Grower Summary

A systems approach to disease resistance against necrotrophic fungal pathogens (BBSRC HAPI)

CP 152

Final Report 2019

Project title:	A systems approach to disease resistance against necrotrophic fungal pathogens (BBSRC HAPI)
Project number:	CP 152 (BBSRC HAPI project BB/M017923/1)
Project leader:	Katherine Denby, University of York
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Key staff:	Adam Talbot (University of York) John Clarkson (University of Warwick) Carol Wagstaff (University of Reading) David Pink, Paul Hand (Harper Adams University) Tozer Seeds
Location of project:	University of York, University of Warwick, University of Reading, Tozer Seeds
Industry Representative:	Melville Miles, Freshtime UK Ltd
Date project commenced:	18th May 2015
Date project completed:	30th September 2018

DISCLAIMER

GROWER SUMMARY

Headline

- Genetic variation in resistance to the diseases Botrytis and Sclerotinia was observed in screened germplasm (diversity sets) of cultivated and wild lettuce.
- Regions of the lettuce genome that confer disease resistance to these pathogens (quantitative trait loci, QTL) were successfully identified from analysing a highly developed mapping population.
- Markers associated with these QTL are being developed for use in commercial breeding programmes and in future research work to narrow down the QTL and identify the underlying causal gene.
- For another mapping population, field trials have shown reproducible field resistance against Sclerotinia for one of the parent lines, demonstrating its value in work to reduce crop losses in field-grown lettuce.

Background

Lettuce is one of the most economically important leafy vegetables worldwide. Two of the most important pathogens of field grown and protected lettuce crops in the UK are *Botrytis cinerea* and *Sclerotinia sclerotiorum* with the former being a particular problem post-harvest while the latter can result in a 50% crop loss pre-harvest. Because their infection mechanism causes losses towards the end of the growing season, many of the costs associated with growing have already been spent, causing unavoidable losses for British growers. Primary disease control relies on agricultural fungicides, however both pathogens have shown some resistance to the control methods. One way to fight back is to produce lettuce varieties that are resistant to the pathogen.

The overall objective of this project was to identify sources of disease resistance in lettuce against *Botrytis cinerea* and *Sclerotinia sclerotiorum*, two economically important fungal pathogens. A wider objective was to demonstrate how systems biology approaches can be exploited to facilitate breeding of quantitative resistance to ubiquitous pathogens in horticultural crops. Network analysis, along with quantitative genetic studies, were used to identify novel alleles (and associated markers) for increasing the resistance of lettuce to both *B. cinerea* and *S. sclerotiorum*.

Summary

Katherine Denby and Adam Talbot (University of York), John Clarkson (University of Warwick), David Pink and Paul Hand (Harper Adams University) worked with the vegetable

breeders A. L. Tozer, Carol Wagstaff from Reading University, AHDB and Freshtime Ltd. to reduce crop losses in lettuce due to fungal pathogens.

Development of host resistance is an ideal solution but breeding for disease resistance to these pathogens has remained an intransigent problem for breeders. It was important to understand how plants respond to (and interact with) the pathogen infection. Having a greater understanding at a molecular level can drive strategies to enhance disease resistance of crop plants. This can include molecular pathways that can be turned on, or even turned off. The team have screened a diversity set of cultivated and wild lettuce and observed genetic variation for resistance to both Botrytis and Sclerotinia. They have successfully identified regions of the genome that confer disease resistance to these pathogens (quantitative trait loci, QTL) from analysing a highly developed mapping population. Markers associated with these QTL are being developed for use in commercial breeding programmes and in future research work to narrow down the QTL and identify the underlying causal gene. For another mapping population, field trials have shown reproducible field resistance against *Sclerotinia* for one of the parent lines, demonstrating its value in work to reduce crop losses in field-grown lettuce.

