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Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding

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Abstract Molecular markers were identified in common wheat for the *Pm24* locus conferring resistance to different isolates of the powdery mildew pathogen, *Erysiphe graminis* DM f. sp. *tritici* (Em. Marchal). Bulked segregant analysis was used to identify amplified fragment length polymorphism (AFLP) markers and microsatellite markers linked to the gene *Pm24* in an F_2 progeny from the cross Chinese Spring (susceptible)× Chiyacao (resistant). Two AFLP markers *XACA*/*CTA-407* and *XACA*/*CCG-420*, and three microsatellite markers *Xgwm106*, *Xgwm337* and *Xgwm458*, were mapped in coupling phase to the *Pm24* locus. The AFLP marker locus *XACA*/*CTA-407* co-segregated with the *Pm24* gene, and *XACA*/*CCG-420* mapped 4.5 cM from this gene. Another AFLP marker locus *XAAT*/*CCA-346* co-segregated in repulsion phase with the *Pm24* locus. *Pm24* was mapped close to the centromere on the short arm of chromosome 1D, contrary to the previously reported location on chromosome 6D. *Pm24* segregated independently of gene *Pm22*, also located on chromosome 1D. An allele of microsatellite locus *Xgwm337* located 2.4±1.2 cM from *Pm24* was shown to be diagnostic and therefore potentially useful for pyramiding two or more genes for powdery mildew resistance in a single genotype.

Keywords AFLPs · Bulked segregant analysis · Marker-assisted selection · Microsatellites · Powdery mildew resistance · *Triticum aestivum*

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Introduction

Powdery mildew, caused by *Erysiphe graminis* DM f. sp. *tritici* (Em. Marchal), is one of the most destructive foliar diseases of common wheat world-wide and occurs in areas with cool or maritime climates. Breeding of resistant cultivars is the most economical and environmentally safe method to eliminate the use of fungicides and to reduce crop losses due to this disease. Up to now, 25 gene loci for resistance to powdery mildew (*Pm1* to *Pm25*) have been described (Huang et al. 1997c; Hsam et al. 1998; Shi et al. 1998), and some of these have been utilised in commercial resistance breeding. The deployment of individual resistance genes often results in the loss of resistance in a relatively short period due to the emergence of virulent pathogen mutants. Hence, combinations of two or more effective resistance genes may afford better genetic control. However, the selection of genotypes with gene combinations is very time-consuming and often not possible due to the lack of pathogen isolates with specific virulence genes. The development of molecular markers that are closely associated with the respective resistance genes, therefore, would expedite the selection of the gene combinations in marker-assisted selection programmes.

Both restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) have been used to tag fungal resistance genes in wheat. RFLP and RAPD markers linked to wheat leaf rust resistance genes *Lr1* (Feuillet et al. 1995), *Lr9* (Schachermayr et al. 1994; Autrique et al. 1995), *Lr19*, *Lr24* and *Lr32* (Autrique et al. 1995) and to wheat stripe rust resistance gene *Yr15* (Sun et al. 1997) were identified by using near-isogenic lines (NILs). Among the 25 gene loci for powdery mildew resistance in wheat, RFLP markers linked to *Pm1*, *Pm2*, *Pm3*, *Pm4* (Hartl et al. 1993; 1995; Ma et al. 1994), *Pm12* (Jia et al. 1996), and *Pm13* (Donini et al. 1995) and RAPD markers linked to *Pm1* (Hu et al. 1997), *Pm13* (Cenci et al. 1999), *Pm21* (Qi et al. 1996; Liu et al. 1999) and *Pm25* (Shi et al. 1998) have been reported. However, RFLP (Chao et al.

1989; Kam-Morgan et al. 1989) and RAPD markers (Devos and Gale 1992) show a low level of polymorphism in wheat, especially among cultivated lines and/or cultivars.

Microsatellites (Litt and Luty 1989), also termed simple sequence repeats (SSRs) (Jacob et al. 1991), have been proposed as additional markers for genetic mapping in plant species. Studies in wheat have indicated that microsatellite markers are chromosome-specific and evenly distributed along chromosomes (Röder et al. 1998). Such markers also reveal a higher level of polymorphism than RFLP markers (Plaschke et al. 1995). Microsatellite markers were used for tagging a stripe rust resistance gene from wild emmer wheat (Peng et al. 1999). Furthermore, amplified fragment length polymorphism (AFLP) developed by Vos et al. (1995) is an universal marker technique enabling the analysis of a large number of marker loci in a single polymerase chain reaction (PCR). This class of molecular markers is being applied widely in plants for the construction of high-density genetic maps (Becker et al. 1995; Keim et al. 1997) and for identifying specific genes (Schwarz et al. 1999; Thomas et al. 1995). Recently, a large number of chromosome-specific AFLP markers were assigned to different chromosomes and chromosomal arms of common wheat (Huang et al. 1999). AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* (Hartl et al. 1999) and associated with quantitative trait loci (QTLs) for wheat flour colour (Parker et al. 1998) have been identified.

