

GENETIC ANALYSIS OF POWDERY MILDEW RESISTANCE GENE USING SSR MARKERS IN COMMON WHEAT ORIGINATED FROM WILD EMMER (*Triticum dicoccoides* Thell)

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ABSTRACT

Common wheat line N0324, derived from the cross wild emmer (*Triticum dicoccoides* Thell) accession 5055 and Shaan 253, provides effective protection to powdery mildew. Analysis of 205 individuals in the segregating F₂ population generated by crossing N0324 with the susceptible cultivar Shaanyou 225 revealed that resistance in N0324 line was controlled by a single recessive gene designated temporarily as *Pm5055*. Bulked segregant analysis (BSA) and simple sequence repeats (SSRs) were used to characterize the powdery mildew resistance gene *Pm5055*. Four SSR markers such as *Xwmc441*, *Xbarc7*, *Xbarc13* and *Xbarc55* were linked to the gene with genetic distances of 10.6, 23, 23 and 23.9 cM, respectively. Chinese Spring nulli-tetrasomic and ditelosomic lines were used to assign the chromosomal locations of the linked markers. The results suggested that this gene might be located on chromosome 2B. *Pm5055* can be used to diversify powdery mildew resistance sources in future wheat breeding programs.

Key words: *Blumeria graminis* f. sp. *tritici*, molecular markers, *Triticum dicoccoides*, wheat breeding

INTRODUCTION

Powdery mildew which is caused by *Blumeria graminis* (DC) Speer f. sp. *tritici* is a major devastating wheat fungal disease around the world especially in cool and humid climates. Powdery mildew has been reported to be responsible for severe grain yield losses, ranging from 13 to 34% (Leath and Bowen, 1989; Griffey et al., 1993), thus affects wheat production around the world. To reduce production losses, practices such as chemical application and crop rotation are often used to manage the disease. However, international wheat breeding programs have given major emphasis on genetic control of the disease by introducing the resistance genes within elite commercial cultivars (Khan et al., 2012). This means that the deployment of cultivars with broad spectrum powdery mildew resistance is the primary viable option to combat the disease.

The identification of resistance genes from wheat wild relatives and their introgression into selected elite wheat lines has gained importance in breeding efforts to develop resistant wheat varieties. To date, more than 60 genes/alleles conferring resistance to powdery mildew have

been identified at 47 gene loci (*Pm1-Pm50*, *Pm18=Pm1c*; *Pm22=Pm1e*; *Pm23=Pm4c*; *Pm31=Pm21*) in wheat and its relatives (Alam et al., 2011; Ma et al., 2011; Gao et al., 2012; Xue et al., 2012; Xiao et al., 2013). Although resistant cultivars are available for minimizing grain yield losses as well as reducing damage of end-use quality, changes in the climatic and environmental conditions under which crops grown besides the genetic mutation phenomenon have resulted in the appearance of new pathogen races that cause the loss of previously effective sources of resistance (Boyd et al., 2012). Therefore, identification and introgression of novel sources of resistance should be a continuous process to combat the ever evolving pathogens (Riar et al., 2012).

From the early 1990s, molecular markers and linkage mapping have become powerful tools in refining the accuracy and efficiency of genetic mapping in wheat (Hua et al., 2009). Molecular markers accelerate the identification and cloning of disease resistance genes in wheat and various markers, including restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs), have been used to map more than 30 powdery

mildew resistance genes (Liu et al., 2011). Among these markers, SSRs or microsatellites present some distinguished characteristics such as broad availability, easy employment, high polymorphism detection and co-dominant inheritance and are commonly used for genetic mapping (Hao et al., 2008).

This study reports the identification and molecular characterization of a recessive powdery mildew resistance gene in common wheat line N0324 derived from wild emmer accession 5055.

MATERIALS AND METHODS

Plant materials

Wheat line N0324 is a resistant line bred by the College of Agronomy, Northwest A&F University, China. It is derived from a single cross (wild emmer 5055/Shaan 253) between the powdery mildew resistance gene donor, wild emmer accession 5055 and a susceptible Chinese elite common wheat line Shaan 253. The cross was followed by subsequent selection in onward generations.

