# Studentship Project: Annual Progress Report 10/2023 to 10/2024

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| **Student Name:** | **William Atkinson** | **AHDB Project Number:** | **SF/TF 170/a** |
| **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:** | 04/10/21 | **End Date:** | 04/10/25 |

## 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. The stomata are pores formed between two specialised Guard cells that permit gas exchange between the internal leaf environment and the external air. They are the gateway for all the carbon that enters the plant and are used in photosynthesis, and water that is lost from the plant. Increasing stomatal density, the number of stomata per unit leaf area, has previously demonstrated an increase in photosynthetic carbon assimilation of up to 30% at the cost of increased transpiration (Tanaka et al., 2013). Increased stomatal density in strawberry will be achieved through the overexpression of the gene Stomagen/Epfl9, an established positive regulator of stomatal development. Overexpressing hexokinase in the guard cells has been shown to accelerate stomatal closure, which led to a reduction in water loss without compromising photosynthesis. Improving stomatal movement kinetics will allow for superior response times to environmental factors so that carbon gain and water loss can be better optimised. Combining both increased stomatal density and accelerated stomatal closure could improve carbon assimilation whilst mitigating water loss.

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.

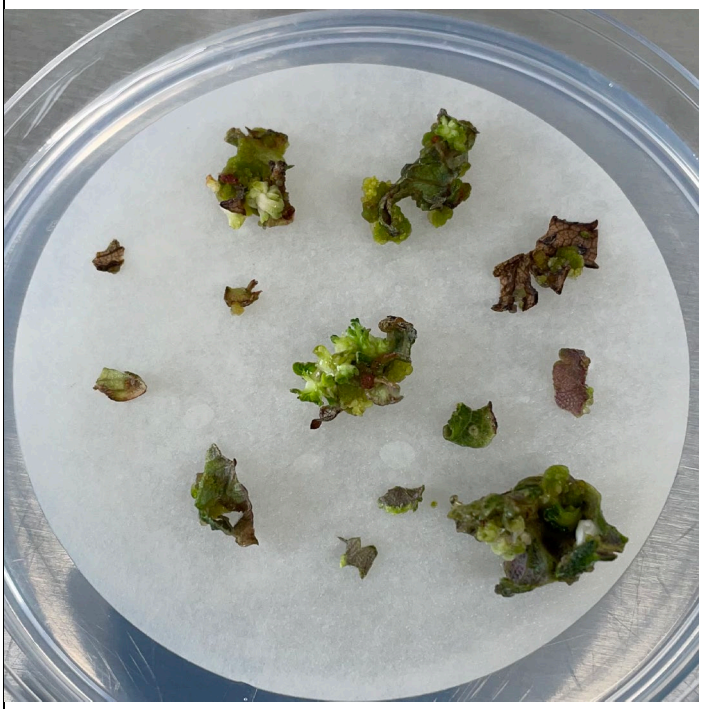
## 2. Key messages emerging from the project

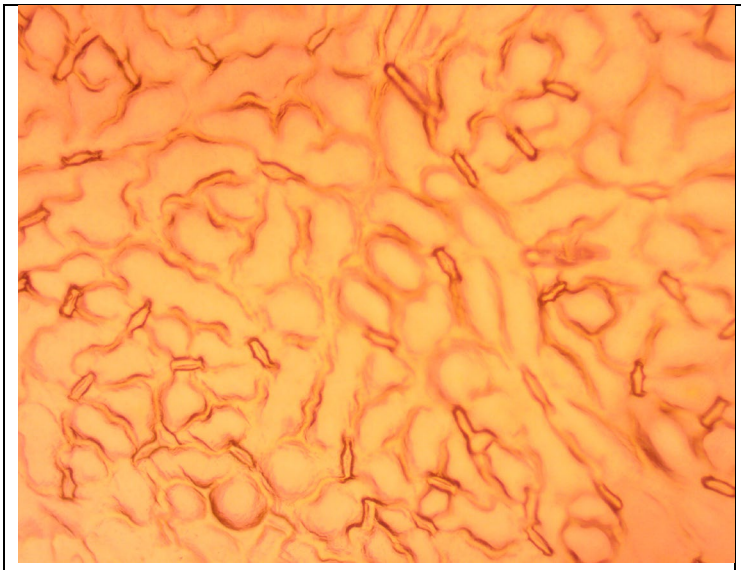
Strawberries are an economically and nutritionally important crop in the UK. Increasing photosynthetic carbon assimilation remains one of the last vestiges for improving crop productivity, and nutritionally important crops, such as fruits and vegetables, have mostly been neglected from this area of research in favour of grains. $CO_2$ enrichment studies on strawberries, which can be used as a proxy for improving

