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Mapping and Characterization of Powdery Mildew Resistance Gene in Synthetic Wheat

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Abstract

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Powdery mildew caused by *Blumeria graminis* f.sp. *tritici* is significantly affecting wheat production worldwide. In the search for new sources of resistance we investigated the powdery mildew resistance in the synthetic hexaploid wheat line „Synthetic 43" with its D genome from *Aegilops tauschii*. This line was developed at CIMMYT and resists a number of common bread wheat diseases. The line was crossed with the powdery mildew susceptible hexaploid wheat cultivar WH542 and a mapping population consisting of 148 RILs was developed. Inheritance studies in the RIL population revealed monogenic inheritance of powdery mildew resistance both at the seedling stage and adult plant stage. This resistance gene was mapped at a distance of 4.8 cM from SSR marker X*wmc150* on chromosome 7D and has been temporarily designated as *PmT*.

Keywords: *Aegilops tauschii*; *Blumeria graminis*; SSR; *Triticum aestivum*

Powdery mildew caused by *Blumeria graminis* f.sp. *tritici* is one of the most important fungal diseases of common wheat (*Triticum aestivum* L.). It predominates in cool or warm regions with humid climates (Priestley & Bayles 1998; Hua *et al.* 2009). In India powdery mildew is gaining momentum in the North Western Plain Zone (Singh *et al.* 2009; Kaur *et al.* 2012) along with other major fungal diseases of this region such as leaf rust and stripe rust. The most effective and environmentally efficient way to prevent infection is to develop the wheat cultivars with resistance genes. Up to now, 50 loci with more than 78 genes/alleles for resistance to powdery mildew have been identified and mapped on different wheat chromosomes (McIntosh *et al.* 2013). However, most of the known resistance genes typically demonstrate a "boom-and-bust" cycle (Todorovska *et al.* 2009) exerting a strong selection pressure resulting in pathotypes with matching virulence (Parks *et al.* 2008). This necessitates regular identification and incorporation of new sources of resistance. *Aegilops tauschii*, the D genome progenitor of wheat, has much variation unutilized in germplasm (Lubbers *et al.*1991). One commonly used pathway for introducing the genetic diversity of D genome into bread wheat is the reconstitution of hexaploid wheat by interspecific crosses of modern tetraploid durum wheats with *Ae. tauschii* (Mujeeb-Kazi *et al.* 2008). CIMMYT developed over thousand synthetics from different *Ae. tauschii* accessions (Muzeeb-Kazi *et al.* 2006). Punjab Agricultural University (PAU) procured several synthetic wheats from CIMMYT and one of these, Synthetic 43, has been found to be resistant to commonly occurring diseases of leaf rust, stripe rust and powdery mildew (Sharma *et al.* 2013). In the present study mapping of powdery mildew resistance gene is being reported.

Synthetic hexaploid wheat, Synthetic 43 (*T. durum* (Yuk) × *Ae. tauschii* (864)), was crossed with WH542 (JUP/BJY"S"//URES), a short-statured hexaploid wheat variety released for cultivation in the North Western Plain Zone of India in 1993. A set of 148 recombinant inbred lines (RILs) was developed by a single seed descent method. Three powdery mildew susceptible hexaploid wheats, Agra local, PBW343 and WL711, were used as susceptible controls. The RIL population was evaluated against powdery mildew at seedling stage (SS) and adult plant stage (APS). Seedlings were raised at 18–20°C and the first leaves of eight to ten days old seedlings

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were inoculated with a mixture of conidia (mixture available naturally) under glasshouse conditions. Powdery mildew infection was recorded 14–15 days after the inoculation. Screening at APS was done at a wheat off-season nursery at Dalang Maidan, Lahul and Spiti District, Himachal Pradesh (32°21'N latitude and 77°14'E longitude, 10 000 ft a.s.l.) for two consecutive years. The disease was recorded on the basis of percentage leaf surface area covered with powdery mildew on a 0–9 scale (Blanco *et al.* 2008). Genomic DNA of Synthetic 43, WH542 and RILs was isolated using the CTAB method (Saghai-Maroof *et al.* 1984). For bulked segregant analysis (BSA) (Michelmore *et al.* 1991), genomic DNA from 20 resistant (score 0–1) and 20 susceptible (score 7–8) RILs was mixed in equal amounts to form resistant bulk (RB) and susceptible bulk (SB). From each of 21 chromosomes of wheat, 10–15 SSRs were selected at equal distance. The position of linked marker was confirmed by amplifying markers on a group of nullitetrasomic lines in the Chinese Spring (CS) background. PCR amplification was performed in a 10µl volume reaction with initial denaturation at 94°C, followed by denaturation at 94°C for 30 s, primer annealing at 50–65°C for 30 s, elongation at 72°C for 30 s followed by final extension at 72°C for 7 min. PCR products were resolved on PAGE gel using a LICOR 4300 DNA analyser (LI-COR, Inc, Lincoln, USA).

