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Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer

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Summary

Powdery mildew caused by *Erysiphe graminis* f. sp. *tritici* is one of the most important wheat diseases in many regions of the world. A powdery mildew resistance gene, originating from wild emmer wheat (*Triticum dicoccoides*) accession 'C20', from Rosh Pinna, Israel, was successfully transferred to hexaploid wheat through crossing and backcrossing. Genetic analysis indicated that a single dominant gene controls the powdery mildew resistance at the seedling stage. Segregating BC_1F_2 progenies of the cross 87-1/C20//2∗8866 were used for bulked segregant analysis (BSA). The PCR approach was used to generate polymorphic DNA fragments between the resistant and susceptible DNA pools by use of 10-mer random primers, STS primers, and wheat microsatellite primers. Three markers, *Xgwm159/430, Xgwm159/460*, and *Xgwm159/500*, were found to be linked to the resistance gene. After evaluating the polymorphic markers in two segregating populations, the distance between the markers and the mildew resistance gene was estimated to be 5–6 cM. By means of Chinese Spring nullisomic-tetrasomics and ditelosomics, the polymorphic markers and the resistance gene were assigned to chromosome arm 5BS and were physically mapped on the gene rich regions of fragment length (FL) 0.41–0.43 by Chinese Spring deletion lines. As no powdery mildew resistance gene has been reported on chromosome arm 5BS, the mildew resistance gene originating from C20 should be a new gene and is designated *Pm30*.

Introduction

Powdery mildew caused by *Erysiphe graminis* (*Blumeria graminis*) f. sp. *tritici* is one of the most important wheat diseases in many regions of the world. Disease resistance has proved an effective and environmentally sound method for control of wheat pathogens. However, major gene resistance tends to become ineffective within a very short period, creating a dilemma for breeders (Zeller & Hsam, 1998; Zhuang & Li, 1993). Diversification of sources of resistance has been suggested as a remedy of the problem, provided that an adequate number of resistance genes are available. So far, 29 genes for resistance to wheat powdery mildew (*Pm1–Pm29*) have been identified and assigned to specific chromosomes or chromosome arms (McIntosh et al., 2000). Some of the genes were transferred from wheat relatives. The wild tetraploid wheat, *Triticum dicoccoides* (2n = 4x = 28; genome AABB), distributed in the Near East Fertile Crescent, is considered the progenitor of cultivated tetraploid and hexaploid wheats (Nevo & Beiles, 1989). Wild emmer wheat was found to be polymorphic for resistance to several pathogens, including stripe rust (Gerechter-Amitai & Grama, 1974; Nevo et al., 1986), powdery mildew (Moseman et al., 1984), leaf rust (Moseman et al., 1985) and stem rust (Nevo et al., 1991). Genes conferring resistance against powdery mildew, yellow rust and leaf rust in wheat derived from wild emmer were reported by Reader & Miller (1991),

Table 1. Chinese Spring deletion lines used for physical mapping

Deletion lines	Description	2n	Fragment length (FL)
Chinese Spring	21″	42	
d5BS-2	20″+1″[d5BS-2]	42	0.42
d5BS-3	19″+1″[d5BS-3]+1′[d2AS-2]+1′[2A]	42	0.41
d5BS-4	18″+1″[d5BS-4]+1″[d7AS-1]+4B‴[4B]	43	0.43
d5BS-5	19″+1″[d5BS-5]+1′[d5AS-8]+1′[5A]	42	0.71
d5BS-6	20″+1″[d5BS-6]	42	0.81
d5BS-8	20″+1″[d5BS-8]	42	0.56
d5BS-9	18″+1″[d5BS-9]+1⁗[1D]	42	0.43

Rong et al. (2000), Gerechter-Amitai et al. (1989), Peng et al. (1999), and Dyck (1994), respectively.

