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Confirmation of Three Quantitative Trait Loci Conferring Adult Plant Resistance to Powdery Mildew in Two Winter Wheat Populations

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Abstract Hypersensitive, race specific genes primarily have been deployed to control powdery mildew (*Blumeria graminis* (DC) EO Speer f. sp. *tritici*) in wheat (*Triticum aestivum* L.); however, recent efforts have shifted to breeding for more durable resistance. Previously, three quantitative trait loci (QTL) for adult plant resistance (APR) to powdery mildew in the winter wheat cultivar Massey were identified in a Becker/Massey (BM) $F_{2:3}$ population. Fourteen new simple sequence repeat (SSR) markers were added to the pre-existing BM $F_{2:3}$ linkage maps near the QTL for APR on chromosomes 1BL (*QPm.vt-1BL*), 2AL (*QPm.vt-2AL*), and 2BL (*QPm.vt-2BL*). Genetic linkage maps comprised of 17 previously and newly mapped SSRs from the BM population on chromosomes 1BL, 2AL, and 2BL were constructed in a USG 3209/Jaypee (UJ) $F_{6:7}$ recombinant inbred line (RIL) confirmation population, wherein the APR resistance of USG 3209 was derived from Massey. Interval mapping analysis of mildew severity data collected in 2002 ($F_{5:6}$) and 2003 ($F_{6:7}$) field experiments with marker genotypic data obtained in 2003 ($F_{6:7}$) confirmed the presence of the three QTL governing APR to powdery mildew in the UJ RILs. The QTL *QPm.vt-1BL*, *QPm.vt-2AL*, and *QPm.vt-2BL* explained 12–13, 59–69, and 22–48% of the phenotypic variance for powdery mildew severity in the UJ confirmation populations, respectively, in two field experiments. The current study verified that the elite wheat cultivar USG 3209 possesses the same QTL for APR as its parent Massey.

Keywords Triticum aestivum · Microsatellites · Partial resistance · Blumeria graminis · Erysiphe graminis

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Introduction

Powdery mildew, caused by *Blumeria graminis* (DC) EO Speer f. sp. *tritici*, is one of the most devastating diseases of common wheat (*Triticum aestivum* L.). Yield losses ranging from 16 to 34% have been reported in the United States (Johnson et al. 1979; Kingsland 1982; Leath and Bowen 1989; Griffey et al. 1993). Utilization of

resistant cultivars has provided the most economical and efficient control of the disease (Bennett 1984). To date, 35 major gene, race-specific loci, designated *Pm1-Pm35*, as well as other non-characterized resistance genes have been reported to confer resistance to powdery mildew in wheat (McIntosh et al. 1998; Hsam et al. 2003; Singrun et al. 2003; Huang and Röder 2004). These genes confer complete resistance to the pathogen throughout the life cycle of the host. However, this resistance is ephemeral and generally has been overcome by rapidly changing, dynamic powdery mildew populations (Roberts and Calwell 1970; Shaner 1973).

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

Simple sequence repeats have become the marker system of choice in wheat because they are highly polymorphic and genome specific. Current coverage of genetic maps is sufficient for densely saturating QTL regions (Röder et al. 1995, 1998; Somers et al. 2004). Utilization of SSRs, known to be associated with QTL for APR, could potentially provide an approach for implementing routine marker-assisted selection (MAS) of resistant plants in segregating breeding populations. However, use of molecular markers, particularly for quantitative traits, has had limited practical impact in plant breeding programs to date.

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

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

Objectives of this current study were to: (1) identify and map additional SSRs in the BM $F_{2:3}$ population near the three QTL regions, (2) validate and map SSRs associated with QTL for APR in cultivar Massey in the confirmation population, USG 3209/Jaypee (UJ), and (3) examine the effect of QTL conferring APR and the potential contribution of defeated race-specific genes possessed by USG 3209 on powdery mildew resistance in the UJ population. The UJ breeding population was selected for this study because the APR of USG 3209 putatively was derived from Massey and USG 3209 is a modern, widely grown commercial cultivar. Effects of the three QTL on APR to powdery mildew, assuming that USG 3209 contains all three QTL from Massey, in a different genetic background was evaluated to determine the feasibility of implementing a MAS breeding program for APR.

Materials and Methods

Original mapping population

DNA from 180 previously developed $F_{2:3}$ lines, derived from the BM cross (Liu et al. 2001) was used in mapping new SSR markers to saturate QTL regions responsible for APR to powdery mildew. Massey (CItr17953) has long exhibited durable powdery mildew resistance and is known to possess APR. Becker (PI494524) does not possess any known genes for powdery mildew resistance and is highly susceptible to powdery mildew populations in the US mid-Atlantic region.

Confirmation population development

Previously mapped and newly added SSR markers in the BM $F_{2:3}$ population were assayed for polymorphism in the UJ RIL confirmation population. USG 3209 (PI617055) was derived from the cross Saluda/4/Massey * 2/3/Massey * 3/Balkan//Saluda (Purdy et al. 1972). Preliminary seedling disease reaction tests, conducted using differential isolates of *B. graminis* (Niewoehner and Leath 1998) at the USDA-ARS Plant Research Unit in Raleigh, NC, USA confirmed that USG 3209 possesses *Pm8* (from Balkan), and other unidentified genes for seedling mildew resistance. Balkan still remains highly resistant to mildew populations in the mid-Atlantic region where virulence to *Pm8* is widespread (Niewoehner and Leath 1998) suggesting that the cultivar may carry additional unidentified race-specific resistance genes. Saluda possesses *Pm3a* for which virulence is prevalent in the mid-Atlantic region (Niewoehner and Leath 1998), while Massey does not possess any known race-specific resistance genes. Jaypee (PI592760), derived from a cross between Arthur 6 and AR39-3 (Doublecrop//Forlani/Garibaldo), is moderately susceptible to *B. graminis* populations prevalent in the mid-Atlantic region.

