

Full Length Research Paper

Chromosomal location and SSR markers of a powdery mildew resistance gene in common wheat line N0308

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Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most devastating diseases of wheat in China and worldwide. A powdery mildew resistance gene, originated from wild emmer accession G25, transferred into a common wheat line N0308. F₂ population was established by crossing N0308, resistance to powdery mildew race Guanzong 4, with susceptible line Shaanyou 225. The segregation of phenotype accorded an expected ratio 3:1. The results indicate that the powdery mildew resistance gene in N0308 is controlled by a single dominant gene, designated temporarily as *PmG25*. SSR markers and the bulk segregant analysis were used to characterize the powdery mildew resistance gene *PmG25*. Six SSR markers such as *Xgpw7425*, *Xwmc75*, *Xgwm408*, *Xwmc810*, *Xbarc232* and *Xbarc142* were located on the distal of resistance gene *PmG25* with genetic distance 7.4, 9.4, 11.2, 22.3, 25.4 and 29.3 cM, respectively. All markers were placed on chromosome 5BL using Chinese Spring nulli-tetrasomic and ditelosomic lines, suggesting this gene might be located on the long arm of chromosome 5B.

Key words: Chromosomal location, SSR markers, powdery mildew, resistance genes, wild emmer.

INTRODUCTION

Wheat powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (*Bgt*) = *Erysiphe graminis* DC. ex *Merat* f. sp. *tritici* Em. Marchal, is one of the most devastating diseases of common wheat worldwide in areas with cool or maritime climates (Bennett, 1984). Generally, the yield losses range from 13 to 34% due to this disease but if the disease attacks are severe to the flag leaf during the heading and filling stage, loss can be up to 50% (Griffey et al., 1993). The development of resistant cultivars is the most effective, economically sound and environmentally friendly approach to escape fungicide use and reduce crop losses caused by this disease. Race-specific or qualitative resistance is the most common type of genetic

resistance followed by seedling and adult-plant-resistance (APR) or quantitative resistance (Huang and Röder, 2004). Until now, 41 loci (*Pm1* to *Pm46*) with more than 60 powdery mildew resistant alleles have been identified and located on different chromosomes in bread wheat and its relatives (Alam et al., 2011; Xue et al., 2012). However, the new *Bgt* races have ability to breakdown the resistances of those genes, because the presence and frequency of virulence genes in the pathogen population changes continuously (Limpert et al., 1987). Control of powdery mildew disease has highly depended on using single gene resistance, and the most common technique has been to replace cultivars when their resistance is no longer effective (Leath and Heun, 1990).

Previously, major resistance genes to powdery mildew have been differentiated by inoculating a set of host genotypes with different pathogen races (Huang et al., 1997), but virulent races are not always available for newly discovered genes (Hsam and Zeller, 2002). Molecular markers such as restriction fragment length

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polymorphisms (RFLPs), random-amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), sequence-tagged sites (STS) and microsatellites, also termed SSRs, provide an alternative tool to accurately identify the resistant gene of interest in a specific chromosome. They can be used to tag genomic region, clone the gene of interest through map-based cloning, near isogenic line development and resistance genes pyramiding into single cultivars by marker assisted selection.

Wild emmer wheat, discovered in northern Israel by Aaronsohn (1910), is an important genetic resource that could be exploited in breeding for resistance to a broad range of diseases and pests (Nevo, 1995). Powdery mildew resistance genes: *Pm16* (Reader and Miller, 1991), *Pm26* (Rong et al., 2000), *Pm30* (Liu et al., 2002), *Pm36* (Blanco et al., 2008), *Pm41* (Li et al., 2009), *Pm42* (Hua et al., 2009), as well as temporarily designated genes: *MIZec1* (Mohler et al., 2005), *MIW72* (Ji et al., 2008), *MI3D232* (Zhang et al., 2010), *MIAB10* (Maxwell et al., 2010), *PmAs846* (Xue et al., 2012), all were introgressed into common wheat from *T. turgidum* subsp. *dicoccoides*.