Molecular markers such as restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), and random amplified polymorphic DNAs (RAPD) have been used to tag powdery mildew resistance genes in wheat. To date, RFLP, AFLP, and RAPD markers linked to some powdery mildew resistance genes, including *Pm1, Pm2, Pm3, Pm4, Pm5, Pm12, Pm13, Pm18, Pm21, Pm24 Pm25* and*Pm26*, have been identified (Hartl et al., 1993, 1995, 1999; Ma et al., 1994; Jia et al., 1996; Qi et al., 1996; Hu et al., 1997; Shi et al., 1998; Cenci et al., 1999; Keller et al., 1999; Rong et al., 2000; Huang et al., 2000).

Microsatellite markers have the advantages of being faster and less expensive, and of requiring smaller DNA samples and less labour than RFLP markers. They tend to show much higher abundance and a higher level of sequence polymorphisms, and they are codominant and highly reproducible (Lagercrantz et al., 1993; Wang et al., 1994). Microsatellite-based maps have been established in many species including *Arabidopsis* (Bell & Ecker, 1994), soybean (Akkaya et al., 1992), rice (Wu & Tanksley, 1993), corn (Senior & Heun, 1993; Taramino & Tingey, 1996), barley (Saghai-Maroof et al., 1994; Becker & Heun, 1995; Liu et al., 1996) and wheat (Röder et al., 1998a). Some important genes in wheat, such as the stripe rust resistance gene *YrH52* (Peng et al., 1999), dwarfing genes *Rht8* (Korzun et al., 1998) and *Rht12* (Korzun et al., 1997), have been tagged by microsatellites.

The present paper reports the identification and molecular mapping of a powdery mildew resistance gene derived from wild emmer accession ‘C20’ using a PCR approach and bulked segregant analysis (BSA) (Michelmore et al., 1991).

Materials and methods

Plant materials

Wild emmer (*T. dicoccoides,* 2n = 4x = 28, AABB) accession ‘C20’ (with IT value 0, unpublished data), from the population of Rosh Pinna, Israel, was the donor of the mildew resistance gene. Susceptible common wheat (*T. aestivum,* 2n = 6x = 42, AABBDD) line ‘87-1’ (with IT value 4) was used as a parent for crossing and another elite line ‘8866’ (with IT value 4) from the Beijing region was selected for backcrossing to improve the agronomic traits. The BC_1F_2 generation of cross 87-1/C20//2∗8866 was used to construct a segregating population for bulked segregant analysis after checking the root tip chromosome number of each plant. Thirty-seven plants with 42 chromosomes and their BC_1F_3 families were screened for powdery mildew reaction. DNA samples from six homozygous resistant and six homozygous susceptible plants of the BC_1F_2 generation were pooled in equal amounts to generate contrasting DNA pools. Another segregating population consisting of 59 individuals from a BC_1F_3 family of the same cross was used to estimate recombination between markers and the resistance gene. Chinese Spring nullisomic-tetrasomics and ditelosomics (kindly provided by Dr Hongjie Li, Hebei Academy of Agricultural and Forestry Science, Shijiazhuang, China) were used for chromosome assignment of the markers. Chinese Spring deletion lines of chromosome arm 5BS (kindly provided by Drs WJ Raupp and BS Gill, Wheat Genetics Resource Centre,

Kansas State University, USA) were used for physical mapping (Table 1).

Powdery mildew test

Seeds of each plant or family (25–40 individuals), the common wheat parents '87-1', '8866' and a highly susceptible cultivar 'Yanda 1817' were planted in 8–10 cm diameter pots. Seedlings were artificially inoculated, when the first leaf was fully expanded, with race 15, a local isolate of *E. graminis* f. sp. *tritici,* virulent to *Pm1, Pm3, Pm5* and *Pm8* but avirulent to the 'C20' accession and its derivatives. Inoculations were performed by dusting or brushing conidia from neighboring sporulating susceptible seedlings of 'Yanda 1817' onto the test seedlings. Reactions were scored 15 days after inoculation, when 'Yanda 1817' was heavily infected, on a 0, 0;, and 1 to 4 scale, with 0 representing no visible symptoms, 0; representing necrotic flecks, and 1, 2, 3, 4 for highly resistant (necrosis with low sporulation), resistant (necrosis with medium sporulation), susceptible (no necrosis with medium to high sporulation), and highly susceptible (no necrosis with full sporulation) reactions, respectively. According to the reaction of BC_1F_3 or BC_1F_4 families, the corresponding BC_1F_2 or BC_1F_3 individual plants were classified into three types, namely homozygous resistant (RR, all plants in BC_1F_3 or BC_1F_4 family being resistant), heterozygous (Rr, most of the plants in an BC_1F_3 or BC_1F_4 family were resistant and the minority were susceptible), and homozygous susceptible (rr, all plants in BC_1F_3 or BC_1F_4 family being susceptible).

