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ORIGINAL PAPER

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Development of wheat near-isogenic lines for powdery mildew resistance

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Abstract Using three Chinese wheat cultivars, 'Bainong 3217', 'Beijing 837' and 'Laizhou 953', as recurrent parents, 33 near-isogenic lines (NILs) carrying 22 powdery mildew resistance genes (*Pm1c*, *Pm2*, *Pm4b*, *Pm12*, *Pm13*, *Pm16*, *Pm20*, *Pm21*, *Pm23*, and 13 undocumented genes) were developed. All NILs had no significant difference to their recurrent parents in the investigated traits of agronomic importance. The results of AFLP analysis indicated Jaccard's genetic similarity of the NILs with their recurrent parents varied from 0.96 to 0.98, and confirmed that the NILs had high genetic similarity with their recurrent parents. The resistance to powdery mildew was stably expressed by the relevant NILs. Eleven of the NILs were tested using molecular markers linked to the resistance genes *Pm1c*, *Pm4b*, *Pm13*, *Pm21*, *PmP*, *PmE*, *PmPS5A*, *PmPS5B*, *PmY39*, *PmY150*, and *PmH*, and they were all found to carry the targeted genes. The potential application of these NILs in gene discovery is discussed.

Introduction

Near-isogenic lines (NILs) are valuable genetic stocks that have been widely used for genetic studies, including the investigation of gene effects, screening of the molecular markers tightly linked with genes of interest, gene expression, and gene isolation. Ferguson et al. (1972) studied the effect of leaf color, chlorophyll concentration, and temperature on photosynthetic rates by exploiting isogenic lines in barley. The effect of *Rht1* and *Rht2* semi-dwarf genes on hybrid vigor and agronomic traits was investigated by Keyes et al. (1989), and the action of leaf rust resistance genes on yield was studied by Seck (1988), using NILs of four wheat isogenic lines. NILs have been widely used for mapping and tagging both qualitative inherited genes and quantitative trait loci (QTL) since the 1990s. For example, Zhang et al. (1996) studied the bacterial blight resistance gene *xa-13* in rice. The dwarf BREIZH (*Bzh*) gene was tagged in *Brassica napus*, using NILs by Foisset (1996). Kandemir et al. (2000) conducted molecular marker-assisted genetic analysis of head shattering in six-rowed barley. The powdery mildew resistance gene *Pm6* was mapped in wheat by Tao et al. (2000). Han et al. (1997) constructed a fine-structure map of the centromere region on barley chromosome 1, which contained malting-quality QTLs. Tuinstra et al. (1998) evaluated drought tolerance of sorghum NILs with associated QTL markers. Monforte and Tanksley (2000a, 2000b) reported the development of a set of NILs and fine mapping of a QTL from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits. In recent years, NILs have been further employed in gene cloning. For example, Wang et al. (2001) was successful in their attempt to isolate the *Xa4* locus in rice, using this strategy.

NILs are generated mainly by crossing a donor line carried the gene of interest to a recurrent parent and then backcrossing to the recurrent parent for six to eight generations, followed by a self-pollination. This is not only laborious but also time-consuming, as it normally takes eight to ten generations. Molecular markers are powerful tools for NIL development by which the backcross period can be significantly shortened to four to five generations (Frisch et al. 1999; Ribaut et al. 2002). Chen et al. (2001) developed an NIL for the bacterial blight resistance gene *Xa21* from a BC_3F_1 population by marker-assisted selection (MAS). Furthermore, with the assistance of molecular markers, development of NILs can be combined with tagging genes of interest (Sanchez et al. 2000).

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Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (syn. *Erysiphe graminis* f. sp. *tritici*) has posed an increasing threat to wheat production worldwide, which resulted in yield loss for 10–30% (Fried et al. 1981). The development of disease-resistant varieties has proved to be the most economical and environmentally safe means to control the disease. There are 45 resistance genes in 29 loci that have been identified in the past 50 years, including five alleles of *Pm1* (*Pm18* = *Pm1c*, *Pm22* = *Pm1e*), seven alleles of *Pm3*, two alleles of *Pm4*, five alleles of *Pm5*, and two alleles of *Pm8*. However, only 19 NILs for eight *Pm* genes were developed, and most of these genes had been overcome by the new races of the fungus. NILs were developed for resistance genes *Pm1*, *Pm2*, *Pm3a*, *Pm3c*, and *Pm4b* in a background of wheat 'Federation' (Pugsley 1961; The et al. 1979), for *Pm1a*, *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3f*, *Pm4a*, *Pm5*, and *Pm7* in a background of wheat 'Chancellor' (Briggle 1969; Zeller et al. 1993; Hu et al. 1997), and for *Pm2*, *Pm5* and *Pm6* in a background of wheat 'Prins' (Hartl et al. 1995). No NILs for other resistance genes have been reported. In the study, the development of 33 NILs for 22 powdery mildew resistance genes was reported.

