Molecular Marker Analysis of Adult Plant Resistance to Powdery Mildew in Common Wheat

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by

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(ABSTRACT)

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* E'm. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the major diseases of wheat (*Triticum aestivum* L.) worldwide. The use of cultivars with resistance to powdery mildew is an efficient, economical and environmentally safe way to control powdery mildew. Race-specific resistance has been extensively used in breeding programs; however, it is ephemeral. Adult plant resistance (APR) to powdery mildew is more durable as demonstrated by the cultivar Massey, which has maintained its APR to powdery mildew since its release in 1981. To develop an efficient breeding strategy, it is essential to understand the genetic basis of APR. The objectives of this study were to identify molecular markers associated with APR to powdery mildew in common wheat Massey and to verify their association using recombinant inbred (RI) lines.

A cross was made between the powdery mildew susceptible cultivar Becker and Massey. One hundred and eighty $F_{2:3}$ lines were rated for disease severity under natural pressure of powdery mildew in field. Using both restriction fragment length polymorphism (RFLP) and microsatellite markers, three quantitative trait loci (QTL), designated as *QPm.vt-1B*, *QPm.vt-2A* and *QPm.vt-2B*, were identified in the Becker × Massey $F_{2:3}$ generation. These loci are located on chromosomes 1B, 2A and 2B, respectively, and explained 17%, 29% and 11% of the total variation among $F_{2:3}$ lines for powdery mildew resistance, respectively. Cumulatively, the three QTLs explained 50% of the phenotypic

variation among $F_{2:3}$ lines in a multi-QTL model. The three QTLs associated with APR to powdery mildew were derived from Massey and displayed additive gene action. *QPm.vt-2B* also fits a recessive model for APR to powdery mildew.

In the second part of this study, 97 RI lines were developed from the Becker × Massey cross. The RI lines were evaluated for APR to powdery mildew under natural disease pressure for three years. Both single marker analysis and interval mapping confirmed the presence of the three QTLs identified in the $F_{2:3}$ generation. The three QTLs, *QPm.vt-1B*, *QPm.vt-2A* and *QPm.vt-2B*, accounted for 15%, 26% and 15% of the variation of mean powdery mildew severity of the RI lines over three years. In a multi-QTL model, the three QTLs explained 44% of the phenotypic variation of the RI lines. The RI lines were grouped according to the genotype of the three QTLs, represented by markers GWM304a, KSUD22 and PSP3100, respectively. The RI lines with Massey alleles at all three loci had a mean disease severity of 3.4%, whereas the RI lines with Becker alleles at all three loci had a mean disease severity of 22.3%. These severity values are similar to those of the corresponding parents.

The molecular markers identified and verified as to their association with APR to powdery mildew in Massey have the potential for use in marker-assisted selection for resistance to powdery mildew and in pyramiding powdery mildew resistance genes, as well as facilitating a better understanding of the molecular basis of APR to powdery mildew.

To my family

To my advisors

Dr. C.A. Griffey

Dr. M.A. Saghai Maroof,

their dedication and excellence

will guide me forever

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TABLE OF CONTENTS

LIST OF TABLES

Pages

LIST OF FIGURES

Pages

CHAPTER I.

Literature Review

Powdery mildew caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* E'm. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the most devastating diseases of wheat (*Triticum aestivum* L.) in areas with maritime or semi-continental climate (Bennett 1984). Grain yield losses ranging from 13% to 34% have been attributed to powdery mildew (Griffey et al. 1993; Leath and Bowen 1989; Johnson et al. 1979). The use of resistant cultivars is an effective, economical, and environmentally safe means of controlling powdery mildew.

Race-specific Powdery Mildew Resistance Genes in Wheat

The widely studied resistance genes for powdery mildew of wheat are qualitative and race-specific. They are effective for some isolates, but ineffective for others. The race-specific resistance genes follow the gene-for-gene model described by Flor (1955). Host resistance requires the simultaneous presence of the resistance allele in the host and the corresponding avirulence allele in the pathogen. Most of the race-specific genes are expressed throughout the entire vegetative life cycle of wheat; however, a few genes such as *Pm5* and *Pm6* are only fully expressed from the four to five leaf growth stage (Lebsock and Briggle 1974).

Currently, 33 alleles at 24 loci for resistance to powdery mildew have been localized on particular chromosomes (Table 1) (McIntosh et al. 1998; Shi et al. 1998). Gene *Pm18* was renamed as *Pm1c* (Hsam et al. 1998). Four, six and two alleles have been identified at the *Pm1*, *Pm3* and *Pm4* loci, respectively. To date, only one allele has been identified at the other 21 loci. Genes *Pm8* and *Pm17*, transferred from rye chromosome 1RS, are located on T1BL·1RS and T1AL·1RS, respectively (Heun et al. 1990). Recent evidence indicates that *Pm8* and *Pm17* are allelic (Hsam and Zeller 1997). Genes *Pm7* and *Pm20*, also transferred from rye, are located on T4BS·4BL-2RL and T6BS·6RL, respectively (Heun and Frieb 1990; Frieb et al 1994). Gene *Pm12* originated from *Aegilops speltoides* is located on T6BS-6SS·6SL (Miller et al. 1988; Jia et al. 1996); *Pm13* derived from *Ae. longissimum* is located on T3B.3BS-3S^{l} or T3D.3DS-3S^{l} (Ceoloni et al. 1988; Ceoloni et al. 1992); and gene *Pm21* introgressed from *Haynaldia villosa* is located on T6VS·6AL

(Chen et al. 1995). In addition to the above mentioned genes introgressed from related species, genes *Pm2* and *Pm19* originated from *Ae. squarrosa* L. (Lutz et al. 1995); *Pm4a* and *Pm5* came from *T. dicoccum* (Briggle 1966; Law and Wolfe 1966); *Pm16* and *Pm25* were derived from *T. dicoccoides* and *T. monococcum*, respectively (Reader and Miller 1991, Shi et al. 1998); *Pm4b* and *Pm6* originated from *T. carthlicum* and *T. timopheevi*, respectively (The et al. 1979; Jorgensen and Jensen 1972); and *Pm1*d was identified in *T. spelta* (Hsam et al. 1998).

Genes *Pm10*, *Pm11*, *Pm14* and *Pm15* were detected using hybrids between *E. g. tritici* and *E. g. agropyri* cultures (Tosa et al. 1987, 1988; Tosa and Sakai 1990). However, these genes are not effective sources of resistance to *B. graminis tritici*. In other words, they are not useful sources of resistance to powdery mildew in wheat breeding programs.

Race-specific resistance is ephemeral (Bennett 1984; Roberts and Caldwell 1970), and breaks down rapidly in the presence of pathogen isolates with matching virulence genes (Bennett 1984). Selection pressure exerted by cultivars with race-specific resistance genes has resulted in the rapid build-up of isolates with matching virulence genes. A virulence survey conducted in the Eastern United States has shown an increase in virulence frequencies and complexity of isolates (Niewoehner and Leath 1998). As early as 1985, virulence genes to the 10 most widely used genes for resistance to wheat powdery mildew already existed in the Southeastern USA (Leath and Murphy 1985).

Adult Plant Resistance to Powdery Mildew in Wheat

Powdery mildew resistance that retards infection and also growth and reproduction of the pathogen in adult plants but not in seedlings has been described as “adult plant resistance” (APR) (Gustafson and Shaner 1982), “slow mildewing” (Shaner 1973) and “partial resistance” (Hautea et al. 1987). This type of resistance can be identified in cultivars with defeated race-specific resistance genes or without known race-specific resistance genes.

Johnson (1984) defined durable resistance as resistance that remains effective during its prolonged and widespread use in an environment favorable for the disease. APR to powdery mildew is durable and remained effective in the winter wheat cultivar Knox and its derivatives during the 20 years in which these cultivars were grown commercially (Shaner 1973). Massey, a derivative of Knox62, was released from Virginia Tech in 1981 (Starling et al. 1984). Its powdery mildew resistance is still effective in adult plants.

To improve the efficiency of wheat breeding for APR to powdery mildew, it is essential to understand the genetic basis of APR. APR to powdery mildew in wheat is much more complex than race-specific resistance because it is a quantitative trait by nature (Shaner and Finney 1975). Using monosomic analysis, Chae and Fischbek (1979) reported that 14 chromosomes were involved in APR to powdery mildew in the cultivar Diplomat. Similarly, Johnson et al. (1998) found that seven chromosomes in Knox 62 were involved in APR to powdery mildew. Chromosomes 5A, 7A, 4B and 5D carry genes showing positive effects on resistance, while chromosomes 3A, 1B and 1D have factors increasing susceptibility. Both qualitative and quantitative genetic studies indicated that APR to powdery mildew was governed by two to three genes, with moderate to high heritability, in four cultivars, Knox 62, Massey, Redcoat and Houser (Griffey and Das 1994; Das and Griffey 1994). Hautea et al. (1987) reported that additive effects were the most important gene action for APR to powdery mildew in four spring wheat crosses. Das and Griffey (1995) also reported that additive gene effects were predominant, but non-additive effects also were significant for APR to powdery mildew.

The current selection methods for APR are not efficient or highly effective. Selection of APR is a time-consuming process involving extensive and precise quantitative measurements (Gustafson and Shaner 1982). Furthermore, it is not easy to select plants with both APR and race-specific resistance. Therefore, development of rapid selection techniques for APR is important for wheat breeding.

Types of DNA Markers

Identification and differentiation of all known powdery mildew resistance genes and phenotypic selection for combinations of highly effective resistance genes are not readily feasible via classical genetics and breeding methods. Fortunately, molecular markers can be used for marker-assisted selection as well as serving as a starting point for map-based cloning of resistance genes (Tanksley et al. 1989). DNA markers are abundant, exhibit no epistatic effects, and possess a much higher level of allelic variation than morphological and isozyme markers. The major types of DNA markers include RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), microsatellite or simple sequence repeat (SSR) and AFLP (amplified fragment length polymorphism).

RFLPs result from point mutation, deletion, insertion, or transposition of DNA sequences. The DNA sequence polymorphisms result in DNA fragment length differences after digestion with DNA restriction enzymes. The different sized DNA fragments, separated by agrose gel electrophoresis, can be visualized by autoradiography. RFLP markers are abundant and codominant. RFLPs have been used in a number of plants and detailed RFLP genetic maps have been constructed for many crop species (O'Brien 1992). A large number of disease resistance genes have been tagged with RFLP markers, including genes conditioning resistance to cyst nematode in wheat (Williams et al. 1994), to powdery mildew in barley (Schonfeld et al. 1996), to bacterial blight in rice (Ronald et al. 1992), to gray leaf spot disease in maize (Saghai Maroof et al. 1996), to *Fusarium oxysporum* in tomato (Sarfatti et al. 1989), and to *Phytophthora infestans* in potato (Leonhard-Shippers et al. 1992).

