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Genetic characterization of powdery mildew resistance in U.S. hard winter wheat

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Abstract Powdery mildew significantly affects grain yield and end-use quality of winter wheat in the southern Great Plains. Employing resistance resources in locally adapted cultivars is the most effective means to control powdery mildew. Two types of powdery mildew resistance exist in wheat cultivars, i.e., qualitative and quantitative. Qualitative resistance is controlled by major genes, is race-specific, is not durable, and is effective in seedlings and in adult plants. Quantitative resistance is controlled by minor genes, is non-race-specific, is durable, and is predominantly effective in adult plants. In this study, we found that the segregation of powdery mildew resistance in a population of recombinant inbred lines developed from a cross between the susceptible cultivar Jagger and the resistant cultivar 2174 was controlled by a major QTL on the short arm of chromosome 1A and modified by four minor QTLs on chromosomes 1B, 3B, 4A, and 6D. The major QTL was mapped to the genomic region where the *Pm3* gene resides. Using specific PCR markers for seven *Pm3* alleles, 2174 was found to carry the *Pm3a* allele. *Pm3a* explained 61% of the total phenotypic variation in disease reaction observed among seedlings inoculated in the greenhouse and adult plants grown in the field and subjected to natural disease pressure. The resistant *Pm3a* allele was present among 4 of 31 cultivars currently being produced in the southern Great Plains. The genetic effects of several minor loci varied with different developmental stages and environments. Molecular markers associated with these genetic loci would facilitate incorporating genetic resistance to powdery mildew into improved winter wheat cultivars.

Keywords Powdery mildew · *Pm3* · Qualitative and quantitative resistance · Molecular markers · Genetic mapping

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Introduction

Powdery mildew is a ubiquitous disease, caused by the biotrophic parasitic fungus *Blumeria graminis* (DC) E.O. Speer f. sp. *tritici*, which affects leaves of wheat in a wide area including the southern Great Plains of the USA. Powdery mildew reduces wheat grain yield and flour quality and can be increased in intensity by excessive use of nitrogen fertilizer (Bennett 1984; Everts et al. 2001; Griffey et al. 1993; Johnson et al. 1979; Leath and Bowen 1989). Although chemical fungicides are available to control

powdery mildew, extensive use of fungicides is expensive and may contribute to the occurrence of resistant pathogen strains and pollution of the environment. Exploitation of natural resistance in locally adapted cultivars represents the most effective, economical, and environmentally safe approach to control this disease (Bennett 1984; Hsam and Zeller 2002).

Two types of powdery mildew resistance exist in wheat: qualitative resistance and quantitative resistance. Qualitative resistance to powdery mildew is controlled by single major genes, which are race-specific, effective in seedlings and adult plants, and usually are of short durability (Bennett 1984; Hsam and Zeller 2002). Resistance conferred by a major gene fits a gene-for-gene model (Flor 1956; Powers and Sando 1960), and the effects from a major gene will not be realized once a virulent pathogen has adapted to the major gene (Menzies and MacNeil 1986; Roberts and Caldwell 1970; Shaner 1973; Wolfe 1984). Therefore, reliance on major gene resistance is undesirable, because loss of resistance in a cultivar may occur rapidly and decisively (Wolfe 1984). In contrast, quantitative resistance to powdery mildew is controlled by minor resistance genes that are effective in adult plants, are non-race-specific, and are of longer durability. Minor resistance genes are usually associated with slow mildewing (Roberts and Caldwell 1970; Shaner 1973), which indicates a delay in the infection process or in the development and/or reproduction of the pathogen (Griffey et al. 1993; Griffey and Das 1994; Hautea et al. 1987; Huang and Röder 2004). Often the level and durability of powdery mildew resistance can be increased by pyramiding qualitative and quantitative resistance genes.

Thirty-six loci may be associated with resistance to powdery mildew in wheat, including 32 loci reviewed by Huang and Röder (2004) and four loci that were recently characterized by Zhu et al. (2005) (*Pm33*) and by Miranda et al. (2006, 2007) (*Pm34*, *Pm35*, and *PmU*, a temporarily designated locus). Most of these loci, however, were not characterized directly in hexaploid common wheat (*Triticum aestivum*; $2n = 6x = 42$; AABBDD) but in diploid or tetraploid progenitors or related species of common wheat. Three powdery mildew resistance genes were transferred from the diploid donor of genome A or its close diploid relative, including *PmU* on chromosome 7AL from *T. urartu* (AA) (Qiu et al. 2005), *Pm25* on 1A from *T. boeoticum* (Shi et al. 1998), and *Pm1b* on 7AL from *T. monococcum* (A^mA^m) (Hsam et al. 1998). Three genes were introduced from potential donors of genome B, including *Pm12* on 6BS (Jia et al. 1996) and *Pm32* on 1BL (Hsam et al. 2003) from *Ae. speltoides* (BB or SS), and *Pm13* on 3BL from *Ae. longissima* (BB or SS) (Ceoloni et al. 1992). Three genes were derived from *Ae. tauschii* (DD), the donor of genome D, including *Pm19* on 7D (Lutz et al. 1995), and *Pm34* and *Pm35* on 5D (Miranda et al. 2006; Miranda et al. 2007). In addition, 16 genes were introduced from tetraploid wheat or wheat relatives, including seven genes from tetraploid species *T. turgidum* (AABB) subsp. *durum*, *dicoccum*, and *dicocoides*; two genes from tetraploid *T. carthlicum* (AABB); two genes from tetraploid *T. timopheevii* (AAGG); four genes from *S. cereale* (S^cS^c); and one gene from *A. ovata* (U^gM^g) (reviewed by Huang and Röder 2004).

