Chicago, IL.
- Starling, T.M., C.W. Roane, and H.M. Camper, Jr. 1984. Registration of Massey wheat. *Crop Sci.* 24:1000.

Tao, W., D. 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.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14:415-421.

Zhou, W.C., F.L. Kolb, G.H. Bai, L.L. Domier, L.K. Boze, and N.J. Smith. 2003. Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. *Plant Breed.* 122:40-46.

Table 1. Seedling reactions of parental lines USG 3209 (APR possessing parent), Jaypee (moderately susceptible parent), Massey (APR source of USG 3209), and Becker (susceptible check with no known resistance genes) tested with *B. graminis* isolates used for adult-plant greenhouse screening tests.

Line	Isolates Used for Greenhouse Screening Test				
	E3-14	101a2	#8	#9	127
USG 3209	av ^a	v	v	av	v
Jaypee	v	av	v	v	av
Massey	v	v	v	v	v
Becker	v	v	v	v	v

^a av=avirulent, v = virulent

Table 2. Seedling reactions to a set of powdery mildew differentials containing genes *Pm1*-*Pm8* and *Pm17* screened with bulked *B. graminis* isolates used in the adult-plant greenhouse screening test. Also reaction of wheat lines used to increase powdery mildew inoculum containing known *Pm* genes in the greenhouse experiment are listed below.

Lines	Powdery Mildew Resistance Genes	Reaction
Chancellor	No known resistance genes	v ^a
Axminster	<i>Pm 1</i>	v
Coker68-15*7/CI 13836	<i>Pm 1</i>	v
Ulka	<i>Pm 2</i>	v
Asosan	<i>Pm 3a</i>	v
Chul	<i>Pm 3b</i>	av
Sonora	<i>Pm 3c</i>	v
Coker68-15*6/Sonora	<i>Pm 3c</i>	v
Coker68-15*6/Trit	<i>Pm 3c</i>	v
Michigan Amber	<i>Pm 3f</i>	v
Yuma	<i>Pm 4a</i>	v
Coker68-15*5/Yuma	<i>Pm 4a</i>	m
Coker68-15*5/Kapli	<i>Pm 4a</i>	v
Ronos	<i>Pm 4b</i>	v
Hope	<i>Pm 5</i>	v
C747	<i>Pm 6</i>	v
Transec	<i>Pm 7</i>	v
Coker68-15*7/Transec	<i>Pm 7</i>	v
Federation/Kavkaz	<i>Pm 8</i>	v
Amigo	<i>Pm 17</i>	av
Wheat Lines for Increasing Inoculum		
Wakefield	<i>Pm1</i>	v
Saluda	<i>Pm3a</i>	v
Roane	<i>Pm4a</i>	v
Pocahontas	<i>Pm4b</i>	v
Massey	No known seedling resistance genes	v
Becker	No known seedling resistance genes	v

^a av = avirulent, v = virulent, m = mixed reaction

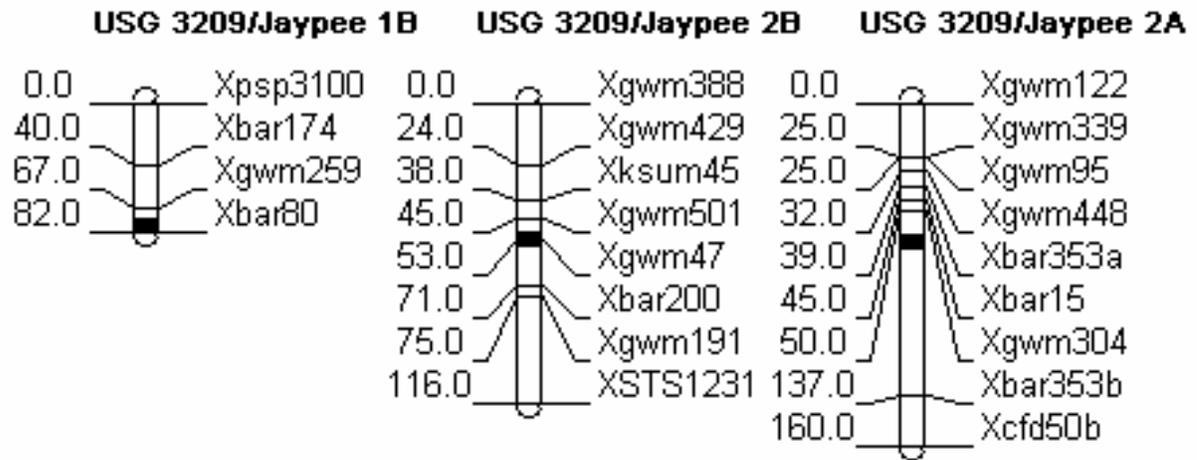


Fig. 1 Linkage maps of wheat chromosomes 1B, 2B, and 2A developed from UJ RIL population. Black blocked areas on the linkage group represent QTL positions.

