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Molecular mapping of powdery mildew resistance genes in wheat: A review

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Summary

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the most important diseases of common wheat (*Triticum aestivum* L.) worldwide. Molecular mapping and cloning of genes for resistance to powdery mildew in hexaploid wheat will facilitate the study of molecular mechanisms underlying resistance to powdery mildew diseases and help understand the structure and function of powdery mildew resistance genes, and permit marker-assisted selection in breeding programs. So far, 48 genes/alleles for resistance to powdery mildew at 32 loci have been identified and located on 16 different chromosomes, of which 21 resistance genes/alleles have been tagged by restriction fragment length polymorphisms (RFLPs), random-amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), sequence characterized amplified regions (SCARs), sequence-tagged sites (STS) or simple sequence repeats (SSRs). Several quantitative trait loci (QTLs) for adult plant resistance (APR) to powdery mildew have been associated with molecular markers. The detailed information on chromosomal location and molecular mapping of these genes has been reviewed. Isolation of powdery mildew resistance genes and development of valid molecular markers for pyramiding resistance genes in breeding programs is also discussed.

Introduction

Wheat is the most widely cultivated and important food crop in the world. It is the staple crop for about 35% of the human population. Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*), is one of the most devastating diseases of common wheat worldwide and occurs in many areas with cool or maritime climates (Bennett, 1984). This disease has caused severe yield losses ranging from 13 to 34% (Griffey et al., 1993; Leath & Bowen, 1989). Growing resistant cultivars is the most economical and environmentally safe approach to eliminate the use of fungicides and to reduce production losses due to this disease. Up to now, 32 loci (*Pm1–Pm32*) with 48 genes/alleles for resistance to powdery mildew have been identified and located on various chromosomes (Hsam et al., 2003; Liu et al., 2002; Xie et al., 2003; Zeller et al., 2002).

Recently, molecular markers such as restriction fragment length polymorphisms (RFLPs), random-amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), sequence characterized amplified regions (SCARs), sequence-tagged sites (STS) and microsatellites, also termed simple sequence repeats (SSRs), have been widely used to tag and identify powdery mildew resistance genes in wheat by using F_2 and back-cross populations, near-isogenic lines (NILs), doubled haploids (DH), recombinant inbred lines (RILs) or bulked segregant analysis (BSA, Michelmore et al., 1991). An important application of tightly linked or co-segregatin molecular markers to resistance genes is to perform marker-assisted selection (MAS) in wheat breeding programs.

Another application is the employment of these markers to screen for yeast artificial chromosomes (YACs) and bacterial artificial chromosomes (BACs) for performing physical mapping of genes and chromosome walking. Subsequent studies will elucidate the structure and function of candidate genes and allow comparative genetic mapping among the different species of cereals (Figure 1). Huang (1999) and Hsam & Zeller (2002) reported on characterization of specific powdery mildew resistance genes and breeding for powdery mildew resistance in common wheat, respectively. In this review, we will summarize the present status of molecular mapping of powdery mildew resistance genes or quantitative trait loci (QTL) for adult plant resistance (APR). Isolation of powdery mildew resistance genes and marker-assisted selection will be also discussed.

Chromosomal location and distribution of powdery mildew resistance genes

Before a new gene is located to a specific chromosome of wheat, it needs to be identified and characterized according to the gene-for-gene hypothesis. Based on the study of genetics of resistance against rust (*Melampsora lini*) in flax, Flor (1955) proposed the gene-for-gene hypothesis for interactions between host and pathogen, which indicated that for each resistance gene in the host there is a corresponding avirulence gene in the pathogen. Powers & Sando (1960) validated the gene-for-gene hypothesis for the host–pathogen relationship between common wheat and its powdery mildew pathogen. By using a set of powdery mildew isolates that can differentiate lines possessing known resistance genes (Table 1), major resistance genes and gene combinations in cultivars or germplasm accessions may be postulated (Huang, 1999; Huang et al., 1997a; Lutz et al., 1992; Zeller et al., 1993b, 1998).

If a line shows a response pattern different from that of the documented resistance genes after inoculation with a set of defined *Bgt* (*Blumeria graminis* f. sp. *tritici*) isolates, then it carries a new resistance gene or a new allele. To assign the new gene involved in this line to a specific chromosome of wheat, monosomic analysis was most frequently used in conventional genetics. A Chinese Spring (CS) monosomic series was developed by Dr. E.R. Sears (Sears, 1954), University of Missouri, USA and these lines are susceptible to powdery mildew. In wheat, each of the 21 monosomic lines is used as the female parent in a cross with the resistant line. The monosomic F_1 plants are cytogenetically identified and then grown to obtain F_2 generations. If a single dominant gene is involved, then in the 20 non-critical crosses a normal ratio of 3 resistant:1 susceptible will be obtained. In the critical cross, the F_1 plants will be R-. When selfed, they will give F_2 plants that are RR (disomic), R- (monosomic) and – (nullisomic). Only nullisomics will be susceptible. The expected segregation ratio with the critical chromosome will be about 97R:3S (Knott, 1989). If the new gene is located on a chromosome that carries one or several known resistance gene(s), a test of allelism should be made between the resistant line and the line possessing the known resistance gene to determine the relationship between the new gene and the known gene (Figure 1). If susceptible recombinants in F_2 generation are not observed when tested with an isolate avirulent to both lines, the new gene is a new allele at an identified locus or closely linked to the known gene. If no susceptible individuals are found in F_3 families when further tested with the same isolate, the new gene is a new allele at an identified locus. Otherwise, the new gene may be independent or linked to the known gene. Using monosomic analysis and allelism tests, most powdery mildew resistance genes/alleles were located on the different chromosomes of wheat (Hsam et al., 1998, 2001; Huang, 1999; Huang et al., 1997b, 2000a,b; Zeller et al., 1993a).

Currently, monosomic analysis is rarely used to locate R-genes (or other genes) to chromosomes. It is more common to screen a segregating population (often used in a bulked segregant analysis, where two DNA bulks are assembled from equal amounts of DNA from 10 homozygous resistant and 10 homozygous susceptible F_3 families, respectively) with a panel of microsatellite markers to identify candidate chromosomal locations (Figure 1), because microsatellite markers are chromosome-specific. Recently, two new powdery mildew resistance genes, *Pm30* (Liu et al., 2002) and *Pm31* (Xie et al., 2003), were located to chromosomes with this strategy.

By using the differential *Bgt* isolates and applying the gene-for-gene hypothesis, a new resistance gene can be identified in species closely related to hexaploid wheat ($2n = 42$, AABBDD), such as the tetraploid emmer wheats (*T. dicoccum*, *T. dicoccoides*) and durum (*T. durum*) ($2n = 28$, AABB), and diploids *T. monococcum* ($2n = 14$, AA) and *T. tauschii* ($2n = 14$, DD), and in more distant relatives like *T. timopheevii* ($2n = 28$, AAGG), *S. cereale* ($2n = 14$, RR), *Ae. speltoides* ($2n = 14$, SS), *Ae longisima* ($2n = 14$, SS)

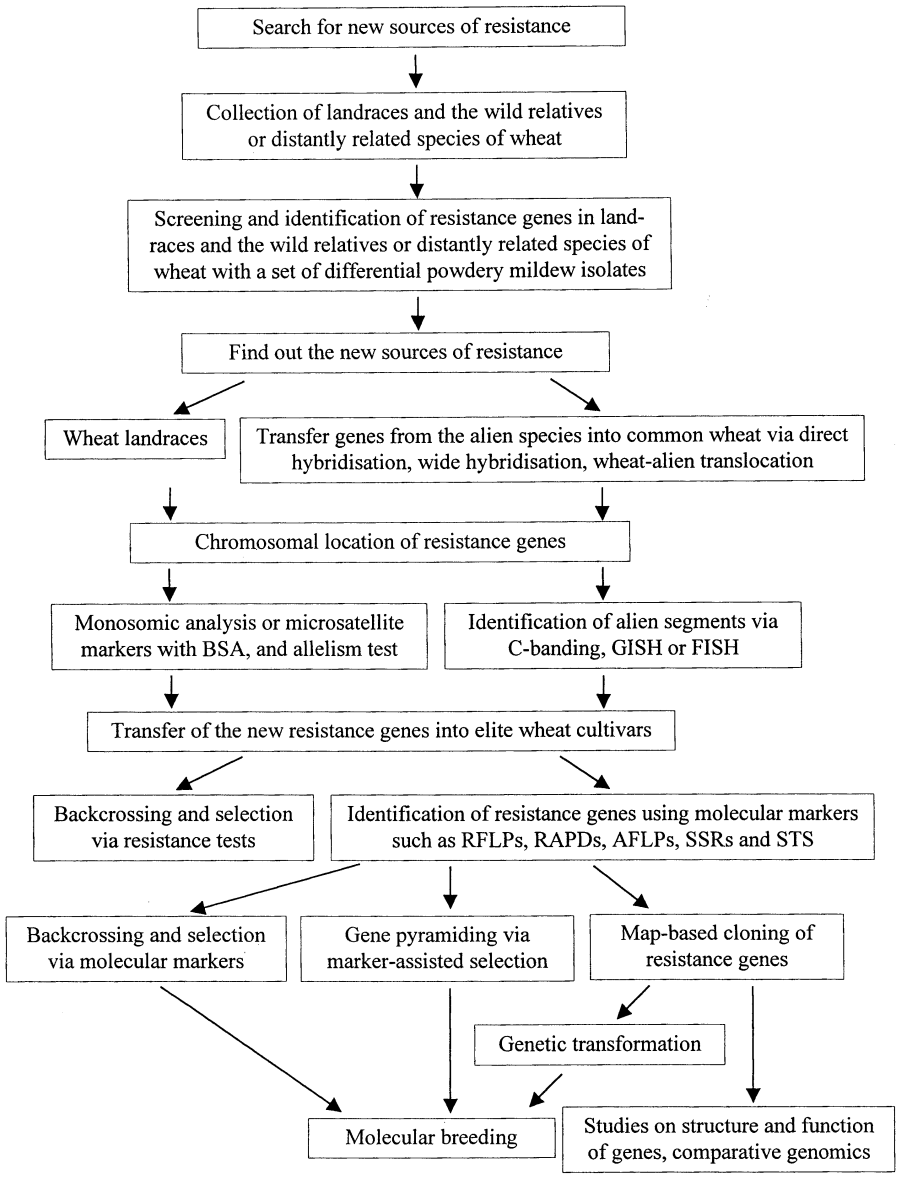

Figure 1. Scheme: from gene identification to molecular breeding.

and *Haynaldia villosa* ($2n = 14$, VV). A new resistance gene found in the closely related species can be successfully introduced into hexaploid wheat by crossing, using embryo rescue (if necessary), and subsequently performing several backcrosses, or by the production of an amphidiploid (AABBDD). Recombinations occur between the homologous chromosomes. Because of four genes *Kr1*, *Kr2*, *Kr3* and *Kr4* on chromosomes 5B, 5A, 5D and 1A whose dominant alleles inhibit crossability (Zheng et al., 1992), the genotypes of the parents are very important in determining the success of a cross. It is well known that hexaploid wheat 'Chinese Spring' and many other Chinese landraces have crossability with other related species (Knott, 1989; Zheng et al., 1992). Eleven genes/alleles *Pm1b*, *Pm3h*, *Pm4a*, *Pm4b*, *Pm5a*, *Pm16*, *Pm19*, *Pm25*, *Pm26*, *Pm30* and *Pm31* were introduced into hexaploid wheat in this way (Table 2). A resistance gene from a distantly related species can be transferred into hexaploid wheat by using a CS *ph1* mutant or radiation-induced translocations. Translocations may involve homoeologous or non-homoeologous chromosomes. Eleven genes *Pm6*,

Table 1. Differential reactions of wheat cultivars/lines possessing powdery mildew resistance genes after inoculation with 15 isolates of *Blumeria graminis* f. sp. *tritici* (according to Huang, 1999; Hsam et al., 2003)