The UJ RIL population was initiated by crossing USG 3209 and Jaypee during the 1997 spring greenhouse season. The population was planted yearly and advanced to the F_4 generation via a modified bulk breeding method, used in our cultivar development program. Briefly, the F_2 and F_3 generations were grown in 20.8 m^2 blocks at Blacksburg and Warsaw, VA, from which spikes, selected at both locations solely on the basis of desirable plant type, were harvested, threshed in bulk and used as a source of seed in subsequent generations. In the F_4 generation, 293 spikes were selected arbitrarily, harvested, and threshed separately to develop 293 individual RILs, which were planted in 1.4 m rows at Warsaw during fall 2001. Subsequent generations (F_5–F_8) were advanced yearly by selecting a single spike from each RIL head row and planting it at Warsaw the following growing season.

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

Microsatellite analysis

Sixty-eight newly identified SSRs located near the three QTL associated with APR to powdery mildew in the BM population were obtained from the following sources: *Xwmc* (Gupta et al. 2003), *Xgwm* (Röder et al. 1998), *Xbarc* (Song et al. 2002), *Xcfd* (Sourdille et al. 2003), *Xcfa* (Guyomarćh et al. 2002), *Xksum* (Yu et al. 2004), and *Xpsp* (Bryan et al. 1997; Stephenson et al. 1998). Markers polymorphic between Becker and Massey were added to the pre-existing BM genetic map (Liu et al. 2001).

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

Microsatellite primer pair SCM09 was used to detect the presence of the 1BL.1RS translocation (Saal and Wricke 1999). DNA from 290 $F_{8:9}$ UJ RILs (three lines were lost in the advancement of population) was extracted as described above for the marker assay. PCR were performed in 12 μl volumes and included 2.0 μl of genomic DNA, 1.2 μl of 10 × PCR buffer with magnesium chloride, 0.96 μl of 10 mM dNTPs, 0.18 μl of Taq DNA polymerase (5 U/μl), 5.26 μl of sterile molecular grade water, 1.0 μl of each 10 μM forward and reverse primer. The forward primer was modified on the 5′ ends to include a 6-FAM fluorescent label. Reactions were carried out in a PTC-200 Thermal Cycler (MJ Research, Watertown, MA, USA). The PCR products were resolved in an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with GeneScan-500 LIZ as an internal size standard (Applied Biosystems). Fragment analysis was performed with GeneMarker Version 1.4 software (SoftGenetics, State College, PA, USA).

Thirty-one SSRs, selected on the basis of newly created linkage maps (Fig. 1) of chromosomes 1BL, 2AL, and 2BL in the BM population, were assayed for polymorphism between USG 3209 and Jaypee in the confirmation population. Two DNA bulks were created by selecting RILs which exhibited homogeneous disease reaction in the $F_{5:6}$ field experiment. The bulks contained equal amounts of DNA from eight resistant and eight susceptible $F_{5:6}$ RILs, respectively. DNA from the two bulks, APR resistance source Massey, and parental lines USG 3209 and Jaypee, were used to survey SSR markers for putative association with APR to powdery mildew in the UJ population. Markers exhibiting a similar pattern of polymorphism between the resistant parents (Massey and USG 3209), the susceptible parent (Jaypee), and the two respective bulks were considered as putative resistance-related markers.

Map construction

MAPMAKER 3.0b (Lander et al. 1987) was used in linkage group map construction in both the BM and UJ populations. Initial grouping of markers in both populations was performed using the ''group'' command at a LOD score of 3.0 with a maximum Haldane distance of 50 cm. The ''order'' and ''compare'' commands were used to determine the most probable marker order in the populations. Markers not meeting the threshold were placed in intervals using the ''try'' command.

Genetic distances and marker order calculated by MAPMAKER 3.0b were imported into QTL CARTOGRAPHER Version 2.0 (Basten et al. 1994) to create linkage group figures. Interval mapping (Lander and Botstein 1989) was used to

