Theor Appl Genet (2012) 124:911–922
DOI 10.1007/s00122-011-1756-8

ORIGINAL PAPER

Identification and characterization of a novel powdery mildew resistance gene *PmG3M* derived from wild emmer wheat, *Triticum dicoccoides*

Weilong Xie · Roi Ben-David · Bin Zeng · Assaf Distelfeld · Marion S. Röder · Amos Dinoor · Tzion Fahima

Received: 12 July 2011 / Accepted: 5 November 2011 / Published online: 8 December 2011

Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*) is one of the most important wheat diseases worldwide. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides,* the tetraploid ancestor (AABB) of domesticated bread and durum wheat, harbors many important alleles for resistance to various diseases, including powdery mildew. In the current study, two tetraploid wheat mapping populations, derived from a cross between durum wheat (cv. Langdon) and wild emmer wheat (accession G-305-3M), were used to identify and map a novel powdery mildew resistance gene. Wild emmer accession G-305-3M was resistant to all 47 *Bgt* isolates tested, from Israel and Switzerland. Segregation ratios of F_2 progenies and F_6 recombinant inbred line (RIL) mapping populations, in their reactions to inoculation with *Bgt*, revealed a Mendelian pattern (3:1 and 1:1, respectively), indicating the role of a single dominant gene derived from *T. dicoccoides* accession G-305-3M. This gene, temporarily designated *PmG3M*, was mapped on chromosome 6BL and physically assigned to chromosome deletion bin 6BL-0.70-1.00. The F_2 mapping population was used to construct a genetic map of the *PmG3M* gene region consisted of six simple sequence repeats (SSR), 11 resistance gene analog (RGA), and two target region amplification polymorphism (TRAP) markers. A second map, constructed based on the F_6 RIL population, using a set of skeleton SSR markers, confirmed the order of loci and distances obtained for the F_2 population. The discovery and mapping of this novel powdery mildew resistance gene emphasize the importance of the wild emmer wheat gene pool as a source for crop improvement.

Communicated by B. Friebe.

First authorship shared by W. Xie and R. Ben-David.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1756-8) contains supplementary material, which is available to authorized users.

W. Xie · R. Ben-David · B. Zeng · T. Fahima (✉)
Department of Evolutionary and Environmental Biology, Institute of Evolution, Faculty of Natural Sciences, University of Haifa, Mt. Carmel, 31905 Haifa, Israel
e-mail: fahima@research.haifa.ac.il

A. Distelfeld
Molecular Biology and Ecology of Plants, The Institute for Cereal Crops Improvement, Tel-Aviv University, 69978 Tel-Aviv, Israel

M. S. Röder
Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), 06466 Gatersleben, Germany

A. Dinoor
Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, 76100 Rehovot, Israel

Introduction

Powdery mildew is a destructive wheat disease caused by a biotrophic parasitic fungus, *Blumeria graminis* f. sp. *tritici* (*Bgt*). *Bgt* is responsible for causing one of the most important wheat diseases worldwide. This pathogen has caused severe yield losses ranging from 5 to 34% (Lipps and Madden 1988). One of the most effective and environmentally safe approaches to control powdery mildew is to develop and use resistant cultivars. To date more than 60 powdery mildew resistance alleles (*Pm1–Pm43*) have been reported in wheat (McIntosh et al. 2008; Hsam and Zeller 2002). However, major resistance genes tend to become ineffective within a short period of time due to the increasing variability of the pathogen (Hsam and

Zeller 2002). Therefore, it is necessary to search for novel disease resistance genes and combine multiple resistance genes into each cultivar, in wheat breeding programs, in order to be able to protect wheat yields and secure the world's food supply. Future wheat genetic improvement lies in exploiting the gene pools of the wild relatives of wheat.

Wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell., also known as *T. dicoccoides*] is a valuable source for resistance to powdery mildew (Nevo et al. 2002). Moseman et al. (1984) tested the reactions of 233 *T. dicoccoides* accessions, collected at 10 sites in Israel and elsewhere, with four isolates of powdery mildew. They found that 114 of 233 (49%) accessions were resistant to these four isolates that possess virulence genes overcoming most of the identified *Pm* resistance genes in cultivated wheat.

The development of molecular markers presents a useful tool for both theoretical genetic analysis and plant breeding practices. Molecular markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), simple sequence repeats (SSR), target region amplification polymorphism (TRAP) and resistance gene analogs (RGA) have been widely used to tag and map agronomically important traits in wheat (Hu and Vick 2003; Feuillet and Keller 2004; van der Linden et al. 2004).

The wild emmer wheat accession G-305-3M was previously reported as the donor of *Pm31,* however, further examination conducted by Xie et al. (2011) revealed that there was an error in the pedigree registration of the *Pm31* mapping population and that the donor of *Pm31* is the 6VS/6AL translocation introgressed from *Hynaldia villosa* (Chen et al. 1995), and not the *T. dicoccoides* accession G-305-3M. Therefore, it was concluded that *Pm31* is not a wild emmer wheat-derived *Pm* gene, as was previously reported, instead, it is actually the *Pm21* locus with the resistance allele originating from the translocated 6VS chromosome of *H. villosa* (Xie et al. 2011). In the current study we show that *T. dicoccoides* accession G-305-3M harbors a novel powdery mildew resistance gene, temporarily designated as *PmG3M*, located on chromosome 6BL.

The overall objective of the current study was to explore the genetic basis of powdery mildew resistance in wild emmer wheat as a source for improving cultivated wheat. In this paper we report on: (1) the development of a new mapping population in tetraploid wheat background by crossing wild emmer accession G-305-3M with *T. durum* cultivar Langdon (LDN); (2) the identification and characterization of a novel powdery mildew resistance gene originating from wild emmer wheat; (3) the identification of PCR-based molecular markers linked to this powdery mildew resistance gene; (4) the construction of genetic and physical maps of the gene region using F_2 and recombinant inbred line (RIL) mapping populations of the same cross.

Materials and methods

Plant materials

Wild emmer wheat accession G-305-3M, resistant to powdery mildew, was crossed with a highly susceptible *T. durum* cultivar Langdon (hereafter LDN; Heyne 1959), to produce a segregating mapping population. Langdon was broadly studied as a tetraploid wheat cultivar and was used extensively for wheat genetic research (e.g., Joppa and Williams 1988). The wild emmer accession G-305-3M was collected in Israel by Gerechter-Amitai et al. (1992). An F_2 mapping population consisting of 164 individuals was developed from a single F_1 plant. One hundred and fifty-one F_2-derived F_3 families were used for the construction of the F_2-based genetic map. Single seed decent (SSD) procedure was implemented to produce 164 F_6 homozygous RILs.

Powdery mildew tests

The parental lines (G-305-3M and LDN) were inoculated with 47 different *Bgt* isolates from Israel and Switzerland (Table 1) and screened for powdery mildew resistance, using detached leaf segments as described by Xie (2006) and by Ben-David et al. (2010). Conditions of inoculation, incubation and disease assessment were as described by Hsam and Zeller (1997). Plant reactions to powdery mildew inoculation was recorded on a scale of 0 to 4, with 0 representing no visible symptoms, 0; for necrotic flecks (hypersensitive response, HR), and values of 1, 2, 3 and 4 for highly resistant, resistant, susceptible and highly susceptible reactions, respectively (Mains and Dietz 1930). A score of 0–2 was recorded as resistant and 3–4 as susceptible. Based on the test results with 42 Israeli *Bgt* isolates, the isolate *Bgt*#15 was selected for further phenotyping of the segregating F_2 population and 151 F_2-derived F_3 families, as well as the 164 RILs using the detached leaf segments' method. Isolate *Bgt*#15, to which G-305-3M was highly resistant with necrotic flecks (0;) and LDN was highly susceptible, is also highly virulent on many other *Pm* genes (e.g., *Pm1a*, *Pm3a*, *Pm3b*, *Pm4b*, *Pm5a*, *Pm5b*, *Pm6*, and *Pm7*; Ben-David et al. 2010).