Chinese landrace Chiyacao carries the dominant powdery mildew resistance gene *Pm24* (Huang et al. 1997b) which was initially localised by monosomic analysis on chromosome 6D (Huang et al. 1997c). However, results obtained using from an AFLP marker linked to *Pm24* in the repulsion phase showed that *Pm24* should be assigned to chromosome 1D. The discrepancy of gene location led to further crosses and monosomic analyses between various accessions of Chinese Spring monosomics 1D and 6D with the landrace Chiyacao. Here we describe the reallocation of the resistance gene *Pm24* to chromosome 1D and the identification of SSR and AFLP markers linked to this gene using bulked segregant analysis (Michelmore et al. 1991).

Materials and methods

Plant materials

A total of 85 F_2-derived F_3 families originating from a cross between the susceptible cultivar Chinese Spring (CS) and the resistant Chinese landrace Chiyacao (kindly provided by X.F. Wang, Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou) were used for analysis of linkage between molecular markers and powdery mildew resistance gene *Pm24*. At least 16 plants of each F_3 family were tested to identify the genotype of corresponding F_2 plants. For SSR and AFLP analysis, two DNA bulks were assembled by using equal amounts of DNA from 10 homozygous resistant and 10 homozygous susceptible F_3 families, respectively.

Nulli-tetrasomics (NTs) and ditelosomics (DTs) (1DS and 1DL) of Chinese Spring, originally obtained from Dr. E.R. Sears (University of Missouri, Columbia, USA), were used for chromosome assignment of AFLP markers.

As powdery mildew resistance gene *Pm22* in common wheat cultivar Virest has also been located on chromosome 1D (Peusha et al. 1996), a test of linkage between the two resistance genes was made using an F_2 population from the cross Chiyacao (*Pm24*)× Virest (*Pm22*).

Powdery mildew evaluation

Erysiphe graminis tritici (Egt) isolate nos. 5, 9, 10 and 12 used for the reaction and allelic tests were collected from different parts of Europe and selected from single-spore progenies. The tests were conducted on primary leaf segments cultured on 6 g/l agar and 35 mg/l benzimidazole in plastic boxes. The methods of inoculation, conditions of incubation and disease assessment were according to Huang et al. (1997a). Two major classes of host reactions were distinguished; r=resistant, s=susceptible, and these were used for genetic and linkage analyses. Chi-squared tests for goodness of fit were used to test for deviation of observed data from theoretically expected segregation ratios.

Microsatellite analysis

Genomic DNA was extracted from young leaf tissue and frozen in liquid nitrogen, as previously described by Huang et al. (1999). Wheat microsatellite (WMS) primer pairs were developed by Röder et al. (1998). One primer of WMS primer pairs was labelled using either 5-carboxy-fluorescein (5-FAM), 4,7,2′,7′-tetrachloro-6-carboxy-fluorescein (Tet) or 4,7,2′,4′,5′,7′-hexachloro-6-carboxyrhodamin (Hex). Each PCR reaction contained 50 ng genomic DNA, 10 pmol of each labelled and unlabelled primer, 0.75 U *Taq* DNA polymerase (Qiagen), 2 µl of 10×PCR buffer containing 15 m*M* $MgCl_2$, 0.3 m*M* dNTPs in a total volume of 20 µl. The PCR reaction was carried out in a PE 9600 thermal cycler for 35 cycles at 95°C for 10 s, 55°C for 10 s and 72°C for 30 s with a final step at 72°C for 10 min. The PCR products amplified with Hex-labelled primer and 5-Fam- or Tet-labelled primer were diluted with water at 1:4 and 1:9, respectively. The samples were mixed with 0.15 µl GeneScan-500 TAMRA internal size standard (PE Biosystems) and 0.85 µl formamide dye (98% formamide, 0.01% dextran blue), denatured at 90°C for 3 min and chilled on ice.

Electrophoresis of 36 samples was carried out using 5% denaturing polyacrylamide gel (Long Ranger™, FMC Bioproducts) in 1× TBE buffer (89 m*M* Tris, 89 m*M* boric acid, 2 m*M* EDTA, pH 8.3) on an ABI Prism™ 377 DNA Sequencer (PE Applied Biosystems) at 1200 V for 1.5 h. ABI collection software version 1.1 was used for raw data collection. Microsatellite fragments were analysed using GeneScan™ analysis software version 2.1 as described in the user's manuals.

