Studentship Project: Annual Progress Report 10/2021 to 10/2022

Student Name:	**William Atkinson**	**AHDB Project Number:**	**SF/TF 170a**
Project Title:	**Manipulating stomatal density to improve carbon assimilation in strawberry**		
Lead Partner:	NIAB East Malling		
Supervisor:	Dr Andrew Simkin, Dr Mark Else, Prof. Tracy Lawson		
Start Date:	4/10/21	**End Date:**	4/10/25

1. Project aims and objectives

This project aims to improve the carbon assimilation ability of strawberries and asses the effects on both plant and fruit physiology, by using transgenic approaches to manipulate stomatal density and stomatal movement kinetics. Increasing stomatal density, previously shown to increase photosynthetic carbon assimilation, will be achieved using two different approaches: the overexpression of the gene stomagen, and the targeted knockout of stomagen antagonist genes EPF1 and 2. Stomagen, EPF1 and EPF2 competitively bind to the same receptor, with stomagen promoting stomatal development and, EPF1 and EPF2 acting to inhibit stomatal development. Altering the expression of these genes will change the balance of interactions with the receptor, through increasing stomagen or decreasing EPF1 and EPF2 will increase stomatal density. Stomatal closure and opening can be sped up through the specific overexpression of the genes Hexokinase and AHA2, respectively, in the guard cells that comprise the stomata. Overexpressing hexokinase in the guard cells has been shown to accelerate stomatal closure, which led to a reduction in water loss without compromising photosynthesis. Overexpressing AHA2, a membrane-bound H+ATPase, in the guard cells resulted in greater stomatal conductance due to accelerated opening of the pore. Improving stomatal movement kinetics will allow for superior response times to environmental factors so that carbon gain and water loss can be better optimised.

I aim to produce plants with a range of increased stomatal density, plants with improved stomatal movement kinetics and finally plants with both increased stomatal density and improved movement kinetics. The effects of the changes on both plant and fruit physiology will be assessed by examining factors such as gas exchange, crop yield and fruit quality.

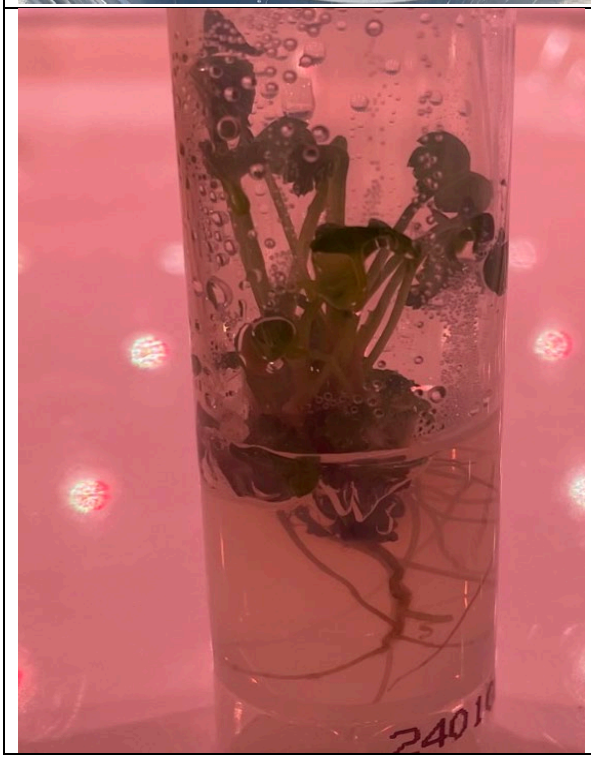
2. Key messages emerging from the project

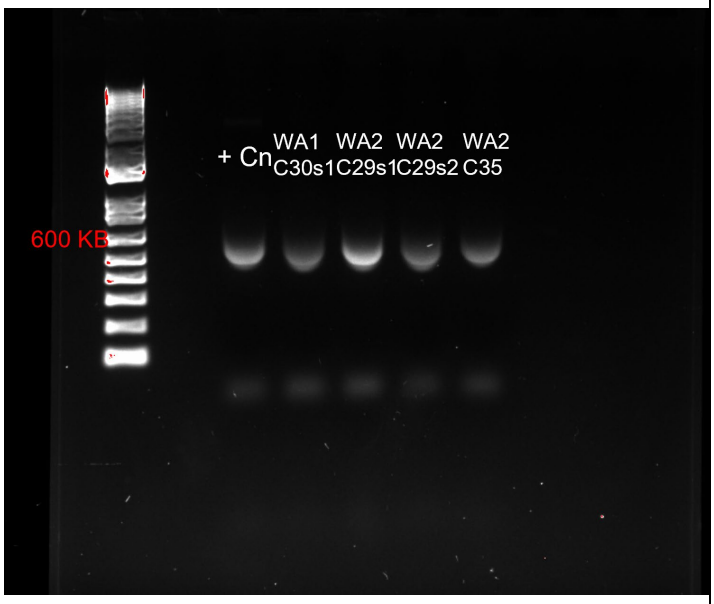
Strawberries are an economically and nutritionally important crop in the UK. Increasing photosynthetic carbon assimilation remains one of the last vestiges for improving crop productivity, and nutritionally important crops, such as fruits and vegetables, have mostly been neglected from this area of research in favour of grains. CO_2 enrichment studies on strawberries, which can be used as a proxy for improving photosynthetic carbon assimilation, have shown not only increased yields, through increased fruit number and size, but also

increased quality of the fruit through increasing the proportion of reducing sugars and thus taste, as well as nutritional quality from increased levels of vitamin C. The improvement of strawberries using transgenic manipulation of stomata has the potential to produce similar improvements.

3. Summary of results from the reporting year

Gene constructs for Stomagen, Guard cell-specific Hexokinase and Stomagen combined with Guard cell-specific Hexokinase overexpression have been constructed and transformed into Strawberry. These plants are at varying stages of positive selection, with Stomagen and Hexokinase individual overexpressions successfully passed through positive selection, as confirmed by PCR against the kanamycin resistance gene they all harbour on extracted plant DNA. The design of guide RNAs for the targeted knockout of EPF1 and 2 for CRISPR/Cas9 gene editing has been completed and assembly of the final gene editing constructs has begun. Assembly of additional constructs containing the H+ATPase, AHA2, are also underway.





4. Key issues to be addressed in the next year

Cloning of remaining constructs and potential new ones. Successive rounds of strawberry transformation to produce putative transgenic plants for remaining and future constructs. Transgenic plants must be weened from in-vitro cultures to growth medium (i.e. verminculite and coir) to be able to assess the expression levels of the transgenes. Upon all transgenic plants being ready, they must be transported to the University of Essex where the vast majority of physiology assessments will take place.

5. Outputs relating to the project

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

Output	**Detail**
Plastid preview conference	**Attended the conference**
CTP summer event	**Delivered presentation on the project**

6. Partners (if applicable)

Scientific partners	University of Essex
Industry partners	CTP FCR
Government sponsor	BBSRC