Genetic stocks

A set of Langdon/Chinese Spring D-genome disomic substitution lines (LDN-DS), kindly provided by Joppa and Williams (1988), and a Langdon/*T. dicoccoides* substitution line LDN (DIC-6B), in which a pair of chromosomes 6B in LDN was substituted by a pair of chromosomes 6B from *T. dicoccoides* accession FA-15-3, and 12 homozygous recombinant substitution lines (RSLs) (Olmos et al.

Table 1 Phenotypic characterization of the two parental lines, Langdon and G-305-3M, for powdery mildew resistance to 47 *Bgt* isolates

Isolate #	Collection site		Wheat species collected from	Wheat cultivar	Plant reaction[a]	
	Country	Site			G-305-3M	Langdon
1	Israel	Hula Valley	*T. aestivum*	Dariel	R[b]	S
4	Israel	Hula Valley	*T. aestivum*	Dganit	R	S
6	Israel	Hula Valley	*T. aestivum*	Atir	R	S
7	Israel	Hula Valley	*T. aestivum*	Shapir	R	S
8	Israel	Hula Valley	*T. durum*	Bareket	R	S
9	Israel	Hula Valley	*T. aestivum*	652	R	S
13	Israel	Hula Valley	*T. aestivum*	519	R	S
15	Israel	Yavor	*T. durum*	Inbar	R	S
16	Israel	Nahal Oz	*T. durum*	Inbar	R	S
20	Israel	Ein Hanatziv	*T. durum*	Inbar	R	S
25	Israel	Bet Dagan	*T. durum*	Inbar	R	R
29	Israel	Ein Hanatziv	*T. aestivum*	Shapir	R	S
30	Israel	Talmei Yafe	*T. aestivum*	Shapir	R	S
33	Israel	Erez	*T. aestivum*	Dganit	R	S
36	Israel	Lahav	*T. aestivum*	Dganit	R	S
37	Israel	Nahal Oz	*T. aestivum*	Dganit	R	S
43	Israel	Yesodot	*T. aestivum*	Bet Hashita	R	S
44	Israel	Negev	*T. aestivum*	Miriam	R	S
47	Israel	Sa'ad	*T. aestivum*	Bet Lehem	R	R
50	Israel	Nahal Oz	*T. aestivum*	Barkai	R	S
52	Israel	Dir El Balakh	–	–	R	S
58	Israel	Ammiad	*T. dicoccoides*	58	R	S
61	Israel	Tabigha	*T. durum*	ZB	R	S
63	Israel	Gilbboa	*T. dicoccoides*	–	R	S
64	Israel	Karei Deshe	*T. dicoccoides*	–	R	S
66	Israel	Ammiad	*T. dicoccoides*	–	R	R
67	Israel	Lahav	*T. durum*	Inbar	R	S
68	Israel	Bet Dagan	*T. aestivum*	Bet Hashita	R	S
70	Israel	Beeri	*T. aestivum*	Dganit	R	S
91	Israel	Sde Eliahu	*T. durum*	Inbar	R	S
92	Israel	Tel Aviv	*T. dicoccoides*	–	R	S
94	Israel	Ein Hanatziv	*T. aestivum*	Dganit	R	S
95	Israel	Ein Hanatziv	*T. durum*	Bareket	R	S
96	Israel	Negba	*T. aestivum*	M50	R	S
97	Israel	Negba	*T. durum*	M	R	S
101	Israel	Nahal Oz	*T. aestivum*	Dariel	R	S
103	Israel	Ammiad	*T. dicoccoides*	–	R	R
106	Israel	Nahal Oz	*T. aestivum*	Atir	R	S
107	Israel	Nahal Oz	*T. aestivum*	Shapir	R	R
108	Israel	Nahal Oz	*T. aestivum*	Bareket	R	S
109	Israel	Tel Aviv	*T. dicoccoides*	–	R	S
113	Israel	Ammiad	*T. dicoccoides*	–	R	S
96229	Switzerland	Ellighausen-Kloten Rd.	*T. aestivum*[b]	–	R	S
96224	Switzerland	Winterthur-Kloten Rd.	*T. aestivum*[b]	–	R	S
96236	Switzerland	Oensingen-Schoetz Rd.	*T. aestivum*[b]	–	R	S
96244	Switzerland	Coppet -Yverdon Rd.	*T. aestivum*[b]	–	R	S
96275	Switzerland	Nyon-Cheseaux Rd.	*T. aestivum*[b]	–	R	S

[a] Plant reaction: *R* resistant (IT = 0–2), *S* susceptible (IT = 3–4)

[b] Approximation, i.e., the wheat species from which the powdery mildew was collected, is uncertain. In order to obtain a set of Swiss representative random mildew samples, a spore trap was mounted on the roof of a car which was driven through the main wheat growing areas of Switzerland. Those areas are dominated by bread wheat cultivars

2003; Distelfeld et al. 2004) were used for chromosome assignment of molecular markers linked to the resistance gene. Two ditelosomics (DT6BL and DT6BS) and seven deletion lines of chromosome 6B (Endo and Gill 1996) were used for physical mapping of molecular markers linked to the resistance gene.

Genomic DNA extraction and BSA analysis

When plants were about 1-month-old, leaves were collected, frozen in liquid nitrogen, and stored in a −80°C freezer. F_2 Genomic DNA was extracted using the plant genomic DNA isolation reagent DNAzol^{TM}ES (Molecular Research Center, Inc., Cincinnati, OH, USA) as described by Peng et al. (1999). Bulked segregant analysis (BSA) (Michelmore et al. 1991) was used to screen for polymorphic markers between resistant and susceptible DNA bulks (composed of F_2 DNA samples, based on the phenotypic test of $F_{2:3}$ families). DNA samples from 10 to 15 homozygous resistant (with plant reaction = 0) and 10 to 15 homozygous susceptible (with plant reaction = 4) F_2 individuals were pooled in equal amounts to generate resistant bulk (R bulk) and susceptible bulk (S bulk), respectively. Genomic DNA samples of F_6 RILs and parental lines were extracted using a large-scale genomic DNA extraction protocol (Kidwell and Osborn 1992; Elbaum et al. 2006) with some modifications, as described by Ben-David (2011).

SSR analysis

Based on the distribution of SSR markers on the A and B genomes of wheat (Röder et al. 1998; Ganal and Röder 2007), 225 SSR markers from A and B genomes (12–20 markers per chromosome) were chosen for testing polymorphism between the two parents and the two (R and S) DNA bulks.