AFLP analysis

AFLP markers were generated from the DNA of the phenotypic pools using 256 *Eco*RI+ANN/*Mse*I+CNN primer combinations as described by Huang et al. (1999). For estimating the level of polymorphic AFLP markers between the Chinese landrace Chiyacao and Chinese Spring the following primer combinations were used: E+AAT/M+CCA, E+AAT/M+CCG, E+AAT/M+CGA, E+ACA/M+CAC, E+ACA/M+CCC, E+ACA/M+CCG, E+ACA/M+CCT, E+ACA/M+CTA, E+ACA/M+CTG, E+ACT/M+CAA, E+ACT/M+CAC, E+ACT/M+CAG, E+ACT/M+CCC, E+ACT/M+CTC, E+AGA/M+CCC, E+AGA/M+CGA, E+AGA/M+CGC, E+AGA/M+CGG, E+AGA/M+CGT, E+AGA/M+CTG.

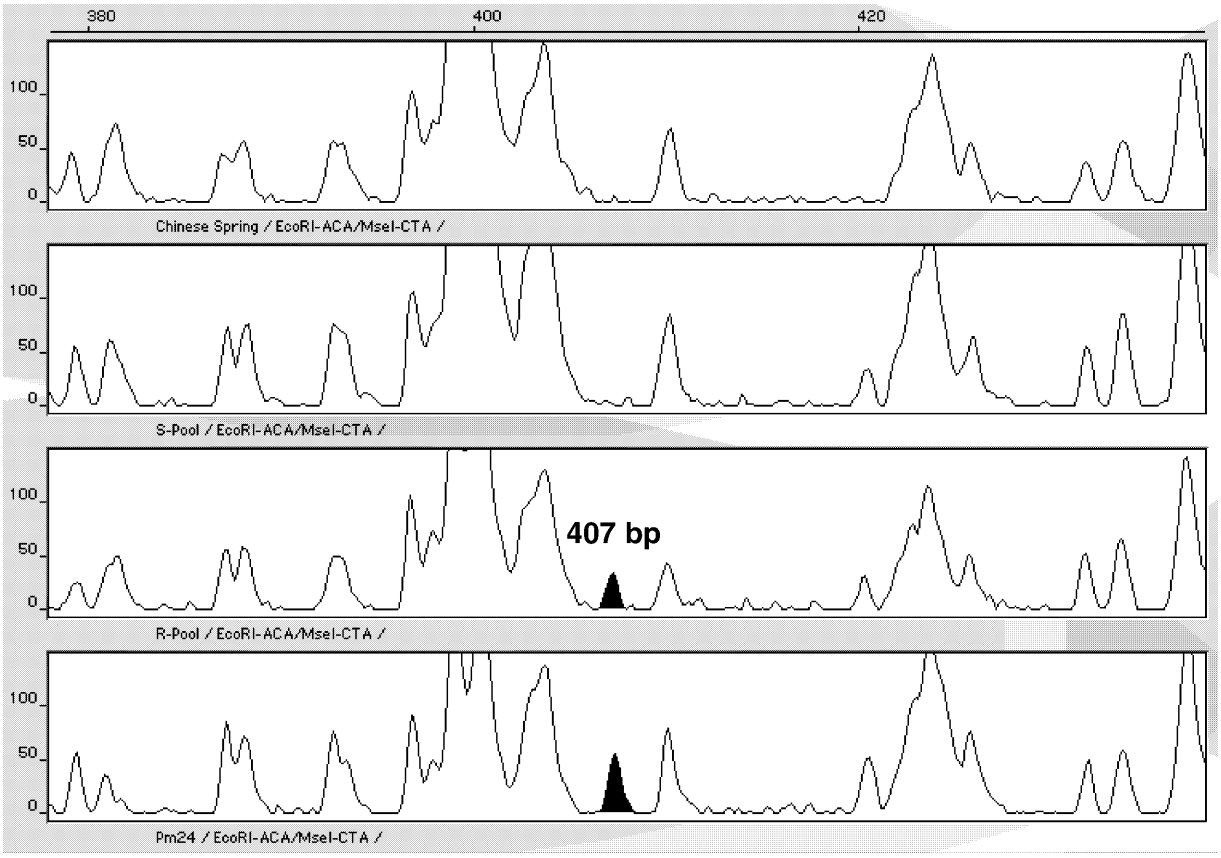

Fig. 1 Electropherograms showing polymorphic (shaded) 407-bp AFLP marker in Chinese Spring, S-Pool, R-Pool and Chiyacao, amplified with the primer combination E+ACA/M+CTA and analysed using GENESCAN™ analysis software version 2.1. The *peaks* represent fragments, whereas *horizontal* and *vertical scales* indicate fragment sizes in base pairs (bp) and relative signal intensity, respectively