Since an introgressed chromosome fragment carrying a powdery mildew resistance gene often also introduces undesirable traits, most of these resistance sources cannot be used directly in breeding programs. An attempt was made to introduce resistance genes from an alien cultivar into local cultivars (Xu et al. 2006), but simultaneous selection of ideal genotypes for the resistance and multiple adaptive traits are difficult and often not possible. When a resistance gene is introduced from one to another cultivar, the effect of this gene may vary in different genetic backgrounds. Resistance conferred by the same gene also can be affected by environmental conditions, resulting in unstable and varying expression of resistance (Keller et al. 1999; Mingeot et al. 2002).

The *Pm3b* gene is the only one that has so far been cloned from hexaploid wheat by subgenome chromosome walking in a diploid and tetraploid wheat species (Yahiaoui et al. 2004). The *Pm3b* gene is a member of the coiled-coil nucleotide binding site leucine-rich repeat (NBS-LRR) class of disease resistance genes, and its susceptible *Pm3b* allele was caused by a single base-pair deletion in the coding region. Availability of this gene sequence has facilitated identifying of polymophisms in seven resistant alleles (*Pm3a–Pm3g*) (Srichumpa et al. 2005; Yahiaoui et al. 2006) and development of specific primers for each of these alleles (Tommasini et al. 2006). The molecular tools have been extensively applied to

identify functional alleles for wheat improvement (Kaur et al. 2008).

In the present study, we explored powdery mildew resistance sources from local cultivars adapted to the southern Great Plains by mapping genetic loci following selection assisted with molecular markers. We developed a population of recombinant inbred lines (RILs) generated from a cross between two winter wheat cultivars, Jagger and 2174. Jagger, which has been planted on a majority of acres in Oklahoma and Kansas since 1997, is susceptible to powdery mildew. 2174 is a cultivar developed in Oklahoma that is highly resistant to powdery mildew. In this paper, we report that the segregation of powdery mildew resistance in the Jagger × 2174 RILs population was controlled by a major QTL *QPm.osu.1A* that associated with the *Pm3a* gene and modified by several other minor QTLs.

Materials and methods

Mapping population and genetic germplasms

Jagger and 2174 were originally used to generate a population of recombinant inbred lines (RILs) segregating for stem elongation and winter dormancy release in winter wheat; a total of 246 SSR markers were mapped in this population (Chen et al. 2009). Based on field observations on adult plants, Jagger is susceptible to powdery mildew whereas 2174 is resistant; therefore, the Jagger × 2174 population of 96 $F_{7:8}$ RILs was used to map genes associated with resistance to powdery mildew. A total of 31 varieties including Jagger and 2174 released in the southern Great Pains in recent years (Edwards et al. 2007) were genotyped using the marker for the *Pm3a* gene.

Powdery mildew evaluation

Initially, powdery mildew was observed on Jagger seedlings but not on 2174 plants when tested in an experiment for nitrogen-use efficiency in a greenhouse. Powdery mildew was much more severe on Jagger plants grown in pots with a higher level of nitrogen fertilizer (27 mg N/kg soil) than on Jagger plants grown in a lower level of nitrogen (13.5 mg N/kg soil). 2174 was highly resistant to powdery mildew under both high and low levels of nitrogen fertilizers. Jagger plants heavily infected with powdery mildew were brushed onto new Jagger seedlings growing in soil in 11.4 cm (diameter) pots. These inoculated seedlings were kept in a moist chamber as described below to promote infection and increase the available powdery mildew for inoculation of the 96 $F_{7:8}$ RILs from the Jagger × 2174 population.

Four plants of each RIL were grown in a 11.4 cm pot containing Sun Gro Redi-earth plug and seeding mix series growing medium (Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA). Seedlings at the 4-leaf stage were placed into a moist chamber constructed of 1.3 cm polyvinyl chloride tubing. Translucent plastic sheeting covered the chamber, and a water-saturated cloth towel was placed inside the chamber to provide high humidity required for infection. Seedlings were placed into the moist chamber for 12 h to allow for saturation and establishment of high humidity and then inoculated by brushing with pots of Jagger seedlings heavily infested with powdery mildew. Chambers were kept at 22–25°C for 24 h, after which the chambers were opened to allow humidity to slowly drop before plants were moved out of the chambers and onto greenhouse benches. Reaction of the plants to powdery mildew was evaluated visually, on days 6, 9, 11, 13, and 15 after inoculation. The phenotype was scored on a 1–6 scale as described by Xu et al. (2006), in which 1 = highly resistant (no visible symptom); 2 = resistant (showing hypersensitive necrotic flecks); 3 = moderately resistant (minute colonies); 4 = moderately susceptible; 5 = susceptible, and 6 = highly susceptible.