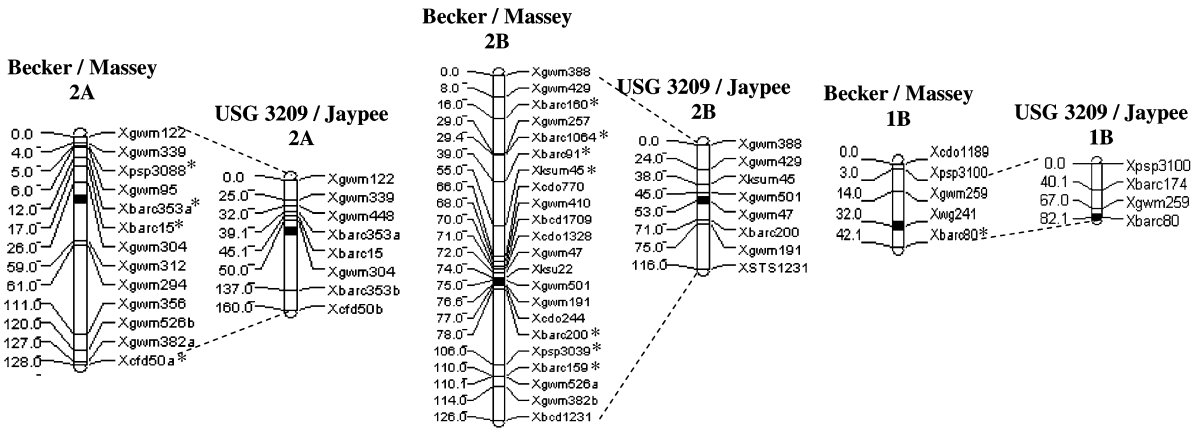

Fig. 1 Molecular map alignment of the original mapping population, Becker/Massey (*BM*) $F_{2:3}$, with the USG 3209/Jaypee $F_{6:7}$ confirmation population. *Black blocked areas* on the linkage groups represent previously and newly mapped QTL on chromosomes 2AL, 2BL, and 1BL. *Markers* denoted with *asterisk* indicate that they were added to the pre-existing BM map in this study

search for QTL in UJ population and compared to QTL resulting from the BM population using QTL CARTOGRAPHER 2.0 software. Significant thresholds for QTL were determined using a 1000 permutation test included in the software (Doerge and Churchill 1996). A mean LOD threshold of 3.0 was selected for all traits to declare a putative QTL significance corresponding to a genome wide α error of 0.05. Estimates of LOD peaks and variance explained were obtained from the Zmapqtl program run with model 3 in QTL CARTOGRAPHER 2.0. Field disease assessments for both BM and UJ populations were analyzed for QTL effects. The QTL were designated according to the guidelines for nomenclature of QTL in wheat as described in McIntosh et al. (1998) and Liu et al. (2001).

Powdery mildew assessment and statistical analysis

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

Statistical analysis was performed using SPSS statistical software Version 12.0 (SPSS Inc. 1998). A one-way ANOVA was used to determine the significance ($P \leq 0.05$) between markers and APR to powdery mildew. Log_{10} was used to transform only the mildew severity data in the $F_{2:3}$ BM population prior to analysis due to a skewed distribution of the data and low phenotypic disease assessments. A pair-wise *t*-test at $P = 0.05$ was used to compare mean mildew severity values.

Seedling disease assessments in the confirmation population

The $F_{7:8}$ UJ RILs were evaluated for the presence of putative hypersensitive genes at the USDA-ARS Plant Science Research Unit as previously described by Niewoehner and Leath (1998). Thirteen isolates of *B. graminis* were used on the basis of their ability to distinguish and identify RILs possessing genes *Pm3a*, *Pm8*, or both, which USG 3209 putatively may have inherited from its parents Saluda (*Pm3a*) and Balkan (*Pm8*) (Table 1). Leaf segments of individual plants were inoculated in the greenhouse and scored on a 0–9 scale (0 = immune, no visible signs or symptoms to 9 = susceptible, completely compatible reaction) in replicated tests. Each $F_{7:8}$ UJ RIL, parental checks, USG 3209, Jaypee, Massey, and Becker were tested in two replications. Lines having a mixed disease reaction type for the two replications were re-screened in additional replicated tests.

Results

Additional mapping in the Becker/Massey population

Of 68 new SSRs assessed for polymorphism in the BM population, 22 (32%) were polymorphic between Becker and Massey. Fourteen of 22 (59%) SSRs cosegregated with markers known to be linked to QTL for APR to powdery mildew in Massey and in this study were added to the pre-existing genetic map of Liu et al. (2001) (Fig. 1). A majority of the new markers mapped near the QTL on chromosomes 2AL and 2BL (*QPm.vt-2AL* and *QPm.vt-2BL*). Nine of the newly added fourteen markers flanked the *QPm.vt-2BL* and were all located in close proximity to the QTL region. Specifically, two closely linked markers, *Xgwm191-2B* and *Xbarc200-2B*, mapped to one side of the QTL where only RFLP markers were located previously. Marker *Xbarc80-1B* was the only SSR

Table 1 Seedling reactions (R = Resistant, S = Susceptible) of parental lines USG 3209 and Jaypee, Becker and Massey (adult plant resistance source), and host differentials CI14120 (*Pm3a*), Kavkaz (*Pm8*), Chancellor (no race-specific genes) tested with *B. graminis* isolates used for identification of hypersensitive genes possessed by recombinant inbred lines of the USG 3209/Jaypee population

Lines	*Pm* genes	*Blumeria graminis* Isolates												
		Arapahoe	Asosan	E3-14	Flat 7–11	St2K-*Pm5*	St2K-*Pm6*	Trego	Yuma	101a2	85063	E2-15	3a	#8
USG 3209	Unknown + *Pm8*	R	R	R	R	R	R	R	R	R	R	R	R	S
Jaypee	Unknown	S	S	S	S	S	S	S	S	R	R	S	S	S
Becker	None	S	S	S	S	S	S	S	S	S	S	S	S	S
Massey	Unknown	S	S	S	S	S	S	S	S	S	R	S	S	S
CI14120	*Pm3a*	S	S	S	S	S	S	S	S	R	R	S	S	R
Kavkaz	*Pm8*	R	R	R	R	R	R	R	R	S	S	S	S	S
Chancellor	None	S	S	S	S	S	S	S	S	S	S	S	S	S

marker mapped to the linkage group of chromosome 1B; however the newly mapped marker, 10 cm away from the QTL (*QPm.vt-1BL*), provided the necessary flanking PCR-based marker on one side of the QTL for efficient MAS.