Table 1 Segregation analysis for the *Pm24* locus and molecular markers in an F_2 population from the cross Chinese Spring×Chiyacao after studying their F_3 families

[a] Genotype: X_1X_1=Chiyacao, X_1X_2=heterozygous, X_2X_2=Chinese Spring
[b] Pooled values from homozygous and heterozygous classes

Gene or markers	Number. of F_2 plants	Observed no.			Expected ratio	χ^2	*P*
		X_1X_1[a]	X_1X_2	X_2X_2			
Pm24	85	15	48	22	1:2:1	2.58	0.20–0.30
E+ACA/M+CTA-407	85	63[b]		22	3:1	0.10	0.70–0.80
E+ACA/M+CCG-420	85	61[b]		24	3:1	0.48	0.40–0.50
E+AAT/M+CCA-346	85	15		70[b]	1:3	2.45	0.20–0.30
gmw106	85	15	51	19	1:2:1	3.78	0.10–0.20
gmw337	84	13	49	22	1:2:1	4.26	0.10–0.20
gmw458	84	18	49	17	1:2:1	2.36	0.30–0.50

Marker nomenclature

Wheat microsatellite loci were designated *Xgwm*, followed by a probe number, according to Röder et al. (1998). AFLP marker designations were based on the primer combination used and the fragment sizes estimated accurately with reference to the internal lane standard GeneScan-500 ROX. The primer combinations are abbreviated by the letters of the three selective nucleotides at the 3′ end of the E+3 primer before, and the M+3 primer behind the slash. Detected loci were marked with an 'X', the basic symbol for molecular marker loci of unknown function in wheat.

Linkage analysis

Linkage between DNA markers and the gene *Pm24* was established with MAPMAKER/EXP version 3.0b (Lander et al. 1987). Markers were placed with a LOD threshold of 3.0. The Kosambi function (Kosambi 1944) was applied to convert recombination fractions into map distances.

Results

Segregation of the resistance gene *Pm24*

A total of 85 F_2-derived F_3 families from the cross between Chinese Spring and Chiyacao were tested with isolate nos. 5, 9 and 12, which were all avirulent to Chiyacao. The observed segregation of 15 homozygous resistant, 48 heterozygous resistant and 22 homozygous