Both at SS and APS, Synthetic 43 was resistant with powdery mildew score of 0 and WH542 was susceptible with score of 7–8. Of the 148 RILs tested at seedling stage, 76 were homozygous for resistance (HR) with powdery mildew score of 1, and 72 were homozygous for susceptibility (HS) with powdery mildew score of 7–8 (Figure 1). At APS, the RIL population again falls into two distinct classes on the basis of powdery mildew score. 76 RILs which were HR at SS remain resistant also at APS. Similarly, 72 RILs which were scored HS at seedling stage remained susceptible also at APS. This segregation of powdery mildew at two different stages gives a perfect fit into the 1HR: 1HS ratio with χ^2 value of 0.1 (P = 0. 75) as expected for a single gene. This segregation indicated a major seedling resistance to powdery mildew in Synthetic 43. This gene has been temporarily designated as *PmT*. Resistance genes effective at seedling stage remain effective through the adult plant stage (Kolmer 2013). In order to assign a molecular marker closely linked to *PmT* in Synthetic 43, 247 SSR markers were amplified on the two parental lines, 105 were found polymorphic. One SSR marker, *Xwmc*150, showed polymorphism among the bulks. This indicated an association of *Xwmc150* with the powdery mildew resistance gene in Synthetic 43. A random sample of 88 RILs was then genotyped using the putatively linked marker *Xwmc*150. Molecular marker data of these RILs (47 HR and 41 HS) were recorded (Figure 2). *Xwmc*150 segregated into 37 (WH542 allele), 43 (Synthetic 43 allele) and six heterozygotes. Subsequent linkage analysis using MapDisto 1.7.5.1 (Lorieux 2012) indicated that *PmT* was linked to *Xwmc150* with a genetic distance of 4.8 cM. According to the consensus map of Somers *et al.* (2004), *Xwmc*150 had been mapped on the long arm of chromosome 7D. The position of *Xwmc150* and thereof linked gene *PmT* was confirmed by amplifying this marker on six CS nullitetrasomics for homoeologous group 7 (N7AT7B, N7AT7D, N7BT7A, N7BT7D, N7DT7A, N7DT7B) (Figure 3). *Xwmc150* amplified similar fragments in CS and WH542. Therefore, the absence of a PCR product in the nullisomics for 7D confirmed the location of *Xwmc*150 on chromosome 7D. This indicated that the powdery mildew resistance of Synthetic 43 was derived from chromosomes 7D of *Ae. tauschii*. Homoeologous group 7 of wheat plays a key role in powdery mildew resistance as of about 60 known alleles of *Pm*, more than 18 alleles have been mapped to homoeologous group 7 chromosomes, which include seven loci on each of chromosome 7A and 7B and four loci (*Pm15*, *Pm19*, *Pm29*, *Pm38*) on chromosome 7D. It is also possible that of the

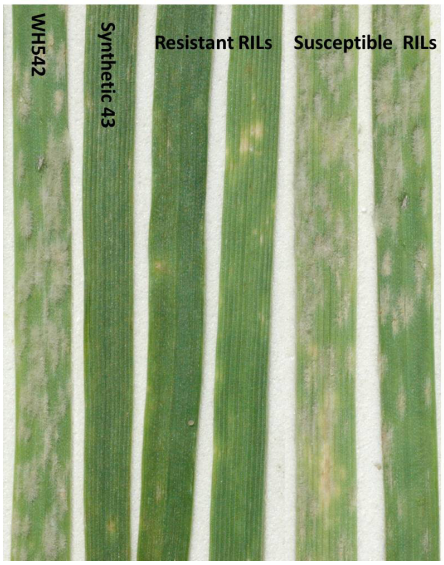

Figure 1. Powdery mildew reaction of Synthetic 43, WH542, resistant RILs and susceptible RILs

