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

A thesis presented in partial fulfillment
of the requirements for the degree of

Doctor of Philosophy
in
Plant Molecular Biology

at Massey University, Palmerston North,
New Zealand.

Vern Eddy Collette

2002

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.

ACKNOWLEDGEMENTS

This acknowledgement is to all who in one way or another have assisted me in completing my study. Firstly, to the government of New Zealand, I am deeply grateful for offering me a Commonwealth scholarship to pursue my doctorate in this wonderfully scenic country. To Kiri Manuera, Margaret Gilbert and your respective teams, I appreciate all the kindness, assistance, interest and support shown to me. I am also thankful for the extension of my scholarship through Massey University, enabling me to produce an excellent thesis.

To my supervisors, Professor Paula Jameson, at Massey University, Dr. Kevin Davies and Dr. Kathy Schwinn of Crop & Food Research (C&FR), it was an honour to be guided by your intellect and proficiency. Many thanks for the mentoring and training provided which I am sure will contribute positively to my development as a scientist. I have always commented to my peers that I was assigned the best combination of supervisors a student could ask for. I hold each one of you in very high esteem and desire for you and your loved ones the very best for the future. My thanks also to Paula and family for allowing me to stay at their home when I first arrived in New Zealand.

To C&FR, I express my sincere thanks for funding my research and to all the staff for providing a most enjoyable work culture. To Dr. Chris Winefield, Steve Arathoon and Jan Mason, many thanks for the countless assistance in the lab and for the friendship and laughter that often characterised our interactions. To the members of the C&FR business house tennis team, thanks for wonderful times of recreation, hopefully you can secure first place in a subsequent tournament. MaryAnne, in the mean time, we would guard zealously our personal title of 'unbeaten mix doubles team over two seasons.'

Many thanks, to the staff at the Massey Plant Growth Unit for the excellent care of my plants, during the thesis. I understood the odds were against us in getting the plants established and thriving but clearly your expertise was more than sufficient.

I can write a thesis of equivalent length describing in detail the contributions made by my wife throughout the duration of this study. Words cannot begin to describe the great debt of love and gratitude I have for my wife and best friend, Lauren. She has joyfully given up the last four years of her life on my behalf. It is her love, encouragement, inspiration, intuition, enthusiasm and prayer that has been integral to my success. Her interest in my work has never diminished and her ability to grasp the concepts of molecular biology, though her background is business studies, is a testimony to the wonderful mind she possesses. I am grateful to her for the detailed corrections of my work. I dedicate this thesis to you my darling and pledge my support in your PhD endeavours.

To all the members of my family and friends both at home in Trinidad and Tobago and around the world. Thanks for the continued support and prayer. This success belongs to us all. Special commendation to my mother, Frances Collette ('the tallest lady'). We both know what this means to us. You are a pillar of strength, a symbol of honour and virtue. Thanks for being my mother, the best in the world.

My appreciation also goes to Dr. Umaharan of the University of the West Indies, St. Augustine, who nurtured my love for genetics and molecular biology. He continues to be an inspiration and a model for me. Also to Kairi Blooms, Trinidad, for their continued support of anthurium research. Such links between industry and university are critical to continued development. I am confident that the results of this work would contribute positively to your company.

Finally, I acknowledge my friend and my God, the ultimate designer of the wonderfully complex biological systems that we explore today through science, and Jesus Christ my Lord, for the multifaceted grace that was given to me at every stage of my thesis. My heart is filled with gratitude and praise to Him.

TABLE OF CONTENTS

ABBREVIATIONS

Chemicals

ANS	Anthocyanin synthase
BSA	bovine serum albumin
CH_3COOH	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	polyvinylpyrrilidone
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

LIST OF FIGURES

On page following

LIST OF TABLES