Detailed RFLP linkage maps have been published for all seven homoeologous groups of wheat chromosomes (Chao et al. 1989; Devos et al. 1993; Xie et al. 1993; Nelson et al. 1995a, b, c; Van Deynze et al. 1995; Marino et al. 1996). About 2000 loci have been either mapped directly or assigned to putative locations inferred by the aligned genomes

of related *Triticum* species. However, the level of RFLP polymorphism is low in wheat (Chao et al. 1989; Cadalen et al. 1997). For this reason, wide crosses have been extensively used for genetic mapping in wheat. Furthermore, RFLP mapping is laborious, time-consuming and requires relatively large amounts of DNA, and is not easily to be automated. Thus, RFLPs have limited application in wheat breeding programs that require large-scale screening of progenies from intraspecific crosses in a short time period.

In 1990, RAPD was proposed to overcome many of the technical limitations of RFLP analysis (Williams et al. 1990; Welsh and McClelland 1990). RAPD is based on the amplification of random DNA segments using a single primer of arbitrary nucleotide sequence. Sequence information and radioactivity are not required for RAPD analysis. It is economical and easy to use. RAPD markers have been used to map agronomically important genes, such as common bunt resistance gene *Bt-10* in wheat (Demeke et al. 1996), genes conferring resistance to *Rhynchosporium secalis* in barley (Barua et al. 1993), *Puccinia graminis* in oat (Penner et al. 1993) and *Pseudomonas syringae* in tomato (Martin et al. 1991). However, most RAPD markers are dominant, and sometimes the results are difficult to reproduce. Fortunately, RAPD markers can be converted to more reliable markers, such as SCARs (sequence characterized amplified regions). For example, the RAPD marker linked to the wheat leaf rust resistance gene *Lr24* was converted to a SCAR marker (Dedryver et al. 1996). Similarly, the RAPD markers tightly linked to rice blast resistance gene *Pi*-10 were also converted to codominant SCAR markers (Naqvi and Chattoo 1996).

Microsatellites, also called simple sequence repeats (SSRs), are tandem repeat sequences with a basic motif of 1 to 6 base pairs. The repeat numbers are variable and can be detected via PCR (polymerase chain reaction). The PCR primers are designed according to flanking sequences of microsatellite. There are three approaches to isolate SSRs: survey of GenBank and EMBL databases; screening of genomic or cDNA libraries; and use of SSR primers from related species. SSRs are abundant and randomly distributed in eukaryotic genomes (Tautz and Renz 1984). They are also highly polymorphic (Saghai

Maroof et al. 1994; Plaschke et al. 1995). Even though isolation of SSRs is time-consuming and expensive, the primer sequences are easy to disseminate among laboratories.

Roder et al. (1998) reported a total of 279 loci amplified by 230 microsatellite primer sets on the wheat genetic map. Another 53 microsatellite loci were mapped in wheat by Stephenson et al. (1998). Most of the wheat SSR primer sets are genome-specific and detect only a single locus in one of the three genomes of bread wheat. SSR markers are highly polymorphic and easy to detect in wheat. Therefore, SSR markers are suitable for mapping agronomically important genes in wheat. For example, dwarfing genes *Rht8* and *Rht12* have been tagged with SSR markers (Korzun et al. 1997, 1998).

AFLP is a novel type of marker (Vos et al. 1995). It is based on selective PCR amplification of restriction fragments from a total digestion of genomic DNA. The technique involves three steps: 1) DNA digestion with two restriction enzymes and ligation of oligonucleotide adapters; 2) selective amplification of a set of restriction fragments, and 3) gel analysis of the amplified fragments. AFLP allows the specific co-amplification of high numbers of restriction fragments without knowledge of nucleotide sequence. Typically 50-100 restriction fragments are amplified and detected on denaturing polyacylamide gels per AFLP reaction.

AFLP has proven to be a powerful alternative molecular marker technique in various crops (Waugh et al. 1997; Maheswaran et al. 1997; Keim et al. 1997). Some agronomically important traits have been tagged with AFLP markers, such as scab resistance in wheat (Bai et al. 1999), virus resistance in soybean (Maughan et al. 1996) and resistance to *Cladosporium fulvum* in tomato (Thomas et al. 1995).

Strategies for Genetic Mapping

When F_2 or backcross populations are used as mapping populations for genes of interest, many markers have to be analyzed for a large number of plants. Furthermore, some traits

are difficult to score on an individual plant basis. Therefore, alternative strategies have been exploited to improve the efficiency of genetic mapping. These include the use of NILs (near-isogenic lines), BSA (bulked segregant analysis) and RI (recombinant inbred) lines or DH (double haploid) populations.

NILs that differ by the presence or absence of the target gene and a small region of flanking DNA, are useful to identify markers linked with the target gene (Young et al. 1988). In principal, genetic markers that are polymorphic between the NIL and its recurrent parent are putatively linked to the target gene (Muehlbauer et al. 1988). Many disease resistance genes have been mapped using NILs, including leaf rust resistance in wheat (Autrique et al. 1995; Schachermayr et al. 1994; Schachermayr et al. 1995), powdery mildew resistance in barley (Hinze et al. 1991; Schuller et al. 1992), blast resistance in rice (Yu et al. 1991), maize dwarf mosaic virus resistance in corn (McMullen and Louie 1989) and soybean mosaic virus resistance in soybean (Yu et al. 1994).

Even though NILs are usually developed via backcrossing, heterogeneous inbred populations are also useful sources for NILs (Haley et al. 1994). This method entails the development of NIL through inbreeding instead of backcrossing. It is especially useful for autogamous crop species.

Although NILs are helpful to map genes of interest, often they are unavailable, and the development of NILs is time-consuming and laborious. To overcome the problems of NILs, Michelmore et al. (1991) successfully used bulked segregant analysis (BSA) to identify RAPD markers tightly linked to genes for resistance to lettuce downy mildew. This strategy involves comparing two pooled DNA samples of individuals from a segregating population. Within each pool, or bulk, the individuals are identical for the trait or gene of interest but are arbitrary for all the other genes. All polymorphic markers between the two DNA pools are putatively linked with the target gene. This strategy has been extensively used to map genes in many crops, such as genes for photoperiod-

sensitive genic male sterility in rice (Zhang et al. 1994) and for nematode resistance in peanut (Garcia et al. 1996).

Recombinant inbred lines or double haploid populations are permanent populations that can be used indefinitely for mapping. They can also be readily disseminated among labs and new data can be continuously added to a pre-existing map. Furthermore, RI lines or DH populations can be evaluated in many different environments. Since each genotype is represented by an inbred line, rather than by an individual plant, a more accurate assessment of the genetic component of variance can be made in studying quantitative traits (Burr et al. 1988). Therefore, RI lines or DH populations are more useful for analysis of quantitative traits or traits that are difficult to characterize on an individual plant basis. RI lines have been used to map genes for resistance to preharvest sprouting (Anderson et al. 1993), aluminum tolerance (Riede and Anderson 1996), and kernel hardness (Sourdille et al. 1996) in wheat. DH populations have been exploited to map genes for resistance to net blotch and spot blotch in barley (Steffenson et al. 1996).

Advances in Mapping Powdery Mildew Resistance Genes in Wheat

Several powdery mildew resistance genes have been tagged with molecular markers (Table 1). Using cultivar Chancellor as the recurrent parent, Briggle (1969) developed NILs for powdery mildew resistance genes *Pm1*, *Pm2*, *Pm3* and *Pm4a*, respectively. These NILs are useful for genetic mapping of powdery mildew resistance genes. Comparing RFLP patterns of NIL possessing gene *Pm1* and the recurrent parent, Ma et al. (1994) reported that the gene *Pm1* cosegregated with RFLP marker CDO347. Hartl et al. (1995) found that RFLP marker Whs178 was 3 cM away from gene *Pm1*. Hu et al. (1997) used RAPD markers to tag gene *Pm1*. Among 1300 arbitrary decamer primers, three primers showed polymorphism between powdery mildew resistant and susceptible pools. RAPD markers $UBC320_{420}$ and $UBC638_{550}$ cosegregated with gene *Pm1* among 244 F_2 plants. Another RAPD marker $OPF12_{650}$ was 5.4 cM away from gene *Pm1*. Recently, Hartl et al. (1999) have used AFLP markers to map gene *Pm1c*. Among 96 primer combinations, 31 polymorphic AFLP fragments between the resistant and

susceptible pools were in accordance with the patterns of the parents. The eight most reliable polymorphic markers were analyzed in a segregating population for the gene *Pm1c*. Two of them cosegregated with the gene *Pm1c* and the other six markers were tightly linked with the gene. One AFLP marker, 18M2, was found to be highly specific for the *Pm1*c gene in diverse genetic backgrounds.

RFLP analysis of NILs possessing the gene *Pm2* and the recurrent parent indicated that 1) RFLP marker BCD1871 was 3.5 cM away from gene *Pm2* (Ma et al. 1994); 2) RFLP marker Whs295 mapped 2.7 cM away from the gene *Pm2* (Hartl et al. 1995); and 3) the gene *Pm2* was also linked with RFLP marker Whs350 (Hartl et al. 1995).

Using RFLP analysis of NILs possessing the gene *Pm3* and the recurrent parent, Hartl et al. (1993) found that RFLP marker Whs179 revealed polymorphism not only between the NILs with and without gene *Pm3*, but also among NILs possessing different alleles of the *Pm3* locus. The genetic distance between probe Whs179 and *Pm3* was 3.3±1.9 cM. Ma et al. (1994) reported that RFLP marker BCD1434 was 1.3 cM away from *Pm3a* or *Pm3b*.

Ma et al. (1994) reported that *Pm4a* cosegregated with RFLP markers BCD1231-2A(2) and CDO678-2A, and was closely flanked by BCD1231-2A(1) and BCD292-2A both with a distance of 1.5 cM. Hartl et al. (1999) found four AFLP markers linked with *Pm4a*. The nearest markers, 4aM1 and 4aM2, were 3.5 cM from the gene *Pm4a*.

Gene *Pm12* was introgressed into wheat from *Aegilops speltoides* (Miller et al. 1988). RFLP analysis indicated that *Pm12* was located on the short arm of translocation chromosome 6BS-6SS.6SL (Jia et al. 1996). Twelve RFLP markers linked with gene *Pm12* were reported (Jia et al. 1996). Due to the lack of recombination between chromosomes 6B and 6S of *Ae. speltoides*, the genetic distance between gene *Pm12* and the RFLP markers is unknown. These markers have potential to select powdery mildew resistant lines with reduced length of the alien chromosome segment.

Gene *Pm13* transferred from *Ae. longissima* was located on the short arms of chromosomes 3B or 3D in different translocation lines. Twenty-nine RFLP clones from homoeologous group 3 were used to identify the breakpoints of the recombinant chromosomes. Only seven markers were polymorphic between translocation lines and the recipient wheat parent. Three RFLP markers can be used to identify the breakpoints of the recombinant chromosome (Donini et al. 1995). Recently, Cenci et al. (1999) reported RAPD and STS (sequence tagged site) markers linked to *Pm13*.