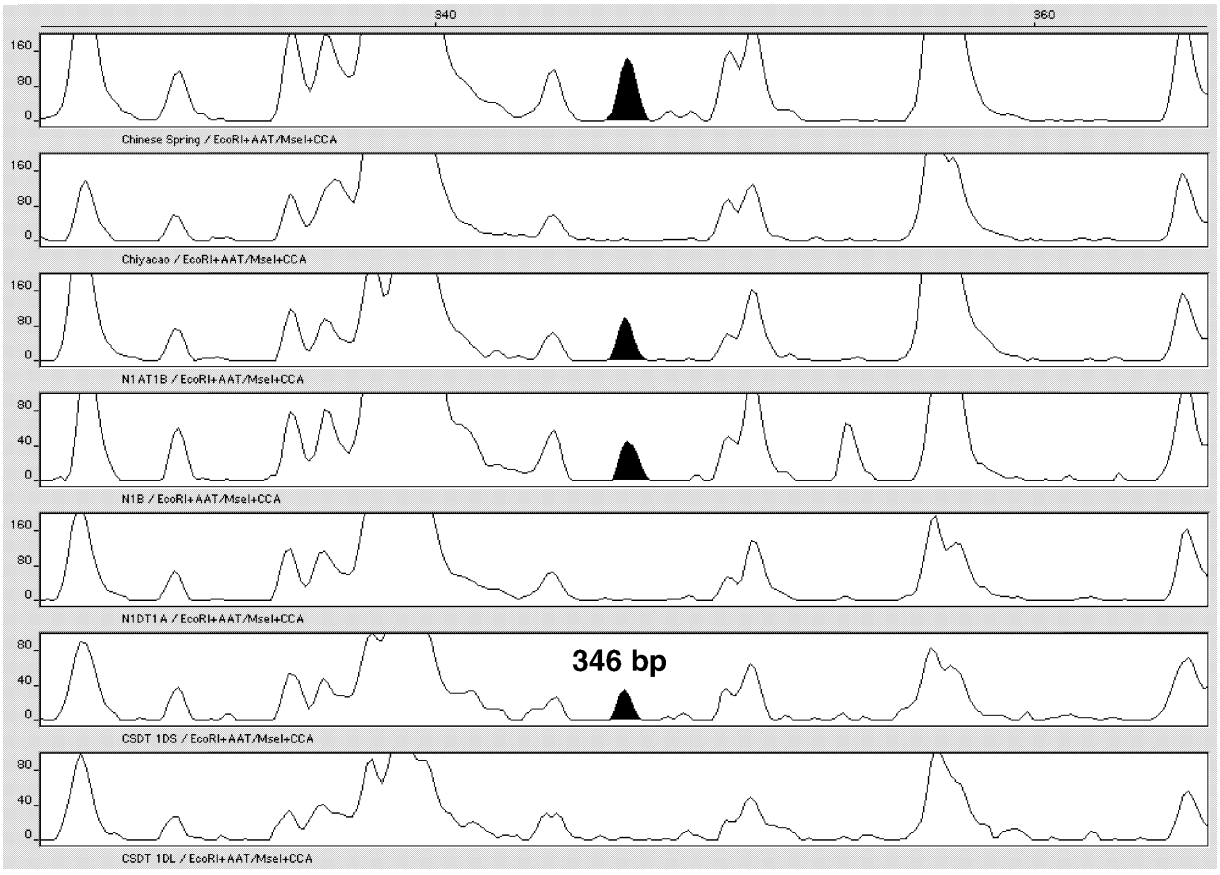

Fig. 2 Electropherograms of AFLP profiles in Chinese Spring, Chiyacao, N1AT1B, N1BT1A, N1DT1A, DT1DS and DT1DL, amplified with the primer combination E+AAT/M+CCA. The marked fragment (*shaded*) with a size of 346 bp was assigned to the short arm of chromosome 1D

susceptible families (Table 1) fitted a 1:2:1 segregation ratio (χ^2=2.58, 0.20<*P*<0.30), supporting segregation of a single locus.

AFLP variation between Chiyacao and Chinese Spring

The 20 AFLP primer combinations used in this study generated a total of 3032 fragments with an average of 152 fragments per primer pair, of which 248 were polymorphic (mean=12.4 per pair) between Chiyacao and Chinese Spring. The polymorphism rate of 8.2% was relatively high.

Identification and mapping of AFLP markers linked to the *Pm24* gene

A total of 256 *Eco*RI+3/*Mse*I+3 primer combinations of 16 possible *Eco*RI+ANN×16 possible *Mse*I+CNN were screened to identify polymorphic AFLP markers between the resistant and susceptible DNA bulks. Only 3 of the 256 primer combinations generated polymorphic AFLP fragments between the bulks.

AFLP markers ACA/CTA-407 and ACA/CCG-420 were present in Chiyacao and the resistant pool, but absent in Chinese Spring and the susceptible pool (Fig. 1). These two AFLP markers were further used to check their linkage to the *Pm24* gene, using a segregating F_2 population from a cross between the two wheats. Both AFLP markers showed a 3:1 segregation (Table 1). No recombination was found between *XACA/CTA-407* and the *Pm24* gene, indicating that *XACA/CTA-407* co-segregated with the *Pm24* gene, whereas *XACA/CCG-420*, was closely linked to the *Pm24* gene, with a map distance of 4.5±1.6 cM. These two markers were linked to *Pm24* in the coupling phase and are therefore 'resistance-dominant' markers. AFLP marker AAT/CCA-346 was amplified from the susceptible cultivar Chinese Spring and the susceptible pool, but not from Chiyacao and the resistant pool. This marker showed also a 3:1 segregation (Table 1). *XAAT/CCA-346* was linked to *Pm24* in repulsion phase and is a 'susceptibility-dominant' marker. No recombination with *Pm24* was observed, indicating co-segregation with susceptibility.

To determine the chromosomal location of the 'susceptibility-dominant' AFLP marker we, amplified DNA from 21 nulli-tetrasomics with the primer combination E+AAT/M+CCA. The fragment AAT/CCA-346 was absent only in N1DT1A, but it was present in the other 20 NTs (Fig. 2). After further analyses using CS DT1DS and DT1DL this marker was assigned to the short arm of chromosome 1D based on absence of the fragment in CS

