Pyramiding of powdery mildew and yellow rust resistance genes introgressed from wild emmer wheat into cultivated wheat

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INTRODUCTION

The rapid evolution of the wheat powdery mildew fungal pathogen *Blumeria graminis* f.sp. *tritici* (*Bgt* hereafter), is forcing scientists to continuously search and enrich wheat reservoirs with novel disease resistance genes (R-genes). Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*; WEW hereafter, Fig. 1), the tetraploid progenitor of cultivated wheat (Nevo et al. 2002), is a valuable source for *Bgt* resistance genes. Previously, we identified two novel dominant powdery mildew resistance genes *PmG16* and *PmG3M*, derived from wild emmer wheat accessions G18-16 and G305-3M, that were genetically mapped on chromosome arm 7AL and 6BL (Xie et al. 2012; Ben-David et al. 2010), and officially named *TdPm60* (Li et al., 2021) and *Pm69*, respectively (Fig. 2). The objective of the current study was to clone the two powdery mildew resistance genes *TdPm60* and *Pm69* from wild emmer wheat and deploy them into wheat breeding programs to improve disease resistance in the bread wheat.

Figure 1. The morphology of wild emmer wheat in its natural habitat, upper Galilee, Israel, with immature (A) and mature (B) disarticulating spikes.

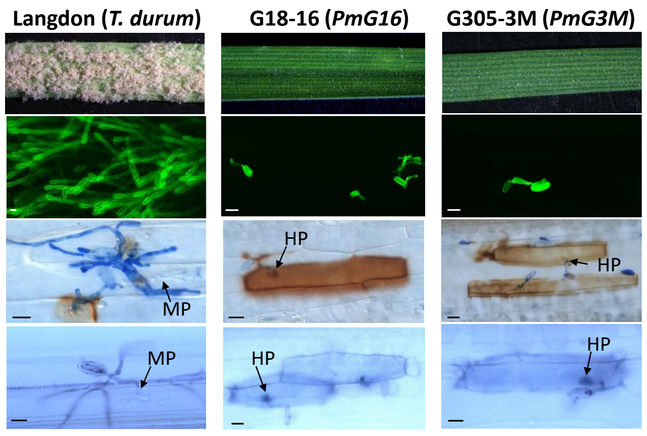

Figure 2. WEW accessions G18-16 and G305-3M were high resistance to powdery mildew with ROS (reddish-brown coloration) and cell death (blue coloration) response. MP, mature haustorium; HP, haustorial primordia. Bars = 20 µm.

RESULTS

***1) TdPm60* confers high resistance to powdery mildew.** We mapped *PmG16* to a 1.4-cM interval on Chromosome 7AL, which resides in the same syntenic region of *TuPm60*, previously cloned from *Triticum urartu* (Zou et al., 2018). The functional molecular marker (FMM) for *TuPm60* co-segregated with *PmG16* and was also associated with resistance to *Bgt* #15 in WEW natural population (Fig. 3A). We used the homologous cloning strategy for cloning the full length (4365 bp) of the corresponding *Pm60* locus (designated as *TdPm60*) from G18-16. Sequence alignment identified only eight SNPs that differentiated between *TdPm60* and *TuPm60* (Fig. 3B). The function of *TdPm60* was validated by the virus-induced gene silencing (VIGS) approach in WEW accessions G18-16 and TD116494 (Fig. 3C).

