Validation of Quantitative Trait Loci and Identification of Diagnostic Markers for Adult-Plant Resistance to Powdery Mildew in Wheat Cultivar Massey

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ABTRACT

Powdery mildew, caused by the pathogen *Blumeria graminis* (DC) Speer (Syn. *Erysiphe graminis* DC) f. sp. *tritici*, is a major disease of wheat (*Triticum aestivum* L.). Race-specific resistance is easily identified in the field due to its qualitative phenotype and it is easy to incorporate because it is inherited as a single gene. Unfortunately, this type of resistance is easily overcome by the pathogen. Traits associated with quantitative trait loci (QTL) such as adult-plant resistance (APR), have become popular with plant breeders because of their durability over a wide geographic range and time. Due to the quantitative nature of these genes, they are difficult to study requiring multiple assessments of disease development under natural conditions in more than one location over a period of several weeks. Numerous QTL for APR to powdery mildew have been mapped in independent studies in different wheat backgrounds. The wheat cultivar Massey has been the subject of several studies due to its APR to powdery mildew that has remained effective for several decades. However, it has been difficult to identity simple sequence repeat (SSR) markers that are tightly linked to the QTL for APR in Massey. Such markers give breeders an advantage by allowing them to quickly identify and select for traits that would be difficult to distinguish in the field among breeding progeny from several backgrounds. Therefore, identification of tightly linked markers associated with APR to powdery mildew is necessary so that these traits can be selected for reliably in progeny.

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LITERATURE REVIEW

INTRODUCTION

Powdery mildew, caused by the pathogen *Blumeria graminis* (DC) Speer (Syn. *Erysiphe graminis* DC) f. sp. *tritici*, is a major disease of wheat (*Triticum aestivum* L.). In the United States, the mid-Atlantic region including Maryland, Virginia, North Carolina, and South Carolina commonly have annual mildew epidemics due to the humid climate accompanied by cool springs. In these regions, powdery mildew is of significant economic importance with yield losses as high as 48 % under severe disease epidemics (Everts and Leath, 1992). Yield losses up to 30 % were correlated with mildew severity on the leaf below the flag leaf (F-1) between the watery and milky ripe growth stages (GS 71-75, Zadoks et al. (1974) (Figure 1.1 and Figure 1.2), with severities up to 27.5 % (Hardwick et al., 1994). If infection occurs on seedlings in the fall, tillering is reduced and tiller mortality increases. McDonald and Linde (2002) describe *B. graminis tritici* as a high-risk pathogen due to its potential for gene mutation and genotype flow and its ability to reproduce sexually followed by several asexual cycles, which increase recombination in the pathogen and rapid proliferation of favorable virulent genotypes. Parks et al. (2008) reported that *B. graminis tritici* populations can maintain a suite of virulence genes without detriment to overall pathogen fitness.

Disease Cycle and Pathogen Diversity

Asexual conidia and sexual ascospores serve as the primary inoculum source and may be dispersed by wind or rain due to splashing upon impact of leaf surfaces. Germination of these spores does not require free moisture and they can persist over a wide range of temperatures and relative humidity (Hau and de Vallavieille-Pope, 2006). Conidia have high water content (63-70

%), which is thought to be the reason for primary germ tube formation and attachment to the leaf surface in the absence of external water (Friedrich and Boyle, 1993). The infection process is initiated upon contact and the fungus produces haustoria inside the wheat cells while the mycelium grows on the leaf surface (Hau and de Vallavieille-Pope, 2006). Within a relatively short period of time, more conidia are produced and serve as secondary inoculum dispersed by wind or rain (Deising et al., 2002). This process may be repeated several times during the host plant's growth cycle. As wheat matures, the lower leaves begin to senesce which cues the pathogen to produce cleistothecia (Gotz et al., 1996). The sexual ascospores are contained in the cleistothecia that protect the ascospores until favorable conditions favor their maturity and release. The mycelium and cleistothecia overwinter on crop debris, autumn sown crops and volunteer wheat plants.

Powdery mildew produces a whitish gray, cottony fungal growth on both the upper and lower leaf surfaces (Daamen, 1989). Pustules begin as small white circular patches of fungal mycelium often surrounded by chlorosis (Cunifer, 2002). When conditions are favorable, pustules often coalesce to form large masses of fungal growth on the leaf surfaces (Figure 1.3). Tissue around older pustules senesces as the diseased area expands and severely diseased leaves subsequently die. Powdery mildew is most prevalent on lower leaves but also can develop on the upper leaves, heads and awns of susceptible cultivars under epidemic conditions. Cleistothecia are small, round and dark in color and are visible during the later stages of disease development. An illustration summarizing the disease cycle is in Figure 1.4.

Optimal conditions for *B. graminis tritici* infection are between 15 to 22 ˚C with high relative humidity. Spore germination can occur over a wide range of temperatures and does not require free moisture. Canopy structure also influences *B. graminis tritici* development as thick

canopies create a microenvironment that is often more humid than in the upper portion of the plant (Aust and Von Hoyningen-Huene, 1986). Occurrences of powdery mildew in regions with less than optimal environmental conditions for *B. graminis tritici* infection are often attributed to adoption of semi-dwarf cultivars and intensive nutrient management practices since the Green Revolution (Bennett, 1984).

Chemical control combined with the use of resistant cultivars is currently the most effective way for producers to control powdery mildew. However, fungicide applications should be properly timed with plant growth stage and economic threshold in order to be cost effective while maintaining yields. Hardwick et al. (1994) reported that a two-spray program at Zadoks growth stages 45 and 59 increased yields up to 21 % in the susceptible cultivar Kanzler. However, in 2005, the Fungicide Resistance Action Committee (FRAC) included *B. graminis tritici* among pathogens at high-risk for development of resistance to fungicides (FRAC, 2005; Russell, 2004). The most economically and environmentally favorable solution to abate the diverse and dynamic *B. graminis tritici* populations in wheat is by developing resistant cultivars that do not require stringent application of fungicides to reach their yield potential.

Sources of Resistance

Resistance genes to powdery mildew (*Pm*) are found in domesticated wheat and its wild relatives. Primary sources of resistance include domesticated hexaploid wheat (2n=6n=42, AABBDD), hexaploid landraces and species that share homologous genomes with common wheat (Jiang et al., 1993). These closely related species include the progenitor of the A genome *Triticum urartu*, the intermediate progenitor of domesticated durum and bread wheat *Triticum turgidum* spp. *dicoccoides*, the progenitor of the D genome *Aegilops tauschii*, as well as *Triticum*

turgidum (durum wheat), *Triticum monococcum* and *Triticum turgidum* spp. *dicoccon* (Cowger et al., 2012). The secondary gene pool includes tetraploid species that share a single homologous and single homoeologous set of chromosomes with domesticated wheat including *Triticum timopheevii* and *Triticum araraticum* or have a single related genome such as *Aegilops speltoides* and *Aegilops longissima* (Cowger et al., 2012; Jiang et al., 1993). The tertiary gene pool consists of distantly related species with homoeologous genomes to domesticated wheat. This group includes *Aegilops caudate*, *Aegilops ovate*, *Aegilops umbellulata*, *Aegilops triuncialis* and *Aegilops variabilis* (Cowger et al., 2012). Lesser-related species such as *Secale cereal*, *Haynaldia villosa* and *Thinopyrum intermedium* have also been used as sources of resistance (Chen et al., 1995; Feldman, 2000; He et al., 2009). Powdery mildew resistance genes have been recently reviewed and a list of these genes and further information can be found in the review by Cowger et al. (2012).

Host Resistance to Powdery Mildew

Disease resistance involving plant host-pathogen interactions is comprised of two types of expression known as race-specific or vertical resistance and race non-specific, horizontal or adult plant resistance (APR). As the name implies, race-specific (vertical) resistance is governed by a single major gene with large effects and follows the gene-for-gene model (Flor, 1971). This model is based on the interaction of pathogen elicitors and plant receptors for those elicitors. When the plant has a receptor that corresponds to and therefore recognizes the pathogen elicitor, a hypersensitive reaction takes place at or near the site of recognition (Parlevliet et al., 1980). This reaction occurs very rapidly and produces small necrotic spots also known as flecking. Although race-specific resistance is easy for plant breeders to work with because it consists of a

single gene, it has limitations when applied over a wide geographical area. Selection pressure is exerted on the pathogen population carrying the recognized elicitor and, depending on the intensity of selection, pathotypes without the recognized elicitor or a mutation from avirulent to virulent elicitors can develop rapidly. This lack of durability is of greater consequence for pathogens with high evolutionary potential (McDonald and Linde, 2002). The intensity, frequency, and geographic region where the resistance gene is deployed will affect how rapidly this "breakdown" may occur (Parks et al., 2008).

A minor gene or several tightly linked genes having an additive effect on a trait are known as quantitative trait loci (QTL). Such QTL typically govern APR, which confers broad-spectrum (horizontal) resistance effective against a large number of pathogen genotypes that occur in a given population. Rather than having a gene for gene/receptor-elicitor relationship, several genes operating in different metabolic or defense pathways that are not continuously expressed in the plant act as inhibitors to the pathogen (McDonald and Linde, 2002). It is possible that these QTL are in close proximity to major genes or may co-localize with these loci (Cowger et al., 2012). The response of APR is much slower than for race-specific major gene resistance, yet also can be expressed during most of the plant's growth stages (Bougot et al., 2006). Infection can occur although it is delayed and has a lower level of infectivity and resulting in smaller and fewer spores than on a susceptible plant. In combination, several minor genes are less likely to be overcome by the pathogen as in race-specific resistance but also may have the potential to become less effective over time (Parlevliet, 2002).

Race-Specific Resistance Genes

Numerous race-specific *Pm* genes and QTL or genes governing APR to powdery mildew have been identified and are located on every wheat chromosome (Cowger et al., 2012; Ma et al., 2011; Marone et al., 2013). More than 70 genes conferring resistance to powdery mildew have been identified at 44 loci designated *Pm1-50* (Alam et al., 2011; Cowger et al., 2012; Ma et al., 2011; Maxwell et al., 2012; McIntosh et al., 2012; Mohler et al., 2013). Analyses using host-pathogen interactions, cytogenetics, and molecular marker techniques have been used to determine the chromosomal location of these *Pm* genes (Alam et al., 2011). Powdery mildew resistance genes reside in clusters of gene-rich regions distributed throughout the genome in a non-random pattern (Faris et al., 1999).

Resistance genes mapped in short intervals can represent allelic series at the same locus, or clusters of R genes (Marone et al., 2013). On the long arm of chromosome 7A, gene *Pm1* has five alleles designated *Pm1a* (Sears and Briggle, 1969), *Pm1b*, *Pm1c*, *Pm1d* (Hsam et al., 1998) and *Pm1e* (Singrun et al., 2003). Gene *Pm3* is located on the short arm of chromosome 1A and has 15 functional allelic forms (Bhullar et al., 2010) designated *Pm3a, Pm3b*, *Pm3c* (Briggle and Sears, 1966), *Pm3d, Pm3e*, *Pm3f* (Zeller et al., 1993) *Pm3g* (Yahiaoui et al., 2006) *Pm3k* (Yahiaoui et al., 2009), and *Pm3l – Pm3r* (Bhullar et al., 2009). On the long arm of chromosome 2A, gene *Pm4* has four alleles designated *Pm4a, Pm4b* (The et al., 1979), *Pm4c* (Hao et al., 2008) and *Pm4d* (Schmolke et al., 2012). Gene *Pm5* on the long arm of chromosome 7B has five alleles designated *Pm5a* (Law and Wolfe, 1966), *Pm5b, Pm5c, Pm5d* (Hsam et al., 2001) and *Pm5e* (Huang et al., 2003). In a recent QTL meta-analysis study performed by Marone et al. (2013), several QTLs conferring resistance to powdery mildew identified in independent studies

were shown to co-localize with race-specific genes. These resulting maps also illustrated the clustering of *Pm* alleles and QTLs.

QTL for Adult Plant Resistance

Some QTL conferring APR to powdery mildew that map near race-specific *Pm* genes may be associated with the residual effect of defeated *Pm* genes (Li et al., 1999). In several studies, QTL for APR mapped at or near the *Pm4* locus and may be the residual effect of *Pm4* since virulence to several of the alleles at this locus is widespread (Bougot et al., 2006; Liu et al., 2001; Mingeot et al., 2002; Muranty et al., 2009). Specifically, *Pm4a* has been shown to continue to restrict the severity of infection by *B. graminis tritici* despite being overcome (Martin and Ellingboe, 1976; Nass et al., 1981).

Gene *Pm6* was the first powdery mildew resistance gene identified on chromosome 2B (Jorgensen and Jensen, 1973). Identification of markers tightly linked to *Pm6* has been the subject of many studies (Ji et al., 2008; Qin et al., 2011; Tao et al., 2000) due to the successful deployment of this gene throughout the world (Purnhauser et al., 2011; Zhao et al., 2013). Several studies have identified QTL for APR located near *Pm6* including QTL mapped in the cultivar Massey (Liu et al., 2001), RE9001 (Bougot et al., 2006) and 'Fukuho-komugi' (Liang et al., 2006) but their relationship to *Pm6* remains unclear.

Gene *Pm6* and the stem rust (*Puccinia graminis*) resistance gene *Sr36* were transferred as a gene cluster to common wheat as part of the t2BS.2GS.2GL.2BL translocation from *T. timopheevii* ssp. *timopheevii* (Allard and Shands, 1954; Jorgensen and Jensen, 1973; McIntosh and Gyarfus, 1971). Lines carrying this translocation occur in approximately 20 % of U.S. Eastern soft winter wheat cultivars (Olson et al., 2010). Wide distribution of *Pm6* has resulted

in corresponding virulence in pathogen populations (Cowger et al., 2012; Parks et al., 2009). Despite widespread virulence to *Pm6*, a QTL in the vicinity of this gene has been associated with APR in several studies (Bougot et al., 2006; Liang et al., 2006; Liu et al., 2001). In addition to the stem rust and powdery mildew resistance, this translocation also contains genes governing numerous important agronomic traits such as improved grain weight, milling quality, and flour yield, (Jiang et al., 2011; Souza et al., 2012; Tsilo et al., 2008).

Other QTL that map near *Pm* genes include *Pm1* (Chantret et al., 2001), *Pm3a* (Liang et al., 2006), *Pm5* (Chen et al., 2009; Keller et al., 1999; Lillemo et al., 2008), *Pm13* (Chen et al., 2009), *Pm35* (Muranty et al., 2009), *Pm38* (Liang et al., 2006; Lillemo et al., 2008; Spielmeyer et al., 2005) and *Pm43* (Lan et al., 2010). Gene *Pm38* is part of a pleiotropic locus (*Lr34*/*Pm38*/*Yr18*) that also confers APR to leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis*), which was cloned by Krattinger et al. (2009).

Cloning of the *Lr34*/*Yr18*/*Pm38* locus allowed for the prediction of a 1401 amino acid protein resembling an adenosine triphosphate binding cassette (ABC) transporter belonging to the pleiotropic drug resistance (PDR) subfamily (Krattinger et al., 2009). Although this protein is not well characterized, the ABC transporter PEN3/PDR8 has been characterized in the model plant *Arabidopsis thaliana* (Cowger et al., 2012). The PEN3/PDR8 transporter is involved in non-host specific resistance to powdery mildew. It contributes to cell wall defense and intercellular defenses residing in the plasma membrane and accumulates at site of pathogen infection (Lipka et al., 2008; Stein et al., 2006). Future cloning of more genes associated with QTL for APR will help researchers understand the complex nature of these genes. A list summarizing the QTL conferring APR to powdery mildew in wheat adapted from Cowger et al. (2012) is presented in Table 1.1.

Breeding Strategies for Resistance

Gene pyramiding is an important strategy in breeding for resistance to powdery mildew in wheat (Hsam and Zeller, 2002). Pyramiding race-specific resistance genes not yet overcome by the pathogen is a common strategy to maintain the durability of these genes because the pathogen would have to undergo multiple mutations to develop virulence to each resistance gene (McDonald and Linde, 2002; Parks et al., 2008). However, gene pyramids of race-specific genes are less likely to remain durable if virulence to the individual *Pm* genes has already been established, because the resistance pyramid would then select for corresponding virulence pyramids in the pathogen population (Brown and Wolfe, 1990; Parks et al., 2008). The speed at which a corresponding virulence pyramid is established in an avirulent pathogen population may be determined by the selection pressure on the population due to resistance gene distribution regardless of whether or not any of the genes have yet been defeated (Bennett, 1984). Ideally, pyramiding minor genes such as QTL for APR with or without race-specific genes should be employed in breeding programs to maintain durable resistance.

Marker Assisted Selection

Marker assisted selection (MAS) uses DNA-based molecular markers linked to specific genes or QTL that govern traits of interest to select progeny with desirable gene combinations. Despite the complex nature of the wheat genome, it has become easier to deal with complex quantitative traits using MAS (Collard and Mackill, 2008). Combining resistance QTL for powdery mildew from different wheat cultivars can increase the level of resistance in the resulting progeny (Bai et al., 2012). Furthermore, combining QTL with additive and complementary effects for mode of action and growth stage of expression should result in

cultivars with high levels of resistance that will remain durable for a longer period of time (Cowger et al., 2012). The use of molecular markers is essential to pyramid QTLs since these traits are not easily assessed phenotypically. Tyagi et al. (2014) successfully combined eight QTLs for seven different traits including resistance to leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis*) and stripe rust (*Puccinia striiformis*), and several grain quality traits in common wheat using MAS over five consecutive generations. The success or reliability of using MAS to pyramid genes is dependent on whether or not the molecular markers are tightly linked (5 cM or less) to the associated trait (Collard and Mackill, 2008).

APR to Powdery Mildew in the Cultivar Massey

Durable resistance is defined as resistance that has remained effective for many years in cultivars possessing genes that have been widely deployed while being grown over a wide geographic area (Johnson and Law, 1975). Adult plant resistance to powdery mildew in several widely grown cultivars has remained effective for more than four decades (Cowger et al., 2012). Roberts and Caldwell (1970) first described the wheat cultivar Knox (CI 12798) as having "slow mildewing" resistance. The APR in Knox and its derivatives 'Knox 62' (CI 13701) (Shaner and Finney, 1975) Massey (CI 17953) (Griffey and Das, 1994), 'USG 3209' (PI 617055), 'USG 3555' (PI 654454, Griffey et al. 2009), and 'Shirley' (PI 656753, Griffey et al. 2010) cumulatively has remained effective for more than 50 years over a wide geographic area.

Massey, a derivative of 'Blueboy' (CI 14031) / Knox 62 (Starling et al., 1984), has been used in several studies investigating APR to powdery mildew due to its durability (Das and Griffey, 1994; Griffey et al., 1993; Liu et al., 2001; Tucker et al., 2006). The effect of APR in Knox and its derivatives is described as having a lower sporulation rate and infection efficiency

as compared to race-specific genes (Shaner, 1973) even under epidemic conditions (Griffey et al., 1993). Massey inherited APR to powdery mildew from Knox 62. Two to three genes were postulated to govern APR to powdery mildew in Knox 62 and Massey (Griffey and Das, 1994). Studies also have reported that APR is highly heritable with a predominance of general combining ability (GCA) and additive effects making it desirable for use in breeding programs (Griffey and Das, 1994).

In a 'Becker' (PI 494524) / Massey mapping population, $F_{2:3}$ plants were assessed for reaction to powdery mildew under natural conditions in the field and exhibited a 1:62:1 genotypic ratio ($P = 0.32$) suggesting three genes conferring APR in Massey (Liu et al., 2001). The distribution of progeny for disease severity was continuous and skewed towards resistance making it likely that more than one gene controlled APR (Liu et al., 2001). Three QTL for APR to powdery mildew in this study were mapped to the long arm of chromosomes 1BL, 2AL and 2BL (Liu et al., 2001).

In the Becker / Massey population, a QTL on chromosome 2AL designated *QPm.vt-2A* had the highest logarithm of odds (LOD) score (9.23) and was located in the microsatellite interval *Xgwm304 – Xgwm312*, 12.0 cM from *Xgwm304*, and explained 29 % of the total phenotypic variation in the $F_{2:3}$ generation. This QTL inherited from Massey, fit an additive model with an LOD score of 8.65. A QTL on chromosome 2BL designated *QPm.vt-2B* was identified by the RFLP marker *WG388* with an LOD score of 4.34 and explained 11 % of the total phenotypic variation. This QTL, which was inherited from Massey, was shown to fit recessive and additive models with LOD scores of 4.30 and 3.64. A QTL on chromosome 1BL, designated *QPm.vt-1B* was located between the microsatellite marker *Xgwm259* and the RFLP marker *WG241* with an LOD score of 5.25. The QTL, inherited from Massey, was located 8.0

cM from *Xgwm259*, explained 17 % of the total variation, and fit an additive model. These results were confirmed in $F_{5:6} – F_{7:8}$ generations under natural field conditions (Liu et al., 2001). An LOD score above 3 is a strong indicator that the loci are linked to the QTL.

A USG 3209 / 'Jaypee' (P1 592760) validation population was developed to confirm the results of the Becker / Massey population (Tucker et al., 2007). USG 3209 is a derivative of Massey from which it putatively inherited the APR to powdery mildew. Mildew severity data was collected from the $F_{5:6}$ and $F_{6:7}$ generations under natural field conditions. Three QTL were identified *QPm.vt-1BL, QPm.vt-2AL* and *QPm.vt-2BL* and explained 12 to 13 %, 59 to 69 %, and 22 to 48 % of the phenotypic variation for powdery mildew severity in two field experiments, respectively. These results verified that the three QTL in the cultivar USG 3209 were inherited from the parent Massey (Tucker et al., 2007).

The *QPm.vt-2AL* QTL was located in the microsatellite marker interval *Xgwm304 – Xbarc353* and was considered to have a major effect on the basis of high LOD scores (10.7 – 20.6) and proportion of explained phenotypic variation (59 – 69 %) in the $F_{5:6}$ and $F_{6:7}$ generations, respectively. The *QPm.vt-2BL* QTL was located in the microsatellite marker interval *Xgwm501 – Xgwm191* and also had high LOD scores (25.2 – 12.6) and explained a large portion of the phenotypic variation (48 – 22 %) in the $F_{5:6}$ and $F_{6:7}$ generations. The location of *QPm.vt-1BL* was shifted towards the end of the chromosome in this study flanked by the microsatellite markers *Xgwm259* and *Xbarc80*. This QTL was consistent in both generations with an LOD of 6.0 and explained 13 % of the phenotypic variation.