Triticum aestivum-Haynaldia villosa disomic 6VS/6AL translocation lines carrying powdery mildew resistance gene *Pm21* were developed from the hybridization between common wheat cultivar Yangmai5 and alien substitution line 6V(6A) (Chen et al. 1995). Eight of 180 RAPD primers were polymorphic between translocation line and Yangmai5. One RAPD marker, OPH17-1900, is specific to chromosome arm 6VS, and could be used as a molecular marker for the detection of gene *Pm21* in breeding programs (Qi et al. 1996).

Gene *Pm25* was transferred to wheat from wild einkorn (*Triticum monococcum* L. subsp. *aegilopoides* (Link) Thell). Shi et al. (1998) found three RAPD markers that were loosely linked to *Pm25*.

APR to powdery mildew in wheat is much more complex than race-specific resistance, and it is a quantitative trait by nature (Shaner and Finney 1975). To my knowledge, there is only one paper on molecular markers associated with APR. Keller et al. (1999) mapped and characterized APR to powdery mildew in a segregating population of 226 recombinant inbred (RI) lines derived from a cross between Swiss wheat variety Forno and Swiss spelt variety Oberkulmer. The RI lines were assessed for APR to powdery mildew in five environments. Quantitative trait loci (QTL) analysis was based on a genetic map containing 182 loci with 23 linkage groups. In single environment, six QTLs, 14 QTLs, six QTLs, 11 QTLs and 13 QTLs for resistance to powdery mildew were detected. Based on mean disease data over five environments, eighteen QTLs for APR to powdery mildew were detected, explaining 77% of the phenotypic variance in a

simultaneous model. However, only two QTLs were consistent over all five environments.

DNA markers associated with resistance to powdery mildew (Table 1) will facilitate the transfer and pyramiding of genes controlling powdery mildew resistance. Due to the lack of sufficient differential isolates of *B. graminis*, combination of highly effective resistance genes is not readily feasible via conventional breeding methods. It is even more difficult to identify plants with both race-specific and adult plant resistance to powdery mildew. Molecular marker-assisted selection will be helpful to develop varieties with more durable resistance to powdery mildew.

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Table 1. Chromosomal locations, sources and type of molecular markers linked with powdery mildew resistance genes in wheat

Gene	Chromosome	Source	Type of Markers
Pm1	7AL	*Triticum aestivum*	RFLP, RAPD, STS, AFLP
Pm2	5DS	*Aegilops squarrosa*	RFLP
Pm3	1AS	*T. aestivum*	RFLP
Pm4a	2AL	*T. dicoccum*	RFLP
Pm4b	2AL	*T. carthlicum*	
Pm5	7BL	*T. dicoccum*	
Pm6	2B	*T. timopheevi*	
Pm7	T4BS.4BL-2RL	*Secalis cereale*	
Pm8	T1BL.1RS	*S. cereale*	
Pm9	7AL	*T. aestivum*	
Pm10	1D	*T. aestivum*	
Pm11	6BS	*T. aestivum*	
Pm12	T6BS-6SS.6SL	*Ae. speltoides*	RFLP
Pm13	T3BL.3BS-3S^{l} T3DL.3DS-3S^{l}	*T. longissimum*	RFLP, RAPD, STS
Pm14	6B	*T. aestivum*	
Pm15	7DS	*T. aestivum*	
Pm16	4A	*T. dicoccoides*	
Pm17	T1AL.1RS	*S. cereale*	
Pm19	7D	*Ae. squarrosa*	
Pm20	T6BS.6RL	*S. cereale*	
Pm21	T6AL.6VS	*Haynaldia villosa*	RAPD
Pm22	1D	*T. aestivum*	
Pm23	5A	*T. aestivum*	
Pm24	6D	*T. aestivum*	
Pm25	1A	*T. monococcum*	RAPD

CHAPTER II.

Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey

ABSTRACT

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* E'm. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the major diseases of wheat (*Triticum aestivum* L.) worldwide. Race-specific resistance has been extensively used in wheat breeding programs, even though it is ephemeral. Adult plant resistance (APR) to powdery mildew is more durable than race-specific resistance. To develop efficient strategies for breeding for resistance to powdery mildew, it is essential to study the genetics of APR to powdery mildew. A cross was made between common wheat cultivars Becker and Massey. Seedlings of Massey are susceptible to the prevalent isolates of powdery mildew found in Virginia, yet resistance is expressed in the adult plant stage. Becker does not have any known genes for powdery mildew resistance and is susceptible to powdery mildew in both seedling and adult plant stages. Powdery mildew severity on F-2 leaves (the second leaf below the flag leaf) of 180 $F_{2:3}$ lines was rated under natural disease pressure in the field. Both RFLP and microsatellite markers were used to identify QTLs (quantitative trait loci) associated with APR to powdery mildew. Three QTLs, designated as *QPm.vt-1B*, *QPm.vt-2A* and *QPm.vt-2B*, were identified. They are located on wheat chromosomes 1B, 2A and 2B, and explained 17%, 29% and 11% of the total variation of $F_{2:3}$ lines for powdery mildew resistance, respectively. The three QTLs associated with APR to powdery mildew were derived from Massey, and displayed additive gene action. *QPm.vt-2B* also fits a recessive model for APR to powdery mildew. In a multi-QTL model, the three QTLs explained 50% of the total variation of $F_{2:3}$ lines for APR to powdery mildew. The molecular markers identified in this study have potential for use in marker-assisted selection and pyramiding of genes for resistance to powdery mildew.

Key words: *Blumeria graminis* f. sp. *tritici*, adult plant resistance (APR), QTL, RFLP and microsatellite

INTRODUCTION

Powdery mildew is one of the major diseases of wheat in the world. It is of economic importance especially in areas with maritime or semi-continental climate (Bennett 1984). Yield losses ranging from 13% to 34% have been reported (Griffey et al. 1993; Leath and Bowen 1989; Johnson et al. 1979). Resistant cultivars offer an effective, economical and environmentally safe way to control powdery mildew. There are two types of resistance to powdery mildew. One is called race-specific resistance, which is effective for some isolates of powdery mildew, but ineffective for others. Race-specific resistance genes are expressed in seedlings and throughout the vegetative cycle of wheat. Currently, thirty-three alleles at 24 loci have been reported for resistance to powdery mildew (McIntosh et al. 1998; Shi et al. 1998). Even though race-specific resistance has been extensively used in wheat breeding programs, selection pressure exerted by cultivars with race-specific resistance genes results in the rapid build-up of isolates with matching virulence genes. Subsequently, race-specific resistance breaks down when confronted by pathogen isolates with matching virulence genes and, therefore, is ephemeral.

Another type of resistance to powdery mildew is called adult plant resistance (APR), which retards infection, growth and reproduction of the pathogen in adult plants but not in seedlings. It is also called “slow mildewing” (Shaner 1973) and “partial resistance” (Hautea et al. 1987). This type of resistance can be identified in cultivars with defeated race-specific genes or lacking known race-specific resistance genes. APR to powdery mildew is more durable than race-specific resistance. For example, APR in wheat cultivar Knox and its derivatives remained effective against powdery mildew infection during the 20 years in which these cultivars were grown commercially (Shaner 1973). Massey, a derivative of Knox62, was released from Virginia Tech in 1981 (Starling et al. 1984), and still has effective powdery mildew resistance in adult plants.

To improve the efficiency of wheat breeding for APR to powdery mildew, it is essential to understand the genetic basis of APR. Being a quantitative trait by nature, APR to powdery mildew in wheat is much more complex than race-specific resistance (Shaner

and Finney 1975). Using monosomic analysis, Chae and Fischbeck (1979) reported that 14 chromosomes were involved in APR to powdery mildew in cultivar Diplomat. Both qualitative and quantitative genetic studies indicated that APR to powdery mildew was governed by two to three genes, with moderate to high heritability, in four cultivars, Knox 62, Massey, Redcoat and Houser (Griffey and Das 1994; Das and Griffey 1994b). Hautea et al. (1987) reported that additive gene effects were the most important for APR to powdery mildew in four spring wheat crosses. Das and Griffey (1995) also reported that additive gene effects were predominant, but non-additive effects also were significant for APR to powdery mildew.

The lack of extensive genetic information hinders the development of effective wheat breeding strategies for APR to powdery mildew. The objectives of this study were to identify molecular markers associated with APR to powdery mildew in common wheat cultivar Massey, to localize the QTLs to wheat chromosomes, and to study the gene action of APR to powdery mildew.

MATERIALS AND METHODS

Plant Materials

A cross was made between common wheat cultivars Becker and Massey. Seedlings of Massey are susceptible to the prevalent isolates of powdery mildew found in Virginia, yet resistance is expressed in the adult plant stage (Starling et al. 1984). Becker does not have any known genes for powdery mildew resistance and is susceptible to powdery mildew in both seedling and adult plant stages. Leaf tissue of 180 $F_{2:3}$ lines (30 to 40 plants per $F_{2:3}$ lines) was used for DNA extraction.

Powdery Mildew Assessment

From the cross between Becker and Massey, 180 $F_{2:3}$ lines with 50 seeds per line were space-planted at Warsaw, Virginia, in October 1994. A row of each parent was planted every 20 rows. Several rows of Becker were planted around the $F_{2:3}$ population to ensure ample powdery mildew inoculum. Average powdery mildew severity on F-2 leaves (the

second leaf below the flag leaf) of each $F_{2:3}$ plant was assessed under natural disease pressure using the James disease assessment key (James 1971). Briefly, the disease severity was rated based on the percentage leaf area covered by powdery mildew. The $F_{2:3}$ plants were rated as 50% when the F-2 leaves were totally covered by powdery mildew. A score of 0% was given for the plants without powdery mildew on F-2 leaves. The disease severity of each $F_{2:3}$ plant, in a $F_{2:3}$ row, was averaged to obtain mean mildew severity for each $F_{2:3}$ line.

A single head of each plant from the putative homozygous resistant and homozygous susceptible $F_{2:3}$ lines was harvested. Twenty randomly selected heads from each putative homozygous $F_{2:3}$ line were planted to test the homogeneity for reaction to powdery mildew of $F_{3:4}$ lines in the adult plant stage. The powdery mildew reaction of $F_{3:4}$ lines was used to select the best $F_{2:3}$ lines for bulked segregant analysis (BSA).

RFLP Analysis

A total of 213 clones including WG (wheat genomic), BCD (barley cDNA), CDO (oat cDNA), ABC (barley cDNA), KSU (wheat cDNA) and RZ (rice cDNA) clones was used to survey polymorphism between Becker and Massey. Two DNA bulks were made by mixing equal amount of DNA from four putative homozygous resistant and from four putative homozygous susceptible $F_{2:3}$ lines, respectively. The two DNA bulks were also included in the polymorphism survey to eliminate from consideration those polymorphic markers that were less likely to be associated with APR to powdery mildew. Markers that showed a similar pattern of polymorphism between the parents and between the two bulks, were considered as putative resistance-related markers (Fig. 1). These markers were used to probe the $F_{2:3}$ lines to test for their association with APR to powdery mildew. According to published wheat and barley genetic linkage maps, more polymorphic markers in the vicinity of the putative resistance-related markers were used to probe the $F_{2:3}$ lines.

