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ORIGINAL PAPER

Rye-derived powdery mildew resistance gene *Pm8* in wheat is suppressed by the *Pm3* locus

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Abstract Genetic suppression of disease resistance is occasionally observed in hexaploid wheat or in its interspecific crosses. The phenotypic effects of genes moved to wheat from relatives with lower ploidy are often smaller than in the original sources, suggesting the presence of modifiers or partial inhibitors in wheat, especially dilution effects caused by possible variation at orthologous loci. However, there is little current understanding of the underlying genetics of suppression. The discovery of suppression in some wheat genotypes of the cereal rye chromosome 1RS-derived gene *Pm8* for powdery mildew resistance offered an opportunity for analysis. A single gene for suppression was identified at or near the closely linked storage protein genes *Gli-A1* and *Glu-A3*, which are also closely associated with the *Pm3* locus on chromosome 1AS. The *Pm3* locus is a complex of expressed alleles and pseudogenes embedded among *Glu-A3* repeats. In the current report, we explain why earlier work indicated that the mildew suppressor was closely associated with specific *Gli-A1* and *Glu-A3* alleles, and predict that suppression of *Pm8* involves translated gene products from the *Pm3* locus.

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Introduction

The maintenance of resistance to obligate pathogens of common wheat (*Triticum aestivum*) over many years has depended on ongoing availability, identification and utilization of resistance genes. As resistance genes became ineffective due to increased virulence in pathogen populations, breeders introduced new genes. These initially came from common wheat, but subsequently there was increasing use of resistance genes introduced from wheats with lower ploidy or from related species. Common wheat is an allohexaploid species and genes introgressed to it from species of lower ploidy often confer lower levels of resistance than in the original source genotypes. In some cases, such introgressions do not have adequate resistance to protect the hexaploid cultivar derivatives from significant losses. In other instances, the original hybrids with wheat, or the amphiploids or partial amphiploid derivatives, are fully susceptible, indicating evidence of genetic suppression of resistance.

Although there have been reports of resistance suppression in wheat hybrids and amphiploids (e.g. Kerber 1983; Bai and Knott 1992; Kema et al. 1995), in no case is there a clear understanding of its genetic basis. In some examples, the actual parents used to create the original hybrids are no longer available. This problem is exacerbated because an amphiploid is the product of single gametes from each parental accession and the parents may not be genetically homogeneous. Indeed, some donors of such amphiploids are outcrossing species, accessions of which are more likely to be genetically heterogeneous.

Several disease resistance genes were simultaneously introduced to hexaploid wheat from cereal rye (*Secale cereale*) cv. Petkus in the 1930s (Zeller 1973), all on a single segment of rye chromosome 1R. The genes include

Sr31 [resistance to *Puccinia graminis* f. sp. *tritici* (*Pgt*), the stem rust pathogen], *Lr26* (resistance to *P. triticina*, the leaf rust pathogen), *Yr9* (resistance to *P. striiformis* f. sp. *tritici*, the stripe rust pathogen) and *Pm8* [resistance to *Blumeria graminis* f. sp. *tritici* (*Bgt*), the powdery mildew pathogen]. Wheat derivatives of Petkus with a 1R(1B) substitution or, more commonly, a 1BL·1RS translocation bearing these genes were exploited worldwide. Pathogen variants with virulence on lines possessing these genes were subsequently reported, the most recent report being that of virulence in *P. graminis tritici* for *Sr31* (Pretorius et al. 2000).

Hanusova (1992) showed that some lines known to carry the 1R(1B) substitution or 1BL·1RS chromosome and/or one or more of the rust resistance genes were susceptible to *Bgt* isolates known to be avirulent on other lines with the same 1RS chromosome. This indicated the likelihood of suppression of *Pm8* in some genotypes. Hanusova et al. (1996) subsequently reported that suppression was controlled by a single dominant gene, and that it was located on chromosome 7D. Ren et al. (1996) serendipitously observed a close genetic association between suppression of *Pm8* and a specific electrophoretic gliadin protein band ('suppressor band'). The gene responsible for the protein band was located on chromosome 1A. This relationship was so close that the presence of the suppressor in a genotype could be predicted in the absence of the 1RS chromosome. Study of a wide array of germplasm indicated that the frequencies of the suppressor gene varied among geographical areas, being relatively low in Europe, but high in breeding populations from the International Maize and Wheat Improvement Center (commonly named by its Spanish acronym CIMMYT for Centro Internacional de Mejoramiento de Maíz y Trigo).

