Dissection of Powdery Mildew Resistance Uncovers Different Resistance Types in the *Triticum Turgidum* L. Gene pool

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ABSTRACT

Powdery mildew caused by the biotrophic pathogen, *Blumeria graminis (DC.) E.O. Speer f. sp. tritici Em. Marchal* (*Bgt* hereafter), is a foliar wheat disease resulting in severe yield losses worldwide. The continuous threat for breakdown of race-specific resistance to powdery mildew is forcing a consecutive effort to enrich the resistance reservoir. In the current study, a large collection of wheat germplasm, consisting of wild and domesticated wheat genotypes, were screened for powdery mildew resistance. The screening was done with two Israeli local *Bgt* isolates collected from contrasting environments and hosts. Two *Triticum Turgidum* L. ssp. lines: #G18-16 (*T. dicoccoides*) and Langdon (*T. durum*), differing in their response to inoculation with those two isolates were used in constructing a mapping population consisting of 152 F_6 recombinant inbred lines (RILs). These RILs were tested for powdery mildew resistance with the two *Bgt* isolates: (**i**) *Bgt*#15, collected from *T. durum* and avirulent on #G18-16; (**ii**) *Bgt*#66, collected from *T. dicoccoides* and generating partial resistance response in Langdon. Segregation ratio of the RIL population in reaction to Bgt#15 showed that the resistance in #G18-16 is controlled by a single dominant gene that was mapped to the distal end of the chromosome arm 7AL. Genetic map of this genomic region was constructed using 25 microsatelites, DArT (Diversity Array Technology) and CAPS (Cleaved Amplified Polymorphic Sequences) markers. QTL (Quantitative Trait Locus) analysis of the reaction to *Bgt*#66, conducted using 690 DNA markers revealed one major QTL on chromosome 1A (PEV 22.7%) and additional four minor QTLs on chromosomes 1B, 2B, 3A and 7A. Host-pathogen evolutionary aspects and future implementation of powdery mildew resistance in wheat breeding programs are discussed.

INTRODUCTION

Powdery mildew caused by the parasitic fungus *B. graminis* is one of the most devastating diseases of wheat with yield losses of up to 34%. So far, 48 genes/alleles for powdery mildew resistance at 37 loci have been identified and located on 16 different wheat chromosome arms. Only three powdery mildew resistance genes were identified in *T. dicoccoides* (Hasm & Zeller, 2002). The earlier finding of wild emmer wheat inoculated with powdery mildew was recorded by Reichert (1940), decades after it was discovered by A. Aaronsohn in 1906. Israel and its vicinity is a center of diversity of wild emmer wheat (Harlan & Zohary, 1966), where it occupies wide eco-geographical range. Hence, it is known to harbour a wide allelic repertoire relevant for the improvement of various economically important traits in cultivated wheats including resistance to powdery mildew (e.g. Gerechter-Amitai et al., 1984). The availability of both host and pathogen *ex-situ* genetic collections enable us to examine issues of plant: pathogen co-evolution through phenotype and genotype assays. The goals of the current study were as following: (1) Characterise the virulence and aggressiveness of *Bgt*#15 and *Bgt*#66; (2) Analyse the phenotyipic reaction of domesticated and wild wheat germplasm to inoculation with those two *Bgt* isolates; (3) Genetically map the genes which are involved in resistance to *Bgt*#15 and *Bgt*#66 using a RIL population.

MATERIALS AND METHODS

Bgt isolate #15 was collected from a durum wheat cultivar at Yavor and *Bgt* isolate #66 was collected from a wild emmer wheat accession at Ammiad, Israel. A collection of 48 domesticated (including *T. aestivum*, *T. durum* and *T. dicoccum*) and 54 wild emmer accessions were inoculated with these two isolates. Disease severity (mildew pustules/cm^2) was determined at seedling stage on detached leaf segments..