Materials and methods

Plant materials

Three wheat cultivars, 'Bainong 3217', 'Beijing 837' and 'Laizhou 953', were used as recurrent parents for NIL development. Although 'Bainong 3217' was a leading cultivar in the 1980s in the Yellow River of China, reaching 1.65 million hectare per annum, it carried no resistance genes against powdery mildew. 'Beijing 837' and 'Laizhou 953', which carried a defeated resistance gene *Pm8*, were elite winter wheat cultivars released in 1980s in northern China.

Twenty-one genotypes were used as donors in this study (Table 1). They were a subset of those genotypes identified previously to carry resistance genes. Of these, nine carried the known resistance gene(s) *Pm1c*, *Pm2*, *Pm6*, *Pm4b*, *Pm12*, *Pm13*, *Pm16*, *Pm20*, *Pm21*, and *Pm23*. The other 12 possessed 13 unknown resistance genes (Am9 with two resistance genes). Eight donor lines were amphidiploid lines synthesized from the author's laboratory (Table 1), three from derivatives of wheat and *Ae. longissima*, *Agropyron cristatum*, and *Erompyron orientale*, respectively; a Chinese landrace, 'Hongquanmang', was collected from the Henan Province, China (Table 1).

The mapping populations were BC_3F_2 or BC_5F_2 segregation populations from the crosses of donor lines and recurrent parents, each consisting of 100–150 individuals.

NIL development

Donor lines were crossed with recurrent parents, followed by backcrossing to the recurrent parents for three to six generations and then self-crossing for two to four generations. A prevalent virulence isolate E09 was inoculated in each generation, and only the plants with resistance to powdery mildew were kept for advancement for the population with dominant genes. Plants in BC populations with recessive resistance genes revealed by self-crossing were also kept for advancement. The AFLP method was used to detect the similarity between the backcrossed plants and their parents in BC_3 and BC_4 generations, and 12 pairs of polymorphic primer combinations were used the assessment of the similarity to

Table 1 Twenty-one donor lines with resistance gene(s) for powdery mildew resistance

Donor lines	Original	Genome
Am1	*Triticum carthlicum* (acc. Ps5)/*Aegilops tauschii* (acc. Ae34)	AABBDD
Am3	*T. carthlicum* (acc. Ps5)/*Ae. tauschii* (acc. Ae38)	AABBDD
Am4	*T. carthlicum* (No.Ps5)/*Ae. tauschii* (No.Ae39)	AABBDD
Am6	*T. durum* (acc. DR147)/*Ae. tauschii* (acc. Ae39)	AABBDD
Am8	*T. durum* (aac. DR181)/*Ae. caudata* (acc.Y45)	AABBCC
Am9	*T. carthlicum* (acc. Ps5)/*Ae. umbellulata* (acc.Y39)	AABBUU
Am16	*Ae. ventricosa* (acc. Ae64)/*T. carthlicum* (acc. Ps5)	AABBDDMM
AmC	*T. durum* (acc. DR147)/*Ae. caudata* (acc. Ae14)	AABBCC
Xiaobing	Derivatives of *T. aestivum*/*Agropyron cristatum*	Unknown
Xiaohan	Derivatives of *T. aestivum*/*Erompyron orientale*	Unknown
Hongquanmang	Chinese landrace, collected from Henan province	AABBDD
Y150	*Ae. Longissima*	SS
Mardler(*Pm2* + *Pm6*)	Common wheat	AABBDD
VPM (*Pm4b*)	Derived from *Ae. ventricosa*	Translocation
Line 31(*Pm12*)	Derived from *Ae. speltoides*	6B/6S translocation
R1A (*Pm13*)	Derived from *Ae.longissima*	3B/3S translocation
Line Pm16 (*Pm16*)	Derived from *T.dicoccoides*	AABBDD
M1N (*Pm1c*)	Undesignated subline of Weihenstephan M1	AABBDD
PI583795(*Pm20*)	Derived from *Secale*	6D/6R translocation
R43(*Pm21*)	Derived from *Heterotheca villosa*	6A/6V translocation
Line 81-7241(*Pm23*)	Common wheat	AABBDD

the recurrent parent. For each generation, about 50 plants of disease resistance were selected for characterization, and two to five individuals with much similarity to their parents were selected for backcrossing or self-crossing. Finally, the NILs were selected from self-crossing populations.

