Investigation of aphid detection in *Arabidopsis thaliana*

Plants sense pathogenic organisms (bacteria, fungi and oomycetes) through perception of conserved pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) in a process known as PAMP-triggered immunity (PTI). Another important plant-biotic interaction, the one occurring between plants and insects, is less well understood. The Hogenhout lab at the John Innes Centre has sought to uncover an analogous detection system in plants to insects such as aphids, an economically important plant pest and vector of many plant viruses.

Evidence that plants do perceive aphids has been demonstrated by measuring signalling events such as calcium and reactive oxygen species (ROS) bursts as well as transcriptional changes in response to exogenously applied aphid extract. A transcriptome analysis carried out in the Hogenhout lab comparing aphid-exposed leaves with non-exposed leaves identified several differentially expressed receptor-like proteins (RLPs), which are likely candidates as aphid-specific PRRs. Amongst these candidate PRRs, a few receptors were identified during ROS screening of knockout mutants. Although these putative RLPs appear to be involved with aphid perception, no known functional role in immunity has been demonstrated.

As a part of my summer project in the lab, I wanted to screen mutant lines of paralogs, interaction partners and closely related genes of the candidate aphid PRRs identified previously for perturbed ROS response to aphid extract. Many of these genes are not characterised, therefore, I designed primers to distinguish mutant and wild-type alleles and to identify homozygous knockout mutants. I managed to optimise PCR amplification reactions using temperature gradients and identified several homozygous lines that can be taken forward in future investigations.

During ROS screening I found that aphid extract appears to partly inhibit the ROS burst. In order to further optimise plant responses to aphid extract to accurately compare mutant lines, I prepared a dilution series of aphid extract and exposed wild-type leaves to each concentration. I found that the optimal aphid extract concentration for use in ROS burst assays was approximately 1 mg/ml, which is lower than currently used concentrations. In addition to testing aphid extract concentrations, I wished to confirm the validity of an Arabidopsis mutant as a positive control in ROS experiments, by exposing this mutant line and wild-type leaves to aphid extract, flg22 and a combination of aphid extract and flg22. I found that the mutant was compromised in ROS response to both aphid extract and flg22 relative to wild-type plants. Moreover, aphid extract partially inhibited the characteristic flg22-induced ROS response. Taken together, these experiments may indicate a biological component in aphid extract that partially inhibits the ROS burst.

I would like to sincerely thank Professor Saskia Hogenhout and Dr Claire Drurey as well as all the members of the Hogenhout lab for the opportunity to work in such a dynamic environment and for their continued guidance and support throughout this project. I have learnt a great deal during the summer and wish to continue investigating plant-insect interactions and contribute to research in this exciting area.

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