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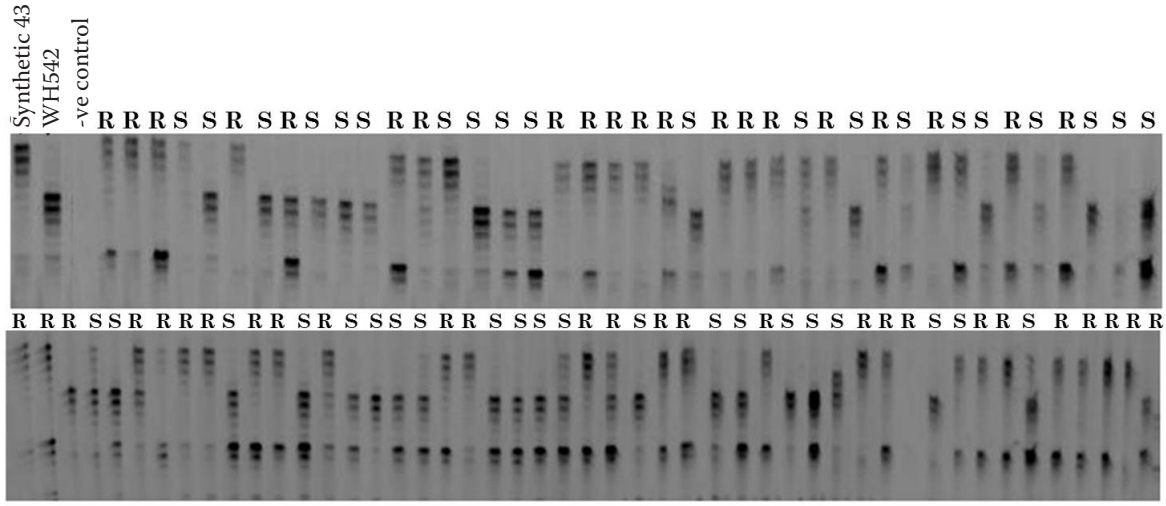

Figure 2. PCR amplification profiles of Synthetic 43, WH542, negative (-ve) control and 88 Synthetic 43 × WH542 recombinant inbred lines (R – resistant, S – susceptible) for SSR marker *Xwmc150*

known genes on homoeologous group 7 some may be homoeoalleles as the long arms of chromosomes 7A and 7D showed high levels of conservation in molecular marker order (Devos *et al.*1994). Till date four *Pm* resistance genes have been reported from *Ae. tauschii* including *Pm2*, *Pm19*, *Pm34* and *Pm35.* Of these only *Pm19* has been reported on chromosome 7D but markers linked to this gene are not known (Lutz *et al.*1995). Similarly, markers linked to gene *Pm15* are not known (Tosa & Sakai 1990). *Pm38* mapped on chromosome 7DS is an APS gene while the gene reported in the present study is a seedling resistance gene. *Pm29*, another gene on chromosome 7DL, has been derived from a non-progenitor species of wheat, *Ae. ovata* (UUMM) (Zeller *et al*. 2002). In India not much work has been done on the powdery mildew resistance and less information is available on its pathogenic variability. With the monocultivation of high-yielding dwarf varieties under the high fertility conditions, powdery mildew is becoming an important disease here. Effectiveness of *PmT* in the natural epidemic of powdery mildew at Dalang Maidan in the Himalayas, which is considered as a hot spot for powdery mildew disease, indicated usefulness of resistance.

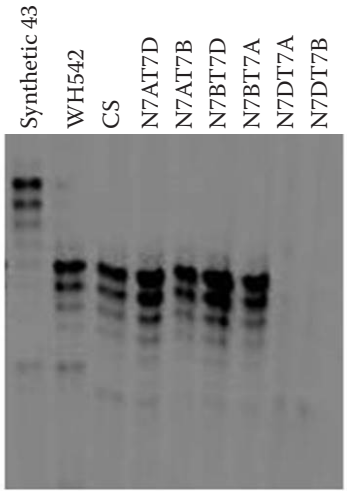

Figure 3. PCR amplification profiles of Synthetic 43, WH542, Chinese Spring (CS) and six nullitetrasomics in the Chinese spring background for SSR marker *Xwmc150* (N – Nullisomic, T – Tetrasomic)

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