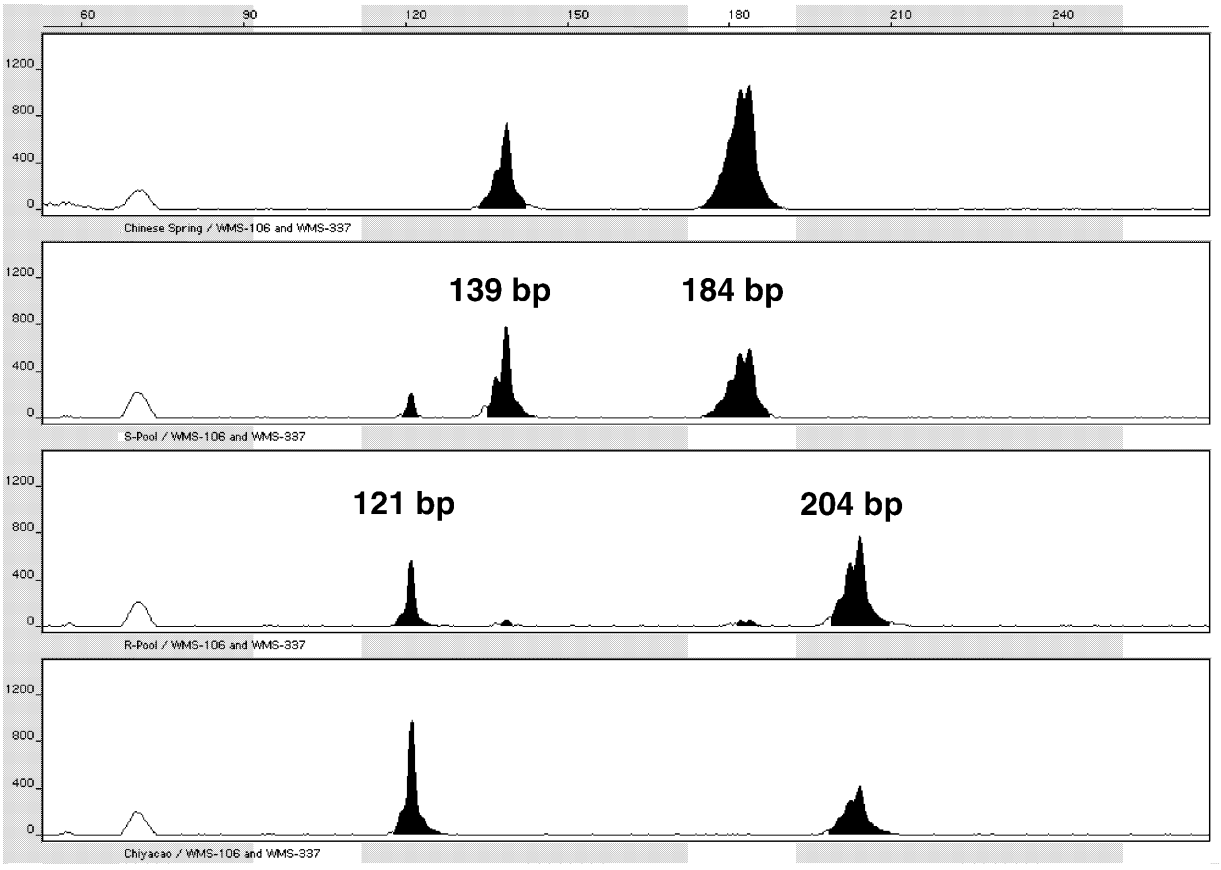

Fig. 3 Polymorphic microsatellite markers in Chinese Spring, S-Pool, R-Pool and Chiyacao, amplified with the primer pairs WMS106 and WMS337. Fragments of 121 bp and 139 bp and those of 184 bp and 204 bp were produced from primer pairs WMS106 and WMS337, respectively

DT1DL. This indicated that the gene *Pm24* is located on chromosome 1DS. Monosomic analysis of 91 F_2 plants from a further cross between CS monosomic 1D and Chiyacao inoculated with Egt isolate nos. 5 and 12 showed a segregation of 88 resistant to 3 susceptible plants. This ratio deviated significantly from the expected 3:1 ratio (χ^2=22.86; P<0.001), confirming that *Pm24* is located on chromosome 1D. Monosomic analysis of an F_2 family between CS monosomic 6D× Chiyacao tested with the same Egt isolates segregated in a 3:1 ratio.

Integration of microsatellite markers around the *Pm24* locus

Three primer pairs, WMS33, WMS106 and WMS337, and one primer pair, WMS458, from the short arm and long arms of chromosome 1D, respectively, were used to screen the bulks and the two parents for polymorphic markers. Two (*Xgwm106* and *Xgwm337*) of the microsatellites for the short arm of chromosome 1D, and *Xgwm458* for the long arm of chromosome 1D were polymorphic between the two bulks and the two parents (Fig. 3). WMS106, WMS337 and WMS458 amplified fragments with sizes of 139 bp, 184 bp and 112 bp in Chinese Spring and 121 bp, 204 bp and 114 bp in Chiyacao, respectively. These three microsatellite markers showed 1:2:1 segregation in the F_2 population from the cross between Chinese Spring and Chiyacao (Table 1), indicating that the three microsatellites are co-dominant markers. *Xgwm337* was very closely linked to *Pm24*, with a linkage distance of 2.4±1.2 cM. *Xgwm106* and *Xgwm458* were also linked to *Pm24*, with map distances of 19.2±3.8 cM and 10.3±2.5 cM, respectively (Fig. 4).

