Genetic and genomic dissection of powdery mildew resistance genes derived from wild emmer (*Triticum dicoccoides*)

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INTRODUCTION

Wild relatives are important germplasm for improving yield, quality and resistance to biotic and abiotic stresses in modern crop cultivars. Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is one of the most important fungal diseases of wheat (*Triticum aestivum* L.) worldwide. The wild emmer wheat, *T. dicoccoides* (2n = 4x = 28; genome AABB), is considered the progenitor of cultivated tetraploid and hexaploid wheats. Wild emmer wheat was found to be highly tolerant to stress and resistant to pathogens, including powdery mildew, stripe rust, leaf rust, and stem rust, as well as having high yield potential for wheat improvement (Nevo *et al* 2002). To date 38 powdery mildew resistance genes have been identified (McIntosh *et al* 2007). Of these five genes, *Pm16* (Reader & Miller 1991), *Pm26* (Rong *et al* 2000), *Pm30* (Liu *et al* 2002), *Pm36* (Blanco *et al* 2008) and *MlZec1* (Mohler *et al* 2005), originated from wild emmer and have been successfully transferred into common wheat or durum wheat backgrounds.

Molecular markers are important tools in tagging powdery mildew resistance genes from wild relatives when introducing them into cultivated genetic backgrounds. RFLP markers linked to *Pm26* on 2BS, SSR markers for *Pm16, Pm30, Pm36* and *MlZec1* have been identified and facilitate the molecular marker assisted introgression of these genes into elite breeding lines.

Wild emmers are found to be highly resistant to most of the prevalent isolates of *Blumeria graminis* f. sp. *tritici* in China. We report here on the introgression of powdery mildew resistance into elite common wheat lines and the molecular analysis of the resistance genes using genomic tools.

MATERIALS AND METHODS

Wild emmer accessions were kindly provided by Drs. E. Nevo and T. Fahima, Institute of Evolution, University of Haifa, Israel and Dr. Z. K. Gerechter-Amitai, Agricultural Research Organization, The Volcani Center, Israel. Powdery mildew susceptible elite Chinese common wheat cultivar 87-1 was used as the parent and the recurrent parent to make crosses with wild emmer accessions after 3 to 4 backcrosses. Powdery mildew resistant derivatives with elite agronomic traits were selected and used in the breeding program. Segregating BC_4F_2 populations and their BC_4F_3 progenies were inoculated with *Blumeria graminis* f. sp. *tritici* isolate E09 for genetic analysis and molecular mapping of the powdery mildew resistance genes. Reactions were scored on a 0, 0;, and 1 to 4 infection type (IT) scale, with 0 representing no visible symptoms, 0; representing necrotic flecks, and 1, 2, 3, 4 for highly resistant, resistant, susceptible, and highly susceptible reactions, respectively. Resistant reactions of 20 seedlings of each BC_4F_3 progenies were tested to classify the BC_4F_2 individuals of mapping populations into three types, namely homozygous resistant (RR), heterozygous resistant (Rr) and homozygous susceptible (rr). SSR, EST-SSR and EST markers were used to tag the powdery mildew resistance genes in each mapping population. Chinese Spring (CS) null-tetrasomics, ditelosomics and deletion lines (kindly provided by Drs. WJ Raupp and BS Gill, Wheat Genetics Resource Centre, Kansas State University, USA) were used for chromosomal arm and bin assignments of the powdery mildew resistance gene and their linked polymorphic markers. Linkage between the resistance genes and their linked markers was determined using MAPMAKER with an LOD threshold of 3.0. The Kosambi mapping function was used to calculate map distances.