To utilize MAS more effectively, the addition of SSR markers in place of RFLPs for these QTL was necessary (Tucker et al., 2007). However, the distance between these markers and the QTL for APR was rather large (*QPm.vt-1BL* 10 cM from *Xbarc80, QPm.vt-2AL* 80 cM

between *Xgwm304 – Xbarc353*, *QPm.vt-2BL* 30 cM between *Xgwm501 – Xgwm191*) and difficult to reliably implement them for MAS.

Further work on the USG 3209 / Jaypee population was conducted to generate a whole genome linkage map and further delineate and refine the chromosomal regions involved in APR to powdery mildew (Hall and Griffey, 2009). This study, performed over 10 environments in Virginia, Maryland and North Carolina under natural field conditions, identified four genomic regions associated with APR to powdery mildew on chromosomes 1BL, 2AL, 2BL and 5AS.

The QTL on chromosome 1BL, *Qpm.vag-1B*, was located near the rye-specific SSR marker *SCM09*, which is the diagnostic marker for the 1RS:1AL and 1RS:1BL translocations containing genes *Pm17* and *Pm8*, respectively. Tucker et al. (2007) determined that USG 3209 has the race specific genes *Pm8* and *Pm3a*. Interestingly, the donor parent Massey does not have the 1RS:1BL translocation but Liu et al. (2001) also identified *QPm.vt-1B* in the Becker / Massey population (Hall and Griffey, 2009). The location of the QTL on 1BL was different in these two populations and this may be explained by the presence of the 1RS:1BL translocation in USG 3209 and thus these are different QTLs.

On chromosome 2AL, the QTL *QPm.vag-2A* was mapped in the SSR marker interval *Xgwm448*, *Xgwm122* and *Xgwm95* spanning a total of 23.5 cM with the peak of this QTL at *Xgwm122*. This QTL was only significant in Virginia and Maryland and had an average LOD score of 4.2 and explained 10 % of the phenotypic variation across the 10 environments. The QTL on 2BL, *QPm.vag-2B*, was the most significant in this study with an LOD score of 7.27 and explained 19 % of the phenotypic variation across the 10 environments. The QTL spans 23.9 cM across the interval of SSR markers *Xbarc200*, *Xgwm47*, and *Xgwm501* with the peak of the QTL

at *Xgwm47*. The marker order on 2AL and 2BL differs between the present study and that of Liu et al. (2001) likely due to the different populations.

The newly identified QTL *QPm.vag-5A* (from USG 3209) was located near the SSR marker *Xbarc56* with an LOD score of 4.9 and explained 9 % of the phenotypic variation across the 10 environments. This QTL was similar to the one identified by Keller et al. (1999) on 5AS in the spelt wheat cultivar Oberkulmer.

In addition to Massey and USG 3209, QTL for APR on 2AL have been mapped in RE714 (Chantret et al., 2001; Chantret et al., 2000; Mingeot et al., 2002) and 'Courtot' (Bougot et al., 2006) 'Forno' (Keller et al., 1999) and the same QTL on 2BL has also been mapped in RE9001 (Bougot et al., 2006), 'Fukuho-komugi' (Liang et al., 2006) 'Lumai 21' (Lan et al., 2010) and 'Festin' (Mingeot et al., 2002). Bougot et al. (2006) noted that the QTL *QPm.sfr-2A* (Keller et al., 1999), *QPm.vt-2A* (Liu et al., 2001) and *QPm.inra-2A* (Bougot et al., 2006) were in similar regions and were in close proximity to the defeated race-specific gene *Pm4a*. In addition to the QTL on 2AL, Bougot et al. (2006) identified a QTL on 2BL in RE9001 (*QPm.inra-2B*) in close proximity to the defeated race-specific gene *Pm6* and *QPm.vt-2B* which Liu et al. (2001) also noted in the same region as *Pm6*. The relationship of these QTL to defeated race-specific genes remains unclear.

Proposed Research

Despite the number of SSR markers added to the map in the three consecutive studies with Massey and its derivative USG 3209, the marker interval identified for these QTL remains large (Hall and Griffey, 2009; Liu et al., 2001; Tucker et al., 2007). For reliable use in MAS, it is important that markers are tightly linked to the QTL. Mapping QTL for APR to powdery

mildew is inherently difficult because of the environmental sensitivity of *B. graminis tritici* populations in addition to the complex and additive nature of these QTL (Keller et al., 1999; Mingeot et al., 2002).

The objectives of this study are to: 1) map and validate QTL for APR to powdery mildew in Massey using a new population containing the susceptible cultivar MPV 57, 2) saturate the QTL regions on chromosomes 2AL and 2BL, 3) to identify diagnostic markers for these QTL that can be used reliably in MAS, and 4) identify new QTL conferring APR to powdery mildew.

REFERENCES

1. Alam, M., F. Xue, C. Wang, W. Ji. 2011. Powdery mildew resistance genes in wheat: Identification and genetic analysis. Journal of Molecular Biology Research 1(1):20-39.
2. Allard, R.W., R.G. Shands. 1954. Inheritance of resistance to stem rust and powdery mildew in cytologically stable spring wheats derived from *Triticum timopheevii*. Phytopathology 44266-274.
3. Alley, M.M., P. Scharf, D.E. Brann, W.E. Baethgen, J.L. Hammons. 2009. Nitrogen management for winter wheat: principles and recommendations, Viginia Cooperative Extension, Communications and Marketing, College of Agriculture and Life Sciences, Virginia Polytechnic and State University Blacksburg, VA. p. 1-6.
4. Aust, H.J., J. Von Hoyningen-Huene. 1986. Microclimate in relation to epidemics of powdery mildew. Annual Review of Phytopathology 24491-510.
5. Bai, B., Z.H. He, M.A. Asad, C.X. Lan, Y. Zhang, X.C. Xia, J. Yan, X.M. Chen, C.S. Wang. 2012. Pyramiding adult-plant powdery mildew resistance QTLs in bread wheat. Crop and Pasture Science 63(7):606-611.
6. Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: A review of its use in agriculture and breeding programs. Plant Pathology 33(3):279-300.
7. Bhullar, N.K., M. Mackay, B. Keller. 2010. Genetic diversity of the *Pm3* powdery mildew resistance alleles in wheat gene bank accessions as assessed by molecular markers. Diversity 2(5):768-786.
8. Bhullar, N.K., K. Street, M. Mackay, N. Yahiaoui, B. Keller. 2009. Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the *Pm3* resistance locus. Proceedings of the National Academy of Sciences 106(23):9519-9524.
9. Börner, A., E. Schumann, A. Fürste, H. Cöster, B. Leithold, M. Röder, W. Weber. 2002. Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (Triticum aestivum L.). Theoretical and Applied Genetics 105(6-7):921-936.
10. Bougot, Y., J. Lemoine, M.T. Pavoine, H. Guyomar'ch, V. Gautier, H. Muranty, D. Barloy. 2006. A major QTL effect controlling resistance to powdery mildew in winter wheat at the adult plant stage. Plant Breeding 125(6):550-556.
11. Briggle, L.W., E.R. Sears. 1966. Linkage of resistance to *Erysiphe graminis* f sp. *tritici* (*Pm3*) and hairy glume (*Hg*) on chromosome 1A of wheat Crop Science 6(6):559-561.
12. Brown, J.K.M., M.S. Wolfe. 1990. Structure and evolution of a population of *Erysiphe graminis* f.sp. *hordei*. Plant Pathology 39(3):376-390.
13. Chantret, N., D. Mingeot, P. Sourdille, M. Bernard, J.M. Jacquemin, G. Doussinault. 2001. A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. Theoretical and Applied Genetics 103(6-7):962-971.
14. Chantret, N., P. Sourdille, M. Roder, M. Tavaud, M. Bernard, 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. Theoretical and Applied Genetics 100(8):1217-1224.
15. Chen, P.D., L.L. Qi, B. Zhou, S.Z. Zhang, D.J. Liu. 1995. Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theoretical and Applied Genetics 91(6-7):1125-1128.

16. Chen, Y., R. Hunger, B. Carver, H. Zhang, L. Yan. 2009. Genetic characterization of powdery mildew resistance in U.S. hard winter wheat. Molecular Breeding 24(2):141-152.
17. Clark, B., R. Bryson, L. Tonguç, C. Kelly, G. Jellis. 2008. Powdery Mildew, *In*: G. Jellis (Ed.), The Encyclopedia of Cereal Diseases, HGCA/BASF. p. 59-62.
18. Collard, B.C.Y., D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences 363(1491):557-572.
19. Cowger, C., L. Miranda, C. Griffey, M. Hall, J.P. Murphy, J.J. Maxwell. 2012. Wheat Pwdery Mildew, *In*: I. Sharma (Ed.), Disease Resistance in Wheat, CAB International, Cambridge, MA. p. 84-119.
20. Cunifer, B.M. 2002. Disaeases and Pests: Powdery Mildew, *In*: B. C. Curtis, Rajaram, S., Gomez Macpherson, H. (Ed.), FAO Plant Production and Protection Series, Food and Agriculture Organization of the United Nations, Rome.
21. Daamen, R.A. 1989. Assessment of the profile of powdery mildew and its damage function at low disease intensities in field experiments with winter wheat. Netherlands Journal of Plant Pathology 95(2):85-105.
22. Das, M.K., C.A. Griffey. 1994. Diallel analysis of adult-plant resistance to powdery mildew in wheat. Crop Science 34(4):948-952.
23. Deising, H.B., S. Reimann, A. Peil, W.E. Weber. 2002. Disease management of rusts and powdery mildews, *In*: K. Esser and J. W. Bennett (Eds.), The Mycota: A comprehensive treatise on fungi as experimental systems for basic and applied research. Agricultural applications. p. 243-269.
24. Everts, K.L., S. Leath. 1992. Effect of Early Season Powdery Mildew on Development, Survival, and Yield Contribution of Tillers of Winter-Wheat. Phytopathology 82(11):1273-1278.
25. Faris, J.D., W.L. Li, D.J. Liu, P.D. Chen, B.S. Gill. 1999. Candidate gene analysis of quantitative disease resistance in wheat. Theoretical and Applied Genetics 98(2):219-225.
26. Feldman, M.W. 2000. Origin of Cultivated Wheat, *In*: A. P. Bojean and W. J. Angus (Eds.), The World Wheat Book, A History of Wheat Breeding, Lavoisier Publishing, Paris. p. 1-56.
27. Flor, H.H. 1971. Current status of the gene for gene concept. Annual Review of Phytopathology(VII):275-296.
28. FRAC. 2005. Pathogen Risk List, Fungicide Resistance Action Committee, Limburgerhof, Germany. p. 1-5.
29. Friedrich, S., C. Boyle. 1993. Effect of varying humidities in vitro uopn the production and germination of mildew conidia from *Erysiphe-graminis* f.sp. *tritici*. Journal of Plant Diseases and Protection 100(2):180-188.
30. Gotz, M., S. Friedrich, C. Boyle. 1996. Development of cleistothecia and early ascospore release of *Erysiphe graminis* DC f. sp. *tritici* in winter wheat in relation to host age and climatic conditions. Journal of Plant Diseases and Protection 103(2):134-141.
31. Griffey, C., M. Das, E. Stromberg. 1993. Effectiveness of adult-plant resistance in reducing grain yield loss to powdery mildew in winter wheat. Plant Disease 77(6):618-622.
32. Griffey, C.A., M.K. Das. 1994. Inheritance of adult-plant reistance to powdery mildew in Knox 62 and Massey winter wheats. Crop Science 34(3):641-646.

33. Griffey, C.A., W.E. Thomason, R.M. Pitman, B.R. Beahm, J.J. Paling, J. Chen, J.K. Fanelli, J.C. Kenner, D.W. Dunaway, W.S. Brooks, M.E. Vaughn, E.G. Hokanson, H.D. Behl, R.A. Corbin, M.D. Hall, S. Liu, J.T. Custis, C.M. Waldenmaier, D.E. Starner, S.A. Gulick, S.R. Ashburn, D.L. Whitt, H.E. Bockelman, E.J. Souza, G.L. Brown-Guedira, J.A. Kolmer, D.L. Long, Y. Jin, X. Chen, S.E. Cambron. 2009. Registration of 'USG 3555' Wheat. Journal of Plant Registration 3(3):273-278.
34. Griffey, C.A., W.E. Thomason, R.M. Pitman, B.R. Beahm, J.J. Paling, J. Chen, P.G. Gundrum, J.K. Fanelli, J.C. Kenner, D.W. Dunaway, W.S. Brooks, M.E. Vaughn, E.G. Hokanson, H.D. Behl, R.A. Corbin, M.D. Hall, S. Liu, J.T. Custis, C.M. Waldenmaier, D.E. Starner, S.A. Gulick, S.R. Ashburn, E.H. Jones, D.L. Whitt, H.E. Bockelman, E.J. Souza, G.L. Brown-Guedira, J.A. Kolmer, D.L. Long, Y. Jin, X. Chen, S.E. Cambron. 2010. Registration of 'Shirley' Wheat. Journal of Plant Registration 4(1):38-43.
35. Hall, M., C. Griffey. 2009. Wheat, 'USG 3209' / 'Jaypee', http://wheat.pw.usda.gov/ggpages/map_summary.html, Graingenes.
36. Hao, Y., A. Liu, Y. Wang, D. Feng, J. Gao, X. Li, S. Liu, H. Wang. 2008. *Pm23*: a new allele of *Pm4* located on chromosome 2AL in wheat. Theoretical and Applied Genetics 117(8):1205-12.
37. Hardwick, N.V., J.E.E. Jenkins, B. Collins, S.J. Groves. 1994. Powdery mildew (*Erysiphe graminis*) on winter wheat: control with fungicides and the effects on yield. Crop Protection 13(2):93-98.
38. Hau, B., C. de Vallavieille-Pope. 2006. Wind-dispersed diseases, *In*: B. M. Cooke, et al. (Eds.), The Epidemiology of Plant Diseases, Springer Netherlands. p. 387-416.
39. He, R., Z. Chang, Z. Yang, Z. Yuan, H. Zhan, X. Zhang, J. Liu. 2009. Inheritance and mapping of powdery mildew resistance gene *Pm43* introgressed from *Thinopyrum intermedium* into wheat. Theoretical and Applied Genetics 118(6):1173-80.
40. Hollaway, G. 2006. Yellow Leaf Spot of Wheat, *In*: F. C. P. Department of Environment and Primary Industries (Ed.), Department of Environment and Primary Industries, Melborne, Victoria.
41. Hsam, S.L.K., X.Q. Huang, F. Ernst, L. Hartl, F.J. Zeller. 1998. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 5. Alleles at the *Pm1* locus. Theoretical and Applied Genetics 96(8):1129-1134.
42. Hsam, S.L.K., X.Q. Huang, F.J. Zeller. 2001. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.) 6. Alleles at the *Pm5* locus. Theoretical and Applied Genetics 102(1):127-133.
43. Hsam, S.L.K., F.J. Zeller. 2002. Breeding for powdery mildew resistance in common wheat, *In*: R. R. Belanger, et al. (Eds.), The Powdery Mildews: A Comprehensive Treatise, APS Press, St Paul, MN. p. 219-238.
44. Huang, X.Q., L.X. Wang, M.X. Xu, M.S. Roder. 2003. Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 106(5):858-65.
45. Jakobson, I., H. Peusha, L. Timofejeva, K. Järve. 2006. Adult plant and seedling resistance to powdery mildew in a Triticum aestivum× Triticum militinae hybrid line. Theoretical and applied genetics 112(4):760-769.
46. Ji, J., B. Qin, H. Wang, A. Cao, S. Wang, P. Chen, L. Zhuang, Y. Du, D. Liu, X. Wang. 2008. STS markers for powdery mildew resistance gene *Pm6* in wheat. Euphytica 163(2):159-165.

47. Jiang, J., B. Friebe, B. Gill. 1993. Recent advances in alien gene transfer in wheat. Euphytica 73(3):199-212.
48. Jiang, Q., J. Hou, C. Hao, L. Wang, H. Ge, Y. Dong, X. Zhang. 2011. The wheat (*T. aestivum*) sucrose synthase 2 gene (*TaSus2*) active in endosperm development is associated with yield traits. Functional and Integrative Genomics 11(1):49-61.
49. Johnson, R., C.N. Law. 1975. Genetic control of durable resistance to yellow rust (*Puccinia striiformis*) in the wheat cultivar Hybride de Bersée. Annals of Applied Biology 81(3):385-391.
50. Jorgensen, J.H., C.J. Jensen. 1973. Gene *Pm6* for resistance to powdery mildew in wheat. Euphytica 22(2):423-423.
51. Keller, M., B. Keller, G. Schachermayr, M. Winzeler, J.E. Schmid, P. Stamp, M.M. Messmer. 1999. Quantitative trait loci for resistance against powdery mildew in a segregating wheat×spelt population. Theoretical and Applied Genetics 98(6-7):903-912.
52. Krattinger, S.G., E.S. Lagudah, W. Spielmeyer, R.P. Singh, J. Huerta-Espino, H. McFadden, E. Bossolini, L.L. Selter, B. Keller. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323(5919):1360-1363.
53. Lan, C., S. Liang, Z. Wang, J. Yan, Y. Zhang, X. Xia, Z. He. 2009. Quantitative Trait Loci Mapping for Adult-Plant Resistance to Powdery Mildew in Chinese Wheat Cultivar Bainong 64. Phytopathology 99(10):1121-1126.
54. Lan, C., X. Ni, J. Yan, Y. Zhang, X. Xia, X. Chen, Z. He. 2010. Quantitative trait loci mapping of adult-plant resistance to powdery mildew in Chinese wheat cultivar Lumai 21. Molecular Breeding 25(4):615-622.
55. Law, C.N., M.S. Wolfe. 1966. Location of genetic factors for mildew resistance and ear emergence time on chromosome 7B of wheat. Canadian Journal of Genetics and Cytology 8(3):462-470.
56. Li, Z.K., L.J. Luo, H.W. Mei, A.H. Paterson, X.H. Zhao, D.B. Zhong, Y.P. Wang, X.Q. Yu, L. Zhu, R. Tabien, J.W. Stansel, C.S. Ying. 1999. A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Molecular and General Genetics 261(1):58-63.
57. Liang, S.S., K. Suenaga, Z.H. He, Z.L. Wang, H.Y. Liu, D.S. Wang, R.P. Singh, P. Sourdille, X.C. Xia. 2006. Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology 96(7):784-9.
58. Lillemo, M., B. Asalf, R. Singh, J. Huerta-Espino, X. Chen, Z. He, Å. Bjørnstad. 2008. The adult plant rust resistance loci *Lr34*/*Yr18* and *Lr46*/*Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theoretical and Applied Genetics 116(8):1155-1166.
59. Lipka, U., R. Fuchs, V. Lipka. 2008. Arabidopsis non-host resistance to powdery mildews. Current Opinion in Plant Biology 11(4):404-11.
60. Liu, S., C.A. Griffey, M.A.S. Maroof. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar massey. Crop Science 41(4):1268-1275.
61. Ma, H., Z. Kong, B. Fu, N. Li, L. Zhang, H. Jia, Z. Ma. 2011. Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat. Theoretical and Applied Genetics 123(7):1099-1106.
62. Marone, D., M.A. Russo, G. Laido, P. De Vita, R. Papa, A. Blanco, A. Gadaleta, D. Rubiales, A.M. Mastrangelo. 2013. Genetic basis of qualitative and quantitative

resistance to powdery mildew in wheat: from consensus regions to candidate genes. BMC Genomics 14(562):1-17.
63. Martin, T.J., A.H. Ellingboe. 1976. Differences between compatible parasite-host genotypes involving *Pm4* locus of wheat and corresponding genes in *Erysiphe-graminis* f. sp. *tritici*. Phytopathology 66(12):1435-1438.
64. Maxwell, J.J., J.H. Lyerly, G. Srnic, J.P. Murphy, C. Cowger, R. Parks, D. Marshall, G. Brown-Guedira, L. Miranda. 2012. *MlNCD1*: A novel *Aegilops tauschii*- derived powdery mildew resistance gene identified in common wheat. Crop Science 52(3):1162-1170.
65. McDonald, B.A., C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology 40349-379.
66. McIntosh, R.A., J. Gyarfus. 1971. *Triticum timopheevi* as a source of resistance to wheat stem rust. Plant Breeding 66240-248.
67. McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, D.C. Somers, R. Appels, K.M. Devos. 2012. Catalogue of gene synbols for wheat, supplement.
68. Mingeot, D., N. Chantret, P.V. Baret, A. Dekeyser, N. Boukhatem, P. Sourdille, G. Doussinault, 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 Breeding 121(2):133-140.
69. Mohler, V., C. Bauer, G. Schweizer, H. Kempf, L. Hartl. 2013. *Pm50*: a new powdery mildew resistance gene in common wheat derived from cultivated emmer. Journal of Applied Genetics 54(3):259-263.
70. Muranty, H., M.T. Pavoine, B. Jaudeau, W. Radek, G. Doussinault, D. Barloy. 2009. Two stable QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 are expressed at different times along the growing season. Molecular Breeding 23(3):445-461.
71. Nass, H.A., W.L. Pedersen, D.R. Mackenzie, R.R. Nelson. 1981. The residual effects of some defeated powdery mildew resistance genes in isolines of winter-wheat. Phytopathology 71(12):1315-1318.
72. Olson, E.L., G. Brown-Guedira, D.S. Marshall, Y. Jin, M. Mergoum, I. Lowe, J. Dubcovsky. 2010. Genotyping of U.S. wheat germplasm for presence of stem rust resistance genes *Sr24*, *Sr36* and *Sr1RS* [Amigo] Crop Science 50(2):668-675.
73. Parks, R., I. Carbone, J.P. Murphy, C. Cowger. 2009. Population Genetic Analysis of an Eastern U.S. Wheat Powdery Mildew Population Reveals Geographic Subdivision and Recent Common Ancestry with U.K. and Israeli Populations. Phytopathology 99(7):840-849.
74. Parks, R., I. Carbone, J.P. Murphy, D. Marshall, C. Cowger. 2008. Virulence structure of the Eastern US wheat powdery mildew population. Plant Disease 92(7):1074-1082.
75. Parlevliet, J.E. 2002. Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124(2):147-156.
76. Parlevliet, J.E., W.H. Lindhout, A. Ommeren, H.J. Kuiper. 1980. Level of partial resistance to leaf rust, *Puccinia hordei*, in West-European barley and how to select for it. Euphytica 29(1):1-8.
77. Purnhauser, L., L. Bóna, L. Láng. 2011. Occurrence of 1BL.1RS wheat-rye chromosome translocation and of *Sr36*/*Pm6* resistance gene cluster in wheat cultivars registered in Hungary. Euphytica 179(2):287-295.