Four restriction enzymes (*Eco*RI, *Eco*RV, *Dra*I and *Hind*III) were used to digest the genomic DNA. DNA isolation and RFLP procedures were as described by Saghai Maroof et al. (1984, 1996).

Microsatellite Analysis

A total of 139 wheat microsatellite primer pairs from published papers (Roder et al. 1998; Stepheson et al. 1998; Ma et al. 1996) was used to survey the polymorphism between Becker and Massey and between the same two bulks used in RFLP analysis. PCR reactions were performed in a total volume of 10 µl in a Perkin-Elmer thermal cycler. After an initial denaturing step for 3 min at 94 ^{0}C, 32 cycles were performed with 0.5 min at 94 ^{0}C, 0.5 min at either 47 ^{0}C, 55 ^{0}C or 60 ^{0}C (depending on the primer pair), and 1 min at 68 ^{0}C, followed by a final extension step of 7 min at 68 ^{0}C. The reaction mixture and polyacrylamide gel electrophoresis were as described by Saghai Maroof et al. (1994).

Statistical Analysis

Software JMP IN 3.1 (Sall and Lehman 1996) was used for statistical analyses. Due to the skewed distribution of disease severity among $F_{2:3}$ lines (see results), $\log_{10}$ transformed data were used in all statistical analyses. One-way ANOVA was conducted to confirm significant ($P< 0.05$) association between putative resistance-related markers and APR to powdery mildew. Chi-square tests were used to test Mendelian segregation ratio (1:2:1) for codominant markers.

Interval Mapping

Genetic linkage maps were constructed using MAPMAKER 3.0b (Lander et al. 1987). A threshold log likelihood ratio (LOD) of 2.0 or greater was used with "ripple" command to assign markers to map positions. Markers not meeting that threshold were placed in the intervals using the MAPMAKER "try" command. Centimorgan (cM) values were calculated using the Haldane mapping function (Haldane 1919). Linkage groups were scanned for the presence of QTLs at a LOD threshold of 3.0 at every 2.0 cM interval using MAPMAKER/QTL 1.1b with a free model (Lincoln et al. 1992; Paterson et al. 1988). The QTLs were designated according to the guidelines for nomenclature of

quantitative trait loci in wheat (McIntosh et al. 1998). Pm and vt (*V*irginia *T*ech) were used for trait designator and laboratory designator, respectively. Gene action was tested by fitting QTLs to particular genetic models, either dominant, recessive or additive. One LOD score less than the free model indicated that a constrained model was unlikely.

RESULTS

Among the 180 $F_{2:3}$ lines derived from the cross Becker/Massey, five lines were verified as homozygous resistant and four lines as homozygous susceptible to powdery mildew based on disease data of the $F_{3:4}$ lines. All other $F_{2:3}$ lines segregated for powdery mildew resistance. The observed segregation pattern fit a 1:62:1 ratio (P = 0.32), indicating the likelihood of three genes conferring APR to powdery mildew in Massey. The distribution of powdery mildew severity of $F_{2:3}$ lines (Fig. 2.) was skewed toward lower disease severity and deviated significantly from a normal distribution. The continuous distribution also indicates that more than one gene likely controls APR to powdery mildew in this population.

Among 213 RFLP markers, 88 (41.3%) were polymorphic between Becker and Massey. The microsatellite markers showed much higher polymorphism between the two parents. Ninety (64.7%) markers were polymorphic among 139 microsatellites surveyed. In total, 178 (50.6%) markers were polymorphic between Becker and Massey. For some of the polymorphic markers, the bulked DNA showed similar banding patterns as that of the corresponding parents. The putative resistance-related markers were used initially to genotype the $F_{2:3}$ lines.

One-way ANOVA was performed to assess the relationship between putative resistance-related markers and APR to powdery mildew. Due to the skewed distribution of disease severity of $F_{2:3}$ lines, $\log_{10}$ transformed data were used for ANOVA. The results are shown in Table 1, and the low P-value indicates that these markers are associated with APR to powdery mildew.

According to the known wheat and barley genetic linkage maps (http://wheat.pw.usda.gov/), more polymorphic markers that were likely linked to the putative resistance-related markers were used to genotype the $F_{2:3}$ lines. Genetic linkage maps (Fig. 3) were constructed using MAPMAKER 3.0. Because most of the microsatellite markers are genome specific, based on the published wheat microsatellite genetic map (Roder et al. 1998), QTLs associated with APR to powdery mildew were located on chromosomes 1B, 2A and 2B.

Most of the markers located on chromosome 2B did not fit the 1:2:1 ratio for codominant markers (Table 2). Segregation distortions favoring the Massey allele were observed. Because of segregation distortion, the genetic distance is significantly reduced when compared with published genetic maps of wheat (Roder et al. 1998). Also, order of the markers shows some difference from the published maps. Devos et al. (1993) reported similar results for the genetic map of chromosome 2B in the cross 'Timgalen' × RL4137. The *T. timopheevi* chromosome segment introgressed into chromosome 2B in Timgalen was preferentially transmitted. Recombination was also greatly reduced in that segment.

Interval mapping was conducted with MAPMAKER/QTL 1.1. The likelihood plots are shown in Fig. 3. The results indicate that chromosome 2A is very important for APR to powdery mildew. The QTL on chromosome 2A, designated as *QPm.vt-2A*, has the highest LOD score (9.23). *QPm.vt-2A* is located at the microsatellite marker interval, GWM304a-GWM312, and is 12.0 cM from GWM304a. It explained 29% of the total variation of the $F_{2:3}$ lines (Table 3). The resistance conferred by *QPm.vt-2A* was derived from Massey. Both the dominant (LOD = 4.47) and recessive models (LOD = 7.82) were deemed unlikely. This QTL fits an additive model with a LOD of 8.65.

The QTL detected on chromosome 2B was designated as *QPm.vt-2B*. This QTL peaked at the RFLP marker WG338 with a LOD score of 4.34 (Table 3). *QPm.vt-2B* explained 11% of the total variation of $F_{2:3}$ lines. Only the dominant mode was deemed unlikely. The LOD scores for recessive and additive models were 4.30 and 3.64, respectively, suggesting recessive or additive gene effects for this QTL.

The third QTL, designated as *QPm.vt-1B*, is located on chromosome 1B. The log-likelihood plot peaked at the interval between microsatellite marker GWM259 and RFLP marker WG241 with a LOD score of 5.25 (Table 3). *QPm.vt-1B* was 8.0 cM from microsatellite marker GWM259 and explained 17% of the total variation of $F_{2:3}$ lines. *QPm.vt-1B* was derived from Massey and fits an additive model with a LOD of 5.24. Both dominant and recessive models were rejected as unlikely. The three QTLs explain 50% of the total variation of $F_{2:3}$ lines in a multi-QTL model.

DISCUSSION

Genetics of APR to Powdery Mildew in Wheat

The results of this study are in agreement with the classical genetic studies of APR to powdery mildew in Massey. Griffey and Das (1994) reported that two to three genes control APR to powdery mildew in Massey. In this study, three QTLs for APR to powdery mildew were detected in the Becker/Massey $F_{2:3}$ mapping population. The three QTLs were located on the chromosomes 1B, 2A and 2B, respectively. Not only the number of genes, but also the gene action is in agreement with the previous studies. The predominance of additive genetic effects for APR to powdery mildew has been previously reported (Hautea et. al 1987; Das and Griffey 1994a, 1995). In the present study, all three QTLs had significant additive effects on APR to powdery mildew. Das and Griffey (1995) reported that susceptibility was dominant in the cross Becker/Massey. Similarly, we found that *QPm.vt-2B* is recessive for APR to powdery mildew in the same cross.

Recently, Keller et al. (1999) reported QTLs for APR to powdery mildew in a segregating wheat/spelt population. With the method of composite interval mapping, 18 QTLs were detected, explaining 77% of the phenotypic variance in a simultaneous fit model. Only two QTLs with major effects were consistent over all five environments. It is interesting to note that the QTL on chromosome 2A identified by Keller et al. (1999) is in a similar genomic region as *QPm.vt-2A* identified in present study. As found in the

current study, additive effects were the predominant factor of inheritance in the mapping population of Keller et al. (1999). However, the number of QTLs is quite different from the current study. This may be explained by the fact that the parents of the two mapping populations are very different. Also, the difference may be due to the different experimental environments in the two studies.

Application of Microsatellite Markers in Wheat Genetic Mapping

Microsatellite markers are highly polymorphic in wheat (Roder et al. 1998). Among 139 microsatellite markers, 90 (64.7%) were polymorphic between Becker and Massey. In contrast to microsatellite markers, only 41.3% of RFLP markers were polymorphic for the same parents. Without microsatellite markers, it would be difficult to identify QTLs using a mapping population derived from an intraspecific cross such as in this study. For example, 213 RFLP markers were tested for polymorphism between Becker and Massey. Only 88 (41.3%) were polymorphic, and none of them mapped to the vicinity of *QPm.vt-2A*. In other words, it was almost impossible to detect *QPm.vt-2A* without microsatellite markers in this mapping population.

Microsatellites are not only highly polymorphic, but most of them are also genome-specific in wheat (Roder et al. 1998) and amplify only a single locus from one of the three genomes. This characteristic is very helpful to assign QTLs to specific chromosomes based on linkage with microsatellite markers. In this study, QTLs associated with APR to powdery mildew were located to wheat chromosomes 1B, 2A and 2B, based on linkage with genome-specific microsatellite markers.

Microsatellites are abundant in the wheat genome (Roder et al. 1995; Ma et al. 1996), easy to detect via PCR and easy to disseminate among laboratories. Currently, at least two microsatellite maps of wheat are available. A total of 279 loci amplified by 230 primer sets was placed onto a genetic framework map composed of RFLPs previously mapped in the reference population of the International Triticeae Mapping Initiative (ITMI) Opata × W7984 (Roder et al. 1998). Another 53 microsatellite loci were also mapped in wheat (Stephenson et al. 1998). Furthermore, microsatellite analyses can be

easily automated and are suitable for large-scale screening. As has been demonstrated by a genetic diversity study among adapted European wheat cultivars (Plaschke et al. 1995), and microsatellite tagging of dwarfing genes in wheat (Korzun et al. 1997, 1998), microsatellite markers will have extensive applications in wheat genetic mapping, especially for the intraspecific crosses among adapted wheat lines.

Marker Assisted-selection for Resistance to Powdery Mildew

Even though APR to powdery mildew is more durable than race-specific resistance, selection of APR to powdery mildew is a time-consuming process involving extensive and precise quantitative measurements (Gustafson and Shaner 1982). The three QTLs for APR to powdery mildew identified in the present study explain 50% of the total phenotypic variation of $F_{2:3}$ lines in a multi-QTL model. The molecular markers, especially the microsatellite markers, associated with the three QTLs have potential for use in marker-assisted selection for APR to powdery mildew.