RESULTS AND DISCUSSIONS

Table 1 Genetic analysis of the powdery mildew resistance genes derived from wild emmer in different $BC_4F_{2:3}$ populations

Pedigree	RR	Rr	rr	Total	$X^2$1:2:1
87-1*4/C20	21	52	26	99	0.76
87-1*4/G-303-1M	28	78	18	124	9.87
87-1*4/G-573-1	46	50	17	113	16.40
87-1*4/G-168-1	40	88	41	169	0.30
87-1*4/I217	28	55	20	103	1.72
87-1*4/I222	29	64	19	112	4.07
87-1*4/G-584-M	20	84	24	128	12.75
87-1*4/G-556-M	32	81	23	136	6.01
87-1*4/G-360-M	24	40	30	94	2.85
87-1*4/G-551-M	29	71	20	120	5.38
87-1*4/G-727-M	20	78	28	126	8.15

Genetic analysis of the powdery mildew segregating BC_4F_2 populations and their BC_4F_3 progenies indicated that different resistance genes have been introduced into common wheat genetic backgrounds. Single dominant

genes are responsible for powdery mildew resistance in all backcrossing derivatives tested except 87-1*4/G-303-1M where a single recessive resistance gene was detected (Table 1). Segregation distortions were observed in several populations (87-1*4/G-303-1M, 87-1*4/G-573-1, 87-1*4/G-584-M etc.) indicating probable low male transmission or presence of segregation distortion loci in the wild emmer chromosomes.

SSR, EST-SSR and EST-STS markers were applied to map the powdery mildew resistance genes in different segregation populations using the bulked segregated analysis (BSA) approach. SSR markers *Xgwm159* and *Xcfd81* were found to be linked to *Pm30*, originating from C20, and the resistance gene was mapped on 5BS. *Pm30*, or its alleles, were also found in other wild emmer derivatives originated from G-275-M, G-722-M, and G-757-M, indicating its prevalence in the wild emmer population. One recessive powdery mildew resistance gene *mlG-303-1M* was identified in population 87-1*4/G-303-1M and mapped on chromosome 2BS by SSR markers. *mlG-303-1M* is 15.2 cM away from RFLP marker *Xwg516* that is co-segregated with another recessive powdery mildew resistance gene *Pm26* derived from wild emmer (Rong *et al* 2000). Another dominant resistance gene *mlG-573-1* was characterized in the population 87-1*4/G-573-1, and subsequently mapped on chromosome 2BL by SSR and EST-SSR markers. It is still not clear if this locus is allelic to *mlZec1*, another powdery mildew resistance gene originated from wild emmer and mapped on 2BL (Mohler *et al* 2005). Dominant mildew resistance loci were also found in populations derived from G-168-1 and I217, and mapped on the distal bin of chromosome 2AL by SSR and EST-STS markers. Linkage maps indicated that *mlG-168-1* and *mlI217* are located in the same chromosome region as that of *Pm4a* and *Pm4b* alleles, indicating they might be new alleles of the *Pm4* loci or members of a closely linked resistance gene cluster on 2AL (McIntosh et al 2007). Powdery mildew resistance loci were also found in populations 87-1*4/I222, 87-1*4/G-584-M, and 87-1*4/G-556-M. SSR, EST-SSR and EST-STS mapping results revealed that *mlI222, mlG-584-M* and *mlG-556-M* were located on bin 5BL 0.59-0.75, the distal side of the *Ph1* gene (Griffiths *et al* 2006). The allelic association of *mlI222, mlG-584-M and mlG-556-M*, as well as *Pm36*, a recently reported powdery mildew resistance gene derived from wild emmer and mapped on 5BL in tetraploid wheat (Blanco *et al* 2008), is still not clear and requires further characterization.

AFLP,EST-SSR,EST-SNP, and RGA markers are being employed to develop more tightly linked DNA markers to these powdery mildew resistance genes. Rice and Brachypodium genome sequences are being used for comparative genomic analysis to identify the corresponding orthologous genomic regions in rice and Brachypodium. Sequence comparison results indicated that orthologous genomic regions harbouring the powdery mildew resistance genes could be found in rice and Brachypodium genomes. High colinearity was observed between RGA-like genes flanking genomic regions in Brachypodium and the rice orthologous region, except that the RGA-like genes were not present in rice. Wheat ESTs homologous to the Brachypodium RGA-like flanking genes are being used to develop SNP markers in constructing fine genetic maps for the target powdery mildew resistance genes. Candidate NBS-LRR genes are being isolated from wild emmer using map based cloning approach. Fine genetic and genomic dissection of these powdery mildew resistance genes will shed light on the molecular evolution of powdery mildew resistance genes in wild emmer.

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