

## ABSTRACT

Characterization of the Anthocyanin Biosynthetic Pathway in *Anthurium andraeanum* (Hort.): Assessment of the Genetic Model for Spathe Color Inheritance

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*Anthurium andraeanum* (Hort.) is a tropical ornamental valued for its colorful spathe. The genetic model for color inheritance in the spathe, suggests three independent loci designated *O*, *R* and *M* control color. To evaluate the genetic model, the full-length coding region of the anthocyanin biosynthetic genes flavonoid 3'-hydroxylase (*F3'H*), flavanone 3-hydroxylase (*F3H*), dihydroflavonol 4-reductase (*DFR*), anthocyanidin synthase (*ANS*) and a putative regulator of the pathway (*AaMyb1*) were determined in cultivars with different states of *O*, *R* and *M*. One *F3'H* allele cloned and sequenced from anthurium was associated with cultivars that accumulated pelargonidins and may not be functional or contain a mutation in its promoter. Different mutations were identified among the other genes that could potentially influence gene expression and/ or activity.

To further investigate the pathway, RT-qPCR assays were developed to quantify the mRNA expression of the anthocyanin genes. Putative reference genes were also cloned from anthurium and the most suitable genes for data normalization were identified. Additionally, the chalcone synthase (*CHS*), *F3H* and *ANS* were cloned into expression vectors and recombinant proteins were

produced. The proteins along with a synthesized DFR peptide were used to generate polyclonal antibodies to determine the corresponding protein levels in the spathe.

The expression of *F3H* and *ANS* were associated with the *R* locus, the expression of *DFR* was associated with the *O* locus and the *M* locus was implicated as encoding *F3'H*. White cultivars which were in the homozygous recessive state for either *O* or *R* or both, exhibited reduced expression of the anthocyanin biosynthetic genes and had negligible levels of anthocyanin.

The intensity of color in red and pink spathe correlated strongly with anthocyanin abundance. Red spathe accumulated anthocyanin throughout development whereas pinks displayed temporal variations in accumulation. Anthocyanin abundance closely mirrored the expression of *F3'H* but did not correspond with the expression of any other gene assayed. The study demonstrated that earlier and higher the rate of *F3'H* expression during spathe development, the greater the accumulation of anthocyanins. Other ancillary mechanisms that down regulate *F3H*, *ANS*, and *DFR* expression levels, were evident in some pink cultivars. A diurnal rhythm in expression of the *CHS*, *F3H*, *DFR*, *ANS*, *F3'H* and *AaMyb1* genes were also noted.

Keywords: *Anthurium andraeanum* (Hort.) spathe; Anthocyanin biosynthetic pathway; Flavonoids; Phenylpropanoid pathway; Flavonoid 3'-hydroxylase; Diurnal.