Project title:	Identifying novel powdery mildew susceptibility/resistance genes in strawberry.
Project number:	
Project leader:	Dr Helen Cockerton
Report:	Report 2 10/2021
Previous report:	10/2020
Key staff:	Professor Jim Dunwell
Location of project:	NIAB EMR
Industry Representative:	Harriet Dunclafe
Date project commenced:	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 disease symptoms (*Podosphaera aphanis*) to identify key mildew resistance/susceptibility genes using a Genome Wide Association Study (GWAS).

Background

Strawberries are the major soft fruit produced in the UK, favored for its sweet fragrant taste. In addition to the strawberry containing compounds that have 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 yield. However a warmer, more humid environment the 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 by regulations, as some products such as Brupirmate, Penconazole or Proquinazid can only be applied a few times a year. The restriction on applications reduces 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 one mode of resistance

that has occurred is caused by to mutations in the CYP51 gene (Sombardier *et al.*, 2010).

Summary

The first year of this PhD required the propagation of 350 different strawberry cultivars. Five replicate plants of each cultivar were propagated and transferred into the field. Genotypic data will be established through DNA extractions from each cultivar and genotyping will be conducted using the istraw35 Affymetrix SNP chip. Year 2 has consisted of collecting phenotypic data on leaves, year 3 will include phenotyping of the fruit and flowers. Phenotypic data has been gathered through scoring of disease symptom 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. Resistance genes are frequently found within QTL regions (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 powdery mildew were identified. Bioinformatics tools were used to establish the evolutionary conservation of Mildew loci 0 (MLO) 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. Using different methods of gene editing these genes will be disrupted to alter gene function and will be validated with pathogenicity tests.

Financial Benefits

No recommendations at this stage

Action Points

No action points are available at this stage of the project

SCIENCE SECTION

Introduction

Plants have evolved three main types of defenses 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 (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, some R genes have direct interaction with effectors whereby they can completely incapacitate the effectors (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 onto 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 (S) 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

The first year focused on propagating 350 strawberry genotypes with five replicates for planting into the field. Additional plants infected with powdery mildew have been introduced to ensure inoculation of the disease over the course of the trial. Disease symptom analysis has been conducted in year 2 for foliage and will be conducted in year 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 is being conducted out using the CRISPR/Cas 9 system. CRISPR technology requires the design of the specific sgRNA primers that meet precise parameters 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). As well as additional online bioinformatic tools for more accurate design (Table 1).

Table 1. Online Bioinformatic tools

Web tool name	Website
Crisprater	https://crispr.cos.uni-heidelberg.de/ -
Micorhomology	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

Initial Denaturation	98°C	30 seconds
30 Cycles	98°C 55°C	10 Seconds 30 Seconds

	72°C	30 Seconds
Final extension	72°C	7 Minutes
Hold	14°C	

Table 3. Topo Cloning Reaction (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 detailed in Table 3. The solution was then mixed gently and incubated for 5 min at room temp. 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 42 °C without shaking. 250 µl S.O.C Medium was added and put on the shaker at 37 °C, 200 rpm for 1 hour. The transformed E. coli was then loaded onto LB medium plates containing 50 µg/ml Kanamycin and incubated overnight at 37 °C. The next day 5 colonies were picked to confirm transformants. Successful colonies were stored in 50% glycerol and kept at -80 °C.

In vitro micropropagation

Fragaria x ananassa cultivars 'Hapil', 'Calypso' and Hawaii 4 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). Strawberry plants for transformations are sub-cultured and maintained monthly to generate the required numbers of plants for transformation.

Strawberry transformations

Selected colonies were grown overnight in low salt LB media with antibiotics. In sterile conditions young leaves were collected and submerged in inoculum for 15 mins. Leaves were removed scored and left to dry on blotting paper, then placed on shoot regeneration media (SRM – MS medium supplemented with α-naphthaleneacetic acid (NAA) and thidiazuron (TDZ). The pH was adjusted to 5.8, Agargel was added before autoclaving, then supplemented with filter sterilised glucose) plates and placed in darkness for 4 days. Leaves were then washed and placed on fresh SRM media with antibiotics and left to produce callus. Plates are replaced with fresh media every three weeks.

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 grams 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. Data on leaves was collected in July, August, and September in year 2. Year 3 will incorporate flower and fruit assessments. Phenotypic data was obtained through disease symptom scoring on a five point scale (Table 4) (Simpson, 1987).