Based on phenotype only, it is difficult to identify plants with both APR and race-specific resistance to powdery mildew. Fortunately, molecular markers linked to genes *Pm1* (Ma et al. 1994; Hartl et al. 1995, 1999; Hu et al. 1997), *Pm2* (Ma et al. 1994; Hartl et al. 1995), *Pm3* (Hartl et al. 1993; Ma et al. 1994), *Pm4* (Ma et al. 1994; Hartl et al. 1999), *Pm12* (Jia et al. 1996), *Pm13* (Donini et al. 1995; Cenci et al. 1999), *Pm21* (Qi et al. 1996) and *Pm25* (Shi et al. 1998) have been reported. With marker-assisted selection, it is also possible to select plants with both APR and race-specific resistance to powdery mildew.

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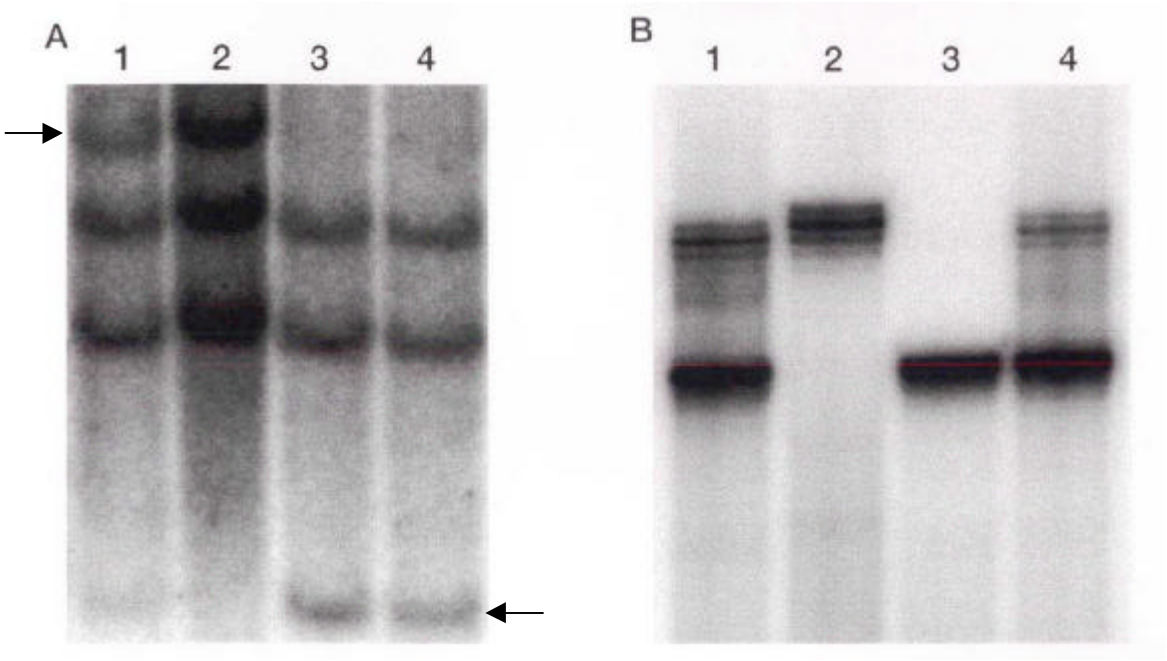


Fig. 1. Autoradiogram of RFLP marker KSUD22 (A) and microsatellite marker GWM261 (B) showing the difference between a putative resistance-related marker and a marker unlinked with resistance, respectively. Lanes: 1, susceptible bulk; 2, susceptible parent, Becker; 3, resistant parent, Massey; 4, resistant bulk. The arrows indicate the polymorphic bands between Becker and Massey.

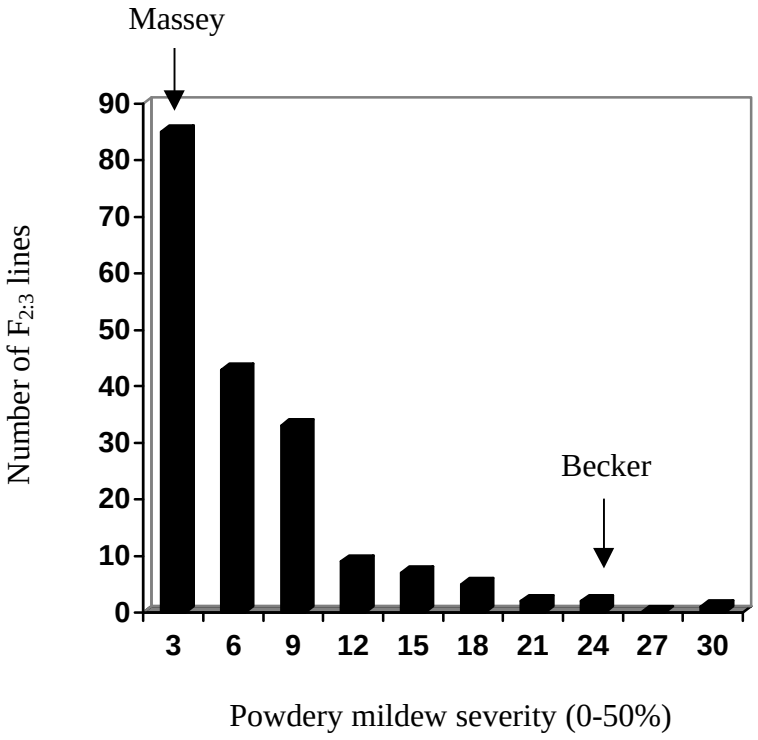
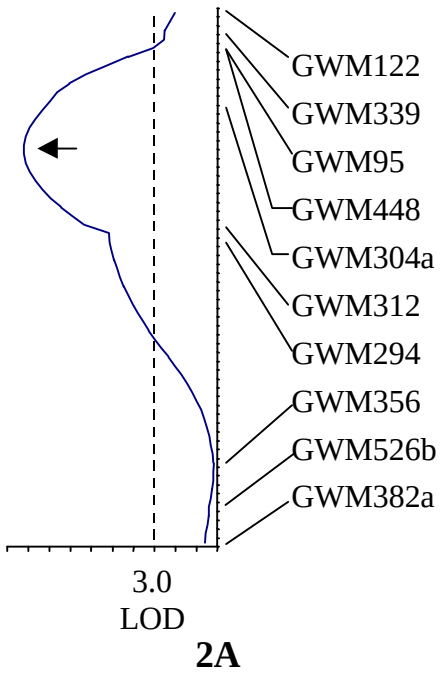
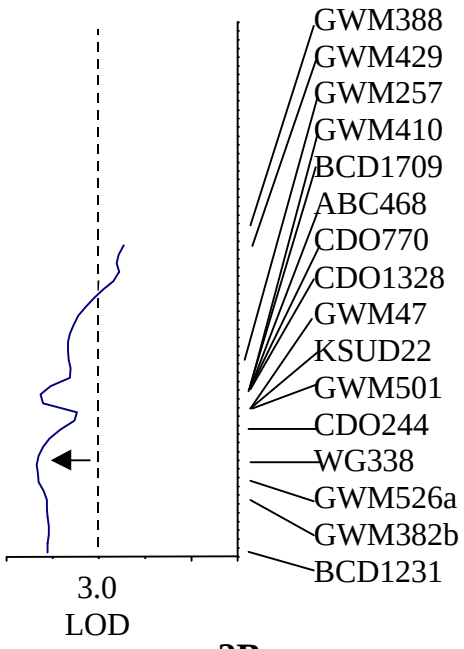


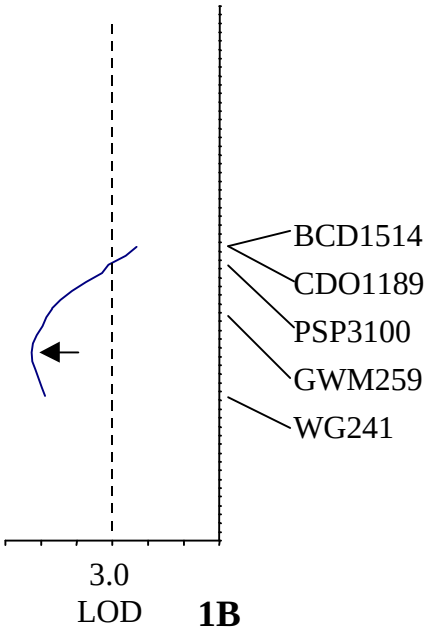
Fig. 2. Distribution of powdery mildew severity of $F_{2:3}$ lines derived from Becker/Massey cross. The powdery mildew severity of the two parents Becker and Massey is indicated by arrows.



2A



2B



1B

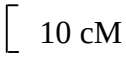


Fig. 3. The likelihood plots of QTLs associated with APR to powdery mildew in a common wheat cultivar Massey. The arrows indicate the most likely positions of the QTLs. The vertical dashed lines at LOD 3.0 represent the minimum LOD required for significance.

Table 1. One-way ANOVA for the putative powdery mildew resistance-related markers in the Becker/Massey $F_{2:3}$ population

Markers	Chromosome	df of error[a]	Probability	R^{2b}
GWM304a	2A	167	<0.0001	18.3
GWM312	2A	176	<0.0001	15.1
WG338	2B	129	0.0003	11.8
GWM526a	2B	160	<0.0001	12.5
PSP3100	1B	112	0.0004	13.0
WG241	1B	130	<0.0001	13.6

[a] Degrees of freedom of error
[b] Coefficient of determination

Table 2. Chi-square test for Mendelian segregation ratio (1:2:1) of markers located on chromosome 2B in the cross Becker/Massey

Markers	A[a]	H[b]	B[c]	P-value
ABC468	28	73	55	0.0068
BCD1709	29	83	52	0.0392
WG338	24	56	52	0.0006
GWM257	27	63	56	0.0008
GWM410	30	86	51	0.0662
GWM501	28	91	55	0.0126
GWM526a	32	62	67	<0.0001
GWM47	28	86	62	0.0013
KSUD22	26	80	62	0.0004

[a] Becker type
[b] Heterozygous type
[c] Massey type

Table 3. QTLs associated with APR to powdery mildew in common wheat cultivar Massey

QTLs	Chro.[a]	Interval	Pos[b]	%Var. [c]	LOD	Add.[d]	Dom.[e]	Mode[f]
QPm.vt-1B	1B	GWM259 /WG241	8.0	17	5.25	-0.2844	-0.0186	A
QPm.vt-2A	2A	GWM304a/ GWM312	12.0	29	9.23	-0.3467	0.1204	A
QPm.vt-2B	2B	WG338 /GWM526a	0.0	11	4.34	-0.1736	0.1319	R,A

[a] Chromosomal locations of QTLs for APR to powdery mildew

[b] Position of the highest LOD score in terms of genetic distance (cM) from the first marker of the support interval

[c] Percentage of variance explained by individual QTL

[d] Additive effect due to substitution of a Becker allele by the corresponding Massey allele

[e] Dominance effect of individual QTL

[f] The possible pure modes of gene action for each QTL (A-additive; R-recessive)

CHAPTER III.

