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IDENTIFICATION AND MOLECULAR MAPPING OF POWDERY MILDEW RESISTANCE GENE *PMG25* IN COMMON WHEAT ORIGINATED FROM WILD EMMER (*TRITICUM TURGIDUM* VAR. *DICOCCOIDES*)

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Abstract

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most economically important wheat diseases in many regions through out the world. N0308, a common wheat line contains powdery mildew resistance gene introgressed from wild emmer accession G25. Genetic analysis of the F_2 populations and F_3 families derived from the cross between N0308 and a susceptible common wheat cultivar Shaanyou 225 indicated a single dominant gene, temporarily designated *PmG25*, conferred resistance to powdery mildew race 'Guanzhong 4'. Bulked segregant analysis and molecular markers were used to characterize the powdery mildew resistance gene *PmG25*. Eleven SSR markers (*Xgpw1082, Xgpw3191, Xfcp1, Xfcp393, Xfcp394, Xgpw7425, Xwmc75, Xgwm408, Xwmc810, Xbarc232* and *Xbarc142*) and two EST-STS markers (*BF482522* and *BF202652*) were linked with *PmG25* on the long arm of chromosome 5B. The resistance gene was flanked by *Xfc1/Xfcp393* and *Xgpw3191*, with genetic distances of 1.3 and 3.3 cM, respectively, and located on the chromosome bin 5BL-14- 0.75-0.76 in the test with a set of deletion lines. The powdery mildew resistance genes *Pm36* and *Ml3D232* have also been mapped to the region. The chromosome location and genetic mapping results suggested that the powdery mildew resistance gene derived from wild emmer G25 may be allelic or closely linked to *Pm36*.

Introduction

Wheat has become an important cereal crop from all perspectives. It provides more calories in diet than any other crop. It is staple food crop and also known as "king" of the cereals (Laghari *et al*., 2010). Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*) is one of the most devastating diseases of common wheat worldwide in areas with cool or maritime climates (Bennett, 1984). Generally yield losses ranging from 13 to 34% due to this disease but if the disease attacks severely to the flag leaf during the heading and filling stage loss can be severe as 50% (Griffey *et al.*, 1993; Leath & Bowen, 1989). International wheat breeding has given major emphasis on genetic control of disease by introducing the new resistant genes within elite commercial cultivars (Khan *et al*., 2012). Until now, 41 loci (*Pm1 to Pm45*) with more than 60 powdery mildew resistant genes/alleles have been identified and located on different chromosomes in bread wheat and its relatives (Ma *et al.*, 2011; Alam *et al*., 2011).

Wild emmer wheat, discovered in northern Israel by Aaronsohn (1910), is a harbor of rich genetic resource that could be exploited in breeding for resistance to a broad range of diseases, pests, grain protein quality and quantity, micronutrient concentrations (Zn, Fe, and Mn) and tolerance to abiotic stress (salt, drought and heat) (Nevo, 1995). Among many agriculturally important characteristics already found in *T. dicoccoides* in its resistance to several diseases, including fusarium head blight, tan spot (*Pyrenophora tritici-repentis*), leaf blotch (*Stagonospora nodorum*), stripe rust, stem rust, leaf rust and powdery mildew. Powdery mildew resistance genes: *Pm16* (Reader & 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: *MlZec1* (Mohler *et al*., 2005), *MlIW72* (Ji *et al.*, 2008), *Ml3D232* (Zhang *et al*., 2010), *MIAB10* (Maxwell *et al*., 2010), 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 & Stubbs, 1970).The inheritance study of G25 in a crosses with a susceptible *T. durum* cultivar, revealed that the resistance in this wild emmer selection is probably conferred by one dominant gene (Gerechter-Amitai & Grama, 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 very broad spectrum resistance among the tetraploid and hexaploid wheat (Gerechter-Amitai & Stubbs, 1970).

Although more than 60 genes/alleles resistant to powdery mildew have been reported, resistance has been broken down many of these genes by pathogen races possessing corresponding virulence genes. Therefore, it is essential to search the new sources of resistance. The common wheat lines known as N0308 were bred by the Key Laboratory for Molecular Biology of Agriculture in Shaanxi Province, Nowrthwest A&F university, Shaanxi, China using tetraploid wild emmer (AABB) line 'G25' as a source of powdery mildew resistance. Therefore, we tried to identify the chromosomal location and mapping of molecular markers linked to the powdery mildew resistance gene in N0308.

