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RESEARCH ARTICLE

# Fine mapping of powdery mildew resistance gene *PmTm4* in wheat using comparative genomics

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**Abstract**

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the most severe wheat diseases. Mining powdery mildew resistance genes in wheat cultivars and their appliance in breeding program is a promising way to control this disease. Genetic analysis revealed that a single dominant resistance gene named *PmTm4* originated from Chinese wheat line Tangmai 4 confers resistance to prevailing isolates of *B. graminis* f. sp. *tritici* isolate E09. Detailed comparative genomics analyses helped to develop closely linked markers to *PmTm4* and a fine genetic map was constructed using large $F_2$ population, in which *PmTm4* was located into a 0.66-cM genetic interval. The orthologous subgenome region of *PmTm4* in *Aegilops tauschii* was identified, and two resistance gene analogs (RGA) were characterized from the corresponding sequence scaffolds of *Ae. tauschii* draft assembly. The closely linked markers and identified *Ae. tauschii* orthologs in the mapping interval provide an entry point for chromosome landing and map-based cloning of *PmTm4*.

**Keywords:** powdery mildew resistance gene, *PmTm4*, genetic mapping, comparative genomic analysis

## 1. Introduction

Common wheat (*Triticum aestivum* L., 2n=6x=42) is one of the most major cultivated crops of the world, along with rice and maize, and this crop alone provides 20% of total food calories and protein that humans need (FAO 2015a). Demand for wheat is estimated to rise more than 50% by 2050 (CRP 2014; FAO 2015b). Many strategies are being applied to meet this demand, including preventing yield losses *via* taking control of wheat diseases. Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most common diseases in wheat worldwide, which can lead to serious yield losses under favorable conditions (Cowger *et al*. 2012). Breeding and growing powdery mildew resistant cultivars are the most effective, economic and environmentally safe approach to control this disease. Marker assisted selection (MAS) used in breeding program is able to accelerate development of new resistant variet-

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ies. Identification of powdery mildew resistance genes and development of molecular markers closely linked to them are essential for MAS processes. So far, more than 60 powdery mildew resistance genes or alleles were identified and genetically mapped on 20 chromosomes in wheat (*Pm1* to *Pm54*, *Pm18=Pm1c*, *Pm22=Pm1e*, *Pm23=Pm4c*, *Pm31=Pm21*) (Alam *et al*. 2011; McIntosh *et al*. 2013), some of which have already been utilized in breeding program. From the catalogued wheat powdery mildew resistance genes, most of them are race-specific and only a few of them, such as *Lr34/Yr18/Pm38*, confer partial resistance or adult plant resistance (APR) in a non race-specific manner. Since race-specific resistance genes tend to be ineffective for the emergence of new virulence *Bgt* isolates, more resistance genes are needed to catch up with the fast pace of pathogen evolution.

Wheat landraces are valuable sources of many agronomically important traits (Cavanagh *et al*. 2013). Breeding for powdery mildew disease resistance in wheat utilizing landraces is an effective way to fight this disease. Rich genetic diversity of powdery mildew resistance was found in Chinese landraces (Huang *et al*. 1997). Presently, several powdery mildew resistance genes or alleles were identified in Chinese landrace, including *Pm5e* in Fuzhuang 30 (Huang *et al*. 2003), *Pm5d* in IGV1–455 (Nematollahi *et al*. 2008), *Pm24* in Chiyacao (Huang and Röder 2011), *Pm24b* in Baihulu (Xue *et al*. 2012), *Pm45* in D57 (Ma *et al*. 2011), *Pm47* in Hongyanglazi (Xiao *et al*. 2013), *pmX* in Xiaohongpi (Fu *et al*. 2013), *MLHLT* in Hulutou (Wang *et al*. 2015), *PmH* in Hongquanmang (Zhou *et al*. 2005), and *Mlxbd* in Xiaobaidong (Xue *et al*. 2009). These genes are beneficial targets of wheat breeding, especially in China. Common wheat line Tangmai 4 is such a breeding line containing a powdery mildew resistance gene *PmTm4* originated from a Chinese wheat landrace (Hu *et al*. 2008). It seems that the *Pm5* locus contains diversified allelic variation in wheat germplasm, especially in Chinese wheat landrace. Developing high-density genetic linkage map of *PmTm4* would be beneficial for cloning the *Pm5* locus and characterizing its allelic variation that would be interested for wheat breeders.

