Ongoing research

Regulation of UV-Induced Flavonoid Production in *Marchantia polymorpha*: a role in the evolution of plants for land colonisation?

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Plants are thought to have colonized the land around 500 million years ago. One of the major challenges the first pioneers faced was protection against UV radiation. UV has severe detrimental effects on plant cells and was at particularly high levels during the period of land colonisation because the ozone layer was not fully developed. Seed plants use a mix of secondary metabolites as UV 'sunscreens', and of these the flavonoid group of phenylpropanoids is of particular importance. UV-B is perceived in seed plants by the UVR8 photoreceptor, whose activation stimulates a cascade of transcription factors that up-regulate flavonoid gene transcription. Of particular importance are the initial target protein, the bZIP HY5, and the direct activators of the flavonoid genes, which together form the MYB-bHLH-WDR (MBW) transcriptional complex. There are no convincing reports of the identification of flavonoids from algae and it is generally accepted that the phenylpropanoid pathway arose in land plants. However, it is an open question whether inducible flavonoid production for UV-B tolerance is a universal system in plants that may have evolved during land colonization, as data is lacking from organisms outside the angiosperms. Indeed, an alternative proposal is that the flavonoid pathway arose for control of auxin action, as flavonols are modulators of auxin activity.

As the closest living relatives of the first land plants, bryophytes can help to inform us about systems that have an early evolutionary origin. With funding from The Marsden Fund of New Zealand, we are defining the UV tolerance mechanisms of the liverwort *Marchantia polymorpha* (marchantia) and comparing them with those of angiosperms, with a focus on the flavonoids. Marchantia is an outstanding model system: it is a small rapidly growing plant, it is easily transformable, the main life stage is haploid, it can be rapidly clonally propagated via gemmae and it has a small, recently sequenced, genome. Marchantia produces flavones, which are a key UV-induced flavonoid of many angiosperms and a red pigment thought to be related to anthocyanins (Riccionidin A). In other liverworts that have been studied, Riccionidin A appears to be bound to the cell wall, as opposed to the usual vacuolar location of anthocyanins in angiosperms. Liverworts also produce a great variety of other small metabolites, including many that are thought to be derived from the phenylpropanoid pathway. Of particular note are the bibenzyls, which are produced in abundance and have great structural variety in liverworts, including marchantia. Bibenzyls show anti-fungal activities and are also induced by general stresses such as ABA treatment or UV-C exposure.

Figure 6.1: *Marchantia polymorpha* plant with an archegonia (female sexual organ) and a close up image of the clonal reproductive organ, the gemmae cup, stained to visualise flavonoids

Nothing is known on the biosynthesis of flavonoids in marchantia. We have used RNAseq analysis of UV-treated plants and Blast interrogation of transcriptome and genome resources to identify biosynthetic and regulatory gene candidates for the marchantia flavonoid pathway. While the early steps of the shikimate and phenylpropanoid pathway can be identified with confidence from sequence similarity to angiosperm genes, the later steps of flavonoid biosynthesis will require functional studies for gene assignment. With regard to finding candidate regulatory genes, these are even more challenging to identify based on phylogenetic analysis alone. Thus, we are characterizing our candidate biosynthetic and regulatory genes using transgenic over-expression, CRISPR mutagenesis and functional analysis in genetically defined angiosperm mutants. Results to date include the identification of a UV-induced transcription factor that when over-expressed in marchantia confers constitutive production of large amounts of flavonoids. Strikingly, the over-expression transgenics are deep red in colour, from production of large amounts of Riccionidin A. When we generate CRISPR lines for this gene, the transgenics lose the ability to form red pigmentation.

A great advantage of marchantia is the ease of transformation combined with its haploid genome, as CRISPR events are obtained in the initial transgenic generation. We are now generating CRISPR knockouts for other genes of interest that may relate to the flavonoid/UV-B system. UVR8 and HY5 have been reported as conserved across plants and this is the case in marchantia, which has single copy candidate genes for each. To test the function of both of these we are currently generating CRISPR mutant lines, and would be interested to collaborate on the functional analysis of these with other researchers. Moreover, studies on evolution are most powerful when data is available from a wide range of organisms, and we would be pleased to hear from other groups that are studying flavonoids and UV-B responses in species beyond seed plants.