DNA isolation

Total DNA was extracted from the healthy leaves of resistant or susceptible plants by the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al., 1984) with minor modifications.

Polymerase chain reaction and electrophoresis

All PCR reactions were performed in 25 μl volumes in a Perkin-Elmer 480 Thermocycler. RAPD reactions contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each deoxynucleotide, 50ng 10-mer Operon (Operon Technologies, Calif., USA) or UBC (University of British Columbia, Canada) primer, 50 to 100 ng genomic DNA, and 1.25 U Taq DNA polymerase. The amplification was performed at 94 °C for 3 min, followed by 45 cycles at 94 °C for 1 min, at 36 °C for 2 min, and at 72 °C for 2 min, with a final extension at 72 °C for 10 min. PCR products were separated on 1.4% agarose gels and visualized under UV light after ethidium bromide staining. Two repeat amplifications for each primer were conducted to ensure the repeatability of RAPD reactions. STS primers from barley and wheat genome mapping projects were kindly provided by Drs TK Blake and LE Talbert (Montana State University, USA), respectively. PCR conditions were the same as those described by Tragoonrung et al. (1992) and Talbert et al. (1994).

Wheat microsatellite primers used were kindly provided by Drs MS Röder (Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany) and MD Gale (John Innes Centre, UK) or synthesized according to the published sequences (Plaschke et al., 1995). Amplification conditions were optimized according to those described by Plaschke et al. (1995) and Bryan et al. (1997). The reaction mixture contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ (sometimes 2.0 to 2.5 mM), 200 μM of each deoxynucleotide, 50 ng of each primer, 50 to 100 ng genomic DNA, and 1.25 U Taq DNA polymerase. The amplifications were performed at 94 °C for 3 min, followed by 45 cycles at 94 °C for 1 min, at 50 to 63 °C (depending on the microsatellite primer pair) for 1 min, and at 72 °C for 2 min, with a final extension at 72 °C for 5 min. PCR products were mixed with one-tenth volume of loading buffer (50 mM Tris-HCl, pH 8.0; 50 mM EDTA, 1% SDS, 0.25% Bromophenol blue, 0.25% xylene cyanol, 50% glycerol) and 12 μl were loaded for electrophoresis in vertical, non-denaturing 8% (ethidium bromide staining) or 10% (silver staining) polyacrylamide gels (Bio-Rad protein cell II, 0.75 mm thick and 20 cm long) in 1 $\times$ TBE (90 mM Tris-borate (pH 8.3), 2 mM EDTA) at 300 v for 4 h.

Linkage analysis

Genetic map distances were calculated by means of the Kosambi mapping function (Kosambi, 1944).

Chromosome arm assignment and physical mapping

Polymorphic loci linked to the mildew resistance gene were detected in a set of Chinese Spring nullisomic-tetrasomics. PCR reactions were then performed on the corresponding ditelosomics in order to locate the polymorphic loci on the specific chromosome arms.

