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Identification of RAPD markers linked to the gene *PM1* for resistance to powdery mildew in wheat

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Abstract Powdery mildew caused by *Blumeria graminis* DC. f. sp. *tritici* Ém. Marchal is an important disease of wheat (*Triticum aestivum* L. em Thell). We report here the identification of three random amplified polymorphic DNA (RAPD) markers closely linked to a gene for resistance to *B. graminis* in wheat. RAPD-PCR (polymerase chain reaction) analysis was conducted using bulked segregant analysis of closely related lines developed from a segregating F_5 family. The F_5 family was derived from a cross between the susceptible cultivar Clark and the resistant line Zhengzhou 871124. Genetic analysis indicated that resistance of Zhengzhou 871124 to powdery mildew is conferred by the gene *Pm*1. After performing RAPD-PCR analysis with 1300 arbitrary 10-mer primers and agarose-gel electrophoresis, two RAPD markers, $UBC320_{420}$ and $UBC638_{550}$, were identified to be co-segregating with the disease resistance. No recombinants were observed between either of the RAPD markers and the gene for resistance to powdery mildew after analysis of 244 F_2 plants. The third RAPD marker, $OPF12_{650}$, was identified with denaturing gradient-gel electrophoresis (DGGE), and was determined to be 5.4 ± 1.9 cM from the resistance gene. $UBC320_{420}$ and $UBC638_{550}$ were present in wheat powdery mildew differential lines carrying the gene *Pm*1, suggesting linkage between these markers and the *Pm*1 resistance gene. Co-segregation between *Pm*1 and the two markers $UBC320_{420}$ and $UBC638_{550}$ was confirmed in a segregating population derived from a cross with CI14114, the wheat differential line carrying *Pm*1. The method of deriving closely related lines from inbred families that are segregating for a trait of interest should find wide application in the identification of DNA markers linked to important plant genes. The RAPD marker $UBC638_{550}$ was converted to a sequence tagged site (STS). RAPD markers tightly linked to target genes may facilitate selection and enable gene pyramiding for powdery mildew resistance in wheat breeding programs.

Key words Powdery mildew · Wheat · RAPD markers · Related inbred lines · Bulked segregant analysis

Introduction

Powdery mildew caused by *Blumeria graminis* DC. f. sp. *tritici* Ém. Marchal is an important disease of wheat (*Triticum aestivum* L. em. Thell). Genes for resistance to powdery mildew from different sources have been identified in wheat at 19 distinct loci (McIntosh 1988; Friebe et al. 1994). Most of these loci have been located on particular wheat chromosomes (McIntosh 1988). Among these loci, the *Pm*1 locus is on the long arm of chromosome 7A (Sears and Briggle 1969). Genetic resistance has been used effectively to control powdery mildew. However, since several of these genes have been effective for only a few years after being widely deployed, it is necessary to search for new sources of resistance and to use available genes in combinations that will provide effective and more durable resistance. Based on Flor's (1955) gene-for-gene hypothesis, Moseman (1959) demonstrated that the presence of a specific gene for mildew resistance can be ascertained by an interaction with the *B. graminis* cultures that possess the corresponding gene for avirulence. As additional resistance genes to new pathogen isolates are required to obtain resistance, identification of different resistance genes and linkage relationships will become difficult. By exploiting linkage between molecular markers and mildew-resistance genes, the presence or absence of

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specific resistance genes can be efficiently determined in breeding lines with more than one resistance gene.

Molecular markers that are tightly linked to genes of interest can be used in plant breeding programs to facilitate selection and as a starting point for the physical localization of such genes (Tanksley et al. 1989; Martin et al. 1994). Marker-facilitated selection would be particularly effective for pyramiding two or more resistance genes to provide effective and potentially stable resistance to powdery mildew in wheat, where simultaneous or even sequential screening of plants with several different pathogen isolates is difficult or impractical.