Mapping in the USG 3209/Jaypee confirmation population

Overall, the UJ confirmation population was significantly less polymorphic than the original BM mapping population. Twenty one of 31 (68%) SSR markers mapped in the BM population were polymorphic in the UJ population. Eighteen of these 21 (86%) SSR markers were putatively linked to APR QTL on the basis of bulk segregant analysis results. These markers were used to genotype the UJ $F_{6:7}$ RILs and linkage groups were constructed by MAPMAKER 3.0B (Fig. 1). Marker order was consistent between the original BM and UJ confirmation population. Only slight rearrangement of marker order was observed on the 2B linkage group for marker intervals *Xgwm501-2B*: *Xgwm47-2B* and *Xgwm191-2B*:*Xbarc200-2B*. Marker order on linkage groups 2A and 1B was entirely conserved between the two populations. Marker interval *Xgwm259-1B* to *Xpsp3100-1B* in the UJ population spanned a region of 67 cm, while the interval in the BM population only measured 11.2 cm. A large gap (80 cm) between markers *Xgwm304-2A* and *Xbarc353B-2A* in the UJ population resulted partially from the absence of six SSRs in the linkage groups due to lack of polymorphisms or association with the QTL. A large gap (33 cm) also exists in the BM population between markers *Xgwm304-2A* and *Xgwm312-2A* where *QPm.vt-2AL* resides.

Three additional markers that were not polymorphic or not located on selected linkage groups in the BM population were added to the UJ linkage groups. Marker *XSTS1231-2B*, is a sequence tagged site (STS) marker designed from the RFLP probe Xbcd1231 (Ma et al. 2004) which was mapped in the BM population (Liu et al. 2001). SSR markers *Xbarc353b-2A* and *Xbarc174-1B* were also added to the UJ linkage groups. Mapped locations of the two SSRs in the

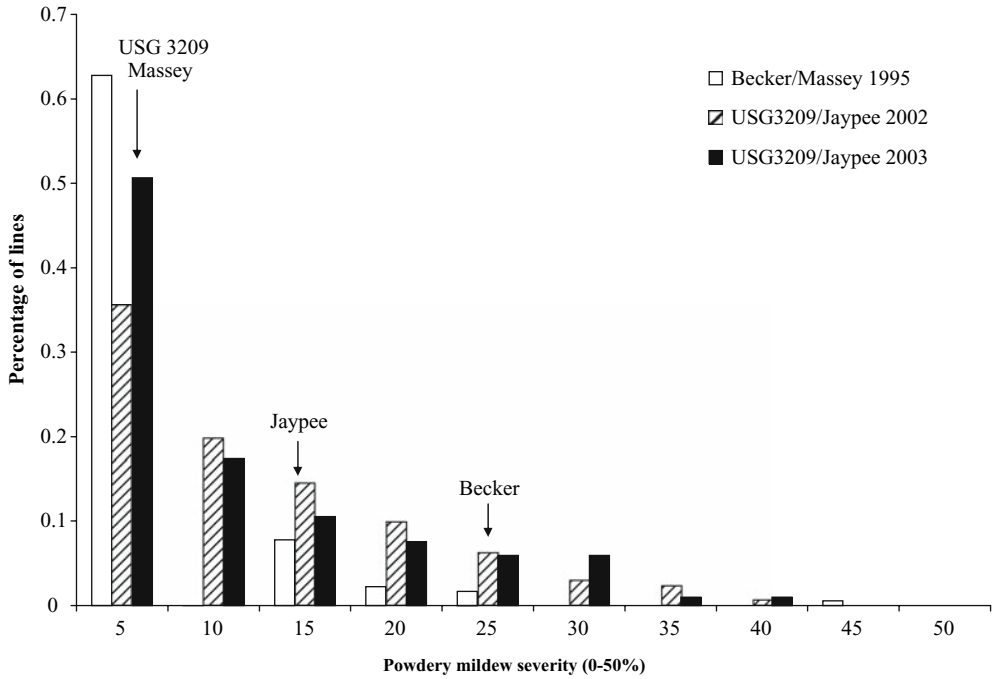

Fig. 2 Distribution of Becker/Massey $F_{2:3}$ (1995) lines (Liu et al. 2001), and USG 3209/Jaypee $F_{5:6}$ (2002) and $F_{6:7}$ (2003) RILs for mean powdery mildew severity assessed under natural epidemics in the field at Warsaw, VA. The average powdery mildew severity of the parental lines is also indicated by *arrows*

UJ population are in agreement with published maps (http://wheat.pw.usda.gov).

Validation of QTL effects in the confirmation population by interval mapping

Field assessments of powdery mildew severity in both BM and UJ populations were skewed toward resistance (Fig. 2). Interval mapping was conducted using QTL CARTOGRAPHER 2.0 to validate and compare QTL effects in BM and UJ populations. A total of 19 and 39 markers on three linkage groups located on chromosomes 1BL, 2AL, and 2BL were analyzed in the UJ and BM populations, respectively.

