Unravelling the molecular basis of wheat powdery mildew virulence patterns through ultraviolet mutagenesis

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Background

Wheat is a major agricultural crop and its production is threatened by a number of diseases, including the biotrophic fungus wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*, *Bgt*).

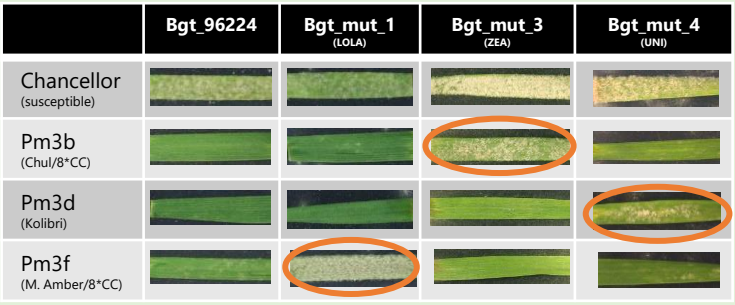
Resistance (*R*) genes play a major role in plant immunity, being able to recognize race-specific avirulence (Avr) effectors that fungal pathogens secrete during infection (3).

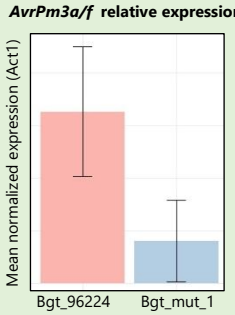
The Pm3 allelic series encode for R proteins that can recognize the corresponding AvrPm3 effectors produced by *Bgt,* thus activating the plant immune response (2). There is evidence of additional components besides *Avrs* can play a role in *Bgt* virulence (2, 4).

We propose ultraviolet (UV) mutagenesis as a method of bypassing the lack of transformability in *Bgt* to identify novel genes involved in virulence (1).

Results

- 24 *Bgt* mutants have been produced (12 were sequenced).
- The gain of virulence of most *Bgt* mutants is race-specific (one mutant is only virulent to one Pm3 allele).
- One Pm3b-virulent *Bgt* mutant carries a single base pair deletion in *AvrPm3b/c* → proof that UV mutagenesis is a suitable method for discovering *Avr* genes.
- Most mutants had no mutations in known *Avr* sequences but lowered their expression → presence of additional regulating components that influence race-specific *Bgt* virulence → need for further investigation.





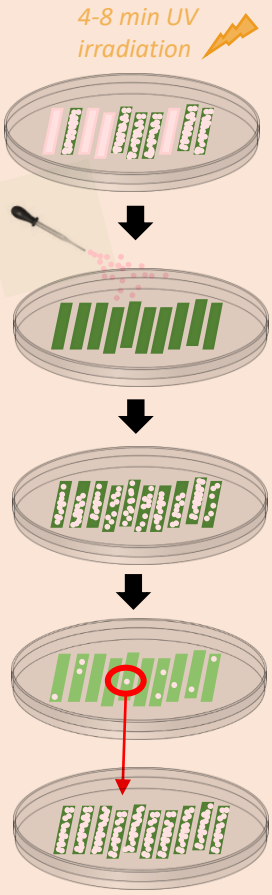
Gain of virulence on ***Pm3f*** — *No mutations in AvrPm3f*

Gain of virulence on ***Pm3b*** — *No mutations in AvrPm3b*

Gain of virulence on ***Pm3d*** — *No mutations in AvrPm3d*

Downregulation of the *Avr* expression: presence of a race-specific regulation component in the *Pm3-AvrPm3* interaction system

Method



1) Mutagenesis: Isolate Bgt_96224 growing on a susceptible cultivar is irradiated multiple times
2) Propagation: «Mutant mixtures» are propagated on a susceptible wheat line
3) Selection: «Mutant mixtures» are used for infection of Pm3 lines (resistant)
4) Single colony isolation: mutants with gain of virulence on Pm3 lines are single-colony isolated
5) Propagation, characterization & sequencing: mass growth, phenotype confirmation, qRT-PCR, DNA extraction, whole genome sequencing (Illumina)
6) Bioinformatics: alignment of *Bgt* mutant sequences to the reference genome (Bgt_96224), SNP-call, identification of regions of high mutation frequency & correlation of SNPs with phenotype

Take-home messages & outlook

- ✓ Development of a mutagenesis approach to create *Bgt* mutants with gain of virulence
- ✓ Potential to discover genetic components such as *Avrs* in the *Bgt* – wheat interaction
- ✓ The results indicate that avirulence of *Bgt* is not only caused by *Avrs* → ongoing discovering novel components of the expression machinery

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References

(1) Barsoum, M. *et al.* (2020) 'Ultraviolet mutagenesis coupled with next-generation sequencing as a method for functional interrogation of powdery mildew genomes', *Molecular Plant-Microbe Interactions*, 33(8), pp. 1008–1021. (2) Bourras, S. *et al.* (2015) 'Multiple Avirulence Loci and Allele-Specific Effector Recognition Control the Pm3 Race-Specific Resistance of Wheat to Powdery Mildew', *The Plant Cell*, 27, pp. 2991–3012. (3) Jones, J. D. G. and Dangl, J. L. (2006) 'The plant immune system', *Nature*, 444(7117), pp. 323–329. (4) Parlange, F. *et al.* (2015) 'Genetic and molecular characterization of a locus involved in avirulence of Blumeria graminis f. sp. tritici on wheat Pm3 resistance alleles', *Fungal Genetics and Biology*. Elsevier Inc., 82, pp. 181–192.