	Blumeria graminis tritici isolate[1]															
Cultivar/line	2	5	6	9	10	12	13	14	15	16	17	76	77	90	117	*Pm*-genes
Axminister/8*Cc[2]	r[1]	s	r	i,s	r	s	s	s	r	s	s	s	r	r	r	*1a*
MocZlatka	r	r	r	r	r	i	r,i	r	r	s	s	r	-	r	-	*1b*
M1 N	r	r	r	r	r	r	r	r	r	r	r	r	-	s	r	*1c*
TSD	r	r	r	r	r	i	r	r,i	r	r,i	r,i	i	r	r	r	*1d*
Virest	r	i	r	r	r	i	i,r	i	r	i,s	i,s	i	r	r	r	*1e*
Ulka/8*Cc	s	r	r	s	r	s	s	s	r	s	s	r	r	s	r	*2*
Asosan/8*Cc	r	s	r	r	r	s	r	r	s	s	r	r	r	r	r	*3a*
Chul/8*Cc	r	s	s	r	r	r	r	r	s	r	i,s	r	r	r	s	*3b*
Sonora/8*Cc	r	s	s	i	r	s	r	i,s	s	s	s	r	s	i,s	s	*3c*
Kolibri	s	s	s	r	s	r	s	r	r	s	r	i	s,i	r	s	*3d*
W150	s	i,s	i,s	i	r	i,s	r	r,s	s	s	s	r	-	i	-	*3e*
Mich. Amb./8*Cc	r	s	s	i	r	s	r	i,s	s	s	s	r	-	s	-	*3f*
Khapli/8*Cc	s	r	s	r	i	r	s	s	i	s	i	i	r	r	s	*4a*
Armada	s	r	s	r	r	r	s	s	r	s	s	r	r	r	s	*4b*
Hope	s	s	s	s	r	s	s	r	s	s	s	r	s	s	s	*5a*
Komoran	s	s	s	s	r	s	s	r	s	s	s	s	r,i	s	-	*5b*
Kolandi	i	s	s	s	r	s	s	r	s	s	s	i	s	-	-	*5c*
IGV 455	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	*5d*
Fuzhuang 30	r	r	r	r	r	r	r	r	r	r	r	r	s	r	r	*5e*
TP114/2*Starke[3]	s	r,i	r,i	r	r,i	s	r,i	r,i	r,i	i	s	r	r	s,i	s	*6*
Transec	i	i	r,i	s	s	i	s	s	s	s	s	s	-	s	-	*7*
Disponent	r	s	s	r	s	r	s	s	s	s	r	i	r	s,i	r	*8*
N14	s	s	i	r	s	s	s	s	i	s	s	i,r	-	s	-	*9*
6BS-6SS/6SL[4]	r	r	r	r	r	r	r	r	r	r	r	-	-	-	r	*12*
BRG 3N[5]	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	*16*
Amigo	i	i	i	i	i	i	r	s	i	r	r	r	r	s	s	*17*
XX 186[6]	s	s	r	i	r	r	i	i	s	i	r	r	r	s	s	*19*
6BS/6RL[7]	r	r	r	r	r	r	r	r	r	r	r	r	r	-	-	*20*
6AL/6VS[8]	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	*21*
Chiyacao	r	r	r	r	r	r	r	r	r	r	r	r	i,r	r	s	*24*
Pova	r	r	r	r	i	r,i	i,s	r	r,i	i,s	s	r	-	-	r	*29*
L501[9]	i	i	i	r	r	i	s	s	i	s	s	-	-	-	r	*32*

[1]Maintained at Freising-Weihenstephan, Germany; r: resistant, s: susceptible, i: intermediate, -: not tested.
[2]Seven times backcrossed to 'Chancellor'.
[3]Once backcrossed to 'Starke'.
[4]Wheat-*Ae. speltoides* translocation line.
[5]BRG 3N/76-F_2-205, a *T. turgidum var. dicoccoides* derivative.
[6]XX 186, a *T. durum Ae. squarrosa* hexaploid synthetic wheat line.
[7]Wheat-Rye translocation line.
[8]Wheat-*Haynaldia villosa* translocation line.
[9]Wheat-*Ae. speltoides* translocation line.

Pm7, *Pm8*, *Pm12*, *Pm13*, *Pm17*, *Pm20*, *Pm21*, *Pm27*, *Pm29* and *Pm32* were transferred into hexaploid wheat by such methods (Table 2). The translocated chromosomes carrying alien resistance genes can be identified through C-banding and GISH (Genomic *in situ* hybridization) (Figure 1; Friebe et al., 1996). Detailed procedures and techniques were reviewed by Knott (1989) and Jones et al. (1995).

Table 2. Chromosomal locations, cultivars/lines, sources and references of the known powdery mildew resistance genes

Gene/allele	Location	Cultivar/line	Source	Reference
Pm1a	7AL	Axminister	*T. aestivum*	Sears & Briggle (1969)
Pm1b	7AL	MocZlatka	*T. monococcum*	Hsam et al. (1998)
Pm1c (formerly *Pm18*)	7AL	Weihestephan M1N	*T. aestivum*	Hsam et al. (1998)
Pm1d	7AL	*T. spelta var. duhamelianum*	*T. spelta*	Hsam et al. (1998)
Pm1e (*Pm22*)	7AL	Virest	*T. aestivum*	Singrün et al. (2003)
Pm2	5DS	Ulka/XX 194	*T. aestivum/Ae. tauschii*	McIntosh & Baker (1970) and Lutz et al. (1995)
Pm3a	1AS	Asosan	*T. aestivum*	Briggle & Sears (1966)
Pm3b	1AS	Chul	*T. aestivum*	Briggle (1966)
Pm3c	1AS	Sonora	*T. aestivum*	Briggle (1966)
Pm3d	1AS	Kolibri	*T. aestivum*	Zeller et al. (1993a)
Pm3e	1AS	W150	*T. aestivum*	Zeller et al. (1993a)
Pm3f	1AS	Michigan Amber	*T. aestivum*	Zeller et al. (1993a)
Pm3g	1AS	Aristide	*T. aestivum*	Zeller & Hsam (1998)
Pm3h	1AS	Abessi	*T. durum*	Zeller & Hsam (1998)
Pm3i	1AS	N324	*T. aestivum*	Zeller & Hsam (1998)
Pm3j	1AS	GUS 122	*T. aestivum*	Zeller & Hsam (1998)
Pm4a	2AL	Khapli	*T. dicoccum*	The et al. (1979)
Pm4b	2AL	Armada	*T. carthlicum*	The et al. (1979)
Pm5a	7BL	Hope	*T. dicoccum*	Law & Wolfe, 1966
Pm5b	7BL	Ibis	*T. aestivum*	Hsam et al. (2001)
Pm5c	7BL	Kolandi	*T. aestivum* ssp. *sphaerococcum*	Hsam et al. (2001)
Pm5d	7BL	IGV 1-455	*T. aestivum*	Hsam et al. (2001)
Pm5e	7BL	Fuzhuang 30	*T. aestivum*	Huang et al. (2003a)
mlxbd (*Pm5* allele)	7BL	Xiaobaidong	*T. aestivum*	Huang et al. (2000a)
Pm6	2BL	TP 114	*T. timopheevii*	Jørgensen (1973)
Pm7	4BS·4BL-2RL	Transec	*S. cereale*	Friebe et al. (1994)
Pm8	1RS·1BL	Disponent	*S. cereale*	Hsam & Zeller (1997)
Pm9	7AL	N14	*T. aestivum*	Hsam et al. (1998)
Pm10[1]	1D	Norin 26	*T. aestivum*	Tosa et al. (1987)
Pm11[1]	6BS	Chinese Spring	*T. aestivum*	Tosa et al. (1988)
Pm12	6BS-6SS·6SL	Trans. line 31	*Ae. speltoides*	Jia et al. (1996)
Pm13	3BL·3SS-3S 3DL·3SS-3S	CS trans. line	*Ae. longissima*	Ceoloni et al. (1992)
Pm14[1]	6BS	Norin 10	*T. aestivum*	Tosa & Sakai (1990)
Pm15[1]	7DS	Norin 26	*T. aestivum*	Tosa & Sakai (1990)
Pm16	4A	Norman rec. line	*T. dicoccoides*	Reader & Miller (1991)
Pm17	1RS·1AL	Amigo	*S. cereale*	Heun et al. (1990)
Pm19	7D	XX 186	*Ae. tauschii*	Lutz et al. (1995)
Pm20	6BS·6RL	KS93WGRC28	*S. cereale*	Friebe et al. (1994)
Pm21	6VS·6AL	Yangmai 5 line	*Haynaldia villosa*	Chen et al. (1995)
Pm23	5°	82-7241	*T. aestivum*	McIntosh et al. (1998)
Pm24	1DS	Chiyacao	*T. aestivum*	Huang et al. (2000b)
Pm25	1A	NC96BGTA5	*T. boeoticum*	Shi et al. (1998)
Pm26	2BS	TTD140	*T. dicoccoides*	Rong et al. (2000)

(*Continued on next page*)

Table 2. (*Continued*)

Gene/allele	Location	Cultivar/line	Source	Reference
Pm27	6B-6G	146-155-T	*T. timopheevii*	Järve et al. (2000)
Pm28	1B	Meri	*T. aestivum*	Peusha et al. (2000)
Pm29	7DL	Pova	*A. ovata*	Zeller et al. (2002)
Pm30	5BS	C20	*T. dicoccoides*	Liu et al. (2002)
Pm31 (*MlG*)	6AL	G-305-M/781//Jing 411*3	*T. dicoccoides*	Xie et al. (2003)
Pm32	1BL·1SS	L501	*Ae. speltoides*	Hsam et al. (2003)

[1]Resistance to *Blumeria graminis* f. sp. *agropyri*.

Chromosomal locations, cultivars/lines, sources and references for the 48 known powdery mildew resistance genes/alleles are listed in the Table 2. A suppressor of both *Pm8* and *Pm17* (an allele of *Pm8*; Hsam & Zeller, 1997) was located on chromosome 7D (Zeller & Hsam, 1996). Ren et al. (1996) reported a suppressor gene (*SuPm8*) on chromosome 1A, which did not inhibit the expression of *Pm17*. The detailed information on characterization and distribution of powdery mildew resistance genes in different countries as well as suppressors of *Pm* genes was reviewed by Huang (1999), and Hsam & Zeller (2002). These powdery mildew resistance genes are non-randomly distributed in the genome (Table 3), but form clusters in gene-rich regions (Gill et al., 1996a,b). Eight gene loci were identified in homoeologous group 1, whereas only one gene (*Pm16*) was found in homoeologous group 4 (i.e. chromosome 4A; Reader & Miller, 1991). Ten different alleles were located at the *Pm3* locus on the short arm of chromosome 1A (Zeller et al., 1993a; Zeller & Hsam, 1998). Recently, Hsam et al. (1998, 2001), Singrün et al. (2003) and Huang et al. (2000a; 2003a) reported five alleles at the *Pm1* locus on chromosome 7AL and six alleles at the *Pm5* locus on chromosome 7BL.

Molecular mapping of powdery mildew resistance genes

Molecular mapping and cloning of resistance genes in plants will facilitate an understanding of molecular mechanisms and evolution of disease resistance, and will permit marker-assisted selection in breeding programs. Several marker systems such as RFLPs and PCR-based markers like RAPDs, SSRs, SCARs, STS and AFLPs are available (Gupta et al., 1999) for mapping. Single nucleotide polymorphisms (SNPs) have not yet been applied to the identification of powdery mildew resistance genes, because only the *Pm3b* gene was recently isolated (Yahiaoui et al., 2004). To date, 21 powdery mildew resistance genes/alleles have been mapped using different molecular marker systems (Table 4). Chromosomal positions of several mapped powdery mildew resistance gene loci are presented in Figure 2.