Table 2. Genetic analysis of the powdery mildew resistance gene *Pm30*

Pedigree	Generation	Genotypes			χ^2 (1:2:1)	P	Segregation data[1]
		RR	Rr	rr			
87-1/C20//2∗8866	BC1F2	6	23	8	2.4054	0.25–0.50	A
87-1/C20//2∗8866	BC1F3	15	29	18	0.5484	0.75–0.90	A
		R-		rr	χ^2 (3:1)		
87-1/C20//2∗8866 - 13	BC1F3	91	#27	0.2825	0.50–0.75	A	
87-1/C20//2∗8866	BC1F3	305	#95	0.3333	0.50–0.75	B	
87-1/C20//2∗8866	BC1F4	665	#218	0.0457	0.75–0.90	B	
87-1/C20//4∗8866 - 2	BC3F2	111	#33	0.3333	0.50–0.75	A	
87-1/C20//4∗8866 - 7	BC3F2	96	#33	0.0233	0.75–0.90	A	
87-1/C20//4∗8866 - 10	BC3F2	102	#35	0.0219	0.75–0.90	A	

[1]: A: Progenies of single plants; B: Pooled data of segregating lines, i.e. RR + Rr: rr.

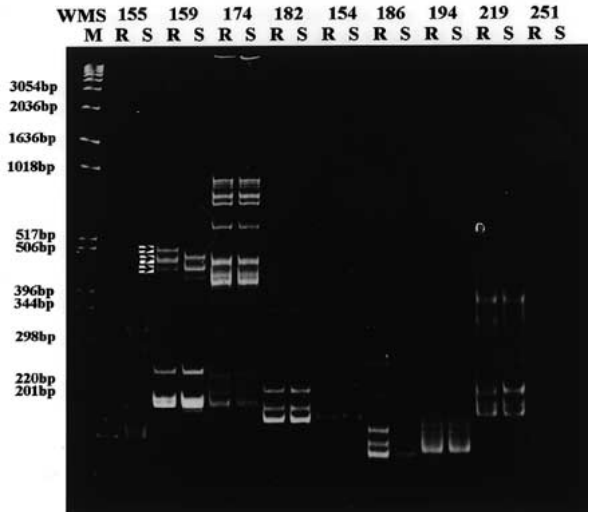

Figure 1. Polymorphisms detected by wheat microsatellite primer *WMS159* between the resistant (R) and susceptible (S) DNA pools. Amplification products were separated on 8% non-denaturing PAGE, stained with ethidium bromide. M: 1kb DNA ladder (Gibco BRL). Arrows indicate the three polymorphic fragments amplified by *WMS159* in resistant DNA pool.

Physical mapping of the molecular markers was conducted by use of a set of Chinese Spring deletion lines of the specific chromosome arm.

Results

Powdery mildew resistance

The resistance gene from wild emmer accession 'C20' was transferred to hexaploid wheat through crossing and backcrossing. The derivatives of 'C20' were highly resistant to race 15 of *E. graminis* f. sp. *tritici* (with IT value 0 and 0;). Genetic analysis indicated that the powdery mildew resistance was controlled by a single dominant gene (Table 2), which is designated *Pm30.*

Table 3. Analysis of linkage between the powdery mildew resistance gene *Pm30* and the polymorphic markers, *Xgwm159/430, Xgwm159/460*, and *Xgwm159/500*, in two segregation populations

Plant genotype	Marker genotype					
	87-1/C20//2∗8866 BC1F2			87-1/C20//2∗8866 BC1F3		
	AA	Aa	aa	AA	Aa	aa
RR	6			14	2	
Rr		20	3	1	23	3
rr		1	7		1	15
Recombination rate (%)		5.41			5.93	
Genetic distance (cM)		5.43			5.96	

Identification of molecular markers linked to Pm30

Of the 400 random 10-mer oligonucleotide primers tested, 321 produced scorable amplification products: 3 to 12 DNA fragments ranging from 200 to 3,000 bp in size were amplified by each primer, resulting in a total of 2,516 fragments. Nine primers showed polymorphisms between the resistant and susceptible DNA 'pools', but none was found to be linked to the resistance gene when tested on segregating pop-