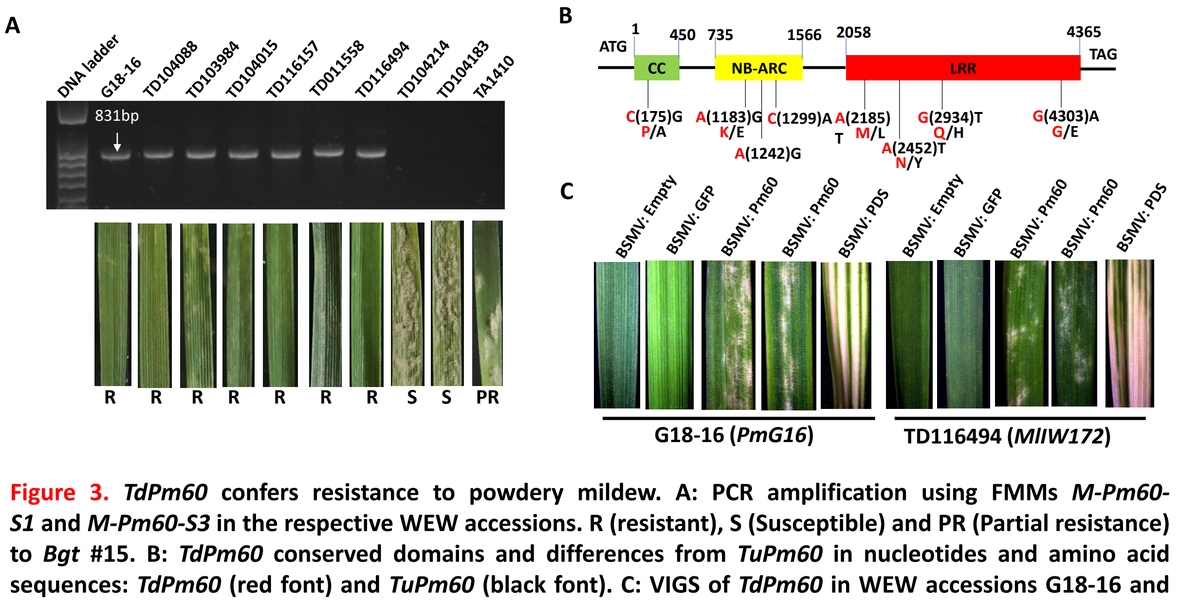

Figure 3. *TdPm60* confers resistance to powdery mildew. A: PCR amplification using FMMs *M-Pm60-S1* and *M-Pm60-S3* in the respective WEW accessions. R (resistant), S (Susceptible) and PR (Partial resistance) to *Bgt* #15. B: *TdPm60* conserved domains and differences from *TuPm60* in nucleotides and amino acid sequences: *TdPm60* (red font) and *TuPm60* (black font). C: VIGS of *TdPm60* in WEW accessions G18-16 and TD116494, which contain the *TdPm60* functional allele.

***2) TdPm60* is a major powdery mildew resistance gene in the WEW natural populations.** *TdPm60* also constitutes a strong candidate for *MlIW72*, *MlIW172* and *MIWE18* based on their genetic location (Fig. 4A) and FMMs analysis. *TdPm60* alleles were identified in approximately 25.2% (58 out of 230) wild emmer wheat accessions (Fig. 4B). Only one accession contained the *TdPm60a* allele, with a 240-bp deletion in the LRR domain compared with *TdPm60*. All of these 59 WEW accessions containing *TdPm60* alleles were highly resistant to *Bgt* #15.

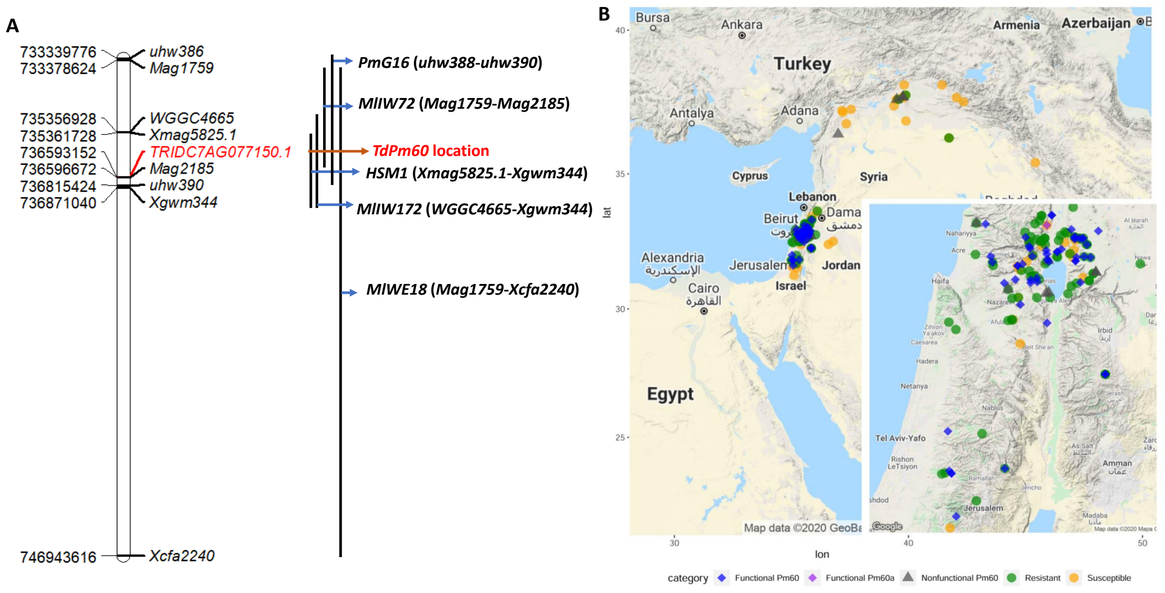

Figure 4. *TdPm60* was a major allele in WEW natural populations. A: Anchoring of the flanking markers of *MlIW72*, *MlIW172*, *PmG16* and *SHM1* to the reference genome WEW Zavitan_v.2.0 (Zhu et al. 2019). B: The geographic distribution of the *TdPm60* (blue) and *TdPm60a* (purple) based on FMM analysis.