Table 3. Distribution of powdery mildew resistance genes among homoeologous chromosomes in wheat, rye and barley

Chromosome	A	B	D	R[1]	H
1	*Pm3*, *Pm25*	*Pm28*, *Pm32*	*Pm10*[2], *Pm24*	*Pm8*, *Pm17*	*Mla*, *Mlk*, *Mli*, *Mlp*, *MlGa*
2	*Pm4*	*Pm6*, *Pm26*		*Pm7*	*MlLa*
3		*Pm13*			
4	*Pm16*				*Mlg*, *mlo*
5	*Pm23*	*Pm30*	*Pm2*		*Mlj*
6	*Pm21*, *Pm31* (*MlG*)	*Pm11*[2], *Pm12*, *Pm14*[2], *Pm27*		*Pm20*	*Mlh*
7	*Pm1*, *Pm9*, *Pm18* (*Pm1c*)	*Pm5*	*Pm15*[2], *Pm19*, *Pm29*		*Mlf*, *mlt*

[1]Genes introgressed into wheat from cereal rye and designated in accordance with wheat genetic nomenclature.
[2]Resistance to *Blumeria graminis* f. sp. *agropyri*.

Table 4. Molecular mapping of the genes/QTLs for resistance to powdery mildew in wheat using molecular markers

Gene/QTL	Location	Marker type	Closest/flanking marker(s)	Linkage/ contribution	Strategy for analysis	Reference
Pm1a	7AL	RAPD, STS	$UBC320_{420}$, $UBC638_{550}$	Both co-segregate	F_5, F_2 lines, BSA	Hu et al. (1997)
		RFLP	WHS178-9.4 kb-*Eco*RI	2.8 ± 2.7 cM	F_2 lines, NILs	Hartl et al. (1995)
		RFLP	CDO347	Co-segregate	F_2 lines, NILs	Ma et al. (1994)
		RFLP, STS	mwg2062, cdo347, psr121, psr148, psr680, psr687, wir148, C607, $STS638_{542}$, ksuh9	All co-segregate	F_2 lines	Neu et al. (2002)
Pm1c		RFLP, RAPD	WHS178-15 kb-*Eco*RI, $OPH\text{-}11_{1900}$	4.4 ± 3.6 cM, 13 cM	F_2 lines, BSA	Hartl et al. (1995)
		AFLP	S19M22-325/200	Co-segregate	$F_3 + F_4$ lines, BSA	Hartl et al. (1999)
			S14M20-137/138	Co-segregate		
Pm1e		SSR, AFLP	GWM344-null–S13M26-372	0.9 cM, 0.2 cM	$F_{2:3}$ lines, BSA	Singrün et al. (2003)
Pm2	5DS	RFLP	WHS350-6.5 kb-*Eco*RV, WHS295	3.8 cM, 2.7 ± 2.6 cM	F_2 lines, NILs	Hartl et al. (1995)
		RFLP	BCD1871	3.5 cM	F_2 lines, NILs	Ma et al. (1994)
		STS	STSwhs350	–	F_2 lines, NILs	Mohler & Jahoor (1996)
Pm3a	1AS	RFLP	WHS179	3.3 ± 1.9 cM	DH, NILs	Hartl et al. (1993)
Pm3b		RFLP	BCD1434	1.3 cM	F_2 lines, NILs	Ma et al. (1994)
Pm3g		RFLP	Gli-A5	5.2 cM	DH	Sourdille et al. (1999)
Pm4a	2AL	RFLP	BCD1231, CDO678	Co-segregate, co-segregate	F_2 lines, NILs	Ma et al. (1994)
		AFLP	4aM1	3.5 cM	$F_3 + F_4$ lines, BSA	Hartl et al. (1999)
		STS	$STS_{bcd1231\text{-}1.7kb}$	Co-segregate	NILs	Liu et al. (1998)
Pm5e	7BL	SSR	GWM1267-136	6.6 cM	$F_{2:3}$ lines, BSA	Huang et al. (2003a)
Pm6	2BL	RFLP	BCD135-9 kb-*Eco*RV	1.6 ± 1.5 cM	F_2 lines, NILs	Tao et al. (2000)
Pm8	1RS·1BL	RFLP	IAG95	Tightly linkage	F_2 lines, BSA	Wricke et al. (1996)
		RAPD	OPJ07-1200, OPR19-1350	–	Translocation lines	Iqbal & Rayburn (1995)
		STS	SEC-1b-412 bp	–	Translocation lines	de Froidmont (1998)
		STS	$STS_{iag95\text{-}1050}$	Co-segregate	DH, $F_{2:3}$ lines	Mohler et al. (2001)
Pm12	6BS-6SS·6SL	RFLP	psr10, psr106, Nor-2, psr141, psr113, psr142, psr149, psr2	Co-segregate	F_2 lines	Jia et al. (1996)
Pm13	3BL·3SS-3S	RFLP	psr305, psr1196	–	Recombinant lines	Donini et al. (1995)
	3DL·3SS-3S					
		RFLP, RAPD, STS	cdo460, utv135, $OPV13_{800}$, UTV13, $OPX12_{570}$, UTV14	–	Recombinant lines	Cenci et al. (1999)
Pm17		RFLP, AFLP	IAG95–	1.5 cM	$F_{2:3}$ lines	Hsam et al. (2000)
			CA/CT-355	1.5 cM		

(*Continued on next page*)

Table 4. (*Continued*)

Gene/QTL	Location	Marker type	Closest/flanking marker(s)	Linkage/ contribution	Strategy for analysis	Reference
Pm21	6VS·6AL	RAPD	$OPH17_{1900}$	Co-segregate	F_2 lines	Qi et al. (1996)
		RAPD, SCAR	$OPH17_{1400}$, $SCAR_{1265}$, $SCAR_{1400}$	All co-segregate	F_2 lines	Liu et al. (1999)
Pm24	1DS	AFLP, SSR	E34/M51-407, gwm337-204	Co-segregate, 2.4 ± 1.2 cM	$F_{2:3}$ lines, BSA	Huang et al. (2000b)
		SSR	gwm1291	Co-segregate	$F_{2:3}$ lines	Huang & Röder (2003)
Pm25	1A	RAPD	$OPA04_{950}$	12.8 cM	BC_1F_1 lines, BSA	Shi et al. (1998)
Pm26	2BS	RFLP	wg516	Co-segregate	RSLs	Rong et al. (2000)
Pm27	6B-6G	RFLP, SSR	psp3131	Co-segregate	F_2 lines	Järve et al. (2000)
Pm29	7DL	RFLP, AFLP	S24M13-233, S19M23-240, S22M26-192, S25M15-145, S13M23-442, S22M21-217, S17M25-226	All co-segregate	F_2 lines, BSA	Zeller et al. (2002)
Pm30	5BS	SSR	gwm159-460, gwm159-500	5–6 cM	BC_2F_2 lines, BSA	Liu et al. (2002)
Pm31	6AL	SSR	psp3029	0.6 cM	BC_2F_2 lines, BSA	Xie et al. (2003)
QTL	1A	RFLP	psr1201b-psr941	7.7%	RILs	Keller et al. (1999)
	1B		CD9b-psr593a	11.6%		
	1D		psr168-glk558b	9.5%		
	2A		psr380-glk293b	7.7%		
	2D		psr932-psr331a	10.0%		
	3A		psr598-psr570	14.4%		
	3D		psr1196a-Lrk10_6	15.7%		
	4A		gwm111c-psr934a	14.7%		
			mwg710b-glk128	14.3%		
	4B		psr593b-psr1112	7.5%		
	4D		glk302b-psr1101a	14.4%		
	5A		psr644a-psr945a	22.9%		
			psr911-psr120a	10.5%		
			psr1194-psr918b	16.6%		
	5B		psr580b-psr143	12.6%		
	6B		psr167b-psr964	8.7%		
	7B		psr593c-psr129c	11.3%		
			glk750-mwg710a	31.8%		
MlRE	6AL	SSR, RFLP	ksuD27	24.1–37.0%	$F_{2:3}$ lines, BSA	Chantret et al. (2000)
QTL	5D	SSR, RFLP	gwm174	16.8–25.3%	$F_{2:3}$ lines, BSA	Chantret et al. (2000)
QTL	5D	SSR, RFLP	cfd26	28.1–37.7%	DH, $F_{2:3}$ lines	Chantret et al. (2001)
			gbxG083c	33.5–37.9%		
	6A		*MlRE*	12.2%		
			gwm427	8.8–13.4%		

(*Continued on next page*)

Table 4. (*Continued*)

Gene/QTL	Location	Marker type	Closest/flanking marker(s)	Linkage/ contribution	Strategy for analysis	Reference
	7A		gwm344	6.4%		
	7B		gwm577	1.7%		
QTL	1A	SSR, RFLP	cdo572b-bcd442	39.3–43.0%		Mingeot et al. (2002)
	2A		*Pm4b*-gbxG303	22.7–39.2%		
	2B		gwm148-gbxG553	23.6–71.5%		
	4A		gbxG036-gbxG542	22.3%		
	5D		gwm639a-gwm174	21.4–54.3%		
	6A		*MlRE*	19.8–53.9%		
	7B		pdaC01-gbxR035b	22.8–33.5%	DH	
QPm.vt-1B	1B	RFLP, SSR	gwm259-wg241	17.0%	$F_{2:3}$ lines, BSA	Liu et al. (2001)
QPm.vt-2A	2A	RFLP, SSR	gwm304a-gwm312	29.0%	$F_{2:3}$ lines, BSA	Liu et al. (2001)
QPm.vt-2B	2B	RFLP, SSR	wg338-gwm526a	11.0%	$F_{2:3}$ lines, BSA	Liu et al. (2001)

Abbreviations: BSA = bulked segregant analysis, DH = doubled haploids, NIL = near-isogenic line, RIL = recombinant inbred line, RSL = recombinant substitution line.

Restriction fragment length polymorphisms

RFLP is the consequence of the variation that exists in the distribution and presence (or absence) of restriction sites recognized by endonucleases. Each restriction endonuclease recognizes a specific and characteristic nucleotide sequence. RFLP is generated whenever there is a loss or creation of restriction sites by base substitutions, insertions/deletions, inversions, duplications, or translocations. Such polymorphisms can be detected by hybridization of a labeled probe to digested total DNA fragments separated by electrophoresis. Single copy genomic sequences, sequences with a low copy number, or cDNA clones can be used as probes for hybridization. RFLP markers are co-dominant. RFLP probes from cereals can often be mapped to all three homoeologous wheat chromosomes (e.g., 1A, 1B or 1D) as well as to syntenic chromosomes of other cereal species. They are therefore very useful for comparative genetics of cereal species (Devos & Gale, 2000; Gale & Devos, 1998). However, RFLP analysis has some disadvantages in wheat, because it is laborious and time-consuming. Moreover, RFLP markers reveal only a very low level of polymorphism (Chao et al., 1989; Devos & Gale 1992). Nevertheless, RFLP markers were used to map several powdery mildew resistance genes. Using near-isogenic lines (NILs), the gene *Pm1* was mapped 3 cM from RFLP marker *Xwhs178-7A* (Hartl et al., 1995). According to Hartl et al. (1993), the RFLP marker *Xwhs179-1A* closely linked to the *Pm3* locus distinguished three different alleles *Pm3a*, *Pm3b* and *Pm3c*. *Pm3g* was mapped to the distal region of the short arm of chromosome 1AS (Sourdille et al., 1999). Similarly, Ma et al. (1994) reported RFLP markers that co-segregated with *Pm1* and *Pm4a* and others that were tightly linked to *Pm2* or *Pm3b*. Neu et al. (2002) found seven RFLP markers forming a cluster that co-segregated with the *Pm1* gene, revealing suppressed recombination on the long arm of chromosome 7A. These authors speculated that the suppressed recombination resulted from an alien introgression of chromatin from an unidentified wild-related species or was due to chromosomal rearrangements. An RFLP marker *Xiag95-1R* was closely linked to *Pm8* (Wricke et al., 1996). Resistance genes *Pm6* (Tao et al., 2000), *Pm12* (Jia et al., 1996), *Pm13* (Cenci et al., 1999, Donini et al., 1995), *Pm26* (Rong et al., 2000) and *Pm29* (Zeller et al., 2002) that were introduced from alien chromosome segments into common wheat were also mapped using RFLP markers. An RFLP marker *Xwg516-2B* co-segregated with the recessive gene *Pm26* (Figure 2, Rong et al., 2000), whereas eleven RFLP loci co-segregated with the gene *Pm12* because of a lack of recombination between *Ae. speltoides* and wheat chromatin (Jia et al., 1996).

