Studentship Project: Annual Progress Report November 2022 to November 2023

Student Name:	**Samantha Lynn**	**AHDB Project Number:**	**SF/TF 170/a**
Project Title:	Identifying Novel Powdery Mildew Susceptibility and Resistance Genes in Strawberry		
Lead Partner:	NIAB		
Supervisor:	Dr Helen Cockerton		
Start Date:	23/09/19	**End Date:**	23/09/23

1. Project aims and objectives

Work package 1: 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 on the foliage and in year 3 on the foliage, fruits and flowers. Phenotypic data will allow assessment of tissue specific disease resistance alleles via a Genome Wide Association Study (GWAS).

Work package 2. Functional validation of candidate susceptibility factors. This project will use the CRISPR/Cas9 gene editing system and Host Induced Gene Silencing (HIGS) to delete/silence candidate susceptibility genes. The tranformants disease phenotypes will be assesed through pathogenicity tests in order to validate gene function. The premise of this project is that by inactivating the strawberry genes associated with powdery mildew interaction we will be able to generate a disease resistant plant.

Work package 3: RNA Sequencing. Investigation of differences in RNA expression during powdery mildew infection.

2. Key messages emerging from the project

- Identify genes associated with powdery mildew disease resistance
- Identify changes in RNA expression during disease infection
- Validate MLO gene function

3. Summary of results from the reporting year

WP1 GWAS Mildew field experiment

In year 2 disease assessments were performed in July, August and September. Figure 1a shows mild mildew disease symptoms were observed in July, with less than 50% mildew coverage within the field. There is a substantial rise in disease symptoms observed in September in figure 1c. With 70% of plants showing

powdery mildew symptoms and a rise in severe leaf curling (scale 4) and leaf necrosis (scale 5). The ANOVA (Table 1) indicates a very significant genetic component associated with powdery mildew disease resistance (*F* value = 3.273; $p<0.0001$).

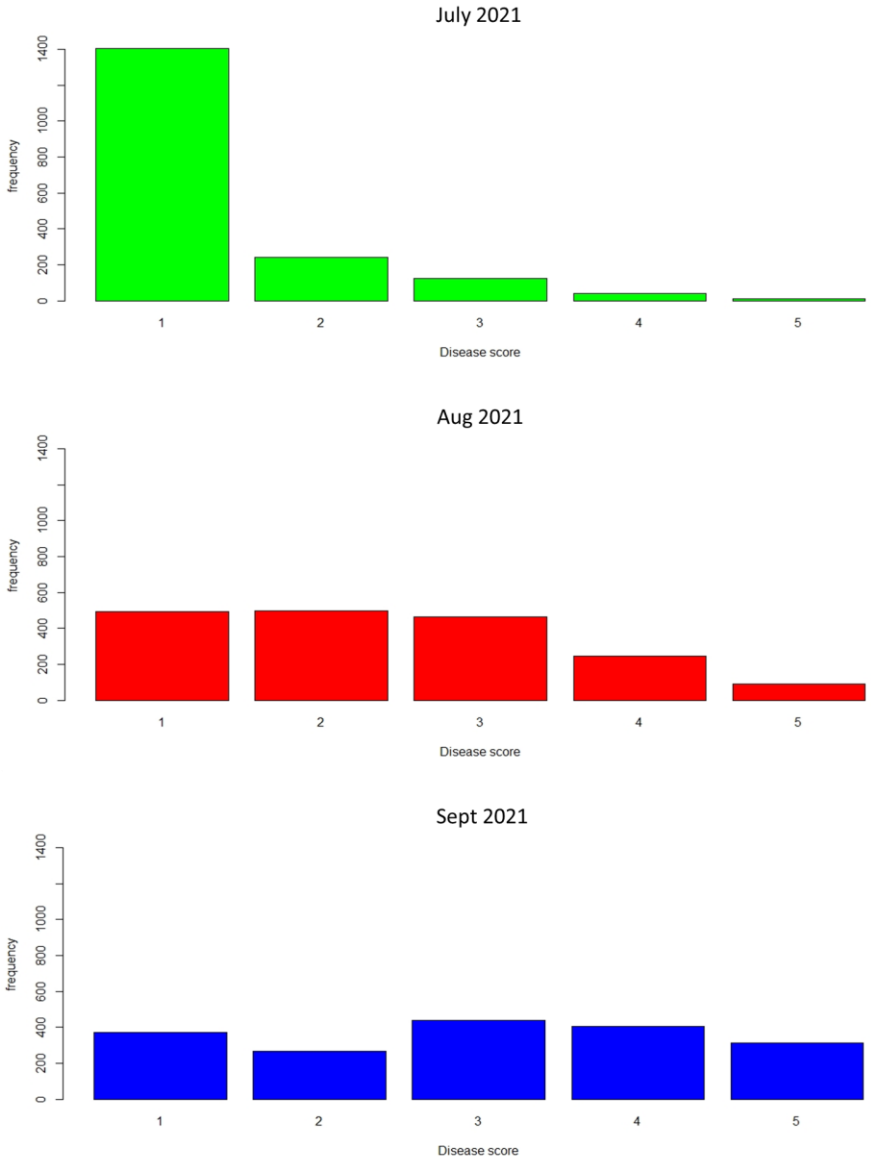

Figure 1. Frequency histogram of disease scores assessed in summer 2021 – 1a July, 1b August and 1c September

ANOVA						
	Df	Sum Sq	Mean Sq	F Value	Pr(<F)	
Cultivar	381	1247	3.273	<2e-16	***	
Residuals	1411	1221	0.865			

Signif. Codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '	1

Table 1. Analysis of Variance (ANOVA) of strawberry powdery mildew disease score data to determine the relative influence of strawberry genotype on disease phenotype.

