Novel Transient Assay for Color Expression in Detached Anthurium Spathes

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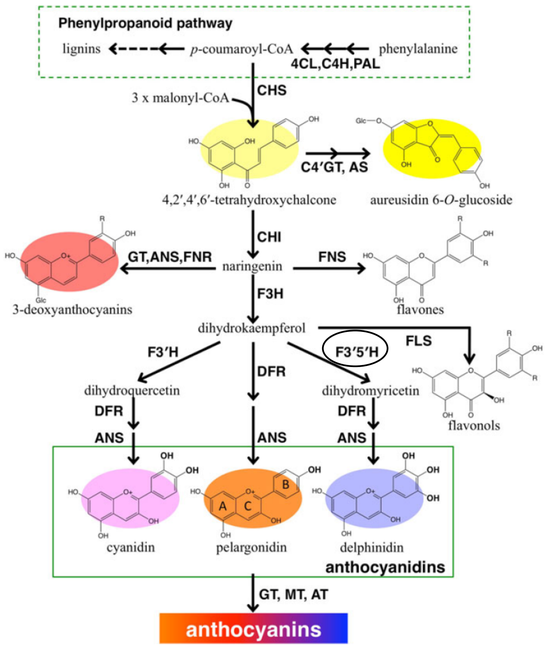
Introduction

Anthuriums are the top grossing cut flowers in the Hawaii floriculture industry. Development of novel colored anthuriums keeps the Hawaii industry competitive globally.

Genetic engineering with the gene Flavonoid 3',5'-hydroxylase (F3'5'H) for expression of anthocyanins in the delphinidin pathway is of interest to create novel, blue colored spathes. The development of stable genetically engineered anthurium plants is a lengthy process. Genetic transformation and selection can take at least a year. First flowering and visualization of gene expression takes nearly two years from planting out.

Transient expression is a rapid alternative to stable transformation for observing gene expression. Transient expression is the rapid but temporary expression of a gene shortly after DNA delivery to cells of target tissues. Generally, transient expression can be observed 48 hours after DNA introduction.

Delivery systems for transient expression include particle bombardment and *Agrobacterium* infiltration (i.e. Agroinfiltration). Particle bombardment is a biolistic system for the delivery of exogenous genes into cells. Agroinfiltration is the infiltration of *Agrobacterium tumefaciens* carrying genes of interest into target tissues. Previously particle bombardment of anthurium spathes resulted in excessive browning of wounded tissue, making transient gene expression difficult to assess.



Generalized flavonoid biosynthetic pathway (Adapted from Nishihara and Nakatsuka, 2011). The delphinidin pathway is of interest for genetic engineering of blue anthurium. F3'5'H is the key enzyme for delphinidin synthesis.

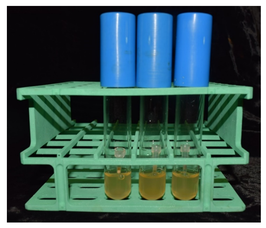
Objective

To optimize agroinfiltration as a gene transfer system for transient expression of the structural color gene F3'5'H in anthurium spathes.

Materials and Methods

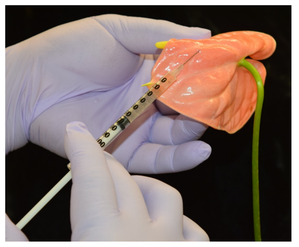
AGROBACTERIUM CULTURE

- Tubes with 2 mL of YEB medium and 2 µL each of rifampicin (50 mg/mL) and kanamycin (50 mg/mL) were inoculated with *Agrobacterium* and grown for two days at 250 rpm on a shaker at 28 °C.
- 5 mL of inoculum was transferred to a flask with 50 mL YEB and 50 µL each of rifampicin (50 mg/mL) and kanamycin (50 mg/mL), placed on a shaker at 250 rpm and 28 °C. (Fitch and He, personal communication)
- The Bacterial suspension was adjusted to a final OD_{600} of 0.8
- Cells were collected by centrifugation for 15 min at 3000 g and resuspended in 10 mM MES (pH5.5) plus 10 mM $MgSO_4$ for agroinfiltration.



AGROINFILTRATION of ANTHURIUM

- Bacterial suspension was injected into spathes using a 1 ml plastic syringe. Approximately 100 µl of bacterial suspension was injected into each spot (typically 3–4 cm^2 in infiltrated area), with 4 to 6 spots in a single spathe. Control spathes were injected with infiltration buffer without *Agrobacterium*.



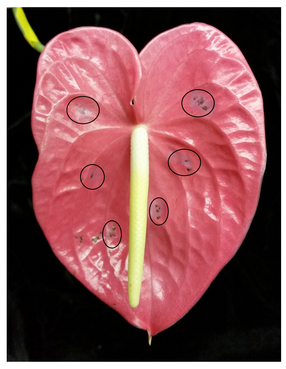
- Spathes were left at room temperature under fluorescent lights. Spathes were observed for color changes near areas of infiltration.

Results

Experiment 1. Assessment of transient expression of F3'5'H on 'Marian Seefurth'. Circles indicate injection sites with color change.

Marian Seefurth

Control
Infiltration Buffer Only



Agroinfiltrated with F3'5'H

Experiment 2. Effect of spathe developmental stage (fully expanded vs. newly unfurled) on transient expression of F3'5'H. Circles indicate injection sites with color change.

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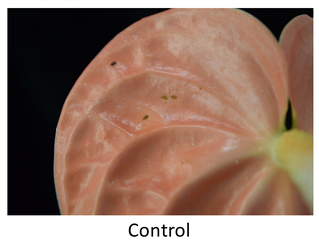

Control
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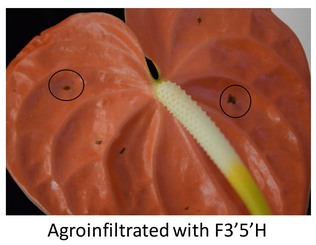
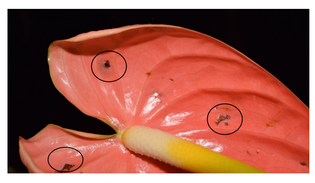


Agroinfiltrated with F3'5'H

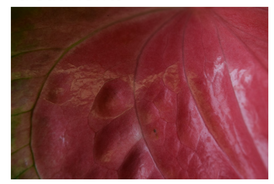
Control
Infiltration Buffer Only



Agroinfiltrated with F3'5'H

Experiment 3. Agroinfiltration with F3'5'H, agroinfiltration without F3'5'H, and only infiltration buffer to determine if color change results from *Agrobacterium* pathogenicity or F3'5'H. Six selections were evaluated. Results were variable among selections.

UH 2010 – Blue spots developed on spathes treated with both agroinfiltration treatments



Control
Infiltration Buffer Only

Control
Agroinfiltrated without F3'5'H

Agroinfiltrated with F3'5'H

UH 2008 -- UH 2008 was the only selection with marked differences among treatments. Brown spots developed at injection sites for the control agroinfiltration without F3'5'H, while blue spots developed on injection sites on spathes injected with F3'5'H

Control
Infiltration Buffer Only

Control
Agroinfiltrated without F3'5'H

Agroinfiltrated with F3'5'H

Conclusions

Agroinfiltration is a promising gene delivery system for transient expression of color genes in anthurium spathes. Further experiments are needed to validate the transient expression of the gene F3'5'H via *Agrobacterium* infiltration.

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