Backcross-derived near-isogenic lines (NILs) have been successfully used for identifying restriction fragment length polymorphism (RFLP) markers tightly linked to disease-resistance genes in several crop species. This has been shown in tomato for the *I2* locus for resistance against *Fusarium oxysporum* (Sarfatti et al. 1989), the *Tm2* locus for resistance against tomato mosaic virus (Young et al. 1988), the *Mi* locus for resistance against root knot nematode *Meloidogyne* spp. (Klein-Lankhorst et al. 1991); in maize for resistance to maize dwarf mosaic virus (McMullen and Louie 1989); in rice for the genes *Pi*-2 and *Pi*-4 for blast resistance (Yu et al. 1991); in barley for the powdery mildew resistance genes *Mla* (Schuller et al. 1992; Jahoor and Fischbeck 1993), *MlLa* (Hilbers et al. 1992; Giese et al. 1993), and *ml*-o (Hinze et al. 1991). Random amplified polymorphic (RAPD) DNA markers (Welsh and McClelland 1990; Williams et al. 1990) have also proven useful for efficiently targeting tightly linked markers using backcross-derived NILs (Martin et al. 1991; Paran et al. 1991; Penner et al. 1993a, b). RAPD markers have the advantages of technical simplicity and a generally higher level of resolved polymorphisms, and thus may be more applicable to plant breeding programs than restriction fragment length polymorphism (RFLP) markers. Both RFLP and RAPD markers have been used to target disease-resistance genes by using backcross-derived NILs in wheat. Using NILs, Schachermayr et al. (1994) identified three RAPD and one RFLP markers that showed complete linkage to the *Lr*9 leaf rust resistance gene in wheat. A RAPD marker associated with the Hessian fly resistance gene *H*9 was identified with NILs in conjunction with denaturing gradient-gel electrophoresis (DGGE) by using two primers in combination in wheat (Dweikat et al. 1994). In wheat using NILs an RFLP marker has been identified to be linked to the *Pm*3 locus for resistance to powdery mildew at a distance of 3.3 ± 1.9 cM (Hartl et al. 1993). Co-segregating RFLP markers for powdery mildew resistance genes *Pm*1 and *Pm*4 and tightly linked RFLP markers for *Pm*2 and *Pm*3b were recently reported by Ma et al. (1994).

Even though backcross-derived NILs have been widely used, the development of pairs of NILs for economically important genes through backcrossing is costly and time consuming and, more importantly, few such pairs of backcross-derived NILs are presently available for marker-based applications in wheat. An alternative strategy, bulked segregant analysis (Michelmore et al. 1991), was proposed to alleviate the problems associated with use of backcross-derived NILs by utilizing DNA bulks of segregating individuals of known genotype for the gene of interest. This approach has been employed successfully to identify RAPD markers for a resistance gene to downy mildew in lettuce (Michelmore et al. 1991), and for crown rust resistance gene *Pc*68 in oats (Penner et al. 1993a). Success has also been realized using bulked segregant analysis in combination with backcross-derived NILs for identifying RAPD markers tightly linked to bean rust resistance genes (Haley et al. 1993; Miklas et al. 1993). Instead of using backcross-derived NILs, Haley et al. (1994) described the use of heterogeneous inbred populations as sources of NILs for targeting RAPD markers tightly linked to a major bean rust resistance locus.

We describe here an approach to develop the closely related inbred lines resistant and susceptible to powdery mildew from a segregating F_5 family. Using these closely related lines in combination with bulked segregant analysis, we identified three RAPD markers tightly linked to a powdery mildew resistance gene in wheat. The method should find wide application in the identification of DNA markers, either RFLP or RAPD, tightly linked to important genes.

Materials and methods

Plant materials

The wheat accessions CI14114, CI14118, CI14120, CI14123, CI14125, PI405718, CI140189, and PI361879 carrying genes *Pm*1, *Pm*2, *Pm*3a, *Pm*4a, *Pm*5, *Pm*2 + *Pm*6, *Pm*7, and *Pm*8, respectively, were crossed en masse as pollen parents to adapted lines segregating for male sterility conditioned by a dominant male-sterile gene at the Henan Academy of Agricultural Science, Zhengzhou, China. Resistant F_1 plants were inter-mated once to combine more than one of the eight powdery mildew resistance genes into the same genotype. The resulting powdery mildew-resistant male-sterile plants were crossed to powdery mildew-susceptible adapted lines, and the resistant line Zhengzhou 871124, used in this study, was developed by pedigree selection of the resulting populations during inbreeding (Z. J. Lin, personal communication). The powdery mildew resistance gene(s) carried by Zhengzhou 871124 could not be determined from its parentage, except that its resistance must involve one or more of the genes *Pm*1 to *Pm*8. Genetic analysis of resistance to powdery mildew was carried out using F_1, BC_1F_1 (F_1 backcrossed to the resistant parent), BC_2F_1 (F_1 backcrossed to the susceptible parent), and F_2 populations from a cross between the susceptible cultivar Morocco and the resistant line Zhengzhou 871124. The F_2 populations of the crosses between Zhengzhou 871124 and each of 11 wheat differential lines with known genes for resistance to powdery mildew were tested for segregation of resistance to powdery mildew.

Development of the closely related resistant and susceptible inbred lines and construction of DNA bulks for RAPD analysis are illustrated in Fig. 1. Two DNA bulks for RAPD analysis were