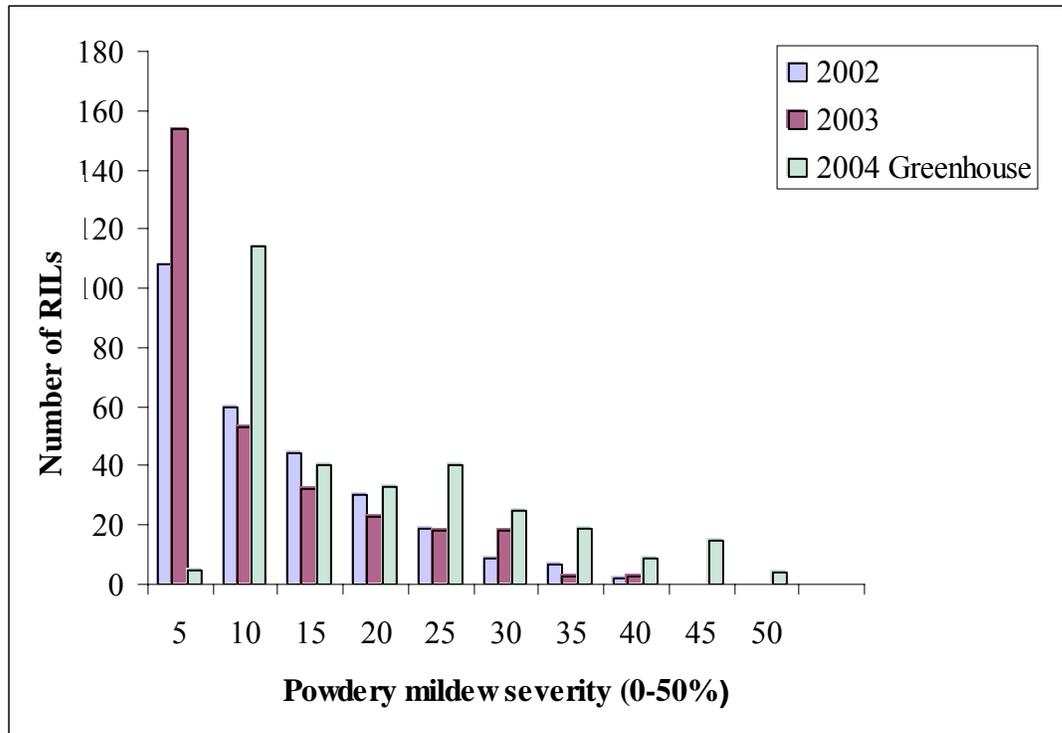


Fig. 2. Histogram of mean powdery mildew disease severity in the field (2002 and 2003) and 2004 greenhouse screening of the 293 RILs derived from the cross of UJ.

