

Project title: A Systems Approach to Disease Resistance Against Necrotrophic Fungal Pathogens in Lettuce

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

Grower Summary

Headline..... 1

Background..... 1

Summary 2

Knowledge Transfer Activity 4

GROWER SUMMARY

Headline

A summary of work going on in the HAPI lettuce diseases project is given below.

Background

The fungal pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum* have broad host ranges and cause serious disease on many horticultural crops. Both fungi can cause substantial losses on field-grown and protected lettuce crops, an industry worth almost £200 M annually in the UK. *B. cinerea* is a particular problem post-harvest, whereas *S. sclerotiorum* can result in up to 50% crop loss pre-harvest. Chemical control is problematic as few effective compounds are available, the number of sprays is restricted and timing is difficult. Moreover, the fungicides are medium to high risk for development of resistance. Development of durable resistance in the crop is a more sustainable solution, but lettuce breeders have found it difficult to achieve this. The objective of this Horticulture and Potato Initiative (HAPI) project is to demonstrate that a novel approach to breeding for pathogen resistance is possible. Genomic and systems biology (computational) approaches will be used in combination with quantitative genetics studies to identify novel genes for increasing the resistance of lettuce to both *B. cinerea* and *S. sclerotiorum*. If successful the methodology will provide a foundation to develop similar resistance to these pathogens in other horticultural crops.

Two hypotheses will be tested in the project. Firstly, that genes which confer resistance to both *B. cinerea* and *S. sclerotiorum*, two necrotrophic fungal pathogens, will be identified. Genome sequencing of these fungi has indicated that they share a range of genes associated with infection and colonization of plants, hence host-resistance mechanisms against one pathogen might also confer resistance to the other. Secondly, the feasibility of applying systems biology research into horticultural crop species will be tested. This is founded on the fact that the researchers have used systems biology approaches to generate network models of how genes interact during the defence response of *Arabidopsis* to infection by *B. cinerea* where they combined large-scale gene expression data with mathematical modelling to predict the key resistance genes. In this work, they will carry out network analysis of the lettuce defence response and test whether the same genes are involved in disease resistance, and/or whether the hub genes in the network are important. This is a completely new approach to crop improvement, relying on gene to gene interactions during defence against pathogen infection. The researchers will also look for conservation of disease

resistance genes in tomato and Brassica, key crops affected by the 2 pathogens. Additionally, they will employ a more traditional quantitative genetic analysis to identify regions of the lettuce genome that influence resistance against both pathogens. The researchers will screen nearly 100 lettuce accessions and cross accessions with the greatest resistance to a standard cultivar to generate mapping populations. A pre-existing mapping population (known to be segregating for disease resistance) will be screened for disease resistance to both *B. cinerea* and *S. sclerotiorum* to identify important genomic regions for these traits. Finally quantitative genetic analysis and results from network analysis will be integrated to generate lettuce lines and markers for use in breeding programmes.

This project is possible because of the lettuce genome sequence that is available to the researchers, as well as the extensive lettuce germplasm and genetic and genomic resources that Warwick has generated. The work will be exploited primarily through A. L. Tozer Ltd. to develop lettuce varieties with increased resistance to *B. cinerea* and *S. sclerotiorum*.

Summary

The key aims of this work are to identify alleles and markers in lettuce for increasing resistance against *B. cinerea* and *S. sclerotiorum*, and to evaluate the ability of systems biology approaches to enable breeding of quantitative resistance in horticultural crops. Lettuce is being used for the majority of this research due to the preliminary data that is already available for this crop and the resources the researchers have built up, including mapping populations from parents with varying levels of resistance; however, they will assess the broad relevance of the findings to other crops. Specific objectives are to:

1. Use network modelling to identify key regulatory genes in lettuce influencing resistance to *B. cinerea* and *S. sclerotiorum*.
2. Generate and screen lettuce populations to map quantitative resistance against both pathogens.
3. Integrate mapping, expression and sequence data to identify markers for beneficial alleles.
4. Assess broad relevance of key regulatory genes and the systems approach in tomato and Brassica.

During the first 10 months of the project three excellent staff (Adam Talbot, Josie Brough and Juliane Graham) were recruited and technicians have been trained by a postdoc in pathogen susceptibility assays and the necessary molecular techniques.

A standardised reproducible infection protocol for both *Botrytis cinerea* and *Sclerotinia sclerotiorum* on lettuce has been established for both a quantitative detached leaf assay and a whole plant assay.

A *B. cinerea* infection was carried out on the Saladin cultivar and tissue harvested every 3 hours for 54 hours post inoculation for transcriptome profiling by RNA sequencing. High quality RNA has been extracted from the replicate samples and these are currently being processed for sending to the sequencing facility.

A lettuce diversity set of 96 lines (developed within the Defra-funded VeGIN project), plus additional cultivated varieties from A.L. Tozer Ltd. were screened for resistance against *B. cinerea* and *S. sclerotiorum* using a randomised complete block design. This has demonstrated variation in susceptibility to both pathogens between lettuce varieties, indicating sources of resistance within the population. Furthermore, there is evidence for correlation between susceptibility to both pathogens in some accessions, suggesting potential broad spectrum resistance. Accessions that will be used to generate new crosses during 2016 (for both additional mapping populations and to bring genetic sources into Tozer's breeding programmes) have been selected.

Prior to the project start date we had carried out preliminary screening of lettuce accessions and crossed accessions to generate some initial mapping populations (F2 generation seed). Phenotyping in year 1 has indicated the parents of one of these mapping populations show contrasting susceptibility to both pathogens, indicating the progeny will likely segregate for resistance and enable discovery of resistance QTLs. During the 2015 season we grew the F2 seed and harvested material for genotyping (towards milestone F). F3 seed were generated from these F2 plants.

In addition to our original objectives, we also screened parents of existing mapping populations generated by Prof. Richard Michelmore for resistance to *B. cinerea* and *S. sclerotiorum*. This has highlighted several informative mapping populations. These populations (at advanced stages of generation, F6-F10) will be screened to identify additional sources of resistance for use by Tozers. The added advantage of these populations is that they are already genotyped (hence resistance QTL can be quickly identified).

A PhD student affiliated with the project (Elspeth Ransom) is working on a transcriptome data set from lettuce leaves infected with *S. sclerotiorum* (generated in the DeFRA funded VeGIN project) and is developing pipelines for analysis of time series RNA sequencing data which will then be exploited to analyse the transcriptome data generated in this project.

Tozer have started making crosses to incorporate genetic sources of resistance into their breeding programmes using information generated by screening the lettuce diversity set. Around 30 crosses are being made this season.

Knowledge Transfer Activity

In September 2015 Katherine Denby gave a talk on “The Impact of Plant Pathogens on Everyday Lives” at the British Society for Plant Pathology President’s meeting. Dr Adam Talbot attended an open day at Elsoms with a poster about the project and also spoke at the February 2016 Lettuce Research Day which was held at Harper Adams University. The day was funded as a VeGIN Leafy Vegetable meeting and was attended by lettuce researchers as well as industry members of VeGIN and technical committees of the British Leafy Salads’ Association and the Baby Leaf Growers’ Association. An article reporting on this project was published in the March 2016 BLSA Newsletter.