Verification of molecular markers associated with adult plant resistance to powdery mildew with recombinant inbred lines

ABSTRACT

Powdery mildew caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* E'm. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is a devastating disease of wheat (*Triticum aestivum* L.). Massey has retained adult plant resistance (APR) to powdery mildew under commercial cultivation since its release in 1981. Three quantitative trait loci (QTLs) associated with APR to powdery mildew in Massey were identified in a previous study. The objective of the present study was to verify the QTLs using 97 recombinant inbred (RI) lines derived from a cross between Massey and a mildew susceptible cultivar Becker. The RI lines were evaluated for APR to powdery mildew under natural disease pressure in the field for three years. Both single marker analysis and interval mapping confirmed the presence of the three QTLs, *QPm.vt-2A*, *QPm.vt-2B* and *QPm.vt-1B*, which explained 26%, 15% and 15% of the variation of mean powdery mildew severity of the RI lines over the three years, respectively. Cumulatively, the three QTLs accounted for 44% of the phenotypic variation of the RI lines. The RI lines were grouped according to genotype for the three QTLs , represented by markers GWM304a, KSUD22 and PSP3100, respectively. The RI lines with Massey alleles at all three loci had a mean disease severity of 3.4%, whereas the RI lines with Becker alleles at all three loci had a mean disease severity of 22.3%. These values are similar to those of the corresponding parents. The molecular markers associated with APR to powdery mildew may facilitate marker-assisted selection for powdery mildew resistance and a better understanding of the molecular basis of APR.

Key words: *Blumeria graminis* f. sp. *tritici*, adult plant resistance (APR), QTL, RFLP, microsatellite, RI line and verification

INTRODUCTION

Powdery mildew caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* E'm. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is a major disease of wheat (Bennett 1984). Extensive studies have been conducted to identify sources of powdery mildew resistance and transfer resistance genes from alien species to wheat. As many as 34 alleles at 24 loci have been reported for resistance to powdery mildew in wheat (McIntosh et al. 1998; Shi et al. 1998). However, most of these resistance genes either have been overcome by the pathogen or are associated with large alien chromosome fragments from the donor species. Thus, durable and easily deployed powdery mildew resistance genes are highly desirable.

Adult plant resistance, which retards infection and also growth and reproduction of the pathogen in adult plants but not in seedlings, has been repeatedly reported as being durable around the world. In USA, APR to powdery mildew in wheat cultivar Knox and its derivatives remained effective during the 20 years in which these cultivars were grown commercially (Shaner 1973). In Switzerland, the cultivars Probus, Zenith and Arina have shown stable intermediate resistance for many years (Keller et al. 1999). Similar examples have also been found in UK (Bennett 1984) and Germany (Chae and Fischbeck 1979).

With the development of molecular marker technologies, high density genetic maps are available for many crop species. It is possible to identify, map and estimate the effects of quantitative trait loci (Tanksley 1993). A number of quantitative traits have been mapped in wheat, such as kernel hardness (Sourdille et al. 1996), high protein content (Mesfin et al. 1999), flour viscosity (Udall et al. 1999), kernel morphology and texture (Campbell et al. 1999), and resistance to leaf rust (Nelson et al. 1997; William et al. 1997), karnal bunt (Nelson et al. 1998), fusarium head blight (Bai et al. 1999; Waldron et al. 1999) and preharvest sprouting (Anderson et al. 1993). There are few studies about QTLs associated with APR to powdery mildew in wheat. Using recombinant inbred (RI) lines derived from a cross between wheat variety Forno and spelt variety Oberkumer, Keller et

al. (1999) reported 18 QTLs associated with powdery mildew resistance. Ten of the 18 QTLs were derived from the common wheat parent, the other eight QTLs were derived from the spelt parent. The 18 QTLs explained 77% of the phenotypic variance in a simultaneous fit model. However, only two QTLs with major effects were consistent over all five testing environments.

Estimates of the presence and effects of QTLs are subject to experimental error. The spurious or biased effect estimate of QTLs could be caused by genotyping and phenotyping errors (Lander and Botstein 1989), statistical fluctuations (Lander and Kruglyak 1995), and errors associated with sampling of progenies used in mapping populations and with the sampling of environments used in QTL experiments. Stuber et al. (1992) and Beavis et al. (1994) identified different yield QTLs for the same maize cross. The latter authors pointed out that sampling of progeny was the most likely explanation for the difference. Using different testers and independent samples in maize, Melchinger et al. (1998) observed a low power of QTL detection and a large bias in estimates of QTL effects. It may, therefore, be necessary to conduct additional experiments to verify the presence and effects of such QTLs.

The advantages of recombinant inbred (RI) lines for detecting QTL have been described (Burr et al. 1988; Lander and Botstein 1989; Burr and Burr 1991). RI lines are permanent mapping populations, which can be used indefinitely for mapping. The phenotype of each RI line is represented by a homozygous line, rather than by an individual plant. Replicated phenotypic measurements can also reduce environmental variance. Greater precision in trait measurements will allow detection of QTLs with smaller effects and provide more accurate estimates of QTL effects. Increased recombination between tightly linked loci also will allow for better genetic resolution of QTL.

Using an $F_{2:3}$ mapping population, three QTLs, *QPm.vt-1B*, *QPm.vt-2A* and *QPm.vt-2B*, were identified for APR to powdery mildew in the common wheat cultivar Massey (Liu

1999). The objective of this study was to verify the three QTLs associated with APR to powdery mildew using RI lines.

MATERIALS AND METHODS

Development of RI Lines and Disease Assessment

A cross was made between common wheat cultivars Becker and Massey. Seedlings of Massey are susceptible to the prevalent isolates of powdery mildew found in Virginia, yet resistance is expressed in the adult plant stage (Starling et al. 1984). Becker does not have any known genes for powdery mildew resistance and is susceptible to powdery mildew in both seedling and adult plant stages. Seed from a single head of each of 97 F_2 plants, randomly selected among 200 F_2 plants, were planted in individual head rows in field at Warsaw, Virginia, in October 1993. A row of each parent was also planted every 10 rows. A randomly selected single head from each row was harvested and planted in a head row in each consecutive year. By the 1998-1999 growing season, 97 $F_{7:8}$ RI lines were obtained.

Several rows of Becker were planted around the head rows to enhance the development of natural powdery mildew inoculum. Average disease severity on F-2 leaves (the second leaf below flag leaf) of each head row was assessed under natural pressure of powdery mildew using the James disease assessment key (James 1971). Briefly, the disease severity was rated based on the percentage leaf area covered by powdery mildew. The plants were rated as 50% when the F-2 leaves were totally covered by powdery mildew. A score 0% was given for the plants without powdery mildew on F-2 leaves. Disease data collected from $F_{5:6}$, $F_{6:7}$ and $F_{7:8}$ head rows were used for verification of the QTLs identified in the previous study. Heritability of APR to powdery mildew was calculated by the variance component method (Fehr 1987). Each of the three year's disease data was considered a replicate for the purpose of obtaining an estimate of heritability.

After random single head selection, the $F_{6:7}$ lines were bulk harvested for each row. Forty seeds of each $F_{6:7}$ line were planted in the greenhouse and the leaf tissue was used for DNA extraction.

Molecular Marker Analysis

The focus of this study was to verify QTLs associated with APR to powdery mildew. Based on the results of molecular marker analysis of APR to powdery mildew in the $F_{2:3}$ generation (Liu1999), only polymorphic markers between the parents, located on chromosomes 1B, 2A and 2B, were used to genotype the RI lines. The RFLP and SSR procedures were as previously described by Liu (1999). RI lines with heterozygous marker pattern were scored as missing data for that marker.

Data Analysis

According to the previous results (Liu1999), one-way ANOVA was conducted to test for significant association ($P < 0.05$) between critical markers (with high R^2 in $F_{2:3}$ generation) and APR to powdery mildew of RI lines. Then, other markers in the vicinity of the critical markers were used to genotype the RI lines. Genetic linkage maps were constructed using MAPMAKER/EXP 3.0b (Lander et al. 1987). A threshold log likelihood ratio (LOD) of 2.0 or greater was used with "ripple" command to assign markers to map positions. Markers not meeting the threshold were placed in intervals using the MAPMAKER "try" command. Centimorgan (cM) values were caculated using the Haldane mapping function (Haldane 1919). Interval mapping was conducted using MAPMAKER/QTL 1.9 (Lincoln et al. 1992; Paterson et al. 1988). Considering that this is a verification study, a LOD score of 2.1 was used as the threshold, which corresponds to a point-wise significance level of $P = 0.001$ (Lander and Kruglyak 1995).

RESULTS

Analysis of Phenotypic Data

Powdery mildew severity on the parents ranged from 0.3 to 3% for Massey and 20 to 45% for Becker among replicates over three years. The average powdery mildew

severity of RI lines was 6, 12 and 7% in 1997, 1998 and 1999, respectively. Higher disease pressure resulted in higher average powdery mildew severity in 1998. The disease data over three years were highly correlated, and the coefficients of correlation were as follows: 1997 and 1998 ($r = 0.76$, $P < 0.001$); 1997 and 1999 ($r = 0.81$, $P < 0.001$); 1998 and 1999 ($r = 0.81$, $P < 0.001$). The heritability of APR to powdery mildew was 0.75. The distribution of average disease severity over three years was continuous and skewed toward lower disease severity (Fig. 1), suggesting quantitative inheritance of APR to powdery mildew.

Single Marker Analysis of APR to Powdery Mildew

Based on the results of molecular marker analysis of the $F_{2:3}$ generation (Liu 1999), the critical markers associated with APR to powdery mildew were initially used to genotype the RI lines. One-way ANOVA for each marker was conducted to determine its effect on APR to powdery mildew (Table 1). Results indicated that all the markers had significant ($P < 0.05$) effects on mean disease severity over three years. The marker GWM304a located on chromosomes 2A had the largest effect on APR to powdery mildew. Except for marker PSP3100, the remaining markers in Table 1 were significantly associated with disease data of each generation. Marker PSP3100 located on chromosome 1B had a slightly higher P-value (0.0826) for disease data of the $F_{7:8}$ lines. Therefore, the single marker analysis of RI lines verified the presence of the three QTLs for APR to powdery mildew identified in the $F_{2:3}$ generation.

Interval Analysis of APR to Powdery Mildew

A total of 29 polymorphic markers located on chromosomes 1B, 2A and 2B was mapped with the RI lines. Genetic maps were constructed using MAPMAKER/EXP 3.0b (Fig. 2). In terms of marker order and genetic distance between markers, the maps were similar to the maps obtained from $F_{2:3}$ data (Liu1999). The mean disease severity of RI lines over three years was analyzed by interval mapping using software MAPMAKER/QTL 1.9. The likelihood plots are shown in Fig. 2.