Adult-plant reaction to powdery mildew of the 96 RILs and their two parental lines was evaluated in the field in 2008 at the Agronomy Research Station, Stillwater, OK, USA. The plants in the field were naturally infected. The 96 RILs were planted in a randomized complete-block design with two replications. Each line was planted in a single two-row plot per replicate, whereas each parent was planted in three-two-row plots per replicate. Adult-plant reaction to powdery mildew was evaluated on 2 May 2008 during the heading stage. Lines were rated using the same scale as that used in the greenhouse evaluation.

Determination of avirulence/virulence formula of *Blumeria graminis* f. sp. *tritici* used in greenhouse inoculations

The avirulence/virulence formula of the *B. graminis* f. sp. *tritici* (BGT) used to test for reaction to powdery mildew in the greenhouse was determined by inoculating a set of 15 single-gene differentials (SGDs; 'Chancellor' background) along with the parent cultivars used to make the population of RILs and two check cultivars (Chancellor, susceptible, and Century, resistant). The pathogen used in this experiment was the same as that was increased for inoculating the population in greenhouse. Two replications of the 15 SGDs were inoculated (2-leaf seedling stage) as described previously and the experiment was conducted twice. In this determination, a scale of 1–4 was used to determine the reaction of the SGDs and cultivars to powdery mildew, with 1 = resistant; 2 = moderately resistant; 3 = moderately susceptible; 4 = susceptible. Ratings of '1' or '2' were considered to indicate a resistant reaction (avirulent pathogen), and ratings of '3' or '4' were considered a susceptible reaction (virulent pathogen).

Molecular mapping

Sixty-six SSR markers were added to our previous linkage maps in the Jagger × 2174 population (Chen et al. 2009). A total of 310 SSR markers were assembled in linkage groups using MapMaker 3.0 program, and QTLs were identified using the WinQTLCart 2.5 program as previously described (Chen et al. 2009). Other unlinked markers were also tested using correlation analysis to examine which markers might be related to powdery mildew resistance using SAS software (SAS 9.1, SAS Institute Inc. Cary, NC, USA).

Identification of *Pm3* alleles

After a major QTL on the short arm of chromosome 1A was found associated with variation in powdery mildew resistance in the population, specific primers for each of seven *Pm3* alleles were used to amplify genomic DNA fragments of *Pm3* genes from Jagger and 2174. PCR conditions were set up as described by Tommasini et al. (2006). PCR products with expected size were purified and sequenced.

Results

Powdery mildew resistance in parental lines and their RILs

Jagger was highly susceptible and 2174 highly resistant to powdery mildew in experiments conducted in the greenhouse, when the plants were grown in soil in pots containing sufficient nutrients including nitrogen. Variation in powdery mildew resistance between the two parental cultivars facilitated mapping the resistance trait based on segregation in the available population of $F_{7:8}$ RILs generated by crossing the parental cultivars.

On day 6 after inoculation, the distribution pattern of the resistance in the 96 RILs followed a bimodal manner (Fig. 1a). One peak indicated susceptibility similar to Jagger, and the other peak indicated resistance similar to 2174. This observation strongly suggested that the variation in powdery mildew resistance at the early stage of infection might be controlled by a single gene with major effect.

On day 15 after inoculation, the distribution pattern of resistance in the RILs was skewed toward greater disease severity (Fig. 1b). Lines previously scored on day 9 with low severity rating were scored progressively higher on the severity scale for day 15. Lines scored as highly susceptible on day 9 did not change in rating on day 15. Jagger was highly and consistently susceptible to powdery mildew, whereas 2174 was consistently resistant at the early stages of post-inoculation, although some powdery mildew did develop on 2174 under the high inoculum level and ideal infection conditions provided in this assay. These observations indicated that the development of the pathogen and/or other minor genes altered the distribution pattern of powdery mildew resistance in the RILs.

In the field, cool and rainy weather for approximately 2 weeks resulted in widespread development of powdery mildew. Quantitative measurement of powdery mildew in the field revealed a different distribution pattern from that characterized in the greenhouse. The resistance pattern in the field was skewed toward a lower disease-severity level (Fig. 1c). This result raises alternative possibilities. One possibility is that the population of *B. graminis* f. sp. *tritici* races in the field was different from the population observed in the greenhouse, and thus the