Table 4. Strawberry powdery mildew disease symptoms five-point scale

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 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 PLINK will be used 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 minutes, 70% ETOH for 1 minute and rinsed three times with sterile distilled water and dried on sterile blotting paper or under the 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 placed on a leaflet. Incubation was performed at 20 °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.

Results

WP1

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.

The validation of gene function with be established by employing CRISPR/Cas 9 and host induced gene silencing (HIGS) techniques**.**

Table 5. Parameters for selecting sgRNA primers

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

CRISPR/CAS 9 cloning

The design of single guided RNA (sgRNA) (Table 5) has been determined using the Genious program (as described in the Methods). The selected sgRNA were sent for synthesising by Eurofins. Using the golden gate approach level 1 components (Cas9, antibiotic resistance, sgRNA) were individually incorporated into vector backbone using restriction enzymes. The next step will be to assemble level 2 which incorporates all components into one vector ready for strawberry transformations. Once introduced into the strawberry the genes of interest will be disrupted and gene function will be lost. Validation of the gene function will be established with pathogenicity tests.

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).

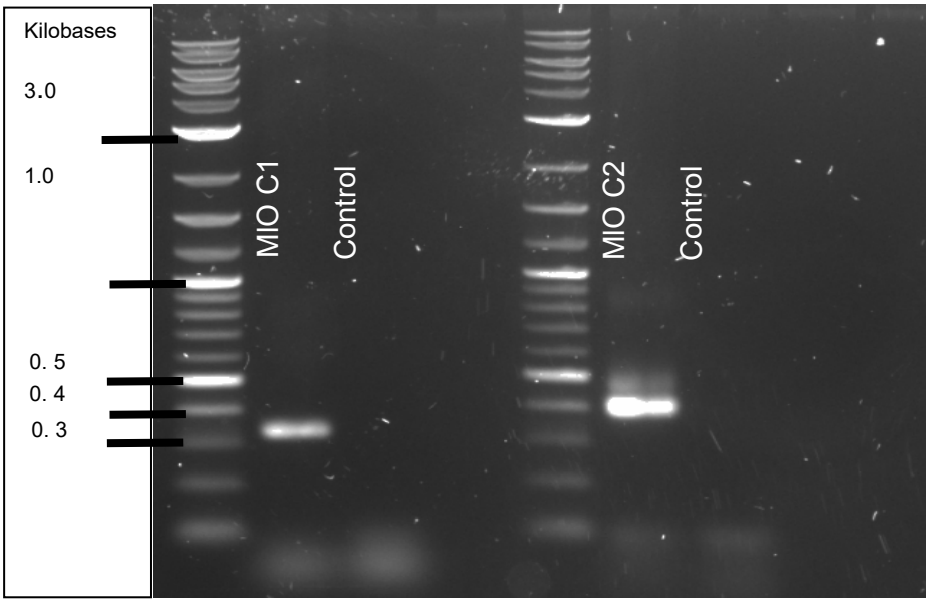


Figure 1. Agarose gel- MLO candidate 1 (C1) - 326 base pairs and MLO candidate 2 (C2) - 384 base pairs

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 to Eurofins for sequencing.

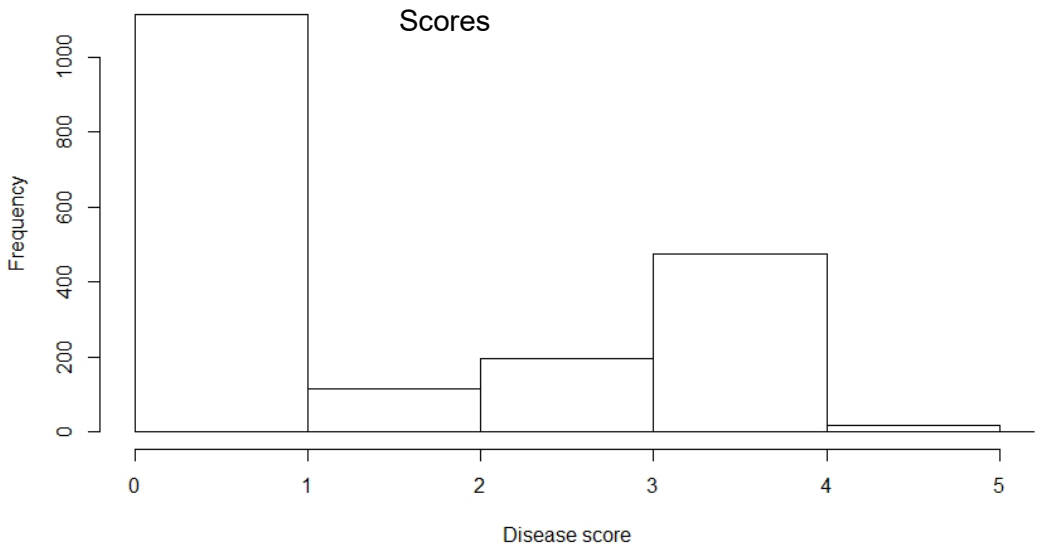
Selected cultures were grown overnight and transformed into young strawberry leaves. Leaves are currently being regularly sub plated waiting for regeneration of calli.