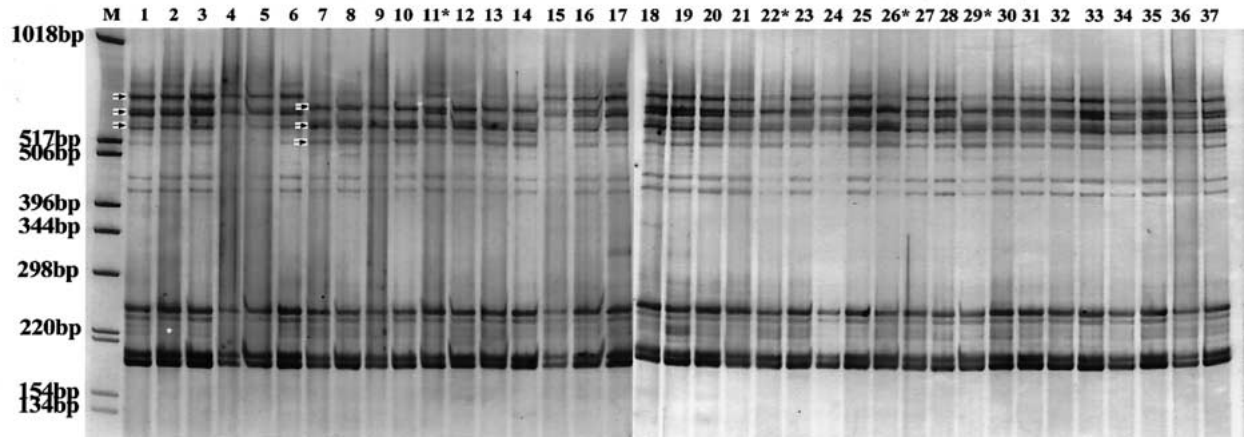

Figure 2. Polymorphic markers, amplified by wheat microsatellite primer *WMS159* in homozygous resistant plants (*Xgwm159/430, Xgwm159/460*, and *Xgwm159/500*, lanes 1 to 6), homozygous susceptible plants (*Xgwm159/400, Xgwm159/430*, and *Xgwm159/470,* lanes 7 to 14) and heterozygous resistant plants (lanes 15 to 37) of the BC_1F_2 segregating population of 87-1/C20//2∗8866. Amplification products were separated on 10% non-denaturing PAGE, silver stained. * Indicates recombinants. M: 1kb DNA ladder.

ulations. Of the 200 STS primers from barley and wheat genome mapping projects, nearly 70 percent yielded amplification products and no polymorphic DNA fragment was detected between the resistant and susceptible DNA 'pools'. Of 80 wheat microsatellite primers, three primer pairs yielded polymorphic DNA fragments between the resistant and susceptible DNA 'pools'. When the polymorphisms were tested in two segregating populations, no linkage was found between the polymorphisms and the resistance gene for two pairs of primers. Only one primer pair, *WMS159*, yielded three polymorphic DNA fragments, *Xgwm159/430, Xgwm159/460*, and *Xgwm159/500*, in resistant plants, which were linked to *Pm30* in segregating populations. Another three DNA fragments, *Xgwm159/400, Xgwm159/430*, and *Xgwm159/470*, amplified by *WMS159*, were found to be linked to the susceptibility allele (Figure 1, Figure 2). The genetic distance between the markers and *Pm30* was estimated to be 5–6 cM after evaluation of the markers in the two segregating populations (Table 3).

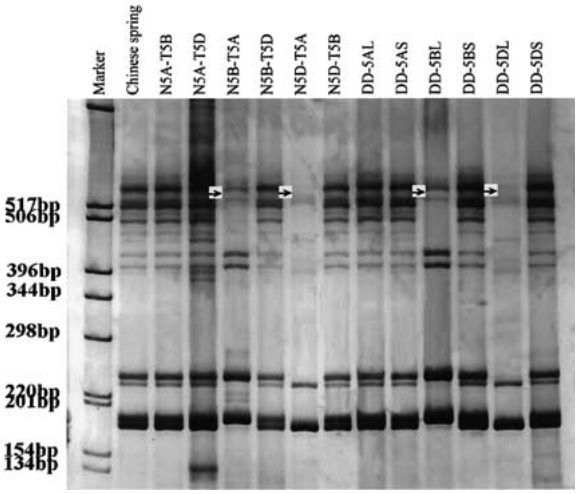

Figure 3. Amplification of *Xgwm159/400, Xgwm159/430*, and *Xgwm159/470* in Chinese Spring nullisomic-tetrasomic and ditelosomic stocks, separated on 10% non-denaturing PAGE and silver stained. Arrows indicate the non-amplification of the target fragments. Marker: 1kb DNA ladder.