Random-amplified polymorphic DNAs

RAPD markers were first described by Williams et al. (1990) and Welsh & McClelland (1990). They are

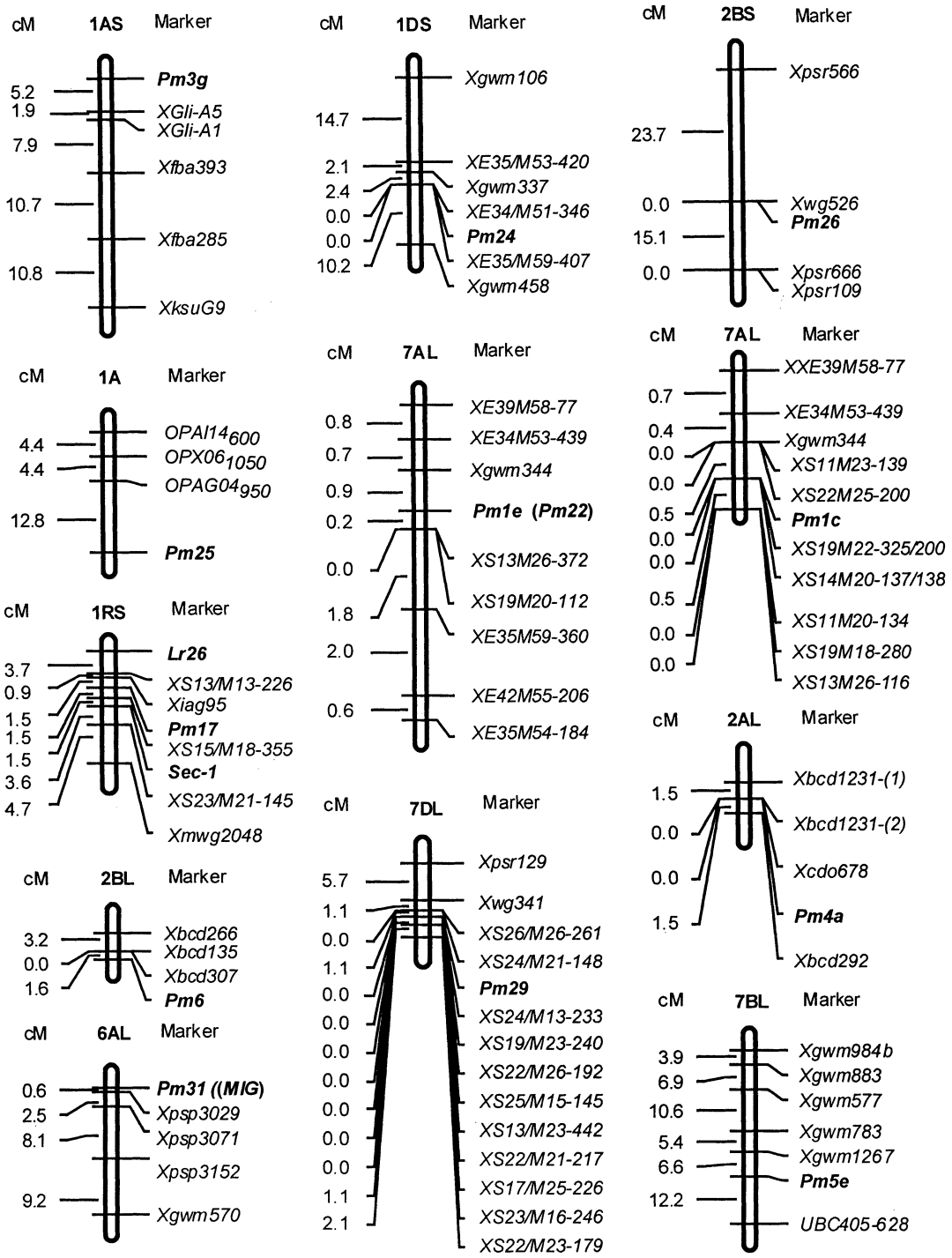

Figure 2. Molecular mapping of genes *Pm1c* and *Pm1e* (Singrün et al., 2003), *Pm3g* (Sourdille et al., 1999), *Pm4a* (Ma et al., 1994), *Pm5e* (Huang et al., 2003a), *Pm6* (Tao et al., 2000), *Pm17* (Hsam et al., 2000), *Pm24* (Huang et al., 2000b), *Pm25* (Shi et al., 1998), *Pm26* (Rong et al., 2000), *Pm29* (Zeller et al., 2002) and *Pm31* (*MlG*, Xie et al., 2003).

revealed using a single DNA primer with random sequences of usually 10 bases and are PCR-based dominant markers. In wheat, a RAPD marker closely linked to *Pm1* and two RAPD markers co-segregating with *Pm1* were identified (Hu et al., 1997). Iqbal & Rayburn (1995) detected specific RAPD markers for *Pm8* and *Pm17* that are located on the 1RS.1BL and 1RS.1AL translocated chromosomes, respectively. Qi et al. (1996) found a specific RAPD marker, $OPH17_{1900}$, for *Pm21*, which is on chromosome 6VS of *Haynaldia villosa*. Three RAPD markers, $OPX06_{1050}$, $OPAG04_{950}$, and $OPAI14_{600}$, were found to be linked to *Pm25* located on chromosome 1A (Shi et al., 1998). RAPD technology has the shortcoming of relatively low reproducibility between laboratories and even between different PCR machines within the same laboratory. Because of their low levels of polymorphism, RAPD markers were not used for the construction of linkage maps in wheat and were only applied for mapping of single genes. RAPD markers can be converted into sequence characterized amplified regions (SCARs) for use in MAS.

Amplified fragment length polymorphisms (AFLPs)

Vos et al. (1995) developed AFLP markers by combining the features of RFLP and PCR. AFLP analysis is a reliable and efficient method and a powerful technique to generate large numbers of markers for the construction of high-density genetic maps (Becker et al., 1995; Keim et al., 1997), identifying specific genes (Kasuga et al., 1997; Schwarz et al., 1999) and map-based cloning of resistance genes (Büschges et al., 1997; Wei et al., 1999). There are two ways to determine chromosomal locations of AFLP markers and linked genes in wheat. The direct method involves the use of crosses of Chinese Spring and the resistance source in bulked segregant analysis to identify repulsion phase markers to anchor unknown linkage groups to wheat chromosomes using CS aneuploid stocks (Huang et al., 2000b,c; Singrün et al., 2003). The indirect method involves linkage of AFLP markers to chromosome-specific markers like microsatellite markers. Hartl et al. (1999) detected several AFLP markers very tightly linked to *Pm1c*, among which one AFLP fragment was specific for *Pm1c*. *Pm4a* was also identified in the same population using AFLP markers (Hartl et al., 1999). Mapping of co-migrating AFLP markers *XE39M58-77* and *XE34M53-439* on chromosome 7A in two wheat crosses segregating for *Pm22* and *Pm1c*, respectively, indicated that *Pm22*, which had earlier been assigned to chromosome 1D in the Italian wheat cultivar 'Virest' (Peusha et al., 1996), was an allele of the complex *Pm1* locus. *Pm22* was therefore re-designated as *Pm1e* (Singrün et al., 2003). Two AFLP markers, *XAG/AG-228* and *XCA/CT-355*, were linked to *Pm17* with genetic distances of 2.4 cM and 1.5 cM, respectively (Hsam et al., 2000). Huang et al. (2000b) found that two AFLP markers associated in coupling with *Pm24*. The AFLP marker locus *XE35M59-407* co-segregated with the *Pm24* allele and *XE35M53-420* mapped 4.5 cM from it. Another AFLP marker locus *XE34M51-346* co-segregated in repulsion with *Pm24* (Figure 2). Similarly, *Pm29* was closely linked to flanking AFLP markers *XS26M26-261* and *XS23M16-246* at 1.1 cM, respectively, whereas seven additional AFLP markers co-segregated with this resistance gene. Three of them, *XS17M26-226*, *XS23M16-246*, *XS26M26-261*, were diagnostic for the *Pm29* allele (Figure 2; Zeller et al., 2002).

Sequence-tagged sites (STS) and sequence characterized amplified regions (SCARs) STS markers (Olson et al., 1989) are 'single copy' sequences amplified using specific primers that match the nucleotide sequences at the ends of a DNA fragment of an RFLP probe. SCAR markers are derived from RAPD fragments from which the ends have been sequenced and used as specific primers for amplification (Paran & Michelmore, 1993). STS or SCARs may be (co)dominant if they display length polymorphism. When the amplified fragments do not reveal length polymorphism, a restriction enzyme is used to cleave the amplified fragments and the resulting fragments are called cleaved amplified polymorphic regions (CAPR; Konieczny & Ausubel, 1993). One drawback of STSs or SCARs is the need for sequence analysis before primers can be designed. The RAPD marker $UBC638_{550}$, which co-segregated with *Pm1,* was converted to STS markers by Hu et al. (1997) and Neu et al. (2002). The STS marker differentiated susceptible cultivars and those carrying the *Pm1a* allele (Hu et al., 1997). An STS marker developed from the RFLP probe IAG95 distinguishes the rye-derived powdery mildew resistance alleles *Pm8* and *Pm17* (an allele of *Pm8*; Hsam & Zeller, 1997) in wheat (Mohler et al., 2001). Mohler & Jahoor (1996) derived an allele-specific STS marker for *Pm2* from the RFLP probe WHS350. This marker identified most lines possessing *Pm2*. Amplification using STS primers designed from the sequence of the RFLP probe BCD1231 generated a specific band which was present in all 11 tested

cultivars and lines containing the *Pm4a* and *Pm4b* alleles (Liu et al., 1998). To rapidly detect the 1BL/1RS wheat-rye translocation in winter wheat breeding lines, de Froidmont (1998) developed a specific co-dominant STS marker. An STS marker *Xutv14-3S*^{l} derived from RAPD marker $OPX12_{570}$ amplified a specific product from the introgressed segment carrying the *Pm13* gene (Cenci et al., 1999). Liu et al. (1999) developed two SCAR markers, $SCAR_{1265}$ and $SCAR_{1400}$, to detect the *Pm21* gene in different genetic backgrounds. The $SCAR_{1265}$ marker was specific for the *Pm21* allele.

Microsatellites or simple sequence repeats (SSRs)

Microsatellites or SSRs are tandem repeats of two to six nucleotides. They are abundant and randomly distributed throughout the genome. Their high level of polymorphism, combined with their ease of detection and transferability between researchers, have made them valuable genetic markers. Polymorphisms are based on size differences between alleles, usually the result of variable numbers of repeats (Di Rienzo et al., 1994; Huang et al., 2002a). Variations in the length of repeat units can be identified by amplification of the region containing the motif via PCR using primers designed to the regions flanking individual microsatellites. Microsatellites can be isolated by screening genomic libraries or screening Expressed Sequences Tags (ESTs) and cDNA sequences using *in silico* searches.

Over 1000 microsatellites from wheat are currently available (Gupta et al., 2002; Guyomarc'h et al., 2002; Huang et al., 2003b; Röder et al., 2004; Song et al., 2002; Stephenson et al., 1998). Microsatellite markers are co-dominant, chromosome-specific and evenly distributed along chromosomes in wheat (Röder et al., 1998a,b). The current level of genome coverage provided by microsatellite markers in wheat (ca. one every 10–15 cM) is sufficient to be useful for studies relating to genetic diversity (Huang et al., 2002a; Plaschke et al., 1995), location of resistance genes, and identification of QTLs for yield and yield components (Huang et al., 2003a,b; Liu et al., 2002; Xie et al., 2003), and marker-assisted breeding (Huang et al., 2000b, 2003a). An allele at the microsatellite locus *Xgwm337-1D* was located 2.4 cM from *Pm24* on chromosome 1DS. The co-dominant microsatellite marker *Xgwm337-1D* displays a specific allele for *Pm24* and therefore can be used as a diagnostic marker for the selection of *Pm24* (Huang et al., 2000b). Recently, a microsatellite marker *Xgwm1291-1D* was found to co-segregate with the *Pm24* gene, and physical mapping using deletion lines revealed that the *Pm24* locus is located in the gene-rich region of the distal region of chromosome 1DS (Huang & Röder, 2003). Similarly, a co-dominant marker *Xgwm1267-7B* was relatively close to *Pm5e* with a linkage distance of 6.6 cM (Figure 2). Marker *Xgwm1267-7B* could not distinguish *Pm5e* from *Pm5d* and *mlxbd* (an undesignated *Pm5* allele), or the susceptible cultivar 'Chinese Spring', but could differentiate lines with the other known powdery mildew genes. Therefore, *Xgwm1267-7B* may be useful for the introgression of the gene *Pm5e* to the European wheat breeding pool (Huang et al., 2003a). Two other resistance genes *Pm30* and *Pm31*, both derived from wild emmer (*T. dicoccoides*), were identified using microsatellite markers and BSA (Liu et al., 2002; Xie et al., 2003). The microsatellite locus *Xgwm159-5B* on the short arm of chromosome 5B was linked to the *Pm30* gene with a genetic distance of 5–6 cM (Liu et al., 2002), whereas four microsatellite markers from chromosome 6AL were mapped in a BC_2F_3 population, in which the SSR locus *Xpsp3029-6A* was closely (0.6 cM) linked to *Pm31* (Figure 2, Xie et al., 2003). Singrün et al. (2003) reported that the microsatellite locus *Xgwm344-7A* mapped 0.9 cM from *Pm1e*.

