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Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.)

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Abstract Powdery mildew, caused by *Erysiphe graminis* DM f. sp. *tritici* (Em. Marchal), is one of the most important diseases of common wheat world-wide. Chinese wheat variety 'Fuzhuang 30' carries the powdery mildew resistance gene *Pm5e* and has proven to be a valuable resistance source of powdery mildew for wheat breeding. Microsatellite markers were employed to identify the gene *Pm5e* in a F_2 progeny from the cross 'Nongda 15' (susceptible) × 'Fuzhuang 30' (resistant). The gene *Pm5e* was mapped in the distal region of chromosome 7BL. Seven microsatellite markers were found to be linked to the gene *Pm5e*, of which two codominant markers *Xgwm783* and *Xgwm1267* were relatively close to *Pm5e* with a linkage distance of 11.0 cM and 6.6 cM, respectively. It is possible to use the 136-bp allele of *Xgwm1267* in 'Fuzhuang 30' for marker-assisted selection during the wheat resistance breeding process for facilitation of gene pyramiding. The mapping information in the present study provides a starting point for fine mapping of the *Pm5* locus and map-based cloning to clarify the molecular structure and function of the different alleles at the *Pm5* locus. A microsatellite linkage map of chromosome 7B was constructed with 20 microsatellite loci, nine on the short arm and 11 on the long arm. This information will be very useful for further mapping of agronomically important genes of interest on chromosome 7B.

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Introduction

Powdery mildew, caused by *Erysiphe graminis* DM f. sp. *tritici* (Em. Marchal), is one of the most-devastating diseases of common wheat world-wide and occurs in many areas with a cool or maritime climate. 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, 30 gene loci (*Pm1–Pm30*) for resistance to powdery mildew have been identified and located on different chromosomes (Järve et al. 2000; Rong et al. 2000; Liu et al. 2002; Zeller et al. 2002), of which the recessive resistance gene *Pm5* was located on the long arm of chromosome 7B (Law and Wolfe 1966; Lebsock and Briggle 1974). *Pm5* is widely contributed in the cultivars and landraces of China and Europe (Huang et al. 1997a; Zeller et al. 1998). Four alleles and two alleles at the *Pm5* locus have been recently reported by Hsam et al. (2001) and Huang et al. (2000a), respectively.

Molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), have been widely used to tag fungal resistance genes in wheat. RFLP, RAPD and AFLP markers linked to wheat powdery mildew resistance genes *Pm1* (Hartl et al. 1995, 1999; Hu et al. 1997), *Pm2* (Ma et al. 1994), *Pm3* (Hartl et al. 1993), *Pm4* (Ma et al. 1994), *Pm6* (Tao et al. 2000), *Pm8* (Hsam et al. 2000), *Pm12* (Jia et al. 1996), *Pm13* (Donini et al. 1995; Cenci et al. 1999), *Pm18* (Hartl et al. 1993), *Pm21* (Qi et al. 1996), *Pm24* (Huang et al. 2000b), *Pm25* (Shi et al. 1998), *Pm26* (Rong et al. 2000), *Pm27* (Järve et al. 2000), *Pm29* (Zeller et al. 2002) and *Pm30* (Liu et al. 2002) have been identified. Microsatellites, also termed simple sequence repeats (SSRs) as a new type of genetic

marker reveal a much higher polymorphism in wheat than any other marker system (Plaschke et al. 1995; Huang et al. 2002). Recently, Röder et al. (1998; unpublished data) developed a large number of microsatellite markers and constructed a microsatellite map of hexaploid wheat (Huang et al. 2001). Because microsatellite markers were chromosome-specific and evenly distributed along chromosomes (Röder et al. 1998), they have been applied for tagging resistance genes in wheat (Peng et al. 1999; Börner et al. 2000) and identifying quantitative trait loci (QTL) alleles for yield and yield components from wild relatives of common wheat (Huang et al. 2003). Because of their co-dominant inheritance, they were most suitable for marker-assisted selection in wheat breeding programmes (Korzun et al. 1998; Huang et al. 2000b).