Population of 152 F_6 recombinant-inbred lines (RILs) was developed via single-seed decent, from a cross between durum wheat *cv*. Langdon (LDN hereafter) and wild emmer accession #G18-16. A genetic linkage map of 2,317 cM was previously developed based on 690 microsatellite and DArT markers (Peleg et al. 2008). The RILs and two parental lines were inoculated with *Bgt* isolates #15 and #66 at the seedling stage. The reaction to *Bgt*#15 was evaluated qualitatively (infection type scale of 0-4, with 0-2 considered as resistant and 3-4 considered as susceptible). The reaction to *Bgt*#66 was characterized quantitatively by counting mildew pustules density. For the *Bgt*#15 reaction, Chi-square test was used to examine segregation deviation from Mendelian inheritance for powdery mildew resistance and a genetic linkage map was established using the MultiPoint package. For the *Bgt*#66 reaction, quantitative trait loci (QTL) analysis was performed with the MultiQTL package using the general interval mapping for a RIL population (http://www.MultiQTL.com) for all 14 chromosomes of tetraploid wheat.

RESULTS AND DISCUSSION

Analysis of variance of the phenotypic test, revealed significant interaction between the pathogen (*Bgt* isolate) and the host (domesticated/wild). Each isolate showed a different reaction pattern in the tested collection. The high variance of disease severity in wild accessions inoculated with *Bgt*#15 generated heterogeneity of variance between levels of tested factors and therefore only descriptive statistics is presented for the four groups (Table 1). *Bgt*#15 is a highly virulent isolate attacking 70.5% of the genotypes in the collection. Nevertheless, the proportion of resistant genotypes differed significantly between domesticated (12.5%) and wild (44.4%) genetic materials ($\chi^2 = 16.1$; $\chi^2 0.05, 2 = 7.8$). Isolate *Bgt*#66 generated reciprocal reaction, showing reduced aggressiveness in domesticated germplasm and causing high disease severity in the wild material (Table 1).

Table 1. Mean number of pustules, standard error (S.E.), confident interval and analysis of variance of 102 wheat genotypes inoculated with *Bgt* isolate #15 and #66.

Bgt isolate[a]	Genetic source	Disease severity (number of pustules/cm^2)		
		Mean	**S.E.**	**Confidence interval (95%)**
#15	Domesticated	7.39	0.33	6.74-8.03
	Wild	5.87	0.96	3.94-7.81
#66	Domesticated	2.47	0.28	1.92-3.02
	Wild	6.25	0.48	5.28-7.22

Analysis of variance[b]

Source	**d.f.**	**Mean square**	**F ratio**	**Probability**
Bgt isolate (*Bgt*)	1	11.64	17.67	<.001
Genetic source (G)	1	2.53	3.84	0.050
Bgt × G	1	35.58	54.03	<.001
Model[c]	3	27.78	42.19	<.001
Experimental error	354	0.66	-	-
C. Total	357	-	-	-

[a] *Bgt* isolate #15 was collected from durum wheat cultivar in Yavor, North-Western Israel; *Bgt* isolate #66 was collected from wild emmer wheat accession in Ammiad, North-Eastern Israel.

[b] The density of pustules was transformed to √(X+1) before analysis of variance.

[c] Tested model: $\mathbf{Y_{ijk}=\mu+\alpha_i+\beta_j+\alpha_i X\beta_j+e_{ijk}}$