Materials and Methods

Plant materials: Wheat line N0308 (G25/Shaan 253) was derived from a single cross between G25 and Shaan 253 a susceptible Chinese elite common wheat line followed by subsequent selection in onward generations. Wild emmer (*T. dicoccoides*) accession 'G25', kindly provided by Dr.

Eviatar Nevo, Institute of Evolution, Haifa University, Haifa 31905, Israel was used in this cross as the donor of powdery mildew resistance gene. Homozygous N0308 line containing powdery mildew resistance was derived with highly susceptible common wheat line Shaanyou 225 to produce F_1 hybrid, F_2 segregating populations and F_3 families.

Powdery mildew test: F_2 individuals, wild emmer G25, N0308, Shaan 253 and Shaanyou 225 were planted in pots. Seedlings were artificially inoculated at the two leaf stage with powdery mildew race 'Guanzhong 4', a local isolate of *Bgt* in Shaanxi Province, avirulent to wild emmer G25 and N0308. The $F_{2:3}$ families (15 seedlings of each $F_{2:3}$ family) were tested to confirm the phenotypes and to establish the resistance genotypes of each F_2 plant. The test results were evaluated about two weeks after inoculation when pustules were fully developed on Shaanyou 225 and susceptible F_2 populations. The infection types were recorded as resistant (with IT value 0, 0; 1, 2) or susceptible (with IT value 3 and 4) where IT 0, represented no visible disease symptom; 0;, 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.

Genomic DNA extraction: Genomic DNA were extracted from leaf tissue of parents (N0308 and Shaanyou 225), individual plants of the F_2 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). Two DNA pools were made by pooling equal amounts of DNA from 10 resistant and 10 susceptible F_2 plants (Michelmore *et al.*, 1991) for bulk segregate analysis (BSA).

PCR amplification and electrophoresis separation: Wheat microsatellite markers (gwm, wmc, cfa, barc, cfd and fcp series) mapped to the A and B genomes were selected for marker analysis. Relevant information to these markers is published on the Grain Genes website (http://www.wheat.pw.usda.gov). In addition, EST-STS markers (Yao *et al*., 2007) were also tested that were closely linked to *Pm36* and *M13D232* located on chromosome 5BL in wild emmer wheat. PCR amplifications were performed in 10μL volume using a Perkin Elmer 480Thermocycler. The reaction mixture contained 10mM/L Tris-HCl, 50mM/LKCl, 2mM/L $MgCl_2$, 200μmol/L of each dNTPs, 250nM/L of each primer, 20 to 40ng genomic DNA, and 0.25U 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, 50–60°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. The PCR products were separated in 8% non-denaturing polyacrylamide gels (39 acrylamide : 1 bisacrylamide). Gels were then silver stained (Xu *et al*., 2002) and photographed.

Chromosomal assignment: To assign chromosome and chromosomal arm locations of the linked microsatellite markers were confirmed using a set of 'Chinese Spring' homoeologous group 5 nulli-tetrasomics, ditelosomics and deletion lines (Table 1). Markers were mapped to chromosomal bins flanked by breakpoints of the largest deletion fragment possessing.

Table 1. Chinese spring 5BL deletion lines used for chromosomal bin mapping.

Deletion line	Description	Fraction length
5BL-11	20 + 1 [d5BL-11]	0.59
5BL-14	18 + 1 [d5BL-14]	0.75
5BL-9	18 + 1 [del5BL-9] +1'[del7AL-11] + 1 [7A] + 1 [del2BS-9L]	0.76
5BL-16	19 + 1 [del5BL-16] + 1[del2DL-12] +1 [2D]	0.79
5BL-13	17 + 1 [d5BL-13]	0.82

Linkage analysis and genetic mapping: Chi-square (χ2) tests for goodness of fit were used to evaluate deviations of observed data with expected segregation ratios. Linkages between molecular markers and the resistance gene were determined using Mapmarker 3.0, with a LOD score threshold of 3.0 (Lincoln *et al.*, 1993).