Because of its co-dominant inheritance and close linkage to *Pm24*, the microsatellite marker gwm337 was chosen to test its applicability to predict *Pm24*. DNA of 4 susceptible cultivars and 31 resistant cultivars/lines with known powdery mildew resistance genes or gene combinations were amplified using the primer pair WMS337 (Table 2). A 188-bp fragment was observed for the Italian cultivar Virest carrying the gene *Pm22* on chromosome 1D. The sizes of the amplified fragments of other genotypes ranged from 157 bp in the synthetic wheat line XX186 to 202 bp in the Chinese cultivars Xiaobaidong and Fuzhuang 30. All were different from the amplified fragment with a size of 204 bp in landrace Chiyacao. This indicated that the 204 bp allele is specific for the *Pm24* locus.

Linkage analysis between *Pm24* and *Pm22*

Genetic linkage between the gene *Pm24* in Chiyacao and the gene *Pm22* in Virest was investigated. A total of 452

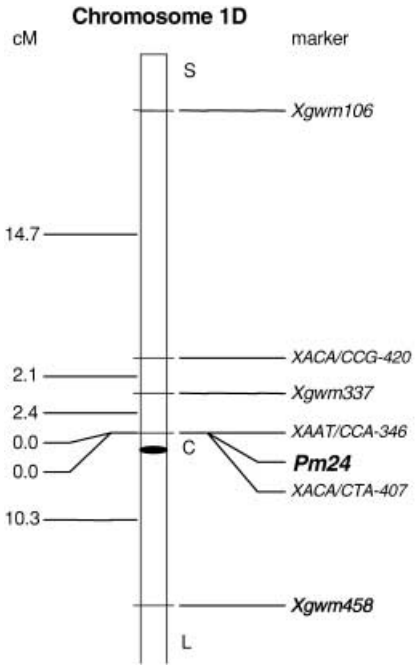

Fig. 4 A genetic map of the *Pm24* region. Three microsatellite loci (*Xgwm106*, *Xgwm337* and *Xgwm458*) and three AFLP loci (*XACA/CCG-420*, *XACA/CCA-407* and *XAAT/CCA-346*) are shown. *C* Relative centromere position, *S* short arm, *L* long arm

F_2 plants from a cross between Chiyacao and Virest was tested with *Egt* isolate no. 10, avirulent for both genes. The F_2 population segregated 424 resistant : 28 susceptible individuals, conforming to a ratio of 15 resistant: 1 susceptible ($\chi 2$ 15:1=0.00, $P>0.95$), this indicates that the gene *Pm24* segregated independently of the gene *Pm22*, shows that the two resistance genes are at different loci on chromosome 1D.

Table 2 Fragment sizes of 35 wheat cultivars and lines without or with known powdery mildew resistance gene(s) after amplification using primer pair WMS337

Cultivar/line	Resistance gene	Fragment size (bp)
Chinese Spring	–	184
Amor	–	186
Kanzler	–	184
Trakos	–	185
Axminster/8*Cc	*Pm1a*	185
M1 N	*Pm1c* (*Pm18*)	185
Ulka/8*Cc	*Pm2*	185
Asosan/8*Cc	*Pm3a*	185
Chul/8*Cc	*Pm3b*	185
Sonora/8*Cc	*Pm3c*	185
Kolibri	*Pm3d*	185
W150	*Pm3e*	200
Michigan Amber/8*Cc	*Pm3f*	185
N324	*Pm3i*	168+190
Khapli/8*Cc	*Pm4a*	185
Clan	*Pm4b+Pm5*	183
Kontrast	*Pm5*	183
IGV 455	*Pm5* allele	187
Xiaobaidong	*Pm5* allele	202
Fuzhuang 30	*Pm5* allele	202
Disponent	*Pm8*	185
Zecoi-4	*Pm16*	186
Amigo	*Pm17*	187
XX186	*Pm19*	157
TAM104-T6BS·6RL	*Pm20*	188
Virest	***Pm22***	**188**
Chiyacao	***Pm24***	**204**
Herzog	*Pm4b+Pm8*	185
Piko	*Pm5+Pm6*	186
Greif	*Pm5+Pm6*	186
Normandie	*Pm1+Pm2+Pm9*	185
Triso	*Pm1+Pm4b+Pm5*	177
Troll	*Pm1+Pm2+Pm4b*	187
Ritmo	*Pm2+Pm5+Pm6*	186
Apollo	*Pm2+Pm4b+Pm8*	184