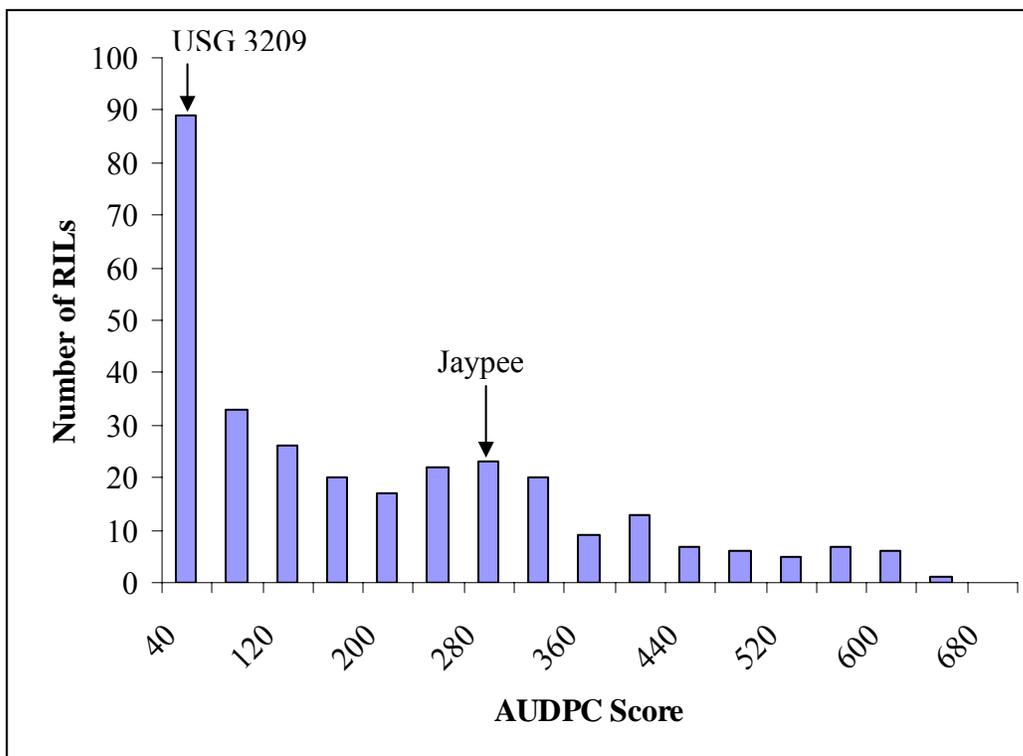


Fig. 3. Distribution of AUDPC values calculated for powdery mildew severity in the 2004 greenhouse screening. Powdery mildew severity of the two parents is noted by arrows.

Table 3. One-way ANOVA to verify molecular markers associated with APR to powdery mildew in UJ RILs.

Marker	Chrom ^a .	2002 Field Severity F _{5:6}			2003 Field Severity F _{6:7}			2004 GH ^d Severity F _{7:8}			2004 GH AUDPC F _{7:8}		
		df ^b	R ^{2c}	P	df	R ²	P	df	R ²	P	df	R ²	P
Xbar15	2A	248	8.3	≤0.001	272	7.6	≤0.001	272	3.9	=0.001	272	3.7	=0.001
Xgwm304	2A	261	9.1	≤0.001	280	8.3	≤0.001	280	4.8	≤0.001	280	4.8	≤0.001
Xbar353b	2A	264	3.2	=0.003	288	3.7	=0.001	288	4.2	=0.001	288	5.4	≤0.001
Xgwm501	2B	274	29.6	≤0.001	292	16.6	≤0.001	292	2.5	≤0.006	292	2.1	0.011
Xgwm47	2B	267	27.9	≤0.001	291	16.0	≤0.001	291	4.0	≤0.001	291	3.6	0.002
Xgwm191	2B	264	14.4	≤0.001	283	6.2	≤0.001	283	0.005	-	283	0.001	-
Xgwm259	1B	265	4.3	≤0.001	283	6.4	≤0.001	283	2.6	=0.006	283	2.3	=0.009
Xbar80	1B	258	9.8	≤0.001	274	8.2	≤0.001	274	4.2	=0.001	274	3.8	=0.001

^a = Chromosome

^b = Degrees freedom of error

^c = Coefficient of determination

^d = Greenhouse

Table 4. Average mildew severity values of RILs in the UJ population selected by single and flanking SSR markers for each QTL. A value in the same column followed by the same letter is not significantly different ($p \leq 0.05$) on the basis of pairwise comparisons (t test).

Marker(s)	Chrom. ^a	Genotypes of F _{6:7} Plants ^b	n ^c	Mean Severity 2002 Field Data (F _{5:6})	Mean Severity 2003 Field Data (F _{6:7})	Mean Severity 2004 GH ^d (F _{7:8})
Xgwm304	2A	M ₁ M ₁	144	7.3ab	6.3a	10.1a
		m ₁ m ₁	136	12.8c	11.7c	14.8b
Xbar353b	2A	M ₂ M ₂	238	9.7bc	8.3b	11.5a
		m ₂ m ₂	50	14.0cd	13.3cd	18.7c
Xgwm191	2B	M ₃ M ₃	133	6.5a	6.4a	12.2ab
		m ₃ m ₃	150	13.3cd	11.1c	12.7b
Xgwm501	2B	M ₄ M ₄	192	6.7a	6.3a	11.2a
		m ₄ m ₄	100	17.0d	14.3d	15.0b
Xgwm259	1B	M ₅ M ₅	138	8.5b	6.5a	10.4a
		m ₅ m ₅	145	12.2c	11.1c	13.9b
Xbar80	1B	M ₆ M ₆	124	8.1b	6.3a	10.2a
		m ₆ m ₆	150	12.2c	11.0c	14.4b
Xgwm304+ Xbar353b	2A	M ₁ M ₁ M ₂ M ₂	114	7.1a	6.0a	10.0a
		m ₁ m ₁ m ₂ m ₂	26	15.7d	15.9d	19.7c
Xgwm191+ Xgwm501	2B	M ₃ M ₃ M ₄ M ₄	116	5.8a	5.8a	12.2ab
		m ₃ m ₃ m ₄ m ₄	78	17.7d	14.4d	14.9b
Xgwm259+ Xbar80	1B	M ₅ M ₅ M ₆ M ₆	96	7.1a	6.0a	10.3a
		m ₅ m ₅ m ₆ m ₆	111	12.7c	11.8c	15.1b