One primer of each primer pair was labeled with fluorescent dye, Cy-5. The PCR reactions were performed as described in Peng et al. (1999). Fragment analysis was carried out in an automated laser fluorescence (ALF) DNA sequencer (Amersham Biosciences, Uppsala, Sweden). Fragment sizes were calculated using the computer program Fragment Manager Version 1.02 (Amersham Biosciences, Uppsala, Sweden). Genotyping of the SSR markers was also preformed on 1–3% SeaKem® or MetaPhor® Agarose (Cambrex Bio Science, Rockland, ME, USA) stained with GelRed^{TM} (Biotium, Hayward, CA, USA).

RGA and TRAP analysis

RGA primers were designed based on conserved NBS, LRR, and Kinase motifs of cloned disease resistance genes, as described by Xie et al. (2011). Altogether 320 combinations of RGA primer pairs were screened for polymorphism between the two parents and between the two opposite bulks. Data regarding RGA primer sequences is available on the supplementary material section of Xie et al. (2011). For TRAP analysis, the sequences of four arbitrary primers were kindly provided by Dr. J. Hu (Liu et al. 2005, see also supplementary material, Table S1).

At a later stage, after the physical location of the gene was verified, 36 fixed primers were designed based on expressed sequence tags (ESTs) mapped on chromosome deletion bin 6BL 0.40–1.00 (http://wheat.pw.usda.gov/; Dilbirligi et al. 2004). We adapted the concept of Hu and Vick (2003) and designed the fixed primers based on the expressed sequences. We also used 36 degenerate or specific oligonucleotide RGA primers as fixed primers. All primers were designed using the program Primer3 (http:/www-genome.wi.mit.edu/cgi-bin/primer/primer.cgi) and synthesized by Sigma (Sigma-Aldrich, St Louis, MO, USA). The four arbitrary primers were fluorescently labeled with Cy-5.

Genetic mapping

For each segregating marker, a χ^2 analysis was performed to test for deviation from the expected segregation ratio in the F_2 (1:2:1 for co-dominant markers or 3:1 for dominant markers) and F_6 RIL (1:1) populations. Linkage analysis and map construction were performed based on the evolutionary strategy algorithm included in the MultiPoint package (Mester et al. 2003a), as described in Peleg et al. (2008). Dominant markers can cause a bias in the estimation of locus orders and recombination rates (e.g., Peng et al. 2000). The approach which was chosen to deal with this obstacle was to divide the marker data into two subsets, as described by Mester et al. (2003b): (1) one set containing the coupling-phase dominant markers and the co-dominant markers; (2) another set containing the repulsion phase dominant markers and the co-dominant markers.

Results

Reactions of *T. dicoccoides* accession G-305-3M to a collection of powdery mildew isolates from Israel and Switzerland

The reaction patterns of *T. dicoccoides* accession G-305-3M and *T. durum* cv Langdon, inoculated with 47 powdery mildew isolates from Israel and Switzerland are described in Table 1. G-305-3M was highly resistant to 39 powdery mildew isolates with necrotic flecks (0;), or no visible

symptoms (0), and intermediately resistant (IT = 1–2) to eight isolates. The parental line LDN was susceptible (IT = 3–4) to 42 isolates, and highly resistant to five isolates.

Inheritance of the powdery mildew resistance derived from *T. dicoccoides* accession G-305-3M

Triticum dicoccoides accession G-305-3M was highly resistant, with small round necrotic flecks (IT = 0;) to the powdery mildew isolate *Bgt#15*, whereas LDN was highly susceptible to this isolate, with fully developed symptoms and sporulation (IT = 4). The phenotypic test of the F_2 population, consisting of 160 individuals, with isolate *Bgt#15* showed the following segregation ratios: 123 resistant (IT = 0–2) and 37 susceptible (IT = 3–4), fitting the theoretical ratio of 3:1 ($\chi^2 = 0.36$; $\chi^2_{0.05,\ 1} = 3.84$). The test of 151 $F_{2:3}$ families showed a segregation ratio of 30 homozygous resistant, 84 heterozygous resistant, and 37 homozygous susceptible, fitting the theoretical ratio of 1:2:1 ($\chi^2 = 2.6$, lower than $\chi^2_{0.05,\ 2} = 5.99$). Based on these results, it can be inferred that the powdery mildew resistance of *T. dicoccoides* accession G-305-3M is controlled by a single dominant gene. Therefore, this powdery mildew resistance gene was temporarily designated as *PmG3M,* in accordance with the rules of gene symbolization of wheat (McIntosh et al. 2008). A segregation of a single dominant gene was confirmed also at the F_6 generation (e.g. Fig. 1). The segregation ratio obtained for the 164 RIL population (89 R: 75 S) was not significantly different from the 1:1 ratio ($\chi^2 = 3.15$; $\chi^2_{0.05,\ 2} = 3.8$) expected for the segregation of a single dominant gene (Table 2).

Screening of polymorphic SSR markers

Among the 225 SSR markers, 172 (76%) generated polymorphisms between the parents, G-305-3M and LDN. Differences in band densities were observed between the R and S bulks for the markers *Xgwm1093* and *Xgwm192* that were previously mapped on the short arm of chromosome 4A (Röder et al. 1998; Ganal and Röder 2007). However, genotyping of the mapping population showed that there was no genetic linkage between these two markers and *PmG3M* gene. This gene is thus not located on chromosome 4A. Since the screening of the DNA bulks with 225 SSR markers did not identify markers that are linked to *PmG3M*, we conducted another screening using at first 60 RGA primer combinations which allowed the screening of several thousands of amplified RGA bands.

Identification of a RGA marker linked to *PmG3M*

A first set of 60 RGA primer combination pairs were used to screen for polymorphism between the parents and the two DNA bulks. Most of the primer pairs generated 50–100 scorable bands. Most of the bands were almost identical between the two bulks both in size and density. However, four out of 60 primer pairs revealed polymorphism between the two opposite bulks. These primer pairs were used to test 30 individuals of the F_2 mapping population. Only one band from primer pair Pto-kin1IN and CF9-F coincided well with the resistance of the tested individuals and the parents G-305-3M and LDN, and showed association with the resistance gene in the repulsion phase. This marker was further used to genotype the whole mapping population. The use of MultiPoint software showed that

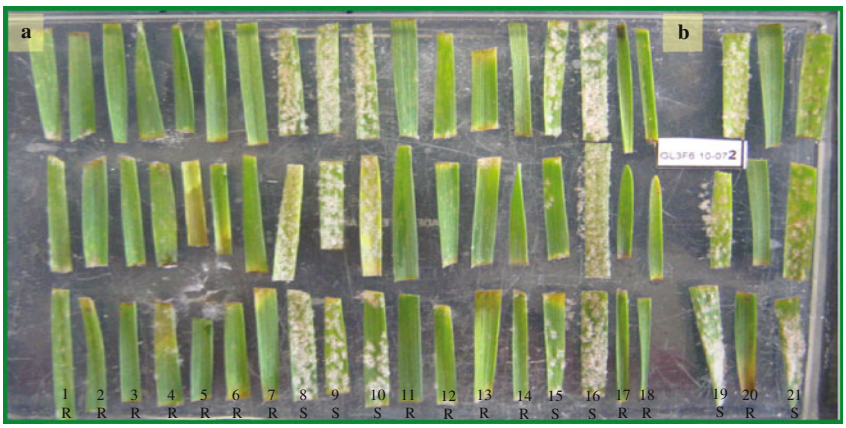

Fig. 1 Phenotypes of the F_6 homozygous RIL mapping population segregating for *PmG3M.* A set of 164 RILs were tested with powdery mildew isolate *Bgt*#15 using detached leaf segments obtained from seedlings. The first leaf of 10–14 days old seedlings was cut into three segments that were arranged in one column and inoculated with *Bgt* spores. **a** An example of 18 segregating F_6 RILs (out of 164) is presented in the figure above. *Each column* of three leaf segments represents one individual RIL (*columns 1–18*); **b** Reference lines are: (*19*) susceptible parent Langdon, (*20*) resistant parent G-305-3M (*21*) susceptible durum cultivar Inbar. The phenotypic plant reaction is marked with *R* for resistant and *S* for susceptible plant at the *bottom* of each column

