Investigating the AM symbiosis signalling pathway (Crop Science Centre)

Rhiannon Durant, Rokos Award report

Over 80% of terrestrial plants are colonised by arbuscular mycorrhizae (AM), including key crop species. This symbiosis is essential for efficient nutrient uptake with up to 100% of the plant's phosphorus being delivered by the fungus as inorganic phosphate. Therefore, understanding the signalling pathway between crops and fungi that allows for the establishment of symbiosis could allow us to optimise it in the future. This could lead to the generation of food crops that require less phosphorus fertiliser, increasing efficiency and reducing environmental impact. The Cereal Symbiosis lab based in the Crop Science Centre, Cambridge is a current leader in research into the molecular processes behind AM symbiosis.

I am very interested in how plant molecular biology can help to solve environmental issues and how we optimise our food supply for a changing climate and growing population. Therefore, this topic attracted my attention and I reached out to Dr Paszkowski, the director of the Cereal Symbiosis lab. Luckily for me, the lab had space to take on summer students and so I applied to the Rokos Award for funding.

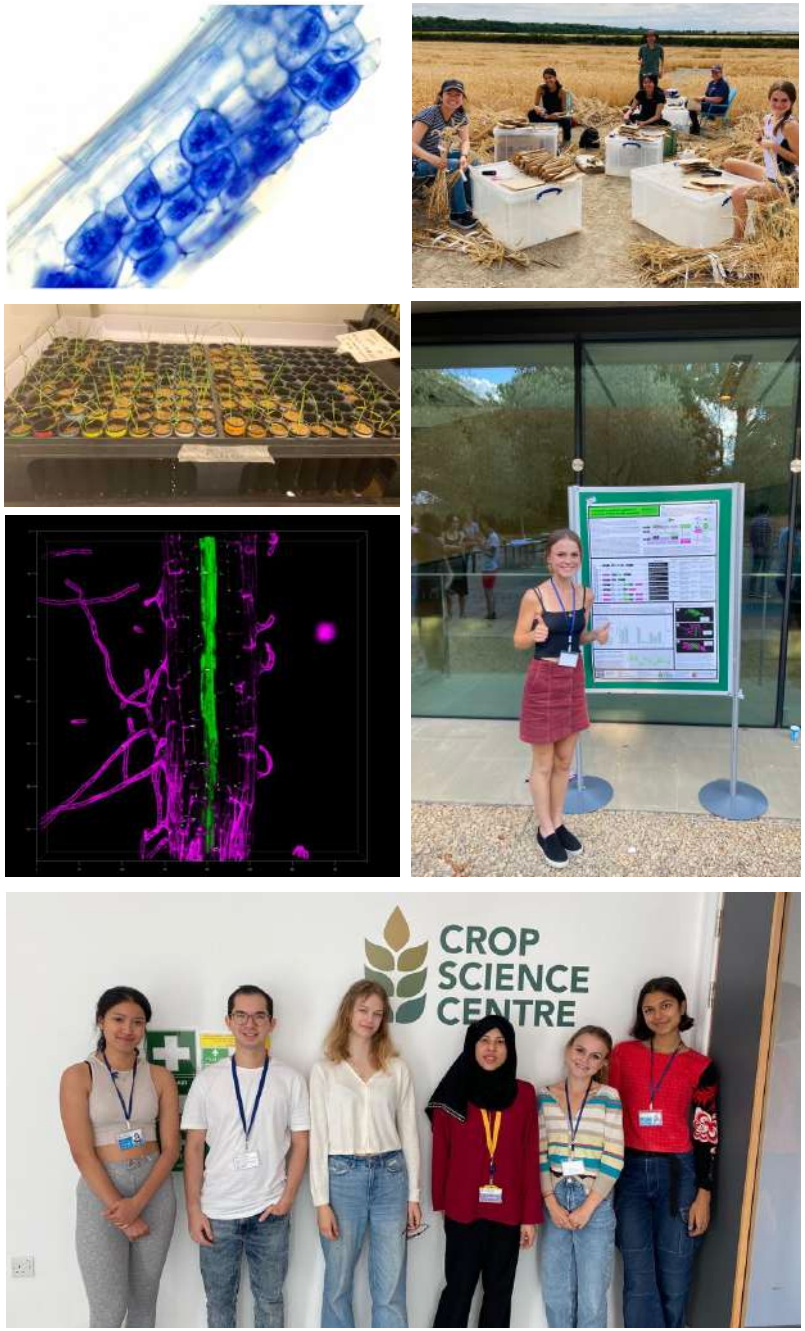
I worked with a PhD student, Gabriel, over the summer. Gabriel's research focuses on spatial localisation of a protein called Dwarf14-like (D14L). This protein is essential for AM symbiosis in rice; it acts upstream in the signalling pathway and creates a 'permissive state' for establishment of colonisation.

The interesting feature of D14L is that its promotor appears to function only in the stele (centre of the root) however it needs to be located in the epidermis to prime the root for colonisation. Single-cell RNAseq analysis shows D14L mRNA present throughout the tissues of the root, suggesting that D14L migrates in mRNA form outwards from the stele. Interestingly, the cDNA sequence with the native promotor alone doesn't rescue the *d14l* AM-negative phenotype, suggesting sequences in the UTRs or intron are required for this transport.

My part of the project was to conduct a side-by-side comparison of 10 rice lines containing different D14L gene constructs, assessing their AM colonisation levels, symbiosis marker gene expression via qPCR, mesocotyl length, and fluorescence localisation using confocal microscopy. The goal was to help confirm previous observations made about D14L's localisation/movement and its ability to function.

During my 9 weeks in the lab, I developed a range of skills which will likely prove very useful for my part II and beyond. I gained experience conducting basic molecular biology techniques such as high-throughput PCRs, gel electrophoresis, DNA & RNA extraction and cDNA synthesis. I also improved my microscopy skills, including use of both brightfield and confocal fluorescence microscopes. I had the opportunity to make a scientific poster and present it at conference for summer students. Most importantly, I learned techniques specific to plant molecular biology, including germinating seeds, protoplasting and tissue grinding. This was significant for me, as my Biochemistry course is mostly focused on human and animal cells and so I have had few opportunities to work with plants, which are my key interest. I even had the opportunity to help harvest a large-scale field trial, which was quite a unique experience!

Overall, I am extremely grateful to both the Cereal Symbiosis lab and to the Rokos award for giving me the chance to undertake this project. I learned so much in a short space of time and got a taste of what real scientific research is like. I feel as though I have contributed (in a very tiny way) to the advancement of research into AM symbiosis, which is extremely rewarding.



Clockwise, from top right: stained arbuscules, harvesting a field trial, my conference poster, me and the other summer students, confocal image of spatially restricted D14L tagged with GFP, rice seedlings in cones