Resistance gene analog (RGA) polymorphism (RGAP)

More than 30 disease resistance genes have been cloned from diverse plant species. The largest group of resistance genes carries leucine-rich repeats (LRRs) and nucleotide-binding site (NBS) (Hulbert et al., 2001). Employing primers derived from the conserved LRRs of the *RPS2* gene of *Arabidopsis thaliana* and the *N* gene of tobacco, Leister et al. (1996) found molecular markers absolutely linked to the nematode resistance locus *Gro1* and the late blight resistance locus *R7*. Similarly, Yu et al. (1996) designed primers based on the NBS of *RPS2* and *N*, and mapped resistance gene analogs (RGAs) to the vicinity of known soybean genes for resistance to potyviruses (*Rsv1* and *Rpv*), Pyhytophtora root rot (*Rps1*, *Rps2* and *Rps3*), and powdery mildew (*rmd*). Recently, RGAs were successfully used for mapping leaf and yellow rust resistance genes in wheat by employing primers designed from the conserved LRR and NBS domains (Shi et al.,

2001; Spielmeyer et al., 2000; Yan et al., 2003). The RGA marker, *rgaYr10b*, co-segregated with the leaf rust resistance genes *Lr21* and *Lr40* (Spielmeyer et al., 2000), whereas three and four resistance gene analog polymorphism (RGAP) markers co-segregated with the yellow rust resistance genes *Yr5* (Yan et al., 2003) and *Yr9* (Shi et al., 2001), respectively. Two candidate genes *T10rga1* and *T10rga2* were isolated in the wheat NIL Thatcher*Lr10* using resistance gene analogs *RGA1* and *RGA2* identified in a *T. monococcum* DV92 BAC contig spanning the *Lr10* locus in *T. aestivum* (Feuillet et al., 2003; Wicker et al., 2001). This strategy should be applied in mapping powdery mildew resistance genes of wheat to detect candidate genes and diagnostic markers. Recently, a candidate gene for *Pm3b* was identified using primers based on a partially conserved LRR region between the resistant variety Chul and *T. monococcum* cv. DV92 (Yahiaoui et al., 2004).

Molecular mapping of quantitative trait loci (QTLs) for adult plant resistance (APR)

Major resistance genes are race-specific. This kind of resistance is generally of short durability, being overcome by pathogen races with matching virulence alleles (Roberts & Caldwell, 1970; Shaner, 1973). In contrast, quantitative resistance to powdery mildew is considered to be non-race-specific and is inherited polygenically. Because this resistance is generally observed at the adult stage and is usually associated with slow mildewing or delayed infection, development and reproduction of pathogen, it was designated adult plant resistance (Griffey et al., 1993; Griffey & Das, 1994), slow mildewing (Roberts & Caldwell, 1970; Shaner, 1973) or partial resistance (Hautea et al., 1987). Some APRs control mildew effectively, but APR should not be confused with partial resistance. Some partial resistance is indeed expressed in adult plants.

APR to powdery mildew is more durable than race-specific resistance. For example, APR in wheat cultivar Knox and its derivative Massey remained effective against powdery mildew infection for 20 years (Griffey & Das, 1994). Molecular markers can be employed to locate QTL for APR. Several QTLs for resistance to powdery mildew have been located using molecular markers (Chartret et al., 2000, 2001; Liu et al., 2001; Keller et al., 1999; Mingeot et al., 2002). The flanking markers for these QTLs are listed in Table 4. Keller et al. (1999) found 18 QTLs, of which two were of major effect. One of them mapped to the *Pm5* locus on chromosome 7B. The other QTL, on chromosome 5A, was derived from the spelt cultivar 'Oberkulmer' and did not correspond to any known *Pm* gene. A QTL detected on chromosome 2B peaked at the RFLP locus *Xwg338* with a LOD score of 4.34. This QTL explained 11% of the total variation between $F_{2:3}$ lines (Liu et al., 2001). Using DH and $F_{2:3}$ lines derived from the crosses involved in the resistant RE714, a major QTL for APR was detected on chromosome 5D. This QTL was stable over time and at two developmental stages (Chantret et al., 2001; Mingeot et al., 2002). With the help of microsatellites combined with BSA, two major QTLs associated with APR were detected: one (*MlRe*) on chromosome 6A and the other on chromosome 5D (Chantret et al., 2000). Another major QTL for APR in cultivar 'Massey' was found on chromosome 2A with a LOD score of 9.23. This QTL was located within the interval *Xgwm304-2A–Xgwm312-2A* and explained 29% of the total phenotypic variation between $F_{2:3}$ lines of a cross with susceptible cultivar 'Becker' (Liu et al., 2001).

The above and two minor QTLs in cultivar 'Massey' were detected in RIL lines from the same cross. Such molecular markers have potential for use in MAS and pyramiding of genes for resistance (Liu et al., 2001). A major QTL for APR in line RE714 was mapped on chromosome 5D using microsatellite markers. This QTL seemed to be expressed throughout development of the plant. Gene *MlRe* on chromosome 6A of RE714 was race-specific even though it is an APR (Robe & Doussinault, 1995). It is therefore unlikely to provide stable resistance. The microsatellite marker associated with the QTL for APR might be more useful because the QTL was stable over 3 years and at different developmental stages (Chantret et al., 2001).

The expression of QTL for APR to powdery mildew in a resistant line is often affected by environmental conditions (different locations and years), different crossing partners and different population types. Of 18 QTLs detected in a RIL population, three showed significant QTL × environment interaction (Keller et al., 1999). Similarly, only two of eight QTLs detected in DH lines from the cross RE714/Festin were consistent over the five environments (Mingeot et al., 2002). Eight QTLs were detected in DH lines from the cross RE714/Festin, whereas only three of them were mapped in DH lines from RE714/Hardi (Mingeot et al., 2002). Two QTLs were found in DH lines from the cross RE714/Hardi in 1998, whereas four QTLs

were detected in $F_{2:3}$ lines from the same cross in the same year. Only the QTL on chromosome 5D was detected in both trials (Chantret et al., 2001).

Conclusions and perspectives

Cloning of powdery mildew resistance genes

As the genome is large (16×10^9 bp) and 80% of it constitutes repetitive sequences (Smith & Flavell, 1975), map-based cloning of resistance genes in wheat is more difficult in rice and tomato which possess much smaller genomes. However, if a gene is located in a gene-rich region in wheat, it is possible to perform map-based cloning, because high levels of recombination occur in those regions (Gill et al., 1996a,b; Sandu et al., 2001). The ratio of physical to genetic distance within the gene-rich regions was estimated to be <200 kb/cM, a 22-fold increase compared to the genome average (Faris et al., 2000). A 211 kb sequence of chromosome $1A^mS$ of *T. monococcum* showed a density of one gene per 42 kb (Wicker et al., 2001). Analysis of a contiguous DNA sequence of a BAC from the gene-rich region of chromosome 1DS revealed high gene density (one gene per 8.9 kb) similar to that of *Arabidopsis* (Brooks et al., 2002). An even higher gene density of one gene per 4–5 kb was reported for the *Lrk* regions which are located in the same gene-rich regions on wheat and barley chromosome group 1 (Feuillet & Keller, 1999). The large genome size of hexaploid wheat makes it difficult to construct a large-insert BAC library. BAC libraries have been constructed from diploid species *T. monococum* (Lijavetzky et al., 1999) and *T. tauschii* (Moullet et al., 1999) whose genomes are related to the A and D genomes of hexaploid wheat, respectively. A BAC library was also constructed from tetraploid durum cv. Langdon (AABB, Cenci et al., 2003). Recently, Huang et al. (2003c) reported map-based cloning of the leaf rust resistance gene *Lr21* which is located in a gene-rich region at the distal end of chromosome arm 1DS. Subgenome chromosome walking in a *T. monococum* DV92 BAC library enabled isolation of a candidate for the *Q* gene conferring the square-headed phenotype and free-threshing character of domesticated wheat (Faris et al., 2003), as well as the leaf rust resistance gene *Lr10* (Feuillet et al., 2003; Stein et al., 2000). Yahiaoui et al. (2004) employed BAC libraries from diploid *T. monococum* and tetraploid *T. turgidum* as simple models for positional cloning of the powdery mildew resistance gene *Pm3b* in hexaploid wheat. These studies demonstrate that subgenome chromosome walking in combination with haplotype analysis makes it feasible to isolate agronomically important genes located in the gene-rich regions of common wheat.

Comparative genetics in grasses indicated that gene content and order are similar (syntenous) within taxonomic families, despite large differences between species in genome size and chromosome number (Gale & Devos, 1998). A high conservation of genome co-linearity exists within the tribe *Triticeace*, which includes wheat, barley, rye and wild relatives such as *Aegilops* spp. But chromosome rearrangements observed between different genomes may be complicating factors. For example, the barley (*Hordeum vulgare*) genome is highly co-linear with that of diploid wheat *T. monococcum*, differing from it by two translocations and two inversions (Dubcovsky et al., 1996). Because the co-linearity between wheat or barley and rice at the micro-level is not perfect and is often interrupted by insertions, deletions and inversions, isolation of genes in large genome species such as wheat and barley using model species like rice with small genomes for map-based cloning have been only partially successful (Kilian et al., 1997; Foote et al., 1997). In barley, many powdery mildew (*Blumeria graminis* f. sp. *hordei*) resistance genes from different origins have been identified (Wei et al., 1999; Schönfeld et al., 1996; Table 3). More than 30 alleles were found at the *Mla* locus (Kintzios et al., 1995). The *Mla* resistance cluster is associated with three NBS-LRR resistance-gene homologue (RGH) families (Wei et al., 1999). Currently, four alleles *Mla1* (Zhou et al., 2001), *Mla6* (Halterman et al., 2001), *Mla12* (Shen et al., 2003) and *Mla13* (Haterman et al., 2003) have been isolated. Although the *Mla* locus in barley and the *Pm3* locus in wheat are located in corresponding gene-rich regions of the short arms of homoeologous group 1 (Sandu et al., 2001), and all four *Mla* alleles and the *Pm3b* encode resistance proteins with NBS and LRR motifs (Yahiaoui et al., 2004), the *Pm3b* gene is not an ortholog of barley *Mla* (designated *TaMla*), but tightly linked to *TaMla* (0.7 cM; Zhou et al., 2001).

Functional analysis

Once a candidate for a resistance gene is identified, its function can be confirmed by genetic transformation, mutant analysis, or SNPs. The genetic transformation of large-insert constructs containing candidate genes via *Agrobacterium tumefaciens*-mediated

transformation has been difficult in cereal species because of the very low efficiency of *Agrobacterium* infection. The direct delivery of DNA by microprojectile bombardment is currently the most reliable and satisfactory method of transformation for production of fertile transgenic wheat plants (Altpeter et al., 1996). A particle inflow gun was reported to transfer a cosmid clone containing a candidate for the leaf rust resistance gene *Lr21* into immature embryos of the susceptible Fielder. One transgenic plant carrying the corresponding fragment was resistant, confirming the cloning of *Lr21* (Huang et al., 2003c). For powdery mildew resistance genes in cereals, particle bombardment was used to deliver large DNA fragments into the epidermal cells of barley and wheat for transient expression to confirm the cloning of *Mla1* (Zhou et al., 2001), *Mla6* (Halterman et al., 2001), *Mla12* (Shen et al., 2003), *Mla13* (Halterman et al., 2003) and *Pm3b* (Yahiaoui et al., 2004), respectively.