Table 2 Segregation ratio in F_{2-3} and RIL mapping populations of the cross between *T. durum* cv. Langdon and wild emmer wheat G-305-3M in response to inoculation with isolate *Bgt*#15

Population type	Size	Segregation						
		Type	Expected ratio	R	H	S	χ^2	$\chi^2_{(0.05,\ 2)}$
F_{2-3} families	151	Dominant	A:H:B = 1:2:1	30	84	37	2.6	5.99
RIL	164	Dominant	A:B = 1:1	89	–	75	3.1	3.8

this RGA marker (*Xuhw213*) with a fragment size of 319 bp, derived from the susceptible parent LDN, was linked to *PmG3M* in the repulsion phase at a genetic distance of 3.5 cM (Table 3; Fig. 2c).

Assignment of *PmG3M* to chromosome 6B

The RGA marker *Xuhw213*, which was linked in repulsion phase to the *PmG3M* gene, was used to test a set of LDN/Chinese Spring D-genome disomic substitution (DS) lines (Joppa and Williams 1988). The 319-bp band amplified by *Xuhw213* was present in all LDN-CS-DS lines, except for LDN-CS-6D (6B) substitution line (Supplementary material: Fig. S2), suggesting that *Xuhw213* and linked-*PmG3M* were located on chromosome 6B. The LDN-derived marker *Xuhw213* was also absent in LDN (DIC-6B). A more accurate localization of *Xuhw213* on chromosome 6B was obtained using a set of 12 RSLs, which contained segments of chromosome 6B from *T. dicoccoides* accession FA-15-3 segregating in the background of LDN (Olmos et al. 2003; Distelfeld et al. 2004). The graphical genotypes of these RSLs with *Xuhw213* and eight other DNA markers (Du and Hart 1998; Distelfeld et al. 2004) are presented in Table 4. The obtained graphical genotypes show that *Xuhw213* is

Table 3 Molecular markers used for the mapping of the powdery mildew resistance gene *PmG3M* located on chromosome 6BL

Locus[a]	Marker type	Primer pair[b]	Segregation		Fragment size (bp) in[c]	
			Type	χ^2	G-305-3M	LDN
Xbarc24	SSR	barc24F/barc24R	Co-dominant	5.4	170	194
Xbarc134	SSR	barc134F/barc134R	Dominant	2.1	Null	193
Xgwm193	SSR	gwm193F/gwm193R	Co-dominant	8.9[d]	180	166
Xgwm219	SSR	gwm219F/gwm219R	Co-dominant	1.5	163	155
Xgpw7262	SSR	gpw7262F/gpw7262R	Co-dominant	3.5	195	201
Xedm149	EST–SSR	edm149F/edm149R	Co-dominant	3.7	207	209
Xuhw213	RGA	Pto-kin1IN/CF9-F	Dominant	4.0[d]	Null	319
Xuhw214	RGA	Pto-kin4/XLRR-INV2	Dominant	0.8	740	Null
Xuhw215	RGA	P-loop/SLEY-2	Dominant	0.8	473	Null
Xuhw216	RGA	P-loop/NBS-R1	Dominant	0.2	471	Null
Xuhw217	RGA	P-loop/GLPLTL-1	Dominant	0.8	472	Null
Xuhw218	RGA	P-loop/NIL-AS	Dominant	0	465	Null
Xuhw219	RGA	P-loop/Xa1NBS-R	Dominant	0.1	462	Null
Xuhw220	RGA	Xa1NBS-F/Pto-kin2IN	Dominant	1.7	491	Null
Xuhw221	TRAP	TRAP003/BM137137	Dominant	2.6	256	Null
Xuhw222	TRAP	TRAP013/BF429057	Dominant	0.4	212	Null
Xuhw223	TRAP–RGA	TRAP003/Pto-R	Dominant	0.0	108	Null
Xuhw224	TRAP–RGA	TRAP013/NBS-F1	Dominant	0.4	119	Null
Xuhw225	TRAP–RGA	TRAP003/MLRR-F	Dominant	4.3[d]	Null	378

[a] The RGA and TRAP markers developed in the present study are designated as the *Xuhw* series. The following markers were previously published: *Xgwm* markers (Röder et al. 1998), *Xbarc* markers (Somers et al. 2004), *Xgpw7262* marker (Sourdille et al. 2004b, Graingenes data base, http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/) and the *Xedm149* marker (Mullan et al. 2005)

[b] RGA primer information is presented in Xie et al. (2011). The sequences of four arbitrary primers for TRAP analysis are presented in Table S1

[c] Fragment sizes of all markers were calculated using the computer program Fragment Manager Version 1.02 (Pharmacia) by comparison with the internal or the external size standards

[d] The markers' segregations deviate significantly from the expected segregation ratios (3:1 or 1:2:1)

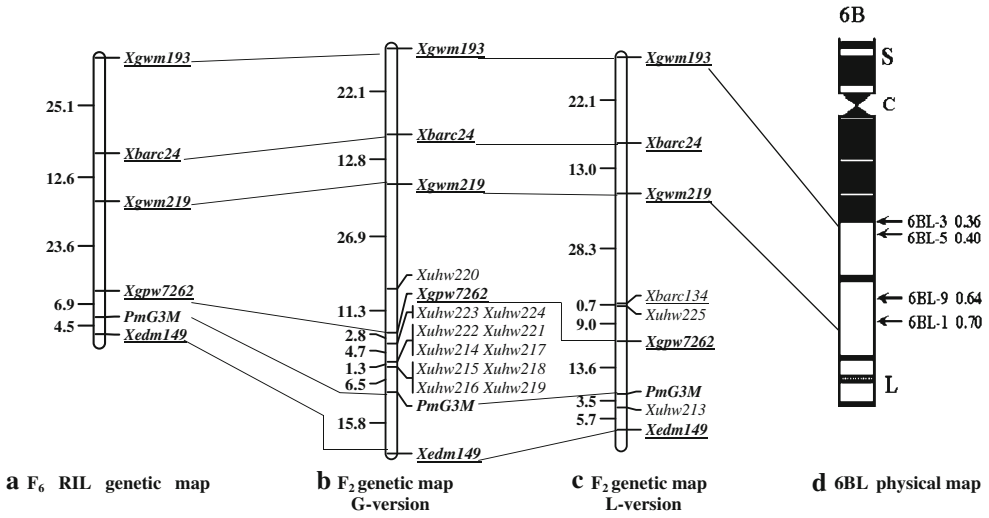

Fig. 2 Genetic maps of chromosome arm 6BL of wheat in two generations of the same cross containing powdery mildew resistance gene *PmG3M* and anchored markers for the physical map of the chromosome arm 6BL. **a**. Genetic map of chromosome 6BL of wheat based on RIL mapping population containing powdery mildew resistance gene *PmG3M*. **b**. The G-version map constructed using co-dominant markers and the dominant markers derived from accession G-305-3M. **c**. The L-version map constructed using co-dominant markers and the LDN-derived dominant markers. **d**. C-banding diagram of chromosome 6B cited from Endo and Gill (1996) and redrawn. *Lines* are connecting between the corresponding markers in the three maps. The co-dominant markers are in *bold*. SSR markers are *underlined*. The RGA and TRAP markers developed in the present study are designated as the *Xuhw* series. Distances between markers are indicated on the *left side* of each map in cM

mapped on the long arm of chromosome 6B, between SSR marker *Xgwm219* and RFLP marker *Xpsr106,* (Table 4).

