Project title:	Identifying novel powdery mildew susceptibility/resistance genes in strawberry.
Project number:	SF/TF 170: CTP PhD Studentship Scheme
Project leader:	Dr Helen Cockerton
Report:	Annual report 2020
Previous report:	N/A
Key staff:	Professor Jim Dunwell
Location of project:	NIAB EMR
Industry Representative:	Harriet Dunclafe
Date project commenced:	October 2019

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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

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

Analysing strawberry powdery mildew (*Podosphaera aphanis*) to identify key mildew resistance/susceptibility genes using a Genome Wide Association Study (GWAS).

Background

Strawberry is a major soft fruit produced in the UK, favored for the sweet fragrant taste and containing compounds with potential health benefits (Amil-Ruiz *et al.*, 2011). The introduction of tunnels benefited strawberry crops through protection from extreme weather conditions, extending seasonal production and improving harvest yield. However in these conditions diseases such as powdery mildew can be more prevalent in warm and humid environments (Asalf *et al.*, 2013; Hall, Jin and Dodgson, 2019).

Podosphaera aphanis is a global pathogen, causing disease on strawberry. The symptoms of the disease include upward curling of the leaf edges and discolored patches as well as powdery white mycelium growth which can cover the leaf, flowers and fruit. Infection of the fungus can impact on strawberry development causing misshapen and damaged fruit leading to severe yield loss due to unmarketable fruit (Hall and Jin, 2017; Hall, Jin and Dodgson, 2019).

Powdery mildew is mitigated by the application of stringent crop management practices and fungicide sprays. The fungicide sprays are applied at regular intervals using chemicals such as myclobutanyl or demethylation-inhibiting fungicide (DMI) (Carisse and Bouchard, 2010). However, the frequency of fungicide application can be restricted, as some products such as Brupirmate, Penconazole or Proquinazid can only be applied a few times a year. These restricted applications reduce the possibility of insensitivity and fungicide resistance (Hall, Jin and Dodgson, 2019). For instance, sterol demethylation inhibitors (DMIs) fungicides like Penconazole are highly effective at controlling the disease and is commonly used in commercial fields. However, over time the powdery mildew fungus has developed resistance to these fungicides, current evidence suggests resistance is caused by to mutations in the CYP51 gene (Sombardier *et al.*, 2010).

Summary

The first year of this PhD studentship has required the propagation of 350 different strawberry cultivars. Five replicate plants of each cultivar were propagated and transferred into the field. The process involved the collection of runner's plants, producing misted tips and planting. Genotypic data will be established through DNA extractions from each cultivar and genotyping on the istraw35 Affymetrix SNP chip. Whilst phenotypic data will be collected in year 2 and 3 through disease symptom scoring and imaging of leaves and fruit. The genotypic and phenotypic data will be used in a Genome Wide Association Study (GWAS). The GWAS will identify key Quantitative Trait Loci (QTLs) associated with disease resistance. Disease resistance in plants can be controlled by either a single gene or multiple alleles which are frequently identified through association with QTLs (Pu *et al.*, 2020). The QTLs are represented by molecular markers that correlate with the disease phenotype. Single nucleotide polymorphisms (SNPs) molecular markers are used due to their high frequency in the genome, ease of identification and cost-effective nature (Zhu *et al.*, 2008).

As part of a literature search, key genes associated with resistance to powdery mildew were identified. Bioinformatics tools were used to establish evolutionary conservation of genes against available strawberry genomes from GDR (Jung *et al.*, 2014) - octoploid *Fragaria* x *ananassa* 'Camarosa' and diploid *Fragaria vesca*. These genes were concluded to have a high evolutionary conservation within the subspecies strawberry genome and therefore good candidates for gene editing to validate gene function. Initial stages for gene editing has involved sub culturing of the strawberry cultivars 'Hapil' for is known susceptibility to powdery mildew and 'Calypso' known for its high transformation success. In addition to the bioinformatic design of primers for vector construction.