QPm.vt-2AL and *QPm.vt-2BL* in the UJ populations explained significantly more of the total variation for mildew severity than estimates reported in the original BM populations (Table 2). The interval mapping method located one major QTL between markers *Xgwm304-2A* and *Xbarc353b-2A*, which was consistent with its

Table 2 Comparison of QTL effects on adult plant resistance (*APR*) to powdery mildew using interval mapping analysis of original datasets of the Becker/Massey $F_{2:3}$ and RILs (Liu et al. 2001) and the USG 3209/Jaypee RILs

APR QTL	Becker/Massey populations			USG 3209/Jaypee populations		
	Population	LOD	R^{2a}	Population	LOD	R^2
QPm.vt-2AL	$F_{2:3}$	9.2	0.29	$F_{5:6}$	10.7	0.59
QPm.vt-2AL	$F_{5:6}$–$F_{7:8}$	4.7	0.26	$F_{6:7}$	20.6	0.69
QPm.vt-2BL	$F_{2:3}$	4.3	0.11	$F_{5:6}$	25.2	0.48
QPm.vt-2BL	$F_{5:6}$–$F_{7:8}$	2.9	0.15	$F_{6:7}$	12.6	0.22
QPm.vt-1BL	$F_{2:3}$	5.3	0.17	$F_{5:6}$	6.1	0.13
QPm.vt-1BL	$F_{5:6}$–$F_{7:8}$	2.5	0.15	$F_{6:7}$	6.2	0.13

[a] Phenotypic variation explained

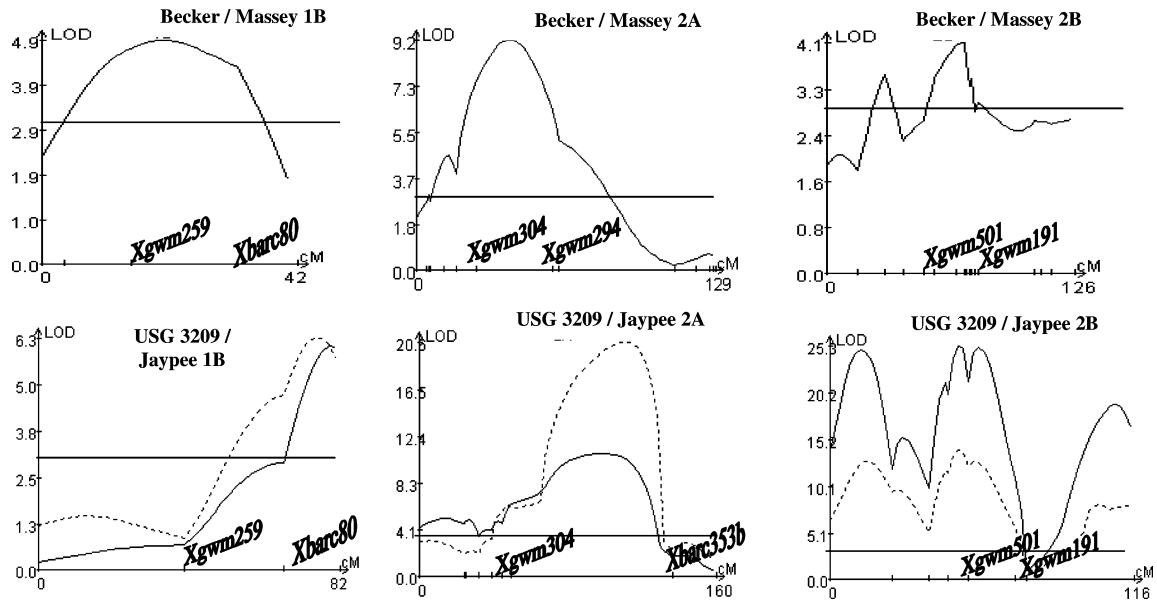

Fig. 3 Comparison of interval mapping analysis with flanking SSR markers for QTL associated with adult plant resistance to powdery mildew for the Becker/Massey $F_{2:3}$ and USG 3209/Jaypee (*UJ*). The *solid line* in graphs of the UJ population represents interval mapping of mildew data taken in the $F_{5:6}$ while the *dashed line* is for mildew data taken in the $F_{6:7}$ generation. The *vertical lines* at LOD 3.0 represent the minimum LOD required for significance

location in the BM population (Fig. 3). Association of *QPm.vt-2AL* with field data in the UJ $F_{6:7}$ population had the highest LOD value (20.6) and explained 69% of the total phenotypic variation. The QTL also was associated, albeit to a lesser extent, with field severity data in the $F_{5:6}$ RIL population with a LOD of 10.7 and explained 59% of the phenotypic variation.

Similar to the BM population, interval mapping located two major peaks on chromosome 2B in the UJ confirmation population (Fig. 3). The major peak identified in analysis of the UJ population was in the marker interval of *Xgwm501-2B* and *Xgwm191-2B* over the 2-year field experiment. QTL analysis of markers and field severity data collected in the UJ $F_{5:6}$ population displayed the largest LOD peak of 25.2 for *QPm.vt-2BL* which explained 48% of the phenotypic variation. The phenotypic effect of *QPm.vt-2BL* in the $F_{6:7}$ RILs significantly decreased but accounted for 22% (LOD = 12.6) of the total variation.

Location of *QPm.vt-1BL* in the UJ populations was shifted towards the end of the chromosome compared to the same QTL in the BM populations (Fig. 3). The QTL effects were consistent for both UJ RIL populations with LOD peaks of ~6.0 explaining 13% of the phenotypic variance in the $F_{5:6}$ and $F_{6:7}$ generations.