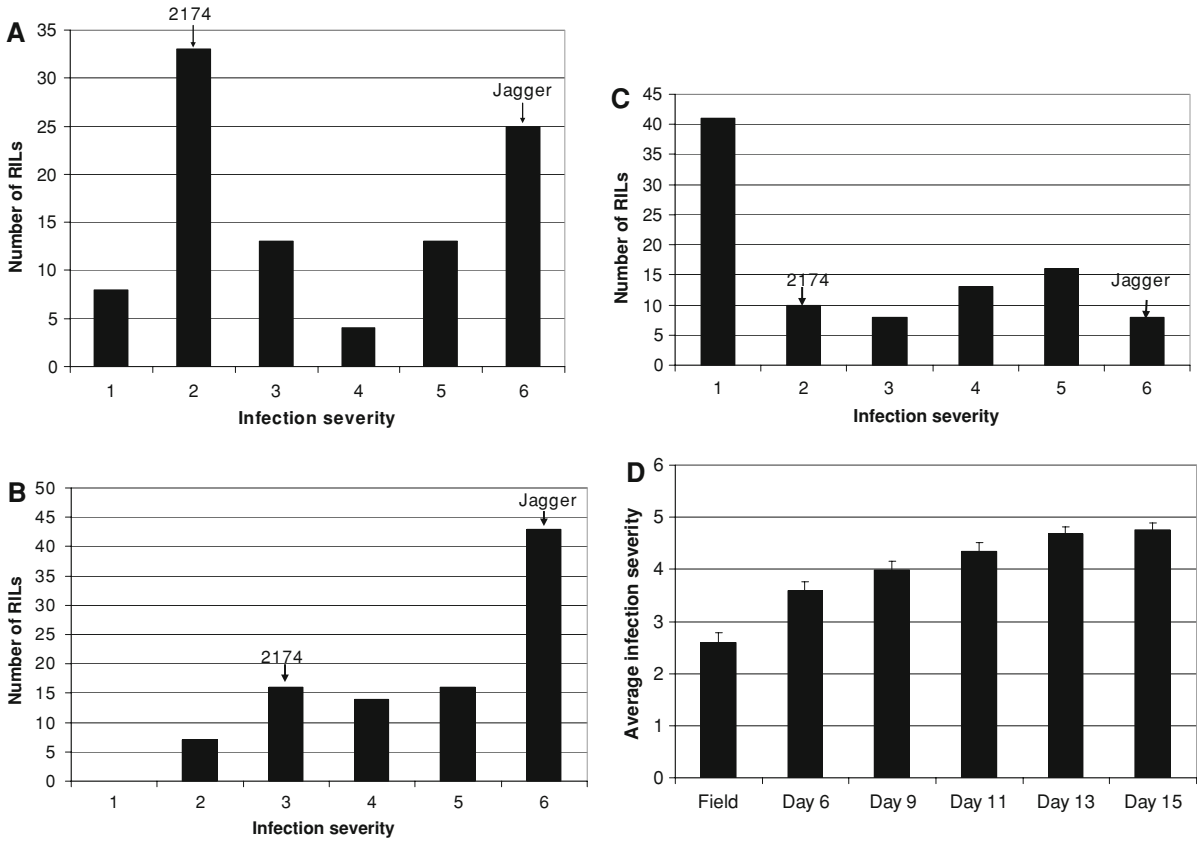

Fig. 1 Analysis of the infection severity of the 96 recombinant inbred lines (RILs). Frequency distribution for powdery mildew severity scored in greenhouse on day 6 post-inoculation (**a**), day 15 post-inoculation (**b**), and in the field (**c**) for the 96 RILs. *Arrows* indicate the reactions of parents Jagger and 2174. (**d**) Mean severity readings of the 96 RILs in field and on days 6, 9, 11, 13, and 15 post-inoculation in the greenhouse. *Arrow bar* indicates standard error

plants had a different response. The other possibility is that the same population of races occurred in the field as in the greenhouse, but the phenotypes were characterized at different stages (i.e., seedling in the greenhouse vs. adult in the field). The average infection severity characterized for the field was the lowest in comparison with severity characterized on different days in the greenhouse (Fig. 1d), suggesting that resistance in the field was characterized at the earlier stage of the powdery mildew infection than in the greenhouse.

Statistical analysis showed a high correlation between ratings scored in the greenhouse and field. Among the 96 RILs, disease reactions recorded on days 9 versus 15 were highly correlated ($r = 0.78$, $P < 0.001$), and their association with field reactions was also significant ($r = 0.75$, $P < 0.001$ and $r = 0.74$, $P < 0.001$), respectively. These results indicate that a major gene might be responsible for segregation of powdery mildew reaction in this population, regardless of the assay performed on seedlings in the greenhouse or adult plants in the field.

QTL analysis

The previously reported 246 SSR markers (Chen et al. 2009) were first analyzed to test their association with powdery mildew resistance. Once a QTL or an individual marker was anchored, more markers flanking the target genomic regions were added to construct a linkage group. As a consequence, a total of 310 markers was mapped and assembled into 36 linkage groups, each containing 3 or more markers. An additional nine linkage groups contained 2 markers, and 18 unlinked individual markers were also analyzed. Based on these mapped markers, a

major QTL and several minor QTLs were found associated with powdery mildew reactions recorded in the greenhouse and field.

A major QTL was mapped on chromosome 1A, thus designated as *QPm.osu.1A* (Fig. 2a). This QTL was initially mapped with SSR marker *Xcfa2153* that was reported to be tightly linked to *Xpsp2999* on 1AS, which was located 0.07 cm to *Pm3b* (Yahiaoui et al. 2004). Ten SSR markers were anchored in this linkage group (Fig. 2a), all of which were previously mapped on the short arm of chromosome 1A, validating the chromosomal location of this QTL. The *QPm.osu.1A* locus was a critical factor in the expression of powdery mildew reaction among seedlings in the greenhouse and adult plants in the field.