Financial Benefits

No recommendations at this early stage

Action Points

No action points are available at this time as this is early stages of the project

SCIENCE SECTION

Introduction

Plants have evolved three main types of defense against pathogens and pests. The first is passive resistance such as wax cuticles or strengthened cell walls, repelling initial infection. The plant's second defense mechanism is non-host resistance (NHR) protecting against specific pathogens at the plasma membrane (Kobayashi and Kobayashi, 2007). This type of defense uses transmembrane pattern recognition receptors (PRRs) that are triggered by pathogen-associated molecular patterns (PAMPs). The immune response is amplified by an increase in stress-related hormones leading to reinforcement in transcriptome reprogramming and a reduction in growth and photosynthesis (Lu *et al.*, 2018). The third type of defense is effector-triggered immunity (ETI). The ETI response mounts against a specific resistant gene (R gene), activated by pathogen effectors (Jones and Dangl, 2006; Qi *et al.*, 2011). R genes are activated in response to signals produced by a pathogen's effectors in the cell (van Schie and Takken, 2014). The R genes can be effective through different mechanisms, for instance, one method is to interact directly with effectors by incapacitating them (Yang *et al.*, 2013).

Diseases in plants can be controlled either by a single gene or multiple genes, which are frequently identified through association with Quantitative Trait Loci (QTLs) (Pu *et al.*, 2020). QTLs are molecular markers that are associated with a trait of interest, examples of molecular markers include restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), amplified fragment length polymorphisms (AFLP) or direct single nucleotide polymorphisms (SNP) assays (Batley and Edwards, 2007; Zhang *et al.*, 2017). SNPs are single base-pair changes in the DNA sequence that occur at a high frequency in the genome. Microarray-based genotyping technology detects SNPs by hybridization of DNA to oligonucleotides spotted on sequencing chips (Huang and Han, 2014; Cockerton *et al.*, 2018). The use of SNP chips in human and crop studies enables direct scanning of allelic variation across the genome covering hundreds of thousands of SNPs for high resolution for mapping of QTLs (Zhu *et al.*, 2008; Huang and Han, 2014).

In contrast to R genes, are susceptibility genes, these genes can facilitate the pathogens entry into the host. A significant S gene family is the trans-membrane Mildew resistant Locus 0 (MLO). The MLO genes were first identified in barley in 1942 and found to act as suppressors of the defense response (Amil-Ruiz *et al*, 2011; Callega, 2011). Since then, MLO genes have been identified in several other species such as peach, wheat and grapevine (Pessina, 2016). Recently Jambagi *et al* (2017) identified 12 MLO genes in the *Fragaria vesca* accession 'Hawaii 4'. Studies have shown that non-synonymous mutations in MLO genes can prompt the loss of function, leading to mildew resistance (Jiwan *et al.*, 2013; Jambagi and Dunwell, 2017). Recessive mutant's of the MLO gene have been identified in barley and Arabidopsis and have resulted in resistance to the penetration of mildew due to the inability of the pathogen to enter the plant cell wall (Pavan *et al.*, 2010).

The initial stage of this project has required the identification of plant candidate genes of interest that are involved in powdery mildew resistance. During the literature review two such genes of interest have been identified that appear to be associated with powdery mildew resistance. The two candidate genes are susceptibility factors; Mildew Loci 0 (MLO) (Sargent *et al.*, 2019; Tapia *et al.*, 2020). MLO genes facilitate the powdery mildew pathogen during infection, resulting in *Fragaria x ananassa* powdery mildew susceptibility. (Pavan *et al.*, 2008; Pessina, Lenzi, *et al.*, 2016).

This project will focus on three work packages:

Work package 1: Functional validation of candidate susceptibility factors. Delete/silence candidate susceptibility genes to validate function. This project will use the CRISPR/Cas9 gene editing systems and Host Induced Gene Silencing (HIGS). The premise of this project is that by inactivating the MLO gene and assessing disease phenotypes gene function can be confirmed.

Work package 2: GWAS Mildew field experiment. Propagate and plant five replicates of 350 strawberry genotypes in the field. Additional plants infected with powdery mildew will be introduced to ensure inoculation of the disease over the course of the trial. Disease symptom analysis will be conducted in year 2 for foliage and 3 for fruits and flowers. Phenotypic data will allow a Genome Wide Association Study (GWAS).

Work package 3: Controlled Pathogenicity Inoculations. Method development to allow the investigation of different tissue susceptibility. Hyper-spectral, multi-spectral, thermal and visual range imaging methods will be used to assist the assessment of disease progression.