3) Cloning of *Pm69* in the whole-genome sequence of G305-3M by using long-read sequencing technology. For cloning of *Pm69*, map-based cloning approach encountered by genome structural variations that suppressed recombination (Fig. 5A-B), and the isolation of targeted 6B chromosome of G305-3M failed for sequencing. Finally, *Pm69* was cloned in the whole-genome sequence of G305-3M by Oxford Nanopore sequencing Technology (ONT), combined with transcriptome sequencing of susceptible mutants (MutRNAseq) (Fig. 5C).

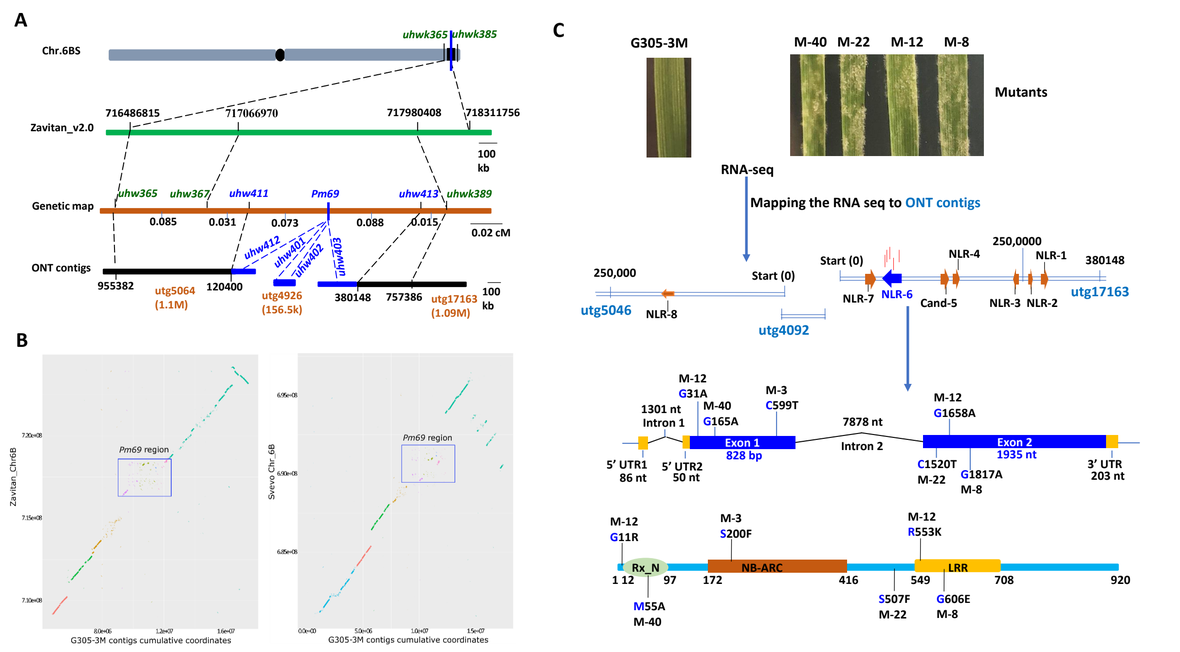

Figure 5. Cloning of *Pm69* from WEW G305-3M. A: fine mapping of *Pm69*. B: Comparison of the G305-3M contigs with the 6B pseudomolecule of WEW_v2.0 and durum wheat Svevo RefSeq Rel. 1.0 around the *Pm69* genetic region. C: The workflow of identification of *Pm69* from the ONT contigs by MutRNAseq.