Fine mapping and map-based cloning of important genes is a challenging task in common wheat not only because its genome is hexaploid (AABBDD), highly receptive (90%) and huge (17 Gb), but also because no reference sequence is available. With the release of wheat expressed sequence tags (EST) database (Mochida *et al*. 2006; Coordinators 2016), reference sequences of wheat closely related species like *Brachypodium* (IBI 2010), rice (IRGSP 2005) and sorghum (Paterson *et al*. 2009), and sequence resource in genome sequencing projects of barley (IBGSC *et al*. 2012; Poursarebani *et al*. 2013; Ariyadasa *et al*. 2014; Zeng *et al*. 2015), *Aegilops tauschii* (Jia *et al*. 2013; Luo *et al*. 2013), *Triticum urartu* (Ling *et al*. 2013), *Triticum aestivum* (Belova *et al*. 2013; Choulet *et al*. 2014; IWGSC 2014), and synthetic wheat w7984 (Chapman *et al*. 2015), new routes to clone genes in wheat are emerging (Borrill *et al*. 2015). One of the routes is comparative genomics analysis, which uses the conserved collinearity between wheat and its related species to facilitate the development of markers linked closer with target genes in wheat (IBI 2010; Catalan *et al*. 2012; Brutnell *et al*. 2015; Mandadi and Scholthof 2015). To date, many powdery mildew resistance genes were genetically mapped by making use of comparative genomics analysis, such as *Pm6* (Qin *et al*. 2011), *Pm41* (Wang *et al*. 2014), *MlIW172* (Ouyang *et al*. 2014), and *MlWE4* (Zhang *et al*. 2015). Recently, the physical map of *Ae*. *taus*chii (Luo *et al*. 2013), the D genome donor of common wheat, and its mainly anchored shotgun genome assembly (Jia *et al*. 2013) were constructed, making comparative genomics analysis in grass families and gene mapping in wheat more informative. With this information in D genome, powdery mildew genes *MlIW170* (Liang *et al*. 2015) and *MLHLT* (Wang *et al*. 2015) were successfully mapped into a narrow genetic interval.

In this study, we applied comparative genomics analysis to develop closely linked markers for fine mapping the powdery mildew resistance gene *PmTm4* in Chinese wheat line Tangmai 4.

## 2. Materials and methods

### 2.1. Plant materials

A powdery mildew resistant common wheat line Tangmai 4 (Li *et al*. 2004; Hu *et al*. 2008) was crossed with a highly susceptible common wheat line Xuezao (Zhang *et al*. 2010) to produce $F_1$ hybrids, $F_2$ segregating populations and their $F_{2:3}$ families for fine mapping of powdery mildew resistance gene *PmTm4* in Tangmai 4. Fuzhuang 30, Xiaobaidongmai, Hongquanmang, Mazhamai, and Laozaomai were used as donors of powdery mildew resistance genes *Pm5e*, *xbd*, *PmH*, *Pmmz*, and *PmTm4*. Partial of the Chinese minicore collections (MCC) were kindly provided by Dr. Zhang Xueyong of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences.

### 2.2. Evaluation of powdery mildew resistance

*Blumeria graminis* f. sp. *tritici* (*Bgt*) isolate E09 is a prevailing pathotype on common wheat at the region of Beijing, and is virulent to resistance genes *Pm1*, *Pm3a*, *Pm3c*, *Pm5a*, *Pm7*, *Pm8*, *Pm17*, *Pm19* and Xuezao (Zhou *et al*. 2005; Zhang *et al*. 2010, 2015). It was used in this study to evaluate the