Since the *Pm3* locus is located in the short arm of chromosome 1A, it naturally and reasonably became one candidate gene for *QPm.osu.1A*. Primers specific to each of seven resistant alleles *Pm3a-Pm3g* were used to amplify the *Pm3* gene from Jagger and 2174. As a result, except for Pm3aF/R, other primers did not produce expected PCR products in either of two parental lines (Fig. 3).

2174 was found to have an expected size of PCR product with a 624-bp band using primers Pm3aF/R, but no product was obtained from Jagger using the same primers. PCR products from 2174 were purified from the agarose gel and directly sequenced. An excellent quality of sequence was obtained, indicating that it was a single gene in 2174. The result of sequence blast in GenBank showed that the 2174 Pm3aF/R sequence was exactly the same as the *Pm3a* allele sequence (AY939880) but differed from other Pm3 alleles (Srichumpa et al. 2005).

Pm3aF/R primers were used to amplyfy the *Pm3a* gene from each of 96 RILs, and the population showed the same pattern of polymorphic bands as detected in the two parental lines. Mapping the *Pm3a* gene showed that it resided between two SSR markers *Xcfa2153* and *Xpsp2999*, confirmed that the *Pm3a* gene was responsible for *QPm.osu.1A*.

The LOD values for *QPm.osu.1A* at *Pm3a* was 19.4 and 18.8 for readings taken on day 6 and day 15 (post-inoculation) in the greenhouse, respectively, and 20.1 for field readings, which on average explained 61% of the total phenotypic variation (Table 1). Hence the majority of the phenotypic variation was controlled by the *QPm.osu.1A* locus, consistent with our conclusion by statistical analysis on distribution of the resistance in RILs that the variation in powdery mildew resistance in the present population was controlled by a single and major gene. From three times of readings, the mean infection severity value of the RILs carrying the Jagger *Pm3* allele was 5.0, whereas those carrying the 2174 allele had a mean value of 2.4, demonstrating a significant difference in powdery mildew resistance between these two alleles.

One minor QTL was detected in the segregation of powdery mildew reaction characterized for both the greenhouse and field populations. It was mapped to the short arm of chromosome 3B, thus designated as *QPm.osu-3B* (Fig. 2b). This locus centered on *Xwms533* and accounted for 10% of the total phenotypic variation in the field population and 13% of the total phenotypic variation in the greenhouse population (day 6 post-inoculation). The RILs carrying the Jagger allele at the *QPm.osu-3B* locus had an infection severity value of 4.2 on day 6, which was significantly higher ($P < 0.001$) than value (2.9) for RILs carrying the 2174 allele. This also indicated the *QPm.osu-3B* locus contained a susceptible allele in Jagger but a resistant allele in 2174.

One minor QTL on chromosome 6D, designated as *QPm.ous.6D*, was found associated with powdery mildew resistance on day 15 post-inoculation in the greenhouse (Fig. 2c). This QTL had a LOD value of 3.0, explaining 13% of the total variation in the greenhouse. The LOD value for this trait determined on day 6 post-inoculation in the greenhouse or in the field did not exceed the threshold value of 2.5, but the difference in the reaction scores was significant between the Jagger and 2174 alleles for the greenhouse population ($P < 0.001$) and for the field population ($P < 0.05$). On day 15 in the greenhouse, the RILs carrying the Jagger allele at *QPm.ous.6D* had a mean severity reading of 5.2, while those carrying the 2174 allele had a mean reading of 4.2, again indicating that Jagger contained a susceptible allele and 2174 contained a resistant allele.

Two additional minor QTLs were detected only in the field. One was on chromosome 4A (designated *QPm.osu-4A*) (Fig. 2d) with LOD value 2.8 and explained 12% of the total variation; another one was on chromosome 1B (designated *QPm.osu-1B*) (Fig. 2e) with LOD value 3.0 and explained 13.8% of the total variation. The LOD value for the greenhouse assay was lower than the common threshold of 2.5

