The role of *Mlo* genes during powdery mildew infection and mycorrhizal colonisation

The British Society for Plant Pathology Bursary Report

Over the summer I had the opportunity to complete a summer project at the John Innes Centre (JIC), working in the lab of Dr Christopher Ridout. My project was built upon some of the work done by Catherine Jacott (PhD student), who is looking at the extent of overlap between the pathways utilised for mildew infection and mycorrhizal colonisation.

The major part of my work at JIC was looking at the relative gene expression of the *Mlo* family of genes after inoculation with both wheat powdery mildew and barley powdery mildew spores. When homozygous recessive, this gene family is seen to confer resistance to powdery mildew disease, caused by the fungal agent *Blumeria graminis*. In addition to looking at the gene expression wild type (WT) barley, I also observed expression in a *mlo-5* mutant. This aimed to investigate the potential for compensation of the *Mlo* gene family members. It was hypothesised that in the *mlo-5* mutant background, we would see stronger basal expression and/or induction of other *Mlo*-family genes as compared to WT if the genes were compensating for one another. To do this, I extracted RNA from inoculated leaves at 2 timepoints (16 hours and 24 hours) post inoculation and took 4 biological replicates through to cDNA generation. Using RT-qPCR, the relative gene expressions were determined for the *Mlo* and *Mlo*-like genes in barley, with reference to the expression of a housekeeping gene, EF1α. The early-response gene Rnr8 acted as a positive control for powdery mildew interaction.

The results of this experiment allowed me to identify 2 candidate genes (allocated names *Mlo-6* and *Mlo-7*) which were significantly expressed at greater levels in the *mlo-5* mutant, both basally (mock inoculation) and at 16 hours post inoculation. Both genes were also significantly induced upon mildew inoculation, suggesting that they may have roles in the plant-pathogen interaction. This provides evidence for potential compensation within the *Mlo*-family members. The data showing *Mlo-6* is upregulated in the *mlo-5* mutant background supports data seen in a previous paper. Other genes showed no difference or significantly lower expression in the mutant background. These results were unsurprising due to the diverse roles of the selected genes. The lab will continue to investigate the roles of the candidate genes identified, through looking at their expression at different time-points during the process of mycorrhization.

To further study the effect of a non-functional *mlo*-gene on mycorrhization, the model plant *Medicago truncatula* will be used. Following previous research, *Mlo*-genes were selected and lines of seeds were collected from transformed plants, which have a TNT retrotransposon insertion in one of 2 potentially interesting genes. One of the aims of my project was to genotype the plants grown from the transformed lines through PCR analysis using WT and TNT primers. This allowed the identification and selection of homozygous mutants for further study.

The last part of my project was to perform an assay where the extent of powdery mildew colonisation was assessed in a diversity set of different barley cultivars. Unfortunately, after growing the plants, inoculating them and then carrying out the staining procedure necessary to visualise the fungus, no colonisation was seen and only 7 spores across several samples. After retracing our steps, we found a mildew fungicide had been sprayed in the growth cabinet used a few days prior to growing the plants here, and this may have affected experimental results.

I have thoroughly enjoyed my time at JIC, it has given me key insights as to how research progresses, and the preparation stages involved in carrying out experiments which I would not have otherwise seen. I have learnt so much about experimental design, and had the chance to use techniques which I had learnt about but never had first-hand experience of. Before I started the project, I was hesitant in considering doing a PhD and embarking on a career in research. The project has allowed me to experience working in a research environment, and I enjoyed it so much I am now seriously considering continuing with academia after graduation. I would like to thank the Chris Ridout Lab for their help and patience in supervising me as I completed the project, and the BSPP for funding my project and providing me with this fantastic opportunity.

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