Results

Inheritance of the powdery mildew resistance in N0308: One-hundred and thirty seven F_2 plants, derived from the cross of N0308×shaanyou 225 along with 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 resistance (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 (χ^2 = 0.55, P>0.05). The $F_{2:3}$ families segregated as 27 homozygous resistant 72 segregating and 38 homozygous susceptible, as expected of a 1:2:1 ratio (χ^2= 2.12, 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.

Molecular marker analysis: Initially, 300 SSR markers mapped to the A and B genomes of wheat were screened for their polymorphism between the parental lines as well as the resistant and susceptible DNA bulks. Three SSR markers, *Xgwm408, Xgpw7425* and *Xwmc75* were polymorphic between the parents, as well as the bulks. These markers proved to be linked to the resistance locus by testing on F_2 individuals. *Xgwm408, Xgpw7425* and *Xwmc75* all were located on the long arm of chromosome 5B. Further SSR markers located on 5BL were screened. Eight SSR markers (*Xfcp1, Xfcp393, Xfcp394, Xgpw3191, Xgpw1082, Xwmc810, Xbarc232* and *Xbarc142*) were polymorphic between the resistant and susceptible bulks, and were closely linked to the resistance gene. Among the eleven polymorphic SSR markers, four (*Xfcp1, Xgpw7425, Xbarc232* and *Xbarc142*) were found co-dominant, and seven (*Xfcp393, Xfcp394, Xgpw3191, Xgpw1082, Xwmc810, Xgwm408* and *Xwmc75*) were dominant (Table 2, Fig. 1). A linkage map including the resistance locus and its closely linked markers was constructed (Fig. 4). To narrow the distance between the adjacent marker and *PmG25,* 20 EST markers in the interval of chromosome 5BL-14 were screened. The result indicated that two EST-STS markers (*BF482522* and *BF202652*) were linked to the resistance gene with genetic distance 3.8 cM and markers were then added to the linkage map (Fig. 2).

Linkage analysis between each microsatellite marker and *PmG25* using MAPMAKER 3.0b indicated the locus order and genetic distance between *PmG25* and the eleven SSR markers. A molecular genetic map involving the *PmG25* region was constructed with a total map length of 39.6 cM (Fig. 4). In the present study, the powdery mildew resistance gene *PmG25* was flanked by the SSR loci *Xfcp1/Xfcp393* and *Xgpw3191* with the genetic distances of 1.3 cM proximal and 3.3 cM distal, respectively. *Xfcp394, Xgpw7425, Xwmc75, Xgpw1082* and *Xgwm408* were relatively closed to *PmG25*, with genetic distances of 2.7, 7.4, 9.4, 10.3, 11.2 cM, respectively. Other *PmG25*-linked SSR loci were *Xwmc810, Xbarc232* and *Xbrac142* through with larger genetic distances of 22.3, 25.4 and 29.3 cM (Fig. 4).

Chromosomal arm and physical bin assignments of markers: Chinese Spring homoeologous group 5 nullisomic-tetrasomics, ditelosomics and deletion lines were used to bin map the *PmG25* linked molecular markers. *Xfcp1, Xfcp393, Xfcp394, Xgpw3191, BF482522* and *BF202652* were mapped on 5BL bin 0.75–0.76; *Xgpw1082* on 5BL bin 0.55–0.75; *Xgpw7425, Xwmc75* and *Xgwm408* on 5BL bin 0.76-0.79 and *Xwmc810, Xbarc232,* and *Xbarc142* on 5BL bin 0.79–0.82 (Fig. 3). Deletion bin mapping thus indicated that *PmG25* is most likely located in 5BL bin 0.75–0.76 (Fig. 4).

Discussion

The cultivar N0308 has showed a high level of resistance to powdery mildew in Shaanxi province, and segregation ratios confirmed the hypothesis that the powdery mildew resistance gene *PmG25* controlled by a single dominant gene. Molecular mapping indicated that *PmG25* was physically located on chromosome 5BL bin 0.75–0.76, close to several markers including *Xfcp1, Xfcp393, Xfcp394, Xgpw3191, BF482522* and *BF202652* (Fig. 4).