Materials and methods

WP1 Design of sgRNA primers for Gene editing

The deletion of candidate genes involved with powdery mildew infection will be carried out using the CRISPR/Cas 9 system. CRISPR technology requires the design of the specific sgRNA primers that meet precise parameters in order to ensure accuracy during gene editing. This was achieved by using bioinformatic tools to identify potential secondary problems such as possibly off target modification sites in the genome (Bortesi and Fischer, 2015; Brazelton *et al.*, 2015; Zhang *et al.*, 2016). The initial step of sgRNA primer design was achieved using the software Geneious (Brazelton *et al.*, 2015; Naito *et al.*, 2015; Zhou, Wang and Liu, 2018; Wilson *et al.*, 2019). The algorithms used by Geneious 10 software was used to design potential sgRNA primers, using the reference genome 'Camarosa' obtained and MLO gene sequences from GDR (Jung *et al.*, 2014; Sargent *et al.*, 2019). Parameters such as on-target sequence scores were generated based on Doench *et al* algorithms and off-target scores based on Cho *et al* algorithms (Cho *et al.*, 2014; Doench *et al.*, 2014). To maximise specificity and accuracy, additional bioinformatics tools were applied in conjunction with Geneious (table 1). The frame-shifts at nuclease target sites caused by indels was determined with software developed by Bae *et al* - microhomology-mediated end joining. The use of this predictive tool can identify the frequency of microhomology associated deletions by determining the length of deletion and size of microhomology, to design primers with the lowest chance of frameshifts (Bae *et al.*, 2014). To avoid the potential of secondary structures in the sgRNA primers, the online tool vector builder was used (Mathews, Turner and Zuker, 2000) and off-target sites were identified using genome BLAST tools against the strawberry genome sequences held in the Genome Database for Rosaceae (GDR) (Jung *et al.*, 2014).

Table 1. Online bioinformatic tools

Web tool name	Website
Crisprater	https://crispr.cos.uni-heidelberg.de/ -
Microhomology	http://www.rgenome.net/mich-calculator/
Out of frame score	http://www.rgenome.net/mich-calculator/
Secondary structure	http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/Predict1.html
Secondary structure	https://en.vectorbuilder.com/tool/dna-secondary-structure.html
Cripsrdirect	https://crispr.dbcls.jp/
GDR	https://www.rosaceae.org/search/genes

HIGS cloning with TOPO kit

Table 2. PCR Conditions

PCR Conditions		
Initial Denaturation	98ºC	30 seconds
30 Cycles	98ºC 55ºC 72ºC	10 Seconds 30 Seconds 30 Seconds
Final extension	72ºC	7 Minutes
Hold	14ºC	

Table 3. TOPO Cloning Reaction (reagents in TOPO kit)

Reagent	Volume
Fresh PCR product	0.5 ul
Salt Solution	1 µL
Sterile Water	add to a final volume of 5 µl
TOPO® vector	1 µL
Final volume	6 µL

Polymerase chain reaction (PCR) was used to generate product matching a 400bp section of the MLO target genes. HIGS primers and 'Hapil' DNA were used as specified in the protocol in table 2 using Q5® High-Fidelity DNA Polymerase. The pENTR™/D-TOPO™ Cloning Kit was used for the cloning reaction performed as in table 3. The solution was then mixed gently and incubate for 5 min at room temperature. The TOPO cloning reaction was placed on ice ready for one shot competent *E. coli*. 2 µl of TOPO cloning reaction was added into a vial of competent *E. coli* and mixed gently. The solution was incubated on ice for 5 min, then heat-shocked for 30 sec at 42ºC without shaking. 250 µl S.O.C Medium (SOC = 1 ml SOB, 10ul 40% glycerol) was added and put on the shaker at 37ºC, 200 rpm for 1 hour. Then loaded onto LB medium with 50 µg/ml Kanamycin and incubate overnight at 37ºC. The next day 5 colonies were picked to confirm transformants.

WP2 Field trial

In year 1, five replicates of 350 different cultivars were collected from stock plants kept under polytunnel conditions (located at NIAB EMR, Kent). Propagation of experimental plants was achieved through the generation of misted tips. Individual runners were trimmed down to one leaf, pinned in module trays (Plantpak Jumbo Tray 54 Cell) containing 50 g of standard compost and grown in the glasshouse for 6 weeks with misting. After two weeks the misting was reduced and Universal Green nutrient feed was introduced. The plants were then transported to the field and planted in a randomised block design. Preliminary phenotypic data was obtained in October year 1 and more in-depth analysis of leaves, flowers and fruit in year 2 and 3. Phenotypic data will be obtained through disease symptom scoring on a five point scale (table 4) (Simpson, 1987).

