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## **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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### **GROWER SUMMARY**

#### Headline

This project aims to improve strawberry fruit yield per hectare and strawberry fruit quality by increasing leaf photosynthetic performance through genetic manipulation of relevant enzymes.

### Background

As the global population continues to rise and climate change threatens current crop yields, new solutions are required to increase agricultural and horticultural productivity. Early studies have suggested that photosynthetic efficiency is a limiting factor on maximised crop growth in C3 plants and thus represents a target for improving yield. Previous work has demonstrated that the overexpression of key regulatory steps in the Calvin Bensom Cycle (CBC) results in an increase in biomass yield and grain yield in tobacco and wheat respectively. This enhanced regeneration of the carbon acceptor RuBP (ribulose-1,5-bisphosphate) in the CBC is therefore demonstrative of a viable and effective method for improving photosynthetic efficiency across species. This work continues this line of research where rate-limiting enzyme activities in the CBC will be increased via genetic modification.

This work is being carried out in cultivated strawberry (*Fragaria x ananassa Duch.*), as current research in this area aims to understand how fundamental research in model plants, such as Tobacco and Arabidopsis, can be applied to crops. We will aim to determine if overexpression of CBC genes, independently or simultaneously, results in changes to total biomass, harvestable yield, and developmental characteristics. Another key area of my research is to ascertain if enhanced photosynthesis, increased total biomass and increased fruit yield affect strawberry fruit quality. We will investigate this using a range of analytical chemistry techniques to study if or how key flavour and nutritional compounds differ between genetically modified and unmodified lines.

## Summary

In the first year of this project, plasmids (loops of DNA) containing the CBC enzyme SBPase (sedoheptulose-1,7-bisphosphatase) and the starch synthesis enzyme AGPase (adenosine diphosphate glucose pyrophosphorylase) were constructed. Insertion of these plasmids (transformation) into leaflet explants and subsequent explant regeneration are being tested in a range of cultivars. Regeneration has shown to be successful for the cultivar Calypso and the experimental line EMR 773. Successfully transformed plantlets of Calypso have been

now been generated after extensive troubleshooting of the transformation method. EMR 773 remains recalcitrant to regeneration, though transformation of the tissue is achievable.

Methods for extracting and analysing flavour components of strawberry fruit have also been developed in preparation for understanding how manipulating primary carbon metabolism in the leaf influences secondary carbon metabolism of strawberry fruit flavour. Initial results have identified the high sensitivity of flavour compounds to different extraction methods, fruit age and cultivar type, highlighting the need for strict controls in this field of analysis.

Experimental work for determining differences in expression (degree of activity of a gene) of photosynthetic genes is currently being carried out. This will give insight as to how photosynthesis is controlled in strawberry on a genetic level and provide vital data for further gene expression analysis later in the project.

Project progress has been delayed due to the impact of COVID-19.

# **Financial Benefits**

While it is difficult to ascertain exact financial payoffs at this stage of the project, it is reasonable to hypothesise that the project will have similar benefits on fruit growth and quality of carbon dioxide enrichment, as this also improves atmospheric carbon assimilation. This method, employed extensively by the Dutch horticultural industry, has been shown to increase strawberry fruit soluble sugar by up to 20 % (Wang and Bunce, 2004) and roughly double fruit dry weight production per plant (Sun et al., 2012). These data imply that genetic manipulation may also be capable of improving both fruit yield and quality, which could result in large monetary returns for growers.

## **Action Points**

There are no action points that must be taken at this stage.