^a = Chromosome

^b = 'M' marker allele from USG 3209 (resistant); 'm' marker allele from Jaypee (susceptible)

^c = Number of F_{6:7} UJ lines

^d = Greenhouse

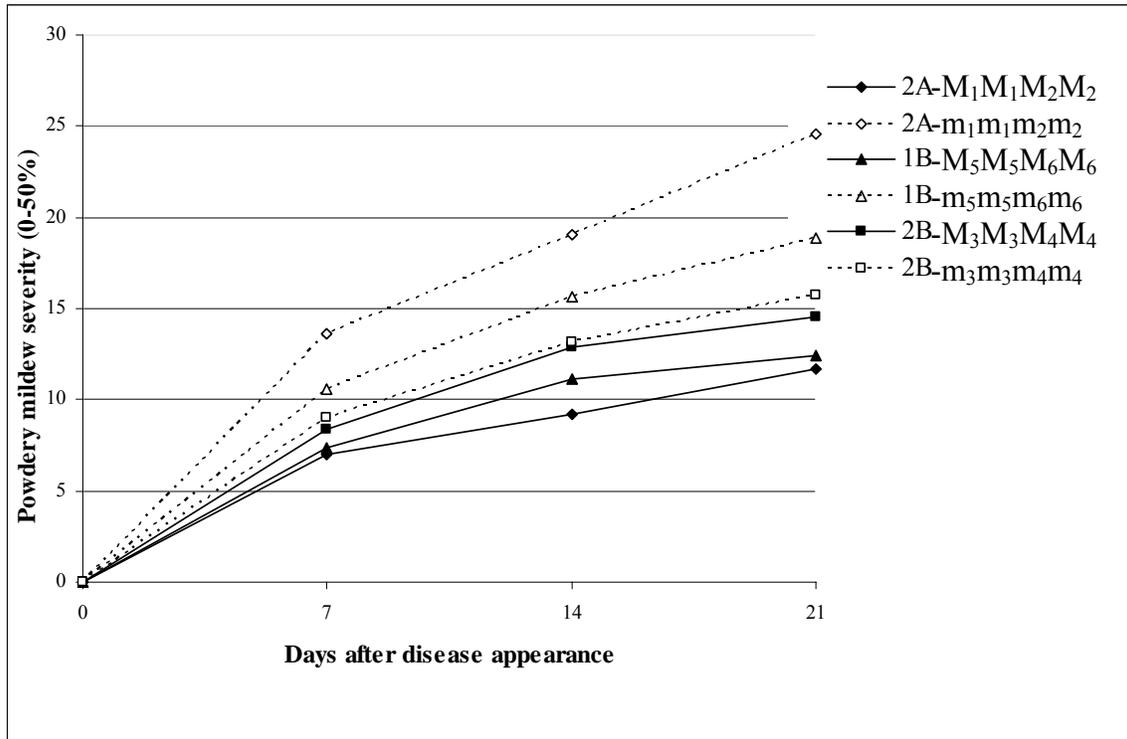


Fig. 4. QTL for APR on chromosomes 2A, 1B, and 2B effects on AUDPC for RILs selected by MAS in the 2004 greenhouse experiment. 'M' refers to the resistant marker allele from USG 3209 and 'm' refers to the marker allele from the susceptible parent Jaypee.