Chromosome arm assignment

The three susceptibility-linked markers, *Xgwm159/400, Xgwm159/430*, and *Xgwm159/470*, were amplified in Chinese Spring (Figure 3), which is in agreement with its susceptibility to powdery mildew. To assign the chromosome and chromosome arm locations of the three polymorphic DNA fragments and the linked resistance gene, a set of Chinese Spring nullisomic-tetrasomic lines (excluding N4BT4A, N4BT4D, and N4DT4B) were used. All three markers were present in all lines tested except N5BT5A and N5DT5A, indicating they were located on chromosomes 5B and 5D. Chinese Spring ditelosomics of chromosome group 5 were then used to amplify the markers, in order to locate them on a specific chromosome arm. All three markers were absent only in ditelosomic lines 5BL and 5DL, suggesting that they were located on chromosome arms 5BS and 5DS (Figure 3). This is in agreement with the findings that the detectable al-

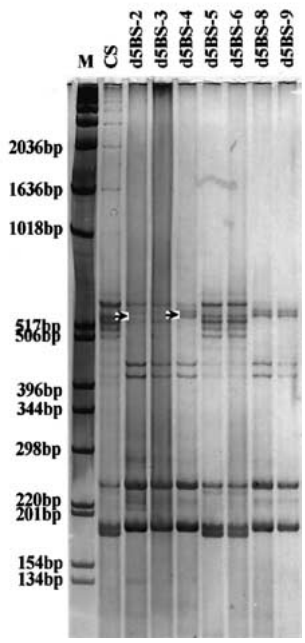

Figure 4. Amplification of *Xgwm159/400, Xgwm159/430*, and *Xgwm159/470* in Chinese Spring deletion lines of chromosome arm 5BS, separated on 10% non-denaturing PAGE and silver stained. Arrows indicated the non-amplification of the target fragments in deletion lines. CS: Chinese Spring. M: 1kb DNA ladder.

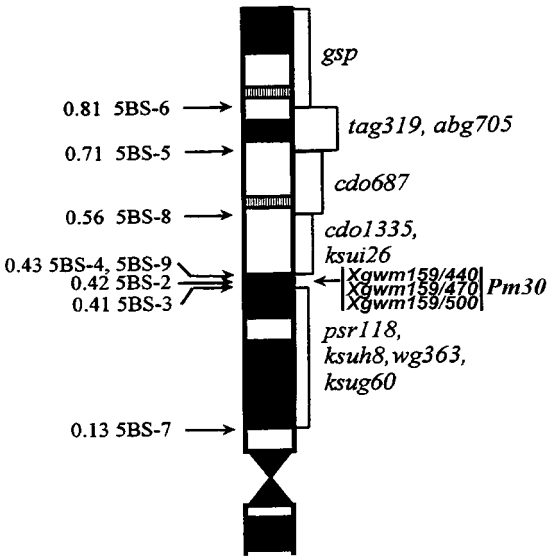

Figure 5. Physical mapping of *Xgwm159/400, Xgwm159/430*, and *Xgwm159/470* in chromosome arm 5BS-deletion map (adapted from Gill et al. (1996) with minor modifications).

leles of *Xgwm159* were located on 5DS (Plaschke et al., 1995) and 5BS (Röder et al., 1998a). Since *Pm30* originated from wild emmer with the AABB genome, the powdery mildew resistance gene and its linked markers should be located on chromosome arm 5BS.