78. Qin, B., A. Cao, H. Wang, T. Chen, F. You, Y. Liu, J. Ji, D. Liu, P. Chen, X.-e. Wang. 2011. Collinearity-based marker mining for the fine mapping of *Pm6*, a powdery mildew resistance gene in wheat. Theoretical and Applied Genetics 123(2):207-218.
79. Roberts, J.J., R.M. Caldwell. 1970. General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in 'Knox' wheat. Phytopathology (abstract)(60):1310.
80. Russell, P.E. 2004. Sensitivity Baselines in Fungicide Resistance Research and Management, *In*: FRAC (Ed.), AIMPRINT, United Kingdom. p. 56.
81. Schmolke, M., V. Mohler, L. Hartl, F. Zeller, S.K. Hsam. 2012. A new powdery mildew resistance allele at the *Pm4* wheat locus transferred from einkorn (*Triticum monococcum*). Molecular Breeding 29(2):449-456.
82. Sears, E.R., L.W. Briggle. 1969. Mapping the gene *Pm1* for resistance to *Erysiphe graminis* f. sp. *tritici* on chromosome 7A of wheat. Crop Science 996-97.
83. Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. Phytopathology 63:867-872.
84. Shaner, G., R.E. Finney. 1975. Inheritance of slow mildewing resistance in wheat. Proceedings of the American Phytopathological Society. 2, 49,
85. Singrun, C., S.L. Hsam, L. Hartl, F.J. Zeller, 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.). Theoretical and Applied Genetics 106(8):1420-4.
86. Souza, E.J., C. Sneller, M.J. Guttieri, A. Sturbaum, C. Griffey, M. Sorrells, H. Ohm, D. Van Sanford. 2012. Basis for selecting soft wheat for end-use quality. Crop Science 52(1):21-31.
87. Spielmeyer, W., R.A. McIntosh, J. Kolmer, E.S. Lagudah. 2005. Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theoretical and Applied Genetics 111(4):731-735.
88. Starling, T.M., C.W. Roane, H.M. Camper. 1984. Registration of Massey Wheat. Crop Science 24(5):1000.
89. Stein, M., J. Dittgen, C. Sanchez-Rodriguez, B.H. Hou, A. Molina, P. Schulze-Lefert, V. Lipka, S. Somerville. 2006. Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell 18(3):731-46.
90. Tao, W., D. Liu, J. Liu, Y. Feng, P. Chen. 2000. Genetic mapping of the powdery mildew resistance gene Pm6 in wheat by RFLP analysis. Theoretical and Applied Genetics 100(3-4):564-568.
91. The, T.T., R.A. McIntosh, F.G.A. Bennett. 1979. Cytogenetical studies in wheat. IX. Monosomic analysis, telocentric mapping and linkage relationship of gene *Sr1*, *Pm4*, and *Mle*. Australian Journal of Biological Sciences 32115-125.
92. Tsilo, T.J., Y. Jin, J.A. Anderson. 2008. Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Science 48(1):253-261.
93. Tucker, D., C. Griffey, S. Liu, G. Brown-Guedira, D. Marshall, M. Maroof. 2007. Confirmation of three quantitative trait loci conferring adult plant resistance to powdery mildew in two winter wheat populations. Euphytica 155(1):1-13.

94. Tucker, D.M., C.A. Griffey, S. Liu, M.A. Saghai Maroof. 2006. Potential for effective marker-assisted selection of three quantitative trait loci conferring adult plant resistance to powdery mildew in elite wheat breeding populations. Plant Breeding 125(5):430-436.
95. Tyagi, S., R.R. Mir, H. Kaur, P. Chhuneja, B. Ramesh, H.S. Balyan, P.K. Gupta. 2014. Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). Molecular Breeding1-9.
96. Yahiaoui, N., S. Brunner, B. Keller. 2006. Rapid generation of new powdery mildew resistance genes after wheat domestication. The Plant Journal 47(1):85-98.
97. Yahiaoui, N., N. Kaur, B. Keller. 2009. Independent evolution of functional *Pm3* resistance genes in wild tetraploid wheat and domesticated bread wheat. The Plant Journal 57(5):846-56.
98. Zadoks, J.C., T.T. Chang, C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research 14(6):415-421.
99. Zeller, F., J. Lutz, U. Stephan. 1993. Chromosome location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.) 1. *Mlk* and other alleles at the *Pm3* locus. Euphytica 68(3):223-229.
100. Zhao, Z., H. Sun, W. Song, M. Lu, J. Huang, L. Wu, X. Wang, H. Li. 2013. Genetic analysis and detection of the gene *MlLX99* on chromosome 2BL conferring resistance to powdery mildew in the wheat cultivar Liangxing 99. Theoretical and Applied Genetics 126(12):3081-9.

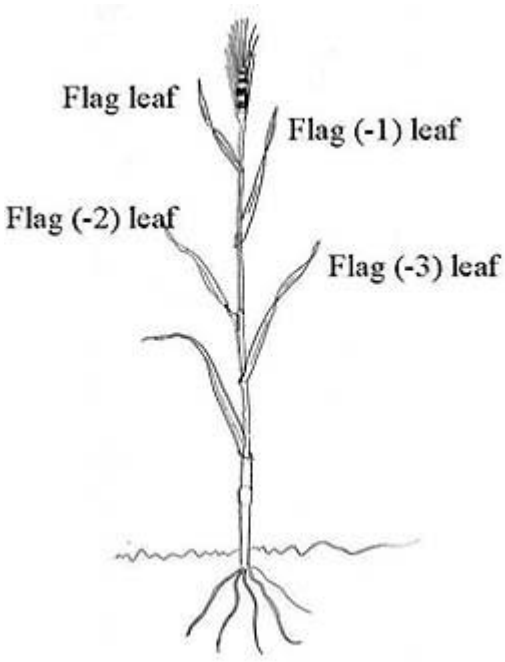

Figure 1.1. Diagram of a wheat plant. Flag (-2) leaf is two leaves below the flag leaf, Flag (-1) leaf is one leaf below the flag leaf. (Hollaway, 2006) Used under fair use guidelines.

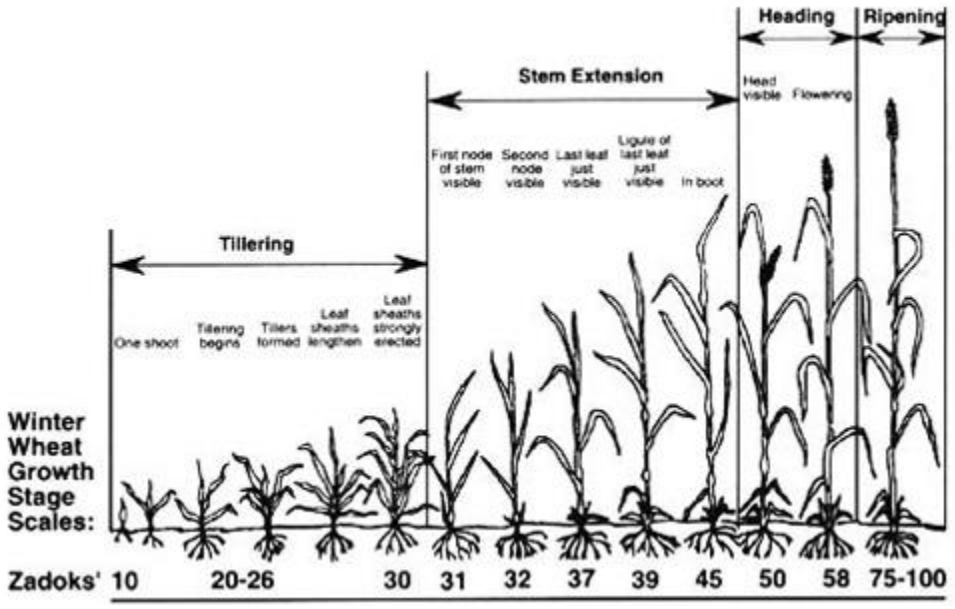

Figure 1.2. Zadoks growth stages of wheat. Adapted from Alley et al. (2009) Used under fair use guidelines.

Figure 1.3. Wheat plants infected with powdery mildew. Photo by Tiffany R. Sikes.

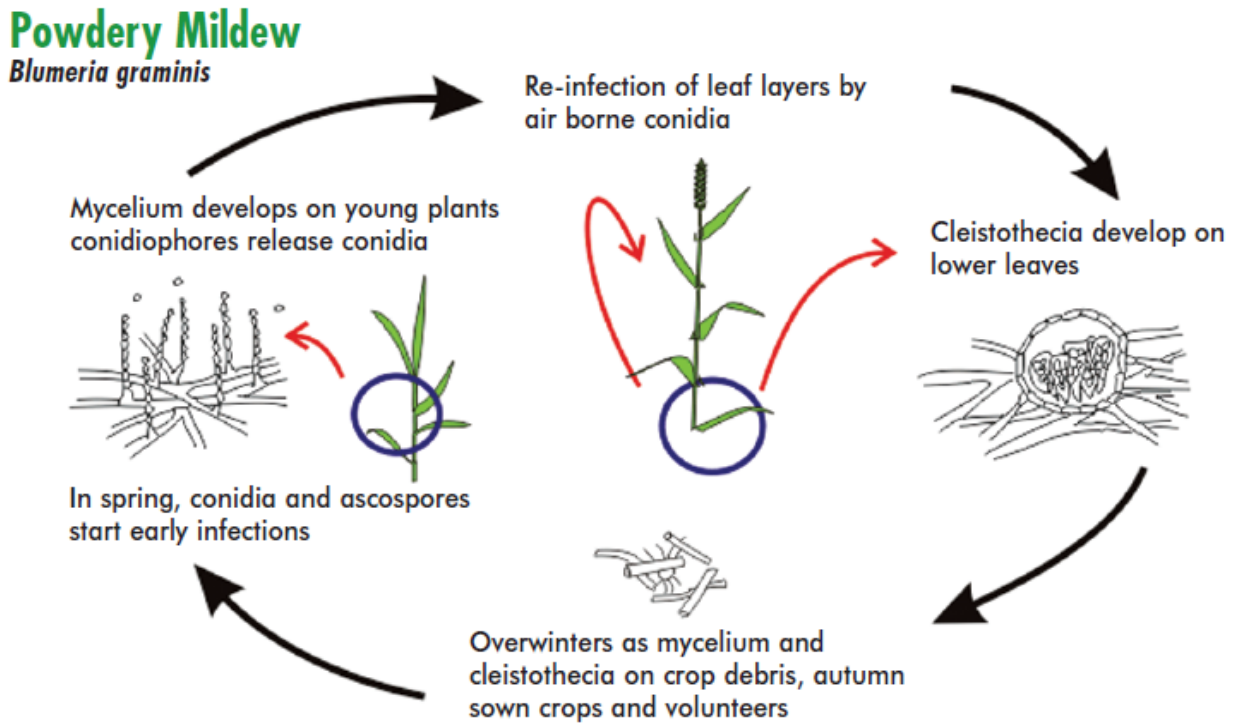

Figure 1.4. Disease cycle of *Blumeria graminis tritici*. From "The encyclopedia of cereal diseases" (Clark et al., 2008) HGCA/BASF 2008, Used with permission, 2014.

Table 1.1. Quantitative trait loci (QTL) conferring adult plant resistance to powdery mildew in different wheat backgrounds and studies.

Chromosome[†]	Cultivar[‡]	Flanking Markers[§]	Reference[¶]
1A	RE714	*Xcdo572b - Xbcd442*	Mingeot et al. (2002)
1A	Bainong 64	*Xbarc148 - Xwmc550*	Lan et al. (2009)
1A	Oberkulmer	*Xpsr1201b - Xpsr941*	Keller et al. (1999)
1B	Massey	*Xgwm259 - Xwg241*	Liu et al. (2001)
1B	Massey	*Xpsp3100 - Xcdo1189*	Liu et al. (2001)
1B	Massey	*Xgwm259 - Xbarc80*	Tucker et al. (2007)
1B	USG 3209	*Xgwm259 - Xbarc80*	Tucker et al. (2007)
1B	USG 3209	*Xscm09 - Xgwm273*	http://wheat.pw.usda.gov/ggpages/map_summary.html
1B	Forno	*CD9b - Xpsr593a*	Keller et al. (1999)
1B	2174	*Xwmc134*	Chen et al. (2009)
1D	RE9001	*Xgwm106*	Bougot et al. (2006)
1D	Oberkulmer	*Xpsr168 - Xglk558b*	Keller et al. (1999)
2A	Massey	*Xgwm304 - Xgwm312*	Liu et al. (2001)
2A	Massey	*Xgwm304*	Liu et al. (2001)
2A	Massey	*Xgwm304 - Xgwm294*	Tucker et al. (2007)
2A	USG 3209	*Xgwm304 - Xbarc353b*	Tucker et al. (2007)
2A	USG 3209	*Xgwm122 - Xgwm95*	http://wheat.pw.usda.gov/ggpages/map_summary.html
2A	Forno	*Xpsr380 - Xglk293b*	Keller et al. (1999)
2A	Courtot	*Xgwm275*	Bougot et al. (2006)
2B	USG 3209	*Xgwm501 - Xgwm191*	Tucker et al. (2007)
2B	USG 3209	*Xgwm47 - Xbarc200*	http://wheat.pw.usda.gov/ggpages/map_summary.html
2B	Massey	*Xwg388 - Xgwm526*	Liu et al. (2001)
2B	Massey	*Xwg388 - XksuD22*	Liu et al. (2001)
2B	Massey	*Xgwm501 - Xgwm191*	Tucker et al. (2007)
2B	RE9001	*Xgwm877*	Bougot et al. (2006)
2B	RE9001	*Xgwm47 - Xbarc200*	Bougot et al. (2006)

2B	Fukuho-komugi	*Xgwm877.1 - Xwmc435.1*	Liang et al. (2006)
2B	Lumai 21	*Xbarc1139 - Xgwm47*	Lan et al. (2010)
2B	Festin	*Xgwm148 - XgbxG553*	Mingeot et al. (2002)
2B	Lumai 21	*Xbarc98 - Xbarc1147*	Lan et al. (2010)
2D	RE9001	*Xgwm102*	Bougot et al. (2006)
2D	RE9001	*Xcfd2e*	Bougot et al. (2006)
2D	Lumai 21	*Xwmc18 - Xcfd233*	Lan et al. (2010)
2D	Oberkulmer	*Xpsr932 - Xpsr331*	Keller et al. (1999)
2D	Synthetic W7984	*XksuH9 - XksuD23*	Börner et al. (2002)
3A	Festin	*Xpsr598 - Xgwm5*	Mingeot et al. (2002)
3A	Saar	*Xstm844tcac - Xbarc310*	Lillemo et al. (2008)
3A	Forno	*Xpsr598 - Xpsr570*	Keller et al. (1999)
3B	Courtot	*Xgwm389*	Bougot et al. (2006)
3B	RE9001	*Xgwm66, Xgwm77*	Bougot et al. (2006)
3B	2174	*Xwms533*	Chen et al. (2009)
3B	Synthetic W7984	*Xcdo105 - Xbg131*	Börner et al. (2002)
3D	Oberkulmer	*Xpsr1196 - Lrk10_6*	Keller et al. (1999)
3D	RE9001	*Xcfd152*	Bougot et al. (2006)
3D	RE9001	*Xgwm707*	Bougot et al. (2006)
4A	*T. militinae 8/1*	*Xgwm232 - Xgwm160*	Jakobson et al. (2006)
4A	Forno	*Xgwm111c - Xpsr934a*	Keller et al. (1999)
4A	2174	*Xwms160*	Chen et al. (2009)
4A	RE714	*XgbxG036 - XgbxG542*	Mingeot et al. (2002)
4A	RE714	*XgbxG036*	Chantret et al. (2001)
4A	Courtot	*Xcfd71b*	Bougot et al. (2006)
4B	Avocet-YrA	*Xgwm251 - Xgwm375*	Lillemo et al. (2008)
4B	Hardi	*Xp36m50b*	Muranty et al. (2009)
4B	Forno	*Xpsr593b - Xpsr1112*	Keller et al. (1999)
4D	Oligoculm	*Xgwm375 - Xgwm251*	Liang et al. (2006)

4D	Synthetic W7984	*Xcdo795 - Xbcd1262*	Börner et al. (2002)
4D	Yumai 57	*Xgwm194 - Xcfa2173*	Zhang et al. (2008)
4D	Forno	*Xglk302b - Xpsr1101a*	Keller et al. (1999)
4D	Bainong 64	*Xbarc200 - Xwmc331*	Lan et al. (2009)
4D	Courtot	*Xwmc25b*	Bougot et al. (2006)
5A	Tahti	*Xgwm186 - Xgwm415*	Jakobson et al. (2006)
5A	Oberkulmer	*Xpsr644a - Xpsr945a*	Keller et al. (1999)
5A	Saar	*Xgwm617b - wmc327*	Lillemo et al. (2008)
5A	RE714	*Xbarc141*	Muranty et al. (2009)
5A	USG 3209	*Xbarc56*	http://wheat.pw.usda.gov/ggpages/map_summary.html
5B	Oberkulmer	*Xpsr580b - Xpsr143*	Keller et al. (1999)
5B	Courtot	*Xgwm790b*	Bougot et al. (2006)
5B	Saar	*Xbarc4 - Xgwm274b*	Lillemo et al. (2008)
5B	RE714	*Xgwm499*	Muranty et al. (2009)
5B	T. militinae 8/1	*Xgwm133.mi6 - Xgwm205.mi1*	Jakobson et al. (2006)
5D	RE714	*Xgwm174*	Chantret et al. (2000)
5D	RE9001	*Xcfd189*	Bougot et al. (2006)
5D	Yumai 57	*Xwmc215 - Xgdm63*	Zhang et al. (2008)
5D	Courtot	*Xcfd8*	Bougot et al. (2006)
5D	Synthetic W7984	*Xfba209 - Xbcd1103*	Börner et al. (2002)
6A	RE714	*Xgpw7388*	Muranty et al. (2009)
6A	RE714	*Xgwm427*	Mingeot et al. (2002)
6A	Synthetic W7984	*Xfba20 - Xfba111*	Börner et al. (2002)
6B	Bainong 64	*Xbarc79 - Xgwm518*	Lan et al. (2009)
6B	Forno	*Xpsr167b - Xpsr964*	Keller et al. (1999)
7A	RE714	*Xp32m51n*	Muranty et al. (2009)
7A	RE714	*Xgwm344*	Chantret et al. (2001)
7A	RE714	*Xgpw2252*	Muranty et al. (2009)

7A	Bainong 64	*Xbarc127 - Xbarc174*	Lan et al. (2009)
7B	Forno	*Xglk750 - Xmwg710a*	Keller et al. (1999)
7B	Saar	*Xwmc581 - XwPt-8807*	Lillemo et al. (2008)
7B	RE714	*XgbxG035b*	Chantret et al. (2001)
7B	RE714	*XpdaC01 - XgbxR035b*	Mingeot et al. (2002)
7B	RE714	*Xgwm577*	Chantret et al. (2001)
7D	Opata	*Xbcd1872 - Xwg834*	Börner et al. (2002)
7D	Courtot	*Xgpw1106*	Bougot et al. (2006)
7D	Courtot	*Xgdm67*	Bougot et al. (2006)

† Chromosome on which the quantitative trait loci (QTL) is located
‡ Cultivar with the resistance QTL
§ Closest flanking marker or markers to the QTL
¶ Reference for further information for each QTL

CHAPTER 2

VALIDATION OF QUANTITATIVE TRAIT LOCI AND IDENTIFICATION OF DIAGNOSTIC MARKERS FOR ADULT-PLANT RESISTANCE TO POWDERY MILDEW IN WHEAT CULTIVAR MASSEY

Tiffany R. Sikes

ABSTRACT

Powdery mildew, caused by the pathogen *Blumeria graminis* (DC) Speer (Syn. *Erysiphe graminis* DC) f. sp. *tritici*, is a major disease of wheat (*Triticum aestivum* L.). Vertical resistance governed by race-specific genes has often been used in breeding programs because it confers complete resistance during all plant growth stages and is easily transferred to progeny in crosses. However, this type of resistance often is easily overcome by the pathogen and remains effective only for a few years. Use of horizontal resistance, such as adult plant resistance (APR), has become increasingly popular due to its durability over a wide geographic region and time. Multiple studies have been conducted to identify simple sequence repeat (SSR) markers tightly linked to the quantitative trait loci (QTL) conferring APR to powdery mildew in the soft red winter (SRW) wheat cultivar Massey and its derivative 'USG 3209', yet large distances between QTL and SSR markers remain. In this study, a new mapping population, Massey / 'MPV 57', was used to further saturate the QTL regions identified previously using new SSR and single nucleotide polymorphism (SNP) markers. Phenotypic evaluations were conducted in two environments on the $F_{2:3}$ generation in the spring of 2012. Genotypic data was collected from the F_2 generation to generate linkage maps for QTL analysis. Four QTL were identified on chromosomes 2AL, 2BL, 2DS and 4DL explaining 8.1 to 14.4 %, 8.4 to 24.5 %, 9.3 to 13.1 % and 6.1 % of the phenotypic variation, respectively. The QTL on 2AL was flanked by the SSR markers *Xcfa2043* and *Xcfa2058* and was only significant in the North Carolina test. The QTL on 2BL was associated with the SNP marker *IWA2571* and was only significant in the Maryland test. The QTL on 2DS flanked by the gene *Ppd-D1b* for photoperiod sensitivity and the SSR marker *Xgwm484* was identified in both environments. A minor QTL on 4DL flanked by the SSR markers *Xwmc331* and *Xcfd71* was only significant for mean mildew severity for date 3 of

the rating on the F-2 leaves (two leaves below the flag leaf) in the North Carolina test. The markers identified in this study are more tightly linked to their associated QTL on chromosomes 2AL and 2BL than in previous studies, and the new QTL on chromosomes 2DS and 4DL were also flanked by tightly linked markers and therefore useful for marker-assisted selection (MAS).