'Fuzhuang 30' is a selection line from the cross of two Chinese landraces, has proven to be a valuable resistance source of powdery mildew for wheat breeding (Huang et al. 1997a) and has been reported to carry a semidominant resistance gene (Tang 1993). But the study on genetic analysis indicated that the resistance in 'Fuzhuang 30' was controlled by a recessive gene (Huang et al. 1997b). The recessive gene temporarily designated *mlfz* was located on chromosome 7B and allelic to the *Pm5* gene by monosomic analysis and an allelism test (Huang et al. 2000a). Recently, a RAPD marker linked to the powdery mildew resistance gene in 'Fuzhuang 30' has been identified (Wang et al. 2000). The gene *mlfz* was designated as *Pm5e* in this report. The major objectives of the present study were to identify microsatellite markers linked to the powdery mildew resistance gene *Pm5e* and to construct a microsatellite linkage map of chromosome 7B.

Materials and methods

Plant materials

Both the susceptible cultivar 'Nongda 15' and the resistant cultivar 'Fuzhuang 30' were kindly provided by T.M. Yang, Department of Plant Genetics and Breeding, China Agricultural University, Beijing. A total of 100 F_2-derived F_3 families originating from a cross between 'Nongda 15' and 'Fuzhuang 30' were used for linkage analysis between molecular markers and powdery mildew resistance gene *Pm5e*. Twenty plants of each F_3 family were tested to identify the genotype of corresponding F_2 plants. For bulked segregant analysis (Michelmore et al. 1991), two DNA bulks were assembled by using equal amounts of DNA from ten homozygous resistant and ten homozygous susceptible F_3 families, respectively.

Three nulli-tetrasomics (N7AT7B, N7BT7A and N7DT7A) and two ditelosomics (DT7BS and DT7BL) of 'Chinese Spring', which were originally obtained from the late Dr. E. R. Sears, Columbia, Missouri, USA, were used for chromosome-arm assignment of SSR markers.

Genomic DNA extraction

Total genomic DNA was extracted from young leaf tissue, frozen in liquid nitrogen, as previously described by Huang et al. (2000c), with some modification. Briefly, 3–5 g of leaf tissue per sample were ground in liquid nitrogen and incubated in 1.5 × CTAB extraction buffer [1.5% (w/v) CTAB, 100 mM Tris–HCl pH 8.0, 20 mM EDTA pH 8.0, 1.05 M NaCl and 1.5% β-mercaptoethanol] at 60 °C for 30 min. Following a single chloroform:isoamyl alcohol (24:1) extraction, the precipitated DNA was dissolved in TE buffer. DNA samples were stored at –20 °C. The DNA was diluted to a concentration of 5–10 ng/μL before use for microsatellite analysis.

Powdery mildew evaluation

The *Erysiphe graminis tritici* (*Egt*) race 15 used for the resistance tests was kindly provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing. Leaf segments were cut from seedling with 3–4 leaves and put on 0.45–0.5% agar and 60 mg/l of 6-BA (pH 5.8) in enamel dishes. The segments were inoculated by gently shaking heavily infected plants over the segments so that conidiospores were uniformly distributed on the leaf segments. The tests were conducted in a growth chamber in which the temperature was maintained at 16 °C with a 12-h photoperiod of white fluorescent light. Reactions to infection were scored 10 days after inoculation and verified after 12 days. The infection types (IT) of powdery mildew on leaf segments were recorded on a scale of 0–4, namely 0 representing no visible symptoms, 0; for necrotic flecks, and 1–4 for resistant, light-susceptible, susceptible and highly susceptible. Two major IT were distinguished: resistant (IT = 0, 0; and 1) and susceptible (IT = 2–4), and were used for genetic and linkage analyses. Chi-square tests for goodness of fit were used to test for deviation of observed data from theoretically expected segregation.

Microsatellite analysis

Forty two microsatellite markers, which were mapped on wheat chromosome 7B in the ITMI population (Röder et al. 1998, unpublished data), were chosen for screening of polymorphism between two parents of the mapping population. The six markers *Xgwm883*, *Xgwm1173*, *Xgwm1175*, *Xgwm1184*, *Xgwm1267* and *Xgwm1498* were kindly provided by Dr. M. Ganal, TraitGenetics GmbH, Am Schwabeplan 1b, 06466 Gatersleben, Germany. The polymorphic SSR markers (Table 1) were used to amplify DNA of two bulks to look for closely linked markers to the gene *Pm5e*. One primer of WMS primer pairs was labelled using either fluorescein or Cy5. The polymerase chain reaction (PCR) contained 50–100 ng of template DNA, 250 nM of each labelled and unlabelled primer, 1 U of *Taq* DNA polymerase, 2.5 μl of 10 × PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs in a total volume of 25 μl. The PCR reaction was carried out in an MJR PTC-225 thermocycler. After 3-min denaturation, 45 cycles were performed with 1 min at 94 °C, 1 min at 50, 55 or 60 °C (depending on the individual microsatellite markers), 2 min at 72 °C and a final extension step of 10 min at 72 °C.