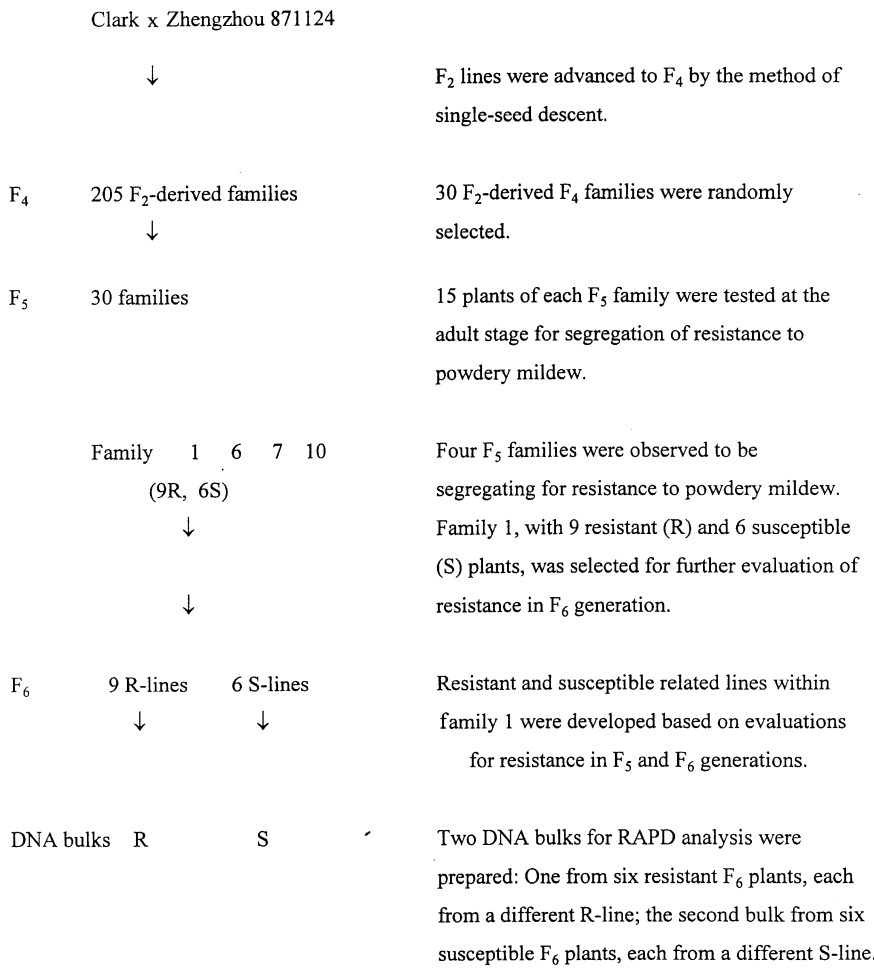

Fig. 1 Strategy for developing closely related inbred lines resistant or susceptible to powdery mildew and DNA bulks for RAPD analysis

constructed by using equal amounts of DNA from each of six F_6 resistant plants, each from a different F_5 line, and six F_6 susceptible plants, each from a different F_5 line, respectively. Two F_2 populations segregating for reaction to powdery mildew were used for analysis of linkage between RAPD markers and powdery mildew resistance. One population consisted of 85 random F_2 plants derived from a cross between the susceptible cultivar Clark and the resistant line Zhengzhou 871124. The second population consisted of 159 random F_2 plants derived from a cross between Morocco and Zhengzhou 871124. Twelve wheat powdery mildew differential lines and 20 accessions with the assumed *Pm*1 gene (McIntosh 1988) were tested for the presence of the markers. These accessions were obtained from the National Small Grains Collection, USDA-ARS, Aberdeen, Idaho.

Powdery mildew evaluations

Powdery mildew evaluations were performed on the first or second leaves of seedlings in the greenhouse, using isolate 94-6 from a single colony of *B. graminis*, with virulence/avirulence as presented in Table 2. An inoculum of the isolate was maintained and produced on seedlings of susceptible Morocco. Plant materials were inoculated by dusting them with conidia from infected seedlings of Morocco. Inoculation was repeated 1 day after the first inoculation to help ensure successful inoculation. About a week after inoculation, infection types of powdery mildew on plants were recorded on a scale of 0–4 (Mains and Dietz 1930) and recorded again after 2 days. From this scale, two major infection type (IT) classes were designated, resistant (IT = 0–2) and susceptible (IT = 3–4), and were used for genetic and linkage analysis.

RAPD analysis

Leaf tissues from 2-week-old seedlings grown in the greenhouse or in a growth chamber were collected prior to inoculation with powdery mildew. Genomic DNA was extracted as previously described by Dweikat et al. (1993). Oligonucleotide primers (10-mers) were purchased from The University of British Columbia (UBC) (Vancouver, Canada) and Operon Technologies (Alameda, Calif.). PCR amplification reactions contained 10 mM Tris-HCl, pH 8.8 at 25°C, 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM 10-mer primer, 10 ng template DNA, and 1.5 units of *Taq* DNA polymerase (Promega). The reaction mixture was overlaid with one drop of light mineral oil. Amplifications were performed in an MJR PTC-100 thermocycler programmed for 36 cycles of 40 s at 94°C, 1 min at 36°C, 1 min at 72°C, and ending with 6 min at 72°C.