Three named genes and one temporarily-designated powdery mildew resistance gene have been characterized on chromosome 5B. *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; Chen *et al*., 2005), *Pm30* was derived from the wild emmer accession C20 originated from the Rosh Pinna population in Israel and mapped on 5BS bin 0.56–0.71 (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 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 bin 0.29-0.76 which was closely linked to EST-SSR marker *BJ261635* (Blanco *et al*., 2008). To characterize the relationship between *PmG25* and *Pm36*, EST marker *BJ261635* was tested in our segregating population but failed to show polymorphisms between the resistant and susceptible plants. However, *Pm36* was located 10.0 cM proximal to *Xwmc75* where we mapped the resistance gene in N0308 to the same region, 9.4 cM proximal to *Xwmc75*. Thus, we were unable to determine the exact relationship between *PmG25* and *Pm36*. *Ml3D232* transferred from the wild emmer accession 1222 into common hexaploid wheat line 3D232 and mapped on 5BL bin 0.59-0.76 (Zhang *et al*., 2010). No polymorphisms between the resistant and susceptible lines were detected for six EST-derived STS markers; *BE494426, BE442763, BE445282, BE407068, CA635388* and *CJ832481* that linked to *MI3D232*. Therefore, these markers could not be mapped in our study. The resistance gene *Ml3D232*, *Pm36* and our study *PmG25* 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 *Pm36*, *Ml3D232* and *PmG25*.

The present work adds to the rich genetic resource with potential value of wild emmer for the improvement of cultivated wheat. Potentially useful traits found in wild emmer can be transferred to cultivated wheat by direct hybridization, backcrossing and selection. Markers closely linked to *PmG25* can be used for transferring powdery mildew resistance gene to cultivated wheat.

Table 2. Segregation ratios for SSR markers linked to the powdery mildew resistance gene *PmG25* evaluated in an F_2 population of N0308/ Shaanyou 225.

SSR marker	No. of F_2 plants	Homozygous resistant	Heterozygous resistant	Susceptible	$\chi2_{(3:1)}$
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
Xfcp394	137	100		37	0.3
Xfcp1	137	30	71	36	0.12
Xgpw1082	137	101		36	0.12
Xgpw3191	137	103		34	0.002
Xfcp393	137	101		36	0.12
BF202652	137	102		35	0.022
BF482522	137	102		35	0.022

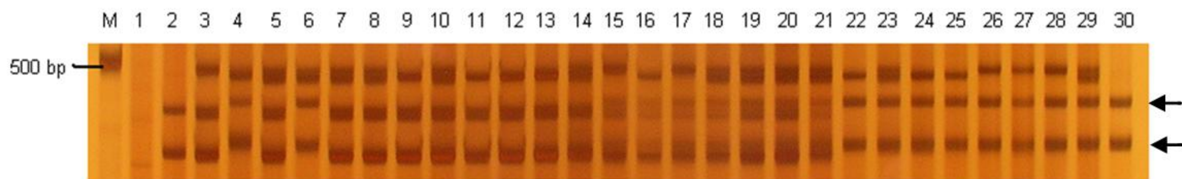

Fig. 1 PCR product of *Xfcp1* in the resistant and susceptible F_2 plants
1: Shaan 253; 2: *T. dicoccoides* accession G25; 3: N0308; 4: Shaanyou 225; 5: Resistance pool; 6: Susceptible pool; 7_14: Homozygous resistant; 15_21: Heterozygous resistant; 22-30: Susceptible F_2 plants; M: 1 kb DNA ladder

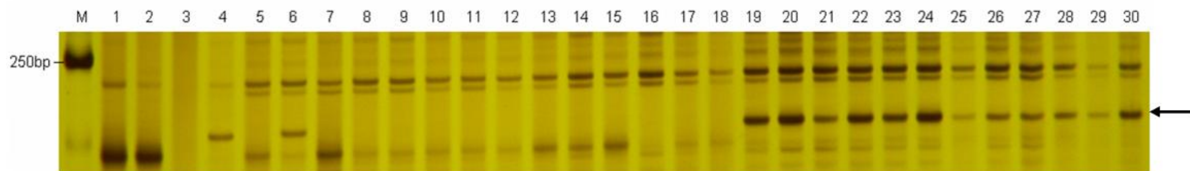

Fig. 2 Amplification pattern of EST-STS marker *BF202652* in parents and F_2 plants
1: Shaan 253; 2: *T. dicoccoides* accession G25; 3: N0308; 4: Shaanyou 225; 5: Resistance pool; 6: Susceptible pool; 7_18: Resistant individual; 19_30: Susceptible individual of F_2 plants; M: 1 kb DNA ladder