Consistent with $F_{2:3}$ data, chromosome 2A had the highest LOD score (4.65) at the interval between microsatellite markers GWM304a and GWM312. The peak was 6 cM from marker GWM304a. *QPm.vt-2A* explained 26% of the phenotypic variation of RI lines. *QPm.vt-2B* was also verified with the RI lines. The LOD score peaked at the interval between RFLP markers KSUD22 and WG338. The peak was 4 cM from marker KSUD22 with a LOD score of 2.89. This QTL accounted for 15% of the phenotypic variation of RI lines. *QPm.vt-1B* had a relatively small LOD score of 2.52 and explained 15% of the phenotypic variation of RI lines. The peak of the LOD score was 4 cM from microsatellite marker PSP3100. In a multi-QTL model, the three QTLs explained 44% of the phenotypic variation of RI lines.

Selection for APR to Powdery Mildew Using Molecular Markers

To demonstrate the selective effect of *QPm.vt-2A* on APR to powdery mildew, the RI lines were grouped based on genotypes of microsatellite marker GWM304a. The distributions of powdery mildew severity of the two genotypic groups of RI lines are presented in Fig. 3. The RI lines with the Massey allele are skewed toward low powdery mildew severity. Most RI lines with the Becker allele had higher powdery mildew severity. Forty RI lines with the Becker allele had a mean powdery mildew severity of 12.8%, whereas 48 RI lines with the Massey allele had a mean powdery mildew severity of 5.3% (Fig. 4A) (Nine RI lines with missing data).

To show the effects of *QPm.vt-2B* and *QPm.vt-1B* on APR to powdery mildew, the RI lines were also grouped according to genotypes revealed by markers KSUD22 and PSP3100, respectively. For RFLP marker KSUD22, RI lines carrying the Becker allele had a mean powdery mildew severity of 12.5%, whereas RI lines carrying the Massey allele had a mean powdery mildew severity of 5.7% (Fig. 4A). For microsatellite marker PSP3100, fifty-one RI lines with the Massey allele had a mean powdery mildew severity of 6.5%, and 40 RI lines with Becker allele had a mean powdery mildew severity of 10.9% (Fig. 4A).

Considering the three above-mentioned loci simultaneously, 19 RI lines with Massey alleles and 7 RI lines with Becker alleles at all three loci had a mean powdery mildew severity of 3.4 and 22.3%, respectively (Fig. 4B), which is similar to that of the corresponding parents. In other words, the three loci adequately explain differences in powdery mildew severity between the two parents.

DISCUSSION

Genetics of APR to Powdery Mildew

Previously, three QTLs associated with APR to powdery mildew in the cultivar Massey were identified in the $F_{2:3}$ generation of the cross Becker/Massey (Liu 1999). The QTL, *QPm.vt-2A*, had the major effect and accounted for 29% of the total variation of $F_{2:3}$ lines. Collectively, the three QTLs explained 50% of the total variation of powdery mildew severity of $F_{2:3}$ lines. In this study, the presence of the three QTLs was verified with RI lines. The gene effects of the three QTLs were similar in both studies. *QPm.vt-2A*, *QPm.vt-2B* and *QPm.vt-1B* accounted for 26%, 15% and 15% of the total variation of RI lines, respectively. The three QTLs cumulatively explained 44% of the total variation of RI lines.

It is interesting to note that the three QTLs are located on chromosomes 1B, 2A and 2B, respectively. Currently, there is no known powdery mildew resistance gene located on chromosome 1B (McIntosh et al. 1998). Based on molecular markers associated with *QPm.vt-1B*, this QTL is likely located on the long arm of chromosome 1B. So, it is unlikely to be homoeologous with powdery mildew resistance gene *Pm*3, which is located on 1AS (McIntosh and Bennett 1978). Genes *Pm10* and *Pm22* are both located on chromosome 1D (Peusha et al. 1996; Tosa et al. 1987), and gene *Pm25* is located on 1A (Shi et al. 1998). Because of the limited information on the precise chromosomal position of these genes, it is not possible to compare them with *QPm.vt-1B* for ortholog at this time. Ma et al. (1994) reported that *Pm4a* located on chromosome 2AL was 1.5 cM from RFLP marker BCD292, which is also in the vicinity of *QPm.vt-2B*. It is speculated that *Pm4* and *QPm.vt-2B* may be homoeologous. Based on seedling powdery mildew

reaction of Massey with isolates of known virulence, there is no indication that Massey carries gene *Pm6* (Griffey and Leath, personal communication), which is located on chromosome 2B (Nyquist 1963). To test whether *QPm.vt-2A* and *QPm.vt-2B* are homoeologous, more common markers between the two chromosomes need to be tested.

Schonfeld et al. (1996) pointed out that the loci for powdery mildew resistance were not only found in homoeologous positions, but also show a high collinearity among wheat, rye and barley genomes. It is amazing to note that gene *MlLa* governing speculated durable resistance to powdery mildew in barley was localized on barley chromosome 2 (Giese et al. 1993), which is homoeologous with wheat chromosomes of group 2. Also, the gene *MlLa* is characterized by an intermediate type of reaction to powdery mildew. This gene has been effective against powdery mildew in barley for a number of years (Jorgenesen 1983). Determining whether *MlLa* is homoeologous with *QPm.vt-2A* and *QPm.vt-2B* should be pursued in the future.

Development of Cultivars with Durable Resistance to Powdery Mildew

Race-specific resistance to powdery mildew has been extensively studied and used in wheat breeding, but it has contributed remarkably little to long-term, effective disease control because populations of the pathogen generally have adapted rapidly to cultivars with this type of resistance. Cultivars with race-specific resistance provide only temporary control of powdery mildew, lasting two to five years (Brown et al. 1997). Johnson (1984) defined durable resistance as resistance that remains effective during its prolonged and widespread use in an environment favorable to the disease. Prior evidence has demonstrated that APR to powdery mildew is durable. APR remained effective in the winter wheat cultivar Knox and its derivatives during the 20 years in which these cultivars were grown commercially (Shaner 1973). Massey, a derivative of Knox, was released from Virginia Tech in 1981 (Starling et al. 1984), and is still effective against powdery mildew in adult plants.

Pyramiding resistance genes of different mechanisms into one variety is a common strategy to develop varieties with more durable disease resistance. However, it has been

difficult to measure the level of APR when race-specific resistance genes are present. Marker-assisted selection may allow genes with overlapping effects to be efficiently combined. The level of APR to powdery mildew can be estimated based on the presence of markers associated with the resistance genes. In the present study, markers GWM304a, KSUD22, PSP3100 and their closely linked markers can be used to detect the QTLs for APR to powdery mildew derived from cultivar Massey. Therefore, the molecular markers associated with APR to powdery mildew may be useful to develop varieties with durable resistance to powdery mildew.

Genetic Mapping of Other Agronomic Traits Using the RI Lines

The RI lines developed in this study are useful for genetic mapping of other agronomic traits, especially for traits that are difficult to identify individually. Since a genotype is represented by an inbred line, rather than by an individual plant, a more accurate assessment of the trait can be obtained. Also, the RI lines can be evaluated in many different environments. Genotype × environment interaction therefore can be assessed.

As many as 217 RFLP markers and 139 microsatellite markers have been tested for polymorphism between Becker and Massey, a total of 178 markers was polymorphic (Liu1999). Furthermore, more than 50 markers have been mapped using the RI lines in this study, which are useful information for mapping other agronomic traits. The amount of effort for future molecular marker analysis of this population will be greatly reduced.

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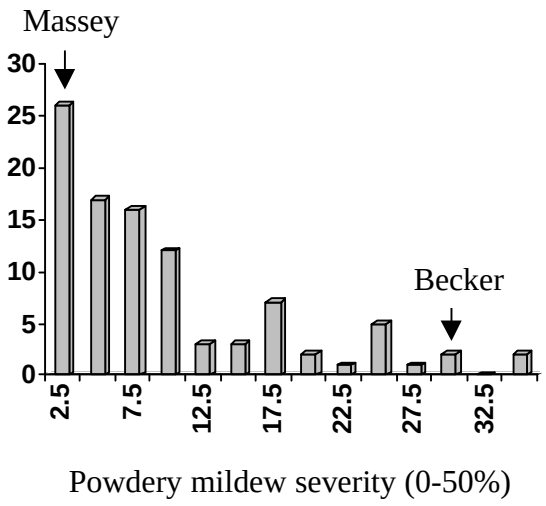
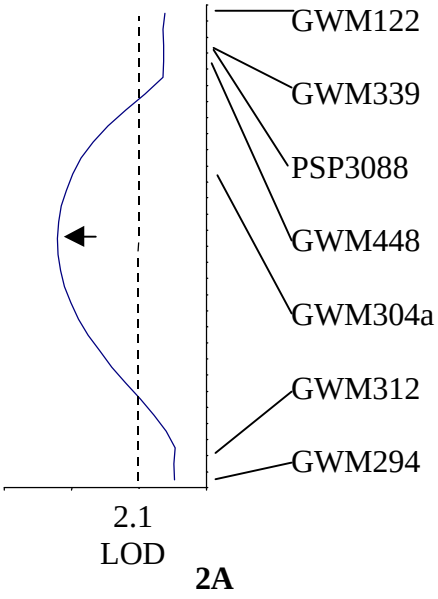


Fig. 1. Histogram of mean powdery mildew severity over three years for 97 RI lines derived from the cross Becker/Massey. The powdery mildew severity of the two parents Becker and Massey is noted by arrows.



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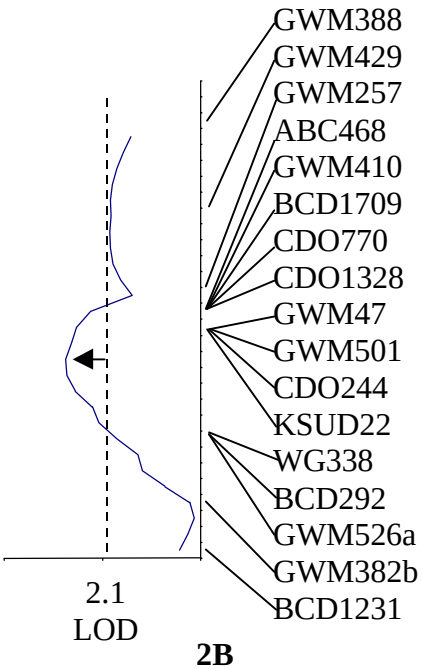

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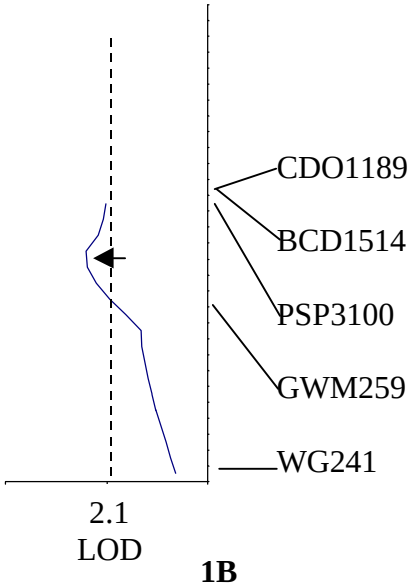


1B

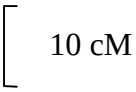


Fig. 2 The likelihood plots of QTLs associated with APR to powdery mildew for Becker/Massey RI lines. The arrows point to the most likely positions of the QTLs. The vertical dashed lines at LOD 2.1 represent the LOD threshold.