WP2

Field experiment

Year 1 of the field study planted 350 strawberry plants with five replicates in a randomized design for field for disease symptom analysis in year 2. This data has provided phenotypic data for a genome wide association study to identify genes associated with powdery mildew.

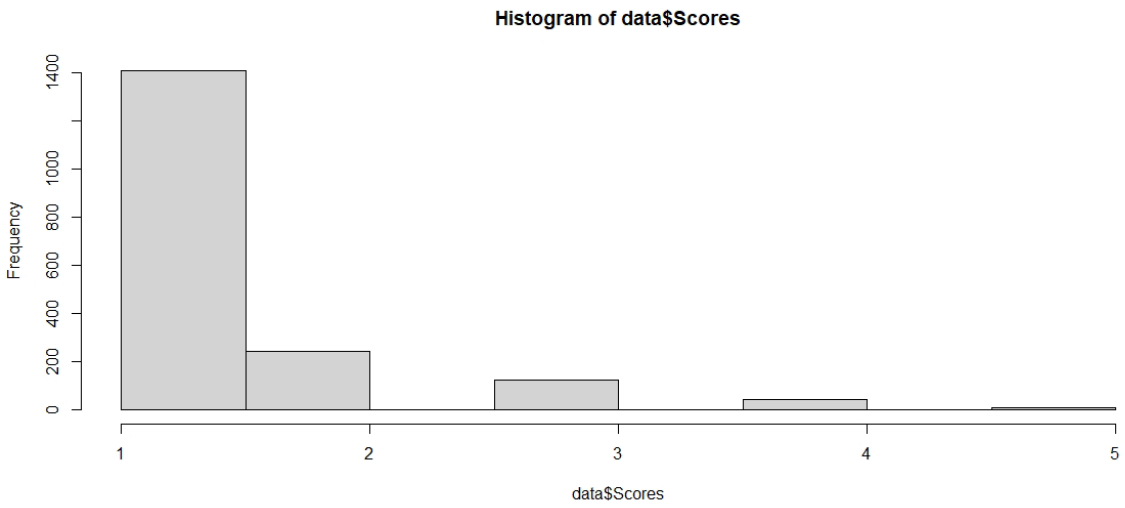
The strawberries were planted in the field in August and preliminary phenotyping in October 2020 (Graph 1).



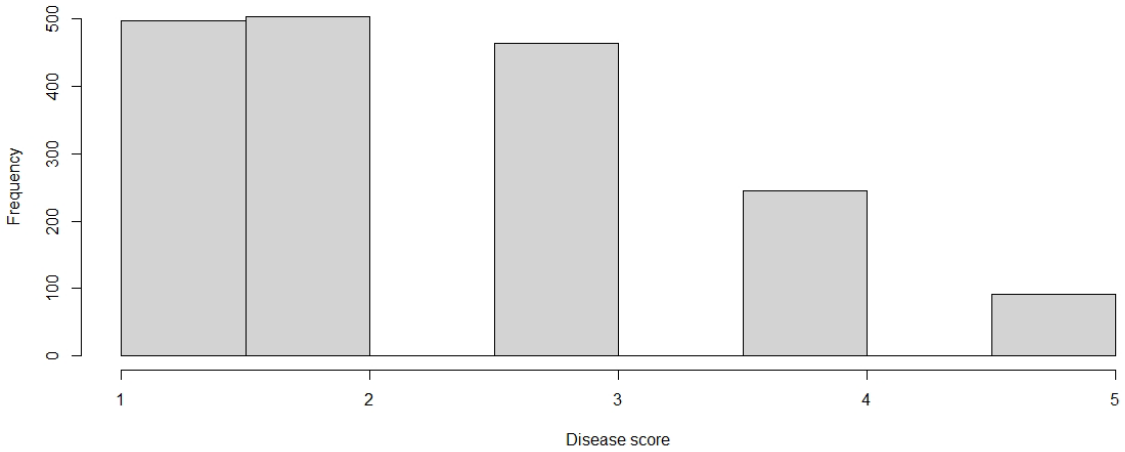
Graph 1 – Histogram of phenotypic scores from October 2020 after initial planting in August.