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The Team We are studying the mechanism of UV-B tolerance in some of our most basic plant species, the liverworts, so it can be compared to the well characterised flavonoid-based systems of angiosperms. The project is based in the Plant Pigments Team of Plant & Food Research. The team of about a dozen staff is led by Kathy Schwinn and located in Palmerston North in the North Island of New Zealand. The group carries out a wide range of both applied and fundamental research on plant pigments. The liverwort project is led by Kevin Davies and Simon Deroles in collaboration with Brian Jordan. Brian was previously the leader of the Plant Pigments team, having joined the team from the Institute of Horticulture Research, UK in 1994. Brian's research expertise has focused on light and plant development and since 1990 he has been recognized as an authority on plant cellular responses to UV-B. Brian is now based at Lincoln University, but has stayed a close friend and colleague of the Palmerston North team. In 2011 the opportunity was taken to develop a research collaboration on UV-B regulation of the flavonoid pathway in the model liverwort species *Marchantia*.

In 2013 a prestigious New Zealand Marsden Grant was awarded and funded the appointment of post-doctoral and PhD fellows. Enter Will and Nick! Will Clayton joined the team after completing his Master's in biotechnology at Massey University, Palmerston North. He started as a PhD student on the project in 2014, studying changes in flavonoid production, plant physiology and gene expression following UV-B exposure of marchantia. Currently based at Lincoln University but working closely with the team in Palmerston North, Will is developing many new skills and enjoying the challenge of working towards unravelling this novel UV-B story. Nick Albert originally completed his PhD with Kathy and the team, and after a few years working elsewhere returned for the Marsden post-doctoral appointment. He has developed molecular tools for marchantia, including CRISPR-based mutagenesis and is identifying the genes that regulate flavonoid production in liverworts.

We are now two thirds through the project with many exciting results and concepts emerging. We very much hope that the UV4Plants community finds our research interesting and we look forward to future collaborations. Best Wishes from New Zealand.

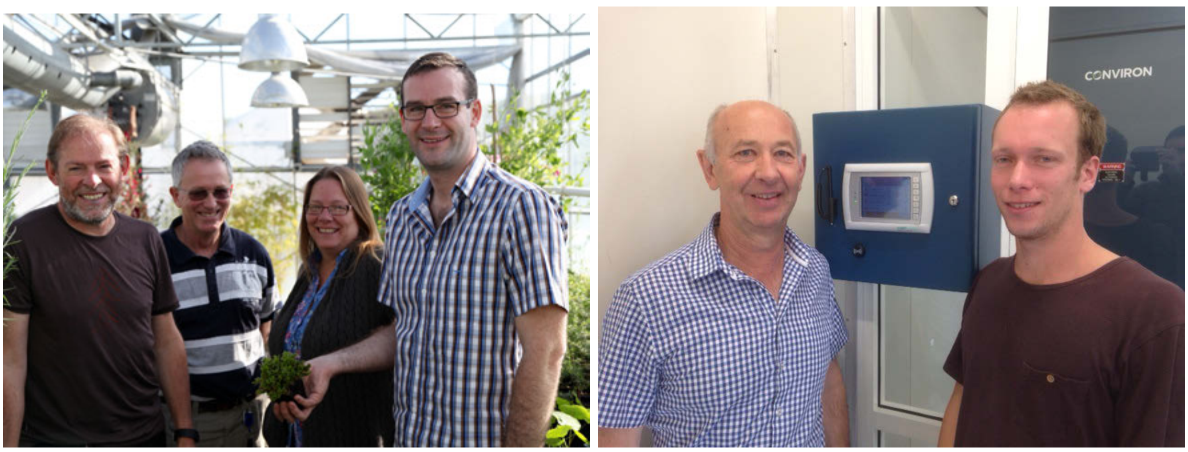

Figure 6.2: On the left, the Palmerston North team, from left to right Kevin Davies, Simon Deroles, Kathy Schwinn and Nick Albert, and on the right Brian and Will.