Table 4. Strawberry powdery mildew disease symptoms (five point scale)

Score	Symptoms
1	No visual symptoms
2	Slight leaf curling, no visual mycelia
3	Leaf curling and mottling
4	Severe leaf curling, discoloration and visible damage to the leaf surface
5	Severe necrosis and partial leaf death

Genotypic data has been generated via DNA extractions using Qiagen DNAeasy plant mini extraction kit (Qiagen Ltd., Manches- ter, UK) and sequencing with Affymetrix Istraw35 Axiom® array (i35k) containing approximately 35,000 potential genetic markers (Verma *et al.*, 2017). This data will be used to conduct a genome wide association analysis in order to identify QTL associated with powdery mildew resistance. The disease phenotype will be calculated for each individual using the Area Under the Disease Progression Curve (AUDPC) (Package and Mendiburu, 2020) and auto spatial correlation will be assessed using the R package sPats (Cockram *et al.*, 2010). Best Linear Unbiased Predication (BLUP) will allow the prediction of genotypic scores for GWAS analysis (Cockram *et al.*, 2010). The analysis will be conducted for end-point disease scores, mean susceptibility scores and the AUDPC. To understand environmental influences, the AUDPC values can be used to look at genotypic and environment interactions on disease severity across blocks using a two way ANOVA (Sargent *et al.*, 2019). SNPs can be used to estimate genome-wide linkage disequilibrium (LD) using a customized R package such as rrBLUP to determine dominance and recessive patterns (Clarke *et al.*, 2011; Li *et al.*, 2019) and the PLINK to calculate association results (Clarke *et al.*, 2011).

WP3 Controlled pathogenicity inoculations

Powdery mildew isolates were collected from cultivated stock plants. Immature trifoliate leaves of resistant and susceptible cultivars were collected from polytunnels. Trifoliate leaves were surface sterilized with sodium hypochlorite (0.5%) for 3 min, 70% ETOH for 1 min and rinsed three times with sterile distilled water and dried on sterile blotting paper or under lamina flow hood. Single leaflets were placed three to a petri dish containing dampened sterile filter paper (Asalf, 2013).

Leaves containing mildew were collected and conidia transferred with a fine paint brush to detach conidia from leaves as described by Asalf *et al* (2014) (Asalf *et al.*, 2014, 2016) . Each selected conidium was placed on a leaflet incubated at 20^{o}C, 80% RH for seven days, 16 hour light (16L/8D h photoperiod). Observations were made over a daily time course for two weeks using a dissection microscope and fluorescent imaging.

Preliminary data was obtained through imaging of powdery mildew infection on upper and lower leaf surface using the red (640 nm), blue (513 nm) and green (572nm) light range with the Azure Biosystem 600.

Results

WP1 Design of sgRNA primers for Gene editing

In order to assess these genes for gene editing, tblastx was used to perform a BLAST search against the complete octoploid genome sequence of *Fragaria x ananassa cv Camarosa* from GDR (Jung *et al.*, 2014). The results demonstrate a 95% similarity to the reference genome, establishing a high evolutionary conservation of these genes in the *Fragaria* genome. Additionally, a BLAST search was performed using the Genome Database for Rosacea (GDR) for wild diploid *Fragaria vesca, F. nubicola, F. nipponica and F. innumae.* Results showed 95% conservation with all *Fragaria* diploid sub species (Jung *et al.*, 2014; GDR, 2019). Overall, these two genes are good candidates to go forward with gene editing.

In vitro micropropagation

Fragaria x ananassa cultivars 'Hapil' and 'Calypso' were chosen for transformation. 'Calypso' was selected for its high transformation rate (Wilson *et al.*, 2019) and 'Hapil' due to its susceptibility to mildew (Cockerton *et al.*, 2018). Plants are sub cultured and maintained on a monthly basis to generate the required numbers of plants for transformation.

To validate gene function the CRISPR/Cas 9 and host induced gene silencing (HIGS) approaches will be used**.**

CRISPR/CAS 9 cloning

Designed single guided RNA (sgRNA) (table 5), have been selected and synthesised by Eurofins.

