Characterisation of phytopathogen-targeted secretion pathways in wheat

The aim of this summer internship was to characterise recombinant wheat lines carrying a fluorescent protein fused to a secretion signal peptide. The protein fusion in question is thought to be part of a very exclusive secretion pathway which goes to sites of attempted fungal entry. This pathway would be perfect for attaching a cargo that you would not want to go anywhere else, such as antifungal peptides. The hope is that by understanding this defensive pathway in wheat it will be possible to engineer biotechnology approaches to protect cereal food supplies from phytopathogenic attack. This could lead to enhancements to world food supply by reducing crop losses to phytopathogens.

My task was to identify recombinant T1 wheat plants that are stably producing transcript encoding the fusion protein using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) experiments. Expression of the construct was predicted to be pathogen-induced. Consequently, leaves from each wheat cultivar were infected with *Blumeria graminis* and incubated for two days before the plant RNA was extracted. cDNA was then synthesised and qPCR performed using Tubulin and Venus primers. The Tubulin primers were used as a control to directly compare the quantities of DNA synthesised with the Tubulin primers, to that synthesised with the Venus primers, which would amplify the relevant protein fusion.

Normally the cultivars would be screened for fluorescence, however when infected with *B. graminis* the wheat tissue produced too much auto-fluorescence and I was unable to isolate fluorescence from the recombinant protein. The RT-qPCR approach identified several candidate positive lines which are now being analysed by the research group using further microscopy approaches and Western blotting.

Alongside the main project I was required to characterise recombinant *Arabidopsis* containing an equivalent protein fusion to that found in wheat, in order to find a positive control for this protein construct. These plants were also infected with *B. graminis* and screened for fluorescence using a Leica SP8 laser scanning confocal microscope. The outcomes of this were successful.

I have gained a helpful insight into this field of study, learning skills that are critical when working in Plant Pathology, including the different planting conditions needed for wheat and Arabidopsis as well as skills that I would not have picked up from the teaching laboratory, such as live-cell imaging.

This project allowed me to gain an enjoyable insight into laboratory work. I would like to thank the BSPP for awarding me the undergraduate vacation bursary, Dr M.J. Deeks for his help and all of the staff at the Mezzanine laboratory who made me feel welcome.

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