Fragment analysis was carried out in an automated laser fluorescence (ALF) or ALF express sequencer (Pharmacia), using a short gel cassette. For the primers labelled with fluorescein, denaturing gels (0.35-mm thick) with 6% polyacrylamide containing 40 slots were prepared using SequaGel XR (Biozym); the gels were run on the ALF sequencer in 1 × TBE buffer [0.09 M Tris-borate (pH 8.3), 2 mM EDTA] at 650 V, 50 mA, 50 W and 50 °C, with 2 mA laser power and a sampling interval of 0.84 s. For the primers labelled with Cy5, denaturing gels (0.35-mm thick) with 8% polyacrylamide containing 40 slots were prepared using ReproGel High Resolution (Pharmacia). The gels were run on the ALF express sequencer in 0.75 × TBE buffer [0.09 M Tris-borate (pH 8.3), 2 mM EDTA] at 850 V, 50 mA, 50 W and 50 °C, with 2-mA laser power and a sampling interval of 1 s. The gels were re-used five and three times, respectively. In 39 lanes, fragments with known sizes were included as internal standards. An external standard with four fragments was loaded in one lane. Fragment sizes were calculated using the computer program Fragment Analyser Version 1.0 (Pharmacia) by comparison with the internal and external size standards.

Linkage analysis

The linkage relationship between DNA markers and the gene *Pm5e* was established with MAPMAKER/Exp version 3.0b

Table 1 Description of the polymorphic microsatellite markers

Locus	Annealing temperature (°C)	Fragment size (bp) in		
		Chinese Spring	Nongda 15	Fuzhuang 30
Xgwm46-7B	60	184	170	145
Xgwm255-7B	55	147	147	139
Xgwm297-7B	55	150	152	170
Xgwm333-7B	55	149	153	147
Xgwm344-7A	55	134	130	128
Xgwm344-7B	55	Null	Null	Null
Xgwm400-7B	60	137	139	135
Xgwm537-7B	60	212	206	214
Xgwm569-7B	50	136	134	138
Xgwm573a-7B	50	213	209	217
Xgwm577-7B	55	132	130	Null
Xgwm611-7B	55	151	150	151
Xgwm783-7B	50	103	103	105
Xgwm883-7B	50	198	196	Null
Xgwm897-7B	50	147	145	113
Xgwm963-7B	50	255	255	253
Xgwm984a-7B	50	183	205	183
Xgwm1144-7B	60	127	129	127
Xgwm1173-7B	55	251	253	273
Xgwm1175-7B	60	245	Null	245
Xgwm1184-7B	55	139	145	143
Xgwm1267-7B	60	136	142	136
Xgwm1498-7B	55	176	164	172

Table 2 Segregation analysis for the *Pm5e* gene and microsatellite markers in an F_2 population from the cross 'Nongda 015' × 'Fuzhuang 30' after studying their F_3 population

