Developing the tools for identifying the activation routes of biotic stress tolerance genes in *Arabidopsis*.

Rationale

Hunger is the leading cause of death globally. Both the WHO and the UN has listed food security as major global health initiatives. Global warming, alongside a growing population, is exacerbating the problem. A major contributor, aided by our changing climate, is the spread of pests and pathogens (biotic stresses). A third of the global rice harvest is lost to the fungal rice blast disease, while pests are responsible for a 40% yearly reduction in crop outputs. Developing new robust crop protection strategies is therefore of critical importance.

Background and previous research

Two common hormones that plants release to activate defence when under biotic stress are salicylic acid (SA) and methyl jasmonate (MeJa). Plants respond to these compounds by altering their gene expression, and this requires the Mediator complex. We require tools to identify the activation routes of different stress tolerance genes in order to have a chance of improving crops' defences through better activatable genes.

Arabidopsis is a tractable model plant species, that can be used experimentally as a single-cell (protoplast) high-throughput screening platform to monitor changes in gene expression following stress treatments. Bioinformatic tools allow direct comparison between *Arabidopsis* and crops. Luciferase is a bioluminescent protein that can be used to study plant responses in real time by linking the gene encoding it (LUC+) to specific DNA promoter sequences (cis-acting motifs).

By linking the LUC+ reporter to a specific promoter motif, Whalley *et al,* (2011) identified the common motif in genes that are activated by calcium concentration. They made luciferase fusions with 4 different promoter motifs: site II, CAM box, CRT, ABRE. Using the latter two reporters, Knight *et al,* (in prep) transformed them into protoplasts of mediator mutants in order to identify which subunits are required for the transcriptional activation of genes by cold-induced hormone ABA. This demonstrated how LUC+ can elucidate gene response to plant hormones.

Reporter constructs and high-throughput protoplast screening can be used to build a picture of plant stress response pathways. This project aims to develop this experimental system further.

Proposal

Part A and B will be worked on in parallel.

Part A: In six weeks, it is feasible to create and test two novel reporters that can later be used widely by the scientific community investigating pathogen response. MeJa and SA are released to induce biotic stress pathways that require the G-box[1] and W-box[2] motif, respectively.

1. Creation: Design two new luciferase reporters with Gibson gene assembly.
2. Transformation: Isolated Arabidopsis protoplasts are transfected with the reporter constructs.
3. Investigation: Screen for altered gene expression in response to stress hormones, measured by bioluminescence.

Part B: I will also use pre-existing motif reporters to investigate how the mediator mutants *med25*, *med16*, and *med14* respond to biotic stresses[3]. These mediator units have all been linked to a variety of stress acclimation pathways including pathogen tolerance.

1. Wild type (WT) *Arabidopsis*: Investigating connections between SA/MeJa/ABA pathway and the pre-existing motifs for abiotic stresses. Do these hormones activate other pathways? How do the biotic stress hormones influence established abiotic pathways?

2. WT, *med14*, *med16* and *med25 Arabidopsis*: Investigate mediator complex roles using pre-existing reporters. Does the lack of any mediator components affect hormone response?

Objectives

- I will have created and tested a transferable biomolecular tool, establishing the link between SA and MeJa and their corresponding motifs.
- Using the floral dip method, I will begin stably transforming *Arabidopsis*. The seed product is ready six to nine months later. When matured, stresses associated with the G-box and W-box can be studied on the whole plant in a realistic way. Both can be used by future researchers.
- Furthermore, informed by pre-existing research, I will have elucidated some of the complex pathways associated with biotic stress hormones SA and MeJa.

This research will produce a greater comprehension of biotic stress pathways and two tools to allow for further investigation. Establishing these tools is a fundamental step towards upregulating gene expression for more robust and productive crops.