Table 5. Parameters for selecting sgRNA primers

MLO candidate 1 vesca						
Name	**# Off-target Sites**	**Off-target Score**	**On-target Activity Score**	**Sequence**	**Paired CRISPR Score**	**PAM**
CRISPR guide 91	2 (0 in CDS)	82.68%	0.74	ATATTGCCGAAAACAAAGGAGGG	82.68%	GGG
CRISPR guide 25	1 (0 in CDS)	83.33%	0.543	AATATTGCCGAAAACAAAGGAGG	79.10%	AGG
CRISPR guide 5	1 (0 in CDS)	83.33%	0.564	ATATGGTTGTTAGAATACTGGGG	83.33%	GGG
MLO candidate 1 Camarosa						
Name	**# Off-target Sites**	**Off-target Score**	**On-target Activity Score**	**Sequence**	**Paired CRISPR Score**	**PAM**
CRISPR guide 48	2 (0 in CDS)	71.43%	0.74	ATATTGCCGAAAACAAAGGAGGG	82.68%	GGG
CRISPR guide 42	4(0 in CDS)	75.34%	0.543	AATATTGCCGAAAACAAAGGAGG	75.51%	AGG
CRISPR guide 36	6 (0 in CDS)	77.02%	0.564	ATATGGTTGTTAGAATACTGGGG	77.02%	GGG
MLO candidate 2 vesca						
Name	**# Off-target Sites**	**Off-target Score**	**On-target Activity Score**	**Sequence**	**Paired CRISPR Score**	**PAM**
CRISPR guide 12	1 (0 in CDS)	83.33%	0.499	AAGTGATTATAACCAAGCTGGGG	83.33%	GGG
CRISPR guide 63	2 (0 in CDS)	83.12%	0.209	AACCTCCCATTAATATAACATGG	82.89%	TGG
CRISPR guide 4	1 (0 in CDS)	83.33%	0.46	AAAAGGATATGATAAGTGCACGG	83.33%	CGG
MLO candidate 2 Camarosa						
Name	**# Off-target Sites**	**Off-target Score**	**On-target Activity Score**	**Sequence**	**Paired CRISPR Score**	**PAM**
CRISPR guide 47	4 (0 in CDS)	62.97%	0.499	AAGTGATTATAACCAAGCTGGGG	62.97%	GGG
CRISPR guide 93	5 (0 in CDS)	55.46%	0.273	AACCTCCCATTAATATAACATGG	57.89%	TGG
CRISPR guide 50	6(0 in CDS)	62.28%	0.46	AAAAGGATATGATAAGTGCACGG	58.91%	CGG
MLO candidate 1 vesca						
Name	**GC %**	**G or A**	**Microhomology Score**	**Out-of-frame Score**	**Efficiency score**	**Secondary structure**
CRISPR guide 91	43.5	Y	667.1	77.784	0.63 MEDIUM	△G:-0.50
CRISPR guide 25	39.1	Y	591.5	65.477	0.76 HIGH	△G:-0.50
CRISPR guide 5	39.1	Y	1018.4	66.04477612	0.75 HIGH	△G:0.00
MLO candidate 1 Camarosa						
Name						
CRISPR guide 48	43.5	Y	667.1	77.784	0.63 MEDIUM	△G:-0.50
CRISPR guide 42	43.5	Y	591.5	65.477	0.76 HIGH	△G:-0.50
CRISPR guide 36	39.1	Y	1018.4	66.04477612	0.75 HIGH	△G:0.00
MLO candidate 2 vesca						
Name						
CRISPR guide 12	43.5	Y	340.4	58.401	0.65 MEDIUM	△G:-1.10
CRISPR guide 63	34.8					
CRISPR guide 4	39.1	Y	1233.2	77.1326	0.61 MEDIUM	△G:0.00
MLO candidate 2 Camarosa						
Name						
CRISPR guide 47	43.5	Y	340.4	58.401	0.65 MEDIUM	△G:-1.10
CRISPR guide 93	34.8	Y	837.8	82.310814	0.61 MEDIUM	△G:-0.50
CRISPR guide 50	39.1	Y	1233.2	77.1326	0.61 MEDIUM	△G:0.00

HIGS cloning – HIGS cloning primers were designed to match approximately 400 bp of the MLO candidate genes using the Geneious 10 software, with an additional 4 bp CACC overhang, recommended for the pENTR TOPO cloning kit. Using 'Hapil' DNA a blunt end product was produced via PCR (Figure 1).