Gene or markers	No. of F_2 plants	Observed no.			Expected ratio	χ^2	*P*
		X_1X_1[a]	X_1X_2	X_2X_2			
Pm5e	100	23	55	22	1:2:1	1.02	0.50–0.60
Xgwm46	100	21	56	23	1:2:1	1.52	0.40–0.50
Xgwm255	100	21	54	25	1:2:1	0.96	0.50–0.60
Xgwm297	100	23	56	21	1:2:1	1.52	0.40–0.50
Xgwm333	100	22	56	22	1:2:1	1.44	0.40–0.50
Xgwm400	100	19	60	21	1:2:1	4.08	0.10–0.20
Xgwm537	100	20	54	26	1:2:1	1.36	0.50–0.60
Xgwm569	100		74[b]	26	3:1	0.05	0.80–0.90
Xgwm573a	100	26	50	24	1:2:1	0.08	>0.95
Xgwm577	100		82[b]	18	3:1	2.61	0.20–0.30
Xgwm783	100	25	57	18	1:2:1	2.94	0.10–0.20
Xgwm883	100		78[b]	22	3:1	0.48	0.40–0.50
Xgwm897	99	23	56	20	1:2:1	1.89	0.30–0.40
Xgwm963	100	23	54	23	1:2:1	0.64	0.60–0.70
Xgwm984a	99	23	55	21	1:2:1	1.30	0.50–0.60
Xgwm1144	99	30	52	17	1:2:1	3.67	0.10–0.20
Xgwm1173	99	25	54	20	1:2:1	1.32	0.50–0.60
Xgwm1175	99	22		78[b]	1:3	0.49	0.40–0.50
Xgwm1184	96	22	44	30	1:2:1	2.00	0.30–0.40
Xgwm1267	99	22	57	20	1:2:1	2.35	0.20–0.30
Xgwm1498	100	19	61	20	1:2:1	4.86	0.05–0.10

[a] Genotype: X_1X_1 = Nongda 15; X_1X_2 = heterozygous; and X_2X_2 = Fuzhuang 30

[b] Pooled values from homozygous and heterozygous classes

(Lander et al. 1987). The commands "group" and "ripple", and LOD scores ≥3.0 were used to develop the linkage map. The Kosambi (1944) mapping function was used to convert recombination fractions into centiMorgans (cM) as map distances.

Results

Segregation of the resistance gene *Pm5e*

For the molecular mapping of the gene *Pm5e* in 'Fuzhuang 30', a total of 100 F_2-derived F_3 families from the cross between 'Nongda 15' and 'Fuzhuang 30' were tested with powdery mildew race no. 15, which was virulent to 'Nongda 15' (IT = 4) and avirulent to 'Fuzhuang 30' (IT = 0; necrotic flecks). The observed segregation of 22 homozygous resistant, 55 heterozygous susceptible and 23 homozygous susceptible families (Table 2) fitted a 1:2:1 segregation ratio (χ^2 = 1.02, $0.50 < P < 0.60$), further supporting the fact that the resistance of *Pm5e* is conferred by a single recessive gene (Huang et al. 2000a).