Ren et al. (1997) showed that the CIMMYT wheat Veery was variable in seedling response to a *Bgt* culture avirulent on Federation*4/Kavkaz which carries the 1BL·1RS translocation. Among 10 Veery selections, only Veery#6 was resistant. Veery was also heterogeneous for grain color, some selections being red-seeded, others white. The present authors subsequently found that different selections of Bobwhite were also heterogeneous in response to powdery mildew and for grain color. Selections in both Veery and Bobwhite could be distinguished on the basis of the presence or absence of the gliadin suppressor band. The *Gli-A1* locus (or cluster of loci) is very closely linked with *Glu-A3*, a cluster of closely linked genes for low molecular-weight glutenins. Ren et al. (1996) also noted the close association of *Pm8* suppression with one particular protein band at the *Glu-A3* locus.

For genetic analysis and fine mapping of the suppressor, we generated recombinant inbred lines (RILs) from crosses

Table 1 Genotypes with and without 1BL·1RS

Genotype	1BL·1RS	Glu-A3 primer LMWP1/LMWP2	5′ primer UP3B/UP1A	3′ primer Pm3MF/Pm3ER1	*Pm3*/*Pm3*-like gene	Mildew response	
						Actual	
Bobwhite Red	+	+	+	+	Yes	S	
Bobwhite White	+	−	−	+	No	R	
Veery#5	+	+	+	+	Yes	S	
Veery#6	+	−	−	−	No	R	
Sarhad 82	+	+	+	+	Yes	S	
Skorospelka 35	+	−	+	+	Yes	R	
Aurora	+	−	−*	−	No	R	
Disponent	+	+	−	−	No	R	
Fed*4/Kavkaz	+	−	−	−	No	R	
Kohinoor 83	+	−	+	−	No	R	
Thatcher + Lr26	+	+	−	−	No	S	
						Predicted 1	Predicted 2
Aroona	−	+	+	+	Yes	S	S
Bayonet	−	+	+	+	Yes	S¶	S¶
Canna	−	+	+	+	Yes	S¶	S¶
Chinese Spring	−	+	+	+	Yes	S	S
Condor	−	+	+	+	Yes	S¶	S¶
CSP44	−	+	+	+	Yes	S¶	S¶
Dagger	−	−	+	+	Yes	R	S

Table 1 continued

Genotype	1BL·1RS	Glu-A3 primer LMWP1/LMWP2	5′ primer UP3B/UP1A	3′ primer Pm3MF/Pm3ER1	*Pm3*/*Pm3*-like gene	Mildew response	
Kelalac	−	+	+	+	Yes	S	S
Lance	−	−	+	+	Yes	R	S
Lark	−	−	+	+	Yes	R	S
Millewa	−	+	+	+	Yes	S[¶]	S[¶]
Osprey	−	+	+	+	Yes	S[¶]	S[¶]
Spear	−	+	+	+	Yes	S[¶]	S[¶]
Tatiara	−	−	+	+	Yes	R	S
TD 1656	−	+	+	+	Yes	S	S
WW31	−	+	+	+	Yes	S[¶]	S[¶]
Wyuna	−	+	+	+	Yes	S	S
Banks	−	−	+	−	No	R[¶]	R[¶]
Bodallin	−	−	−	−	No	R	R
Bindawarra	−	−	+	−	No	R[¶]	R[¶]
Cook	−	−	+	−	No	R	R
Federation	−	−	−*	−	No	R	R
Gutha	−	−	+	−	No	R	R
Halberd	−	+	−*	−	No	S[¶]	R[¶]
Hartog	−	−	+	−	No	R[¶]	R[¶]
Jacup	−	−	+	−	No	R	R
Lowan	−	−	−	−	No	R	R
Miling	−	−	+	−	No	R[¶]	R[¶]
Mokoan	−	−	−	−	No	R[¶]	R[¶]
Oxley	−	−	+	−	No	R	R
Teal	−	−	−	−	No	R	R
Thatcher	−	+	−	−	No	S	R

The presence of *Pm3* or a *Pm3*-like gene was predicted by amplification of both primer pairs UP3B/UP1A and Pm3MF/Pm3ER1. Powdery mildew responses "1" were predicted from amplification by the LMWP primer pairs, and indicated similar responses reported by Ren et al. (1996) using backcross derivatives with *Lr26* or *Sr31*. Powdery mildew responses "2" were predicted from the presence of *Pm3*/*Pm3*-like gene. Amplification products with a variant size were treated as negative and marked with "*". [¶]Instances where backcross derivatives with 1BL·1RS were tested by Ren et al. (1996), producing the predicted responses

of powdery mildew resistant and susceptible lines of Veery and Bobwhite (Table 1). The respective lines for intercrossing were also chosen on the basis of differences in grain color. Obviously the choice of these particular parents, which could be considered near-isogenic, for fine mapping restricted the likelihood of molecular marker differences between the parents. However, for fine mapping, we required closely linked markers and assumed we could undertake focused searches for polymorphic associations during development of the RILs. The suppressor band generated by the *Gli-A1* locus was one such polymorphic marker that could be utilized, but we sought molecular alternatives. In hexaploid wheat *Gli-A1* is completely linked with *Glu-A3* for which a potential PCR marker was available (Devos et al. 1995).