Two hundred and five F₂ plants derived from the cross between the resistant line N0324 and the locally adapted common wheat cultivar Shaanyou 225 were used in this study. Shaanyou 225 does not carry any known resistance

gene and is highly susceptible to powdery mildew in Shaanxi province (China).

Powdery mildew test

F₂ plants along with the two parents (N0324 and Shaanyou 225) were sown in the experimental field of College of Agronomy, Northwest A&F University (Shaanxi, China), where the local race of *Blumeria graminis* f. sp. *tritici* (*Bgt*) named ‘Guanzhong 4’ occurs frequently. Around the field, the spreader variety, Chancellor (Suppl. Figure 1), which is often used in the laboratory to maintain some *Bgt* isolates (Xue et al., 2012), was also planted to facilitate the disease propagation. The objective was to evaluate these lines for their resistance gene reaction to powdery mildew by natural infection. The test results were scored at adult plant stage when pustules of powdery mildew were fully developed on the spreader variety (Chancellor), the susceptible parent Shaanyou 225 and some susceptible F₂ plants. Infection types (IT) of the parental lines and the segregating F₂ population were scored on a scale of zero to four (Sheng, 1988) and powdery mildew resistance reactions were classified into two groups, resistant and susceptible. Plants with IT value 0, 1 and 2 were distinguished as resistant whereas those with IT value 3 and 4 were susceptible.



Figure 1. Polymorphic DNA fragments detected by SSR marker *Xwmc441* in N0324 × Shaanyou 225 F₂ population M: 2 kb DNA ladder; 1: N0324; 2: Resistant bulk; 3: Shaanyou 225; 4: Susceptible bulk. H, R and S stand for heterozygous susceptible, homozygous resistant and homozygous susceptible plants, respectively.

DNA isolation

Genomic DNA was extracted from the uninfected seedling leaves of parental lines (N0324, Shaanyou 225) and individual plants of the F₂ progeny by using cetyltrimethylammonium bromide (CTAB) method (Saghai-Marouf et al., 1984) with some modifications. After disease evaluation, two DNA pools were made separately by combining equal amounts of DNA from seven resistant and seven susceptible F₂ plants for bulked segregant analysis (BSA) (Michelmore et al., 1991).

Molecular markers and PCR amplification

About four hundred and thirty wheat microsatellite markers mapped to the A and B genomes were synthesized according to the published sequences (Suppl. Table 1). Genomic-SSRs used in this study included primer sets of GWM (Röder et al., 1998), WMC (Somers et al., 2004) and BARC (Song et al., 2005). STS, EST-STS, EST-SSR

markers, AFLP and AFLP-derived SCAR markers linked to the resistance gene *MI170* (Liu et al., 2011) were also screened. Information about these markers was published on the GrainGenes website (<http://wheat.pw.usda.gov>) or mentioned in reference articles. The SSR analysis procedure was carried out as described by Bryan et al. (1997) with some modifications.

PCR amplifications were performed in a 10 µL volume using either a 480 thermal cycler (Perkin Elmer, Norwalk, CT, USA) or an S1000 thermal cycler (Bio-Rad, California, USA). The reaction mixture contained 10 mM L⁻¹ Tris-HCl, 50 mM L⁻¹ KCl, 2 mM L⁻¹ MgCl₂, 200 µM L⁻¹ of each dNTP, 250 ng L⁻¹ of each primer, 60 to 100 ng of template DNA, and 0.25 U *Taq* DNA polymerase. All PCR programs started with an initial denaturation at 95°C for 3 min, then followed by 35 amplification cycles at 94°C for 30 s, 50–61°C (marker dependent) for 45 s and 72°C for 50 s, and ended by a final extension at 72°C for 10 min.

Table 1. Sequence and annealing temperature of the SSR markers linked to the powdery mildew resistance gene *Pm5055*.