Cloning and sequencing of RAPD fragments

The amplification products of primers UBC320 and UBC638 were resolved by agarose-gel electrophoresis, and the polymorphic bands were excised from the gel. The excised bands were placed in a microfuge tube containing 100 μl of TE buffer (pH 8.0) and heated for 5 min at 95°C. The dissolved DNA was amplified by PCR with

UBC320 and UBC638 primers, respectively, under the above conditions. The amplified products were then cloned using a TA cloning kit (Invitrogen Corp., San Diego, Calif.). Identity of the cloned RAPD products was verified by a digest with the restriction enzyme *Eco*RI, which revealed the vector DNA fragment and the insert fragment of the expected size, and by PCR amplification with UBC320 and UBC638, which produced one fragment of the expected size. DNA sequencing was carried out by the dideoxy chain-termination method using a Sequenase Version 2.0 kit (US-Biochemical Corp., Cleveland, Ohio, USA) with M13 forward and reverse primers.

STS design and analysis

A pair of 20-pb oligonucleotides for each of the two cloned amplification products $UBC320_{420}$ and $UBC638_{550}$ were designed as sequence-tagged site (STS, Olson et al. 1989) primers based on the sequence data (Table 5). Each of these STS primers contains the original ten bases of the RAPD primer plus the next ten internal bases. Primers (20-mer) were synthesized by Operon Technologies (Alameda, Calif.). PCR amplification of genomic DNA with STS primers was carried out in 26 µl of the same reaction mix as for the RAPD amplifications except that STS primers were used. The reactions were performed in an MJR PTC-100 thermocycler programmed for 2 min at 94°C as denaturing step, then followed by 36 cycles of 1 min at 94°C, 1 min at 61°C, 1 min at 72°C, and with a final extension of 72°C for 6 min.

Following amplification, 25 µl of the amplified products were transferred to a new microfuge tube and digested with each of 16 restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Sac*I, *Pst*I, *Hin*fI, *Taq*I, *Bam*HI, *Msp*I, *Rsa*I, *Mbo*I, *Ava*I, *Ban* II, *Bc*II, *Dde*I, and *Hae*III) under conditions recommended by the manufacturer. The amplified digested products were then resolved on a 1.2% agarose gel.

Gel electrophoresis

The PCR amplification products (30 µl) were fractioned on 1.2% agarose gel using 0.5 × TBE buffer (0.045 M Tris-borate and 1.0 mM EDTA pH 8.0), at 3 V/cm for 3 h. The denaturing gradient gels consisted of 12% polyacrylamide (37.5:1 acrylamide:bisacrylamide) in TAE buffer (40 mM Tris-acetate, 20 mM sodium acetate, 1 mM EDTA; pH 7.7). The gel denaturant gradients of 10–50% (100% denaturant concentration of 7 M urea, 40% formamide) were prepared with a gradient maker (Hoefer). The gels were run in 1 × TAE buffer with pH 7.7 at 60°C for 7 h, stained with ethidium bromide (1 µg/ml) for 15 min, destained in deionized water for 2 h, and photographed over a long-wave UV light source.

Linkage analysis

Linkage estimation was based on the maximum-likelihood method using the appropriate formulas of Allard (1956). This recombination fraction was transformed into cM according to Kosambi (1944).

Results

Inheritance and verification of powdery mildew resistance

Segregation patterns of F_1, BC_1F_1, BC_2F_1, and F_2 generations from the cross of Morocco × Zhengzhou 871124 indicated that the resistance of Zhengzhou 871124 was conferred by a single dominant gene (Table 1). Infection types of isolate 94-6 of *B. graminis* f. sp. *tritici* on Zhengzhou 871124 and 12 wheat differential lines are presented in Table 2. No susceptible segregants were observed among 593 F_2 plants from the cross between Zhengzhou 871124 and CI14114 (Table 3), which indicated that Zhengzhou 871124 possesses the *Pm*1 resistance gene. F_2 progenies of all other crosses produced susceptible recombinants. These segregation ratios confirmed that the resistance of Zhengzhou 871124 is conferred by a single gene. The unexpected F_2 segregation ratios involving CI14120, CI14124, and PI405718 (Table 3), are most likely due to misclassification as "susceptible" of several resistant plants, because resistance may not be fully expressed, particularly in seedlings heterozygous for these genes. The cross between Zhengzhou 871124 and CI14125, carrying *Pm*5, was not tested because the recessive nature of *Pm*5 (Bennett 1984) is indicative that it is not the gene for resistance in Zhengzhou 871124.

Identification of tightly linked RAPD markers

A total of 1300 random 10-mer primers (800 UBC primers and 500 Operon primers) were screened to identify polymorphisms between the resistant and susceptible DNA bulks. On average, each primer amplified about five fragments that ranged from 300 to 2000 bp. Of these primers, only three – UBC98, UBC320 and UBC638 – generated DNA fragments that appeared in the resistant bulk, but not in the susceptible bulk, after agarose-gel electrophoresis. The polymorphic fragment (approximately 1600 bp) generated by the primer UBC98 was not investigated

Table 1 Reaction and segregation patterns of F_1, BC_1F_1, BC_2F_1, and F_2 populations to isolate 94-6 of *B. graminis* f. sp. *tritici* from the cross Morocco × Zhengzhou 871124 (BC_1F_1, F_1 backcrossed to the resistant parent; BC_2F_1, F_1 backcrossed to the susceptible parent; R, resistant; S, susceptible)