Discussion

Chromosomal location of the gene *Pm24*

Monosomic analysis in combination with chromosome-specific molecular markers is an efficient protocol approach for locating the chromosomal position of resistance genes in wheat. In the present study, AFLP marker AAT/CCA-346, which co-segregated in repulsion with *Pm24*, was placed on the short arm of chromosome 1D, thereby enabling the localisation of *Pm24* to the short arm of chromosome 1D. Two microsatellite loci *Xgwm106* and *Xgwm337* from the short arm and microsatellite locus *Xgwm458* from the long arm of chromosome 1D were linked to the gene *Pm24*, further supporting this result. These results were also supported by monosomic analysis. According to the wheat microsatellite map (Röder et al. 1998), *Xgwm337* is located on the short arm near the centromere, while *Xgwm458* is on the long arm. The gene *Pm24* lies between *Xgwm337* and *Xgwm458* (Fig. 4). It may be concluded, therefore, that *Pm24* is located on the short arm of chromosome 1D, close to the centromere.

Linkage relationships among *Pm10*, *Pm22* and *Pm24*

The genes *Pm10* in the Japanese cultivar Norin 29 (Tosa et al. 1987) and *Pm22* in the Italian cultivar Virest (Peusha et al. 1996) are located on chromosome 1D. Because *Pm10* confers resistance to the goat grass powdery mildew pathogen *E. graminis* f. sp. *agropyri*, it can be studied only under restricted circumstances. Linkage analysis of *Pm24* and *Pm22* revealed that the two genes are genetically independent.

According to the consensus map of chromosome 1 of the *Triticeae* (Van Deynze et al. 1995), wheat *Pm3* locus and barley *Mla* locus are located in the distal regions of the short arms of wheat chromosome 1A and barley chromosome 1H, respectively. The rye-derived gene *Pm8* is at the end of 1RS of the wheat-rye translocated chromosome T1BL·1RS (Börner and Korzun 1998). As the gene *Pm24* is located on the short arm of chromo-

some 1D, in the vicinity of the centromere, there appears to be no orthologous relationship between *Pm24* and the other powdery mildew resistance genes distally located in the short arms of chromosome 1.

Marker assisted selection

Using an F_2-derived F_3 family in combination with bulked segregant analysis, we identified three dominant AFLP markers, of which two, *XACA*/*CTA-407* and *XAAT*/*CCA-346*, co-segregated with *Pm24* in the coupling and repulsion phases, respectively. One AFLP marker, *XACA*/*CCG-420*, mapped 4.5±1.6 cM from the *Pm24*. Three co-dominant microsatellite loci, *Xgwm106*, *Xgwm337* and *Xgwm458*, were linked to *Pm24*. The order of the microsatellite loci in the vicinity of *Pm24* agreed very well the published microsatellite map of chromosome 1D (Röder et al. 1998). The closely linked microsatellite marker gwm337 was monitored in different genetic backgrounds, and a *Pm24*-specific marker allele could be detected.

Among 25 documented powdery mildew resistance loci, several genes have been characterised using DNA markers such as RFLP, RAPD, AFLP and sequence-tagged site STS markers. Shi et al. (1998) reported that it was difficult to use three RAPD markers for the selection of *Pm25* because of excessive genetic distance between the markers and *Pm25*. Ma et al. (1994) found co-segregating RFLP markers for *Pm1* and *Pm4a*, and closely linked RFLP markers for *Pm2* and *Pm3b*. However, they did not investigate the diagnostic value of these RFLP markers. Using different RFLP markers closely linked to *Pm1* and *Pm2*, Hartl et al. (1995) could not identify all of the cultivars possessing *Pm1* and *Pm2*, respectively. A similar result was reported using a STS marker that was converted from the corresponding RFLP marker linked to *Pm2* (Mohler and Jahoor 1996). Two RAPD markers co-segregating with *Pm1* could be used for the differentiation of susceptible cultivars and cultivars carrying the *Pm1* gene (Hu et al. 1997). Qi et al. (1996) and Cenci et al. (1999) detected a specific RAPD marker for gene *Pm21* from *Haynaldia villosa* and a specific STS marker for the gene *Pm13* from *Aegilops longissima*. Moreover, Hartl et al. (1999) reported an AFLP marker specific for the allele *Pm1c* (Hsam et al. 1998). However, because of their dominant inheritance these markers cannot be applied to differentiating homozygous and heterozygous individuals in segregating progenies.

The problems (e.g. related sequences) associated with the applicability of molecular markers for wheat breeding can be circumvented by the availability of microsatellite markers. Microsatellite gwm337 is a PCR-based, genome-specific, co-dominant marker that displays a specific allele for *Pm24* which confers highly effective resistance to powdery mildew in Europe and China (Huang et al. 1997a, c). Therefore, it can be used as a diagnostic marker for the selection of *Pm24*.

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