Resistance evaluation

Twenty isolates of *B. graminis* f. sp. *tritici* with different virulence types were selected to identify the resistance genes. Seedlings of the 21 donor cultivars/lines, recurrent parents, and NILs were inoculated at the two-leaf stage with the 20 isolates of *B. graminis* f. sp. *tritici*. The seedlings of each segregating population were inoculated with isolate E09, to which all the donor parents were resistant and all the recurrent parents were susceptible. The isolate E09 is a prevalent virulence type in the Beijing area, with virulence to *Pm1*, *Pm3a*, *Pm3c*, *Pm5*, *Pm7*, *Pm8*, *Pm17*, and *Pm19*. The infection types (IT) of hosts were recorded on a scale of 0–4, with 0 representing no visible symptoms, 0; for necrotic flecks, and 1–4 for highly resistant, moderately resistant, moderately susceptible, and highly susceptible. Two major IT were distinguished: resistant (IT = 0, 0;, 1, and 2) and susceptible (IT = 3–4).

Microsatellite marker analysis

Wheat microsatellite markers developed by Röder et al. (1998), Stephenson et al. (1998) and the International Wheat Microsatellite Consortium were used in mapping and tagging the powdery mildew resistance genes. PCR and electrophoresis were carried out as described by Röder et al. (1998).

AFLP analysis

AFLP analysis in the detection of NILs background was performed as described by Vos et al. (1995), with minor modifications. Genomic DNA was digested with *Mse*I and *Pst*I, and the adapters were ligased with T4 ligase. The ligased restriction fragments were preamplified and selective amplified with the *Mse*I primer and the *Pst*I primer. The PCR product was diluted 15-fold in ddH_2O and amplified with the selective primers containing two and/or three additional bases in 3′ end.

Data analysis

Chi-square tests were applied to the inheritance data to establish goodness of fit to the postulated ratios. The recombination frequencies were calculated using JoinMap, version 1.4. Jaccard's genetic index was used for similarity analysis. The formula was as follows: $J = a/a + b$, with a being the fragment number of NILs and b being the fragment number of the recurrent parent.

Results

In total, 33 putative NILs carrying 22 powdery mildew resistance genes were developed in this study (Table 2). Thirteen of these were produced with 'Bainong 3217' as the recurrent parent, thirteen with 'Beijing 837', and the other seven with 'Laizhou 953'. Twenty-two of these NILs derived from generation BC_6 or BC_5, and the other from BC_4 or BC_3. Twenty-two of these NILs, including the 12 from BC_4 or BC_3, were characterized with AFLP markers for the recovering in the background of the recurrent parents, and only the individuals with much similarity to the parent were kept.

Agronomic traits of the NILs and the similarity of NILs to their recurrent parent

Six agronomic traits, plant height, spike length, number of spikelet per spike, grain number per spike, 1,000-grain weight, and heading data were investigated on 16 of the NILs, their recurrent parents, and donor parents in field for 2 years. Fungicide was applied to recurrent parents. Significant differences in agronomic traits were found between the donor genotypes and the recurrent parents (data not shown). For example, most donor lines were tall and late in maturity. Contrasting

Table 2 Number of generations used for the development of the 34 near-isogenic lines (NILs)

Donor lines (genes)	Recurrent parent		
	'Bainong 3217'	'Beijing 837'	'Laizhou 953'
Mardler (*Pm2*)	BC_6F_4	BC_6F_4	
VPM (*Pm4b*)	BC_6F_4	BC_6F_4	
Line 31 (*Pm12*)	BC_6F_4	BC_6F_4	
R1A (*Pm13*)	BC_6F_4	BC_6F_4	
Line Pm16 (*Pm16*)		BC_6F_4	
M1N (*Pm1c*)	BC_5F_2	BC_5F_2	
PI583795 (*Pm20*)	BC_4F_2	BC_4F_2	
R43 (*Pm21*)	BC_6F_3	BC_6F_3	
Line 81–7241 (*Pm23*)	BC_4F_2	BC_5F_2	
Am1 (*PmAm1*)			BC_3F_2
Am3 (*PmAm3*)			BC_5F_2
Am4 (*PmAm4*)	BC_5F_2	BC_4F_2	
Am6 (*PmAm6*)	BC_6F_2	BC_5F_2	
Am8 (*PmAm8*)		BC_5F_2	
Am9 (*PmY39*)			BC_4F_3
Am9 (*PmPS5B*)			BC_4F_3
Am16 (*PmAm16*)			BC_3F_3
AmC (*PmAmC*)			BC_3F_3
Y150 (*PmY150*)			BC_3F_5
Hongquanmang (*PmH*)	BC_6F_2	BC_5F_2	
Xiaobing (*PmP*)	BC_5F_2		
Xiaohan (*PmE*)	BC_3F_2		

