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**A Molecular Analysis of Flower Colour Development
in an Ornamental Monocot
(*Anthurium andraeanum*)**

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of the requirements for the degree of

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in
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ABSTRACT

Colour in *Anthurium andraeanum* spathe and spadix was investigated at the molecular level. A cDNA library was constructed from poly (A)⁺ RNA isolated from different stages of spathe tissue of the red-flowered anthurium cultivar, Altar. Full-length clones for the flavonoid biosynthetic genes, chalcone synthase, flavanone 3-hydroxylase, dihydroflavonol 4-reductase (*DFR*) and anthocyanidin synthase were isolated by heterologous screening. The expression pattern of these genes implicates *DFR* as a prime regulatory target in the spathe, having an independent regulatory mechanism to that of the other three genes. In the spadix, other regulatory targets are suggested. Additional analysis of *DFR* expression in the spathe revealed a diurnal rhythm to its transcript profile and a model of the possible functional significance of this is presented.

Molecular analysis of the genetic model for anthurium spathe colour was performed with three genotypically defined white lines recessive at the *O* and *M* loci, revealing a more complex genetic model than that originally proposed. The hypothesis that the *O* locus encodes a regulatory protein with specific targets is discussed along with various possible identities for *M*.

Several partial *Myb* cDNA clones were isolated, representing six distinct *Myb* groups in the anthurium spathe. A full-length cDNA clone for one *Myb* gene, *AaMyb1*, was obtained. *AaMyb1* encodes a R2R3 *Myb* protein. It had all the structural features in its DNA binding domain that are conserved in R2R3 *Myb* proteins as well as an acidic domain in the C-terminus that is a potential activation domain. In sequence comparisons with other *Myb* proteins, AaMYB1 had high similarity to anthocyanin related *Mybs* from *Zea mays* (maize). However, in transient assays, AaMYB1 was unable to restore wild type phenotype in an *Antirrhinum majus* line, mutated at the anthocyanin *Myb* locus *Rosea1*. The expression pattern of AaMYB1, in fact, suggests a role in regulating flavone production in the anthurium spathe.

Analyses were done to further investigate the regulation of the anthurium *DFR* promoter. Specific conserved *cis*-elements recognised by anthocyanin Myb regulators were found in the promoter fragment. However, transient expression assays showed that the anthurium *DFR* promoter was activated independently of ROSEA1. The possibility that *DFR* expression is controlled by several regulatory mechanisms, involving various signal transduction cascades, is discussed.

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ABBREVIATIONS

Chemicals

ANS	Anthocyanin synthase
BSA	bovine serum albumin
CH ₃ COOH	acetic acid
CHS	Chalcone synthase
CTAB	Cetyltrimethylammonium bromide
DFR	Dihydroflavonol 4-reductase
DMSO	dimethyl sulphoxide
DTT	dithiothreitol
EtBr	ethidium bromide
F3H	Flavanone 3-hydroxylase
IPTG	isopropyl-β-D-thiogalactoside
KAc	potassium acetate
L-Broth	Luria Broth
Liquid N ₂	liquid nitrogen
2β ME	2β mercaptoethanol
MnCL ₂	manganese chloride
PEG	polyethylene glycol
PMSF	phenyl methyl sulfonyl fluoride
PVP-40	polyvinylpyrrolidone
PVPP	polyvinylpolypyrrolidone
SSC	standard saline citrate
TBE	tris borate EDTA buffer
TE	tris EDTA buffer
TLC	thin layer chromatography
X-Gal	5'-bromo-4-chloro-3-indoyl-β-D-galactopyranoside
X-Gluc	5'-bromo-4-chloro-3-indoyl-β-D- glucuronide

Terms/Techniques

CaMV	cauliflower mosaic virus
GUS	β -glucuronidase
GFP	green fluorescent protein
h	hour
min	minute
PLACE	plant cis-acting regulatory elements
rpm	revolutions per minute
s	second
TRANSFAC	transcription factor database
v/v	volume/volume
w/v	weight/volume

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