F_1	BC_1F_1		BC_2F_1						F_2					
	R	S	R	S	Total	Ratio	$\chi 2$	*P* value	R	S	Total	Ratio	$\chi 2$	*P* value
R	102	0	59	52	111	1:1	0.44	0.507	128	55	183	3:1	2.49	0.115

Table 2 Wheat powdery mildew differentials and their infection types to isolate 94-6 of *B. graminis* f. sp. *tritici*

Differential line	Gene	Source[a]	Infection type
Zhengzhou 871124	–	–	0
CI14114[b]	*Pm*1	Axminster	0
CI14118[b]	*Pm*2	Ulka	4
CI14119[b]	*Pm*2	CI 13632	4
CI14120[b]	*Pm*3a	Asosan	0
CI15887[b]	*Pm*3b	*T. sphaerococcum*	0
CI14122[b]	*Pm*3c	Sonora	0
CI14123[b]	*Pm*4a	Khapli	1
CI14124[b]	*Pm*4a	Yuma	1
CI14125[b]	*Pm*5	Hope	0
PI405718	*Pm*2 + *Pm*6	TP114	4
CI14189[b]	*Pm*7	Transec	4
PI361879	*Pm*8	Kavkaz	1

[a] Original source of the resistance gene
[b] Near-isogenic lines developed after seven generations of backcrossing, with cultivar Chancellor as recurrent parent and the respective source lines as donor parents

Table 3 Reactions of F_2 plants from the crosses of Zhengzhou 871124 with 11 wheat powdery mildew differentials to isolate 94-6 of *B. gramminis* f. sp. *tritici* (R, resistant; S, susceptible)

Zhengzhou 871124 × differential	F_2 plants		Ratio (R:S)	χ^2	*P* value
	R	S			
CI15114 (*Pm*1)	593	0			
CI14118 (*Pm*2)	257	84	3:1	0.02	0.888
CI14119 (*Pm*2)	228	72	3:1	0.16	0.689
CI14120 (*Pm*3a)	147	103	9:7	0.67	0.413
CI15887 (*Pm*3b)	271	20	15:1	0.19	0.663
CI14122 (*Pm*3c)	200	15	15:1	0.20	0.655
CI14123 (*Pm*4a)	277	24	15:1	1.53	0.216
CI14124 (*Pm*4a)	239	65	13:3	1.38	0.240
PI405718 (*Pm*2 + *Pm*6)	200	103	45:19	2.69	0.101
CI14189 (*Pm*7)	239	67	3:1	1.57	0.210
PI361879 (*Pm*8)	230	61	13:3	0.92	0.337

further because the band was weak and not clearly reproducible. Primer UBC320 (5′-CCGGCATAGA-3′) generated one polymorphic product of 420 bp (designated as $UBC320_{420}$), and primer UBC638 (5′-GCGGTGACTA-3′) generated one polymorphic product of 550 bp (designated as $UBC638_{550}$), each of which was polymorphic between the two bulks.

Previous studies indicated that denaturing gradient-gel electrophoresis (DGGE) improved the resolution of DNA fragment polymorphism compared to agarose-gel electrophoresis (He et al. 1992; Dweikat et al. 1993). After examining the PCR-amplified DNA fragments from 1300 primers in agarose gels, 46 primers from which ambiguous bands were generated were selected to screen the DNA bulks by using DGGE. Of these primers, OPF12 (5′-ACGGTACCAG-3′) generated a DNA fragment (designated as $OPF12_{650}$) that was present in the resistant bulk but absent from the susceptible bulk after DGGE (Fig. 2); yet this fragment was not detected in agarose gels. On further analysis, it was observed that the polymorphic DNA fragment generated from each of the three primers (UBC320, UBC638, and OPF12) by bulked segregant analysis appeared in all six resistant plants individually but not in any of the susceptible plants (data not presented), suggesting linkage of the RAPD markers and powdery mildew resistance.

Linkage of powdery mildew resistance and RAPD markers

All three RAPD markers, $UBC320_{420}$, $UBC638_{550}$, and $OPF12_{650}$ co-segregated with the resistance of Zhengzhou 871124. No recombination was found between any of the three markers and powdery mildew resistance among 85 F_2 plants from a cross between Clark and Zhengzhou 871124 (data not shown). In a second population of 159 F_2 plants from a cross between Morocco and Zhengzhou 871124, complete co-segregation was observed between resistance and the two markers $UBC320_{420}$ and $UBC638_{550}$, as shown in Fig. 3A, B. Analysis of the second population, however, revealed nine recombinants (four resistant