sharply with the donor genotypes, the recurrent parents showed desirable agronomic traits, including early maturity and semi-dwarfness. None of the derivatives from BC_5 or BC_6 showed any significant difference from their recurrent parents in any of the six traits checked (data not shown). This was also the case for those derivatives of BC_3 or BC_4 when MAS was employed, indicating that molecular markers were powerful tools for NIL development. These results demonstrated that these derivatives were NILs for powdery mildew resistance at the phenotype level.

Previously, six powdery mildew resistance genes, *Pm12*, *Pm13*, *Pm20*, *Pm21*, *PmY150*, and *PmY39*, carried by alien chromosomes or chromosomal fragments had been transferred into wheat from wild relatives of wheat. Of these, *Pm12* was located on 6S of the wheat/*Aegilops speltoides* 6B/6S translocation chromosome (Jia et al. 1996), *Pm13* on $3S^l$ of the wheat/*Ae. longissima* 3B(D)/$3S^l$ translocation chromosome (Friebe et al. 1996), *Pm20* on 6RL of the wheat/*Secale* translocation chromosome (Friebe et al. 1996), *Pm21* on 6V of the wheat/*Haynaldia* 6A/6V translocation chromosome (Qi et al. 1996), *PmY150* on $6S^l$ of the wheat/*Ae. longissim* 6D/$6S^l$ substitution chromosome (Zhu 2003), and *PmY39* on 2U of the wheat/*Ae. umbellulata* 2B/2U substitution chromosome (Zhu 2003). Recombination between any of these alien chromosomes with those of wheat was not detected during backcrossing. No significant difference in agronomic traits between the recurrent parents and the NILs with the alien chromosome or chromosomal segment was observed, indicating that these alien chromosomes/segments had no negative effect on the agronomic traits.

Results of the AFLP analysis for 22 of the NILs are summarized in Table 3. As shown, the Jaccard genetic similarities between NILs and their recurrent parents were higher than 0.96. The highest similarity (0.98) was found in 11 of the 24 lines. Some NILs developed by backcrossing for four generations with MAS owned similarity as high as those from backcrossing of six or more generations, which substantiated the efficiency of MAS.

Powdery mildew resistance of the donor lines, recurrent parents, and NILs

The reactions of the 19 wheat donor lines and three recurrent varieties against 20 isolates of powdery mildew were shown in Table 4. Of nine known resistance genes, *Pm1c*, *Pm12*, *Pm13*, *Pm16*, *Pm20*, and *Pm21* showed resistance to all of the 20 isolates, indicating that the six genes were efficient for breeding powdery mildew resistance. Of the 8 synthetic genotypes, Am9 and AmC were resistant to all 20 isolates; Am1 and Am3 were resistant to all but one; Am4 was resistant to 18 isolates; Am6 was resistant to 17 isolates; and Am8 and Am16 to the 16 isolates. The Chinese landrace 'Hongquanmang' was resistant to 19 isolates except isolate E11. Out of two wheat relatives, *E. orientale* was resistant to all 20 isolates, and *Ae. longissima* acc.Y150 was resistant to the 19 isolates except isolate E16. Recurrent parent 'Bainong 3217' was susceptible to all 20 isolates, indicating that it contained no resistance genes, thus potentially useful as an ideal recurrent parent. Both 'Beijing 837' and 'Laizhou 953' showed the same resistance pattern, as it was resistant to the isolates E30 and E32, but susceptible to all the other 18 isolates.