INTRODUCTION

Powdery mildew, caused by the pathogen *Blumeria graminis* (DC) Speer (Syn. *Erysiphe graminis* DC) f. sp. *tritici*, is a major disease of wheat (*Triticum aestivum* L.) particularly in regions with cool or maritime climates (Bennett, 1984). The pathogen occurs in many wheat growing regions but in the mid-Atlantic region of the United States, epidemics frequently occur due to a humid climate accompanied by a cool spring. Hardwick et al. (1994) reported yield losses up to 30 % that were correlated with mildew severity on the leaf below the flag leaf (F-1) between the watery and milky ripe growth stages (GS 71-75, Zadoks et al. 1974), with severities up to 27.5 %. Chemical control combined with the use of resistant cultivars is currently the most effective way for producers to control powdery mildew. However in 2005, the Fungicide Resistance Action Committee (FRAC) included *B. graminis tritici* among pathogens at high-risk for development of resistance to fungicides (FRAC, 2005; Russell, 2004).

Race-specific resistance, governed by a single major gene, may be easily defeated by *B. graminis tritici* because of its high evolutionary potential (McDonald and Linde, 2002). The intensity, frequency, and geographic region where the resistance gene is deployed will affect how rapidly this "breakdown" may occur (Parks et al., 2008). In contrast, adult plant resistance (APR) to powdery mildew in several widely grown cultivars has remained effective for more

than four decades (Cowger et al., 2012). Quantitative trait loci (QTL) typically govern APR to powdery mildew and have been identified in multiple wheat backgrounds (Cowger et al., 2012).

The effect of APR on powdery mildew in the wheat cultivar Knox (CI 12798) and its derivatives is described as a reduction in sporulation rate and infection efficiency as compared to a hypersensitive reaction of race-specific genes (Shaner, 1973), even under epidemic conditions (Griffey et al., 1993). Similar QTLs for APR to powdery mildew have been mapped in the French wheat lines RE714 (Chantret et al., 2001; Chantret et al., 2000; Mingeot et al., 2002) and RE9001 (Bougot et al., 2006), the Chinese wheat cultivars Bainong 64 (Lan et al., 2009) and Lumai 21 (Lan et al., 2010), and in the Japanese cultivar Fukuho-komugi (Liang et al., 2006). Massey (CI 17953), a derivative of 'Blueboy' (CI 14031) / Knox 62 (CI 13701) (Starling et al., 1984), has been used in several studies investigating APR to powdery mildew due to its durability (Das and Griffey, 1994; Griffey et al., 1993; Hall and Griffey, 2009; Liu et al., 2001; Tucker et al., 2007).

In a 'Becker' (PI 494524) / Massey mapping population, three QTL for APR to powdery mildew were mapped to chromosomes 1BL (*QPm.vt-1B*), 2AL (*QPm.vt-2A*) and 2BL (*QPm.vt-2B*) (Liu et al., 2001) using restriction fragment length polymorphism (RFLP) and single sequence repeat (SSR) markers. A USG 3209 (PI 617055) / 'Jaypee' (P1 592760) validation population was developed to confirm results of the Becker / Massey population and to identify additional SSR markers to make marker assisted selection (MAS) more feasible (Tucker et al., 2007). USG 3209, a derivative of Massey putatively inherited its APR to powdery mildew from Massey. Three QTL were again identified on chromosomes 1BL (*QPm.vt-1BL*), 2AL (*QPm.vt-2AL*) and 2BL (*QPm.vt-2BL*) which verified that the three QTL in the cultivar USG 3209 were inherited from the parent Massey (Tucker et al., 2007).

Further work on the USG 3209 / Jaypee population was conducted to generate a whole genome linkage map using SSR and Diversity Arrays Technology (DArT) markers (that use microarray hybridizations to detect the presence or absence of genomic fragments) to further delineate and refine the chromosomal regions involved in APR to powdery mildew (Hall and Griffey, 2009). This study, performed over 10 environments in Virginia, Maryland, and North Carolina under natural field conditions, identified four genomic regions associated with APR to powdery mildew on chromosomes 1BL (*QPm.vag-1B*), 2AL (*QPm.vag-2A*), 2BL (*QPm.vag-2B*) and a new QTL located on chromosome 5AS (*QPm.vag-5A*). However, the QTL locations on chromosomes 1BL and 2AL in this study were in different chromosomal regions than in the Becker / Massey population (Hall and Griffey, 2009). The results contradicted the QTL locations identified earlier by Liu et al. (2001) and Tucker et al. (2007) thus, require further investigation to validate the QTL on chromosomes 1BL, 2AL and 2BL.

Despite the number of SSR and DArT markers added to the map in the three consecutive studies using Massey and its derivative USG 3209, the marker intervals identified for these QTL remains large (> 5 cM) (Hall and Griffey, 2009; Liu et al., 2001; Tucker et al., 2007). To be reliable for use in MAS, it is important for markers to be tightly linked to the QTL. Mapping QTL for APR to powdery mildew is inherently difficult because of the environmental sensitivity of *B. graminis tritici* populations and the complex and additive nature of these QTL (Keller et al., 1999; Mingeot et al., 2002).

The objectives of this study are to: 1) map and validate QTL for APR to powdery mildew in Massey using a new mapping population with the cultivar MPV 57 as the susceptible parent, 2) saturate the QTL regions for APR to powdery mildew on chromosomes 2AL and 2BL since these two QTL have the highest level of variance for APR to powdery mildew, 3) to identify

diagnostic markers for these QTL that can be used reliably in MAS, and 4) to identify new QTL that confer APR to powdery mildew.

MATERIALS AND METHODS

Plant Materials

Crosses were made between the soft red winter (SRW) wheat cultivars Massey (CI 17953) and MPV 57 (PI 639506) in the greenhouse in 2007. Massey is known to possess three QTL located on chromosomes 1BL, 2AL and 2BL that confer adult plant resistance (APR) to powdery mildew. MPV 57 was selected as the male parent because it is moderately susceptible to powdery mildew and differs from Massey for marker genotypes flanking the 2AL and 2BL QTLs. Two different Massey plants were crossed to three different MPV 57 plants. Seed from each of the three crosses was planted in the greenhouse in 2008 and produced 47 F_1 plants. Progeny rows of the population were grown in the field since each of these rows consisted of seed selected from a single plant and self-pollinated.

F_2 Seed Grown in the Field 2010-2011

Ten F_2 seed from each of the 47 Massey / MPV 57 F_1 plants were planted in 1.2 m progeny rows at Warsaw, VA on October 24, 2010. The parents were included twice and a set of host differentials having known *Pm* genes (*Pm1a – Pm1e, Pm2, Pm3a – Pm3g, Pm4a, Pm4b, Pm5a, Pm5b, Pm5d, Pm6, Pm7, Pm8, Pm9, Pm13, Pm16, Pm17, Pm20, Pm21, Pm24, Pm25, Pm29, Pm32, Pm34, Pm35, Pm36* and *Pm37*) was planted in head rows adjacent to the test to assess the virulence / avirulence spectrum of the *B. graminis* population in the field. Field management practices included the application of 30-60-60-5 (N-P-K-S) fall fertilizer on

October 12, 2010; 11.34 kg ha^{-1} of 12-0-0-1.5 (N-P-K-S) and 0.19 L ha^{-1} Starane® (Fluroxypyr) herbicide on Feburay 7, 2011; 0.051 L ha^{-1} Harmony Extra SG® (Thifensulfuron methyl) herbicide on Febuary 16, 2011; 11.34 kg ha^{-1} 12-0-0-1.5 on March 14, 2011; and 11.34 kg ha^{-1} 24-0-0-3 on March 30, 2011.

At the 3 to 4 leaf stage, plants within each row were thinned to 3 to 5 plants per row. Spacing the plants ensured that each individual plant would have optimal potential for growth. Tissue was collected from individual F_2 plants and the parents in the field at the 3 to 4 leaf stage by taking an entire leaf and storing it in a paper envelope at -80 ˚C. The samples would later be cut into 2.5 cm sections and placed in a 2 mL microcentrifuge tube containing a stainless steel bead for DNA extraction and subsequent use in marker genotyping and QTL mapping. At maturity, 375 F_2 plants were harvested individually. Seed from each plant was threshed and bulked to generate $F_{2:3}$ families.

$F_{2:3}$ Field Experiments 2011-2012

In the fall of 2011, the 375 $F_{2:3}$ families were planted in three environments at Raleigh, NC (Latitude 35° 44' 04.0" Longitude 78°42'11.1"W), Salisbury, MD (Latitude 38°21' 37.2" Longitude 75° 46' 23.8") and Warsaw, VA (Latitude 37˚ 59' 24.5" Longitude 76˚ 46' 29.2") in two randomized complete blocks comprised of 1.2 m progeny rows. Each $F_{2:3}$ row contained approximately 50 seed. The two Massey and three MPV 57 parents were planted in 33 head rows each throughout the tests at the beginning, middle and end of each respective population. The susceptible cultivars Saluda (PI 480474) and Tribute (PI 632689), the moderately resistant cultivar USG 3209 (PI 617055) and the resistant cultivar Shirley (PI 656753) were planted on average every 77 head rows as checks. The susceptible cultivar Becker (PI 494524) was planted

as a border in each environment. Wheat *Pm* host differential lines were also planted in head rows adjacent to the tests at each of these locations.

Assessment of Powdery Mildew

Evaluation of the $F_{2:3}$ families in different environments were conducted to account for potential variation in disease development and for variation in QTL expression of individual genotypes to naturally occurring *B. graminis* populations in different environments. Mildew severity was assessed using the James scale (James, 1971) at the onset of disease epidemics and repeated every 10 to 12 d to provide data for analysis of Area Under the Disease Progress Curve (AUDPC) for the population. The James scale uses a 0 to 50 % scale based on coverage of mildew infection on one side of the leaf. The side of the leaf with the largest area covered by mildew infection was used in the assessment. A rating of 50 meant the entire leaf area was covered by powdery mildew on one side of the leaf.

The plants at the beginning and end of each row were excluded from assessment because of differences in plant microenvironments and the potential for seed mixing in these adjacent areas during planting. Each row was visually divided into five sections to avoid rating tillers from the same plant. A single plant representative of the average phenotype of its section was selected and assessed for mildew severity on the F-2 (two leaves below the flag leaf), F-1 (one leaf below the flag leaf) and flag leaves. This was performed for each rating date and plants were randomly selected based on the criteria specified above. The plants that were used to assess mildew severity were not labeled and it was unlikely the same plant was rated at the different dates.

Raleigh, NC

The population was planted on October 27, 2011. The field was fertilized 3 d prior to planting using 90.72 kg ha^{-1} of 10-10-10 (N-P-K). On December 5, 2011, the head rows were treated with 0.058 L ha^{-1} Harmony Extra SG (Thifensulfuron methyl) herbicide and again on February 9, 2012 using 0.051 L ha^{-1}. On December 13, 2011, the test plots were sprayed with Karate® (Lambda-cyhalothrin) insecticide at 0.94 L ha^{-1}. On March 7, 2012, the head rows were top dressed using 31.75 kg ha^{-1} of 34-0-0 (N-P-K). Mildew severity ratings were taken on March 14-16 (GS 35 – 40), March 26-29 (GS 45 – 56), April 4-8 (GS 58 – 65), and April 16-20 (GS 70 – 75). Weather and environmental conditions favored epidemic levels of infection throughout this period of time.

Salisbury, MD

The population was planted October 17, 2011. Fall fertilizer was not applied to the field site prior to planting. The field was fertilized subsequently on March 6, 2012 with 136 kg ha^{-1} of 15-05-15 (N-P-K) and 0.044 L ha^{-1} Harmony Extra SG (Thifensulfuron methyl) herbicide was applied on March 15, 2012. On April 17, 2012, the head rows were sprayed with Warrior® (Lambda-cyhalothrin) insecticide at 0.22 L ha^{-1}. Conditions at Salisbury were initially unfavorable for mildew infection due to severe drought. The plots were drip irrigated on April 12 using irrigation tape with approximately 15,140 L of water. Mildew severity ratings were taken on April 10-13 (GS 42 – 54), April 23-25 (GS 58 – 65) and May 5-8 (GS 68 – 74). The F-2 leaves were severely damaged due to drought and disease severity assessments were only possible for the first rating date.

Warsaw, VA

The population was planted on October 22, 2011. The field was fertilized 10 d prior to planting with 30-80-80-5 (N-P-K-S). Subsequent test management included applications of 11.34 kg ha^{-1} of 12-0-0-1.5, 0.48 L ha^{-1} Starane (Fluroxypyr) and 0.055 Harmony Extra SG (Thifensulfuron methyl) herbicides on December 20, 2011; 11.34 kg ha^{-1} of 12-0-0-1.5 on January 30, 2012, and 18.14 kg ha^{-1} of Nitamin® slow release N fertilizer (60% methylene ureas and triazone 40% urea) on March 23, 2012. As in Maryland, climatic conditions at Warsaw were excessively droughty and unfavorable for powdery mildew development, and irrigation at this test site was not possible. Barley yellow dwarf and leaf rust infections were severe on susceptible plants and were prevalent throughout the population. Data from the Virginia location were excluded in phenotypic analyses. At maturity, 12 heads were harvested from different plants within each row at the Warsaw test site, and threshed separately to generate the $F_{3:4}$ families.

Molecular Markers and Genotyping of F_2 Generation

Tissue samples approximately 2.5 cm in length from 375 F_2 plants were placed individually in 2 mL tubes each containing a stainless steel bead. The samples were frozen overnight at -80 ˚C and ground for 15-20 s using the Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ). DNA extraction from the F_2 plants was performed using the CTAB extraction method as outlined by Saghai Maroof et al. (1994). The DNA was resuspended using 200 µL molecular grade water in 1.5 mL tubes. Quantification of DNA was performed using the NanoDrop (Thermo Fisher Scientific Inc., Wilmington, DE) from which four 96-well dilution plates were made containing 50 ng μL^{-1} DNA concentrations. The parents and a blank sample

were included in each plate. Each well in the four 96-well plates contained the DNA of an individual F_2 plant, Massey, MPV 57 or blank water sample for all genotyping reactions performed. Two µL of the DNA samples were pipette into multiple 96-well plates numbered 1 to 4 and dried in a 60 ˚C oven for approximately one hour. Each plate was covered with aluminum foil and stored in a drawer until used for genotyping reactions.

Polymerase chain reaction (PCR), modified from Röder et al. (1998), was performed using the microsatellites *Xgwm* (Röder et al., 1998), *Xbarc* (Song et al., 2005), *Xwmc* (Gupta et al., 2002; Somers et al., 2004), *Xcfa* (Sourdille, 2001), *Xcfd* (Guyomarc'h et al., 2002), *Xgdm* (Pestsova et al., 2000), *Xpsp* (Bryan et al., 1997; Stephenson et al., 1998) and sequence-tagged site (STS) markers $NAU/STS_{BCD135\text{-}1}$ and $NAU/STS_{BCD135\text{-}2}$ (Jianhui et al., 2008).

The primers were either directly labeled with the fluorescent dyes FAM (blue), VIC/HEX (green), NED (yellow), PET (red) or contained an indirect fluorescent M13 tail (5' – ACGACGTTGTAAAACGAC – 3' or 5' – CACGACGTTGTAAAACGAC – 3'). A single PCR reaction volume totaled 10 µl containing 100 ng DNA.

Direct label PCR reaction included: 1 x standard reaction buffer, 1.5 mmol mL^{-1} $MgCl_2$, 0.20 mmol mL^{-1} dNTP, 0.20 µmol mL^{-1} forward primer, 0.20 µmol mL^{-1} reverse primer, 0.5 units *Taq* polymerase (Apex, Genesee Scientific, San Diego, CA). The initial denaturing step, 5 min at 95 ˚C, was followed by 34 cycles of 0.45 s at 95 ˚C, 1 min at 50, 52, 55, 57, 58 or 60 ˚C depending on annealing temperature of the direct labeled primer pair, 72 ˚C for 1.30 min and final extension of 5 min at 72 ˚C. The primers *Xbarc55*, *Xbarc349*, *Xbarc76*, *Xgwm388*, *Xgwm484*, *Xwmc317*, *Xwmc331*, *Xwmc332*, and *Xwmc382* were run using a different protocol with an initial denaturing step of 2 min at 95 ˚C, followed by 35 cycles of 0.30 s at 94 ˚C, 0.30 s

at 52, 55, 58, 60 or 61 ˚C depending on primer pair, 0.45 s at 72 ˚C ending with final extension at 72 ˚C for 5 minutes.

The M13 reaction included: 1 x standard reaction buffer, 1.5 mmol mL^{-1} $MgCl_2$, 0.20 mmol mL^{-1} dNTP, 0.080 µmol mL^{-1} forward primer, 0.60 µmol mL^{-1} reverse primer, 0.60 µmol mL^{-1} m13 fluorescent tail, and 0.50 units *Taq* polymerase (Apex, Genesee Scientific, San Diego, CA). The initial denaturing step, 5 min at 95 ˚C, 0.45 s at 95 ˚ C and 0.45 s at 60 ˚C (-2˚C every 3 cycles), was followed by 1.30 min at 72 ˚C for 15 cycles, 0.45 s at 95 ˚C, 0.45 s at 50 ˚C and 1.30 min at 72 ˚C for 20 cycles, and a final extension for 10 min at 72 ˚C. The M13 primers *Xcfa2058, Xgwm193* and *Xwmc361* used a different protocol with the initial denaturing step, 5 min at 95 ˚C, followed by 0.45 s at 94 ˚C and 0.45 s at 65 ˚C (-1 ˚C every cycle) and then 1 min at 72 ˚C for 15 cycles, followed by 25 cycles at 94 ˚C for 0.45 s, at 50 ˚C for 0.45 s, and 1 min at 72 ˚C with a final extension at 72 ˚C for 5 min.

PCR products were observed using Gene Scan™ – 500 LIZ ® (Applied Biosystems, Foster City, CA) by capillary electrophoresis on Applied Biosystems 3130xl or 3730xl Genetic Analyzers (Applied Biosystems) to compile genotypic data with the GeneMarker software by SoftGenetics (State College, PA, USA). Five samples were discarded due to potential contamination or low DNA quality leading to poor amplification.

Bulk Segregant Analysis (BSA) (Michelmore et al., 1991) was performed by pooling equal amounts of F_2 DNA from five most resistant and five most susceptible individuals based on the average $F_{2:3}$ phenotypes. Using the SSR markers that were identified as polymorphic in the parents, BSA allowed for rapid identification of those markers most likely associated with QTLs for powdery mildew resistance and susceptibility. These areas would be targeted for further saturation based on primary QTL analysis.

KBiosciences competitive allele-specific PCR (KASPar) is a SNP genotyping system that is easy and cost-effective (Chen et al., 2010). Each KASPar assay was performed in a total volume of 10 µL: 5 µL DNA (7 ng μL^{-1}), 5 µL 2x KASP reaction mix and 0.14 µL assay mix of primers (two 5' fluorescent labeled FAM and HEX allele-specific primers, one for each SNP allele, and one common reverse primer) optimized by the manufacturer and completed according to manufacturer's instructions (LGC Genomics, Beverly, MA). Plates were read on a PHERAstar Plus microplate reader (BMG LABTECH, Inc., Cary, NC) and data were analyzed using KlusterCaller software (LGC Genomics, Beverly, MA).

Detection of Polymorphic Markers

The two Massey and three MPV 57 parents were screened with 440 SSR and 26 SNP markers to detect polymorphisms. This screen identified 150 of the 440 (34 %) SSR markers as polymorphic and advanced to screen the BSA samples which narrowed this number down to 34 SSR markers that were most likely associated with resistance or susceptibility based on the genotype of the bulk sample. Of the 26 SNP markers, 21 were polymorphic and used in the construction of the genetic map.

Analysis of $F_{2:3}$ phenotypic and F_2 genotypic data

Phenotypic data for powdery mildew severity were analyzed for the mean AUDPC of the F-2, F-1 and flag leaves according to Shaner and Finney (1977) using the following formula:

$$((0 + M_1)/2) * 12 + ((M_1 + M_2)/2) * (D_1 - D_2) + ((M_2 + M_3)/2) * (D_2 - D_3)$$

Where M represents the mean mildew severity for a given date and leaf, 12 was used as the first date rather than starting from zero since mildew development began prior to the first rating and

D represents the date the rating took place. The mean mildew assessments were combined to determine the mean AUDPC (MAUDPC) for the F-2, F-1 and flag leaves.

The phenotypic data from each environment for individual $F_{2:3}$ families were compared to their corresponding F_2 genotypic data using the mean AUDPC and the mean mildew severity for each rating date for the F-2, F-1 and flag leaves. The F-2, F-1 and flag leaves were kept as separate classes in order to determine if one of the leaves was more significant for expression of a QTL at a particular date. Linkage maps were created using MapDisto software (Lorieux, 2012) and Kosambi's mapping function (Kosambi, 1944) with an LOD threshold of 3.0. Marker order was verified using consensus maps of Somers et al. (2004) and Marone et al. (2013). QTL Cartographer V2.5 (Wang et al., 2007) was used in QTL analysis of the Raleigh and Salisbury $F_{2:3}$ phenotypic and F_2 genotypic data. Statistical analysis included composite interval mapping to give forward and backward stepwise regression of the linkage groups (Zeng, 1994). The LOD threshold was determined with 1,000 permutations at a 0.001 significance level with a window size of 20, using model 6 for each mean mildew assessment and mean AUDPC at each location for the F-2, F-1 and flag leaves. The SAS procedure PROC GLM (SAS, 2009) was performed on the 2012 data from Raleigh and Salisbury to obtain ANOVA with replication and line as fixed effects. MapChart (Voorrips, 2002) was used to draw the linkage maps with the identified QTL.

RESULTS

Phenotypic Assessment of the $F_{2:3}$ Generation

Assessment of powdery mildew severity at different locations was necessary because the environment influences the pathogen population and the expression of the QTL for APR in response to powdery mildew infection. Environmental conditions favored high levels of

powdery mildew infection and development in Raleigh, NC throughout the rating period. Initially, conditions in Salisbury, MD were not favorable and very dry, which caused the F-2 leaves to senesce prematurely allowing for only one assessment from the first date on those leaves. Conditions improved by the second rating and moderate levels of infection developed but the intensity of infection was less than observed in Raleigh, NC.