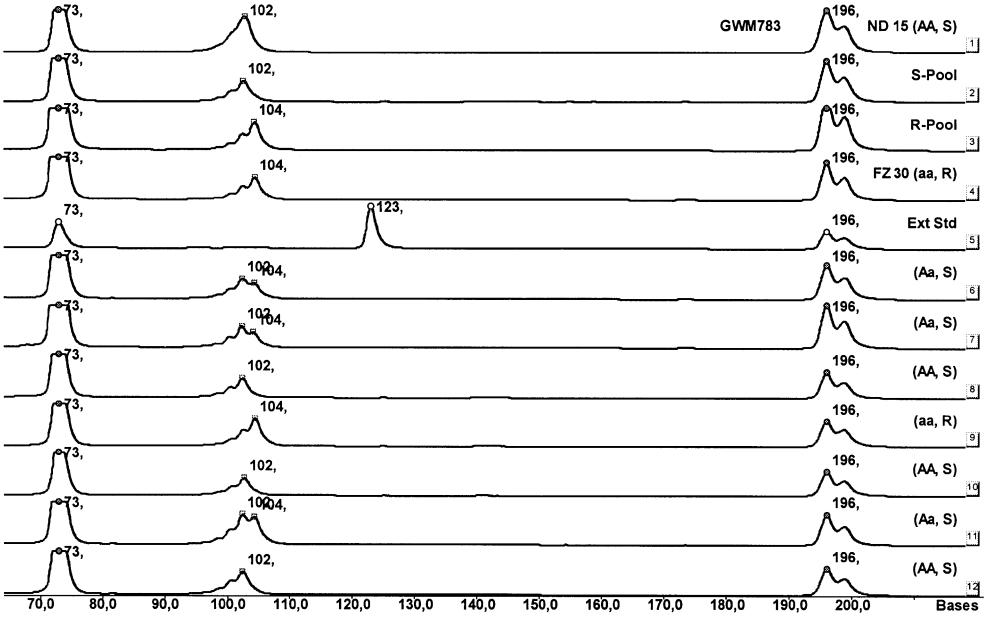

Fig. 1 Polymorphic microsatellite marker *Xgwm783* analysed using Fragment Analyser version 1.02. The peaks represent fragments, whereas the horizontal scale indicates fragment sizes in base pairs calculated from internal standards (73 bp and 196 bp) and external standards (73 bp, 123 bp and 196 bp). The fragments 102 bp and 104 bp were produced by Nongda (ND) 15, S-Pool, R-Pool, Fuzhuang (FZ) 30 and some F_2 plants, respectively. *Lanes 6, 7 and 11* were heterozygous susceptible plants; *lanes 4, 10 and 12* were homozygous susceptible plants; *lane 9* involved homozygous resistant plants

Microsatellite polymorphism between 'Nongda 15' and 'Fuzhuang 30'

Forty two microsatellite markers which were mapped on wheat chromosome 7B (Röder et al. 1998, unpublished data), were chosen to identify polymorphism between 'Nongda 15' and 'Fuzhuang 30'. Among the 42 markers, 22 (52.4%) produced polymorphism between two parents, indicating that microsatellite markers revealed a high level of polymorphism in wheat cultivars. The fragment sizes of the 22 polymorphic microsatellite markers amplified in 'Nongda 15' and 'Fuzhuang 30', respectively, were listed in Table 1.

Identification of microsatellite markers linked to the *Pm5e* gene

The 22 polymorphic microsatellite markers were screened to identify polymorphic markers between the resistant and susceptible DNA bulks. Six out of 22 polymorphic microsatellite markers, namely *Xgwm577*, *Xgwm611*, *Xgwm783*, *Xgwm883*, *Xgwm984a* and *Xgwm1267*, generated polymorphic fragments between the bulks. These markers should be very closely linked to the *Pm5e* gene and were used at first to genotype the F_2 mapping population. Then the other polymorphic microsatellite markers were applied for genotyping. The fragment size of the marker *Xgwm611* amplified in 'Nongda 15' and 'Fuzhuang 30' was 150 bp and 151 bp, respectively (Table 1). This marker was very difficult to genotype the F_2 population and was not used for mapping. As an example, Fig. 1 presents the segregation pattern of microsatellite locus *Xgwm783*, detected by an automated laser fluorescence (ALF) sequencer.

Among the 21 marker loci, seven marker loci were found to be linked to the *Pm5e* gene, with recombination frequencies ranging from 0.06 to 0.25, and LOD scores ranging from 3.18 to 25.63. Two marker loci *Xgwm783* and *Xgwm1267* were relatively close to *Pm5e*, with a linkage distance of 11.0 cM and 6.6 cM, respectively (Fig. 2). Of the seven microsatellite loci, four revealed codominant inheritance, whereas the other three were dominant with the single fragment present in one parent but absent in another (Table 2).

In order to investigate the validation of *Xgwm1267* for marker-assisted selection, DNA of five susceptible cultivars and 15 resistant cultivars/lines with known powdery mildew resistance genes or gene combinations were amplified using the microsatellite marker *gwm1267* (Table 3). The same fragment with a size of 136 bp was present in susceptible 'Chinese Spring', and in IGV455 and 'Xiaobaidong' which possess the genes *Pm5d* and *mlxbd* (*Pm5* allele), respectively (Huang et al. 2000a; Hsam et al. 2001). This indicated that the 136-bp allele was not specific for the gene *Pm5e*. Nevertheless, the sizes of the amplified fragments in other genotypes carrying known powdery mildew resistance genes or gene combinations were different from the amplified fragment with a size of 136 bp in 'Fuzhuang 30'.

Microsatellite linkage map of chromosome 7B

The other 13 microsatellite markers, *Xgwm46*, *Xgwm255*, *Xgwm297*, *Xgwm333*, *Xgwm400*, *Xgwm537*, *Xgwm569*, *Xgwm573a*, *Xgwm897*, *Xgwm963*, *Xgwm1184*, *Xgwm1173* and *Xgwm1498*, that showed polymorphism between 'Nongda 15' and 'Fuzhuang 30' were mapped on chromosome 7B. Except for *Xgwm569*, the other marker loci exhibited a 1:2:1 segregation ratio in the F_2 population (Table 2). The microsatellite marker *Xgwm569* amplified the fragments with a size of 134 bp and 138 bp in