The resistance of 14 NILs is listed in Table 4. Among these, ten showed the same resistance pattern as *Pm1c*, *Pm2*, *Pm4b*, *Pm12*, *Pm13*, *Pm16*, and *Pm21*, indicating that these NILs carry the seven resistance genes. However, the other four displayed different resistance patterns when compared to their donor parent. NIL Am9/3*Lai, derived from Am9, was susceptible to five isolates to which Am9 was resistant. The latter results revealed that two resistance genes may exist in Am9, and the NILs tested here only carried one of them (Zhu 2003). A similar result was found in the derivatives of *E. orientale* and 'Hongquanmang'. NIL Xiaohan/4*Bai was susceptible to four isolates to which *E. orientale* was resistant; NIL (Hong/6*Bai)-1 and (Hong/6*Bai)-2 were both derived from 'Hongquanmang', which was resistant to all races but one. However, these two NILs were resistant to nine and seven isolates, respectively, indicating that there were more than two resistance genes in 'Hongquanmang'.

Table 3 Similarity between NILs and their recurrent parents

NILs[a]	Jaccard genetic similarity	NILs[a]	Jaccard genetic similarity
(Mar/Bai) BC_6F_4-1	0.98	(R43/Bai) BC_6F_4	0.98
(Mar/Bai) BC_6F_4-2	0.98	(R43/Bai) BC_6F_4	0.98
(VPM/Bai) BC_6F_4-1	0.98	(R43/Bai) BC_6F_4	0.98
(VPM/Bai) BC_6F_4-2	0.98	(L81-7241/Bai) BC_6F_4	0.97
(Line 31/Bai) BC_6F_4-1	0.97	(L81-7241/Bai) BC_6F_3	0.97
(Line 31/Bai) BC_6F_4-2	0.97	(L81-7241/Bai) BC_6F_3	0.98
(R1A/Bai) BC_6F_4-1	0.97	(Line Pm16/Bai) BC_6F_3	0.97
(R1A/Bai) BC_6F_4-2	0.97	(Hong/Bai) BC_4F_4	0.98
(R1A/Bai) BC_6F_4-3	0.97	(Hong/Bai) BC_4F_3	0.98
(R1A/Bai) BC_6F_4-4	0.97	(Am6/Bai) BC_4F_2	0.97
(R43/Bai) BC_6F_4	0.98	(Am6/Bai) BC_4F_2	0.96

[a] *Mar* 'Mardler', *Bai* 'Bainong 3217', *Hong* 'Hongquanmang'

Table 4 Reaction of donor lines, recurrent parents and NILs after inoculation with 20 isolates of *Blumeria graminis* f. sp. tritici. *R* Resistant, *S* susceptible

Cultivars/lines	Gene	*B. graminis* f. sp. *tritici* isolate																			
		E1	E2	E3	E5	E6	E7	E9	E11	E13	E15	E16	E17	E18	E20	E21	E23	E25	E26	E30	E32
CI14118	*Pm2*	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	R	R	R	R	S
VPM	*Pm4b*	R	S	R	R	R	R	R	R	R	S	S	S	S	S	S	R	R	S	R	R
Line 31	*Pm12*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Line Pm16	*Pm16*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
M1N	*Pm1c*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
PI583795	*Pm20*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R1A	*Pm13*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R43	*Pm21*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
L81-7241	*Pm23*	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S	R	R
Am1	*PmAm1*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
Am3	*PmAm3*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
Am4	*PmAm4*	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
Am6	*PmAm6*	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	R	R	R
Am8	*PmAm8*	R	R	R	R	S	R	R	R	R	R	R	S	R	R	R	R	S	S	R	R
Am9	*PmAm9*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Am16	*PmAm16*	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	R	S	R	R
AmC	*PmAmC*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Hongjuanmeng	*PmH*	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R
Y150	*PmY150*	R	R	R/S	R	R	R	R	R	R	R	S	R/S	R/S	R	R	R	R/S	R	R	R/S
Eremopyron	*PmE*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Bainong 3217	–	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Beijing 837	*Pm8*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
Laizhou 953	*Pm8*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
Mar/7*Bai	*Pm2*	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	R	R	R	R	S
Mar/7*Bei	*Pm2*	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	R	R	R	R	R
VPM/7*Bai	*Pm4b*	R	S	R	R	R	R	R	R	R	S	S	S	S	S	S	R	R	S	R	R
VPM/7*Bei	*Pm4b*	R	S	R	R	R	R	R	R	R	S	S	S	S	S	S	R	R	S	R	R
Line 31/7*Bei	*Pm12*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R1A/7*Bei	*Pm13*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pm16/7*Bei	*Pm16*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R43/7*Bai	*Pm21*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R43/7*Bei	*Pm21*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Am6/5*Bai		R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
Am9/3*Lai		R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	R	R	S	R	R
Xiaohan/4*Bai		R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S	R	R
Hong/6*Bai-1		R	S	R	S	S	R	S	S	R	S	S	R	S	R	R	S	R	S	S	R
Hong/6*Bai-2		R	S	R	S	S	R	S	S	R	R	S	R	S	S	S	S	S	S	S	R