We were also aware from genetic maps that the *Gli-A1*/*Glu-A3* loci must be closely linked with the *Pm3* locus (Sourdille et al. 1999) and it was tempting to speculate on a role of that locus in *Pm8* suppression. The cloning of *Pm3b* (Yahiaoui et al. 2004) and other *Pm3* alleles included one allele that does not confer powdery mildew resistance (Yahiaoui et al. 2006). The development of PCR markers based on the *Pm3* sequences (Tommasini et al. 2006) opened the possibility of additional molecular polymorphisms for our mapping populations. The experiments to be reported in this paper describe the various steps that led to our prediction that *Pm8* could be a *Pm3* ortholog, and that products of translated *Pm3* alleles are possible suppressors of *Pm8* in wheat. This mechanism of genetic suppression, which may be unique to polyploid species, could explain at least some of the instances of absence of resistance in wheat wide crosses derived from resistant relatives. We provide data on the genetics of *Pm8* and its suppressor and demonstrate that *Pm8* resistance to a *Pm8*-avirulent isolate was not expressed in the presence of the suppressor. Preliminary reports on some of the results were given in McIntosh et al. (2008).

Materials and methods

Wheat materials

Various lines reported in Ren et al. (1996, 1997) provided a basis for the present studies. Four F_5 RIL populations derived from two F_1 plants of Veery#6/Veery#5 and two of Bobwhite White/Bobwhite Red were developed during 2004–2007. Selections of Veery (Kvz/Buho//Kal/BB, CM33027) were released worldwide under more than 60 cultivar names, whereas Bobwhite (Au//Kal/BB/3/WOP, CM33203) selections were grown under at least 13 names in Asia and South America (Skovmand et al. 1997). The sources of *Pm8* were the Russian cultivars Kavkaz and Aurora, respectively. Resistance conferred by *Pm8* is expressed in both cultivars.

In anticipation of the need for a large segregating population for fine mapping, 817 RILs were developed. Molecular tests and powdery mildew response determinations were conducted on F_5 families used to establish F_4 genotypes. In a RIL population of this type segregating for powdery mildew resistance at a single locus, the expected genotypic ratio is 7 homozygous resistant:2 segregating:7 homozygous susceptible.

Responses to powdery mildew

Tests at Plant Breeding Institute (PBI) Cobbitty

All RILs were genotyped by seedling response tests using a *Bgt* isolate (culture NAR) that was avirulent on seedlings of Federation*4/Kavkaz (Fed*4/Kavkaz), the standard reference line for *Pm8* used at PBI Cobbitty. NAR was collected at Narrabri, NSW, in 1995 and was continuously maintained in isolation on seedlings of Morocco wheat. For some experiments described here, *Pm8*-virulent inoculum (designated 'Resident' strain) was collected from the PBI Cobbitty greenhouses and increased on Morocco. Both cultures were avirulent on seedlings of Asosan/3*Federation (Asosan/3*Fed), a reference genotype with *Pm3a*, and virulent on seedlings of Federation.

About 20 seedlings of RILs, segregating lines and parents were grown in 9-cm pots, and were inoculated at the 1–2 leaf stage with the appropriate pathogen culture by dusting conidia from diseased Morocco seedlings. RILs were scored as the numbers of seedlings resistant or susceptible, or simply as homozygous resistant (HR), segregating (Seg) or homozygous susceptible (HS), using a 0–4 scale: resistant (0–3) and susceptible (3+ to 4). Scoring was performed about 10 days after inoculation, or when there were clear response differences among the controls, Fed*4/Kavkaz and Federation, and relevant parents.

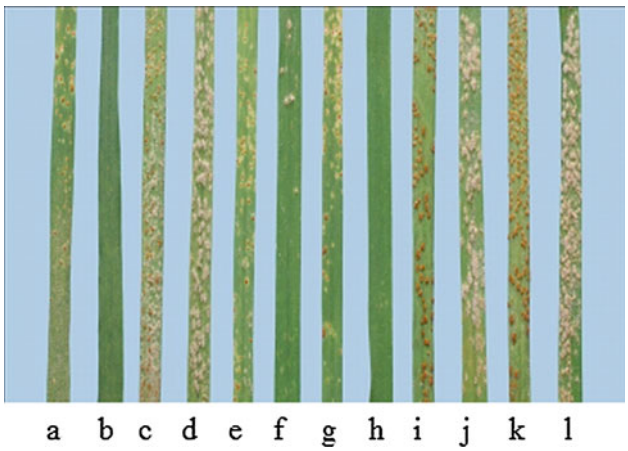

Fig. 1 Powdery mildew and leaf rust responses on paired first and second leaves of (**a**, **b**) Fed*4/Kavkaz, which carries *Lr26* and *Pm8* on 1BL·1RS; (**c**, **d**) Federation, which lacks 1BL·1RS; and (**e**–**h**) two resistant F_2 plants and (**i**–**l**) two susceptible F_2 plants from the intercross of Fed*4/Kavkaz//Federation. Powdery mildew inoculation was with *Bgt* strain 'NAR' (avirulent to *Pm8*). The few powdery mildew lesions on leaf **f** were produced by contamination with the 'Resident' strain

In instances when a conclusive genotype could not be established, tests were repeated.