Effects of defeated hypersensitive *Pm* genes on resistance of RILs in the UJ population

While Massey and USG 3209 possess the same three QTL governing APR to powdery mildew, USG 3209, which also possesses gene *Pm8* and another unidentified gene, expresses a higher level of resistance under natural field epidemics of powdery mildew than its parent Massey. Seedling tests conducted at the USDA-ARS Plant Science Research Unit clearly indicated segregation of gene *Pm8* and another unknown gene among the UJ RILs on the basis of observed resistant versus susceptible reaction patterns of RILs to 13 different isolates of *B. graminis* in comparison to reaction patterns of a set of host differential wheat lines having known *Pm* resistance genes (Table 1). While it was initially postulated that the unidentified gene in USG 3209 was likely *Pm3a*, derived from its parent Saluda, disease reaction patterns of these two cultivars differed for three (E2-15, 3a, and #8) of

the 13 *B. graminis* isolates having virulence for gene *Pm8*. Haplotype analysis was conducted with USG 3209, Jaypee and check lines Saluda and CI14120, known to possess *Pm3a*, using a primer pair specific for *Pm3a* (Tommasini et al. 2006). A fragment of expected size (624 bp) was amplified from Saluda and CI14120 confirming the presence of *Pm3a* in these lines; whereas, no fragment was amplified from DNA of USG3209 and Jaypee. This result confirmed that USG 3209, unlike its parent Saluda, does not possess gene *Pm3a* (Table 1).

Potential residual and epistatic effects of genes *Pm8* and the unidentified gene, derived from USG 3209, were assessed via comparison of mean mildew severity of UJ RILs lacking either *Pm* gene, having the unidentified USG 3209 gene or *Pm8* or both (Table 3). RILs having both race specific genes in addition to QTL for APR consistently had lower mean mildew severities than RILs having either gene alone or lacking these race-specific genes. This indicates that these hypersensitive genes in combination likely contributed in an additive manner to overall powdery mildew resistance of RILs having QTL for APR.

Discussion

In this study, 17 of 31 SSRs associated with three QTL for APR to powdery mildew in Massey were successfully mapped in a confirmation population, comprised of 293 RILs of UJ, where Massey was the resistance source of APR in the parental line USG 3209. Marker order was highly conserved between respective maps resulting from the two populations. Presence of the three QTL for APR in USG 3209 was confirmed in analysis of marker and powdery mildew severity data assessed in the $F_{5:6}$ and $F_{6:7}$ UJ RIL populations in two field experiments. An additional 14 SSR were added to the pre-existing BM genetic map. The newly mapped SSR markers in the BM population replaced the closely linked RFLP markers on one side of *QPm.vt-1BL* and *QPm.vt-2BL*, allowing for more efficient selection for these QTL for APR to powdery mildew utilizing PCR-based markers on both sides of the QTL. A significant contribution to overall powdery mildew resistance of the RILs in the field experiments was also conferred by combination of defeated hypersensitive genes such as *Pm8* and an unknown gene in USG 3209 with QTL for APR in the UJ population.

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

Table 3 Average mean mildew severity of RILs in the USG 3209/Jaypee population having known race-specific *Pm* gene combinations postulated in greenhouse seedling resistance tests

1BL.1RS translocation	*Pm* gene combinations	n[a]	2002 ($F_{5:6}$) mean mildew severity of RILs	2003 ($F_{6:7}$) mean mildew severity of RILs
Absent		129	11.8cd	11.7c
	No known race specific genes	77	14.1d	12.4c
	Unidentified gene	52	9.1b	11.1c
Present		107	9.2b	6.2b
	Pm8 only	70	11.0bc	7.8bc
	Unidentified gene + *Pm8*	37	5.7a	4.0a

Also included are lines shown to have the 1BL.1RS rye translocation by microsatellite marker SCM09 (Saal and Wricke 1999). A number in the same column followed by the same letter is not significantly different ($P \leq 0.05$) on the basis of a pair-wise comparison (*t*-test)

[a] Number of RILs

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

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

The QTL effects in the UJ populations differed in magnitude from those in the original BM mapping population. *QPm.vt-2AL* explained significantly more of the total phenotypic variance for APR to powdery mildew in the UJ population. However, it is likely that an over estimation of the QTL effect occurred in the UJ population due to the large gap between markers *Xgwm304-2A* and *Xbarc353b-2A* where the QTL resides. As additional markers become available they should be added to this region to increase the efficiency of MAS. The closely mapped *QPm.vt-2BL* in the UJ population explained significantly more of the total phenotypic variation for APR whereas the *QPm.vt-1BL* explained proportionally less when compared to the values reported in the original BM mapping population (Liu et al. 2001). Castro et al. (2003) evaluated three QTL for APR to barley (*Hordeum vulgare*) stripe rust (*Puccinia striiformis*) in different genetic backgrounds and found that one QTL with a small genetic effect in the mapping population explained significantly more of the variation in a different genetic background. Therefore, it is likely that effects of QTL governing APR to powdery mildew will vary in different genetic backgrounds due to both positive and negative epistasis among genes affecting host resistance and susceptibility.

Several studies have reported a contribution to APR from defeated race specific genes *Pm4a* (Martin and Ellingboe 1976; Nass et al. 1981), *Pm4b* (Mingeot et al. 2002), *Pm5* (Keller et al. 1999), and *MlRE* (Chantret et al. 2000; Mingeot et al. 2002) in different populations. USG 3209 possesses race specific genes *Pm8* (1BL.1RS translocation), and another unidentified hypersensitive resistance gene. The combination of defeated race-specific gene *Pm8* and the unknown gene with QTL governing APR, clearly had a residual effect on powdery mildew resistance in the UJ population (Table 3). In contrast, the original mapping population developed by Liu et al. (2001) consisted of a susceptible parent, Becker that does contain any known hypersensitive genes, and the APR cultivar Massey, that is susceptible in the seedling stage to the prevalent mildew populations found in the mid-Atlantic region, and likewise does not contain any known hypersensitive resistance genes. Therefore, occurrence of such an interaction was unlikely in the original BM mapping population.