Wild emmer accession G25 from Rosh Pinna, Israel was exposed to be highly resistant to more than 21 stripe rust races both in the seedling and adult plant stages (Gerechter-Amitai and Stubbs, 1970). Using *T. durum* as a bridge, and also by direct crosses with *T. aestivum*, showed that the resistance can be transferred into bread wheat (Grama and Gerechter-Amitai, 1974). However, there is no published report of transferring powdery mildew resistance from this species (G25) to a common wheat chromosome. *T. dicoccoides* G25 appears to have very broad spectrum resistance among the tetraploid and hexaploid wheats (Gerechter-Amitai and Stubbs, 1970).

Till now, more than 60 alleles responsible for resistant to powdery mildew have been reported, but the resistance of many of these genes has been broken down by the pathogen races possessing corresponding virulence genes. So exploring the new sources of resistance is of great demand. In the Key Laboratory for Molecular Biology of Agriculture in Shaanxi Province, Northwest A&F university, Shaanxi, China, the powdery mildew resistance line N0308 was developed by using tetraploid wild emmer (AABB) line 'G25' as a source of powdery mildew resistance. Therefore, the present study was conducted to identify the chromosomal location of powdery mildew resistance gene in N0308.

MATERIALS AND METHODS

Plant materials

The powdery mildew resistance line N0308 (G25/Shaan 253) was derived from a single cross between powdery mildew resistant wild emmer (*T. dicoccoides*) accession 'G25' and a susceptible Chinese elite common wheat line 'Shaan 253', followed by subsequent selection in onward generations. The G25 was obtained from Dr.

Eviatar Nevo, Institute of Evolution, Haifa University, Haifa 31905, Israel. Homozygous N0308 line containing powdery mildew resistance was crossed with highly susceptible common wheat line Shaanyou 225 (Xiaoyan No.6/Ns2761) to produce F₁ hybrid and F₂ segregating populations.

Powdery mildew test

F₂ individuals, wild emmer G25, N0308, Shaan 253 and Shaanyou 225 were planted in pots. 137 F₂ plants were evaluated at the two leaf stage inoculated by powdery mildew race 'Guanzhong 4'- a local isolate of *Bgt* in Shaanxi Province, avirulent to wild emmer G25 and N0308. The test seedlings were inoculated by dusting conidia from sporulated seedlings of Shaanyou 225. The infections were evaluated about two weeks after inoculation when pustules were fully developed on Shaanyou 225 and susceptible F₂ populations. The infection types (IT) were recorded as resistant (with IT value 0, 0₁, 1, 2) or susceptible (with IT value 3 and 4) where IT 0, represented no visible disease symptom; 0₁, hypersensitive necrotic flecks; 1, minute colonies with few conidia; 2, colonies with moderately developed hyphae and moderate conidial production; 3, colonies with well-developed hyphae and abundant conidia, but not coalesced colonies; 4, colonies with well-developed hyphae and abundant conidia and coalesced colonies (Niu et al., 2006).

Genomic DNA extraction

Total DNA was extracted from leaf tissue of parents (N0308 and Shaanyou 225), individual plants of the F₂ population, powdery mildew resistance gene donor wild emmer G25 and common wheat cultivar Shaan 253 by the CTAB method described by Stein et al. (2001). Equal amounts of DNA were pooled from 10 resistant and 10 susceptible F₂ plants to make two DNA pools (Michelmore et al., 1991) for bulk segregate analysis (BSA).