To generate lines combining *Pm8* and *Pm3a*, Fed*4/Kavkaz was crossed with Asosan/3*Fed. F_3 lines from this cross were selected for homozygosity for *Lr26*, and therefore the presence of *Pm8* because all four genes in the 1RS chromosome arm behave as a linkage block in the wheat background, using PBI Cobbitty *P. triticina* pathotype 162-1,2 (PBI culture 95). The methods of seedling leaf rust testing were those in routine use at PBI Cobbitty (McIntosh et al. 1995). The homozygous lines were then tested with the 'Resident' *Bgt* culture which is avirulent on seedlings of Asosan/3*Fed, and virulent on seedlings Fed*4/Kavkaz. Seed of different lines was despatched to North Carolina as non-disclosed *Pm3* genotypes.

In experiments where leaf rust and powdery mildew tests were simultaneously conducted on the same materials, seedlings were firstly inoculated with *P. triticina* and then after 5–7 days were dusted with *Bgt* conidia. This allowed scoring of responses to both pathogens to occur at the same time. An example of results of this kind of test is shown in Fig. 1 for an F_2 family from Fed*4/Kavkaz//Federation. First and second leaves are shown in the figure; the first leaves are infected with both pathogens and the second leaves show the powdery mildew responses more clearly although the responses on both leaves are the same.

Tests at USDA-ARS, Raleigh, NC

Seeds of 21 selected F_3 lines homozygous for *Pm8* and with known (but non-disclosed) *Pm3a* genotype were

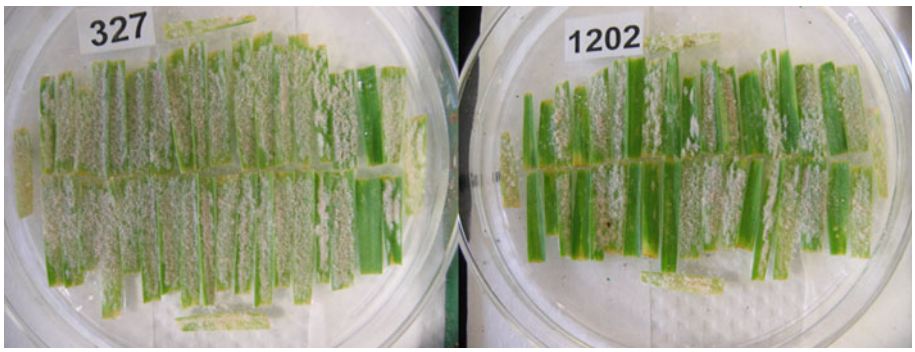

Fig. 2 Responses of paired leaf segments from individual seedlings of F_3 lines 327 and 1202 from Asosan/3*Fed//Fed*4/Kavkaz. Line 327, genotype *Pm8Pm8 Pm3aPm3a*, was homozygous susceptible and line 1202, genotype *Pm8Pm8 Pm3apm3a*, segregated. The lines were tested with the *Bgt* culture AN2-4, which was virulent for *Pm3a* and avirulent for *Pm8*. Each pair, consisting of one leaf segment above and one below, is from the same seedling. The 15 leaf pairs starting from the left side are all from the tested line. The pairs starting from the right side of each dish are Federation, Fed*4/Kavkaz (*Pm8Pm8*), and Asosan/3*Fed (*Pm3aPm3a*), respectively. The single segments at the extreme *right*, *left*, *top* and *bottom* are of the susceptible control Chancellor

provided to USDA-ARS researchers in North Carolina. These lines were tested with *Bgt* isolate AN2-4, which was virulent to *Pm3a* but avirulent to *Pm8*. AN2-4 originated from wheat debris collected in Headland, Alabama, in 2005, and had been single-pustuled and maintained since that time by transfers on detached leaves of the universally susceptible cv. Chancellor on 0.005% benzimidazole water-agar. The purpose of these tests was to determine if *Pm8* conferred resistance in the presence of an ineffective but presumably translated *Pm3* allele, namely *Pm3a*.