Map position of the *QPm.vt-1BL* in the UJ populations was shifted towards the end of the chromosome and marker distances were distorted when compared to the BM genetic map. This distortion and shift in the location of the QTL likely was due to suppressed recombination near the QTL rather than due to the reduced transmission of the rye (*Secale cereale*)-derived 1RS chromosomal segment, carrying the resistance gene *Pm8*, possessed by USG 3209 (Hsam and Zeller 1997). Analysis of the RILs for presence of the 1BL.1RS translocation using the marker SCM09 indicated a 1:1 segregation ratio. Only 107 of 161 RILs carrying the 1BL.1RS translocation exhibited a reaction pattern similar to *Pm8* resistance in response to differential isolates of *B. graminis* in seedling tests. Seventy of these 107

RILs expressing resistance governed by *Pm8* and lacking the unknown gene in USG 3209 had lower average mildew severity values in the 2002 (11.0%) and 2003 (7.8%) experiments than the 77 RILs lacking any know seedling resistance genes with corresponding average severity values of 14.1 and 12.4% (Table 3). Fifty-two RILs having the unknown seedling resistance gene derived from USG 3209 and lacking 1BL.1RS (*Pm8*) also had average severity values (9.1 and 11.1%) that were lower than the 77 RILs lacking any know seedling resistance gene. Thirty-seven RILs in which seedling resistance gene *Pm8* and the unknown gene derived from USG 3209 were expressed had the lowest average mildew severity values in 2002 (5.7%) and 2003 (4.0%). This data indicates that the two seedling resistance genes contributed to the overall level of powdery mildew resistance in an additive manner in conjunction with the QTL for APR.

Fifty-four of 161 RILs carrying the 1BL.1RS did not exhibit a reaction pattern similar to Kavkaz (*Pm8*) in response to differential isolates of *B. graminis* in seedling tests (Data for these RILs not included in Table 3). Ren et al. (1996, 1997) reported a dominant suppressor of gene *Pm8* on chromosome 1AS, designated as *SuPm8*. The hypersensitive gene *Pm3a* has also been reported to reside on chromosome 1AS (Hartl et al. 1993). While data in the current study suggest that *SuPm8* or another unknown suppressor gene is present in the UJ RILs, data for seedling reaction of USG 3209 to 13 differential isolates of *B. graminis* and marker assay indicated that USG 3209 does not contain the *Pm3a* allele of its parent Saluda (Table 1). If Saluda possesses *SuPm8*, USG 3209 may have inherited this gene independent of *Pm3a* via recombination if the two genes are greater than 5 cm apart. Alternatively, a different suppressor gene may be segregating in the population. The identity and chromosome location of the unknown race-specific gene in USG 3209 and validation of the putative presence of the *SuPm8* suppressor gene on chromosome 1AS in USG 3209 needs to be elucidated to gain a better understanding of the interaction and contribution of race-specific seedling and adult-plant genes conferring resistance to powdery mildew.

In summary, three previously mapped QTL for APR to powdery mildew in cultivar Massey were validated in the UJ RIL populations, where Massey is the source of APR in USG 3209. The SSR markers mapped in the BM populations were used to develop a genetic linkage map comprised of the same chromosomal regions in the UJ confirmation population. Many of the markers mapped in the BM population were not polymorphic in UJ population. Therefore, without mapping additional markers, the QTL regions responsible for APR in USG 3209 would have lacked SSR markers that flanked both *QPm.vt-1BL* and *QPm.vt-2BL*. Simply applying the SSRs mapped in the original BM population to MAS in the UJ population would likely not be as effective without having adjacent flanking markers for all three QTL. Genetic effects varied between the original BM mapping and UJ breeding populations, and likely resulted from the presence of fewer polymorphic markers and from interaction and/or contribution of defeated hypersensitive genes with QTL for APR in the UJ population. Nevertheless, SSRs mapped near the three QTL responsible for APR to powdery mildew can be transferred and selected for in other breeding populations where Massey or derivatives of Massey are the parental source of APR. Recent studies also suggest these markers can be effectively utilized for MAS in the UJ confirmation population (Tucker et. al. 2006). As additional PCR markers become available, they should be added to the existing BM genetic map to improve selection effectiveness and increase the density of polymorphic markers available for MAS in other breeding populations where Massey is the ancestral source of APR to powdery mildew.

References

Basten CJ, Zeng ZB, Weir BS (1994) Zmap-a qtl cartographer. In: Smith C, Gavora JS, Chesnais BBJ, Fairfull W, Gibson JP, Kennedy BW, Burnside EB (eds) Proceedings of the 5th world congress on genetics applied to Livestock production: computing strategies and software. Organizing Committee, 5th World Congress on Genetics Applied to Livestock Production, Guelph, ON, Canada, pp 65–66

Bennett FGA (1984) Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programs. Plant Pathol 33:279–300

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterization of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

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

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

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

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294

Fasoula VA, Harris DK, Baily MA, Phillips DV, Boerma HR (2003) Identification, mapping, and confirmation of a soybean gene for bud blight resistance. Crop Sci 43:1754–1759

Griffey CA, Das MK (1994) Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey winter wheats. Crop Sci 34:641–646