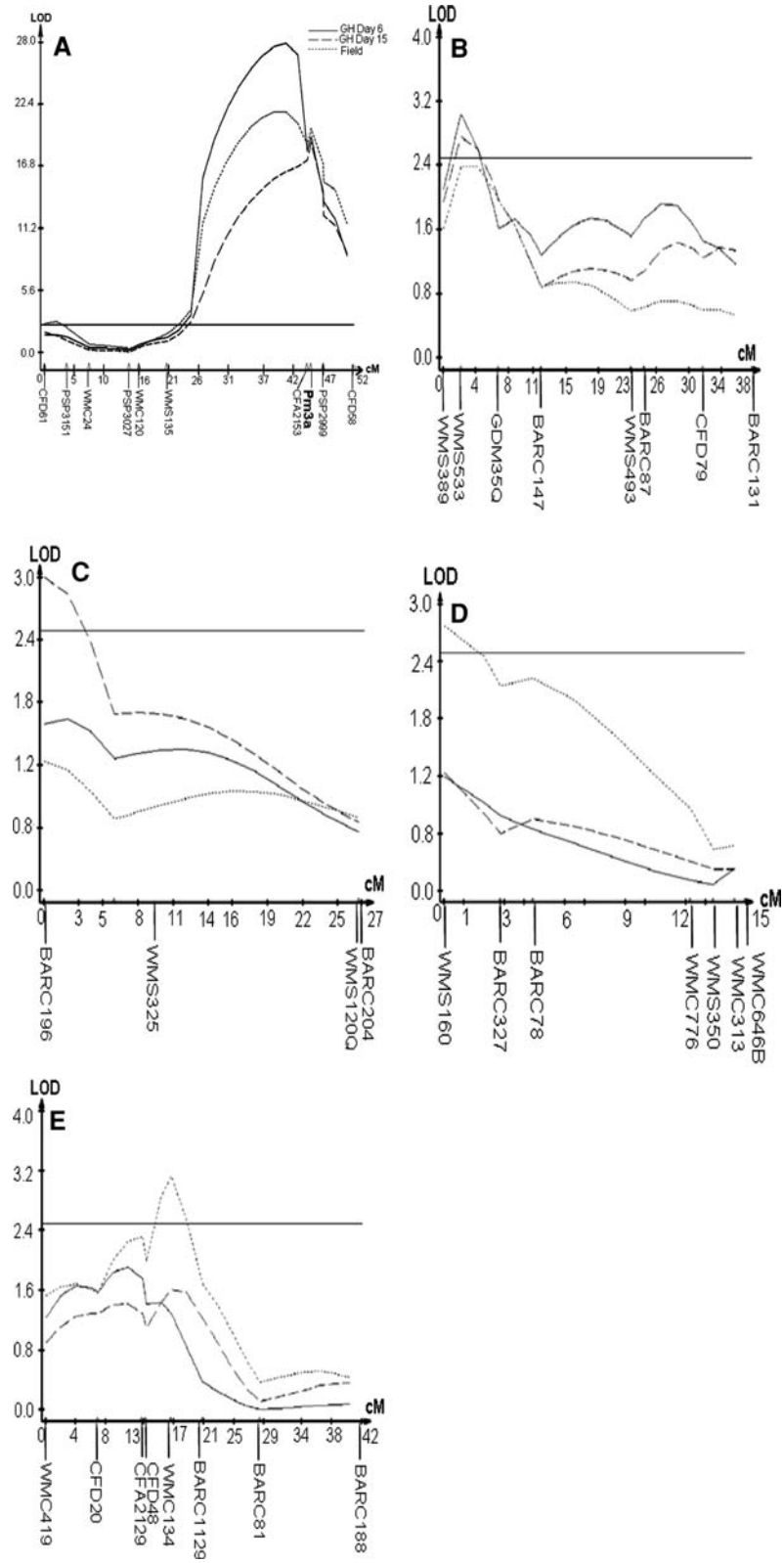

Fig. 2 Chromosomal location of QTLs for powdery mildew reaction in the recombinant inbred lines (RILs). (**a**) A major QTL *QPm.osu-1A* on chromosome 1AS; (**b**) a minor QTL *QPm.osu-3B* on 3B; (**c**) a minor QTL *QPm.osu-6D* on 6D; (**d**) a minor QTL *QPm.osu-41A* on 4A; (**e**) a minor QTL *QPm.osu-1B* on 1B. **a**–**e**, a *solid line* indicates QTL characterized on day 6 post-inoculation in the greenhouse (GH day 6); a *dashed line* indicates QTL on day 15 in the greenhouse (GH day 15); a *dotted line* indicates a QTL characterized in the field population. The *horizontal line* represents a common threshold value of 2.5 LOD

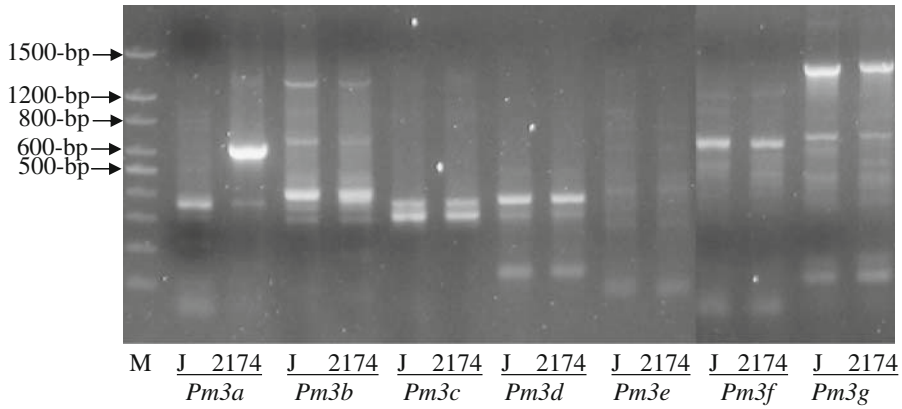

Fig. 3 Genotypes of *Pm3* in local cultivars. *M* represents DNA marker. The letter *J* denotes Jagger. Except for a 624-bp band from *Pm3a* appeared in 2174, no expected size of products from other *Pm3* alleles in either of two parental lines. Expected size is 1382-bp for *Pm3b*, 846-bp for *Pm3c*, 1109-bp for *Pm3d*, 524-bp for *Pm3e*, 624-bp for *Pm3f*, and 540-bp for *Pm3g*