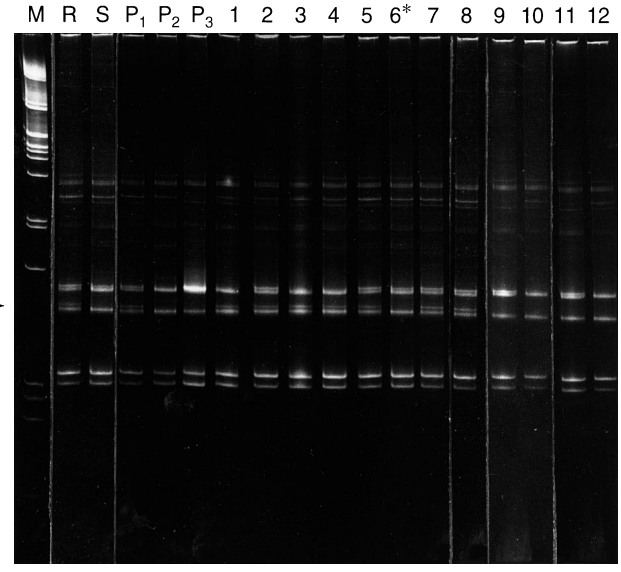

Fig. 2 PCR amplification of bulked DNA (*R* and *S*) of the closely related wheat lines derived from inbreeding, and genomic DNA of cultivars Zhengzhou 871124 (*P1*), Clark (*P2*), Morocco (*P3*), eight resistant F_2 plants (*1–8*), and four susceptible F_2 plants (*9–12*) from the cross of Morocco Zhengzhou 871124 with primer OPF12. *M*, *Pst*I-λ DNA. *R*, resistant; *S*, susceptible. * Indicates an F_2 recombinant between resistance and the indicated RAPD band. Amplification products (20 μl) were fractionated by DGGE

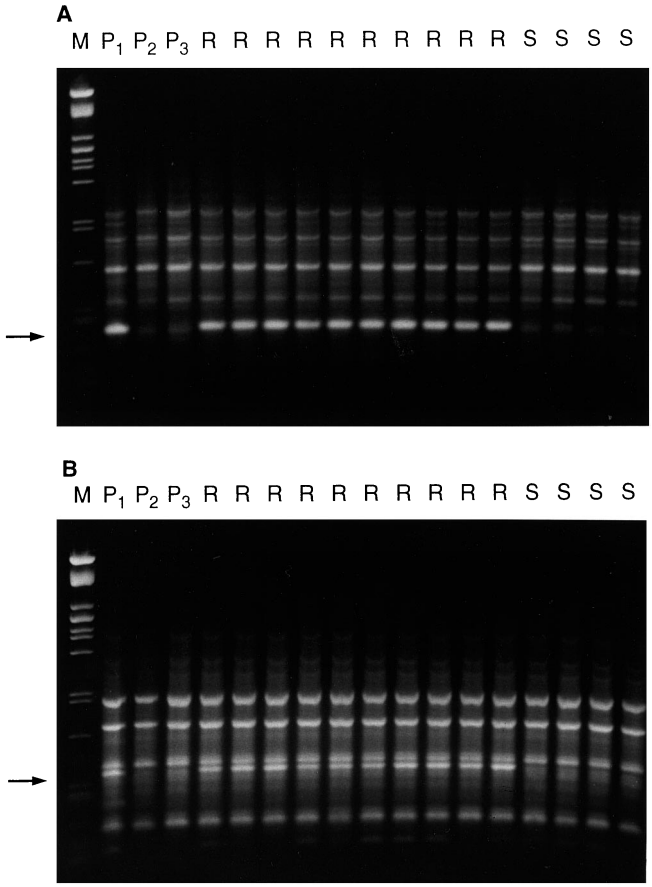

Fig. 3A, B Ethidium bromide-stained agarose gel demonstrating co-segregation between $UBC320_{420}$, $UBC638_{550}$ and powdery mildew resistance (*R*) and susceptibility (*S*) in F_2 individuals from the wheat cross of Morocco × Zhengzhou 871124. **A** RAPD data for primer UBC320; **B** RAPD data for primer UBC638. *M*, *Pst*I-λ DNA. *P1*, Zhengzhou 871124; *P2*, Clark; *P3*, Morocco. *Arrows* indicate resistance-specific RAPD bands

plants without the marker, five susceptible plants with the marker) between $OPF12_{650}$ and resistance among the 159 individuals segregating in the population (Fig. 2). The genetic distance between $OPF12_{650}$ and the resistance gene was estimated to be 5.4 ± 1.9 cM.

Detection of *Pm*1 with the RAPD markers

Markers $UBC320_{420}$ and $UBC638_{550}$ were present in the differential line CI14114, which carries the gene *Pm*1, but not in any of the other differential lines listed in Table 2, indicating linkage between the markers and *Pm*1. The presence of the RAPD markers in Axminster, the original source of *Pm*1 (Briggle 1969), and in the other three *Pm*1-containing accessions (data not presented), strongly suggests their general applicability in the detection of the *Pm*1 resistance gene. Complete co-segregation between *Pm*1 and the two markers $UBC320_{420}$ and $UBC638_{550}$ was confirmed in a segregating population of 25 plants derived from the cross Chancellor * 2/Axminster. Both markers were present in the 16 resistant plants and neither marker was present in the nine susceptible plants (data not presented). These results, together with those in Table 3 showing no susceptible progenies from the cross Zhengzhou 871124 × CI14114 (*Pm*1), confirm that the resistance gene in Zhengzhou 871124 is *Pm*1.