Identification of RGA and TRAP markers linked to *PmG3M*

Further screening of 260 RGA primer pair combinations for polymorphism between the two parents and the two opposite bulks resulted in the identification of seven additional RGA markers *Xuhw214–220* that showed linkage with *PmG3M* gene (Table 3; Fig. 2).

A total of 288 TRAP (or TRAP–RGA) primer pair combinations were used to screen for polymorphism between the parents and the two bulks. Most of the primer pairs generated 30–100 scorable bands. Most of the bands were almost identical between the two bulks both in size and density. Twenty-four out of 288 primer pair combinations (6.3%) revealed polymorphism between the two opposite bulks. However, if we take into account that each primer pair amplified 60 bands on average, and only one of them was polymorphic, then the real proportion of polymorphic bands is 0.129%. These primer pairs were used to test 30 individuals of the mapping population. Five bands from five primer pairs (0.029% of total 17280 bands amplified by 288 primer pair combinations) coincided well with the resistance of the tested individuals, parents G-305-3M and LDN. These five primer pairs were used to genotype the whole mapping population. Fig. S3 demonstrates the segregation pattern of the TRAP marker *Xuhw221* detected by the ALF express fragment analyzer. All five TRAP markers are dominant, with four of them (*Xuhw221, Xuhw222, Xuhw223*, and *Xuhw224*) linked to *PmG3M* in coupling phase, and one in repulsion phase (*Xuhw225*) at genetic distances ranging from 5 to 14.2 cM (Fig. 2). Out of the five TRAP markers, two (*Xuhw221* and *Xuhw222*) were generated from ESTs (BM137173, BF429057) as fixed primers, and the three others (*Xuhw223–Xuhw225*) were generated from RGA primers as fixed primers (Table 3).

SSR markers linked to *PmG3M* gene

After the *PmG3M* gene was localized on chromosome 6BL, additional SSR markers mapped to chromosome 6B were screened for polymorphism between the two parents and the two opposite bulks. Three SSR markers *Xgwm219*, *Xbarc134* and *Xgpw7262* (Röder et al. 1998; Somers et al. 2004; and Sourdille et al.2004b), Graingenes data base, http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/), and one EST–SSR *Xedm149* (Mullan et al. 2005) showed polymorphism between R and S bulks and were genetically linked to *PmG3M*, therefore confirming the location of *PmG3M* on chromosome 6BL. Two SSR markers, *Xgwm193* (Röder et al. 1998) and *Xbarc24* (Somers et al. 2004), which showed polymorphism only between the parents, were also used to genotype the mapping population.

Table 4 Graphical genotypes of 12 recombinant substitution lines (RSLs) used for mapping of RGA marker *Xuhw213*

RSL	Marker[a]									
	Xpsr167	*Xtam60*	*Xgwm508*	*Xmwg79*	*C*	*Xcdo507*	*Xpsr2*	*Xgwm219*	*Xuhw213*	*Xpsr106*
5	L	L	L	L		D	D	D	D	D
8	L	L	L	L		D	L	L	L	L
11	D	D	D	L		L	L	L	L	L
14	L	L	L	L		L	L	D	D	D
19	D	D	D	L		L	L	L	D	D
36	D	D	L	L		L	L	L	L	D
41	D	D	L	L		L	L	L	L	D
47	L	D	D	D		D	D	D	D	D
50	D	D	D	D		L	L	L	L	L
67	L	D	D	D		D	D	D	D	D
68	L	D	D	D		L	L	L	L	L
77	D	L	L	L		D	D	D	D	D

[a] *D T. dicoccoides* allele, *L* LDN allele and *C* centromere. *Xuhw213* is a RGA marker derived from susceptible LDN and linked to *PmG3M* gene in repulsion phase at a distance of 3.5 cM. The genotypes of other markers were cited from Du and Hart (1998) and Distelfeld et al. (2004). Markers on the left of centromere were mapped on 6BS, and the markers on the right side of centromere were mapped on 6BL (Du and Hart 1998; Distelfeld et al. 2004). Based on the graphical genotypes of all markers, *Xuhw213* was located on the long arm of chromosome 6B, between SSR marker *Xgwm219* and RFLP marker *Xpsr106*

Physical mapping of the molecular markers

Chinese Spring ditelosomics (DT) and seven deletion (del) lines for chromosome 6B were used for physical mapping of some of the molecular markers. The bands of SSR marker *Xbarc134* and RGA marker *Xuhw213* were absent in Chinese Spring, therefore, these two markers could not be physically mapped. The *PmG3M*-linked SSR marker *Xgwm219* was physically mapped in the current study on chromosome deletion bin 6BL-1 0.70–1.00 (Figs. 2d, 3). In addition SSR marker *Xgwm193*, previously mapped on 6BS by Röder et al. (1998), was mapped both in this study and by Sourdille et al. (2004a) on 6BL, in the region of FL 0.00–0.36 (Fig. 2d). Based on the genetic map and the deletion bin-based physical map, we concluded that the powdery mildew resistance gene *PmG3M* derived from *T. dicoccoides* accession G-305-3M is located on chromosome bin 6BL-1 0.70–1.00 (Fig. 2d).

Construction of high-density molecular maps of *PmG3M* region

Two versions of genetic linkage map of the *PmG3M* region involving five SSR, one EST–SSR, eight RGA, and five TRAP markers, as well as the *PmG3M* resistance gene were constructed (Fig. 2b, c). One version of the map, G-version, was constructed using co-dominant markers and the *T. dicoccoides* accession G-305-3M derived dominant markers (Fig. 2b). The second map, L-version map, was based on co-dominant markers and the *T. durum* cv. Langdon derived dominant markers (Fig. 2c). The total lengths of the two versions of the map are 104.2 and 95.9 cM, respectively. The orders of loci on the two map versions constructed in the present study are highly consistent when comparing the order of the shared co-dominant SSR markers (Fig. 2b, c), indicating that these two maps are highly reliable. The linkage between *PmG3M* and its closest marker on chromosome arm 6BL is highly