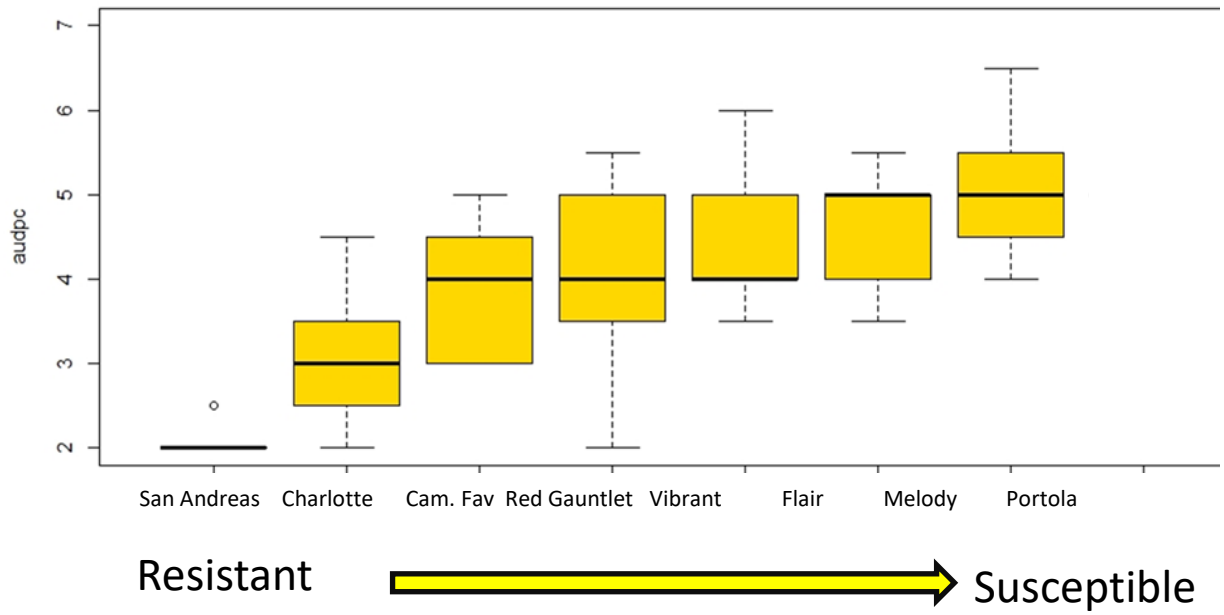

Figure 2. Box plot showing individual cultivar resistance/suspceptibity to Powdery Mildew

The Box plot in figure 2 shows collection of individual cultivars exhibiting a range of phenotypes: from resistance to suseptibility. The seasonal data for 2023 has been collected and the GWAS analysis is currently being processed.

WP2 Functional validation of candidate susceptibility factors

Candidates were identified to take forward for gene editing.

The validation of gene function with be established by employing CRISPR/Cas 9 and Host Induced Gene Silencing (HIGS) techniques**.**

CRISPR/CAS 9 cloning

The design of single guided RNA (sgRNA), has been determined using Genious. The selected sgRNA were synthesisied by Eurofins. The golden gate approach level 1 components (Cas9, antibiotic resistance, sgRNA) were incorporated into a destination vector using restriction enzymes. The next step will be to transform the vector into strawberry. Once introduced into strawberry the genes of interest will be disrupted and gene function will be lost. Validation of the gene function will be established using pathogenicity experiments.

HIGS cloning

HIGS cloning primers were designed to match approximately 400 bp of the candidate genes using the Geneious 10 software, with an additional 4 bp CACC overhang recommended for the pENTR TOPO cloning kit. A blunt end product was produced via PCR using 'Hapil' DNA. The PCR product and pENTR/D-Topo vector were combined and transformed into chemically competent *E. coli* cells. The *E.coli* were then plated on LB including Kanamycin and plates were grown overnight. Successful colonies were then selected, and the plasmid extracted for PCR confirmation of transformations to ensure the vector containing the product has been successfully transformed into agrobacterium.

Agrobacterium cultures containing constructs were grown overnight and transformed into a selection of strawberry cultivars. These leaves are currently being regularly sub-cultured until regeneration of calli is observed.

WP3 RNA sequencing

Strawberry RNA was extracted and sent to Novogene for sequencing, the data is currently being analysed.

4. Key issues to be addressed in the next year

Data Analysis

5. Outputs relating to the project

(events, press articles, conference posters or presentations, scientific papers):

Output	**Detail**
Growers Magazine	**Short article about project**
SocBio Conference	**Poster and Presentation**
East Kent Fruit Society	**Presentation**
BSPP Conference	**Poster**
SCI Meeting	**Presentation**
CTP Event x2	**Presentation**
CDT Conference	**Presentation**

6. Partners (if applicable)

Scientific partners	Prof Jim Dunwell
Industry partners	Harriet Dunclafe
Government sponsor	