Distribution of the phenotypes among the $F_{2:3}$ lines was continuous in both environments for the mean AUDPC (MAUDPC) and the mean mildew severity of each rating date (MMSDate) for the F-2, F-1 and flag leaves. The distributions were skewed toward resistance, and transgressive segregation was observed for both resistance and susceptibility (Figure 2.1). Transgressive segregants are individuals whose phenotypes are the extreme of either parental phenotype and may indicate the presence of multiple QTL with additive effects or other gene action such as epitasis (Rieseberg et al., 1999). These observations are consistent with results reported for the Becker / Massey and USG 3209 / Jaypee populations (Liu et al., 2001; Tucker et al., 2007). In Maryland, the MAUDPC values for the flag leaves of the parents were similar (figure not shown).

The wheat host differentials for powdery mildew typically had similar virulence reactions in North Carolina and Maryland in 2012 (Table 2.1). These tests were rated using a common 0 to 9 ordinal scale with 0 being most resistant and 9 most susceptible. Pathogen populations in both locations had partial to complete virulence to genes *Pm2, Pm3a*, *Pm3b*, *Pm3c*, *Pm3f, Pm4a*, *Pm5a*, *Pm5b, Pm6*, *Pm7*, *Pm8, Pm9*, *Pm17* and *Pm20*. Resistance genes *Pm1a – Pm1e*, *Pm3d*, *Pm13, Pm16, Pm29, Pm34, Pm35, Pm36* and *Pm37* were effective at both locations. Notable differences between locations for virulence based on disease severity ratings were observed for

genes *Pm2*, *Pm3g*, *Pm4a*, *Pm4b*, *Pm5b*, *Pm6*, *Pm7*, and *Pm24*. This indicated that the virulence spectrum of *B. graminis tritici* populations varies between these two locations.

Genotyping F_2 Generation and Linkage Map Construction

A total of 440 SSR and 26 SNP markers were screened for polymorphism between the parents. Of those, 150 polymorphic SSRs were used to screen the BSA samples. Results from the BSA analysis revealed that 34 SSR markers were most likely associated with resistance or susceptibility to powdery mildew. Additional SSR markers were selected and evaluated based on the location of markers identified in initial linkage maps and QTL analyses. In the previous Becker / Massey and USG 3209 / Jaypee studies, SNP markers were not mapped to the identified QTL regions on chromosomes 2AL or 2BL. The SNP markers used in the current study were selected to cover both chromosomes and potentially identify those closest to the QTLs for further saturation. Initially, 24 SNP markers were selected based on these criterion and 19 of these were polymorphic. Subsequently, two additional SNP markers were added based on linkage map and QTL analyses. In total, 21 SNP and 67 SSR markers were used to construct the final linkage map. Thirteen linkage groups were created with 18 SNP and 55 SSR markers with the remainder being unlinked. The linkage groups included chromosomes 2A, 2B, 2D, 3B, 3D, 4A, 4B, 4D, 5A, 6B, and 7D but only those linkage groups with QTL for APR are shown (Figures 2.2, 2.3, 2.4 and 2.5).

The markers on chromosome 2BL did not exhibit the expected 1:2:1 ratio for co-dominant markers (Table 2.2). Segregation distortion favored the Massey alleles and genetic distances were reduced because of the lower recombination in this region. These results were also consistent with those reported for the Becker / Massey population (Liu et al., 2001).

Comparison of the marker order to previously published consensus maps (Cavanagh et al., 2013; Marone et al., 2013; Somers et al., 2004) show disagreement between marker distances for the SSRs and for marker order of the SNPs on 2B. The SNP order on 2AL also did not conform to the consensus map (Cavanagh et al., 2013).

QTL Analysis

There was significant correlation ($P < 0.05$) between powdery mildew assessment data at and between the two locations (Tables 2.3, 2.4 and 2.5). The correlation coefficients ranged from 0.28 to 0.68 between the two locations for corresponding growth stages (eg. NC MMSDate2 F-1 and MD MMSDate1 F-1). Correlation coefficients above 0 indicate that the two assessments may be influenced by the same QTL or QTLs. The MMSDates and MAUDPC values for the F-2, F-1 and flag leaves in the correlation tables for either North Carolina (Table 2.3), Maryland (Table 2.4) or both (Table 2.5) with higher correlation coefficients and probabilities ($P > 0.05$) are more likely influenced by the same genetic factors.

Test of homogeneity of variances was significant; hence, separate ANOVA's were calculated for each location, MAUDPC and mean mildew severities for the F-2, F-1 and flag leaves. The lack of homogeneity between the two locations is likely due to the different *B. graminis* populations and environmental conditions. Broad-sense heritability for the QTL also could not be calculated because of the lack of homogeneity. Significant differences ($P < 0.05$) were detected among genotypes for the MAUDPC (Table 2.6) and mean mildew assessments (Table 2.7) in North Carolina and MAUDPC (Table 2.8) and mean mildew assessments (Table 2.9) in Maryland. The mean mildew severity date 1 (MMSDate1) in North Carolina only

includes the F-2 leaves because the level infection on the F-1 and flag leaves was minimal with a high number of missing data and is not included in the ANOVA.

For each type of phenotypic data, 1,000 permutations at a 0.001 significance level were run to obtain LOD threshold for QTL significance. Of the 24 assessments including the MAUDPC values, QTL were associated with 15 of the powdery mildew assessments at the designated thresholds (Table 2.10). All of the QTL for APR to powdery mildew were inherited from the resistant parent Massey based on additive effects (Table 2.10). A QTL on the long arm of chromosome 2AL was consistent in seven different assessments and only significant in the North Carolina test. This QTL was located between the SSR markers *Xcfa2043* and *Xcfa2058* (Figure 2.2). The total distance between the two flanking markers was 13.4 cM. The phenotypic variation explained by this QTL ranged from 8.1 to 14.4 % with LOD scores from 6.7 to 11.7 and additive and dominant effects ranging from -71.0 to -1.2 and 0.5 to 29.7 respectively. The QTL peak was 5 cM from *Xcfa2043* and 8.4 cM from *Xcfa2058*. The highest LOD score and phenotypic variation explained by this QTL was observed for the NC MMSDate3 F-2 (Table 2.10). This QTL was associated with seven of the fourteen powdery mildew assessments in the NC data set and only with disease ratings on the F-2 and F-1 leaves (Table 2.10). The SSR marker *Xcfa2058* acted in a dominant manner in this population.

A QTL on the long arm of chromosome 2B was consistent in seven assessments and only significant in the Maryland test and was located at the SNP marker *IWA2571*. The phenotypic variation explained by this QTL ranged from 8.8 to 24.5 % with LOD scores from 7.8 to 25.3 and additive and dominant effects ranging from -33.6 to -0.54 and -3.6 to 1.1, respectively (Table 2.10). This QTL was associated with seven of the ten powdery mildew assessments in the Maryland data set. Limited data could be collected from the F-2 leaves in Maryland due to the

initial dry conditions, which might explain the lack of association of the QTL on 2BL with powdery mildew assessments on the lower leaves. The highest LOD score and phenotypic variation explained by this QTL was observed for the MD MMSDate3 F-1 assessment. The SNP marker *IWA2571* was mapped with a group of tightly linked markers associated with the powdery mildew resistance gene *Pm6* and the stem rust resistance gene *Sr36* (Figure 2.3).

A QTL on the short arm of chromosome 2D was associated with powdery mildew severity at both locations. In North Carolina, the QTL was only associated with mean mildew severity data for the F-1 leaves at the 2^{nd}, 3^{rd} and 4^{th} rating dates and explained 9.3 to 13.1 % of the phenotypic variation with LOD scores from 8.0 to 10.6 and additive and dominant effects ranging from -3.3 to -1.3 and 0.5 to 0.7, respectively. In Maryland, the QTL was associated with data from the flag leaves including MAUDPC, and the mean mildew severity at all dates. Phenotypic variation explained by this QTL varied from 10.2 to 12.3 % and LOD scores ranged from 7 to 9.9 with additive and dominant effects ranging from -23.8 to -0.37 and -6.0 to -0.16, respectively. The QTL was located between the photoperiod locus *Ppd-D1* and SSR marker *Xgwm484*. The peak of the QTL was 6 cM from *Ppd-D1* and 9.5 cM from *Xgwm484* with a total distance of 15.5 cM between flanking markers (Figure 2.4).

A single minor QTL on the long arm chromosome 4D was associated with the powdery assessment data for NC MMSDate3 F-2. This QTL was located between the SSR markers *Xcfd71* and *Xwmc331* and explained 6.1 % of the phenotypic variation with and LOD score of 5.5 and additive and dominant effects of -2.3 and -0.03. The QTL peak was closest (2.2 cM) to marker *Xwmc331* and 6 cM from *Xcfd71* (Figure 2.5). Fragment sizes of the SSR markers mapped to the QTL on chromosomes 2AL, 2DS and 4DL are provided in Table 2.11.

DISCUSSION

Linkage Maps, QTL and Diagnostic Markers

Chromosome 2AL

There is general agreement in marker order and distance between the Massey / MPV 57 population and the Becker / Massey population mapped by Liu et al. (2001). In the Becker / Massey $F_{2:3}$ generation, the QTL on 2AL, *QPm.vt-2AL* mapped between the SSR markers *Xgwm304* and *Xgwm312* with the QTL peak being 12 cM from *Xgwm304* and spanning a total distance of 33 cM. In this same population, the $F_{5:6}$, $F_{6:7}$, and $F_{7:8}$ generations had the same SSR interval (*Xgwm304 – Xgwm312*) for the QTL but the peak was 6 cM from *Xgwm304*. In the current study, this QTL was confirmed and new SSR markers, *Xcfa2043 and Xcfa2058*, were added to the map, which have a shorter interval of 13.4 cM and the peak located at 5 cM from *Xcfa2043*. The SSR marker *Xgwm304* did not map to 2AL in this population. The results indicated that *Xcfa2043* could be a more diagnostic marker for *QPm.vt-2AL*.

In the USG 3209 / Jaypee population, a lack of polymorphism between the markers mapped by Liu et al. (2001) left a large gap (80 cM) between the flanking SSR markers *Xgwm304 – Xbarc353* (Tucker et al., 2007). However, the QTL on 2AL later identified by Hall and Griffey (2009) in the USG 3209 / Jaypee population was mapped 73.4 cM above *QPm.vt-2AL* and associated with the SSR markers *Xgwm448* and *Xgwm122* and spanned a total distance of 10.1 cM. This QTL lies 50 cM from the proximal end of the chromosome on 2AL based on Somers et al. (2004) consensus map whereas *QPm.vt-2AL* mapped by Liu et al. (2001) is 74 cM from the proximal end of the consensus map. It appears that this QTL on 2AL in the USG 3209 /

Jaypee population mapped by Hall and Griffey (2009) is different from the one previously mapped in the Becker / Massey population and in the current study.

The phenotypic variation explained by *QPm.vt-2AL* is much larger in the studies by Liu et al. (2001) and Tucker et al. (2007) than that observed in the current study. These differences are likely due to the differences in mapping populations, test environments and years, pathogen populations, and onset and magnitude of the powdery mildew epidemics. Quantitative traits, such as APR to powdery mildew, are often affected significantly by genotype by environment interactions. Tucker et al. (2006) performed a greenhouse experiment with *B. graminis* isolates and noted epidemic levels of infection that were more intense than observed in the field. Under these conditions, the QTL on 1BL and 2BL were not significant and only the QTL on 2AL was associated with resistance (Tucker et al., 2006). This study illustrates the environmental sensitivity of both *B. graminis* populations and the expression of QTL conferring APR to powdery mildew and may explain why the QTL on 2AL was only significant in North Carolina, where powdery mildew epidemics were severe.

In the recent QTL meta-analysis study performed by Marone et al. (2013), the QTL on 2AL identified by Liu et al. (2001) in both the $F_{2:3}$ and $F_{5:6}$, $F_{6:7}$, and $F_{7:8}$ generations are shown to group as MQTL4. No other QTLs are included in this group and the QTL mapped by Tucker et al. (2007) is located above this group (Marone et al., 2013). The MQTL4 is located in a gene-rich region that includes the postulated dominant gene *PmHNK54* that was identified in the Chinese cultivar Zheng 9754 and flanked by the SSR markers *Xgwm372* and *Xgwm312* (Xu et al., 2011). Although gene *PmHNK54* is located in the same marker interval as *QPm.vt-2AL* identified in the current study and by Liu et al. (2001), the band sizes for *Xgwm312* differ between Zheng 9754 and Massey. Xu et al. (2011) mapped *Xgwm312* at 6 cM from *PmHNK54*

with a band size of 100 bp; whereas, the distance of this marker in the current study is 12.8 cM from the peak of *QPm.vt-2AL* and has a band size of 215 bp. It appears that *PmHNK54* is different and not a part of *QPm.vt-2AL*.

Alleles at the *Pm4* gene locus (*Pm4a*, *Pm4b*, *Pm4c* and *Pm4d*) also have been mapped in this region (Hao et al., 2008; Ma et al., 2004; The et al., 1979). However, Liu et al. (2001) mapped SSR markers (*Xgwm526* and *Xgwm356*) associated with *Pm4* that were outside the region of *QPm.vt-2AL*. Tucker et al. (2006) reported that Massey and USG 3209 do not possess *Pm4a* or *Pm4b* on the basis of unique reactions observed to differential isolates. Also, the PCR reactions for the corresponding SSR markers (*Xgwm526*, and *Xbarc122*) (Hao et al., 2008; Schmolke et al., 2012) and the STS marker *ResPm4* (Schmolke et al., 2012) failed to amplify in the current population. The SSR *Xgwm526* was polymorphic between the parents but failed to express properly in the population, while the other markers failed to amplify in both the parents and population. Further investigation and saturation of markers to this region will be helpful to understand the relationship of this QTL to the genes known to reside nearby.

Chromosome 2BL

Significant segregation distortion observed with SSR, STS and SNP markers mapped to the 2BL region associated with APR to powdery mildew in the current study confirms the presence of the *Triticum timopheevii* introgression carrying the genes *Pm6* and *Sr36* from Massey. Preferential segregation and, therefore, reduced recombination of the Massey alleles resulted in shortened genetic distances between the markers than in previously reported maps (Cavanagh et al., 2013; Ji et al., 2008; Liu et al., 2001; Somers et al., 2004; Tsilo et al., 2008;

Tucker et al., 2007). Segregation distortion also was reported in the Becker / Massey population (Liu et al., 2001).

Several markers tightly linked to the SNP marker *IWA2571* were mapped less than 5 cM and were within one-LOD from the peak. The presence of genes *Pm6* and *Sr36* was confirmed by the *Pm6* specific STS markers $NAU/STS_{BCD135\text{-}1}$ and $NAU/STS_{BCD135\text{-}2}$ (Ji et al., 2008) and the SNP marker *IWA8085* specific to the *Pm6*/*Sr36* region (Dr. Gina Brown-Guedira, personal communication, 2013). The *Pm6* specific markers flank the SNP marker *IWA2571* and *IWA8085* (*Pm6*/*Sr36*) maps with $NAU/STS_{BCD135\text{-}2}$ confirming the presence of *Pm6* in Massey.

Two SSR markers (*Xgwm47* and *Xgwm388*) mapped by Liu et al. (2001) and an additional SSR (*Xbarc200*) added by Tucker et al. (2007) were mapped to 2BL in the current study. The marker order is inverted and the distances in this study do not agree with those of previous studies. However, the SSR marker *Xgwm47* mapped near the QTL in all three studies and also was identified by Hall and Griffey (2009) as the marker at the QTL peak. This SSR lies 1 cM from the current QTL peak and SSR marker *Xbarc200* is only 0.3 cM further away. The results indicated that markers *Xbarc200* and *Xgwm47* could also be diagnostic for *QPm.vt-2BL*.

The phenotypic variation explained by the QTL in the Maryland location was within the range (11 – 15 %) reported by Liu et al. (2001) and on average lower than that (22 – 48 %) observed by Tucker et al. (2007). Again, the inconsistency in results may be attributed to differences among parents in the mapping populations, virulence spectra of the pathogen populations, and variability in the disease epidemics. Disease onset was initially slow in Maryland and progressed later and to a lesser extent than was observed in North Carolina.

Interestingly, the RFLP marker *Xcdo244* that mapped 5 cM from *Xgwm47* in the Becker / Massey population (Liu et al., 2001) corresponds to a nucleotide-binding site leucine-rich repeat

(NBS-LRR) receptor-like gene (Marone et al., 2013). The receptor-like NBS-LRR family is the largest class of R-genes that are responsible for pathogen detection in plants (DeYoung and Innes, 2006). An LRR receptor-like protein kinase cluster was identified in collinear regions of *Brachypodium* and rice (*Oryza sativa* L.) by Qin et al. (2011) with conserved wheat STS markers flanking *Pm6*. Qin et al. (2011) recommended this putative resistance gene cluster as the potential site for further fine-mapping and cloning of *Pm6*.

The relationship between QTL for APR to powdery mildew and the race-specific gene *Pm6* is not clear. The QTL on 2BL was significant only in Maryland where mildew epidemics occurred later, were less severe, and where virulence to gene *Pm6* was lower (with a severity rating of 4) than in North Carolina (with a severity rating of 7). Resistance conferred by *Pm6* is only expressed when seedlings reach the fourth leaf stage as is characteristic of many APR genes (Ji et al., 2008; Qin et al., 2011). The French line RE9001 has a QTL in the same region as the SSR marker *Xgwm47* (Bougot et al., 2006) and the Japanese cultivar Fuhuko-komugi with a QTL flanked by *Xwmc877.1 – Xwmc435.1* (Liang et al., 2006). The Marone et al. (2013) QTL meta-analysis study reported that the QTL on 2BL identified by Liu et al. (2001) and Bougot et al. (2006) are in the same location and these were grouped as MQTL8 and the QTL identified by Liang et al. (2006) as MQTL7. Comparison of these QTL regions to the wheat composite map (GrainGenes.org) depict marker *Xwmc435.1* next to *Xgwm47* and *Xwmc877.1* and near *Sr36*, which disagrees with the QTL meta-analysis study.

The complex and confounding nature of the *T. timopheevii* translocation is apparent considering the number of studies aimed at mapping this region in different populations of varying size with different types of markers. The addition of markers identified in the studies discussed above and further saturation of the chromosome in the current population may help

explain some of these anomalies. A better understanding of the size, location and impact of the translocation on recombination in other regions of the 2B chromosome and whether or not the QTL for APR and *Pm6* are independent of one another requires further investigation. Whether or not the genes are encoded by an NBS-LRR or LRR-PRK resistance gene will also help clarify their relationship.

Chromosomes 2DS and 4DL

Identification of a QTL for APR to powdery mildew on the short arm of chromosome 2D has not been reported in previous studies. Lan et al. (2010) reported a QTL for APR to powdery mildew in the cultivar Lumai 21 flanked by the SSR markers *Xgwm18 – Xcfd233* which are outside the QTL region in the current study. The *Ppd-D1* locus is involved in photoperiod sensitivity and initiation of flowering in wheat. Massey has the allele *Ppd-D1b* and is photoperiod sensitive, thus its flowering stage is night length sensitive. MPV 57 has the *Ppd-D1a* allele and is photoperiod insensitive and, therefore, flowering is primarily dependent on temperature. The QTL *Qfhs.vt-2DS*, associated with Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, in Massey was mapped to the same region in the Becker / Massey $F_{7:14}$ population (Liu et al., 2013). However, Liu et al. (2013) indicated that the *Ppd-D1* locus has a pleiotropic effect when combined with the dwarfing genes *Rht-B1* and *Rht-D1* which increases susceptibility to FHB. It is possible that a gene or multiple genes conferring resistance to APR to powdery mildew might co-segregate or have a pleiotropic effect with the *Ppd-D1b* locus. Early flowering time has been associated with increased susceptibility with necrotrophic plant pathogens and the relationship between disease resistance and flowering time for this specific type of plant-pathogen interaction is well correlated (Poland et al., 2009). However, the

relationship of *Ppd-D1b* in Massey and its association with resistance to powdery mildew requires further investigation.

The QTL on 4DL was only associated with mean powdery mildew severity on the F-2 leaves at the third rating date in North Carolina. The SSR marker *Xwmc331* linked to this QTL in the current study also was mapped to a QTL for APR to powdery mildew on 4D in the cultivar Bainong 64 (Lan et al., 2009). However, the other flanking markers (*Xgwm165* and *Xbarc200*) identified by Lan et al. (2009) do not map to the QTL region in the Massey / MPV 57 population. The QTL *QPm.caas-4DL* in Bainong 64 was stable across multiple environments with a high LOD score and large genetic effects (Lan et al., 2009), but in the current study the effect of this QTL is minor and inconsistent. Both studies have the same SSR marker *Xwmc331* tightly linked to their respective QTL (2.2 cM Massey / MPV 57 and 0.8 cM Bainong 64 / 'Jingshuang 16' DH population), so it is possible these may be the same QTL.

Pyramiding QTL

Comparisons of the mean mildew assessment data for QTL having different combinations of the Massey and MPV 57 alleles were performed using a t-test for LSD. Genotypes having Massey alleles at more than one QTL had lower mean mildew severities in North Carolina (Table 2.12) and Maryland (Table 2.13). For example, the RIL genotype having the lowest mean mildew severity for the F-1 leaves on the second rating date (MMSDate2 F-1) at North Carolina has two QTL (2AL and 2DS). In combination, the mean mildew severity of individuals with Massey alleles at both QTL (2AL and 2DS) are lower than those individuals having Massey alleles at only one of these QTL. The use of MAS to pyramid multiple QTL

derived from Massey and other unique APR sources will aid in the development of highly resistant lines.

Conclusions

The results of the current study confirm the QTL identified by Liu et al. (2001) in the Becker / Massey population on chromosomes 2AL and 2BL. The addition of SSR markers to the linkage map in the current study has shortened the marker interval (13.4 cM compared to 33 cM) on chromosome 2AL and the SSR marker *Xcfa2043* is 5 cM from the QTL peak should be more reliable for use in MAS. The marker interval for the QTL on 2BL was further characterized with the addition of SNP markers in the current study, and the SNP marker *IWA2571* should be more diagnostic for MAS of this QTL from Massey. Liu et al. (2001) identified the RFLP *WG338* as the diagnostic marker for this QTL but implementing RFLP markers in MAS is not preferred. Tucker et al. (2007) later identified the SSR markers *Xgwm501* and *Xgwm191* as the flanking markers and spanning a distance of 30 cM. Hall and Griffey (2009) subsequently reported a 23.5 cM distance with the SSR marker *Xgwm47* being closest to the QTL peak. Since this QTL is associated with a translocation from *T. timopheevii* carrying the *Pm6*/*Sr36* gene cluster, markers associated with these genes such as *IWA8085*, the STS markers for *Pm6* and the SSR markers *Xgwm47* and *Xbarc200* may also be utilized for MAS.