PCR amplification and electrophoresis separation

520 wheat microsatellite markers (gwm, wmc, cfa, barc and cfd series) were chosen as kindly provided by Professor Don Marshall (Plant Breeding Center, University of Sydney, Australia) or synthesized according to the published sequences (Röder et al., 1998). In the Grain Genes website (<http://www.wheat.pw.usda.gov>), the relevant information to these markers is available. PCR amplifications were performed in 10 µL volume using a Perkin Elmer 480 Thermocycler. The reaction mixture contained 10 mM/L Tris-HCl, 50 mM/L KCl, 2 mM/L MgCl₂, 200 µmol/L of each dNTPs, 250 nM/L of each primer, 20 to 40 ng genomic DNA, and 0.25 U Taq DNA polymerase. The PCR amplification was as follows: one cycle of 95°C for 3 min; 35 cycles of 94°C for 1 min, 50 to 60°C (depending on the specific primers) for 1.5 min and 72°C for 1 min; and a final extension at 72°C for 10 min. Reaction products were mixed with one fifth volume of loading buffer (100 mM/L EDTA pH 8.0, 10 mM/L Tris-HCl pH 7.5, 5% Ficoll 400; 0.05% bromophenol, 0.05% xylene cyanol) and 8 µL were loaded vertically, for electrophoresis 8% denaturing polyacrylamide gels in 1 × TBE (90mM/L Tris borate pH 8.3, 2mM/L EDTA) at 50 mA for 2 to 3 h (Wang et al., 2007). Gels were then silver stained (Xu et al., 2002) and photographed.

Chromosomal arm assignment

The markers linked to the powdery mildew resistance were tested on DNA samples of Chinese Spring homoeologous group 5 nullisomic-tetrasomics and ditelosomics to assign chromosome and

Table 1. Thirteen primer sets with chromosome/arm detected *T. dicoccoides* accession G25 specific DNA fragments in N0308.

| Primer set | Chromosome |
|------------|--------------------|
| Xwmc422 | 7A |
| Xgwm282 | 7A |
| Xbarc7 | 2B |
| Xbarc13 | 2B |
| Xbarc20 | 4B, 7B |
| Xcfa2147 | 1A,1B,1D |
| Xwmc44 | 1B |
| Xgwm408 | 5B |
| Xgpw7425 | 5B |
| Xwmc75 | 5B |
| Xwmc810 | 5B |
| Xbarc232 | 5AL, 5BL, 5DL |
| Xbarc142, | 5AL, 5BL, 2DL, 6AL |

chromosome arm locations.

Linkage analysis

MAPMAKER/EXP v3.0b (Lincoln et al., 1993) was used to establish a linkage map, with map distances calculated in the Kosambi mapping function (Kosambi, 1944). A LOD score of 3.0 was used as the significance threshold for the declaration of linkage.

RESULTS

Inheritance of the powdery mildew resistance in N0308

There were a total of 137 F_2 plants, derived from the cross of N0308×Shaanyou 225. The F_2 populations along with their parents were inoculated with powdery mildew race Guanzhong 4. Seven days after inoculation, N0308 was highly resistant (IT 0), whereas Shaanyou 225 was highly susceptible (IT 4). All F_1 seedlings were highly resistant (IT 0), indicating that the powdery mildew resistance in N0308 is dominant. The F_2 individuals segregated as 99 resistant and 38 susceptible, which fits 3:1 single Mendelian ratio ($\chi^2 = 0.55$, $P > 0.05$). These results suggest that a single dominant powdery mildew resistance gene has been transferred into the common wheat line N0308 from the wild emmer accession G25.

SSR analysis of the resistance gene

520 microsatellite primer pairs screened for resistant and susceptible parents and 86 primer pairs showed clear polymorphism between N0308 and Shaanyou 225. Out of 86 polymorphic microsatellite primer pairs, 57 (66%) amplified distinguishable polymorphic fragments between

wild emmer G25 and Shaan 253. 12 primer pairs yielded *T. dicoccoides* specific DNA fragments. 13 primer pairs detected *T. dicoccoides* accession G25 specific DNA fragments in N0308 (Table 1). Eight primer pairs (*Xgwm408*, *Xwmc75*, *Xgpw7425*, *Xwmc810*, *Xbarc232*, *Xbarc142*, *Xcfa2147* and *Xwmc44*) were found polymorphic between the resistant and susceptible DNA bulks. When the polymorphisms were tested in segregating populations, six primer pairs such as *Xgpw7425*, *Xwmc75*, *Xgwm408*, *Xwmc810*, *Xbarc232* and *Xbarc142* were located on the distal of resistance gene *PmG25* with genetic distance 7.4, 9.4, 11.2, 22.3, 25.4 and 29.3 cM, respectively (Figure 3). Among the six polymorphic SSR markers, three (*Xgpw7425*, *Xbarc232* and *Xbarc142*) were found co-dominant, and three (*Xwmc810*, *Xgwm408* and *Xwmc75*) were complete dominant (Table 2, Figure 1). All markers were placed on chromosome 5BL using Chinese Spring nulli-tetrasomic and ditelosomic lines, suggesting this gene might be located on long arm of chromosome 5B (Figure 2).