Year 2 assessments were performed in July, August, and September. Graph 2 shows mild mildew symptoms with less than 50% coverage within the field, though there is a significant rise when compared to August in graph 3. With 70% showing powdery mildew symptoms and a rise in severe leaf curling (scale 4) and leaf necrosis (scale

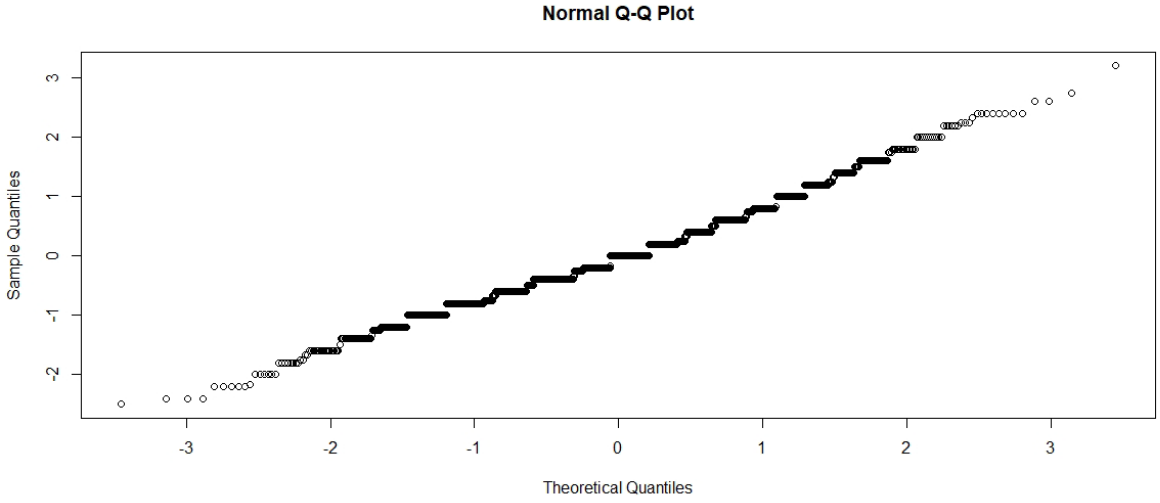
5) Statistical analysis with a QQ plot shows data has normal distribution of cultivars (graph 3). The Anova (table 6) with a F value score of 3.273 and low P<0.0001 score indicates a very significant genetic link to the powdery mildew.



Graph 2 – Histogram of phenotypic scores for July



Graph 3 – Histogram of phenotypic scores from August 2020.



Graph 4 – QQ plot from July 2020

Table 6. One way ANOVA

```
             Df Sum Sq Mean Sq F value Pr(>F)
Cultivar    381   1247   3.273   3.783 <2e-16 ***
Residuals  1411   1221   0.865
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
127 observations deleted due to missingness
```

WP3

Controlled pathogenicity experiments

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.

Leaves were collected from 'Hapil', 'Cultivar 1' and 'Cultivar 2'. Each cultivar with five replicate leaves were inoculated and one non-inoculated control. Cultivars were assessed for the presence of the SNP Affx.88882971 which has previously been associated with powdery mildew disease resistance in strawberry (Cockerton et al., 2018) suggests one cultivar was homozygous resistant and two heterozygous susceptibility (Table 7) [51]. The leaves were placed into a randomized block design in a tray and inoculated (Table 8).