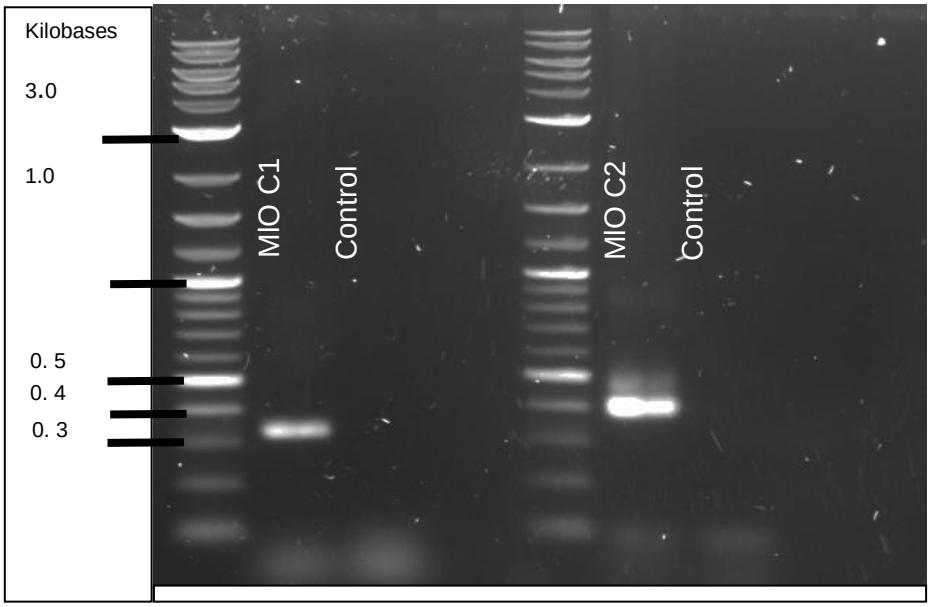

Figure 1. Agarose gel- MLO candiate 1 (C1) - 326 base pairs and MLO candidate 2 (C2) - 384 base pairs

The PCR product and pENTR/D-Topo vector was transformed into chemically competent *E. coli* cells. They were then plated overnight on LB/Kanamycin plates. Successful colonies were then selected and the plasmid extracted for PCR confirmation of transformation to ensure the vector containing the product has been successfully transformed. Successful candidates were sent off to Eurofins for sequencing.

WP2 Field trial

Preparation for the field study planting of 1750 strawberry plants in the field for disease symptom analysis in year 2 and 3, to provide phenotypic data for a genome wide association study.

WP3 Controlled pathogenicity inoculations

Controlled pathogenicity experiments to investigating different tissue susceptibility. Initial experiments have refined a protocol from Belachew *et al* (Asalf, 2013) to perform pathogenicity experiments investigating tissue susceptibility e.g. leaves, of different known resistant and susceptible cultivars.

Results showed that the powdery mildew fungi did not auto-fluoresce in the red range, the green range showed the mildew highly auto-fluoresced. The blue range showed partial auto-fluorescence suggesting certain aspects of the mildew fluoresce in this range, future investigate will be required to identify these possible components in mildew (Figure 2). It was also shown that the lower surface of the infected leaf had a higher level of fluorescence; this may be linked to the fact that mildew is believed to infect the lower leaf more efficiently (Asalf *et al.*, 2014).