Two new QTLs were identified in Massey on chromosomes 2DS and 4DL that are associated with APR to powdery mildew. The QTL on 2DS has not previously been associated with resistance to powdery mildew. Further investigation on the photoperiod sensitive gene *Ppd-D1b* from Massey and its effect on increased resistance to powdery mildew and Fusarium head blight are necessary to elucidate this relationship. The QTL on 4DL is tightly linked to the SSR

marker *Xwmc331*, but its effects were minor, growth-stage specific and inconsistent across locations. Pyramiding the QTL identified in Massey with those from other unique sources will aid in the development of highly resistant lines.

REFERENCES

1. Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: A review of its use in agriculture and breeding programs. Plant Pathology 33(3):279-300.
2. Bougot, Y., J. Lemoine, M.T. Pavoine, H. Guyomar'ch, V. Gautier, H. Muranty, D. Barloy. 2006. A major QTL effect controlling resistance to powdery mildew in winter wheat at the adult plant stage. Plant Breeding 125(6):550-556.
3. Bryan, G.J., A.J. Collins, P. Stephenson, A. Orry, J.B. Smith, M.D. Gale. 1997. Isolation and characterisation of microsatellites from hexaploid bread wheat. Theoretical and Applied Genetics 94(5):557-563.
4. Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen, S. Kiani, K. Forrest, C. Saintenac, G.L. Brown-Guedira, A. Akhunova, D. See, G. Bai, M. Pumphrey, L. Tomar, D. Wong, S. Kong, M. Reynolds, M.L. da Silva, H. Bockelman, L. Talbert, J.A. Anderson, S. Dreisigacker, S. Baenziger, A. Carter, V. Korzun, P.L. Morrell, J. Dubcovsky, M.K. Morell, M.E. Sorrells, M.J. Hayden, E. Akhunov. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proceedings of the National Academy of Sciences 110(20):8057-8062.
5. Chantret, N., D. Mingeot, P. Sourdille, M. Bernard, J.M. Jacquemin, G. Doussinault. 2001. A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. Theoretical and Applied Genetics 103(6-7):962-971.
6. Chantret, N., P. Sourdille, M. Roder, M. Tavaud, M. Bernard, 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. Theoretical and Applied Genetics 100(8):1217-1224.
7. Chen, W., J. Mingus, J. Mammadoc, J.E. Backlund, T. Greene, S. Thompson, S. Kumpatla. 2010. KASPar: A simple and cost-effective system for SNP genotyping. Proceedings of plant and animal genomes XVII conference.
8. Cowger, C., L. Miranda, C. Griffey, M. Hall, J.P. Murphy, J.J. Maxwell. 2012. Wheat Pwdery Mildew, *In*: I. Sharma (Ed.), Disease Resistance in Wheat, CAB International, Cambridge, MA. p. 84-119.
9. Das, M.K., C.A. Griffey. 1994. Diallel analysis of adult-plant resistance to powdery mildew in wheat. Crop Science 34(4):948-952.
10. DeYoung, B.J., R.W. Innes. 2006. Plant NBS-LRR proteins in pathogen sensing and host defense. Nature Immunology 7(12):1243-1249.
11. FRAC. 2005. Pathogen Risk List, Fungicide Resistance Action Committee, Limburgerhof, Germany. p. 1-5.
12. Griffey, C., M. Das, E. Stromberg. 1993. Effectiveness of adult-plant resistance in reducing grain yield loss to powdery mildew in winter wheat. Plant Disease 77(6):618-622.
13. Gupta, K., S. Balyan, J. Edwards, P. Isaac, V. Korzun, M. Roder, M.F. Gautier, P. Joudrier, R. Schlatter, J. Dubcovsky, C. De La Pena, M. Khairallah, G. Penner, J. Hayden, P. Sharp, B. Keller, C. Wang, P. Hardouin, P. Jack, P. Leroy. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theoretical and Applied Genetics 105(2-3):413-422.

14. Guyomarc'h, H., P. Sourdille, G. Charmet, K. Edwards, M. Bernard. 2002. Characterisation of polymorphic microsatellite markers from Aegilops tauschii and transferability to the D-genome of bread wheat. Theoretical and Applied Genetics 104(6-7):1164-1172.
15. Hall, M., C. Griffey. 2009. Wheat, 'USG 3209' / 'Jaypee', http://wheat.pw.usda.gov/ggpages/map_summary.html, Graingenes.
16. Hao, Y., A. Liu, Y. Wang, D. Feng, J. Gao, X. Li, S. Liu, H. Wang. 2008. *Pm23*: a new allele of *Pm4* located on chromosome 2AL in wheat. Theoretical and Applied Genetics 117(8):1205-12.
17. James, W.C. 1971. An illustrated series of assessment keys for plant diseases, their preparation and usage 51:39-65.
18. Ji, J., B. Qin, H. Wang, A. Cao, S. Wang, P. Chen, L. Zhuang, Y. Du, D. Liu, X. Wang. 2008. STS markers for powdery mildew resistance gene *Pm6* in wheat. Euphytica 163(2):159-165.
19. Jianhui, J., Q. Bi, W. Haiyan, C. Aizhong, W. Suling, C. Peidu, Z. Lifang, D. Yu, L. Dajun, W. Xiue. 2008. STS markers for powdery mildew resistance gene *Pm6* in wheat. Euphytica 163(2):159-165.
20. Keller, M., B. Keller, G. Schachermayr, M. Winzeler, J.E. Schmid, P. Stamp, M.M. Messmer. 1999. Quantitative trait loci for resistance against powdery mildew in a segregating wheat×spelt population. Theoretical and Applied Genetics 98(6-7):903-912.
21. Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann Eugenics 12(3):172-175.
22. Lan, C., S. Liang, Z. Wang, J. Yan, Y. Zhang, X. Xia, Z. He. 2009. Quantitative Trait Loci Mapping for Adult-Plant Resistance to Powdery Mildew in Chinese Wheat Cultivar Bainong 64. Phytopathology 99(10):1121-1126.
23. Lan, C., X. Ni, J. Yan, Y. Zhang, X. Xia, X. Chen, Z. He. 2010. Quantitative trait loci mapping of adult-plant resistance to powdery mildew in Chinese wheat cultivar Lumai 21. Molecular Breeding 25(4):615-622.
24. Liang, S.S., K. Suenaga, Z.H. He, Z.L. Wang, H.Y. Liu, D.S. Wang, R.P. Singh, P. Sourdille, X.C. Xia. 2006. Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology 96(7):784-9.
25. Liu, S., C. Griffey, M. Hall, A. McKendry, J. Chen, W. Brooks, G. Brown-Guedira, D. Sanford, D. Schmale. 2013. Molecular characterization of field resistance to Fusarium head blight in two US soft red winter wheat cultivars. Theoretical and Applied Genetics 126(10):2485-2498.
26. Liu, S., C.A. Griffey, M.A.S. Maroof. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar massey. Crop Science 41(4):1268-1275.
27. Lorieux, M. 2012. MapDisto: fast and efficient computation of genetic linkage maps. Molecular Breeding 301231-1235.
28. Ma, Z.Q., J.B. Wei, S.H. Cheng. 2004. PCR-based markers for the powdery mildew resistance gene Pm4a in wheat. Theoretical and Applied Genetics 109(1):140-145.
29. Marone, D., M.A. Russo, G. Laido, P. De Vita, R. Papa, A. Blanco, A. Gadaleta, D. Rubiales, A.M. Mastrangelo. 2013. Genetic basis of qualitative and quantitative resistance to powdery mildew in wheat: from consensus regions to candidate genes. BMC Genomics 14(562):1-17.

30. McDonald, B.A., C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology 40349-379.
31. Michelmore, R.W., I. Paran, 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. Proceedings of the National Academy of Sciences 88(21):9828-9832.
32. Mingeot, D., N. Chantret, P.V. Baret, A. Dekeyser, N. Boukhatem, P. Sourdille, G. Doussinault, 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 Breeding 121(2):133-140.
33. Parks, R., I. Carbone, J.P. Murphy, D. Marshall, C. Cowger. 2008. Virulence structure of the Eastern US wheat powdery mildew population. Plant Disease 92(7):1074-1082.
34. Pestsova, E., M.W. Ganal, M.S. Roder. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43(4):689-97.
35. Poland, J.A., P.J. Balint-Kurti, R.J. Wisser, R.C. Pratt, R.J. Nelson. 2009. Shades of gray: the world of quantitative disease resistance. Trends in Plant Science 14(1):21-29.
36. Qin, B., A. Cao, H. Wang, T. Chen, F. You, Y. Liu, J. Ji, D. Liu, P. Chen, X.-e. Wang. 2011. Collinearity-based marker mining for the fine mapping of *Pm6*, a powdery mildew resistance gene in wheat. Theoretical and Applied Genetics 123(2):207-218.
37. Rieseberg, L.H., M.A. Archer, R.K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83(4):363-372.
38. Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.-H. Tixier, P. Leroy, M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149(4):2007-2023.
39. Russell, P.E. 2004. Sensitivity Baselines in Fungicide Resistance Research and Management, *In*: FRAC (Ed.), AIMPRINT, United Kingdom. p. 56.
40. Saghai Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang, R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proceedings of the National Academy of Sciences 91(12):5466-5470.
41. Schmolke, M., V. Mohler, L. Hartl, F. Zeller, S.K. Hsam. 2012. A new powdery mildew resistance allele at the *Pm4* wheat locus transferred from einkorn (*Triticum monococcum*). Molecular Breeding 29(2):449-456.
42. Shaner, G. 1973. Evaluation of slow-mildewing resistance of Knox wheat in the field. Phytopathology 63:867-872.
43. Shaner, G., R.E. Finney. 1977. Effect of nitrogen-fertilization on expression of slow-mildewing resistance in Knox wheat. Phytopathology 67(8):1051-1056.
44. Somers, J.D., I. Peter, E. Keith. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics.
45. Song, Q.J., J.R. Shi, S. Singh, E.W. Fickus, J.M. Costa, J. Lewis, B.S. Gill, R. Ward, P.B. Cregan. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theoretical and Applied Genetics 110(3):550-560.
46. Sourdille, P., Guyomarc'h, H., Baron, C., Gandon, B., Chiquet, V., Artiguenave, F., Edwards, K., Foisset, N., Dufour, P., . 2001. Improvement of the genetic maps of wheat using new microsatellite markers. Plant and Animal Genome IX Abstracts. PAG-01
47. Starling, T.M., C.W. Roane, H.M. Camper. 1984. Registration of Massey Wheat. Crop Science 24(5):1000.

48. Stephenson, P., G. Bryan, J. Kirby, A. Collins, K. Devos, C. Busso, M. Gale. 1998. Fifty new microsatellite loci for the wheat genetic map. Theoretical and Applied Genetics 97(5-6):946-949.
49. The, T.T., R.A. McIntosh, F.G.A. Bennett. 1979. Cytogenetical studies in wheat. IX. Monosomic analysis, telocentric mapping and linkage relationship of gene *Sr1*, *Pm4*, and *Mle*. Australian Journal of Biological Sciences 32115-125.
50. Tsilo, T.J., Y. Jin, J.A. Anderson. 2008. Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Science 48(1):253-261.
51. Tucker, D., C. Griffey, S. Liu, G. Brown-Guedira, D. Marshall, M. Maroof. 2007. Confirmation of three quantitative trait loci conferring adult plant resistance to powdery mildew in two winter wheat populations. Euphytica 155(1):1-13.
52. Tucker, D.M., C.A. Griffey, S. Liu, M.A. Saghai Maroof. 2006. Potential for effective marker-assisted selection of three quantitative trait loci conferring adult plant resistance to powdery mildew in elite wheat breeding populations. Plant Breeding 125(5):430-436.
53. Voorrips, R.E. 2002. MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. Journal of Heredity 93(1):77-78.
54. Wang, S., C. Basten, Z. Zeng. 2007. Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC.
55. Xu, W., C. Li, L. Hu, H. Wang, H. Dong, J. Zhang, X. Zan. 2011. Identification and molecular mapping of *PmHNK54*: a novel powdery mildew resistance gene in common wheat. Plant Breeding 130(6):603-607.
56. Zadoks, J.C., T.T. Chang, C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research 14(6):415-421.
57. Zeng, Z.-B. 1994. Precision mapping of quantitative trait loci. Genetics 136(4):1457-1468.

Table 2.1. Reaction of wheat host differentials to *B. graminis* populations for tests conducted in North Carolina and Maryland during the 2011-2012 growing season based on a 0 to 9 rating scale where 0 is most resistant and 9 is most susceptible.

Host Differential	*Pm* gene	Mildew Severity [†]	Mildew Severity [‡]
		0-9	0-9
CI 14114	*Pm1a*	1	2
MocZlatka	*Pm1b*	0	0
Pm1c	*Pm1c* [§]	0	0
W.W.	*Pm1d*	0	0
Pm1e	*Pm1e* [¶]	0	0
CI 14118	*Pm2*	4	7
CI 14120	*Pm3a*	5	7
CI 14121	*Pm3b*	7	8
CI 14122	*Pm3c*	5	7
Ralle	*Pm3d*	0	0
W150	*Pm3e*	- [#]	5
CI 15888	*Pm3f*	6	8
Aristide	*Pm3g*	0	5
CI 14124	*Pm4a*	4	8
Ronos	*Pm4b*	1	6
CI 14125	*Pm5a*	6	7
Kormoran	*Pm5b*	3	7
I5	*Pm5d*	4	3
Coker 747	*Pm6*	4	7
Transec	*Pm7*	4	7
Kavkaz	*Pm8*	5	8
N14	*Pm9*	5	9
Pm13	*Pm13*	0	T [††]
Pm16	*Pm16*	0	0
Amigo	*Pm17*	5	8
Tam W-104	*Pm20*	8	8
DH2	*Pm21*	4	3
Pm24	*Pm24*	5	0
NC96NGTA5-Ab	*Pm25*	-	4
Pm29	*Pm29*	0	0
Pm32	*Pm32*	-	2
NC96NGTD7-Ab	*Pm34*	0	2
NC96NGTD3-Ab	*Pm35*	1	3
5-BIL29 (Durum)	*Pm36*	2	0
NC96NGTAG11-Ab	*Pm37*	1	0

NC96NGTA4-Ab	unknown	1	T
NC96NGTA6-Ab	unknown	2	0
NC96NGTD8-Ab	unknown	1	1
NC96NGTAB9-Ab	unknown	0	1
NC96NGTAB10-Ab	unknown	2	2
NC06BGTAG12	unknown	0	T
NC06BGTAG13	unknown	0	T

[†] Mildew severity from Maryland 2012
[‡] Mildew severity from North Carolina 2012
[§] Formerly *Pm18*
[¶] Formerly *Pm22*
[#] - no data due to poor germination or stand
[††] T trace amount of infection

Table 2.2. Chi-square for Mendilian segregation ratio (1:2:1) for the markers on chromosome 2B in the cross Massey / MPV 57.

Marker Order	Marker	Type[†]	Position cM	B[‡]	H[§]	A[¶]	*P*-value
1	*Xgwm374*	co	0.0	106	182	55	<0.001
2	*IWA7371*	a-	6.8	109	0	211	<0.001
3	*IWA1389*	co	6.8	134	187	24	<0.001
4	*IWA535*	co	6.8	134	184	28	<0.001
5	*IWA2924*	a-	6.8	118	0	232	<0.001
6	*Xwmc474*	co	6.8	137	194	33	<0.001
7	*Xbarc200*	co	7.1	139	194	34	<0.001
8	*Xgwm47*	co	7.3	140	195	34	<0.001
9	*Sr36*	co	8.1	135	183	31	<0.001
10	*IWA2872*	co	8.1	134	184	31	<0.001
11	*NAU/STS*$_{BCD135-1}$	co	8.1	135	183	32	<0.001
12	*IWA2571*	co	8.3	132	183	32	<0.001
13	*NAU/STS*$_{BCD135-2}$	co	8.5	135	183	31	<0.001
14	*Xgwm319*	co	9.2	135	176	31	<0.001
15	*Xwmc477*	co	11.3	134	167	45	<0.001
16	*Xgwm388*	a-	12.1	125	0	224	<0.001
17	*Xwmc332*	a-	12.1	128	0	222	<0.001
18	*IWA3252*	a-	12.1	68	0	247	0.16
19	*Xwmc317*	a-	12.1	88	0	262	1.0
20	*Xwmc361*	a-	13.6	69	0	254	0.12

[†] Marker type co-dominant (co) dominant (a-)
[‡] Massey type
[§] Heterozygous type
[¶] MPV 57 type

Table 2.3. Correlation coefficients for the 2012 North Carolina mean area under the disease progress curve and mean mildew severity assessments for powdery mildew.

	MAUDPC † F-2	MAUDPC F-1	MAUDPC F	MMSDate1 F-2	MMSDate1 F-1	MMSDate2 F-2	MMSDate2 F-1	MMSDate2 F	MMSDate3 F-2	MMSDate3 F-1	MMSDate3 F	MMSDate4 F-2	MMSDate4 F-1
MAUDPC F-1	0.72***												
MAUDPC F	0.54***	0.74***											
MMSDate1 F-2	0.60***	0.68***	0.53***										
MMSDate1 F-1	0.53*	0.59**	0.63**	0.41NS									
MMSDate2 F-2	0.81***	0.80***	0.54***	0.64***	0.52*								
MMSDate2 F-1	0.65***	0.93***	0.71***	0.66***	0.49*	0.78***							
MMSDate2 F	0.44***	0.73***	0.89***	0.50***	0.64**	0.49***	0.73***						
MMSDate3 F-2	0.77***	0.80***	0.53***	0.55***	0.46*	0.88***	0.69***	0.44***					
MMSDate3 F-1	0.70***	0.96***	0.69***	0.61***	0.59*	0.76***	0.84***	0.65***	0.83***				
MMSDate3 F	0.50***	0.67***	0.94***	0.48***	0.56*	0.50***	0.63***	0.77***	0.52***	0.65***			
MMSDate4 F-2	0.84***	0.72***	0.51***	0.49***	0.68*	0.76***	0.66***	0.44***	0.76***	0.67***	0.47***		
MMSDate4 F-1	0.69***	0.94***	0.69***	0.65***	0.52*	0.76***	0.84***	0.66***	0.75***	0.86***	0.60***	0.76***	
MMSDate4 F	0.54***	0.66***	0.92***	0.49***	0.62**	0.51***	0.61***	0.73***	0.52***	0.59***	0.81***	0.53***	0.66***

† Mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; mean mildew severity = MMS; Date: Date1 = March 14 to 16 does not include the flag leaves because minimal infection was present, Date2 = March 26 to 29, Date3 = April 4 to 8, Date4 = April 16 to 20; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F

* Significant at 0.05 probability level

** Significant at 0.01 probability level

*** Significant at 0.001 probability level

NS Not significant at 0.05 probability level

Table 2.4. Correlation coefficients for the 2012 Maryland mean area under the disease progress curve and mean mildew severity assessments for powdery mildew.

	MAUDPC † F-2	MAUDPC F-1	MAUDPC F	MMSDate1 F-2	MMSDate1 F-1	MMSDate1 F	MMSDate2 F-1	MMSDate2 F	MMSDate3 F-1
MAUDPC F-1	0.62***								
MAUDPC F	0.53***	0.90***							
MMSDate1 F-2	0.69***	0.67***	0.62***						
MMSDate1 F-1	0.64***	0.82***	0.73***	0.78***					
MMSDate1 F	0.54***	0.74***	0.83***	0.66***	0.78***				
MMSDate2 F-1	0.57***	0.94***	0.84***	0.60***	0.74***	0.67***			
MMSDate2 F	0.47***	0.85***	0.96***	0.57***	0.67***	0.73***	0.84***		
MMSDate3 F-1	0.48***	0.90***	0.82***	0.48***	0.56***	0.57***	0.76***	0.74***	
MMSDate3 F	0.48***	0.87***	0.94***	0.54***	0.64***	0.71***	0.75***	0.84***	0.87***

† Mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; mean mildew severity = MMS; Date: Date1 = April 10 to 13, Date2 = April 23 to 25, Date3 = May 5 to 8; two leaves below the flag leaf = F-2; F-2 ratings were not taken for Dates 2 and 3 because of premature senescence of the leaves due to dry conditions, one leaf below the flag leaf F-1; flag leaf = F

***Significant at 0.001 probability level

Table 2.5. Correlation coefficients for the 2012 Maryland and 2012 North Carolina mean area under the disease progress curve and mean mildew severity assessments for powdery mildew.

	MAUDPC ‡ F-2	MAUDPC F-1	MAUDPC F	MMSDate1 F-2	MMSDate1 F-1	MMSDate2 F-2	MMSDate2 F-1	MMSDate2 F	MMSDate3 F-2	MMSDate3 F-1	MMSDate3 F	MMSDate4 F-2	MMSDate4 F-1	MMSDate4 F
MAUDPC † F-2	0.28***	0.33***	0.43***	0.28***	0.54*	0.28***	0.34***	0.39***	0.22***	0.28***	0.40***	0.25***	0.29***	0.38***
MAUDPC F-1	0.47***	0.58***	0.66***	0.47***	0.62**	0.48***	0.54***	0.61***	0.41***	0.54***	0.63***	0.43***	0.55***	0.58***
MAUDPC F	0.45***	0.65***	0.68***	0.50***	0.50*	0.45***	0.60***	0.65***	0.42***	0.61***	0.66***	0.41***	0.61***	0.58***
MMSDate1 F-2	0.27***	0.42***	0.47***	0.34***	0.46*	0.32***	0.41***	0.45***	0.25***	0.36***	0.44***	0.30***	0.39***	0.40***
MMSDate1 F-1	0.33***	0.45***	0.49***	0.40***	0.60**	0.34***	0.44***	0.48***	0.25***	0.39***	0.46***	0.31***	0.43***	0.42***
MMSDate1 F	0.32***	0.48***	0.50***	0.40***	0.47*	0.33***	0.45***	0.49***	0.26***	0.43***	0.48***	0.30***	0.46***	0.41***
MMSDate2 F-1	0.42***	0.51***	0.58***	0.42***	0.55*	0.44***	0.48***	0.55***	0.36***	0.47***	0.55***	0.40***	0.50***	0.51***
MMSDate2 F	0.39***	0.58***	0.62***	0.44***	0.40NS	0.40***	0.54***	0.59***	0.36***	0.54***	0.60***	0.37***	0.56***	0.52***
MMSDate3 F-1	0.49***	0.57***	0.65***	0.44***	0.56*	0.47***	0.52***	0.59***	0.45***	0.55***	0.63***	0.42***	0.53***	0.58***
MMSDate3 F	0.51***	0.70***	0.72***	0.52***	0.53*	0.50***	0.64***	0.68***	0.49***	0.67***	0.69***	0.45***	0.65***	0.62***

† Data in the rows are for the North Carolina test; mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; mean mildew severity = MMS; Date: Date1 = March 14 to 16 does not include the flag leaves due to minimal infection, Date2 = March 26 to 29, Date3 = April 4 to 8, Date4 = April 16 to 20; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F

‡ Data in the columns are for the Maryland test; Date: Date1 = April 10 to 13, Date2 = April 23 to 25, Date3 = May 5 to 8; F-2 ratings were not taken for Dates 2 and 3 because of premature senescence of the leaves due to dry conditions

* Significant at 0.05 probability level

** Significant at 0.01 probability level

*** Significant at 0.001 probability level

NS Not significant at 0.05 probability level

Table 2.6. Analysis of variance for the 2012 North Carolina mean area under the disease progress curve for powdery mildew.