DISCUSSION

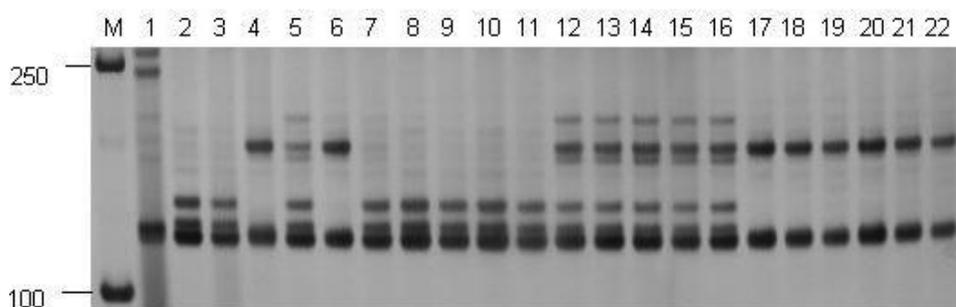
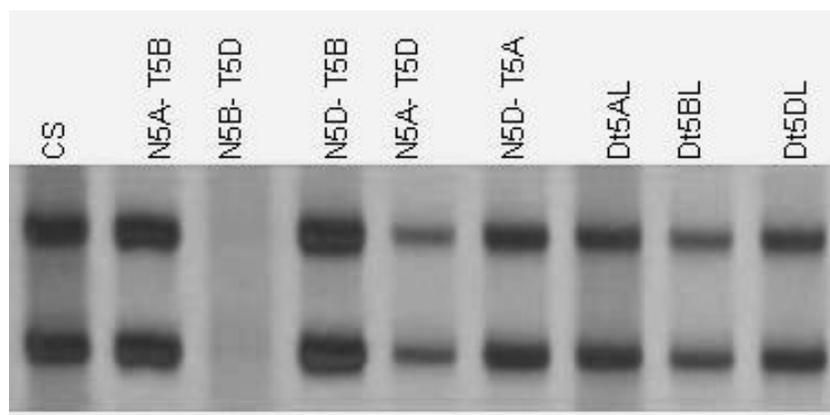
Wild emmer genotypes originated from Israel were highly resistance to wheat powdery mildew in China (Xie et al., 2003). In the present study, a new wheat genotype N0308 resistant to powdery mildew has been introgressed from wild emmer. To gain an insight about the inheritance of the powdery mildew resistance derived from wild emmer, a segregating population was developed by crossing the resistance line N0308 with a susceptible line Shaanyou 225. Segregation ratios confirmed the hypothesis that the powdery mildew resistant in N0308 is controlled by a single dominant gene.

The SSR markers *Xbarc232* and *Xbarc142* have multiple loci in common wheat genome. *Xbarc232* was detected at three loci on chromosome arms 5AL, 5BL and 5DL (Somers et al., 2004) and *Xbarc142* was found at four loci on chromosome arms 5AL, 5BL, 2DL and 6AL (Somers et al., 2004). However, other four markers were single-locus markers that only mapped on chromosome 5BL. These findings and the physical mapping results with Chinese Spring nulli-tetrasomics and ditelosomics lines enabled us to locate the powdery mildew resistance gene *PmG25* on long arm of chromosome 5BL.