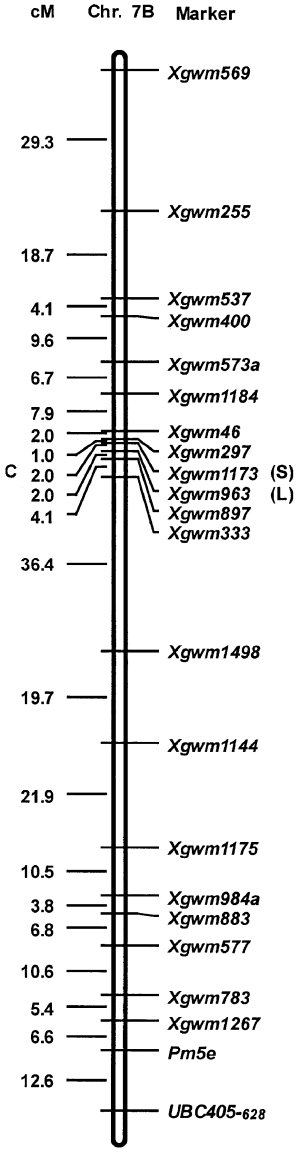

Fig. 2 A microsatellite linkage map involving the gene *Pm5e* on chromosome 7B. Locus names are indicated on the right side of the map. Kosambi map distances (cM) are shown on the left side. C = centromere position, S = short arm, and L = long arm

Table 3 Fragment sizes of 20 wheat cultivars and lines without or with the known powdery mildew resistance gene (s) after amplification using the microsatellite marker *Xgwm1267*

Cultivar/line	Resistance gene	Fragment size (bp)
Chinese Spring	–	136
Nongda 15	–	142
Amor	–	Null
Kanzler	–	152
Trakos	–	Null
Asosan/8*Cc	*Pm3a*	142
Kolibri	*Pm3d*	162
Michigan Amber/8*Cc	*Pm3f*	148
IGV 455	*Pm5d*	136
Xiaobaidong	*mlxbd* (*Pm5* allele)	136
Fuzhuang 30	*Pm5e*	136
Zecoi-4	*Pm16*	Null
Amigo	*Pm17*	162
TAM104-T6BS·6RL	*Pm20*	162
Chiyacao	*Pm24*	156
Herzog	*Pm4b+Pm8*	Null
Piko	*Pm5a+Pm6*	144
Normandie	*Pm1a+Pm2+Pm9*	162
Ritmo	*Pm2+Pm5a+Pm6*	144
Apollo	*Pm2+Pm4b+Pm8*	144

'Nongda 15' and 'Fuzhuang 30', respectively (Table 1). But this marker locus showed a dominant inheritance (Table 2), because only the allele with a size of 134 bp from the parent 'Nongda 15' could be amplified in the heterozygous F_2 plants. The microsatellite marker *Xgwm344* which was polymorphic between two parents (Table 2) could not be assigned to chromosome 7B. A genetic map consisting of 19 microsatellite loci, a RAPD marker and the gene *Pm5e* was constructed with a total length of 221.9 cM. The RAPD marker UBC405-628 was linked to the gene *Pm5e* with a linkage distance of 12.6 cM in the previous study (Wang et al. 2000). The gene *Pm5e* was located between *Xgwm1267* and UBC405-628.