Source	df	Mean Squares		
		MAUDPC F-2 †	MAUDPC F-1	MAUDPC F
Replication	1	38033.4^{NS}	46325.0*	5028.3**
Line	373	48127.8***	26402.7***	2863.9***
Error		17848.8	5413.7	639.5

† Mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F

* Significant at 0.05 probability level

** Significant at 0.01 probability level

*** Significant at 0.001 probability level

^{NS} Not significant at 0.05 probability level

Table 2.7. Analysis of variance for the 2012 North Carolina mean mildew severity assessments for powdery mildew.

Source	df	Mean Squares									
		MMSDate1[†] F-2	MMSDate2 F-2	MMSDate2 F-1	MMSDate2 F	MMSDate3 F-2	MMSDate3 F-1	MMSDate3 F	MMSDate4 F-2	MMSDate4 F-1	MMSDate4 F
Replication	1	27.9[*]	192.9[***]	130.3[***]	1.5[NS]	79.1[NS]	0.3[NS]	15.0[***]	175.9[**]	13.1[NS]	21.5[*]
Line	373	11.5[***]	38.4[***]	17.4[***]	2.3[***]	73.7[***]	37.9[***]	3.9[***]	99.3[***]	80.9[***]	10.7[***]
Error		6.7	14.7	5.6	0.8	23.3	9.8	1.3	24.6	23.4	3.5

[†] Mean mildew severity = MMS; Date: Date1 = March 14 to 16, Date2 = March 26 to 29, Date3 = April 4 to 8, Date4 = April 16 to 20; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F; MMSDate1 does not include the F-1 or flag leaf data because infection was minimal at this date

[*] Significant at 0.05 probability level

[**] Significant at 0.01 probability level

[***] Significant at 0.001 probability level

[NS] Not significant at 0.05 probability level

Table 2.8. Analysis of variance for the 2012 Maryland mean area under the disease progress curve for powdery mildew.

Source	df	Mean Squares		
		MAUDPC F-2 †	MAUDPC F-1	MAUDPC F
Replication	1	5016.5***	59290.0***	99637.3***
Line	373	511.8***	3973.5***	4622.6***
Error		214.7	1147.0	1025.4

† Mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F

*** Significant at 0.001 probability level

Table 2.9. Analysis of variance for the 2012 Maryland mean mildew severity assessments for powdery mildew.

Source	df	Mean Squares						
		MMSDate1[†] F-2	MMSDate1 F-1	MMSDate1 F	MMSDate2 F-1	MMSDate2 F	MMSDate3 F-1	MMSDate3 F
Replication	1	136.8[***]	59.5[***]	8.7[***]	130.9[***]	505.5[***]	54.0[*]	54.5[***]
Line	373	3.5[*]	2.1[***]	1.3[***]	4.0[***]	6.3[***]	21.7[***]	22.3[***]
Error		2.7	1.2	0.5	1.7	2.1	5.7	4.6

[†] Mean mildew severity = MMS; Date: Date1 = April 10 to 13, Date2 = April 23 to 25, Date3 = May 5 to 8; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F; F-2 ratings were not taken for Dates 2 and 3 because of premature senescence of the leaves due to dry conditions

[*] Significant at 0.05 probability level

[***] Significant at 0.001 probability level

Table 2.10. Quantitative trait loci associated with adult plant resistance to powdery mildew in the winter wheat cultivar Massey.

Mildew Assessment	Chr [†]	Marker Interval	Pos cM [‡]	LOD	%Var [§]	Add [¶]	Dom [#]
NC MAUDPC F-2[††]	2AL	*Xcfa2043 - Xcfa2058*	5	7.9	10.3	-71.0	29.7
NC MAUDPC F-1	2AL	*Xcfa2043 - Xcfa2058*	5	7.9	9.2	-49.2	21.9
NC MMSDate2 F-2	2AL	*Xcfa2043 - Xcfa2058*	5	8.1	10.4	-1.9	1.2
NC MMSDate2 F-1	2AL	*Xcfa2043 - Xcfa2058*	5	6.9	8.3	-1.2	0.5
NC MMSDate3 F-2	2AL	*Xcfa2043 - Xcfa2058*	5	11.7	14.4	-3.2	1.7
NC MMSDate3 F-1	2AL	*Xcfa2043 - Xcfa2058*	5	7.8	9.2	-1.9	0.9
NC MMSDate4 F-1	2AL	*Xcfa2043 - Xcfa2058*	5	6.7	8.1	-2.5	1.4
MD MAUDPC F-1[‡‡]	2BL	*IWA2571*	0	20.5	20.0	-33.6	-3.0
MD MAUDPC F	2BL	*IWA2571*	0	11.5	11.1	-25.5	1.1
MD MMSDate1 F-1	2BL	*IWA2571*	0	7.8	8.8	-0.5	-0.1
MD MMSDate2 F-1	2BL	*IWA2571*	0	12.9	13.7	-0.9	-0.1
MD MMSDate2 F	2BL	*IWA2571*	0	9.7	9.9	-0.9	0.1
MD MMSDate3 F-1	2BL	*IWA2571*	0	25.3	24.5	-2.8	-0.4
MD MMSDate3 F	2BL	*IWA2571*	0	13.9	12.9	-1.9	0.1
NC MMSDate2 F-1	2DS	*Ppd-D1 - Xgwm484*	6	8.0	9.3	-1.3	0.5
NC MMSDate3 F-1	2DS	*Ppd-D1 - Xgwm484*	6	9.4	10.7	-2.1	0.7
NC MMSDate4 F-1	2DS	*Ppd-D1 - Xgwm484*	6	10.6	13.1	-3.4	0.5
MD MAUDPC F	2DS	*Ppd-D1 - Xgwm484*	6	9.8	12.3	-23.8	-6.0
MD MMSDate1 F	2DS	*Ppd-D1 - Xgwm484*	6	7.0	11.7	-0.4	-0.2
MD MMSDate2 F	2DS	*Ppd-D1 - Xgwm484*	6	7.6	10.2	-0.8	-0.2
MD MMSDate3 F	2DS	*Ppd-D1 - Xgwm484*	6	9.9	11.3	-1.6	-0.3
NC MMSDate3 F-2	4DL	*Xwmc331 - Xcfd71*	2.2	5.5	6.1	-2.3	0.0

[†] Chromosome locations of quantitative trait loci (QTL) for adult plant resistance (APR) to powdery mildew
[‡] Position of the highest LOD score in terms of genetic distance from the first marker of the support interval
[§] Percentage of variance explained by the individual QTL
[¶] Additive effect
[#] Dominance effect
[††] Mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; mean mildew severity = MMS; North Carolina = NC; Date: Date1 = March 14 to 16, Date2 = March 26 to 29, Date3 = April 4 to 8, Date4 = April 16 to 20; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F
[‡‡] Maryland = MD; Date: Date1 = April 10 to 13, Date2 = April 23 to 25, Date3 = May 5 to 8
Each assessment has a QTL at the designated chromosome. Some assessments have more than one QTL.

Table 2.11. Fragment sizes of Massey alleles for simple sequence repeat (SSR) markers associated with quantitative trait loci (QTL) for adult plant resistance to powdery mildew.

Chr †	Marker	Fragment size ‡
2AL	*Xcfa2043*	213
2AL	*Xcfa2058*	226
2DS	*Xgwm484*	172
4DL	*Xwmc331*	134
4DL	*Xcfd71*	206

† Chromosome on which the simple sequence repeat (SSR) marker was mapped
‡ The fragment size of the marker allele in Massey

Table 2.12. Comparison of the mean area under the disease progress curve and mean mildew severity assessment values of wheat genotypes having different parental alleles and combinations of quantitative trait loci identified in the 2012 North Carolina test.

QTL combination	Number of Lines	Allele[†]	MAUDPC F-2 [‡¶]	MAUDPC F-1	MMSDate2 F-2	MMSDate2 F-1	MMSDate3 F-2	MMSDate3 F-1	MMSDate4 F-1
2AL	93	A	354.3[a]	242.8[a]	11.1[a]	5.7[b]	16.8[b]	9.1[c]	13.2[b]
2AL	88	B	232.8[a]	165.2[a]	7.6[a]	3.8[c]	11.3[d]	6.2[cd]	9.3[e]
2DS	86	A	- [§]	-	-	5.9[ab]	-	9.4[ab]	14.5[a]
2DS	86	B	-	-	-	3.5[cd]	-	5.8[de]	8.6[e]
2AL+2DS	22	AA	-	-	-	6.7[a]	-	10.4[a]	15.5[a]
2AL+2DS	20	AB	-	-	-	4.0[c]	-	6.4[cd]	10.4[d]
2AL+2DS	22	BA	-	-	-	4.4[c]	-	6.9[c]	11.6[c]
2AL+2DS	23	BB	-	-	-	2.7[d]	-	5.0[e]	6.8[f]
4DL	77	A	-	-	-	-	17.2[b]	-	-
4DL	95	B	-	-	-	-	12.6[cd]	-	-
2AL+4DL	15	AA	-	-	-	-	20.5[a]	-	-
2AL+4DL	22	AB	-	-	-	-	14.1[b]	-	-
2AL+4DL	18	BA	-	-	-	-	12.5[cd]	-	-
2AL+4DL	25	BB	-	-	-	-	9.3[e]	-	-

[†] B allele = Massey type; A allele = MPV 57 type

[‡] Mean phenotype of different quantitative trait loci (QTL) combinations sharing the same letter are not significantly different ($P < 0.05$)

[§] - The QTL was not present for the mildew assessment.

[¶] Mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; mean mildew severity = MMS; Date: Date1 = March 14 to 16, Date2 = March 26 to 29, Date3 = April 4 to 8, Date4 = April 16 to 20; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F

Only assessments that contain QTL are listed

Table 2.13. Comparison of mean area under the disease progress curve and mean mildew severity assessment values of wheat genotypes having different parental alleles and combinations of quantitative trait loci identified in the 2012 Maryland test.

QTL combination	Number of Lines	Allele †	MAUDPC ‡¶ F-1	MAUDPC F	MMSDate1 F-1	MMSDate1 F	MMSDate2 F-1	MMSDate2 F	MMSDate3 F-1	MMSDate3 F
2BL	32	A	109.1^{a}	92.8^{b}	1.9^{a}	-	2.8^{a}	3.2^{ab}	8.3^{a}	7.1^{b}
2BL	132	B	41.3^{b}	40.7^{cd}	0.8^{a}	-	1.1^{b}	1.5^{cd}	2.9^{b}	3.2^{d}
2DS	86	A	- §	80.4^{b}	-	0.9^{a}	-	2.8^{b}	-	6.1^{c}
2DS	86	B	-	40.6^{cd}	-	0.3^{a}	-	1.5^{cd}	-	3.2^{d}
2BL+2DS	9	AA	-	107.4^{a}	-	-	-	3.6^{a}	-	8.6^{a}
2BL+2DS	9	AB	-	81.8^{b}	-	-	-	3.2^{ab}	-	5.6^{c}
2BL+2DS	21	BA	-	52.6^{c}	-	-	-	2.0^{c}	-	3.7^{d}
2BL+2DS	32	BB	-	27.6^{d}	-	-	-	0.9^{d}	-	2.3^{e}

† B allele = Massey type; A allele = MPV 57 type

‡ Mean phenotype of different quantitative trait loci (QTL) combinations sharing the same letter are not significantly different ($P < 0.05$)

§ - The QTL was not present for the mildew assessment

¶ Mean area under the disease progress curve = MAUDPC was calculated with the mean of each mildew assessment for the F-2, F-1 and flag leaves; mean mildew severity = MMS; Date: Date1 = April 10 to 13, Date2 = April 23 to 25, Date3 = May 5 to 8; two leaves below the flag leaf = F-2; one leaf below the flag leaf F-1; flag leaf = F

Only assessments that contain QTL are listed

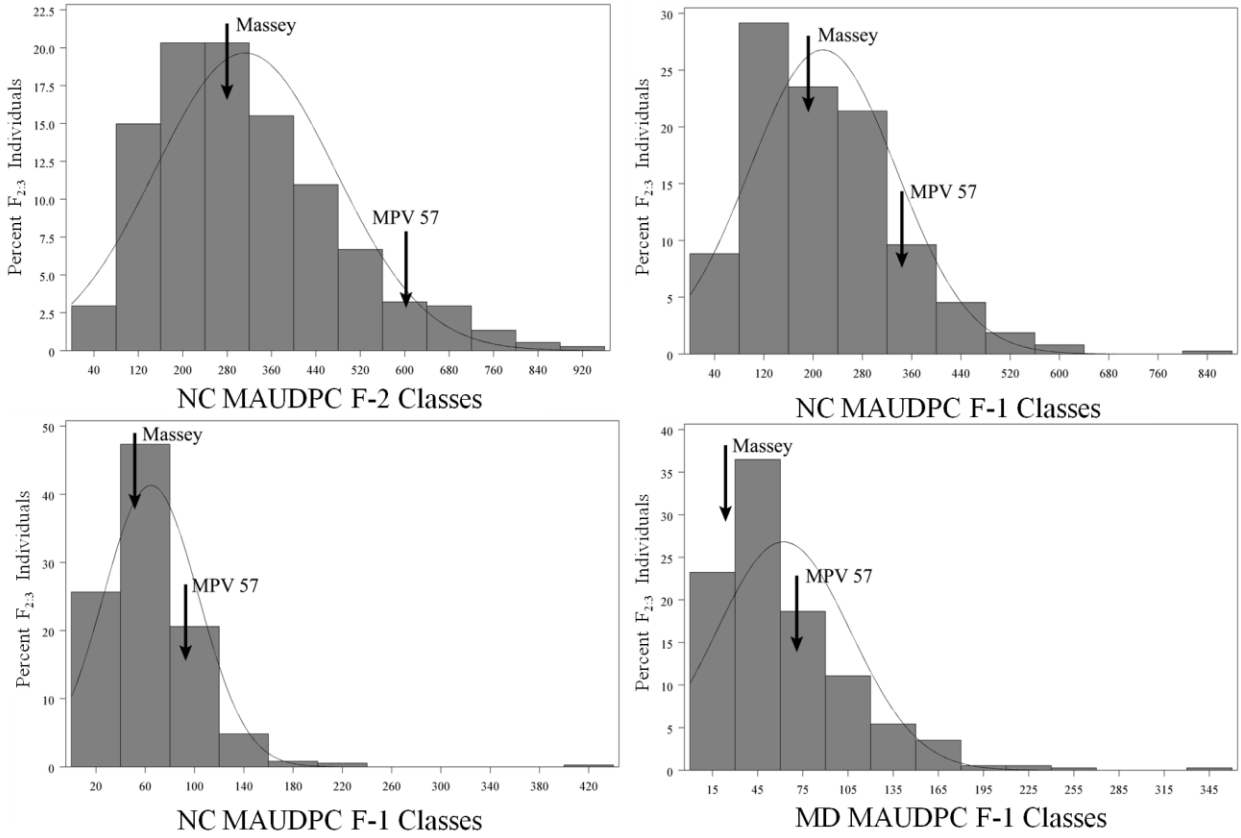

Figure 2.1. Distribution of phenotypes for the Massey / MPV 57 population in the 2011 to 2012 growing season represented by percent of individuals for each mean area under the disease progress curve (MAUDPC) class in each chart. Arrows indicate the MAUDPC class of the resistant parent Massey and the susceptible parent MPV 57. A. North Carolina (NC) MAUDPC for the two leaves below the flag leaf (F-2). B. NC MAUDPC for the one leaf below the flag leaf (F-1). C. NC MAUDPC for the flag leaf (F). D. Maryland (MD) MAUDPC F-1.

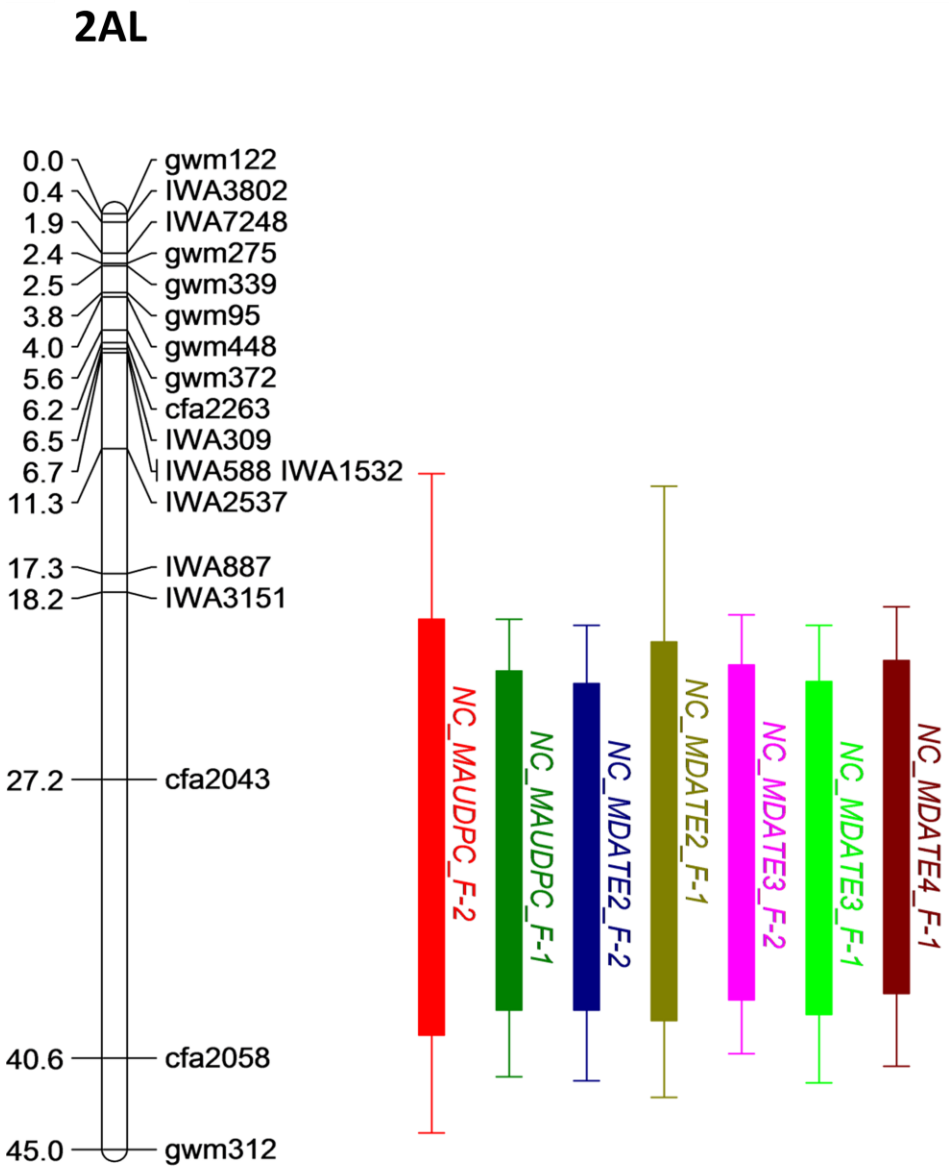

Figure 2.2. Genetic map of chromosome 2AL in the Massey / MPV 57 population. The quantitative trait loci (QTL) for each mildew assessment is represented by a different color. The QTL peak is indicated by the thick bar and the confidence interval by the lines on either side of the bar. NC = North Carolina; mean area under the disease progress curve (MAUDPC) was calculated with the mean of each mildew assessment for the F-2 (two leaves below the flag leaf), F-1 (one leaf below the flag leaf) and flag (F) leaves. Mean mildew severity was calculated for each date (MDATE) for each F-2, F-1 and flag leaves.