Three powdery mildew resistance genes have been characterized on chromosome 5B; *Pm16*, *Pm30* and *Pm36*. *Pm16* originally mapped on chromosome 4A by monosomic analysis (Reader and Miller 1991), was reassigned on 5BS via SSR marker mapping and possibly allelic to *Pm30* (Liu et al., 2002); *Pm30* was derived from the wild emmer accession C20 originated from the Rosh Pinna population in Israel and mapped on 5BS (Liu et al., 2002). *Pm16* and the *Pm30* both are *T. turgidum* subsp. *dicoccoides*-derived powdery mildew resistance genes placed on the short arm of chromosome

Table 2. Segregation ratios for SSR markers linked to the powdery mildew resistance gene *PmG25* evaluated in an F₂ population of N0308/ Shaanyou 225.

| SSR marker | Number of F ₂ plants | Homozygous resistant | Heterozygous resistant | Susceptible | $\chi^2_{(3:1)}$ |
|------------|---------------------------------|----------------------|------------------------|-------------|------------------|
| Xgwm408 | 137 | 97 | | 40 | 1.29 |
| Xbarc232 | 137 | 35 | 65 | 37 | 0.3 |
| Xwmc810 | 137 | 99 | | 38 | 0.55 |
| Xgpw7425 | 137 | 42 | 53 | 42 | 2.34 |
| Xbarc142 | 137 | 28 | 70 | 39 | 0.88 |
| Xwmc75 | 137 | 96 | | 41 | 1.77 |

**Figure 1.** PCR product of *Xgpw7425* in the resistant and susceptible F₂ plants. 1, Shaan 253; 2, *T. dicoccoides* accession G25; 3, N0308; 4, Shaanyou 225; 5, resistance pool; 6, susceptible pool; 7-11, homozygous resistant; 12-16, heterozygous resistant; 17-22, susceptible F₂ plants; M: 1 kb DNA ladder.**Figure 2.** Amplification pattern of *Xwmc75* in Chinese Spring homoeologous group 5 nulli-tetrasomics, ditelosomics lines.

5B. The resistance gene in N0308, located on the long arm of chromosome 5B, is different from *Pm16* or *Pm30*.

Pm36 was transferred into durum wheat line 5BIL-29 and 5BIL-42 from wild emmer accession MG29896 and mapped on 5BL which is closely linked to EST-SSR marker *BJ261635* (Blanco et al., 2008). Powdery mildew resistance gene *Pm36* was observed to be linked with

SSR marker *Xwmc75* that is located on chromosome 5BL with a genetic distance of 10.0 cM. *PmAs846* transferred from the wild emmer accession As846 into common hexaploid wheat line N9134 and mapped on 5BL, and the genetic distance of *PmAs846* from *Xwmc75* was found 14.2 cM (Xue et al., 2012). In this study, *PmG25* was also found to be linked with the same SSR marker (*Xwmc75*)

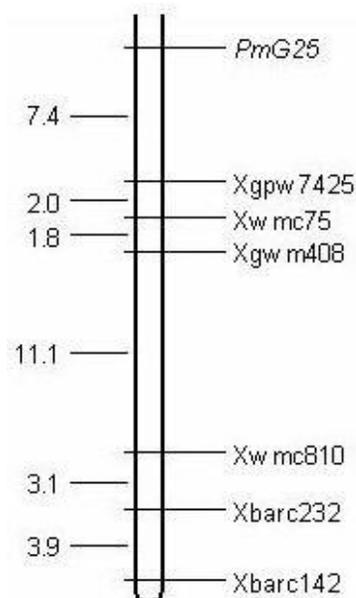


Figure 3. Linkage map of powdery mildew resistance genes *PmG25*.

with a distance of 9.4 cM. Although all the *PmG25*, *Pm36* and *PmAs846* were found to be located in same genetic distance from *Xwmc75*, allelism tests would be necessary to clarify the exact relationships among them.

The present work enriches the genetic resource with potential value of wild emmer for the improvement of cultivated wheat. Potentially useful genes found in wild emmer can be transferred to common wheat by direct hybridization, backcrossing and selection. The molecular markers closely linked to the resistance gene *PmG25* can be used for marker-assisted selection in wheat breeding for powdery mildew resistance.

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