The tests were done on detached leaves floated on benzimidazole water-agar Petri plates (Fig. 2). Detached leaf segments of the F_3 lines were floated on agar and inoculated with AN2-4. Leaf material from a single plant was cut into four segments. For each line, two identical (non random) replicate plates were set up, each containing two replicate leaf segments from each of 15 plants of a single line. This was done in order to determine if the line was segregating or homozygous in response. Each plate also contained the following controls: two segments each of Asosan/3*Fed, Fed*4/Kavkaz, and Federation; and four segments of Chancellor. Inoculated leaves were incubated in a growth chamber for eight days at 17°C with 12 h of light per day. Ratings were made on day 9 after inoculation, using a 0–9 scale: resistant (0–3), intermediate (4–6), and susceptible (7–9).

DNA isolation and PCR protocols

Genomic DNA from approximately 100 mg of plant material collected from 8 to 10 plants of each genotype or line was isolated using the CTAB method (Doyle and Doyle 1987). DNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and diluted to approximately 50 ng/µl.

Three PCR primer pairs were used: LMWP1/LMWP2, which amplifies a sequence of *Glu-A3* associated with the mildew suppressor identified in Ren et al. (1996); UP3B/UP1A, which amplifies a segment in the *Pm3* promoter region in plants that produce an open reading frame and a full length *Pm3* transcript (Tommasini et al. 2006); and Pm3MF/Pm3ER1, which amplifies a sequence in the 3′ region of *Pm3* (Tommasini et al. 2006). These latter primers became available only after the cloning of *Pm3* (Yahiaoui et al. 2004).

Each 10 µl of PCR solution contained 25 ng of DNA, 5 µl of HotStarTaq Plus Master Mix (Qiagen Pty Ltd, Vic., Australia) (with a final concentration of 1 U of HotStarTaq Plus DNA polymerase, 1× PCR buffer, and 200 µM of each dNTP), and 0.5 µM of each primer. DNA amplification was performed in a Mastercycler (Eppendorf AG, Hamburg, Germany) programmed for 5 min at 95°C for initial activation and 35 cycles, each consisting of 30 s at 94°C, 1 min at annealing temperature (60°C for LMWP1/LMWP2, 56°C for UP3B/UP1A, and 58°C for Pm3MF/Pm3ER1), and 1 min at 72°C, followed by a final extension of 10 min at 72°C.

After amplification, PCR products were separated in either 2% agarose gels for primer pairs UP3B/UP1A and Pm3MF/Pm3ER1, or 3% agarose gels for primer pair LMWP1/LMWP2. For LMWP1/LMWP2, the gel was run longer in order to obtain a clear resolution of the two bands.

Results

Phenotypic descriptions of the parental and related materials

Cytological, molecular and powdery mildew response data for wheat genotypes used in the present work are

summarized in Table 1. The Bobwhite and Veery selections initially chosen on the basis of their contrasting powdery mildew responses make up the first group. Powdery mildew susceptible Bobwhite Red and Veery#5 produced amplification products with all three primer pairs, whereas the resistant lines Bobwhite White and Veery#6 did not.

The second group included wheat lines for which information on powdery mildew response had accumulated over a number of years (Ren et al. 1996, 1997; McIntosh, unpublished). Sarhad 82 is a selection of Bobwhite released in Pakistan in 1982. Fed*4/Kavkaz is a backcross derivative selected for powdery mildew resistance, and/or the linked leaf rust resistance, with Kavkaz as a resistant donor. Kohinoor 83 (a Pakistani variety selected from the CIMMYT line Teeter) is one of relatively few CIMMYT 1BL·1RS wheat selections in which *Pm8* is expressed (Ren et al. 1996). Thatcher + Lr26, RL6078, which had the 1BL.1RS translocation due to prior selection for *Lr26*, was included because of its isogenicity with Thatcher. Comparing these lines with the first group, it was evident that the presence of all three amplification products (LMWP1/LMWP2, UP3B/UP1A, and Pm3MF/Pm3ER1) was closely associated with suppressed powdery mildew resistance. Aurora, a resistant line, amplified only with the *Pm3* promoter region primer, but the product differed in size from those of the genotypes in which resistance was suppressed. If amplification with only the *Glu-A3* primer pair is taken as a predictor of suppression the only exception is Disponent. If presence of both *Pm3* primer amplifications is the indicator of suppression, the exceptions are Skorospelka 35 and Thatcher + Lr26. Skorospelka 35 is related to Kavkaz through their common parent Bezostaya 1. Both carry the *Pm3_Kavkaz* allele that has a truncation at the 3′ end (Yahiaoui et al. 2006), presumably preventing translation of the suppressor of *Pm8*. We assume that Skorospelka 35 has the same allele. Yahiaoui et al. (2006) reported that Chinese Spring and Thatcher possessed identical *Pm3* DNA sequences, and therefore like Chinese Spring Thatcher should be suppressive. The two Thatcher genotypes used in the current study behaved identically with the three primer pairs. They were the original University of Sydney accession (W1201 = AUS3444, introduced from the University of Minnesota in 1936), and the Thatcher + Lr26 NIL (near-isogenic line), derived from the Thatcher accession used by Dr P. L. Dyck (Agriculture and Agri-Food Canada, Winnipeg, Canada) in generating NILs. It is predicted that these sources of Thatcher are unlikely to carry *Pm3CS*, although they are *Pm8*-suppressive. The Thatcher source used by Yahiaoui et al. (2006) was given as 'FAL Reckenholz'.