SSR marker	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing temperature (°C)
<i>Xbarc7</i>	GCGAAGTACCACAAATTTGAAGGA	CGCCATCTTACCCTATTTGATAACTA	50
<i>Xbarc13</i>	GCAGGAACAACCACGCCATCTTAC	GCGTCGCAATTTGAAGAAAATCATC	52
<i>Xbarc55</i>	GCGGTCAACACACTCCACTCCTCTCTC	CGCTGCTCCCATTGCTCGCCGTTA	55
<i>Xwmc441</i>	TCCAGTAGAGCACCTTTCATT	ATCACGAAGATAAACAAACGG	60

PCR products were mixed with 2.5 µL volume of loading dye (100 mM L⁻¹ EDTA pH 8.0, 10 mM L⁻¹ Tris-HCl pH 7.5, 5% Ficoll 400, 0.05% bromophenol, 0.05% xylene cyanol) and loaded for electrophoresis in vertical, non-denaturing 8% polyacrylamide gels (PAGE) in 1×TBE (90 mM L⁻¹ Tris borate pH 8.3, 2 mM L⁻¹ EDTA) at 50 mA for 2.5 to 3 h. Gels were then silver stained (Xu et al., 2002) and photographed.

Chromosomal assignment

The markers linked to the powdery mildew resistance gene were tested on DNA samples of Chinese Spring homoeologous group 2 nullisomic-tetrasomics and ditelosomics to assign chromosomal location.

Data analysis

Deviation of observed data from theoretically expected ratios were evaluated using Chi-squared (χ^2) tests to establish goodness-of-fit. Linkages between markers and the resistance gene were performed using JoinMap 4.0 (<http://www.kyazma.nl/index.php/mc.JoinMap/sc.General/>), with a LOD threshold of 3.0. The genetic map was drawn with the software Mapdraw V2.1 (Liu and Meng, 2003).

RESULTS AND DISCUSSION

Inheritance of powdery mildew resistance in the line N0324

At adult plant stage, N0324 was highly resistant with IT value 0-1 whereas Shaanyou 225 was highly susceptible with IT value 4. There were a total of 205 F₂ plants, derived from the cross N0324/Shaanyou 225. All F₁ seedlings and adult plants were highly susceptible. The F₂ individuals segregated as 160 susceptible and 45 resistant, which fits 3:1 single Mendelian ratio ($\chi^2_{3:1} = 1.016$; $P = 0.313$).

These results indicated that a single recessive powdery mildew resistance gene has been transferred into the common wheat line N0324 from the wild emmer accession 5055.

SSR markers analysis

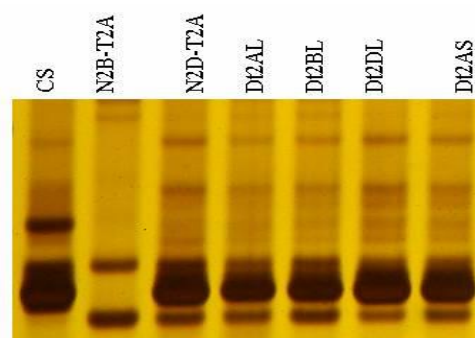
Initially, about 430 SSR markers mapping to the A and B genomes of wheat were screened for their polymorphism between the parental lines (N0324 and Shaanyou 225) as well as between the resistant and susceptible DNA bulks. Thirty seven markers displayed polymorphism (Suppl. Table 1) and were retained for further analysis. Among these, six SSR markers (*Xbarc7*, *Xbarc13*, *Xbarc55*, *Xbarc98*, *Xwmc25* and *Xwmc441*) showed polymorphism between the parents, as well as between the bulks. When the polymorphisms were tested in segregating population, four primer pairs (markers *Xbarc7*, *Xbarc13*, *Xbarc55* and

Xwmc441) were linked to the resistance gene (Table 1). These markers showed co-dominant inheritance (Table 2 and Figure 1), and were placed on chromosome 2B using Chinese Spring nulli-tetrasomic and ditelosomic lines, suggesting that this gene might be located on chromosome 2B (Figure 2). Thus, a linkage map was constructed and *Xwmc441*, *Xbarc7*, *Xbarc13* and *Xbarc55* were linked to the resistance gene *Pm5055* with genetic distances of 10.6, 23, 23 and 23.9 cM, respectively (Figure 3).