[a] *Mar* 'Marlder', *Bei* 'Beijing 837', *Bai* 'Bainong 3217', *Lai* 'Laizhou 953', *Hong* 'Hongquanmang'

Molecular detection of powdery mildew resistance gene in NILs

The resistance genes in 11 NILs were tagged using AFLP and SSR markers.

*M1N/3*Bainong 3217*

A segregation population with 96 individuals from an M1N/3*Bainong 3217 F_2 generation showed a dominant gene for powdery mildew resistance in M1N/3*Bainong 3217. An AFLP marker with the same pattern of donor parent 'M1N' was found to co-segregate with the resistance in the 96 individuals. The result implied that the resistance gene in the NIL was *Pm1c*.

*VPM/7*Bainong 3217*

A pair of STS-PCR primers was designed based on the sequence of an RFLP probe, *BCD1231*, which was found to co-segregate with *Pm4* (Ma et al. 1994). A polymorphic fragment STS$-_{410}$ was amplified with this primer in the donor line VPM, but not in the recurrent 'Bainong 3217'. There were 109 resistant and 35 susceptible plants found in a population of VPM/7*Bainong 3217 F_2s. All of the 109 resistant individuals had the marker STS$-_{410}$. Of the 35 susceptible individuals, four were with, and 31 without, this marker. The genetic distance between *Pm4b* and marker STS$-_{410}$ was about 3.0 cM from the data, which was slightly different with the previous results. The difference should derive from the different mapping population. The results revealed that a powdery mildew resistance gene, *Pm4b*, existed in NIL VPM/7*Bainong3217.

*R1A/7*Bainong 3217*

Pm13 in line R1A was mapped on chromosomes 3B and 3D in previous studies with the cytogenetics method (Friebe et al. 1996). Based on these results, 33 of SSR

primer mapped on chromosomes 3B and 3D were employed to detect the gene *Pm13* in the present study. One of the SSR markers, *Xgwm533*, showed polymorphism between resistant NILs and 'Bainong 3217', exhibiting presence in resistant NIL and absence in 'Bainong 3217'. An F_2 population from R1A/7*Bainong 3217 consisted of 109 individuals and was used to map the gene *Pm13*. The genetic distance was 1.0 cM between *Pm13* and the SSR marker *Xgwm533*. The SSR marker loci *Xgwm533* was mapped on the short arm of wheat chromosome 3B by Röder et al. (1998), so *Pm13* in R1A/7*Bainong 3217 should be on chromosome 3BS.

*R43/7*Bainong 3217*

Pm21 was transferred from *Haynaldia villosa* and located on the translocation chromosome 6AL/6VS of wheat and *Haynaldia*. Line R43 was a resistant line with the gene *Pm21*. Two AFLP marker loci, about 300 bp and 500 bp, produced by the primer combinations M-CGA/P-ACT and M-CGA/P-ACT, respectively, were found present in line R43 and all resistant NILs, whereas they were found absent in recurrent parent 'Bainong 3217'. The two loci were confirmed to co-segregate with *Pm21* in a population of 100 individuals from an R43/7*Bainong 3217 F_2 generation.

*Xiaobing/4*Bainong 3217*

'Xiaobing' was a derivative of Fuco/*Agropyron*. Two hundred and sixty-four resistant and 106 susceptible individuals were scored in the F_2 population of Xiaobing/3*Bainong 3217. Data from segregation ratio indicated that a dominant gene for powdery mildew resistance was present in 'Xiaobing'. Three hundred and sixty-eight AFLP primer combinations were screened for tagging the resistance gene. Twenty-four markers were found linked to the resistance locus. Two of these marker loci, *XM55P66* and *XM55P37*, flanked the locus and were located 0.8 cM and 2.4 cM, respectively, from the locus. The markers revealed that the resistance gene was derived from 'Xiaobing'. However, no evidence for it being derived from 'Xiaobing''s resistant parent, *Agropyron*, was found. The gene was temporarily named *PmP*, and additional work would be required to clarify its origin.