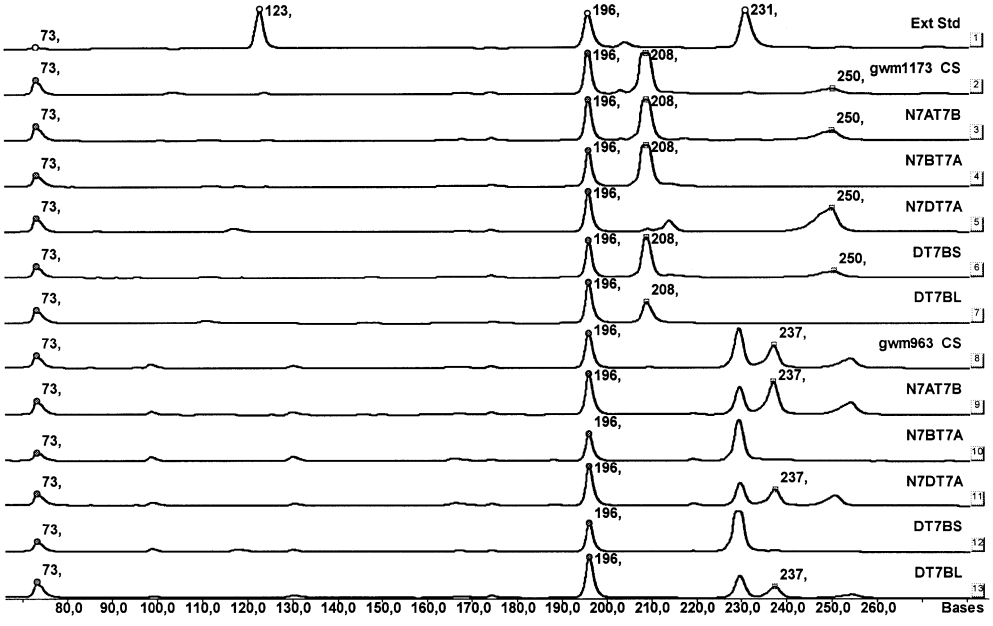

Fig. 3 Electropherograms of microsatellite analysis in Chinese Spring, N7AT7B, N7BT7A, N7DT7A, DT7BS and DT7BL, amplified with the microsatellite markers *Xgwm1173* and *Xgwm963*. The fragment of size 250 bp and 237 bp was assigned to the short arm and the long arm of chromosome 7B, respectively. Another 208-bp allele from *Xgwm1173* was assigned to chromosome 7D

To determine the chromosomal-arm location of the microsatellite markers mapped and the position of centromere, DNA from Chinese Spring, three nulli-tetrasomic lines (N7AT7B, N7BT7A and N7DT7A) and two ditelosomic lines (DT7BS and DT7BL) was amplified with all the mapped primer pairs. It was found that nine microsatellite markers, *Xgwm569*, *Xgwm255*, *Xgwm537*, *Xgwm400*, *Xgwm573a*, *Xgwm1184*, *Xgwm46*, *Xgwm297* and *Xgwm1173*, were located on the short arm of chromosome 7B, while the other 11 marker loci, *Xgwm963*, *Xgwm897*, *Xgwm333*, *Xgwm1498*, *Xgwm1144*, *Xgwm1175*, *Xgwm984a*, *Xgwm883*, *Xgwm577*, *Xgwm783* and *Xgwm1267*, were on the long arm of chromosome 7B (Figs. 2 and 3). This indicates that the position of the centromere is between *Xgwm1173* and *Xgwm963*. Figure 3 also indicated that another fragment with a size of 208 bp was assigned to chromosome 7D. The gene *Pm5e* was mapped on chromosome 7BL. The map distance of the gene *Pm5e* to the centromere is about 128 cM. It is worth noting that the allele with the size of 134 bp in 'Chinese spring' amplified using the microsatellite marker *Xgwm344* was assigned to chromosome 7A. The smaller fragment (104 bp) in 'Opata' amplified with *Xgwm344* was mapped on chromosome 7B in the ITMI population. The larger fragment was not polymorphic between 'Opata' and W-7984 (Röder et al. 1998; personal conmunication).

Discussion

Mains (1933) first described the resistance of wheat cultivar 'Hope' to powdery mildew. Through testing F_3 populations of crosses between 'Hope' and susceptible cultivars, Mains (1934) found that 'Hope' carried a single recessive gene. The origin of resistance in 'Hope' can be traced to the emmer wheat cultivar 'Yaroslov'. By means of the substitution line Law and Wolfe (1966) located the resistance gene to powdery mildew in 'Hope' on the long arm of chromosome 7B. Similarly, McIntosh et al. (1967) localised a recessive gene for resistance to powdery mildew in the cultivar 'Renown', a derivative of the cultivar 'H-44' whose parentage is identical with that of 'Hope', on chromosome 7B. The gene was linked to the stem rust resistance gene *Sr17* with recombination estimates of 6.0% and 2.5% in two different crosses, respectively. Through genetic analysis of crosses between 'Hope' and four near-isogenic lines possessing *Pm1*, *Pm3*, *Pm3* and *Pm4*, respectively, Lebsock and Briggle (1974) confirmed the recessive inheritance mode of the resistance gene in 'Hope' and replaced the symbol *Mlh* with *Pm5*. Recently, *Pm5* was changed into *Pm5a* because of several alleles found at the *Pm5* locus (Huang et al. 2000a; Hsam et al. 2001).