Table 2. Segregation ratios for microsatellite (SSR) markers among F₂ individuals in the N0324 × Shaanyou 225 population.

SSR marker	AA	H	BB	Total	$\chi^2_{1:2:1}$	P-value
<i>Xbarc7</i>	41	118	46	205	4.93	0.085
<i>Xbarc13</i>	41	118	46	205	4.93	0.085
<i>Xbarc55</i>	41	116	48	205	4.04	0.133
<i>Xwmc441</i>	44	108	53	205	1.66	0.436

AA: Homozygous for N0324 allele H: Heterozygous
BB: Homozygous for Shaanyou 225 allele

**Figure 2.** Amplification pattern of *Xbarc13* in Chinese Spring homoeologous group 2 nulli-tetrasomics, ditelosomics lines

At present, introgression of disease resistance genes from wild relatives into wheat has become crucial in developing resistant genotypes (Perugini et al., 2008). Wild emmer has been reported as a valuable resource of several important traits for wheat improvement. It has diverse resistance to many pathogens, including stripe rust (Gramma and Gerechter-Amitai, 1974), stem rust (Nevo et al., 1991), leaf rust (Moseman et al., 1985) and powdery mildew (Moseman et al., 1984).

In this study, the identified recessive gene designated *Pm5055* derived from wild emmer is effective against powdery mildew in the field. The gene was mapped on chromosome 2B based on the location of its polymorphic microsatellite markers. It is linked to the SSR marker *Xwmc441* with a genetic distance of 10.6 cM. Gadaleta et al. (2009) when constructing the genetic and physical maps of new EST-derived SSRs on the A and B genomes chromosome of wheat, located *Xwmc441* on chromosome

arm 2BL. To our knowledge, none of the *Pm* genes mapped to chromosome 2B was linked to *Xwmc441*.