Among 20 wheat accessions, representing ten cultivars, indicated as carrying *Pm*1 (McIntosh 1988) and obtained from the USDA-ARS National Small Grains Collection, only five accessions were resistant to isolate 94-6 of *B. graminis* (Table 4). RAPD markers $UBC320_{420}$ and $UBC638_{550}$ were present in all five of the resistant accessions. Neither of the two markers were present in the susceptible accessions. The results also suggest that the resistance gene in Zhengzhou 871124 is indeed *Pm*1.

STS tagging

One product of the expected size, 420 bp, was amplified from both resistant and susceptible bulks with the pair of 20-bp oligonucleotides, C320-1 and C320-2 (Table 5), that were designed based on primer UBC320 (Fig. 4). Amplified DNA from resistant and susceptible bulks

Table 4 Application of the RAPD markers in the detection of the *Pm*1 gene in different wheat cultivars (R, resistant; S, susceptible; +, presence of the marker; −, absence of the marker)

Accession	Cultivar[a]	Reaction to *B. graminis* isolate 94-6	Marker	
			$UBC320_{420}$	$UBC638_{550}$
PI228307	Axminster	R	+	+
CI8195	Axminster	R	+	+
PI278495	Birdproof	S	−	−
PI352313	Bonus	S	−	−
CI4141	Converse	R	+	+
PI192763	Eborense	S	−	−
CI3315	Huron	S	−	−
PI192099	Kenya	S	−	−
PI192182	Kenya	S	−	−
PI192615	Kenya	S	−	−
PI192672	Kenya	S	−	−
PI192674	Kenya	S	−	−
PI268326	Kenya	S	−	−
CI13836	Mexico	S	−	−
CI13130	Norka	R	+	+
CI4377	Norka	S	−	−
CI4741	Thew	R	+	+
CI5002	Thew	S	−	−
PI93241	Thew	S	−	−
PI93988	Thew	S	−	−

[a] All of these cultivars are reported to carry *Pm*1

Table 5 Sequences of 20-mer primers for the STS derived from the RAPD markers linked to the *Pm*1 resistance gene

Primer	Sequence[a]
C320-1	5′-CCGGCATAGATCGAGAATAG-3′
C320-2	5′-CCGGCATAGAACTTTAAGCG-3′
C638-1	5′-GCGGTGACTAACACATATGA-3′
C638-2	5′-GCGGTGACTAGACATCTTGT-3′

[a] The underlined sequence represents the sequence of the original RAPD primers

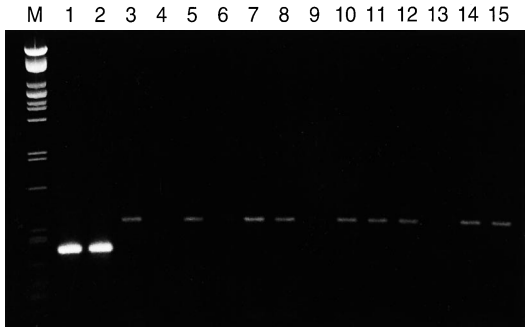

Fig. 4 PCR amplification products produced from the bulked DNA samples of closely related wheat lines: resistant bulk (*1, 3*), susceptible bulk (*2, 4*), and genomic DNA of cultivars Zhengzhou 871124 (*5*), Morocco (*6*), seven resistant F_2 plants (*7–8*, *10–13*, and *14–15*), and two susceptible F_2 plants *9 and 13*) from the cross of Morocco × Zhengzhou 871124 with the sets of STS primers. *Lanes 1–2* are amplified with the primer set of C320-1 and C320-2; *lanes 3–15* are amplified with the primer set of C638-1 and 638-2. *M*, *Pst*I-*λ* DNA marker

was treated with the 16 restriction enzymes described in the Materials and methods section. None of these enzymes revealed a polymorphism between the two bulks. However, when the concentration of each primer was decreased to 0.08 μM, the primer set of C638-1 and C638-2 amplified one DNA fragment of the expected size, 550 bp, in the resistant bulk, but not in the susceptible bulk. Genomic DNA from F_2 plants segregating for powdery mildew resistance from the cross Morocco × Zhengzhou 871124 was also amplified with the primer set of C638-1 and C638-2 (Fig. 4). The specificity of the amplification was increased when the annealing temperature was raised from 55°C to 61°C. Thus, primers C638-1 and C638-2 identified a sequence-tagged site in wheat that is associated with *Pm*1.