Using the F_2-derived F_3 family together with microsatellite markers, we found seven marker loci on chromosome 7BL that were linked to *Pm5e* in the present study. This result further confirmed those obtained using monosomic analysis in the previous study (Huang et al. 2000a). The gene *Pm5e* was mapped in the distal region of chromosome 7B with a map distance of approximately 128 cM to the centromere. Recently, Keller et al. (1999) reported that one major QTL which corresponded to the *Pm5* locus was mapped on chromosome 7BL, about 100-cM apart from the centromere. This discrepancies was probably due to different population type and size. Keller et al. (1999) used 226 recombinant inbred lines, whereas our mapping was performed using 100 F_2-derived F_3 families. So far, six alleles have been found at the *Pm5* locus (Huang et al. 2000a; Hsam et al. 2001). The mapping information in the present study provides a starting point for fine mapping of the *Pm5* locus and map-based cloning to clarify the molecular structure and function of the different alleles at the *Pm5* locus.

Codominant microsatellite markers closely linked to genes of interest are very useful for backcross-assisted selection and gene pyramiding in wheat breeding programs. In the present study, the closest microsatellite marker was *Xgwm1267* with a linkage distance of 6.6 cM. The microsatellite marker *Xgwm1267* could not differentiate *Pm5e* with *Pm5d* in IGV455 and *mlxbd* (the *Pm5* allele) in 'Xiaobaidong', but could distinguish the other known powdery mildew genes such as *Pm1a*, *Pm2*, *Pm3a*, *3d*, *3f*, *Pm5a*, *Pm6*, *Pm16*, *Pm24* and so on (Table 3). Therefore, the 136 bp-allele may not be suitable for use in the Asian wheat gene pool, since the same allele was also detected in 'Chinese Spring'; however, it may be useful for introgression of the gene *Pm5e* in the European wheat breeding pool. The flanking marker UBC405-628 has been used for selection of resistant plants with *Pm5e* in the progenies (L.X. Wang, personal communication). Therefore, use of *Xgwm1267* in combination with UBC405-628 would be more efficient for marker-assisted breeding. Recently, Huang et al. (2000b) found that the microsatellite marker *Xgwm337* tightly linked to the powdery mildew resistance gene *Pm24* on chromosome 1DS was specific and diagnostic for *Pm24*. Both the genes *Pm5e* and *Pm24* originated from wheat land races and exhibited the excellent resistance to powdery mildew (Huang et al. 1997a, 2000a). No doubt, combination of both genes in one cultivar could provide the durable resistance for wheat breeding. The codominant microsatellite markers *Xgwm337* and *Xgwm1267* allow wheat breeders to accelerate the breeding process for selection of the homozygous plants with both *Pm24* and *Pm5e*.

The microsatellite linkage map of chromosome 7B was constructed in the present study. It spanned 221.9 cM in length and consisted of one powdery mildew resistance gene *Pm5e*, one RAPD marker UBC405-628 and 20 microsatellite markers, of which nine were on the short arm and 11 on the long arm. The order of the marker loci was basically consistent with that of the microsatellite linkage map of chromosome 7B in the ITMI population (M.S. Röder, unpublished data). The difference between them was that the marker loci *Xgwm537* and *Xgwm400* were mapped on the top of chromosome 7BS, apart from *Xgwm569* in the ITMI map, but located between *Xgwm255* and *Xgwm573a* in the present study (Fig. 2).

The reason for that was that nonhomoeologous translocation involving chromosome arms 4AL, 5AL and 7BS occurred during the evolution of hexaploid wheat (Naranjo et al. 1987) and might lead to segment inversion of chromosome 7BS. The stem rust resistance gene *Sr17* was closely linked to the gene *Pm5a* on chromosome 7BL (McIntosh et al. 1967). Recently, Li et al. (1999) found that five defense response (DR) genes were clustered in the distal region of chromosome 7BL. The microsatellite markers linked to the gene *Pm5e* were located in this region and hence may be very useful for developing a diagnostic marker to select the desirable DR gene alleles for wheat resistance breeding.

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