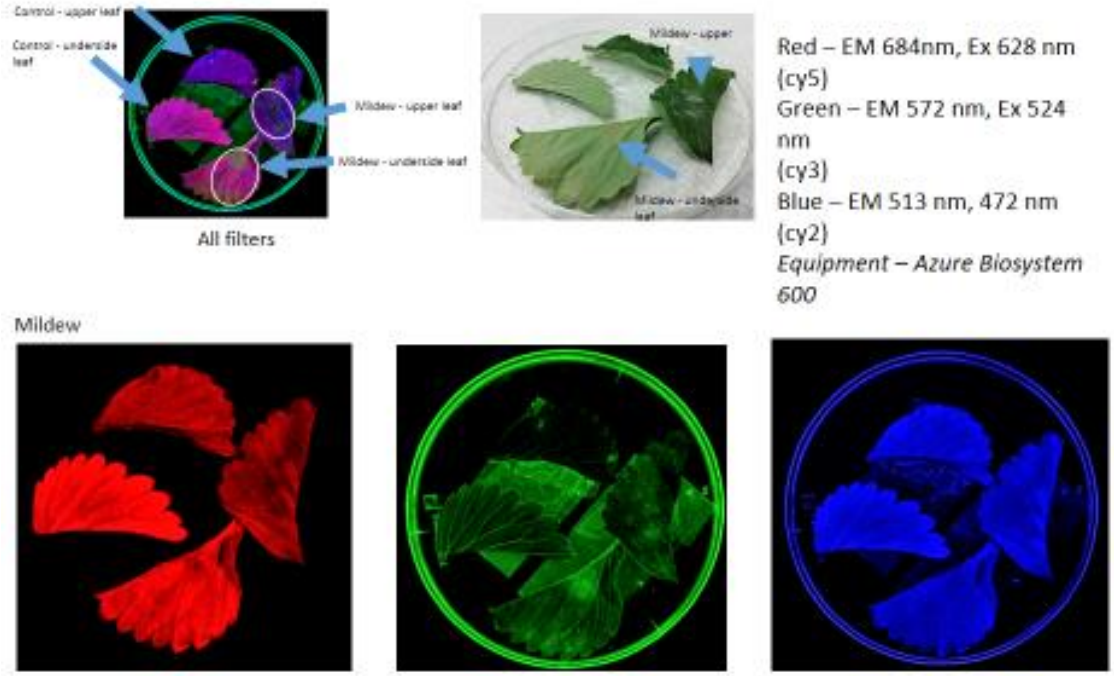

Figure 2. Imaging data of powdery mildew in strawberries

Discussion

WP1 Design of sgRNA primers for Gene editing

Two candidate genes were identified from the literature for gene editing and silencing. The two genes were compared to the octoploid 'Camarosa' and diploid vesca sequences obtained from GDR (Shulaev *et al.*, 2010; GDR, 2019) genome and determined the candidate genes were highly conserved within the Fragaria genus and good candidates for gene editing. Primers for both the CRISPR/Cas 9 and HIGS systems have been designed to run both methods in tandem to validate gene function of the MLO genes.

WP2 Field trial

Year 1 of this project has been preparing 1750 strawberries cultivars for the filed trial. The strawberries were planted in the field in August and preliminary phenotyping will begin in October 2020 with data collection on fruit, leaves and flowers in Year 2 and 3.

WP3 Controlled pathogenicity inoculations

Preliminary data shows autofluorescence in the powdery mildew at the 512 nm wavelength, this work will be followed up with a more in depth analysis that will focus on early development. Future work will look at powdery mildew developmental attributes and compare with known resistant and susceptible cultivars to determine if there is any tissue specificity.

Conclusions

- sgRNA primers and HIGS primers designed and synthesised.
- HIGS - PCR product inserted into vector and ready for the next stage of transformation into strawberry
- Field study planted and ready for assessment in Year 2 and 3
- Controlled pathogenicity inoculations achieve however preliminary data requires further analysis

Knowledge and Technology Transfer

BCPC meeting – presentation and poster

AHDB PhD conference - presentation

CTP conference - presentation x2

University of Reading PhD symposium - presentation x2

NIAB EMR department meeting – presentation x2

NIAB EMR PhD meeting – presentation

AHDB fruit day - poster

Glossary

AFLP – Amplified Fragment Length Polymorphism

DMIs - Sterol Demethylation Inhibitors

DNA – Deoxyribonucleic Acid

ETI – Effector Triggered Immunity

GDR - Genome Database for Rosacea

GWAS – Genome Wide Association Study

HIGS - Host Induced Gene Silencing

QLT - Quantitative Trait Loci

MLO - Mildew Loci 0

NHR – Non Host Resistance

PAMPs - Pathogen-Associated Molecular Patterns

PRR – Pattern Recognition Receptors

R gene – Resistance gene

RAPD – Random Amplified Polymorphic DNA

RFLP – Restriction Fragment Length Polymorphism

S gene – Susceptibility Gene

sgRNA – Single Guided RNA

SNP – Single Nucleotide Polymorphism

SSR – Simple Sequence Repeats

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