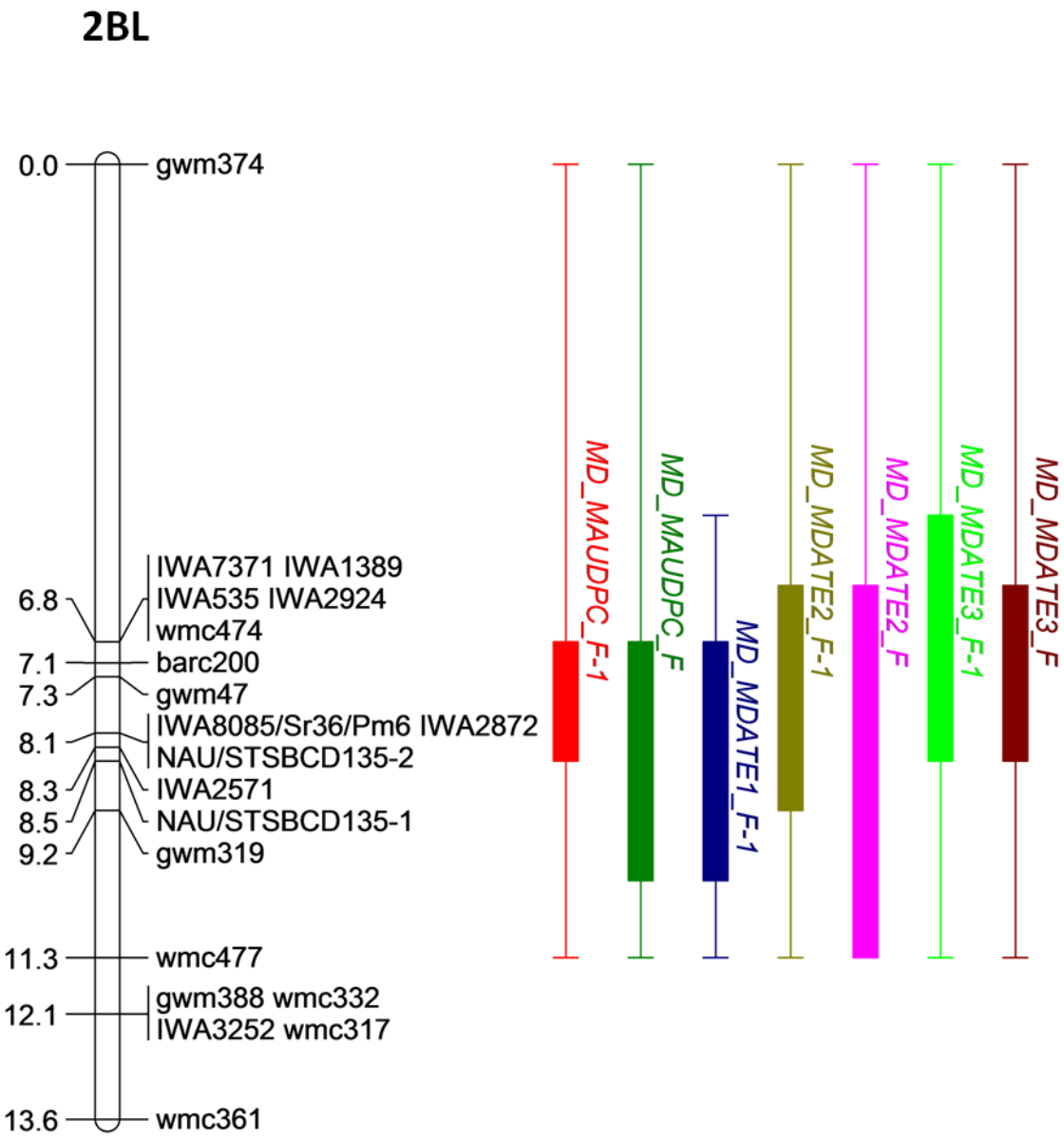

Figure 2.3. Genetic map of chromosome 2BL in the Massey / MPV 57 population. The quantitative trait loci (QTL) for each mildew assessment is represented by each color. The QTL peak is indicated by the thick bar and the confidence interval by the lines on either side of the bar. MD = Maryland; mean area under the disease progress curve (MAUDPC) was calculated with the mean of each mildew assessment for the F-1 (one leaf below the flag leaf) and flag (F) leaves. Mean mildew severity was calculated for each date (MDATE) for each F-1 and flag leaves.

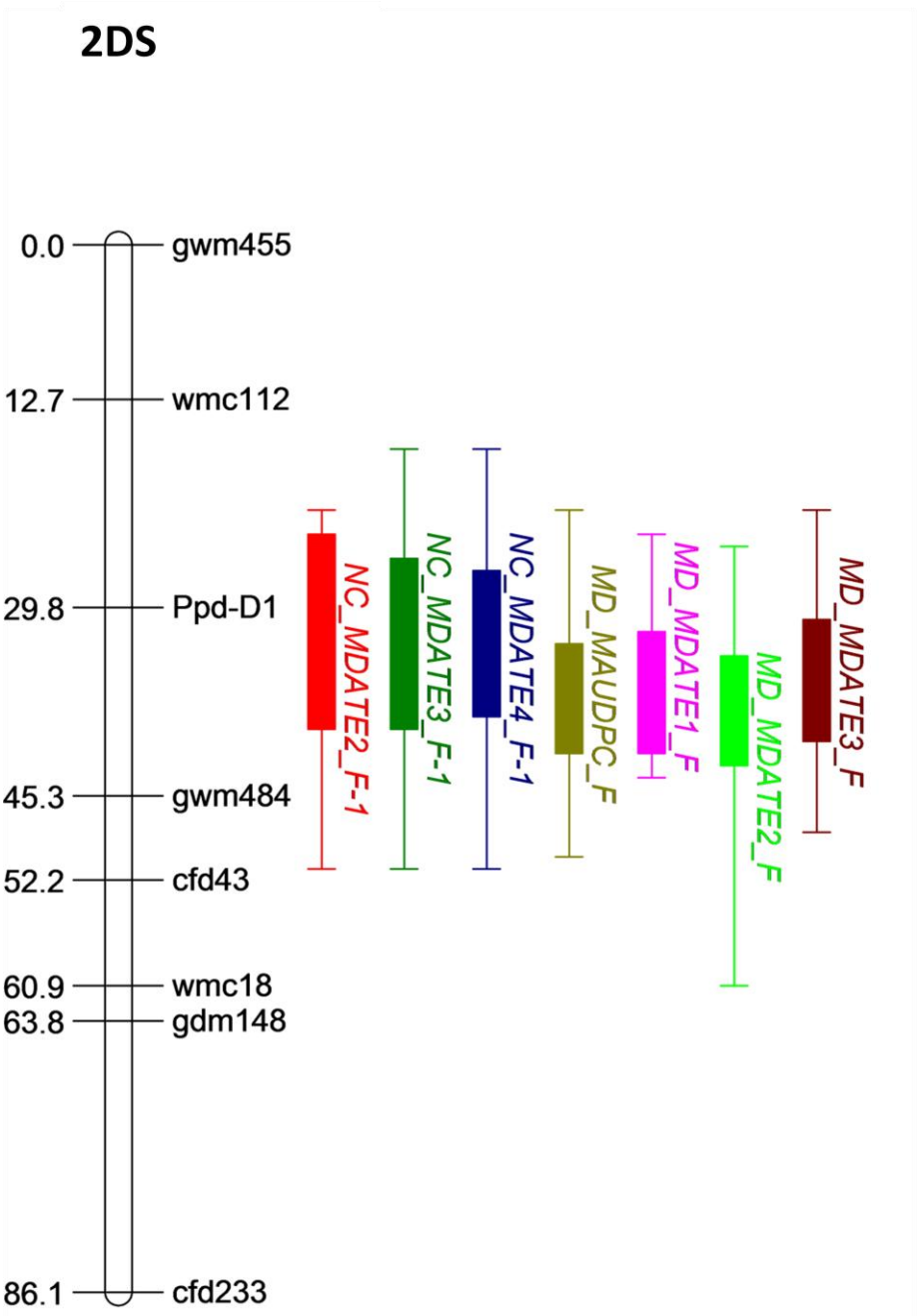

Figure 2.4. Genetic map of chromosome 2DS in the Massey / MPV 57 population. The quantitative trait loci (QTL) for each mildew assessment is represented by each color. The QTL peak is indicated by the thick bar and the confidence interval by the lines on either side of the bar. NC = North Carolina and MD = Maryland; mean area under the disease progress curve (MAUDPC) was calculated with the mean of each mildew assessment for the F-1 (one leaf below the flag leaf) and flag (F) leaves. Mean mildew severity was calculated for each date (MDATE) for each F-1 and flag leaves.

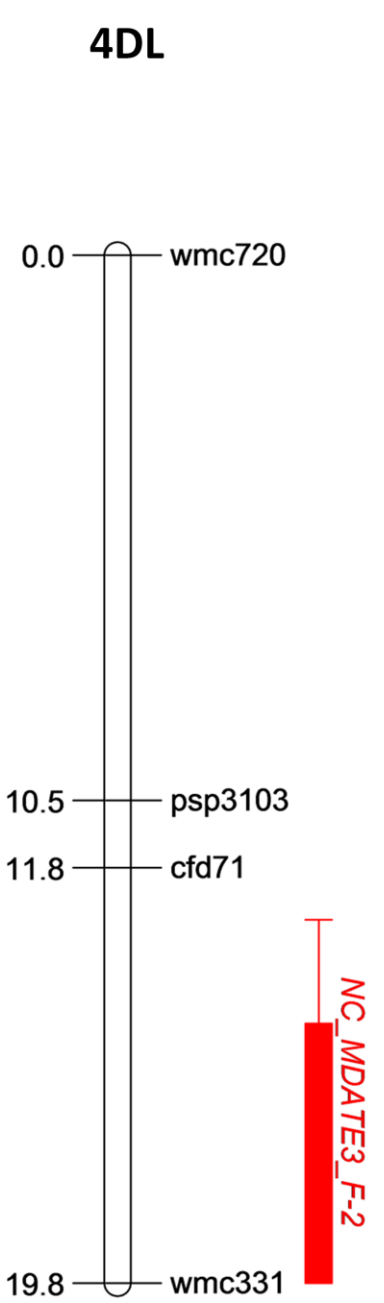

Figure 2.5. Genetic map of chromosome 4DL in the Massey / MPV 57 population. The quantitative trait loci (QTL) for the mildew assessment is represented by the red bar. The QTL peak is indicated by the thick bar and the confidence interval by the lines on either side of the bar. NC = North Carolina; mean mildew severity was calculated for the assessment date (MDATE) for the F-2 leaf (two leaves below the flag leaf).

APPENDIX

DEVELOPMENT OF NEAR ISOGENIC LINES FOR FINE-MAPPING QUANTITATIVE TRAIT LOCI CONFERRING ADULT-PLANT RESISTANCE TO POWDERY MILDEW IN THE WHEAT CULTIVAR MASSEY

Tiffany R. Sikes

INTRODUCTION AND BACKGROUND

Near isogenic lines (NILs) can be useful for fine-mapping quantitative trait loci. These lines are typically developed with backcross methods using two lines that do not share common alleles at loci for the trait or traits of interest. The donor parent containing desirable allele(s) for a gene or genes of interest is crossed with a parent (the recurrent parent) lacking desirable allele(s) for these genes. When the F_1 is crossed back to the recurrent parent, the resulting backcross (BC) generation is denoted as BC_1F_1. The generations are advanced by self-pollinating the BC_1F_1 and subsequent generations to produce near isogenic lines.

Near isogenic lines usually differ in short chromosomal regions of 10 to 30 cM therefore, in a susceptible background, differences in genetic composition near the resistance loci of interest account for a greater proportion of the variation in gene expression making it easier to identify the target region for fine-mapping (Salvi and Tuberosa, 2005). The winter wheat cultivar Massey (CI 17953) possesses QTL that confer adult-plant resistance (APR) to powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*) on chromosomes 2AL and 2BL (Liu et al., 2001). Massey inherited these QTL from its parent 'Knox 62' (CI 13701) (Shaner and Finney, 1975). This APR to powdery mildew is highly heritable (Griffey and Das, 1994) thus, progeny of Massey that inherit this resistance also make good parental material.

A validation population comprised of the parents 'USG 3209' (PI 617055), a derivative of Massey, and the susceptible cultivar Jaypee (P1 592760) was developed to confirm the location of these QTL for APR (Tucker et al., 2007). Further marker work was performed on the USG 3209 / Jaypee population to refine the chromosomal regions and markers governing APR to powdery mildew (Hall and Griffey, 2009). Three simple sequence repeat (SSR) markers were identified for each QTL interval in this study: *Xgwm448*, *Xgwm122* and *Xgwm95* for

chromosome 2AL and *Xbarc200*, *Xgwm47* and *Xgwm501* for chromosome 2BL (Hall and Griffey, 2009).

The SSR marker *Xgwm448* is 10.1 cM from *Xgwm122* which is 13.4 cM from *Xgwm95* spanning a total distance of 23.5 cM. The SSR marker *Xbarc200* is 17.8 cM from *Xgwm47* which is 6.1 cM from *Xgwm501* spanning a total distance of 23.9 cM. With the marker intervals ranging from 6.1 to 17.8 cM, these SSR markers are ideal for the development of backcross lines carrying different introgressed segments of these chromosomal regions.

A new population derived from crosses between Massey and the susceptible cultivar MPV 57 (PI 639506) was developed for use in fine mapping because the two cultivars differ in marker alleles at these chromosomal regions. The development of backcross lines using MPV 57 as the recurrent parent potentially increases the probability of identifying recombinant progeny having unique combinations of alleles for flanking markers of the QTL identified in Massey using the SSR markers previously identified by Hall and Griffey (2009).

The goal in developing this population was to identify NILs having a single Massey allele at each of the three marker loci for the QTL on 2AL and 2BL in the susceptible background of MPV 57. The selected BC_1F_1 progeny will be self-pollinated and characterized for APR phenotype and marker genotype each generation to identify homozygous recombinants with different marker allele combinations of the Massey and MPV 57 parents for the six SSR markers. The recombinant individuals will be assessed for their reaction to powdery mildew in the field and this data will be analyzed with marker genotypes to fine-map the QTL regions for APR. Eventually, fine-mapping might facilitate cloning of the gene regulating expression of APR to powdery mildew, hence elucidating the genes for adult-plant resistance.

MATERIALS AND METHODS

Plant Materials

Crosses were made in the greenhouse between the soft red winter wheat cultivars Massey and MPV 57 in the spring of 2007. Crosses were made between one Massey plant and two different MPV 57 plants and between a second Massey plant with a third MPV 57 plant. The three crosses resulted in 47 F_1 plants of which 21 were used as females to develop BC_1F_1's with the three MPV 57 plants from the initial cross. From these crosses, 399 BC_1F_1 plants were genotyped with SSR markers linked to the two QTL on chromosomes 2AL (*Xgwm448*, *Xgwm122* and *Xgwm95*) and 2BL (*Xbarc200*, *Xgwm47* and *Xgwm501*) to select heterozygous progeny that would likely yield unique homozygous recombinant types at these loci in future generations. Seed from each plant was harvested in bulk and stored until further use, and each generation thereafter was self-pollinated and advanced using the pedigree breeding method.

Genotyping and Marker Assisted Selection

Leaf tissue was collected from individual plants in each generation (BC_1F_1 to BC_1F_4) for DNA isolation using the CTAB extraction method as outlined by Saghai Maroof et al. (1994) in 2 mL microcentrifuge tubes or using SDS in the 96-well plate format modified from Pallotta et al. (2003). In each method, stainless steel beads were placed in each tube, the samples frozen overnight at -80 ˚C and ground using the Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ) for 15-30 s or until finely ground.

Simple sequence repeat markers, identified in the USG 3209 / Jaypee mapping population (Hall and Griffey, 2009), were used to identify the recombinant individuals.

Polymerase chain reaction (PCR), modified from Röder et al. (1998), was performed using the microsatellites *Xgwm448*, *Xgwm122* and *Xgwm95* for chromosome 2AL and *Xgwm47* and *Xgwm501* for chromosome 2BL (Röder et al., 1998) and *Xbarc200* for chromosome 2BL (Song et al., 2005) to identify unique marker allele combinations linked to the QTL for APR.

The primers were directly labeled with the fluorescent dyes FAM (blue), VIC/HEX (green), NED (yellow) and PET (red). Each PCR reaction contained 100 ng DNA and totaled 10 µL. PCR reactions were performed in 96-well plates and included: 1 x standard reaction buffer, 1.5 mmol mL^{-1} $MgCl_2$, 0.20 mmol mL^{-1} dNTP, 0.20 µmol mL^{-1} forward primer, 0.20 µmol mL^{-1} reverse primer, 0.5 units *Taq* polymerase (Apex, Genesee Scientific, San Diego, CA). The initial denaturing step, 5 min at 95 ˚C, was followed by 34 cycles of 0.45 s at 95 ˚C, 1 min at 52 ˚C (*Xbarc200*), 57 ˚C (*Xgwm448*), or 60 ˚C (*Xgwm122*, *Xgwm95*, *Xgwm501* and *Xgwm47*), 72 ˚C for 1.30 min and final extension of 5 min at 72 ˚C.

PCR products were observed using Gene Scan™ – 500 LIZ ® (Applied Biosystems, Foster City, CA) by capillary electrophoresis on Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems) to compile genotypic data with the GeneMarker software by SoftGenetics (State College, PA, USA).

Development of Homozygous Recombinants

Based on the marker data, 263 BC_1F_1 families were selected and advanced to potentially yield unique recombinants with different combinations of alleles for the three markers associated with each QTL on 2AL and 2BL. Each generation was genotyped with the same SSR markers *Xgwm448*, *Xgwm122* and *Xgwm95* linked to the QTL on chromosome 2AL and *Xbarc200*, *Xgwm47* and *Xgwm501* linked to the QTL on chromosome 2BL. Homozygous recombinants

were subsequently grown and advanced in the greenhouse or in head rows in the field. Heterozygous types segregating for alleles that could potentially yield additional unique genotypes were advanced and planted in the greenhouse or in the field.

Ten to five seed from each of the 263 families was planted based on genotype and generation. Earlier generations segregate at more loci thus ten seed were planted from each BC_1F_1 and BC_1F_2 family, while five seed were planted from each BC_1F_3 and BC_1F_4 family as these typically segregate at fewer loci. Each plant was designated by its' familial BC_1F_1 plant number followed by the seed number for each generation. If multiple sets of the same family were grown at different times using the same generation, the numbers continued from the last plant number assigned in earlier plantings. Every seed planted for each generation was catalogued so that it could be traced back to its' original familial seed and previous generation's genotype.

In the greenhouse, seeds were planted in 96-well flats that replicated the 96-well format for DNA extraction and genotyping from the BC_1F_2, BC_1F_3 or BC_1F_4 seed. Before anthesis, at least one head from each plant was covered to ensure cross-pollination did not occur. Heads from each plant were harvested and threshed separately noting whether or not the heads were covered prior to anthesis.

In the field at Warsaw, VA, families were planted in individual 1.2 meter rows containing ten BC_1F_2 (2009) or BC_1F_3 (2010) seed per row that were subsequently transplanted and spaced apart at the 3 to 4 leaf stage so that identification of individual plants could be made quickly. At the same time the plants were spaced apart, tissue was collected in 2 mL microcentrifuge tubes for subsequent DNA extraction. Based on genotype, plants were selected for harvest and the BC_1F_3 (2010) and BC_1F_4 (2011) seed from each plant was bulked.

RESULTS AND DISCUSSION

In total, 4,518 plants were genotyped and 179 homozygous recombinants were selected as the near isogenic lines (NILs) that will be used for fine-mapping the QTL for APR to powdery mildew on chromosomes 2AL and 2BL identified in Massey. From the BC_1F_2, BC_1F_3, BC_1F_4 generations 93, 70 and 16 individuals were identified as homozygous recombinants for alleles at the six marker loci, respectively. In total, 1,655 BC_1F_2 plants, 2,363 BC_1F_3 plants and 500 BC_1F_4 plants were grown and evaluated to obtain the unique homozygous recombinants.

Ideally, a single Massey allele was preferred at one of the six loci with the rest being from the MPV 57 parent. This would allow assessment of the effect that each allele alone has on the APR response to powdery mildew. Such information will make it easier to identify where further saturation of molecular markers is required in order to localize the QTL at less than 1 cM on chromosomes 2AL and 2BL. However, identification of such genotypes was rare, and additional combinations of NILs having Massey and/or MPV 57 alleles were also selected in addition to individuals that became fixed at loci having either all MPV 57 or Massey alleles after the BC_1F_2 generation. The allelic combinations of NILs identified are provided in Table A.1.

Between the six markers, 64 different allelic combinations are possible but only 14 were obtained during the duration of this study. Since the SSR markers are relatively close to one another, recombination between them is rare. This resulted in the identification of numerous individuals that became fixed with all Massey or all MPV 57 alleles at all SSRs on both chromosomes or all Massey alleles on 2AL and all MPV 57 alleles on 2BL and vice versa.

Currently, the identification of unique homozygous recombinants in addition to those identified here is underway in a subsequent study. Use of such NILs with markers tightly linked

to the QTL of interest may prove more powerful and offer higher resolution than a recombinant inbred line (RIL) population.

REFERENCES

1. Griffey, C.A., M.K. Das. 1994. Inheritance of adult-plant reistance to powdery mildew in Knox 62 and Massey winter wheats. Crop Science 34(3):641-646.
2. Hall, M., C. Griffey. 2009. Wheat, 'USG 3209' / 'Jaypee', http://wheat.pw.usda.gov/ggpages/map_summary.html, Graingenes.
3. Liu, S., C.A. Griffey, M.A.S. Maroof. 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar massey. Crop Science 41(4):1268-1275.
4. Pallotta, M.A., P. Warner, R.L. Fox, H. Kuchel, S.J. Jefferies, P. Langridge. 2003. Marker assisted wheat breeding in the southern region of Australia. Proceedings of the Tenth International Wheat Genetics Symposium.
5. Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.-H. Tixier, P. Leroy, M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149(4):2007-2023.
6. Saghai Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang, R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proceedings of the National Academy of Sciences 91(12):5466-5470.
7. Salvi, S., R. Tuberosa. 2005. To clone or not to clone plant QTLs: present and future challenges. Trends in Plant Science 10(6):297-304.
8. Shaner, G., R.E. Finney. 1975. Inheritance of slow mildewing resistance in wheat. Proceedings of the American Phytopathological Society. 2, 49,
9. Song, Q.J., J.R. Shi, S. Singh, E.W. Fickus, J.M. Costa, J. Lewis, B.S. Gill, R. Ward, P.B. Cregan. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theoretical and Applied Genetics 110(3):550-560.
10. Tucker, D., C. Griffey, S. Liu, G. Brown-Guedira, D. Marshall, M. Maroof. 2007. Confirmation of three quantitative trait loci conferring adult plant resistance to powdery mildew in two winter wheat populations. Euphytica 155(1):1-13.

Table A.1. Allele combinations of simple sequence repeat markers linked to quantitative trait loci for APR to powdery mildew on chromosomes 2AL and 2BL developed using the backcross population Massey / MPV 57 // MPV 57 identified in the present study.

Number of Plants	2AL[†]			2BL		
	Xgwm448	*Xgwm122*	*Xgwm95*	*Xbarc200*	*Xgwm47*	*Xgwm501*
37	s	s	s	s	s	s
2	s	s	s	s	s	m
3	s	s	s	s	m	m
6	s	s	s	m	s	s
2	s	s	s	m	m	s
24	s	s	s	m	m	m
20	s	m	s	s	s	s
1	s	m	s	m	s	s
6	s	m	s	m	m	m
3	m	s	s	s	s	s
26	m	s	m	s	s	s
2	m	s	m	m	m	m
33	m	m	m	s	s	s
14	m	m	m	m	m	m

[†]Quantitative trait loci (QTL) on chromosome 2AL and 2BL represented by s allele = MPV 57 and m allele = Massey