Generating New Resources and Pilot Data for Novel Strategies to Increase Future Food Security

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Aims

To contribute to research on certain proteins and small molecular modulators impact's on a plant's water use, disease resistance and photosynthetic behaviour.

Project 1: Identify transgenic *Arabidopsis thaliana* lines to investigate the molecular basis of a strategy to improve crop water use efficiency (WUE).

Project 2: Contribute to proof-of-concept data for the development of treatment strategies for *Botrytis* infection.

Project 3: Access photosynthetic behaviour in Cyt *b*561 T-DNA mutants through Chlorophyl fluorescence imaging.

Introduction

In the light of rapid population growth and ever-increasing environmental pressures, using plant biotechnology innovation to improve agricultural practises is becoming increasingly important. This project focuses on contributing to research that could potentially reduce water and broad-spectrum fungicides use, whilst increasing crop yields, improving both agricultural productivity and sustainability

Project 1: Lopez et al. (1) found recently tobacco plants with increased levels of SBPase activity combined with the expression of the algal electron transport protein CytC6 displayed increased carbon assimilation levels and water use efficiency (WUE). Thus, to investigate the possibility of replicating this phenotype in other species and growth conditions, these genes were transformed into *Arabidopsis thaliana* plants. Using selective media and PCR screening, the project aimed to identify successful transformants for future phenotype analysis.

Project 2: Research has found Phytosulfokine signalling, such as PSKR1, can induce immunity to *Botrytis cinerea* (2), a grey mould causing crop losses worth $10-100 billion a year(3). The project aimed to carry out RNA extraction and cDNA synthesis on samples from an assay prepared, based on the work of Suarez et al. (4), to evaluate the effect of the selected small molecular PSKR modulators on tomato leaf disks.

Project 3: The photosynthetic behaviour of Cyt *b*561 T-DNA mutants was investigated using Chlorophyl fluorescence imaging.

Results and Discussion

Project 1

Selective media was used to identify positive transformants, as seen in Figure 1, with two circled in plate B and A showing no positives. Overall, 21 positive transformants were identified, to undergo PCR screening and be run on 0.8% agarose gels (Figure 2). The figure confirms RE2 203.1-.3 as transgenic lines, however, RE2 204.4 was not confirmed lacking the ~3kb band. The faint bands at ~1Kb for RE2 203.1-3 and RE2204.4 also suggest the primers may have bound elsewhere; confirming these as escapes. Increasing the annealing temperature in the reactions might decrease these unspecific bands.

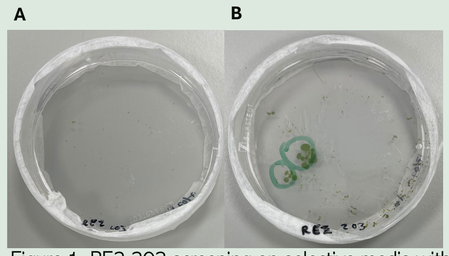

Figure 1. RE2 203 screening on selective media with hygromycin.
A had no positive transformants. B had two positive transformants (circled).

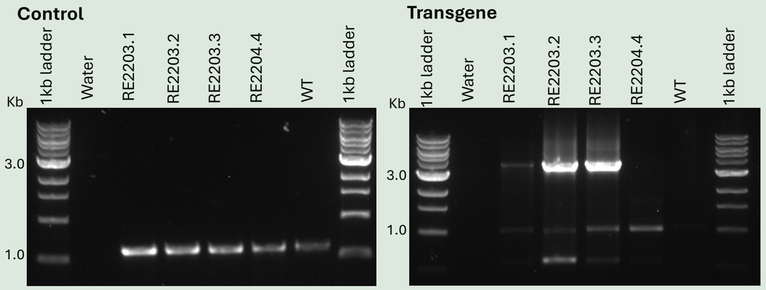

Figure 2. The molecular screening of CYB561 mutants for the gene of interest
The 0.8% agarose gels with SYBR Safe DNA stain and the New England Biolabs 1kb DNA ladder N32325 for a control and transgene PCR of samples RE2203.1-3 and RE2204.4.

Project 2

RNA extractions were performed on 26 tomato leaf samples for future RT-qPCR analysis with their concentrations and purity being measured using a NanoDrop Spectrophotometer. This is shown in Figure 3, with an image of the quantitative analysis of the CF Bot 1.1 RNA extraction given by the Spectrophotometer. In addition to this, a 0.8% agarose gel was run to visualise a random selection of samples, as shown in Figure 4. The figure shows bands in all lanes thus indicating successful RNA extraction has occurred. Finally, selected samples underwent cDNA synthesis for future analysis.

Figure 3. Quantitative analysis of CF Bot 1.1 RNA extraction
The NanoDrop Spectrophotometer readings of the RNA extraction of CF bot 1.1.

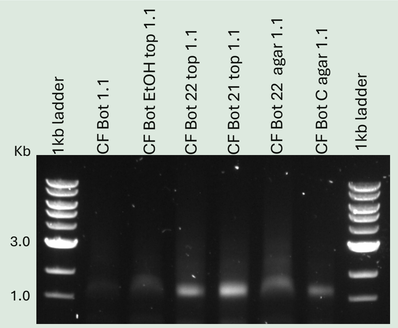

Figure 4. The RNA extraction of *Costoluto fiorentino* samples under varying pressures
The 0.8% agarose gels with SYBR Safe DNA stain and the New England Biolabs 1kb DNA ladder N32325 and samples were loaded with Gel Loading Dye Purple 6x B7024a.

Project 3

Project 3 involved the growth of Cyt *b*561 T-DNA mutants for the collection of raw data using Chlorophyll fluorescence imaging techniques overtime. This data was then combined using R-studio techniques to undergo future analysis, determining the mutant's photosynthetic activity overtime.

Summary

Project 1. 21 *Arabidopsis thaliana* positive transformants were identified with selective media screening, 5 of which were confirmed with PCR screening. These plants can now be used for future phenotypic analysis of the mechanism responsible for changes in intrinsic Water Use Efficiency (iWUE).

Project 2. 26 tomato leaf samples underwent RNA extractions and cDNA synthesis which can be used for future qPCR analysis of small molecular modulators' effect on tomato's response to *Botrytis* Infection.

Project 3. Raw data was collected on the photosynthetic activity of Cyt *b*561 T-DNA mutants' overtime, using Chlorophyl fluorescence imaging techniques, contributing to the understanding of the photosynthetic behaviour of the mutants.

Thereby, contributing to research developing more sustainable, productive farming practises for more resilient crops, whilst reducing water and fungicide use.

References

1) López-Calcagno, P. et al., 2020. Stimulating photosynthetic processes increases productivity and water-use efficiency in the field. Nature Plants volume, Volume 6, p. 1054–1063. **2)** Zhang, H. et al. A Plant Phytosulfokine Peptide Initiates Auxin-Dependent Immunity through Cytosolic Ca^{2+} Signalling in Tomato. The Plant Cell 30, 652-667 (2018). **3)** Poveda, J., Barquero, M. & González-Andrés, F., 2020. Insight into the Microbiological Control Strategies against *Botrytis cinerea* Using Systemic Plant Resistance Activation. MDPI-Agronomy, 10(11), p. 1822. **4)** Suarez, M. B. et al. Development of real-time PCR (TaqMan (R)) assays for the detection and quantification of *Botrytis cinerea* in planta. Plant Physiology and Biochemistry 43, 890-899 (2005).