In this project we took a novel approach to pin-point genes/alleles that confer resistance - examining gene expression profiles during fungal infection of lettuce and using novel computational approaches to predict key regulators of the plant defence response. The team (along with an associated PhD student, Elspeth Ransom) have successfully generated time series transcriptome data from lettuce infected with *Botrytis cinerea* and *Sclerotinia sclerotiorum*. A systems biology approach was used to infer gene regulatory networks from these large data sets. Gene regulatory network models predict how the expression of genes (in this case genes responding to fungal infection) is regulated and analysis of these models highlights candidate genes important in plant immunity against disease. The candidate genes from this “big data” approach are being tested by overexpressing these genes in the model plant Arabidopsis, as well as overexpressing them in lettuce. Two of the lettuce genes have been shown to reduce susceptibility to *Sclerotinia* in Arabidopsis when overexpressed. The team are waiting for results on the transgenic lettuce, as well as from gene-edited lettuce lacking these candidate genes. Integrating the defence network models with gene expression data after *Sclerotinia* and *Botrytis* infection in the mapping population parents and twenty of the diversity set lines, will enable us to examine correlation of candidate gene expression with disease resistance levels.

Our systems biology network approach holds tremendous promise for driving more rapid gene discovery and identification of genes responsible for QTL, and hence dramatically reducing the time required for incorporation of new traits into leafy vegetable varieties.

"*It has been a great opportunity to network with leading academics in this space – and talk directly about the ways we can find innovative solutions to our growing challenges. This has been particularly important during a year as agronomically challenging as 2018 has been for the horticultural sector*." Melville Miles, Freshtime Ltd.

The project has provided the first significant insights into the response of lettuce to these fungal pathogens, the similarity of the lettuce response to the two pathogens (raising the possibility of the same mechanisms increasing resistance against both pathogens), vital information on the timing of the response and predictions of the genes that are critical in determining an effective immune response. In addition to these key scientific insights, the project has stimulated a breadth of new research directions, as well as projects to take these findings and progress them further into active use by vegetables breeders and commercialisation.

The team are now working with A L Tozer to test additional candidate genes using gene-editing of lettuce and to narrow down the QTL conferring resistance for incorporation into elite breeding lines. A BBSRC CASE student (within the White Rose Mechanistic Biology DTP) will progress this further, as well as enhance the network modelling strategy, starting in Oct 2019. The team have applied for a Knowledge Transfer Partnership together to exploit their gene-editing expertise and develop gene editing technology for other key vegetable crops at A L Tozer; this would open up breeding opportunities enormously, especially given the biennial generation time of vegetables such as celery and parsnip.

The time series gene expression profiling provided information not only on expression of lettuce genes during infection, but also on expression of pathogen genes during infection. An application by Prof Denby to the BBSRC Follow on Fund combines this data for *Botrytis cinerea* with results from a BBSRC award to Prof. Simon McQueen-Mason (also at the University of York) for development of novel crop protection products. Our network approach has also attracted the attention of other lettuce breeding companies through the annual HAPI

workshops, and we are in discussion with another company on pursuing specific aspects of fungal resistance using different genetic resources.

Kim Parker of AHDB updated "*There is close collaboration between the researchers and breeder, as well as interest in the project from Freshtime. AHDB have kept growers aware of project details through report summaries, AHDB Grower articles, as well as an excellent presentation by Katherine Denby at the British Leafy Salads R&D meeting in November 2017. While initially sceptical of use of AHDB funds for fundamental genetics, the presentation showed growers the potential benefit in investing in novel breeding techniques as a step towards more sustainable disease control*."

Katherine Denby said of the project "*The ability to combine our cutting-edge systems biology tools and large data sets with traditional quantitative genetics has been fantastic to really make progress on the challenge of breeding for resistance to fungal diseases in lettuce. The HAPI initiative has driven a very productive collaboration – one that has expanded beyond lettuce – with the commitment of A L Tozer to innovation being key.*"

Financial Benefits

Halving the levels of Botrytis and Sclerotinia on lettuce would save £10M per year, given an average crop loss of 10%. In addition, 90% of lettuce crops are treated with fungicides with 2-3 sprays per crop targeted at these pathogens. Using resistant varieties could reduce the fungicide sprays by half. Reduced crop losses and more efficient resource use would be of environmental and public benefit.

Terminology

Mapping population: A population of lettuce lines arising from a cross between two lettuce varieties, or between a cultivated lettuce and a wild relative. The population of lines each contain a different combination of the parent genome and can be used to identify regions of the genome responsible for specific traits.

QTL (Quantitative trait loci) are regions of the genome (DNA sequence of an organism) responsible for a particular trait