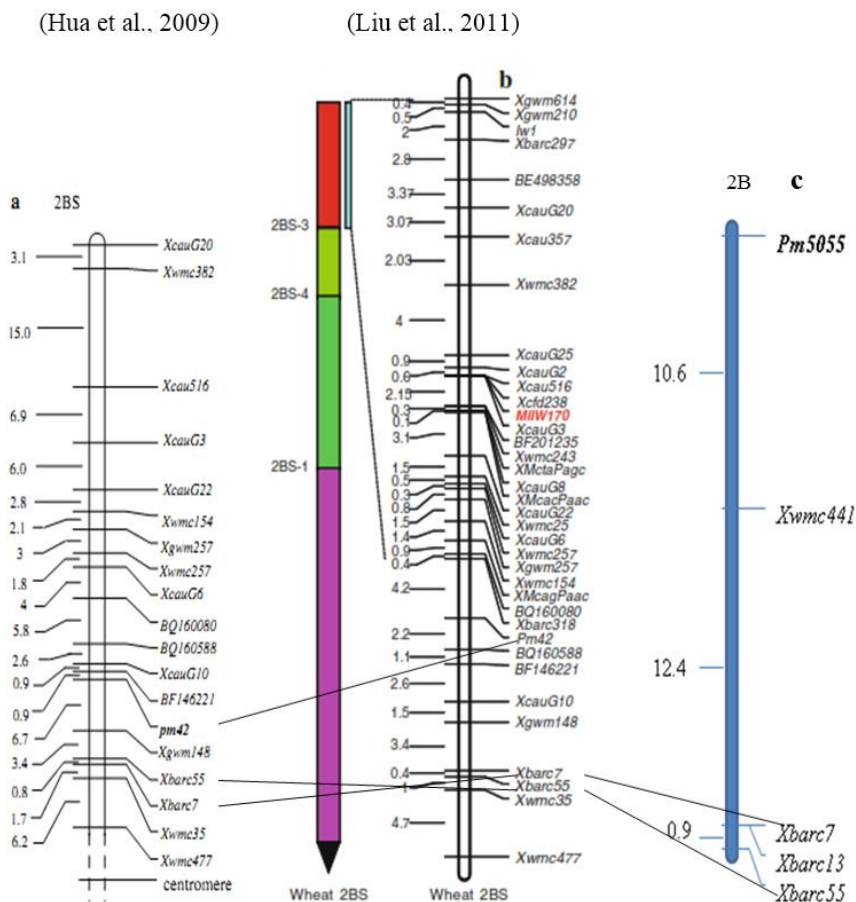


Figure 3. Comparison of *pm42*, *MIIW170* and *Pm5055* loci and linked markers on wheat chromosome 2B.

Among the reported genes conferring resistance to powdery mildew, two recessive genes *Pm26* and *pm42* (Rong et al., 2000; Hua et al., 2009), an incomplete dominant gene *MIIW170* (Liu et al., 2011), and a dominant gene *MI5323* (Piarulli et al., 2012) were mapped to chromosome 2BS. Furthermore, three genes *Pm6*, *Pm33* and *MIZec1* (Jorgensen and Jensen, 1973; Zhu et al., 2005; Mohler et al., 2005) were located on chromosome arm 2BL.

The relationship of *Pm5055* to reported *Pm* genes on chromosome 2B was investigated through their linked primers. Thus, no polymorphisms between our resistant and susceptible lines were detected for an RFLP-derived STS marker *Xcau516* that linked to the recessive powdery mildew resistance gene *Pm26*. Therefore, the marker could not be mapped in our study. However, two SSR markers, *Xbarc7* and *Xbarc55* linked to *Pm5055*, cosegregated with *pm42* and *MIIW170*; two resistant genes originated from wild emmer, *Triticum turgidum* var. *dicoccoides*.

Hua et al. (2009) found that both *Xbarc7* and *Xbarc55* linked to *pm42* with genetic distances of 10.9 and 10.1 cM, respectively (Figure 3a). On the other hand, Liu et al. (2011) integrated *pm42* to the linkage map of an incomplete dominant gene designated *MIIW170* (Figure 3b). On that map, *Xbarc7* and *Xbarc55* were 27.95 and 28.35 cM distant

to *MIIW170* which was in turn 17.15 cM away from *pm42*. In this study, *Pm5055* linked to *Xbarc7* and *Xbarc55* with 23 and 23.9 cM, respectively (Figure 3c). The marker pattern of the three genes (*pm42*, *MIIW170* and *Pm5055*) was the same and the two markers *Xbarc7* and *Xbarc55* gave close map distances to *Pm5055* and *MIIW170*. The resistance genes *pm42* and *MIIW170* and the gene *Pm5055* in this study, suggest that they may belong to the same gene clusters. Clusters of genes conferring resistance to disease on wheat chromosomes are not randomly distributed (Dilbirligi et al., 2004). Genes within a cluster can be allelic or closely linked, for example, the powdery mildew resistance genes at the *Pm1* (Singrün et al., 2003) and *Pm3* loci (Bhullar et al., 2009). Nevertheless, allelism tests would be necessary to clarify the exact relationships between *pm42*, *MIIW170* and *Pm5055*. Meanwhile, the gene *Pm5055* could be useful in future wheat breeding either for its effective reaction to powdery mildew disease or to combine it with other *Pm* genes into a single genotype for gene pyramiding.

CONCLUSION

The employment of molecular markers to characterize the gene of interest has gained importance in crop breeding. Marker assisted selection facilitates the identification of

useful genes for the rapid development of improved crop varieties. In this study, wheat line N0324 carrying powdery mildew resistance gene temporarily designated as *Pm5055*, showed promise resistance in the field to the local race of *Blumeria graminis*, Guanzhong 4, which occurs frequently in Shaanxi province (China). The gene was mapped on wheat chromosome 2B using SSR markers. The linked SSR markers identified in this study and the gene location suggest that *Pm5055* might be closely related to *MIIW170* or *pm42*. This result paves the way for further analysis to investigate the relatedness between the three genes (*pm42*, *MIIW170* and *Pm5055*) in future research.

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