Discussion

Using closely related inbred lines developed from a segregating F_5 family in combination with bulked segregant analysis, we have identified two RAPD markers, $UBC320_{420}$ and $UBC638_{550}$, tightly linked to a gene for resistance to powdery mildew with no recombinants observed between these two markers and the resistance gene after an analysis of 244 F_2 plants. A third RAPD marker, $OPF12_{650}$, was identified with DGGE, and was found to be 5.4 ± 1.9 cM from the resistance gene. The presence of $UBC320_{420}$ and $UBC638_{550}$ only in the wheat differential line with *Pm*1, the detection of the markers in Axminster (the donor parent of *Pm*1 for the differential line CI14114) and in other *Pm*1-containing lines with different genetic backgrounds, and the co-segregation of the markers with *Pm*1 in resistant progenies of CI14114, all confirm that the markers obtained with resistant line Zhengzhou 871124 are linked to *Pm*1, and that the resistance gene of Zhengzhou 871124 is *Pm*1. These results were confirmed by the genetic analysis of F_2 populations of the crosses between Zhengzhou 871124 and 11 wheat differential lines for resistance to powdery mildew, in which no susceptible F_2 plants were observed in the cross of Zhengzhou 871124 and CI14114. These results also infer the general application of RAPD markers for the identification of specific resistance genes to powdery mildew in wheat.

The *Pm*1 gene has been shown to be located on the long arm of chromosome 7A (Sears and Briggle 1969). Co-analysis of the markers employed by these authors and other markers developed for the same chromosome arm should generate a more detailed map of these loci. RFLP markers have been identified to be co-segregating with *Pm*1 (Ma et al. 1994; Hartl et al. 1995). However, these markers were not tightly linked to *Pm*1. Detection of RFLPs by Southern-blot hybridization is laborious and time consuming, which makes this assay undesirable for plant breeding with high sample-throughout requirements. RAPD markers in the present study provide a new protocol for the successful identification of the *Pm*1 locus irrespective of genetic background and, therefore, may facilitate selection of the genotypes carrying *Pm*1 and enable pyramiding *Pm*1 with other resistance genes. Tightly-linked RAPD markers might also prove to be useful for map-based cloning of powdery mildew resistance genes.

We have converted the RAPD markers to sequence tagged sites for greater reliability as a selection tool. These STSs are easier and less sensitive to variations in PCR conditions and DNA quality compared to RAPD analysis. One drawback of STSs is the need for sequence analysis before primers can be designed. Since STSs are more reliable than RAPD markers, and frequently only one or two DNA fragments are amplified, they can be placed on the genetic map of wheat more readily.

Success in using backcross-derived NILs for targeting tightly linked DNA markers relies on the genetic divergence between a pair of NILs near the target locus and genetic identity in the remainder of the genome. In

addition to backcrosses, any other method that serves to maintain genetic divergence at the target locus and progress toward genetic identity elsewhere in the genome should provide genetic material that may be used in the same fashion as backcross-derived NILs. Heterogeneous inbred populations were used successfully as sources of NILs for targeting RAPD markers linked to a rust-resistance gene in common bean (Haley et al. 1994). In our study, we have shown that closely related inbred lines can be utilized in combination with bulked-segregant analysis to identify RAPD markers tightly linked to a disease-resistance gene in a crop species with both a large genome and a high percentage of repetitive DNA. The efficiency of this method was indicated by the fact that close linkage between three DNA markers and the target gene, with no false-positives, was identified in the products of 1300 primers. This efficiency in targeting tightly linked markers and in reducing the occurrence of false-positives may be attributed to the narrow genetic divergence between two DNA bulks where few polymorphisms are expected. The closely related inbred lines developed in our study were derived from a single self-pollinated F_4 plant. Thus, the proportions of heterozygous gene pairs unrelated to the target gene are small in an F_4-derived F_5 family. This residual heterozygosity may have been masked by our bulking strategy. The method should find wide application in the identification of DNA markers, whether RFLP or RAPD, tightly linked to important plant genes.

The underlying idea of developing closely related inbred lines provides a technically simple alternative for deriving NILs for identifying DNA markers linked to specific genes. A pair of NILs can be developed by a process of characterizing an inbred family in many autogamous crop species if the characterization is done in inbred generations. This form of NILs has advantages over backcross-derived NILs. Backcrossing results in the transfer of not only the genes of interest, but also of regions unlinked to the target gene. For this reason, false-positives have often been observed with backcross-derived NILs (Michelmore et al. 1991; Yu et al. 1991. In contrast, a pair of NILs derived from a single plant self-pollinated for several generations is genetically uniform except near the heterozygous target gene. There is minimal chance that regions unlinked to the target region will differ between a pair of NILs. Backcross-derived NILs require many backcrosses and several generations of testing to develop and, therefore, are laborious to generate. The development of NILs from an inbred family requires only one crossing event and as few as two generations of testing. More practically, various inbred families are available in breeder's nurseries, and are potential sources of closely related lines for traits of interest. These inbred families are convenient sources of closely related lines developed from inbred single plants for targeting important plant genes.

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