The next group of lines in Table 1, starting with cv. Aroona, lacked the 1BL·1RS translocation. Chinese Spring which produces a full length transcript (*Pm3CS*, a 'susceptible' allele) that cannot be recognized with any known *Bgt* culture (Yahiaoui et al. 2006), amplified all three primer pairs. Federation produced only a variant product (of different fragment size) with the promoter region primers, consistent with a non-suppressing background. Thatcher, the parent of Thatcher + Lr26, was amplified only by the *Glu-A3* primer pair.

Based on the amplification patterns the presence (S) or absence (R) of suppression in a range of wheat genotypes was predicted firstly on the basis of the result with the LMWP primer pair and secondly on the basis of the combined results with the two *Pm3* primer pairs. The actual mildew responses of *Pm8* NILs of 14 of the genotypes were also available. In all cases the powdery mildew responses of these lines were consistent with both sets of predictions. Among lines for which no NIL was available there were several exceptions. In addition to Thatcher, four (Dagger, Lance, Lark and Tatiara) were predicted to be non-suppressive based on the LMWP primer pair, but suppressive with the *Pm3* primer pairs, and Halberd was predicted to be suppressive based on the LMWP primer pair, but non-suppressive based on *Pm3*.

Genetics of resistance

Inheritance of Pm8

F_2 seedlings of Federation//Fed*4/Kavkaz, inoculated with *Bgt* culture NAR, segregated 110 resistant [infection type (IT) 0–1]:41 susceptible (IT 3 + 4), indicating segregation of a single dominant gene (*Pm8*) for resistance ($\chi^2 = 0.37$; $P > 0.5$). Co-infection with *P. triticina* pathotype 162-1,2, which is avirulent to the dominant *Lr26*, confirmed the presence of at least one 1BL·1RS translocation chromosome in resistant plants (Fig. 1).

Inheritance of the Pm8 suppressor

Segregation data for 817 RILs derived from the Veery and Bobwhite crosses are shown in Table 2. The pooled results for the four populations were initially thought to be genetically heterogeneous. One of the Bobwhite populations deviated significantly from the predicted ratio of 7 homozygous resistant:2 segregating:7 homozygous susceptible, and the ratio of homozygous resistant:homozygous susceptible lines deviated from an expected 1:1 ratio ($\chi^2 = 9.58$; $P < 0.01$). However, a contingency table chi squared analysis of the pooled data indicated no heterogeneity in the data, but rather a deficiency of homozygous resistant lines across the first three populations.

To obtain data on single plant segregation for the suppressor, 50 segregating F_5 lines, each with 7–32 seedlings,

Table 2 Frequencies of F_4 RILs from reciprocal crosses of Bobwhite and Veery selections classified for response to *Bgt* culture 'NAR'

No. of RILs	Bobwhite White/Bobwhite Red		Veery#6/Veery#5		Pooled
	Cross 1	Cross 2	Cross 3	Cross 4	
Homozygous res.	64	92	83	82	321
Segregating	23	17	32	25	97
Homozygous sus.	82	128	110	79	399
Total	169	237	225	186	817
χ^2 (7:2:7)	2.38	12.40**	4.31	0.21	8.80*

$\chi^2_{\text{heterogeneity}} = 10.50^*$, 4 df
* $P < 0.05$, ** $P < 0.01$

but generally 19–32, were tested with *Bgt* culture NAR. The pooled segregation ratio of 335 resistant (IT 0–1+2):904 susceptible (IT 4) indicated a single gene for resistance ($\chi^2_{1:3} = 2.74$; $P_{1df} > 0.05$, $\chi^2_{het} = 28.03$; $P_{48df} > 0.99$) or, because all plants possessed the 1BL·1RS chromosome and therefore *Pm8*, a dominant suppressor of *Pm8*, designated *SuPm8*. In this series of tests, there was no consistent deficiency of resistant individuals.

Molecular analysis of the RILs

Primer pair LMWP1/LMWP2 amplified a sequence of *Glu-A3* (Devos et al. 1995) associated with the mildew suppressor identified in Ren et al. (1996). In total, 264 lines from the two Bobwhite populations were tested using this primer pair. A perfect match was found between the absence of the band (lower fragment) and non-suppression of *Pm8* when lines were homozygous resistant (Fig. 3a). This dominant marker was amplified in lines that were segregating or homozygous susceptible (Fig. 3a).