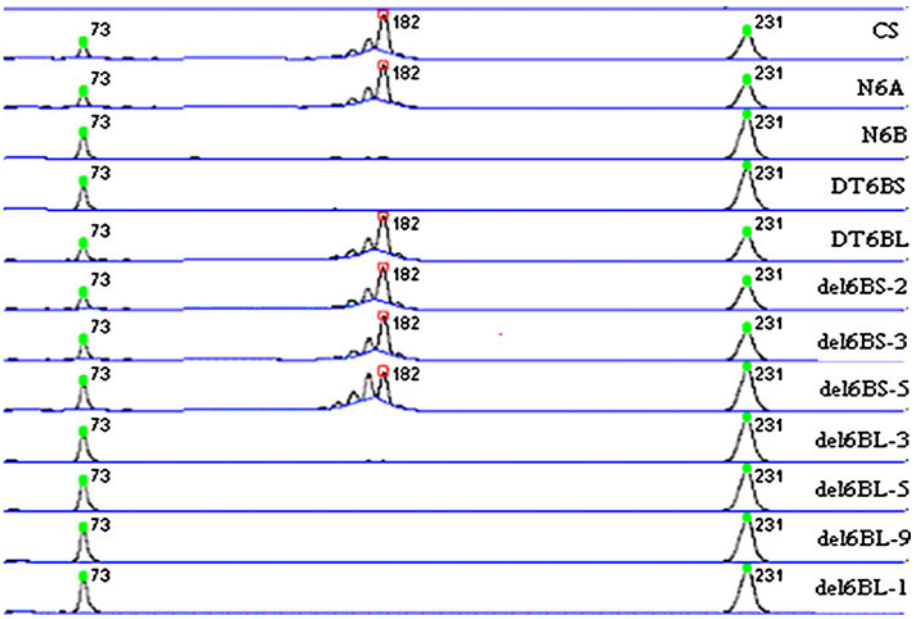

Fig. 3 Amplification of SSR marker *Xgwm219* in Chinese Spring ditelosomics and in seven deletion lines of chromosome 6B. A *curve* is shown from computer program Fragment Manager Version 1.02 (Pharmacia). *CS* Chinese spring, *N6A* nullisomic–tetrasomic line N6A/T6B, *N6B* nullisomic–tetrasomic line N6B/T6B, *DT* ditelosomic line, *del* deletion lines used to assign *Xgwm219* to deletion bin. *Xgwm219* is showing a peak at 182 bp. The two peaks at 73 and 231 bp are internal molecular weight markers added to each of the lanes

significant (LOD value 25.8, $p < 10^{-7}$). Since many G-305-3M derived dominant markers were clustered in a small chromosome region of the G-version map (Fig. 2b), a reliable skeleton map was first constructed with nine interspersed, mainly co-dominant, markers. Using MultiPoint software, the rest of the markers were then added to the most probable intervals on the skeleton map (Fig. 2b). In the G-version map, *PmG3M* was flanked by a cluster of four RGA markers (*Xuhw215, Xuhw216, Xuhw218* and *Xuhw219*) on the proximal side at a distance of about 6.5 cM, and by EST–SSR marker *Xedm149* on the distal side at a distance of 15.8 cM (Fig. 2b). In the L-version map *PmG3M* was flanked by SSR marker *Xgpw7262* and RGA marker *Xuhw213* with genetic distances of 13.6 cM and 3.5 cM from *PmG3M*, respectively (Fig. 2c).

A primary RIL genetic map of *PmG3M* was constructed using four SSR markers (*Xgwm219, Xgwm193, Xbarc24* and *Xgpw7262*) and one EST–SSR (*Xedm149*). Based on marker order and distances along the 6BL chromosome arm the RIL map is in full accordance with the F_2 genetic map (Fig. 2a).

Discussion

PmG3M confers an excellent resistance against powdery mildew

In the current study, Wild emmer wheat accession G-305-3M was resistant to all 47 powdery mildew isolates collected from different wheat species at different locations in Israel and Switzerland. In addition, G-305-3M exhibited full resistance to eight different *Bgt* isolates from five countries located in North America (USA), South America (Chile, Paraguay), Europe (the Netherlands), and Asia (China) (Gerechter-Amitai et al. 1992). The phenotypic response of the F_2 and RIL mapping population to isolate *Bgt*#15 point out a single dominant gene with the resistance conferred by G-305-3M. This resistance gene is temporarily designated as *PmG3M*.

Chromosomal location and genetic mapping of*PmG3M*

Molecular markers, such as RFLPs, RAPDs, AFLPs, SSRs, and RGAs, were widely used to map disease resistance genes in wheat. Among all molecular markers, SSR markers are very popular because of their many advantages over other marker systems (Röder et al. 1998; Fahima et al. 1998, 2002). Several powdery mildew resistance genes have been tagged in wheat (e.g., *Pm24*, *Pm27*, *Pm30*, and *PmG16*) by SSR markers (Huang et al. 2000; Järve et al. 2000; Liu et al. 2002; Ben-David et al. 2010). In the present study, we first used the bulk segregant analysis to screen a large number of SSR markers (225 markers), but failed to identify SSR markers linked to *PmG3M*. A possible reason for this failure is that the gene is located on the distal region of chromosome 6BL, where only a few SSR markers are available. Therefore, we have used the RGA and the TRAP approaches that allowed us to screen the R and S DNA bulks with thousands of amplified DNA fragments in order to identify markers that are linked to *PmG3M*. A RGA marker linked to the target gene was used to assign *PmG3M* to chromosome arm 6BL by testing a set of LDN substitution lines. Finally, *PmG3M* gene was successfully mapped to chromosome 6BL by RGA, TRAP and additional SSR markers (Fig. 2).

By the aid of MultiPoint software (Mester et al. 2003a) two reliable linkage maps of 6BL consisting of seven SSRs, eight RGA and five TRAP markers and the *PmG3M* gene, were constructed (Fig. 2). In both maps *PmG3M*was located in a genetic interval distal to the SSR loci *Xgwm219* and *Xgwm193* (Fig. 2), which were genetically mapped on chromosome 6B by Röder et al. (1998). Based on these two markers it could be concluded that the *PmG3M* gene was located in the distal region of 6BL (FL 0.70–1.00) (Fig. 2d). Additionally, the marker order found in the current study is highly similar to previously published wheat maps (e.g., Somers et al. 2004; Ganal and Röder 2007). The *PmG3M*-linked SSR marker *Xgwm219* was physically mapped in the current study on chromosome deletion bin 6BL-1 0.70–1.00 (Figs. 2d, 3) and was assigned to a smaller chromosome region than previous mapping conducted by Sourdille et al. (2004a), who physically mapped *Xgwm219* onto chromosome bin 6BL-5 0.40–1.00.

The comparative mapping of two mapping populations, F_2 and F_6 RILs, of the same cross produced two genetic maps in which the markers' order and distances are in full agreement (Fig. 2). The primary RIL map could now serve as the main platform for further genetic mapping of the *PmG3M* locus.

The markers *Xuhw213* and *Xgwm193* showed a distorted segregation pattern (Table 3), which has been repeatedly observed in wheat (Blanco et al. 1998; Peng et al. 2000, 2008). The possible causes for segregation deviations of molecular markers are chromosomal rearrangement and gametic or zygotic selection competition manifested in preferential fertilization or from the abortion of a gamete or zygote (Lyttle 1991).

The two co-dominant SSR markers, *Xgpw7262* and *Xedm149*, which flank the *PmG3M* in an interval of about 31.1 and 22.8 cM according to the G-version and the L-version maps, respectively (Fig. 2b, c), could be used as flanking markers for screening recombinants for further high-resolution mapping and positional cloning with a large size of mapping population.

TRAP marker system is efficient to tag resistance genes

The TRAP marker approach was developed by Hu and Vick (2003), and successfully used to characterize wheat genome (Chu et al. 2008). TRAP markers approach is simple, quick and reproducible. The major advantage of this approach is that it uses the available extensive EST information to target the putative candidate gene regions (Hu and Vick 2003), by amplification of open reading frames. In the present study, five TRAP markers based on wheat ESTs were successfully identified. Furthermore, we exemplify the use of fixed primers designed from cloned disease resistance gene sequence, making the use of these markers even more applicable. The fixed primer designed from the cloned resistance gene sequence, can be specific or degenerated (Xie 2006). The TRAP marker system should be useful and efficient for tagging other resistance genes using information of ESTs and cloned resistance genes.