Susceptible mutants of lines carrying resistance genes can be produced through irradiation (Zhou et al., 2001; Büschges et al., 1997), ethylmethane sulfonate (EMS) (Feuillet et al., 2003; Yahiaoui et al., 2004) or fast neutron irradiation (Faris et al., 2003). It is likely that fast neutrons induced very large deletions spanning 6–70 cM of genetic distance in wheat (Faris et al., 2003), whereas γ-ray or EMS induced point mutations or very small insertions/deletions (InDels) in barley and wheat (Büschges et al., 1997; Feuillet et al., 2003; Yahiaoui et al., 2004). Two candidate genes, *T10rga1* and *T10rga2*, were isolated for *Lr10*. Three point mutations (SNPs) in three independently derived *lr10* mutants induced by EMS were identified only in *T10rga1*, indicating that *T10rga1* was *Lr10* (Feuillet et al., 2003). Similarly, a single base deletion in the coding region was detected in an EMS-induced susceptible *Pm3b* mutant, which creates a premature stop codon (Yahiaoui et al., 2004). It is likely that the premature stop codon in the *pm3b* sequence resulted in the loss of function. Huang et al. (2003c) found three InDels and seven nucleotide polymorphisms (SNPs) by comparison of sequences of amplified fragments from two parents WI (*lr21*) and WGRC2 (*Lr21*). Eight SNPs and three small deletions were identified in 11 γ-ray- or EMS-induced *mlo* mutant alleles compared to the *Mlo* wild-type gene (Büschges et al., 1997). Ten alleles were identified at the *Pm3* locus (Table 2; Zeller & Hsam, 1998). Using gene-specific primers for *Pm3b* and genomic DNA from lines with different *Pm3* alleles as a template for PCR amplification, SNPs and InDels may be detected in the amplified fragments. These may help elucidate resistance specificities and functions of different alleles at the *Pm3* locus.

Marker-assisted selection (MAS)

Molecular markers which are closely linked to, or segregate with, target genes, provide a useful tool for breeding programs since they (1) can help to detect/identify resistance genes of interest without the need to perform disease tests; and (2) may shorten the breeding time in marker-assisted breeding compared to traditional breeding. Efficient application of molecular markers in plant breeding will depend on the development cost-effective and automated diagnostic technologies. There are four important requirements for marker-assisted selection in a breeding program.

- Molecular markers should be user-friendly and need to be easy and cheap to use for efficient screening of large populations. At present, easy analysis is based on PCR technology.
- Molecular markers should co-segregate or be tightly linked to traits of interest.
- Molecular markers should have high reproducibility across laboratories and transferability between researchers.
- Molecular markers should be co-dominant for differentiation of homozygous and heterozygous individuals in segregating progenies.

Combination of several effective resistance genes, or even different alleles of the same gene, in a single cultivar (gene pyramiding) may provide a longer period of protection, but with an intermittent sexual reproduction in a haploid pathogen, any extension of effectiveness will be for a limited period. Molecular markers will facilitate the selection process. There are several applications of molecular markers which are indeed useful in breeding and which complement current best practice. For example, (1) selection of genes controlling significant fractions of variation in partial resistance (see section on mapping of QTLs for APR); (2) identification of effective major resistance genes in breeding programs where good levels of partial resistance are already present, so breeders can make informed decisions on whether or not to select lines that carry them; (3) selection for effective major genes where there is currently no other means of controlling disease, as a temporary expedient while levels of partial resistance are increased by field selection.

Pm21 from wheat-*Haynaldia villosa* translocation 6VS/6AL (Chen et al., 1995) confers resistance to all existing mildew pathotypes in China (Qi et al., 1996) and Europe (Huang et al., 1997a). Using RFLP markers that are tightly linked to *Pm2* and *Pm21* or co-segregate with *Pm4a*, three two-gene combinations, *Pm2* + *Pm4a*, *Pm2* + *Pm21*, *Pm4a* + *Pm21* were successfully integrated into an elite wheat cultivar 'Yang158' and double homozygotes were selected from a small F_2 population (Liu et al., 2000).

PCR-based markers are more attractive for MAS, due to the small amount of template required and more efficient handling of large population sizes. Because of their dominant inheritance, AFLP, RAPD and STS markers cannot be applied for differentiation of homozygous and heterozygous individuals in segregating progenies. Among the DNA marker systems of wheat, microsatellites are currently the optimal marker for MAS, because of their co-dominant inheritance and ease of automation. The large numbers of microsatellite markers that have become available (Gupta et al., 2002; Guyomarc'h et al., 2002; Huang et al., 2001; Röder et al., 2004; Song et al., 2002; Stephenson et al., 1998) provide a sound basis for molecular mapping of broad-spectrum powdery mildew resistance genes and development of diagnostic markers for molecular breeding. Because several specific markers for some *Pm* genes have only recently been reported, many markers have not yet been used in breeding programs. It is important to mention that cross-overs may occur between a marker and the target gene, if the marker used for selection is not completely linked to the gene. This leads to a low proportion of false-positive/negative selections in the screening process. It is therefore preferable to develop the marker from the gene itself.

Advanced backcross (AB)-QTL analysis proposed by Tanksley & Nelson (1996) was successfully used for the development of near-isogenic lines (NILs) with QTL and for the isolation of QTL. Once the NIL of a QTL is developed, the QTL is inherited as single Mendelian factor (Yamamoto et al., 1998). A QTL, *fw2.2*, which is responsible for tomato fruit size, was cloned by applying a map-based approach. A high-resolution map was created from a cross between two NILs differing for alleles at *fw2.2* (Frary et al., 2000). A major QTL controlling response to photoperiod of rice, *Hd1*, was isolated using advanced backcross progeny and a map-based cloning strategy (Yano et al., 2000). In order to better genetically dissect QTL for APR to powdery mildew at the molecular level, and for better use of marker-assisted breeding, the advanced backcross approach can be used for the development of NILs with QTL for APR to powdery mildew and for the cloning of such genes.

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References

Altpeter, F., V. Vasil, V. Srivastasa, E. Stöger & I.K. Vasil, 1996. Accelerated production of transgenic wheat (*Triticum aestivum* L.) plants. Plant Cell Rep 16: 12–17.

Becker, J., P. Vos, M. Kuiper, F. Salamini & M. Heun, 1995. Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 249: 65–73.

Bennett, F.G.A., 1984. Resistance to powdery mildew in wheat: A review of its use in agriculture and breeding programmes. Plant Pathol 33: 279–300.

Briggle, L.W., 1966. Three loci in wheat involving resistance to *Erysiphe graminis* f. sp. *tritici*. Crop Sci 6: 461–465.

Briggle, L.W. & E.R. Sears, 1966. Linkage of resistance to *Erysiphe graminis* f. sp. *tritici* (*Pm3*) and hairy glume (*Hg*) on chromosome 1A of wheat. Crop Sci 6: 559–561.

Brooks, S.A., L. Huang, B.S. Gill & J.P. Fellers, 2002. Analysis of 106 kb of contiguous DNA sequence from the D genome of wheat reveals high gene density and a complex arrangement of genes related to disease resistance. Genome 45: 963–972.

Büschges, R., K. Hoollricher, R. Panstruga, G. Simons, M. Wolter, A. Frijters, R. Van Daelen, T. Van der Lee, P. Diergaarde, J. Groenendijk, S. Töpsch, P. Vos, F. Salamini & P. Schulze-Lefert, 1997. The barley *Mlo* gene: A novel control element of plant pathogen resistance. Cell 88: 695–705.

Cenci, A., N. Chantret, X. Kong, Y. Gu, O.D. Anderson, T. Fahima, A. Distelfeld & J. Dubcovsky, 2003. Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp. *durum*). Theor Appl Gent 107: 931–939.

Cenci, A., R. D'Ovidio, O.A. Tanzarella, C. Ceoloni & E. Porceddu, 1999. Identification of molecular markers linked to *Pm13*, an *Aegilops longissima* gene conferring resistance to powdery mildew in wheat. Theor Appl Genet 98: 448–454.

Ceoloni, C., G. Del Signore, L. Ercoli & P. Donini, 1992. Locating the alien chromatin segment in common wheat-*Aegilops longissima* mildew resistance transfers. Hereditas 116: 239–245.

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

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

Chao, S., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner & M.D. Gale, 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78: 495–504.

Chen, P.D., L.L. Qi, B. Zhou, S.Z. Zhang & D.J. Liu, 1995. Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet 91: 1125–1129.

de Froidmont, D., 1998. A co-dominant marker for the 1BL/1RS wheat-rye translocation via multiplex PCR. J Cereal Sci 27: 229–232.

Devos, K.M. & M.D. Gale, 1992. The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84, 567–572.

Devos, K.M. & M.D. Gale, 2000. Genome relationships: The grass model in current research. Plant Cell 12: 637–646.

Di Rienzo, A., A.C. Peterson, J.C. Garza, A.M. Valdes, M. Slatkin & N.B. Freimer, 1994. Mutational processes of simple-sequence repeat loci in human populations. Proc Natl Acad Sci USA 91: 3166–3170.

Donini, P., R.M.D. Koebner & C. Ceoloni, 1995. Cytogenetic and molecular mapping of the wheat-*Aegilops longissima* chromatin breakpoints in powdery mildew resistant introgression lines. Theor Appl Genet 91: 738–743.

Dubcovsky, J., M.C. Luo, G.Y. Zhong, R. Bransteitter, A. Desai, A. Kilian, A. Kleinhofs & J. Dvorak, 1996. Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143: 983–999.

Faris, J.D., J.P. Fellers, S.A. Brooks & B.S. Gill, 2003. A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164: 311–321.

Faris, J.D., K.M. Haen & B.S. Gill, 2000. Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics 154: 823–835.

Feuillet, C. & B. Keller, 1999. High gene density is conserved at syntenic loci of small and large grass genomes. Proc Natl Acad Sci USA 96: 8265–8270.

Feuillet, C., S. Travella, N. Stein, L. Albar, A. Nublat & B. Keller, 2003. Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100: 15253–15258.

Flor, H.H., 1955. Host–parasite interaction in flax rust—its genetics and other implications. Phytopathology 45: 680–685.

Frary, A., T.C. Nesbitt, A. Frary, S. Grandillo, E. van der Knaap, B. Cong, J.P. Liu, J. Meller, R. Elber, K.B. Alpert & S.D. Tanksley, 2000. *fw2.2*: A quantitative trait locus key to the evolution of tomato fruit size. Science 289: 85–88.

Friebe, B., M. Heun, N. Tuleen, F.J. Zeller & B.S. Gill, 1994. Cytogenetically monitored transfer of powdery mildew resistance from rye to wheat. Crop Sci 34: 621–625.

Friebe, B., J. Jiang, W.J. Raupp, R.A. McIntosh & B.S. Gill, 1996. Characterization of wheat-alien translocation conferring resistance to diseases and pests: Current status. Euphytica 91: 59–87.

Foote, T., M. Roberts, N. Kurata, T. Sasaki & G. Moore, 1997. Detailed comparative mapping of cereal chromosome regions corresponding to the *Ph1* locus in wheat. Genetics 147: 801–807.

Gale, M.D. & K.M. Devos, 1998. Comparative genetics in the grasses. Proc Natl Acad Sci USA 95: 1971–1974.

Gill, K.S., B.S. Gill, T.R. Endo & E.V. Boyko, 1996a. Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143: 1001–1012.

Gill, K.S., B.S. Gill, T.R. Endo & T. Taylor, 1996b. Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144: 1883–1891.

Griffey, C.A. & M.K. Das, 1994. Inheritance of adult-plant resistance to powdery mildew in Massey and Knox 62 winter wheats. Crop Sci 34: 641–646.