Physical mapping of Xgwm159/400, Xgwm159/430, and *Xgwm159/470*

A set of Chinese Spring deletion lines for chromosome arm 5BS was used for physical mapping of the susceptibility-linked markers *Xgwm159/400*, *Xgwm159/430*, and *Xgwm159/470*. Among the seven deletion lines tested, two lines, d5BS-5 (FL 0.71) and d5BS-6 (FL 0.81), yielded the same amplification pattern as that of Chinese Spring (Figure 4), indicating that *Xgwm159/400*, *Xgwm159/430*, and *Xgwm159/470* were not located on the distal part of chromosome arm 5BS. The three markers were absent in deletion lines d5BS-8 (FL 0.56), d5BS-4 (FL 0.43), d5BS-9 (FL 0.43), d5BS-2 (FL 0.42), and d5BS-3 (0.41), but minor differences were observed in the amplification pattern between the first three deletion lines and the last two. The markers *Xgwm159/400, Xgwm159/430*, and *Xgwm159/470* could then be physically mapped to the d5BS–3 to d5BS–4 regions of FL 0.41–0.43 (Figure 5).

Discussion

Microsatellite markers based on simple sequence repeats are abundant, highly polymorphic, and evenly distributed over the genome (Lagercrantz et al., 1993; Wang et al., 1994). The development of wheat microsatellite markers in recent years (Röder et al., 1998a; Bryan et al., 1997) provides readily detectable markers for mapping important genes such as yellow rust resistance gene *YrH52* (Peng et al., 1999) in chromosome arm 1BS, and dwarfing genes *Rht8* (Korzun et al., 1998) and *Rht12* (Korzun et al., 1997) in chromosome arms 2DS and 5AL, respectively. In this study, polymorphic markers *Xgwm159/430, Xgwm159/460*, and *Xgwm159/500* were found to be linked to the powdery mildew resistance gene *Pm30* in chromosome arm 5BS. By contrast, no RAPD and STS markers linked to *Pm30* were found.

Xgwm159 contains a $(GT)_{15}$ motif, and the expected sizes of the amplified fragments detected in Chinese Spring, Opata, and Synthetic, and mapped on chromosome arms 5BS and 5DS, were 192bp, 189bp, and 187bp, respectively (Plaschke et al., 1995; Röder et al., 1998a). Plaschke et al. (1995) and Röder et al. (1998a) also observed that fragments on non-

homoeologous chromosomes or of unexpected sizes were amplified in wheat with microsatellite primers, but were usually monomorphic. In this study no linkage was detected between a single locus of *Xgwm159* and the mildew resistance locus, but three fragments of unexpected size, *Xgwm159/430, Xgwm159/460*, and *Xgwm159/500* were found to be linked to *Pm30*. It is not known if the three polymorphic markers of unexpected size, which also mapped on 5BS and 5DS, contain the $(GT)_{15}$ core repeat motif. However, amplification of microsatellite fragments longer than expected was also observed in barley (Senior & Heun, 1993), and these fragments did not contain the targeted repeat.

High-density genetic and physical maps of chromosome group 5 of wheat have been established (Xie et al., 1993; Nelson et al., 1995; Gill et al., 1996). Gill et al. (1996) pointed out that breakages were semi-random and the marker-clustered-regions were the preferred sites in group 5 chromosomes, although the deletions were equally frequent in both heterochromatinic and euchromatinic regions (Endo & Gill, 1996). They also showed that a high proportion of actively transcribing wheat genes and agronomically important genes are present in these marker-rich regions. The distributions of microsatellite markers are similar to those of RFLP markers (Röder et al., 1998b), suggesting that most microsatellite markers lie in genetically active regions of chromatin. The position at FL 0.42 of chromosome arm 5BS was a breakage hotspot (Gill et al., 1996) and the powdery mildew resistance gene *Pm30* was physically mapped to these gene-rich regions. Accordingly, microsatellite markers tend to be very powerful in mapping agronomically important genes in wheat.

Two powdery mildew resistance genes, *Pm16* and *Pm26,* also originating from wild emmer, were located on chromosomes 4A (Reader & Miller, 1991) and 2BS (Rong et al., 2000), respectively. Resistance genes *Pm2* and *Pm23* were assigned to chromosomes 5DS and 5A, respectively (McIntosh et al., 2000), but no powdery mildew resistance gene has been reported on chromosome 5B. The wild emmer-derived gene *Pm30* appears to be a new gene, which can be used to diversify the resistance resources for wheat powdery mildew.

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