Primer pair UP3B/UP1A amplified a segment in the *Pm3* promoter region in plants predicted to produce a full length *Pm3* transcript (Tommasini et al. 2006). The band was absent in the resistant parents and all homozygous resistant lines, but was present in the susceptible parents and all homozygous susceptible and segregating lines, again due to the dominant nature of the marker (Fig. 3b).

Because the primer pair UP3B/UP1A is sufficient to correlate the *Pm3* band with the mildew response phenotype, Pm3MF/Pm3ER1 was not run on the lines in the populations. Instead, only cultivars and genetic stocks were screened using this primer pair (Fig. 4; Table 1). Primer pair Pm3MF/Pm3ER1 amplifies a sequence in the 3′ region of *Pm3* (Tommasini et al. 2006).

Powdery mildew tests at USDA-ARS

All 21 lines tested by USDA-ARS were homozygous for *Pm8*. Both powdery mildew tests at PBI Cobbitty using the local 'Resident' culture and molecular marker tests using the primer pairs UP3B/UP1A showed that nine lines were homozygous *Pm3aPm3a*, six were heterozygous *Pm3apm3a*, and six were homozygous *pm3apm3a*.

Bgt culture AN2-4, used for tests at Raleigh, was virulent on genotypes with *Pm3a* and avirulent on those with *Pm8*. Tests using culture AN2-4 showed that the nine homozygous *Pm3aPm3a* lines were susceptible; the six lines heterozygous for *Pm3a* had segregating responses, and the six lines homozygous for *pm3* were resistant due to expression of *Pm8*. This clearly indicated that *Pm8* did not

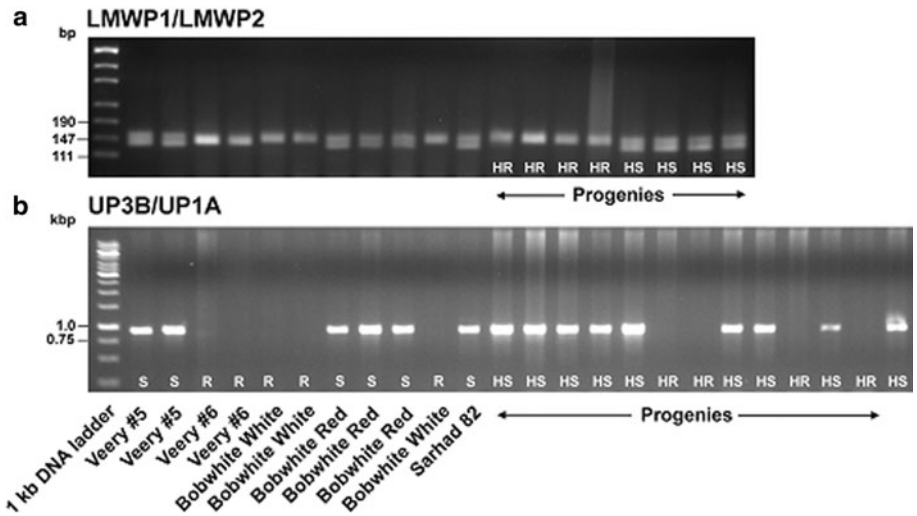

Fig. 3 Amplification of two PCR fragments in wheat RIL parents, Sarhad 82 (=Bobwhite 'S'), and segregating lines. Homozygous powdery mildew responses are indicated by HR and HS. The RILs in each panel are not the same. **a** Primer pair LMWP1/LMWP2, a dominant marker; HR lines have a single band whereas HS lines have a double band. **b** Primer pair UP3B/UP1A, a dominant marker; the band is absent in HR lines, but is present in the HS lines

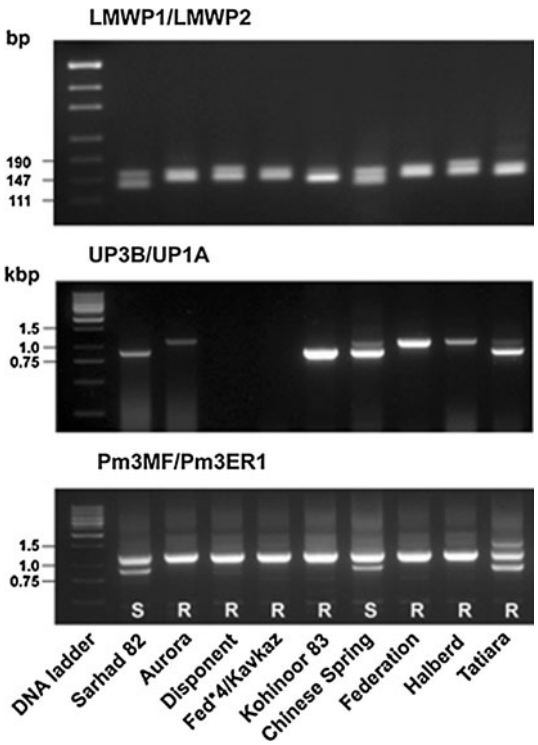

Fig. 4 Amplification with all three primer pairs of five lines with, and four lines without, 1BL·1RS. Powdery mildew responses in the last four genotypes were predicted based on the presence of all three amplification products

confer resistance in the presence of an ineffective *Pm3a* allele. The method of testing and responses of two lines (327, genotype *Pm3aPm3a*; and 1202, genotype *Pm3apm3a*) are shown in Fig. 2.