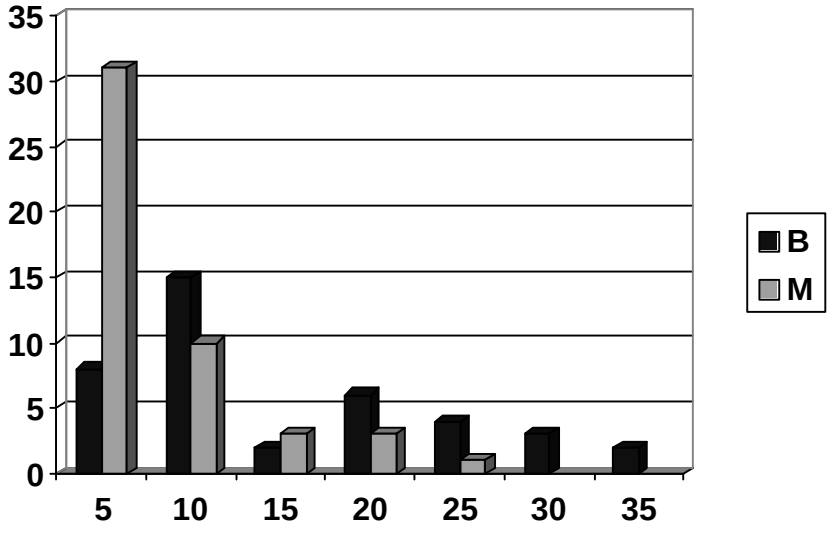
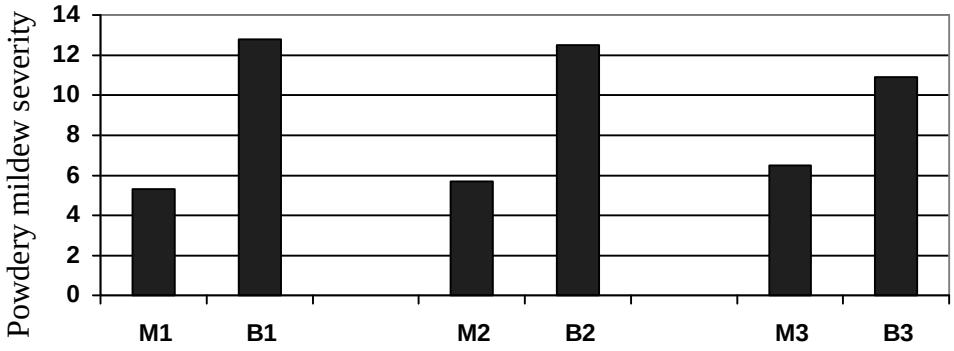
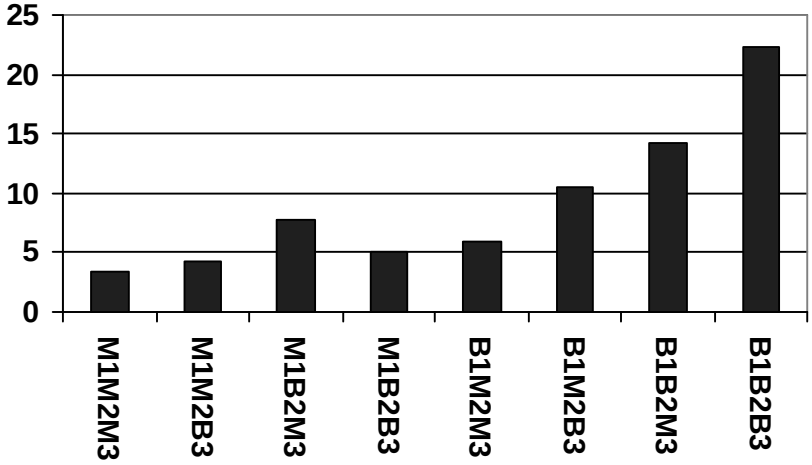


Fig. 3. Distribution of powdery mildew severity of RI lines with the Becker allele (B) or the Massey allele (M) for marker GWM304a.



A



B

Fig. 4. Mean powdery mildew severity (0-50%) over three years of RI lines derived from the cross Becker/Massey. A: grouped by markers GWM304a, KSUD22 and PSP3100, respectively; B: grouped by the three markers simultaneously. B and M represent Becker and Massey alleles, respectively. The markers GWM304a, KSUD22 and PSP3100 are represented by 1, 2 and 3, respectively.

Table 1. One-way ANOVA to verify molecular markers associated with APR to powdery mildew in Becker/Massey RI lines

Markers	Chromosomes	$F_{5:6}$			$F_{6:7}$			$F_{7:8}$			Mean		
		df[a]	R^{2b}	P	df	R^2	P	df	R^2	P	df	R^2	P
GWM304a	2A	74	14.5	0.0007	86	22.6	<0.0001	85	12.3	0.0008	86	20	<0.0001
WG338	2B	66	7.2	0.0275	79	9.8	0.0044	79	13.5	0.0007	79	11	0.0024
KSUD22	2B	69	18.6	0.0002	82	11.5	0.0016	82	16.5	0.0001	82	15.8	0.0002
PSP3100	1B	76	6.0	0.0312	89	9.2	0.0035	88	3.4	0.0826	89	7.3	0.0095
CDO1189	1B	71	8.7	0.0115	84	11.7	0.0013	84	5.9	0.0248	84	10.3	0.0026

[a] Degrees of freedom of error
[b] Coefficient of determination

CHAPTER IV.

Summary and Future Research

Adult plant resistance (APR) to powdery mildew is durable as demonstrated by common wheat cultivar Massey, which has retained its APR to powdery mildew since its release in 1981. To develop an effective strategy for breeding for resistance to powdery mildew, it is essential to understand the genetics of APR to powdery mildew. In the present study, molecular markers associated with APR to powdery mildew in Massey were identified and verified. The major conclusions are summarized as follow:

1. Three QTLs (quantitative trait loci), designated as *QPm.vt-1B*, *QPm.vt-2A* and *QPm.vt-2B*, were identified for APR to powdery mildew in $F_{2:3}$ lines derived from the cross Becker/Massey. They are located on chromosome 1B, 2A and 2B, respectively.
2. The three QTLs, *QPm.vt-1B*, *QPm.vt-2A* and *QPm.vt-2B*, explained 17%, 29% and 11% of the total variation of $F_{2:3}$ lines for powdery mildew resistance, respectively. Cumulatively, the three QTLs accounted for 50% of the total variation of $F_{2:3}$ lines for APR to powdery mildew.
3. The three QTLs associated with APR to powdery mildew were derived from Massey, and displayed additive gene action. *QPm.vt-2B* also fits a recessive model for APR to powdery mildew.
4. The presence of the three QTLs identified in the $F_{2:3}$ generation was confirmed by both single marker analysis and interval mapping of recombinant inbred (RI) lines evaluated for APR to powdery mildew over three years.
5. The three QTLs, *QPm.vt-1B*, *QPm.vt-2A* and *QPm.vt-2B*, explained 15%, 26% and 15% of the variation of mean powdery mildew severity of RI lines over three years. Collectively, the three QTLs accounted for 44% of the phenotypic variation of the RI lines.
6. The three QTLs adequately explain differences in powdery mildew severity between the two parents, as the RI lines with Becker or Massey alleles at all the three QTLs have similar mean disease severity to that of the corresponding parents.
7. The molecular markers identified and verified as to their association with APR to powdery mildew have the potential for use in marker-assisted selection for resistance to powdery mildew.
8. Microsatellites are highly polymorphic in wheat.

9. Severe segregation distortion favoring Massey alleles was observed for markers located on chromosome 2B.

Recommendations for marker-assisted selection of APR to powdery mildew

Microsatellites or SSRs (simple sequence repeats) are highly polymorphic and easy to detect via PCR (polymerase chain reaction) in wheat. Also, microsatellite analysis can be automated, which is necessary for large-scale screening. For marker-assisted selection for APR to powdery mildew, microsatellite marker GWM304a and PSP3100 are the best markers to detect *QPm.vt-2A* and *QPm.vt-1B*, respectively. Microsatellite marker GWM501 or GWM526a are recommended to trace *QPm.vt-2B*. However, other microsatellite and RFLP markers in the vicinity of the above-mentioned markers are also useful for marker-assisted selection for APR to powdery mildew. For example, GWM312 and GWM448 on chromosome 2A; GWM47, KSUD22 and WG338 on chromosome 2B; and GWM259, BCD1514 and WG241 on chromosome 1B could be used in marker-assisted selection.

Future research is proposed as follow:

1. Determining whether the three QTLs associated with APR to powdery mildew in Massey also contribute to APR to powdery mildew in other cultivars, such as Redcoat and Houser, will provide important information for pyramiding powdery mildew resistance genes.
2. The genetic relationship between race-specific resistance genes and genes conferring APR to powdery mildew is also an important project to study in the future. For example, *QPm.vt-2A* and *QPm.vt-2B* are located on the same chromosomes as race-specific resistance genes *Pm4* and *Pm6*, respectively. The genetic relationship between *QPm.vt-2A* and *Pm4* and between *QPm.vt-2B* and *Pm6* should be addressed in the future.
3. Fine mapping of QTLs associated with APR to powdery mildew should be pursued in the future. Based on published wheat genetic maps, there are only limited polymorphic RFLP and microsatellite markers in the vicinity of the three QTLs.

AFLP (amplified fragment length polymorphism) markers are likely to be useful for further fine mapping of the QTLs associated with APR to powdery mildew. Also, a large mapping population is necessary to increase the resolution of QTL mapping. To reduce the cost and time to genotype a large population, selective mapping can be considered. Furthermore, severe segregation distortion affects the fine mapping of *QPm.vt-2B* located on chromosome 2B. Segregation patterns for markers on chromosome 2B should be checked in crosses between Massey and other powdery mildew susceptible cultivars.

VITA

Sixin Liu was born on April 29, 1966 in Zhengding county, Hebei Province, People's Republic of China. After graduating from high school, he was admitted to Agricultural University of Hebei in 1984. Mr. Liu received a Bachelor of Science degree with a major in Agronomy in 1988. In the same year, he was admitted to the Graduate School of Chinese Academy of Agricultural Sciences (CAAS), where he received a Master of Science degree in plant breeding and genetics in 1991. He then joined a plant biotechnology lab as a research assistant in the Institute of Crop Breeding and Cultivation, CAAS, until he was admitted to the graduate program in the Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, in January 1995. He is a member of the Crop Science Society of America and American Association for the Advancement of Science.

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