Griffey CA, Das MK, Stromberg EL (1993) Effectiveness of adult plant resistance in reducing loss to powdery mildew in winter wheat. Plant Dis 77:618–622

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003) Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol Genet Genomics 270:315–323

Gustafon GD, Shaner G (1982) Influence of plant age on the expression of slow-mildewing resistance in wheat. Phytopathology 72:746–749

Guyomarćh H, Sourdille P, Edwards KJ, Bernard M (2002) Studies of the transferability of microsatellites derived from *Triticum tauschii* to hexaploid wheat and to diploid related species using amplification, hybridization and sequence comparisons. Theor Appl Genet 105:736–744

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

Hautea RA, Coffman WR, Sorrells ME, Bergstrom GC (1987) Inheritance of partial resistance to powdery mildew in spring wheat. Theor Appl Genet 73:609–615

Hsam SLK, Zeller FJ (1997) Evidence of allelism between genes *Pm8* and *Pm17* and chromosomal location of powdery mildew and leaf rust resistance genes in the common wheat cultivar 'Amigo'. Plant Breed 116:119–122

Hsam SLK, Lapochkina IF, Zeller FJ (2003) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell). Gene *Pm32* in a wheat-*Aegilops speltoides* translocation line. Euphytica 133:367–370

Huang XQ, Röder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica 137:203–223

James C (1971) A manual of assessment keys for plant diseases, Can. Dep. Agric. Publ. No. 1458. American Phytopathological Society, St. Paul, MN

Johnson JW, Bäenziger PS, Yamazaki WT, Smith RT (1979) Effects of powdery mildew on yield and quality of isogenic lines of 'Chancellor' wheat. Crop Sci 19:349–352

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat x spelt population. Theor Appl Genet 98:903–912

Kingsland GC (1982) Triadimefon for control of powdery mildew of wheat. Plant Dis 66:139–141

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Langridge P, Chalmers K (1998) Techniques for marker development. In: Slinkard AE (ed) Proceedings of the 9th international wheat genets symp. University of Saskatchewan Extension Press, SK, Canada, pp 107–117

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

Li Z, Jakkula L, Hussey RS, Tamulonis JP, Boerma HR (2001) SSR mapping and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode. Theor Appl Genet 103:1167–1173

Liu S, Griffey CA, Saghai Maroof MA (2001) Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. Crop Sci 41:1268–1275

Ma ZQ, Wei JB, Cheng SH (2004) PCR-based markers for the powdery mildew resistance gene *Pm4a* in wheat. Theor Appl Genet 109:140–145

Martin T, Ellingboe A (1976) Differences between compatible parasite/host genotypes involving the *Pm4* locus of wheat and the corresponding genes in *Eryisphe graminis* f. sp. *tritici*. Genetics 66:1435–1438

McIntosh RA, Hart GE, Devos KM, Gale MD (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed) Proceedings of the 9th international wheat genet symp, vol 5. University of Saskatchewan Extension Press, SK, Canada

Mingeot D, Chantret N, Baret PV, Dekeyser A, Boukhatem N, Sourdille P, Doussinault G, Jacquemin JM

(2002) Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible genetic backgrounds. Plant Breed 121:133–140

Nass H, Pedersen D, MacKenzie D, Nelson R (1981) The residual effects of some 'defeated' powdery mildew resistance genes in isolines of Chancellor winter wheat. Phytopathology 71:1315–1318

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

Purdy LH, Loegering WQ, Konzak CF, Peterson CS, Allan RE (1972) A proposed method for illustrating pedigrees of small grain varieties. Crop Sci 8:405–406

Ren SX, McIntosh RA, Sharp PJ, The TT (1996) A storage-protein marker associated with the suppressor of *Pm8* for powdery mildew resistance in wheat. Theor App Genet 93:1054–1060

Ren SX, McIntosh RA, Lu ZJ (1997) Genetic suppression of the cereal rye-derived gene *Pm8* in wheat. Euphytica 93:353–360

Roberts JJ, Calwell RM (1970) General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in ''Knox'' wheat (abstract). Phytopathology 60:1310

Röder MS, Plaschke J, Konig SU, Borner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42:964–972

Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91:5466–5470

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

Singrun C, Hsam SLK, Hartl L, Zeller FJ, Mohler V (2003) Powdery mildew resistance gene *Pm22* in cultivar Virest is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L. em Thell.). Theor Appl Genet 106:1420–1424

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Fickus EW, Cregan PB (2002) Characterization of trinucleotide SSR motifs in wheat. Theor Appl Genet 104:286–293

Sourdille P, Cadalen T, Guyomarćh H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot x Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet 106:530–538

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

Starling TM, Roane CW, Camper HM Jr (1984) Registration of massey wheat. Crop Sci 24:1000

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

Tanksley SD (1983) Molecular markers in plant breeding. Plant Mol Biol Rep 1:3–8

Tommasini L, Yahiaoui N, Srichumpa P, Keller B (2006) Development of functional molecular markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. Theor Appl Genet DOI 10.1007/s00122-006-0420-1

Tucker DM, Griffey CA, Liu S, Saghai Maroof MA (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 Breed 125:430–436

Yu JK, La Rota M, Kantety RV, Sorrells ME (2004) EST derived SSR markers for comparative mapping in wheat and rice. Mol Genet Genomics 271:742–751

Zhou WC, Kolb FL, Bai GH, Domier LL, Boze LK, Smith NJ (2003) Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. Plant Breed 122:40–46