Discussion

The possibility of genetic suppression of phenotypic effects in wide crosses of wheat has been a recurrent topic for many years, but there is no plausible explanation of how suppression actually occurs, assuming the presumed genes are present. Initially, we had only the knowledge that suppression of *Pm8* was closely linked with a gliadin locus on chromosome 1A. The *Gli-A1* gene is a very closely linked prolamin gene cluster family and these in turn are closely linked to the *Glu-A3* locus, another cluster of repeats linked but not as closely as the *Gli-A1* group. These protein loci were the obvious initial targets of our fine mapping work. Real progress in the analysis came with the cloning of *Pm3* alleles and the availability of markers based on the *Pm3* sequences and their upstream region (Yahiaoui et al. 2004; Tommasini et al. 2006). It is now known that *Pm3* is indeed very closely linked with the *Glu-A3* locus and is likely embedded among various repeats of this gene (Wicker et al. 2003; Dong et al. 2010). This would be highly consistent with the results of Ren et al. (1996, 1997) who showed very close relationships between the suppressor and specific alleles of both *Gli-A1* and *Glu-A3*.

It has also been a common occurrence that disease resistance genes, when moved to hexaploid wheat from diploid or tetraploid wheat or other related species, become less effective in the hexaploid background. Well known examples include *Sr21* for resistance to *Pgt*, originally present in diploid *T. monococcum* (The and Baker 1975), and *Lr23* for resistance to *P. triticina*, originally present in tetraploid *T. turgidum*. Both McIntosh and Dyck (1975) and Nelson et al. (1997) provided evidence of specific partial suppression of the resistance conferred by *Lr23*. In that case, there was evidence that suppression was conferred by a region of chromosome 2D possibly homoeologous with the location of *Lr23* in chromosome 2BS. Moreover, specificity of suppression was likely a factor, as the same population of F_3 lines was scored 1:2:1 in Australia using an Australian *P. triticina* pathotype and 1:8:7 in Canada using a Canadian pathotype (McIntosh and Dyck 1975). The data of Kema et al. (1995) for wheat stripe rust were also suggestive of specificity of suppression. Examples of complete suppression are less clearly defined because presence of the genes in the original gametic source may be uncertain. Observations of suppression are usually retrospective and in some instances the parents may not be clearly defined or may not be available. In other cases, putative parental sources, such as Petkus rye in the case of *Pm8*, may carry additional genes for resistance that prevent phenotypic identification of the gene of interest.

The discovery of suppression of the rye-derived *Pm8* in wheat offered an opportunity to investigate a well defined example of suppression, but effective tools for its molecular analysis were limited until the cloning of *Pm3*. We provide evidence that *Pm8* was phenotypically expressed in wheat only when an allele at the *Pm3* locus was not translated. To validate the phenomenon we constructed a *Pm8Pm3a* genetic stock. To test the latter it was necessary to locate a *Bgt* isolate that was virulent for *Pm3a* and avirulent for *Pm8*. Such a genotype was available in southeastern USA, where the gene *Pm3a* had been utilized in resistant cultivars and where the corresponding virulence gene was common as a consequence (Parks et al. 2008). It was interesting that no published literature had reported combinations of an effective *Pm3* allele and *Pm8* despite very common worldwide occurrence of wheats with the 1BL·1RS translocation. However, the use of *Pm3* alleles in wheat breeding internationally has been sporadic, presumably due to virulence polymorphisms in the pathogen population and lack of selection by breeders, as well as the fact that although sometimes described as a significant disease, powdery mildew resistance is seldom given high

priority in wheat breeding. Of course until *Pm3* was cloned, combinations of *Pm8* with genotypes, such as Chinese Spring having translated but not identified *Pm3* alleles could not be identified. Separate studies are underway to demonstrate the same effects by transient expression. The results of that work will be reported elsewhere.

Obviously, our finding that *Pm8* is suppressed by translated alleles at the *Pm3* locus has implications for resistance breeding strategies that employ introgressed alleles from wild relatives. It suggests the utility of determining homology between alien and other native resistance genes in the wheat genotype being used as the recipient parent. We have yet to confirm that all translated *Pm3* alleles confer similar suppression. Obviously more work is needed to elucidate the molecular mechanism of suppression.

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