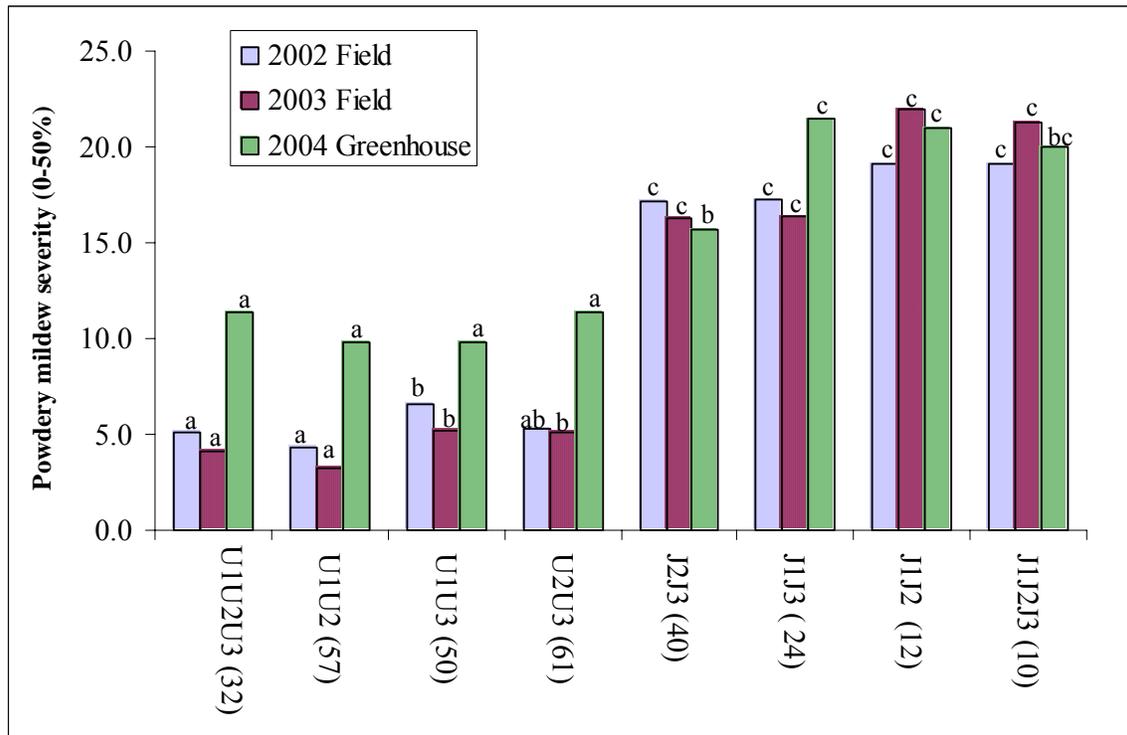


Fig. 5. Mean powdery mildew severity data from two field and one greenhouse experiments grouped by flanking markers for QTL on chromosomes 2A, 2B, and 1B simultaneously. U and J represent USG 3209 and Jaypee alleles at the QTL. Flanking markers Xgwm304 and Xbar353b; Xgwm501 and Xgwm191 and; Xgwm259 and Xbar80 are represented by 1, 2, and 3, respectively. Numbers in the parenthesis indicate the number of RILs with combined QTL. Values of mean mildew severities in an experiment (2002, 2003, 2004) followed by the same letter are not significantly different ($p \leq 0.05$) on the basis of a pairwise comparison (t test).

VITA

Dominic Michael Tucker was born on November 23, 1979 in Toledo, Ohio to the parents of Duane and Elaine Tucker. Dominic's residence from birth through high school graduation in June 1998 was in Temperance, Michigan. He completed high school graduation and enrolled at Michigan State University (Lansing, Michigan) and completed a Bachelor of Science Degree in Crop and Soil Sciences with a minor in Biotechnology in May 2002. Within the undergraduate degree program in May 2000, he participated in the Michigan State University abroad program traveling to New Zealand and Australia studying the agriculture, environment, and social systems of the respective countries. Numerous valuable undergraduate research experiences were also gained through part time employment as a laboratory assistant at the Soil Testing and Plant Nutrition Laboratory and Soil Fertility and Fertilizer Use Management Laboratory at Michigan State University. Dominic was selected for an internship at Pioneer Hi-Bred International Seed Company, Inc. (Ithaca, Michigan) in the summer of 2001 researching hybrid seed corn production and completed an independent project on *Gibberella* (*Gibberella zeae*) ear rot of corn. The Pioneer Hi-Bred summer internship provided beneficial research experiences while working with pollination crews throughout the state of Michigan. These experiences greatly sparked a personal interest in and future dedication to the field of plant breeding and genetic research. A graduate research assistantship was awarded at Virginia Polytechnic Institute and State University (Blacksburg, VA) following Dominic's graduation in May 2002. Dominic's research began under the guidance of Dr. Carl A. Griffey and Dr. Mohammad A. Saghai Maroof. Invaluable research experiences were gained while working side by side with Dr. Griffey in the wheat fields of Eastern Virginia, specifically the stepwise procedure of cultivar development. Dr. Saghai Maroof mentored laboratory skills and research procedures necessary to complete his MS project. Dominic's research focused on mapping and marker-assisted selection of quantitative trait loci for adult plant resistance to powdery mildew. He was involved in the Graduate Student Assembly and served as an officer in the Crop, Soil, and Environmental Graduate Organization coinciding with his graduate studies.

Dominic Michael Tucker