PmG3M is located in a gene-rich region

Deletion line-based high-density maps have revealed that the wheat genome consists of gene-rich and gene-poor regions (Sandhu and Gill 2002). Because wheat has a large polyploid genome (16,000 Mb) that contains more than 80% of repetitive sequences (Dubcovsky and Dvorak 2007; Wicker et al. 2009), the information of whether a gene is located in a gene-rich region and in a high recombination spot, is an essential information for any genomic study. Previous mapping efforts revealed that chromosome regions 6BL 0.70–1.00 and 6BL 0.90–1.00 are among the minor and the major gene-rich regions, respectively (Erayman et al. 2004; Dilbirligi et al. 2004) and contain a high density of ESTs (Qi et al. 2004). In the current study *PmG3M* was physically mapped to chromosome deletion bin 6BL 0.70–1.00. Based on the G-version map, four RGA markers co-segregated with *PmG3M* on the proximal neighboring map interval (Fig. 2b). Since the RGA and TRAP markers were mostly amplified from the coding regions, the cluster revealed in the present maps are in agreement with the R-gene candidate map constructed by Dilbirligi et al. (2004).

The Novelty of *PmG3M*

Three powdery mildew resistance genes were previously mapped on chromosome 6B. *Pm12* is a resistance gene that was transferred from *Aegilops speltoides* to wheat (Jia et al. 1996), and mapped on chromosome 6BS (Qi et al. 2004). *Pm20*, located on rye chromosome 6RL, was translocated to chromosome 6B of common wheat, forming a T6BS.6RL wheat-rye translocation (Friebe et al. 1994). *Pm27*, originated from *T. timopheevii*, was assigned to a 6B–6G translocation in a wheat line background (Järve et al. 2000). This gene co-segregated with the SSR locus *Xpsp3131* that was genetically mapped near the centromere (Bryan et al. 1997; Järve et al. 2000). Furthermore, none of the ten genes originated from wild emmer germplasm were mapped to chromosome 6B [i.e.*Pm16* (Reader and Miller 1991), *Pm26* (Rong et al. 2000), *Pm30* (Liu et al. 2002), *Pm36* (Blanco et al. 2008), *MlZec1* (Mohler et al. 2005),*MlIW72* (Ji et al. 2008), *Pm41* (Li et al. 2009), *Pm42* (Hua et al. 2009), *PmG16* (Ben-David et al. 2010) and *MlAB10* (Maxwell et al. 2010)].

So far, no *Bgt* isolate was clearly virulent on accession G-305-3M of wild emmer wheat. In the current study, a cross between this promising wild emmer wheat accession and domesticated wheat was used to map a novel powdery mildew resistance gene temporarily designated as *PmG3M* to a genetic interval on the distal end of chromosome arm 6BL. Based on its host origin and its genomic location it was concluded that *PmG3M* is a novel wheat powdery mildew resistance gene. This gene is an additional example for the importance of wild germplasm, such as the genetic resources of wild emmer wheat, as a valuable source for novel disease resistance genes for crop improvement.

Acknowledgments This study was supported by The Israel Science Foundation grants #608/03 and #205/08. We also acknowledge The Israel Science Foundation equipment grants #048/99, #1478/04 and #1719/08. The authors wish to thank J. Dubcovsky for providing the 6BS RSLs, and A. Fahoum and O. Shalish for their skillful assistance in the genotyping.

References

Ben-David R (2011) Molecular mapping of powdery mildew resistance genes derived from the *Triticum turgidum* gene pool. Ph.D. thesis, The University of Haifa, Haifa, Israel

Ben-David R, Xie W, Peleg Z, Saranga Y, Dinoor A, Fahima T (2010) Identification and mapping of powdery mildew resistance gene *PmG16*, derived from wild emmer wheat, *Triticum dicoccoides*. Theor Appl Genet 121:499–510

Blanco A, Bellomo MP, Cenci A, De Giovanni C, D'Ovidio R, Iacono E, Laddomada B, Pagnotta MA, Porceddu E, Sciancalepore A, Simeone R, Tanzarella OA (1998) A genetic linkage map of durum wheat. Theor Appl Genet 97:721–728

Blanco A, Gadaleta A, Cenci A, Carluccio AV, Abdelbacki AMM, Simeone R (2008) Molecular mapping of the novel powdery mildew resistance gene *Pm36* introgressed from *Triticum turgidum* var. *dicoccoides* in durum wheat. Theor Appl Genet 117:135–142

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

Chen PD, Qi LL, Zhou B, Zhang SZ, Liu DJ (1995) Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet 91:1125–1128

Chu CG, Xu SS, Friesen TL, Faris JD (2008) Whole genome mapping in a wheat doubled haploid population using SSRs and TRAPs and the identification of QTL for agronomic traits. Mol Breed 22:251–266

Dilbirligi M, Erayman M, Sandhu D, Sidhu D, Gill KS (2004) Identification of wheat chromosomal regions containing expressed resistance genes. Genetics 166:461–481

Distelfeld A, Uauy C, Olmos S, Schlatter AR, Dubcovsky J, Fahima T (2004) Microcolinearity between a 2-cM region encompassing the grain protein content locus Gpc-6B1 on wheat chromosome 6B and a 350-kb region on rice chromosome 2. Funct Integr Genomics 4:59–66

Du C, Hart GE (1998) *Triticum turgidum* L. 6A and 6B recombinant substitution lines: extended linkage maps and characterization of residual background alien genetic variation. Theor Appl Genet 96:645–653

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316:1862–1866

Elbaum R, Melamed-Bessudo C, Boaretto E, Galili E, Lev-Yadun S, Levy AA, Weiner S (2006) Ancient olive DNA in pits: preservation, amplification and sequence analysis. J Archaeol Sci 33:77–88

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger PS, Gill KS (2004) Demarcating the gene-rich regions of the wheat genome. Nucleic Acids Res 32:3546–3565

Fahima T, Roder M, Grama A, Nevo E (1998) Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor Appl Genet 96:187–195

Fahima T, Roder M, Wendehake V, Kirzhner V, Nevo E (2002) Microsatellite polymorphism in natural populations of wild emmer wheat, *Triticum dicoccoides*, in Israel. Theor Appl Genet 104:17–29

Feuillet C, Keller B (2004) Molecular markers for disease resistance: the example wheat. In: Lörz H, Wenzel G (eds) Molecular marker systems in plant breeding and crop improvement (Biotechnology in Agriculture and Forestry), vol 55. Springer, Berlin, pp 353–364

Friebe B, Heun M, Tuleen N, Zeller FJ, Gill BS (1994) Cytologically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci 34:621–625

Ganal MW, Röder MS (2007) Microsatellite and SNP markers in wheat breeding. In: Varshney RK, Tuberosa R (eds) Genomics-assisted crop improvement. Genomics applications in crops, vol 2. Springer, Berlin, pp 1–24

Gerechter-Amitai ZK, Grama A, Kleitman F (1992) Improvement of cultivated wheat by transfer of the high protein potential and resistance to powdery mildew and yellow rust from wild emmer wheat. A final report 1974–1992, submitted to the Netherlands Ministry for Development Cooperation, The Hague, The Netherlands, pp 62

