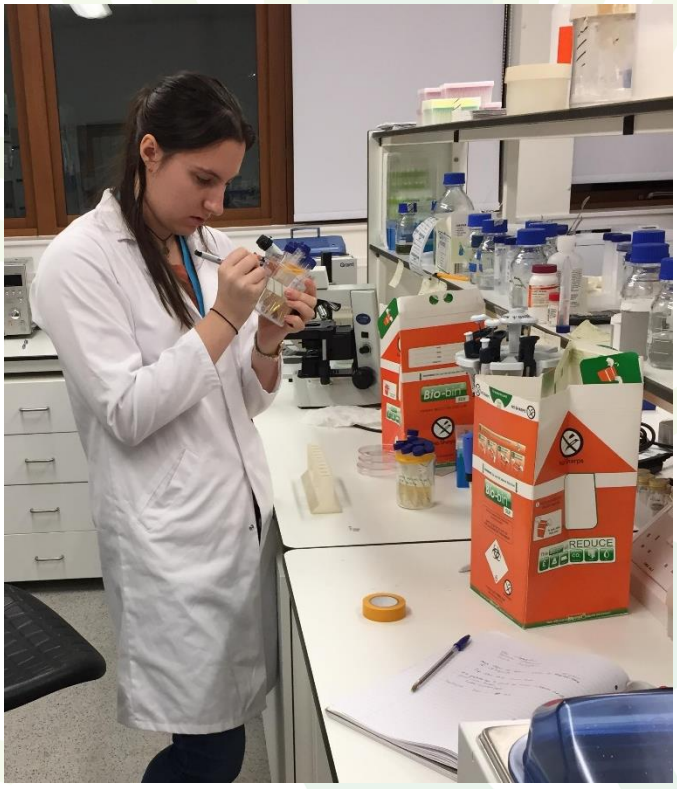
Identifying the functional R-gene(s) in a genetic interval in *Arabidopsis thaliana* using CRISPR Technology

The British Society for Plant Pathology
Bursary Report

In the face of a booming population and rapidly changing climate, development of new techniques for battling plant disease is fast becoming one of the principal issues faced by scientists internationally. With current disease management practices in place, an average of 10-16% of global harvests are still lost to plant pathogens annually, highlighting an urgent need to upgrade agricultural practices with novel methods for optimising crop production. One such method, the CRISPR/Cas9 gene editing technique, was implemented in my MRes project with *A. thaliana* to demonstrate its potential for increasing knowledge of resistance gene (*R*-gene) function through targeted editing.

My study focused on a previously mapped interval of the *A. thaliana* accession RMX-A02 chromosome 4, which was known to confer resistance to *Hyaloperonospora parasitica* (*Hpa*), the causal agent of downy mildew. Using bioinformatic tools I identified seven *R*-genes present in the interval and designed both single and multiplex CRISPR targets (sgRNAs) for silencing each gene. Following this, the sgRNAs were successfully cloned using the Golden Gate Cloning technique, into bacterial vectors containing the Cas9 complex. The resultant 7 single target constructs (1 sgRNA/gene in pDGE063) and 7 multiplex constructs (3 sgRNA/gene in pDGE002) were transformed into *E. coli*, followed by *A. tumefaciens,* using electroporation. These constructs were further transformed into RMX-A02 using the floral dip method and the resultant T_0 seeds were harvested for analysis. Following BASTA selection to confirm transformation success, transgenes were also confirmed by PCR in the selected plants. The seedlings were then inoculated with the relevant *Hpa* isolate and observed for any differences in pathogenesis. For the remaining 11 constructs, glycerol stocks were made to allow further study.



< Eleanor working in the lab

Little to no difference was observed in the virulence of the disease post-transformation for any of the experimental groups, suggesting the three genes that were targeted are not the sole causal genes for resistance to *Hpa* in RMX-A02. To reveal the involvement of the remaining 4 genes and gain further confirmation of the role of the three tested genes, the multiplex constructs could be utilised to increase the certainty of gene silencing. It is also possible for *R*-genes to function in pairs, or larger groups, and this could be explored by simultaneous targeting of gene combinations.

I greatly enjoyed the time I spent completing my MRes at the University of Worcester and am extremely grateful to the members of the Tor Research Group (particularly Prof. Mahmut Tor!). I gained invaluable experience working in a laboratory environment and learning countless molecular techniques. My special thanks goes to the BSPP for providing funding to help me develop my scientific ability, which in turn allowed me to further my academic career. I am currently studying for a PhD working in a similar area on Wheat at Montana State University and without the support provided, I would not have been able to achieve this goal.

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