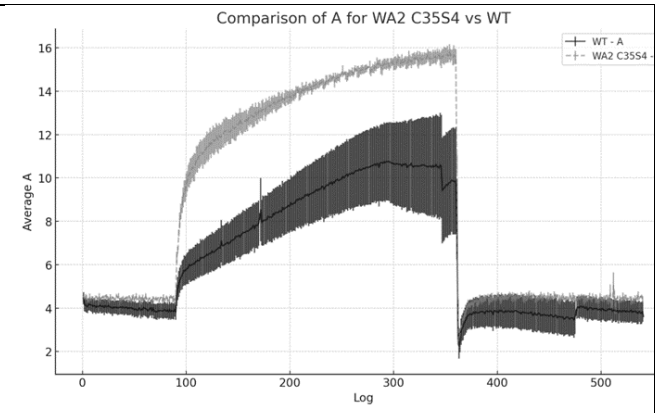
photosynthetic carbon assimilation, have shown not only increased yields, through increased fruit number and size, but also increased quality of the fruit through increasing the proportion of reducing sugars and thus taste, as well as nutritional quality from increased levels of vitamin C. The improvement of strawberries using transgenic manipulation of stomata has the potential to produce similar improvements.

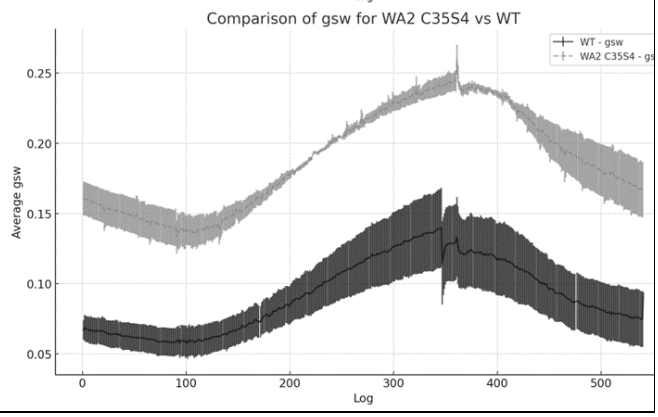
### 3. Summary of results from the reporting year

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 have undergone full *in vitro* regeneration and have been weaned onto soil. The plants have passed transgene screening via PCR against the kanamycin resistance gene they all harbour on extracted plant DNA. Physiology measurements of stomatal patterning through microscopy and analysis of photosynthetic parameters via gas exchange measurements. Work currently underway includes: Molecular analysis of gene expression and analysis of stomatal patterning. An interesting stomatal clustering phenotype has been identified in lines overexpressing stomagen and will be further investigated as to its effect on efficient gas exchange.








## 4. Key issues to be addressed in the next year

Finalise all experimental data, thorough analysis and begin thesis writing.

## 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 22, 23, 24** | **Delivered presentation on the project** |
| **FireBio Uni of Kent** | **Presentation** |
| **Essex graduate forum 22** | **Poster** |
| **Essex graduate forum 23** | **presentation** |
| **SEB centenary conference** | **Presentation** |
| **AgriTechE event** | **Presentation** |
| **CTP Winter event 22,23,24** | **Presentation** |

## 6. Partners (if applicable)

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