Table 7. Cultivars used in the preliminary path test

Cultivar	Mildew Status based on SNP
Hapil	Heterozygous Susceptibility
Cultivar 1	Heterozygous Susceptibility
Cultivar 2	Homozygous Resistant

Table 8. Randomized design for the pathogenicity experiment (C1- cultivar 1, C2 – cultivar 2)

	1	2	3	4	5	6
A	Hapil	C2	C1 Control	Hapil	C2 Control	C1
B	C2	Hapil Control	C2	C1	C1	Hapil
C	C1	C1	Hapil	C2	Hapil	C2

Table 9. Results of pathogenicity experiment – the position and cultivar names for replicates fungal conidia chains

Cultivar	Mildew infection	Infected replicate numbers
C2	60 %	1B, 2A, 3B, 5A
C1	10 %	4B
Hapil	2 %	6B

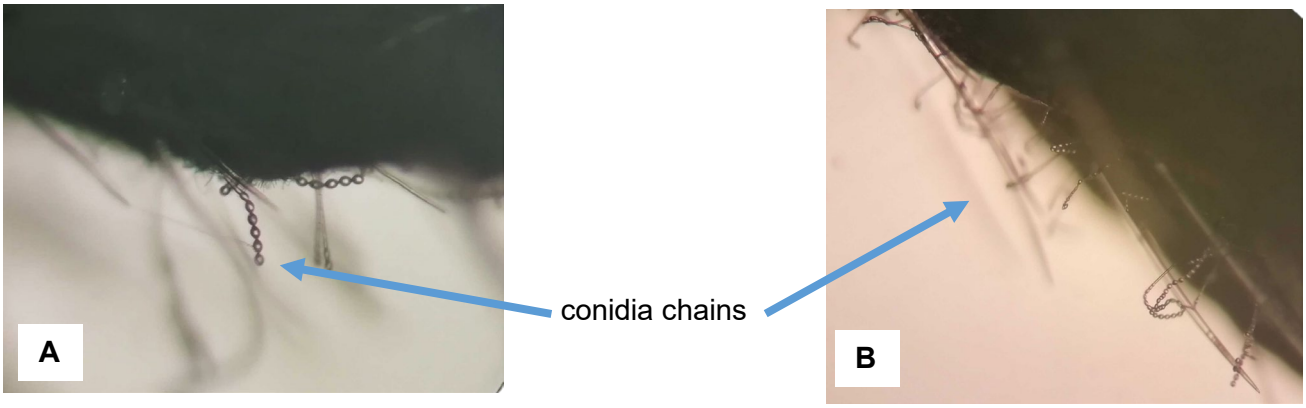


Figure 2a shows microscope image of cultivar 1B with conidia chains, **2b** shows image of cultivar 2A with conidia chains.

Discussion

WP1

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

Preliminary results showed presence of powdery mildew symptoms, even at such early stages of planting

Year two data collection of the leaves has been performed in July, August, and September. With data collection on fruit, leaves, and flowers in Year 3.

Results show that mid-summer phenotyping has significant presence of powdery mildew within the trial. With the QQ plot revealing residues are normally distributed with statistical analysis. A preliminary analysis using a one-way ANOVA revealed a very significant genetic component influencing powdery mildew infection level.

WP3

The results for the preliminary path test showed that only one replicate from each block was found to have conidia chains (Figures 2a and 2b). In the blind test the numbers correspond to the cultivars in the tray (as seen in the layout in Table 9) - 1B, 2A, 3B and 5A (Table 10) with conidia chains observed were Cultivar 2. Results are interesting and further analysis is required with consideration of tissue specificity.

Future work will look at powdery mildew developmental attributes and compare known resistant and susceptible cultivars. In addition to determine if there is any disease tissue specificity by utilizing different advanced imaging techniques.

Conclusions

- sgRNA level 1 ready to construct golden gate level 2 ready for strawberry transformations.
- HIGS constructs are transformed into strawberry and awaiting growth of callus
- Year 2 phenotyping on leaves completed. Field study assessment for Year3 will include flower and fruit phenotyping.
- Controlled pathogenicity inoculations on a range of different cultivars

Knowledge and Technology Transfer

BCPC meeting – presentation and poster

AHDB PhD conference - presentation

CTP conference - presentation x3

University of Reading PhD symposium - presentation x4

NIAB EMR department meeting – presentation x2

NIAB EMR PhD meeting – presentation x2

AHDB fruit day - poster x2

AHDB soft fruit presentation

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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