Griffey, C.A., M.K. Das & E.L. Stromberg, 1993. Effectiveness of adult-plant resistance in reducing grain yield loss to powdery mildew in winter wheat. Plant Dis 77: 618–622.

Gupta, P.K., R.K. Varshney, P.C. Sharma & B. Ramesh, 1999. Molecular markers and their applications in wheat breeding. Plant Breed 118: 369–390.

Gupta, P.K., H.S. Balyan, K.J. Edwards, P. Isaac, V. Korzun, M. Röder, M.-F. Gautier, P. Joudrier, A.R. Schlatter, J. Dubcovscy, R.C. De la Pena, M. Khairallah, G. Penner, M.J. Hayden, P. Sharp, B. Keller, R.C.C. Wang, J.P. Hardouin, P. Jack & P. Leroy, 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105: 413–422.

Guyomarc'h, H., P. Sourdille, G. Charmet, K.J. Edwards & M. Bernard, 2002. Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104: 1164–1172.

Halterman, D.A., F. Wei & R.P. Wise, 2003. Powdery mildew-induced *Mla* mRNAs are alternatively spliced and contain multiple upstream open reading frames. Plant Physiol 131: 558–567.

Halterman, D., F. Zhou, F. Wei, R.P. Wise & P. Schulze-Lefert, 2001. The MLA6 coiled-coil, NBS-LRR protein confers *AvrMla6*-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. Plant J 25: 335–348.

Hartl, L., H. Weiss, U. Stephan, F.J. Zeller & A. Jahoor, 1995. Molecular identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). Theor Appl Genet 90: 601–606.

Hartl, L., V. Mohler, F.J. Zeller, S.K.L. Hsam & G. Schweizer, 1999. Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat. Genome 42: 322–329.

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

Heun, M., B. Friebe & W. Bushuk, 1990. Chromosomal location of the powdery mildew resistance gene of Amigo wheat. Phytopathology 80: 1129–1133.

Hautea, R.A., W.R. Coffman, M.E. Sorrells & G.C. Bergstrom, 1987. Inheritance of partial resistance to powdery mildew in spring wheat. Theor Appl Genet 73: 609–615.

Hsam, S.L.K., X.Q. Huang, F. Ernst, L. Hartl & F.J. Zeller, 1998. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em. Thell.). 5. Alleles at the *Pm1* locus. Theor Appl Genet 96: 1129–1134.

Hsam, S.L.K., X.Q. Huang & F.J. Zeller, 2001. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em. Thell.). 6. Alleles at the *Pm5* locus. Theor Appl Genet 102: 127–133.

Hsam, S.L.K., I.F. Lapochkina & F.J. Zeller, 2003. Chromosomal location of genes for resistance to powdery mildew in common

wheat (*Triticum aestivum* L. em Thell.). 8. Gene *Pm32* in a wheat-*Aegilops speltoides* translocation line. Euphytica 133: 367–370.

Hsam, S.L.K., V. Mohler, L. Hartl, G. Wenzel & F.J. Zeller, 2000. Mapping of powdery mildew and leaf rust resistance genes on the wheat-rye translocated chromosome T1BL.1RS using molecular and biochemical markers. Plant Breed 119: 87–89.

Hsam, S.L.K. & F.J. Zeller, 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, S.L.K. & F.J. Zeller, 2002. Breeding for powdery mildew resistance in common wheat (*Triticum aestivum* L.). In: R.R. Belanger, W.R. Bushnell, A.J. Dik & T.L.W. Carver (Eds.), The Powdery Mildews, A Comprehensive Treatise, pp. 219–238. St. Paul, MN, USA.

Hu, X.Y., H.W. Ohm & I. Dweikat, 1997. Identification of RAPD markers linked to the gene *Pm1* for resistance to powdery mildew in wheat. Theor Appl Genet 94: 832–840.

Huang, X.Q., 1999. Location and identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.) using monosomic analysis and molecular markers. Ph.D. Thesis, Technical University Munich, Freising-Weihenstephan, Germany (in German).

Huang, L., S.A. Brooks, W. Li, J.P. Fellers, H.N. Trick & B.S. Gill, 2003c. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164: 655–664.

Huang, X.Q., A. Börner, M.S. Röder & M.W. Ganal, 2002a. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor Appl Genet 105: 699–707.

Huang, X.Q., H. Cöster, M.W. Ganal & M.S. Röder, 2003b. Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). Theor Appl Genet 106: 1379–1389.

Huang, X.Q., S.L.K. Hsam & F.J. Zeller, 2002b. Chromosomal location of genes for resistance to powdery mildew in Chinese wheat lines Jieyan 94-1-1 and Siyan 94-1-2. Hereditas 136: 212–218.

Huang, X.Q., S.L.K. Hsam & F.J. Zeller, 1997a. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). IX. Cultivars, landraces and breeding lines grown in China. Plant Breed 116: 233–238.

Huang, X.Q., S.L.K. Hsam & F.J. Zeller, 1997b. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em. Thell.). 4. Gene *Pm24* in Chinese landrace 'Chiyacao'. Theor Appl Genet 95: 950–953.

Huang, X.Q., S.L.K. Hsam & F.J. Zeller, 1997c. Genetic analysis of powdery mildew resistance of four common wheat cultivars. In: L. Tvaruzek (Ed.), Protection of Cereal Crops against Harmful Organisms, pp. 190–193. Kromeriz, Czech Republic.

Huang, X.Q., S.L.K. Hsam & F.J. Zeller, 2000a. Chromosomal location of two novel genes for resistance to powdery mildew in Chinese landraces (*Triticum aestivum* L. em. Thell.). J Genet & Breed 54: 311–317.

Huang, X.Q., S.L.K. Hsam, F.J. Zeller, G. Wenzel & V. Mohler, 2000b. Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding.Theor Appl Genet 101: 407–414.

Huang, X.Q. & M.S. Röder, 2003. High-density genetic and physical mapping of the powdery mildew resistance gene *Pm24* on chromosome 1D of wheat. In: H.N. Pogna, M. Romano, E.A. Pogna and G. Galterio (Eds.), Proceedings of the 10th International Wheat Genetics Symposium, September 1–6, 2003, Paestum, Italy, pp. 961–964.

Huang, X.Q., M.S. Röder, E. Pestsova, A. Börner & M.W. Ganal, 2001. Development and use of wheat microsatellite markers for the characterization of germplasm of hexaploid wheat (*Triticum aestivum* L.). In: Proceedings of the Plant & Animal Genome IX Conference, January 13–17, 2001, P260, San Diego, CA, USA.

Huang, X.Q., L.X. Wang, M.X. Xu & M.S. Röder, 2003a. Microsatellite mapping of the powdery mildew resistance gene*Pm5e* in common wheat (*Triticum aestivum* L.). Theor Appl Genet 106: 858–865.

Huang, X.Q., F.J. Zeller, S.L.K. Hsam, G. Wenzel & V. Mohler, 2000c. Chromosomal location of AFLP markers in common wheat utilizing nulli-tetrasomic stocks. Genome 43: 298–305.

Hulbert, S.H., C.A. Webb, S.M. Smith & Q. Sun, 2001. Resistance gene complexes: Evolution and utilization. Annu Rev Phytopathol 39: 285–312.

Iqbal, M.J. & A.L. Rayburn, 1995. Identification of the 1RS rye chromosomal segment in wheat by RAPD analysis. Theor Appl Genet 91: 1048–1053.

Järve, K., H.O. Peusha, J. Tsymbalova, S. Tamm, K.M. Devos & T.M. Enno, 2000. Chromosomal location of a *Triticum timopheevii* – derived powdery mildew resistance gene transferred to common wheat. Genome 43: 377–381.

Jia, J., K.M. Devos, S. Chao, T.E. Miller, S.M. Reader & M.D. Gale, 1996. RFLP-based maps of the homoeologous 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.

Jones, S.S., T.D. Murray & R.E. Allan, 1995. Use of alien genes for the development of disease resistance in wheat. Annu Rev Phytopathol 33: 429–443.

Jørgensen, J.H., 1973. Gene *Pm6* for resistance to powdery mildew in wheat. Euphytica 22: 43.

Kasuga, T., S.S. Salimath, J. Shi, M. Gijzen, R.I. Buzzell & M.K. Bhattacharyya, 1997. High resolution genetic and physical mapping of molecular markers linked to the *Phytophthora* resistance gene *Rps*1-k in soybean. Mol Plant-Microbe Interact 10: 1035–1044.

Keim, P., J.M. Schupp, S.E. Travis, K. Clayton, T. Zhu, L.A. Shi, A. Ferreira & D.M. Webb, 1997. A high-density soybean genetic map based upon AFLP markers. Crop Sci 37: 537–543.

Keller, M., B. Keller, G. Schachermayr, M. Winzeler, J.E. Schmid, P. Stamp & M.M. Messmer, 1999. Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. Theor Appl Genet 98: 903–912.

Kintzios, S., A. Jahoor & G. Fischbeck, 1995. Powdery mildew resistance genes *Mla29* and *Mla32* in *H. spontaneum*-derived winter barley lines. Plant Breed 114: 265–266.

Kilian, A., J. Chen, F. Han, B. Steffenson & A. Kleinhofs, 1997. Towards map-based cloning of the barley stem rust resistance genes *Rpg1* and *rpg4* using rice as an intergenomic cloning vehicle. Plant Mol Biol 35: 187–195.

Knott, D.R., 1989. The Wheat Rusts: Breeding for Resistance. Springer-Verlag, Berlin and Heidelberg.

Konieczny, A. & F.M. Ausubel, 1993. A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype specific PCR-based markers. Plant J 4: 403–410.

Law, C.N. & M.S. Wolfe, 1966. Location of genetic factors for mildew resistance and ear emergence time on chromosome 7B of wheat. Can J Genet Cytol 8: 462–479.

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

Lebsock, K.L. & L.W. Briggle, 1974. Gene *Pm5* for resistance to *Erysiphe graminis* f. sp. *tritici* in Hope wheat. Crop Sci 14: 561–563.

Leister, D., A. Ballvora, F. Salamini & C. Gebhardt, 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genet 14: 421–429.

Lijavetzky, D., G. Muzzi, T. Wicker, B. Keller, R. Wing & J. Dubcovsky, 1999. Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. Genome 42: 1176–1182.

Liu, S.X., C.A. Griffey & M.A. Saghai Maroof, 2001. Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. Crop Sci 41: 1268–1275.

Liu, D.J., J.Y. Liu, W.J. Tao & P.D. Chen, 1998. Molecular markers and breeding wheat for powdery mildew resistance. In: A.E. Slinkard (Ed.), Proceedings of the 9th International Wheat Genet Symposium, University Extension Press, Saskatoon, Canada, Vol. 3, pp. 128–131.

Liu, J., D. Liu, W. Tao, W. Li, S. Wang, P. Chen, S. Cheng & D. Gao, 2000. Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. Plant Breed 119: 21–24.

Liu, Z., Q. Sun, Z. Ni, E. Nevo & T.M. Yang, 2002. Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer. Euphytica 123: 21–29.

Liu, Z., Q. Sun, Z. Ni & T. Yang, 1999. Development of SCAR markers linked to the *Pm21* gene conferring resistance to powdery mildew in common wheat. Plant Breed 118: 215–219.

Lutz, J., S.L.K. Hsam, E. Limpert & F.J. Zeller, 1995. Chromosomal location of powdery mildew resistance genes in *Triticum aestivum* L. (common wheat). 2. Genes *Pm2* and *Pm19* from *Aegilops squarrosa* L. Heredity 74: 152–156.

Lutz, J., E. Limpert, P. Bartos & F.J. Zeller, 1992. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). I. Czechoslovakian cultivars. Plant Breed 108: 33–39.

Ma, Z.Q., M.E. Sorrells & S.D. Tanksley, 1994. RELP markers linked to powdery mildew resistance genes *Pm1, Pm2, Pm3*, and *Pm4* in wheat. Genome 37: 871–875.

McIntosh, R.A. & E.P. Baker, 1970. Cytogenetical studies in wheat. IV. Chromosome location and linkage studies involving the *Pm2* locus for powdery mildew resistance. Euphytica 19: 71–77.