Heyne EG (1959) Registration of improved wheat varieties, XXIII. Agron J 51:689–692

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, Zeller FJ (2002) Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). In: Belanger RR, Bushnell WR, Dik AJ, Carver TLW (eds) The powdery mildews, a comprehensive treatise. APS press, St. Paul, pp 219–238

Hu J, Vick BA (2003) Target region amplification polymorphism: a novel marker technique for plant genotyping. Plant Mol Biol Reptr 21:1–6

Hua W, Liu Z, Zhu J, Xie C, Yang T, Zhou Y, Duan X, Sun Q, Liu Z (2009) Identification and genetic mapping of *Pm42*, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). Theor Appl Genet 119:223–230

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V (2000) Molecular mapping of the wheat powdery mildew resistance gene Pm24 and marker validation for molecular breeding. Theor Appl Genet 101:407–414

Järve K, Peusha HO, Tsymbalova J, Tamm S, Devos KM, Enno TM (2000) Chromosomal location of a *Triticum timopheevii*-derived powdery mildew resistance gene transferred to common wheat. Genome 43:377–381

Ji X, Xie C, Ni Z, Yang T, Nevo E, Fahima T, Liu Z, Sun Q (2008) Identification and genetic mapping of a powdery mildew resistance gene in wild emmer (*Triticum dicoccoides*) accession IW72 from Israel. Euphytica 159:385–390

Jia J, Devos KM, Chao S, Miller TE, Reader SM, Gale MD (1996) RFLP-based maps of the homoelogous group-6 chromosomes of wheat and their application in the tagging of Pm12, a powdery mildew resistance gene transferred from *Aegilops speltoides* to wheat. Theor Appl Genet 92:559–565

Joppa LR, Williams ND (1988) Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat. Genome 30:222–228

Kidwell KK, Osborn TC (1992) Simple plant DNA isolation procedures. In: Beckmann JS, Osborn TC (eds) Plant genomes: methods for genetic and physical mapping. Kluwer Academic Publishers, Dordrecht, pp 1–13

Li G, Fang T, Zhang H, Xie C, Li H, Yang T, Nevo E, Fahima T, Sun Q, Liu Z (2009) Molecular identification of a new powdery mildew resistance gene *Pm41* on chromosome 3BL derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). Theor Appl Genet 119:531–539

Lipps PE, Madden L (1988) Effects of triadimenol seed treatment and tridimefon foliar treatment on powdery mildew epidemics and grain yield of winter cultivars. Plant Dis 72:887–892

Liu ZY, Sun QX, Ni ZF, Nevo E, Yang T (2002) Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer. Euphytica 123:21–29

Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD (2005) A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor Appl Genet 111:782–794

Lyttle TW (1991) Segregation distorters. Annu Rev Genet 25:511–557

Mains E, Dietz S (1930) Physiologic forms of barley mildew, *Erysiphe graminis hordei* Marchal. Phytopathology 20:229–239

Maxwell JJ, Lyerly JH, Srnic G, Parks R, Cowger C, Marshall D, Brown-Guedira G, Murphy JP (2010) MlAB10: a *Triticum turgidum* subsp. *dicoccoides* derived powdery mildew resistance gene identified in common wheat. Crop Sci 50:2261–2267

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers WJ, Morris C, Somers DJ, Appels R, Devos KM (2008) Catalogue of gene symbols for wheat. http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/. Verified 17 March 2010

Mester D, Ronin Y, Minkov D, Nevo E, Korol AB (2003a) Constructing large scale genetic maps using evolutionary strategy algorithm. Genetics 165:2269–2282

Mester D, Ronin, Hu Y, Peng J, Nevo E, Korol AB (2003b) Efficient multipoint mapping: making use of dominant repulsion-phase markers. Theor Appl Genet 107:1102–1112

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by BSA: a rapid method to detect markers in specific genome regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Mohler V, Zeller FJ, Wenzel G, Hsam SLK (2005) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.em Thell). 9. Gene *MIZec1* from *Triticum dicoccoides*-derived wheat line Zecoi-1. Euphytica 142:161–167

Moseman JG, Nevo E, El-Morshidy MA, Zohary D (1984) Resistance of *Triticum dicoccoides* collected in Israel to infection with *Erysiphe graminis tritici*. Euphytica 33:41–47

Mullan DJ, Platteter A, Teakle NL, Appels R, Colmer TD, Anderson JM, Francki MG (2005) EST-derived SSR markers from defined regions of the wheat genome to identify *Lophopyrum elongatum* specific loci. Genome 48:811–822

Nevo E, Korol AB, Beiles A, Fahima T (2002) Evolution of wild emmer and wheat improvement: population genetics, genetic resources, and genome organization of wheat's progenitor, *Triticum dicoccoides*. Springer-Verlag, Berlin, p 364

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, Dubcovsky J (2003) Precise mapping of a locus affecting grain protein content in durum wheat. Theor Appl Genet 107:1243–1251

Peleg Z, Saranga Y, Suprunova T, Ronin Y, Röder MS, Kilian A, Korol AB, Fahima T (2008) High-density genetic map of durum wheat × wild emmer wheat based on SSR and DArT markers. Theor Appl Genet 117:103–115

Peng JH, Fahima T, Röder MS, Li YC, Dahan A, Grama A, Ronin YI, Korol AB, Nevo E (1999) Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B. Theor Appl Genet 98:862–872

Peng JH, Korol AB, Fahima T, Röder MS, Ronin YI, Li YC, Nevo E (2000) Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res 10:1509–1531

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD et al (2004) A chromosome bin map of 16, 000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Reader SM, Miller TE (1991) The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer wheat. Euphytica 53:57–60

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

Rong JK, Millet E, Manisterski J, Feldman M (2000) A new powdery mildew resistance gene: Introgression from wild emmer into common wheat and RFLP based mapping. Euphytica 115:121–126

Sandhu D, Gill KS (2002) Gene-containing regions of wheat and the other grass genomes. Plant Physiol 128:803–811

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

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004a) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Sourdille P, Gandon B, Chiquet V, Nicot N, Somers D, Murigneux A, Bernard M (2004b) Wheat génoplante SSR mapping data release: a new set of markers and comprehensive genetic and physical mapping data (http://wheat.pw.usda.gov/ggpages/ssrclub/geneticphysical)

Van der Linden CG, Wouters DCAE, Mihalka V, Kochieva EZ, Smulders MJM, Vosman B (2004) Efficient targeting of plant disease resistance loci using NBS profiling. Theor Appl Genet 109:384–393

Wicker T, Taudien S, Houben A, Keller B, Graner A, Platzer M, Stein N (2009) A whole-genome snapshot of 454 sequences exposes the composition of the barley genome and provides evidence for parallel evolution of genome size in wheat and barley. Plant J 59:712–722

Xie W (2006) Molecular mapping of powdery mildew resistance genes derived from wild emmer wheat, *Triticum dicoccoides* Koern. Ph.D. thesis, The University of Haifa, Haifa, Israel

Xie W, Ben-David R, Ronin YI, Dinoor A, Xie C, Sun Q, Röder MS, Fahoum A, Fahima T (2011) Suppressed recombination rate on 6VS/6AL translocation region carrying the *Pm21* locus introgressed from *Haynaldia villosa* into hexaploid wheat. Mol Breed. doi: 10.1007/s11032-011-9557-y (in press)