Table 1 Effects of QTLs on the variation of powdery mildew resistance

QTL	Marker	Phenotyping time	LOD	R^2	Mean severity level	
					Jagger	2174
QPm.osu-1A	Pm3a	Day 6, greenhouse	19.4	0.61	5.0	2.3
		Day 15, greenhouse	18.8	0.60	5.9	3.8
		Field	20.1	0.63	4.0	1.2
QPm.osu-1B	WMC134	Day 6, greenhouse	1.2	0.05	4.0	3.1
		Day 15, greenhouse	1.5	0.06	5.1	4.4
		Field	3.0	0.14	3.2	1.9
QPm.osu-3B	WMS533	Day 6, greenhouse	2.9	0.13	4.2	2.9
		Day 15, greenhouse	2.7	0.12	5.2	4.3
		Field	2.3	0.10	3.1	2.0
QPm.osu-4A	WMS160	Day 6, greenhouse	1.2	0.05	4.0	3.2
		Day 15, greenhouse	1.2	0.05	5.1	4.4
		Field	2.8	0.12	3.2	2.0
QPm.osu-6D	BARC196	Day 6, greenhouse	1.6	0.07	4.0	3.0
		Day 15, greenhouse	3.0	0.13	5.2	4.2
		Field	1.2	0.05	3.0	2.1

LOD value, but the difference in severity values scored in the greenhouse was significant between the Jagger allele and 2174 allele for both QTLs ($P < 0.05$). At both *QPm.osu-4A* and *QPm.osu-1B*, the Jagger allele was susceptible and the 2174 allele was resistant to powdery mildew.

The resistant *Pm3a* allele present in local cultivars

A total of 31 locally adapted cultivars, including Jagger and 2174, were genotyped using the specific marker for the *Pm3a* allele. Four of these cultivars (2174, Okfield, Centerfield, Ok102) had the same *Pm3a* allele as 2174. The *Pm3a* allele is absent in the remaining 27 cultivars (Above, Cimarron, Cutter, Custer, Danby, Deliver, Doans, Duster, Endurance, Fannin, Fuller, Guymon, Hatcher, Intrada, Jagalene, Jagger, JEI 110, Lakin, Neosho, OK Bullet, Overley, Santa Fe, Shocker, TAM 110, TAM 111, TAM 112, Trego).

Discussion

2174, a hard red winter wheat variety released by the Oklahoma Agricultural Experiment Station in 1997, is resistant to powdery mildew (Carver et al. 2004). Conversely, Jagger, a hard white winter wheat variety

released by Kansas State University in 1994, is moderately susceptible to powdery mildew. The complementary trait in powdery mildew resistance in these two cultivars has facilitated discovery of quantitative trait loci (QTL) responsible for the variation in the trait in the RIL population. This is a first step in understanding the biological process underlying a complex quantitative trait. Availability of sequences for different *Pm3* alleles has facilitated mapping of the candidate gene for the major QTL. The discovery of the coincident locations between *Pm3a* and the major QTL *QPm.osu.1A* locus for powdery mildew resistance has laid the foundation for molecular characterization of the *QPm.osu.1A* locus.

Our study showed that powdery mildew resistance effects of the *Pm3a* allele were detected not only at the seedling stage in the greenhouse but also at the adult plant stage under natural disease pressure in the field. Therefore, the *Pm3a* allele may play a major role in resistance to powdery mildew during both seedling and adult stages of the resistant cultivar 2174. Resistance to powdery mildew in 2174 has remained effective since its release in 1997. Similar durability was reported (Liu et al. 2001) for the cultivar Massey, which remained effective until 2001 following its release in 1981, and for the cultivar Knox and its derivatives, which remained effective against powdery mildew infection for 20 years (Shaner 1973).

High susceptibility to powdery mildew in Jagger was attributable not only to the major gene(s) on *QPm.osu-1A* locus but also to four minor QTL loci on *QPm.osu-1B*, *QPm.osu-3B*, *QPm.osu-4A*, as well as *QPm.osu-6D*. If powdery mildew reaction follows the gene-for-gene hypothesis, then Jagger lacks the dominant alleles for resistance at these QTLs, or the pathogen has the corresponding gene for virulence and thereby can overcome any resistance genes in these QTLs. The molecular markers for these QTLs may facilitate selection for the resistance genes from 2174 in wheat breeding programs. Although the 2174 allele at all of these QTLs conferred resistance, 2174 did not show immunity to powdery mildew. It is not known if any other QTLs were not yet found due to the presence of gaps between linkage groups. It will be unlikely, however, that any uncharacterized QTL in the gaps contained a major gene, since *QPm.osu.1A* plus four minor QTLs found in this study have accounted for more than 85 and 94% of the total phenotypic variation in the population tested in the field and in the greenhouse, respectively.