***4) Pm69* is a very rare allele, located within a rapidly evolving NLR cluster.** We screened 310 WEW accessions, as well as 228 accessions of other wheat relatives, with *Pm69* marker *uhw403*. Only G305-3M yielded positive PCR amplification. We went back to the original G305-3M collection site south of Kadita, Northern Israel, and collected additional 64 WEW accessions in a radius of less than 1km from the original collection site (Fig. 6). Only three accessions yielded the same PCR products as G305-3M by marker *uhw403*, suggesting that the *Pm69* is a very rare allele. The WEW G305-3M and Zavitan genome assemblies contained more than 40 NLRs around the *Pm69* locus, indicating the presence of an NLR cluster (Fig. 7). The *Pm69* NLR cluster showed high polymorphism among different wheat genome assemblies, while the *OPR11* gene inside the cluster and the further flanking regions of the cluster were more conserved, suggesting that oppositive evolutionary selection pressures operated within this genetic interval (Fig. 7). Therefore, it seems that *Pm69* is located within a rapidly evolving NLR cluster.

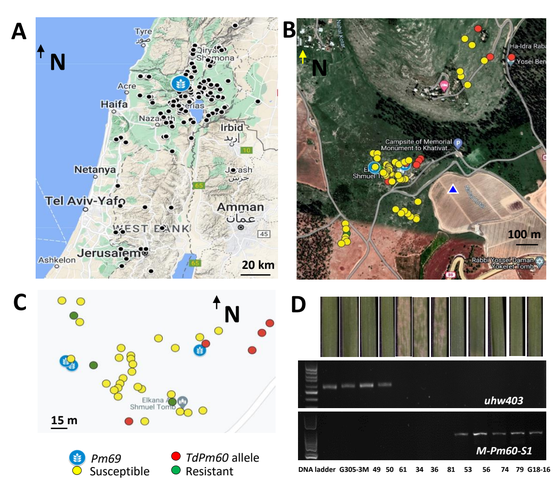

Figure 6. The geographic distribution of the *Pm69* allele in WEW collections.

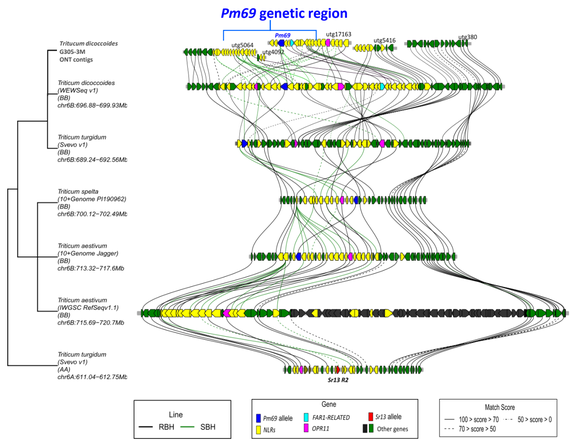

Figure 7. Micro-Collinearity analysis of the *Pm69* genetic region among different *Triticeae* genomes.

5) Introgression of the *TdPm60* and *Pm69* into the cultivated wheat and pyramiding with several yellow rust resistance genes. As part of the aim to develop resistant pre-breeding genetic resources, we transferred *TdPm60* and *Pm69* into serval elite Israeli bread wheat varieties ('Ruta', 'BarNir' or 'Zahir') following the "durum as a bridge" approach (Klymiuk et al. 2019), based on marker-assisted selection. We also pyramided *Pm69* with three yellow rust resistance genes (*Yr5*, *Yr15* and *Yr24*). These selected lines showed high resistance to *Bgt* and *Pst*, demonstrating the high potential of these genes for future wheat resistance breeding (Fig. 8).

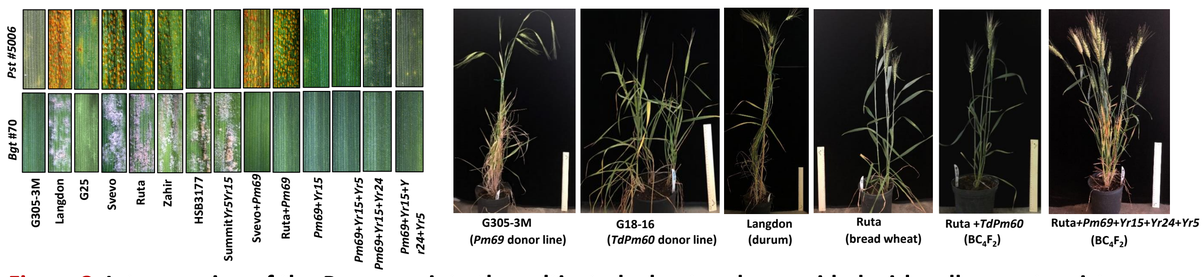

Figure 8. Introgression of the *Pm* genes into the cultivated wheat and pyramided with yellow rust resistance genes.

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