McIntosh, R.A., G.E. Hart, M.D. Gale & W.J. Rogers, 1998. Catalogue of gene symbols for wheat. In: A.E. Slinkard (Ed.), Proceedings of the 9th International Wheat Genet Symposium, University Extension Press, University of Saskatchewan, Saskatoon, Canada, Vol. 5, pp. 1–235.

Michelmore, R.W., I. Paran & R.V. Kesseli, 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828–9832.

Mingeot, D., N. Chantret, P.V. Baret, A. Dekeyser, N. Boukhatem, P. Sourdille, G. Doussinault & J.M. Jacquemin, 2002. Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible genetic backgrounds. Plant Breed 121: 133–140.

Mohler, V., S.L.K. Hsam, F.J. Zeller & G. Wenzel, 2001. An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the *Pm8/Pm17* locus of common wheat. Plant Breed 120: 448–450.

Mohler, V. & A. Jahoor, 1996. Allele specific amplification of polymorphic sites for the detection of powdery mildew resistance loci in cereals. Theor Appl Genet 93: 1078–1082.

Moullet, O., H.B. Zhang & E.S. Lagudah, 1999. Construction and characterisation of a large DNA insert library from the D genome of wheat. Theor Appl Genet 99: 303–313.

Neu, C., N. Stein & B. Keller, 2002. Genetic mapping of the *Lr20–Pm1* resistance locus reveals suppressed recombination on chromosome arm 7AL in hexaploid wheat. Genome 45: 737–744.

Olson, M., L. Hood, C. Cantor & D. Doststein, 1989. A common language for physical mapping of the human genome. Science 254: 1434–1435.

Paran, I. & R.W. Michelmore, 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85: 985–993.

Peusha, H., T. Enno & O. Priilinn, 2000. Chromosome location of powdery mildew resistance genes and cytogenetic analysis of meiosis in common wheat cultivar Meri. Hereditas 132: 29–34.

Peusha, H., S.L.K. Hsam & F.L. Zeller, 1996. Chromosome location of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em. Thell.). 3. Gene *Pm22* in cultivar Virest. Euphytica 91: 149–152.

Plaschke, J., M.W. Ganal & M.S. Röder, 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91: 1001–1007.

Powers, H.R. & W.J. Sando, 1960. Genetic control of the host–parasite relationship in wheat powdery mildew. Phytopathology 50: 454–457.

Qi, L.L., M.S. Cao, P.D. Chen, W.L. Li & D.J. Liu, 1996. Identification, mapping, and application of polymorphic DNA associated with resistance gene *Pm21* of wheat. Genome 39: 191–197.

Reader, S.M. & T.E. Miller, 1991. The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer. Euphytica 53: 57–60.

Ren, S.X., R.A. McIntosh, P.J. Sharp & T.T. The, 1996. A storage-protein marker associated with the suppressor of *Pm8* for powdery mildew resistance in wheat. Theor Appl Genet 93: 1054–1060.

Robe, P. & G. Doussinault, 1995. Genetic analysis of powdery mildew resistance of a winter-wheat line, RE714, and identification of a new specific-resistance gene. Plant Breed 114: 387–391.

Roberts, J.J. & R.M. Caldwell, 1970. General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in 'Knox' wheat. Phytopathology 60: 1310.

Röder, M.S., X.Q. Huang & M.W. Ganal, 2004. Wheat microsatellites in plant breeding – Potential and implications. In: H. Lörz & G. Wenzel (Eds.), Molecular Markers in Plant Breeding and Crop Improvement. Springer-Verlag, Heidelberg, in press.

Röder, M.S., V. Korzun, B.S. Gill & M.W. Ganal, 1998a. The physical mapping of microsatellite markers in wheat. Genome 41: 278–283.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy & M.W. Ganal, 1998b. A microsatellite map of wheat. Genetics 149: 2007–2023.

Rong, J.K., E. Millet, J. Manisterski & M. Feldman, 2000. A new powdery mildew resistance gene: Introgression from wild emmer

into common wheat and RFLP-based mapping. Euphytica 115: 121–126.

Sandhu, D.J., A. Champoux, S.N. Bondareva & K.S. Gill, 2001. Identification and physical localization of useful genes and markers to a major gene-rich region on wheat group 1S chromosomes. Genetics 157: 1735–1747.

Schwarz, G., W. Michalek, V. Mohler, G. Wenzel & A. Jahoor, 1999. Chromosome landing at the *Mla* locus in barley (*Hordeum vulgare* L.) by means of high-resolution mapping with AFLP markers. Theor Appl Genet 98: 521–530.

Sears, E.R., 1954. The aneuploids of common wheat. Res Bull 572, Univ Miss, Coll Agric Exp Stn. 59 pp.

Sears, E.R. & L.W. Briggle, 1969. Mapping the gene *Pm1* for resistance to *Erysiphe graminis* f. sp. *tritici* on chromosome 7A of wheat. Crop Sci 9: 96–97.

Schönfeld, M., A. Ragni, G. Fischbeck & A. Jahoor, 1996. RFLP mapping of three new loci for resistance genes to powdery mildew (*Erysiphe graminis* f. sp. hordei) in barley. Theor Appl Genet 93: 48–56.

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

Shen, Q.H., F. Zhou, S. Bieri, T. Haizel, K. Shirasu & P. Schulze-Lefert, 2003. Recognition specificity and *RAR1/SGT1* dependence in barley *Mla* disease resistance genes to the powdery mildew fungus. Plant Cell 15: 732–744.

Shi, Z.X., X.M. Chen, R.F. Line, H. Leung & C.R. Wellings, 2001. Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. Genome 44: 509–516.

Shi, A.N., S. Leath & J.P. Murphy, 1998. A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathology 88: 144–147.

Singrün, Ch., S.L.K. Hsam, L. Hartl, F.J. Zeller & V. Mohler, 2003. Powdery mildew resistance gene *Pm22* in cultivar Virest is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L. em Thell.). Theor Appl Genet 106: 1420–1424.

Smith, D.B. & R.B. Flavell, 1975. Characterization of the wheat genome by renaturation kinetics. Chromosoma 50: 223–242.

Song, Q.J., E.W. Fickus & P.B. Cregan, 2002. Characterization of trinucleotide SSR motifs in wheat. Theor Appl Genet 104: 286–293.

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

Spielmeyer, W., L. Huang, H. Bariana, A. Laroche, B.S. Gill & E.S. Lagudah, 2000. NBS-LRR sequence family is associated with leaf and stripe rust resistance on the end of homoeologous chromosome group 1S of wheat. Theor Appl Genet 101: 1139–1144.

Sourdille, P., P. Robe, M.-H. Tixier, G. Doussinault, M.-T. Pavoine & M. Bernard, 1999. Location of *Pm3g*, a powdery mildew resistance allele in wheat, by using a monosomic analysis and by identifying associated molecular markers. Euphytica 110: 193–198.

Stein, N., C. Feuillet, T. Wicker, E. Schlagenhauf & B. Keller, 2000. Subgenome chromosome walking in wheat: A 450 kb physical contig in *Triticum monococcum* L. spans the *Lr10* resistance locus in hexaploid wheat (*T. aestivum* L.). Proc Natl Acad Sci USA 97: 13436–13441.

Tanksley, S.D. & J.C. Nelson, 1996. Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191–203.

Tao, W., D. Liu, J. Liu, Y. Feng & P. Chen, 2000. Genetic mapping of the powdery mildew resistance gene *Pm6* in wheat by RFLP analysis. Theor Appl Genet 100: 564–568.

The, T.T., R.A. McIntosh & F.G.A. Bennett, 1979. Cytogenetical studies in wheat. IX. Monosomic analysis, telocentric mapping and linkage relationship of gene *Sr21*, *Pm4* and *Mle*. Aust J Biol Sci 32: 115–125.

Tosa, Y. & K. Sakai, 1990. The genetics of resistance of hexaploid wheat to the wheatgrass powdery mildew fungus. Genome 33: 225–230.

Tosa, Y., H. Tokunaga & H. Ogura, 1988. Identification of a gene for resistance to wheatgrass powdery mildew fungus in the common wheat cultivar Chinese Spring. Genome 30: 612–614.

Tosa, Y., H. Tsujimoto & H. Ogura, 1987. A gene involved in the resistance of wheat to wheatgrass powdery mildew fungus. Genome 29: 850–852.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper & M. Zabeau, 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res 21: 4407–4414.

Wei, F., K. Gobelman-Werner, S.M. Morroll, J. Kurth, L. Mao, R. Wing, D. Leister, P. Schulze-Lefert & R.P. Wise, 1999. The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153: 1929–1948.

Welsh, J. & M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 19: 861–866.

Wicker, T., N. Stein, L. Albar, C. Feuillet, E. Schlagenhauf & B. Keller, 2001. Analysis of a contiguous 211 kb sequence in diploid wheat (*Triticum monococcum* L.) reveals multiple mechanisms of genome evolution. Plant J 26: 307–316.

Williams, G.K., A.R. Kubelik, J.L. Kenneth, A. Rafalski & V.T. Scott, 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535.

Wricke, G., P. Dill & P. Senft, 1996. Linkage between a major gene for powdery mildew resistance and an RFLP marker on chromosome 1R of rye. Plant Breed 115: 71–73.

Xie, C., Q. Sun, Z. Ni, T. Yang, E. Nevo & T. Fahima, 2003. Chromosomal location of a *Triticum dicoccoides*-derived powdery mildew resistance gene in common wheat by using microsatellite markers. Theor Appl Genet 106: 341–345.

Yahiaoui, N., P. Srichumpa, R. Dudler & B. Keller, 2004. Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J 34: 528–538.

Yamamoto, T., Y. Kuboki, S.Y. Lin, T. Sasaki & M. Yano, 1998. Fine mapping of quantitative trait loci *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97: 37–44.

Yan, G.P., X.M. Chen, R.F. Line & C.R. Wellings, 2003. Resistance gene-analog polymorphism markers co-segregating with the *Yr5* gene for resistance to wheat stripe rust. Theor Appl Genet 106: 636–643.

Yano, M., Y. Katayose, M. Ashikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto, Y. Umehara, Y. Nagamura & T. Sasaki, 2000. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12: 2473–2484.

Yu, Y.G., G.R. Buss & M.A. Saghai Maroof, 1996. Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93: 11751–11756.

Zeller, F.J. & S.L.K. Hsam, 1996. Chromosomal location of a gene suppressing powdery mildew resistance genes *Pm8* and *Pm17* in common wheat (*Triticum aestivum* L. em. Thell.). Theor Appl Genet 93: 38–40.

Zeller, F.J. & S.L.K. Hsam, 1998. Progress in breeding for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). In: A.E. Slinkard (Ed.), Proceedings of the 9th International Wheat Genet Symposium, University Extension Press, Saskatoon, Canada, Vol. 1, pp. 178–180.

Zeller, F.J., X.Q. Huang, E.V. Paderina, A. Collaku, K. Kowalczyk, M. Aslam, H. Peusha & S.L.K. Hsam, 1998. Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em. Thell.). XII. Cultivars and landraces grown in Mediterranean countries. Plant Genet Resources Newslett 116: 5–8.

Zeller, F.J., L. Kong, L. Hartl, V. Mohler & S.L.K. Hsam, 2002. Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 7. Gene *Pm29* in line Pova. Euphytica 123: 187–194.

Zeller, F.J., J. Lutz, E.I. Reimlein, E. Limpert & J. Koening, 1993b. Identification of mildew resistance genes in common wheat (*Triticum aestivum* L.). II. French cultivars. Agronomie 13: 201–207.

Zheng, Y.L., M.C. Lou, C. Yen & J.L. Yang, 1992. Chromosome location of a new crossability gene in common wheat. Wheat Inform Service 75: 36–40.

Zeller, F.J., J. Lutz & U. Stephan, 1993a. Chromosome location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L.). 1. *Mlk* and other alleles at the *Pm3* locus. Euphytica 68: 223–229.

Zhou, F., J. Kurth, F. Wei, C. Elliot, G. Valè, N. Yahiaoui, B. Keller, S. Somerville, R. Wise & P. Schulze-Lefert, 2001. Cell-autonomous expression of barley *Mla1* confers race-specific resistance to the powdery mildew fungus via a *Rar1*-independent signaling pathway. Plant Cell 13: 337–350.