*Xiaohan/4*Bainong 3217*

'Xiaohan' was a derivative of Chinese Spring/*Erompyron*. One hundred and fifteen individuals from an F_2 population of Xiaohan/4*Bainong 3217 were characterized. The population was composed of 47 resistant and 108 susceptible individuals. χ^2 tests suggested that the resistance was dominated by a recessive gene. Three microsatellite marker loci, *Xgwm265*-2AL, *Xgwm311*-2AL, and *Xgwm382*-2AL, located on the long arm of wheat chromosome 2A, were found to be linked to the resistance gene at distances of 2.9, 3.6 and 4.4 cM, respectively. Based on its origin, the novel gene was temporarily named *PmE*.

*Am4/4*Bainong 3217*

The segregation ratio of resistance to susceptible fitted the 3:1 ratio in the F_2 generation of Am4/4*Bainong 3217 and 1:2:1 ratio in the F_3 generation, respectively, which indicated that a dominant resistance gene existed in line Am4. This gene was mapped on 2AL at 10.2 cM apart from the SSR marker locus *Xgwm356*. Am4 was an amphidiploid from *Triticum carthlicum* acc. PS5 and *Ae. tauschii* acc. Ae39, so this gene originated from *T. carthlicum*. It was temporarily named *PmPs5A*, and its relation with *Pm4b* needs to be confirmed.

*Am9/3*Laizhou 953-1*

Am9 was an amphidiploid synthesized with *T. carthlicum* acc. PS5 *and Ae. umbellulata* acc. Y39. In a segregating population derived from Am9/3*Laizhou 953, the ratio of the resistant individuals with the susceptible individuals to powdery mildew was tested to fit in with 3:1 ratio in the F_2 generation of Am9/4*Laizhou 953 and 1:2:1 in the F_3 generation, respectively, indicating that a dominant resistance gene to powdery mildew existed in line Am9. This gene linked to four microsatellite marker loci, *Xgwm111*, *Xgwm382*, *Xgwm526*, and *Xwmc317*, with the nearest distance of 1.1 cM to *Xwmc317* of chromosome 2BL (Röder et al. 1998). *Pm6* was the only gene for powdery mildew resistance mapped on chromosome 2B previously, and these two genes were more than 30 cM apart based on the mapping position. So, we deduced that this gene was a new gene and temporarily named it *PmPs5B*.

*Am9/3*Laizhou 953-2*

Three SSR marker loci, *Xgwm257*, *Xgwm296*, and *Xgwm319*, mapped on chromosome 2B, co-segregated with the resistance of Am9/3*Laizhou 953 in the F_2 population with 100 individuals, and all these markers had the same pattern with the one of the Am9 parents, *Ae. umbellulata*, indicating that the derivative was a wheat/*Ae. umbellulata* 2B/2U translocation or substitution line. The resistance gene was located on chromosome 2U of *Ae. umbellulata*. To our knowledge, no resistance gene for powdery mildew had ever been transferred into wheat from *Ae. umbellulata*. Thus, this novel gene was temporarily named *PmY39*.

*Y150/3*Laizhou 953*

The ratio of resistance to susceptible for powdery mildew fits in with the 3:1 in the F_2 generation of *Ae. longissima*/3*Laizhou 953, indicating that the resistance was conditioned by a dominant gene. Three SSR marker

loci, *Xgwm325*, *Xwmc382*, and *Xwmc397*, were found to be linked to the resistance gene. The markers revealed that the resistance gene was transferred to wheat from $6S^{l}$ of *Ae. longissima*, and it was located on the 6B/6S translocation/substitution chromosome. This gene was temporarily named *PmY150*.

*Hong/5*Bainong 3217-1*

A ratio of 1:3 for resistant and susceptible individuals was found in the segregation analysis on BC_5F_2, suggesting that a recessive gene governs the resistance in 'Hongquanmang''s derivatives. This gene was mapped on 7BL at 5.9 cM apart from SSR marker loci *Xgwm611* and 13.2 cM from loci *Xpsp3033*. The resistance gene *Pm5e* had been mapped on this region previously (Huang et al. 2003); however, the reactions to isolates of *B. graminis* f. sp. *tritici* were different between *Pm5e* and the gene in Hong/5*Bainong 3217-1. Thus, this new gene was temporarily named *PmH*. It was required to reveal the relationship of these two genes.