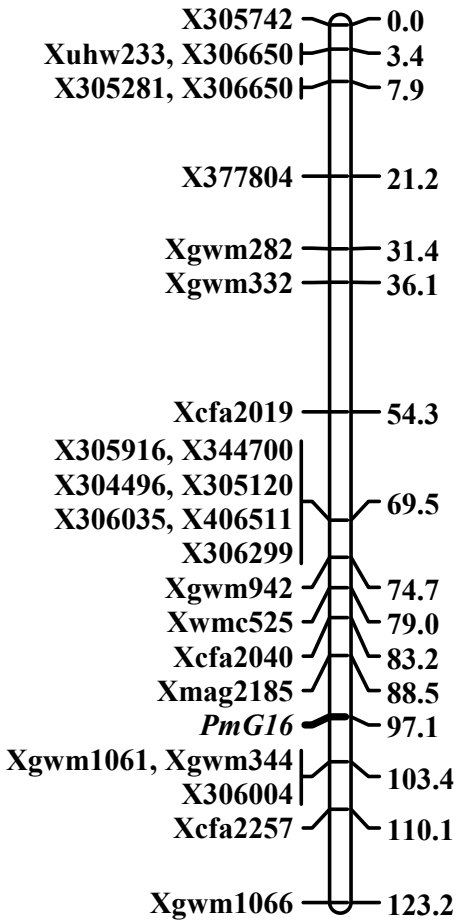

Figure 1. Genetic map of the *Pm*G16 gene region on chromosome 7AL of wheat. Markers are shown on the right with map distances on left. The markers *Xgwm332*, Xcfa2019 and *Xcfa2040* assign this region to 7AL-16 0.86 deletion bin.

This reciprocal pattern which is expressed through the interaction of wild versus cultivated host species tested with two *Bgt* isolates from different origins, is probably the result of long term plant:pathogen co-evolution. Isolate *Bgt*#15 which was originally sampled from a cultivated durum wheat field has virulence preference to domesticated wheat, while isolate *Bgt*#66 which was collected from a wild emmer wheat plant at its natural habitat is showing higher aggressiveness on wild host.

The segregation ratio for the powdery mildew reaction of the RIL population after inoculation with *Bgt*#15 established the roll of a single gene with the resistant allele donated by the #G18-16. A linkage map of wheat chromosome 7A consisting of 25 microsatellite, DArT and CAPS markers and the *Pm*G16 gene was constructed (total chromosome length 123.2 cM; Fig. 1). *Pm*G16 was genetically mapped to the long arm of chromosome 7A, 6.3 cM proximal to marker *Xgwm1061* and 8.6 cM distal to marker *Xmag2185*. By means of

genetic markers the gene was physically mapped on wheat chromosome deletion bin 7AL-16 0.86-1.00. This dense genetic mapping of the *Pm*G16 region promote implementation of marker assisted selection (MAS) in order to implant this gene in advanced breeding material. This genomic location harbors a cluster of powdery mildew R genes with the most known *Pm*1 locus. Further mapping and phenotypic test are needed to determine whether *Pm*G16 is allelic to *Pm*1 or it might be a novel R gene which was not reported before.

A total of five significant QTLs were associated with phenotypic reactions to inoculation with *Bgt*#66, with LOD score range from 4.5-15.4, explaining totally 53.6% of the variance. A major QTL was detected on 1A with LOD of 15.4 explaining 22.7% of the variance of disease severity. Additional four minor QTLs were found on chromosomes 1B, 2B, 3A and 7A. In all QTLs the higher resistance to the *Bgt*#66 was conferred by the domesticated allele (LDN). This finding is exceptional in the sense that so far only a single *Pm* gene is known to be originated from *T. durum* background (Hasm & Zeller, 2002).

To summarize, the genetic analysis of the phenotypic reactions of the RIL population at the seedling stage to the two *Bgt* isolates describes two different resistance mechanisms. The first is monogenic: a wild emmer wheat allele in a single locus confering complete resistance to *Bgt*#15. The second is polygenic: a set of *T. durum* alleles, in five independent QTLs, controls partial resistance to *Bgt*#66 in the RIL population.

A. Aaronsohn the discoverer of wild emmer wheat suggested that utilizing wild emmer wheat genetic resources will help to improve wheat crops (Aaronsohn, 1910). Identifying and mapping of a new powdery mildew resistance gene from *T. dicoccoides* is a small step adding to the implementation of Aaronsohn's vision. In addition, mapping of new resistance genes from *T. durum*, a genetic source which is underrepresented in the current published *Pm* reservoir could also contribute to wheat crop resistance against the pathogen *B. graminis*.

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