Each resistance gene against the powdery mildew pathogen in common wheat should have a corresponding avirulence gene in the pathogen (Flor 1956; Powers and Sando 1960). The evaluation of the infection severity of the limited tester lines available in this study indicated an avirulence/virulence formula for the BGT used in these tests as: 1a 3a 3c 3f 4a 4b 5a 8 17 25 Century 2174/2a 3b 3e 6 7 Chancellor Jagger (Table 2). As a result, *Pm3a*, *Pm3c* or *Pm3f* lines showed good resistance against the mildew mixture used, suggesting that it is likely that 2174 has the same *Pm3* allele as one of three tester lines or that it is unlikely that 2174 has a different *Pm3* allele than the *Pm3b* and *Pm3e* lines. This resting experiment phenotypically confirmed molecular characterization of *QPm.osu.1A* containing the *Pm3a* allele.

Table 2 Reaction of 15 single-gene differentials and four wheat cultivars to powdery mildew

Cultivar/lines	Infection type		
	Trial 1	Trial 2	Mean
Chancellor	3.5	3.5	3.5
Jagger	3.75	3.75	3.75
Pm1a	2	1.5	1.75
Pm2a	4	4	4
Pm3a	1	1	1
Pm3f	1	1	1
Pm3c	1.5	1.5	1.5
Pm3e	2.5	2.5	2.5
Pm3b	3	3.5	3.25
Pm4a	1.5	2	1.75
Pm4b	1	1.5	1.25
Pm5a	1.5	1	1.25
Pm6	4	4	4
Pm7	4	4	4
Pm8	1	1	1
Pm17	1	1	1
Pm25	2	2	2
Century	1	1.67	1.34
2174	1.67	1.67	1.67

Cultivars/lines were rated on a scale of 1–4, where 1 = resistant; 2 = moderately resistant; 3 = moderately susceptible; 4 = susceptible. Two reps of each entry were inoculated in each trial

The resistant *Pm3a* allele has been successfully applied in wheat breeding in the southern Great Plains. All of three cultivars, Ok102, Okfield and Centerfield that were identified carrying the resistant *Pm3a* allele were derived from a cross between 2174 and another parental line. Ok102 was derived from the cross '2174'/'Cimarron' (PI 536993) (Carver et al. 2004). Okfield resulted from a single cross using HBZ374C as one of the parental lines, which was eventually released as 2174 (Carver et al. 2007). Centerfield has the pedigree 'TXGH12588-105*4/FS4//2*2174', which was released by the Oklahoma Agricultural Experiment Station in 2006. All of these 2174 derives have a range from intermediate to resistant to powdery mildew in the field and greenhouse (Carver et al. 2004, 2007), demonstrating that wheat breeders in this region have successfully incorporated this resistant allele into contemporary wheat cultivars.

QPm.osu-3B was located on the 3B short arm, where a resistance gene *Pm13* was previously mapped using RFLP markers (Donini et al. 1995). Whether *Pm13* is the same as *QPm.osu-3B* is still yet to be genetically determined. Coincidently, a major QTL for resistance to *Fusarium* head blight (FHB) in wheat has also been identified in the region where *QPm.osu-3B* resides, based on comparison of SSR markers such as *Xgwm533*, *Xgwm493*, and *Xbarc87* (Liu and Anderson 2003). Genomic sequences in a collinear region of rice showed that there were several protein kinase genes homologous to gene *Lr10* (Feuillet et al. 1997). Most of the other genes do not have a description for their function, and a few genes with predicted function do not have any clear relation to resistance traits (Liu and Anderson 2003). Further identification of the candidate genes in this region for *QPm.osu-3B* would result in cloning of related powdery mildew resistance genes in wheat.

It was difficult to identify candidate genes for *QPm.osu-1B*, *QPm.osu-4A*, or *QPm.osu-6D* based on the comparison of SSR markers with previously reported genes or QTL in a specific arm or chromosome. Further study is needed on the individual resistance genes by constructing a fine map using additional RILs of the current population and/or utilizing progeny from backcrosses of a particular RIL with parental lines. Effects of a single minor gene will be enhanced in a backcross population where other QTLs, such as *QPm.osu-1A*, are fixed for the same allelic state for resistance.

In summary, the locally adapted cultivars Jagger and 2174 are susceptible and resistant to powdery mildew, respectively. Segregation of reaction types in the RIL population derived from their single cross strongly indicates that the resistant *Pm3a* allele in 2174 plays a critical role in the resistance expressed in seedlings and adult plants. Severe susceptibility in Jagger was caused by the absence of the resistant *Pm3a* allele as well as at least four minor genes. Availability of molecular markers for resistant alleles characterized for the major gene and minor genes will facilitate marker assisted selection (MAS) for wheat breeding programs. Near-complete resistance in a wheat cultivar is expected to be obtained by pyramiding the major and minor resistance genes.

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