Discussion

Selection of recurrent parents and molecular maker system for NIL development

Selection of recurrent parents is very important for NIL development. Most recurrent parents selected previously were leading varieties. They had more-desirable traits with no tagged gene(s). If an NIL with the background of a leading variety and gene(s) of interest could be developed quickly, it would be released as a new variety. However, almost no NILs were released as new varieties in the past 30 years. The possible reasons may lie in the lengthy process for NIL development. Therefore, by the time NILs were developed, the variety selected as a recurrent parent might have already been replaced by new ones. Marker-assisted backcrossing could not only shorten generations of backcrossing from six to three, but also replace the offspring selection based on genotypes instead of on phenotypes, which are usually affected by the environments. So, multiple generations of backcrossing and selection could be performed in a year. To take advantage of MAS, an early maturing variety is required. We had selected four extremely early varieties with a two-and-half-month life cycle and with favorable agronomical traits. Because four or five generations could be completed in a year, development of NILs can be reduced to 1 or 2 years with MAS.

There are two applications of molecular markers in NIL development: (1) to accelerate the recovery of the recurrent parent genome in backcross lines and (2) to select the lines with genes of interest from backcrossing populations. Several marker systems have been developed in recent years, such as RFLP, AFLP, RAPD, SSR, and STS. Each marker system has its advantages and disadvantages, depending on applications. In theory, the difference between NILs and the recurrent parent lines only in the gene of interest or donor gene. Therefore, it is very important to detect the genetic background of the backcrossing derivatives during the development of NILs. To do this, a number of marker data points are required in the backcrossing program. The AFLP method was proved to be more efficient in monitoring the background as its capable of detecting up to 100 loci per reaction.

Phenotype and genotype of the NILs

No phenotypic differences were observed among any resistant NILs and their recurrent parents. The genetic similarity value of each NIL to its recurrent parent could be calculated by the formula: $1-(1/2)^{1+n}$, where n is the number cycles backcrossed. In theory, the similarity value to the recurrent parent could reach 0.99 for the materials after six generations of backcrossing. However, the values of similarity detected in this study were 0.96–0.98, which was lower than expected. There were three possible reasons for this result. First, it might be caused by a genetic drag, especially for the gene of interest located on a fragment of alien chromosome. Such situations could be found in the NILs of *Pm12* and *Pm13*, which were carried by the short arms of *Ae. speltoides* and *Ae. longissima* chromosomes, respectively. Second, it might stem from the method of sampling. The expected similarity was calculated on a large population, but the similarity calculated in our experiment was based on only a few lines, which in fact resulted in lower similarity. Third, it might result from the AFLP markers. As most marker loci are dominant, AFLP could not distinguish the heterozygous and homozygous genotypes. The samples used in the experiment were all from F_2 or F_3 progenies of the backcrosses, and a half or a quarter of the individuals were heterozygous in the polymorphic loci. To resolve the above problems, a suitably sized population should be employed when AFLP molecular makers are used to detect genetic similarity in early backcrossing generations.

The use of NILs developed

The NILs and the linked markers developed in present study had been used in gene pyramiding, wheat breeding, and gene expression analysis. No obvious phenotypic segregations were observed in the segregating generations because of a very similar background of the NILs. Using the NILs of *Pm2*, Luo et al. (2002) constructed a conventional cDNA library and a suppression subtractive hybridization (SSH) cDNA library. The SSH cDNA library was found to have obvious advantages in gene expression profiling of disease resistance. Li et al. (2003) employed a set of NILs with *Pm2*, *Pm4b*, *Pm12*, and *Pm13*, and screened the genes related to disease resistance. Three polymorphic fragments were amplified

by using the primers that were designed based on sequences of disease resistance genes.

Prospective

The term gene in NILs is virtually phenotypic ''trait'' or ''phenotypic marker,'' instead of a real gene in the sense of molecular biology. In fact, all the NILs developed previously were based on a special trait. In other words, they are phenotypic NILs. Thousands of gene sequences are available in public gene banks, and the number is increasing daily, from which we can find many allelic variations. However, the functions of most of these genes are still unknown. New types of NILs can be developed based on the natural allelic variation of gene sequences. To differentiate the two types of NILs, we suggest the former to be called ''phenotypic NILs'' (abbreviated as ''P-NILs'') and the latter ''Genotypic NILs'' (abbreviated as ''G-NILs''). The functions of unknown genes may be revealed by comparing phenotypes or traits of the G-NILs. This would be a new approach for functional genomics. Development of thousands of G-NILs is under way using the new efficient strategy developed.

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