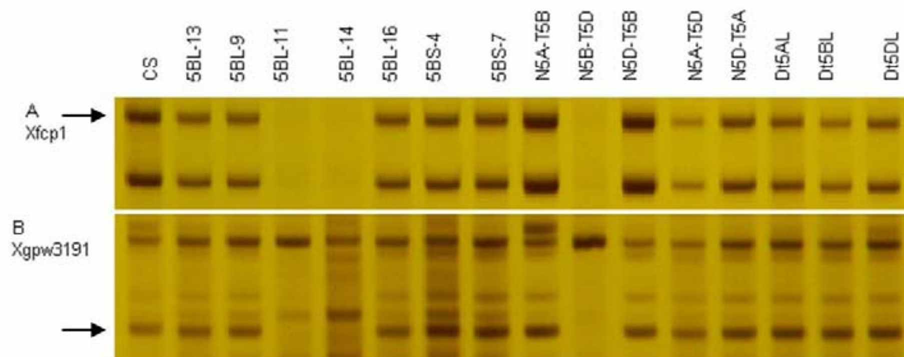

Fig. 3. Amplification pattern of *Xfcp1* (A) and *Xgwm3191* (B) in Chinese Spring homoeologous group 5 nulli-tetrasomics, ditelosomics, 5BL deletion lines

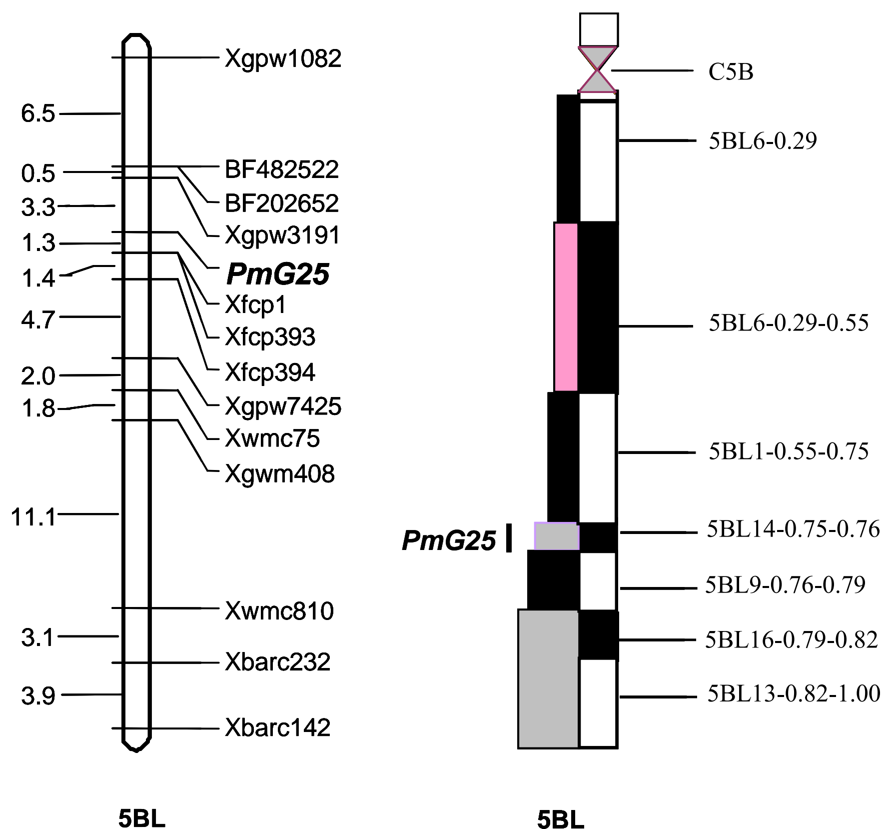

Fig. 4. Linkage and physical bin map of powdery mildew resistance gene *PmG25*.

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