



**UNIVERSITI PUTRA MALAYSIA**

**ISOLATION AND CHARACTERIZATION OF UPREGULATED FLORAL  
TRANSCRIPTS FROM MANGOSTEEN (*Garcinia mangostana* L.)**

**CHAN KAM LOCK**

**FBSB 2008 8**



**ISOLATION AND CHARACTERIZATION OF UPREGULATED FLORAL  
TRANSCRIPTS FROM MANGOSTEEN (*Garcinia mangostana* L.)**

**By**

**CHAN KAM LOCK**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in fulfilment of the Requirements for the Degree of Master of Science**

**July 2008**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**ISOLATION AND CHARACTERIZATION OF UPREGULATED FLORAL  
TRANSCRIPTS FROM MANGOSTEEN (*Garcinia mangostana L.*)**

By

**CHAN KAM LOCK**

**July 2008**

**Chairman : Ho Chai Ling, PhD**

**Faculty : Biotechnology and Biomolecular Sciences**

Mangosteen (*Garcinia mangostana L.*) is one of the slowest-growing and longest living tropical fruit trees. Besides long juvenile period, lack of profuse flowering and irregular fruiting during early maturing stage are some of the major problems associated with growing mangosteen as an export fruit or for fruit products. The initiation of flowering process, development and maturation of flower in mangosteen are largely unknown. The understanding of these processes is important to solve some of the problems associated with growing mangosteen as one of the major fruits. Thus, the objectives of this study were to isolate, identify and sequence the mangosteen transcripts that were upregulated in the floral tissues, and study the gene expression and gene copy number of the selected upregulated floral transcripts. In this study, NSTEP method was found to be the best total RNA isolation method for mangosteen tissues. A subtracted cDNA library was constructed to facilitate the isolation of upregulated transcripts from mangosteen flower. Reverse northern screening and sequence analysis revealed that 28.5 % (149/522) of



these transcripts were upregulated in mangosteen flower. Among these transcripts, 82 of them were assembled into 30 contigs whereas 67 were singletons. A total of 63.9 % of these unigenes had non-significant matches to sequences in the non-redundant protein database in GenBank, 19.6 % had significant matches to unknown proteins and the remaining 16.5 % had putative functions that were further classified into six categories according to their biological functions. A total of three transcripts were selected for further characterization by real time reverse-transcription polymerase chain reaction and southern hybridization analysis. They were GmAGmbp (protein with GATA-type zinc finger domain), GmHsa32 (phosphosulfatase related protein) and GmbZIP (bZIP transcription factor). The 3' untranslated region (UTR) of these three transcripts were isolated from a cDNA library constructed using flower of 0.5-1.0 cm. All of these transcripts were verified to be expressed predominantly in the mangosteen flower tissue. GmAGmbp and GmHsa32 were found to be single copy genes in the mangosteen genome. The subtracted cDNAs isolated in this study might be used as expression markers for crop improvement in the future. However, further characterization of expression patterns and functional analyses are required to gather more valuable information on how these transcripts function during the flowering process.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMENCILAN DAN PENCIRIAN TRANSKRIPT YANG DINAIK-ATURKAN DI  
BUNGA DARIPADA MANGGIS (*Garcinia mangostana L.*)**

Oleh

**CHAN KAM LOCK**

**July 2008**

**Pengerusi : Ho Chai Ling, PhD**

**Fakulti : Bioteknologi and Sains Biomolekular**

Manggis (*G. mangostana L.*) adalah salah satu buah-buahan tropika yang mempunyai pertumbuhan yang sangat lambat dan hayat hidup yang lama. Selain daripada tempoh juvenil yang lama, antara masalah-masalah yang berkaitan dengan penanaman manggis untuk diekspot atau untuk penghasilan produk buah-buahan adalah pembungaan yang kurang dan ia jarang berbuah pada peringkat awal kematangan. Proses permulaan pembungaan, perkembangan dan kematangan bunga manggis adalah kurang diketahui. Pemahaman tentang proses-proses tersebut adalah penting untuk mengatasi masalah-masalah yang terlibat dalam penanaman manggis sebagai salah satu buah-buahan yang penting. Oleh itu, objektif-objektif untuk kajian ini ialah untuk memencil, mengenalpasti dan menjujuk transkript-transkript manggis yang dinaik-aturkan dalam tisu-tisu bunga, dan mengkaji penzahiran gen dan bilangan salinan gen transkript-transkript bunga dinaik-aturkan yang terpilih. Dalam kajian ini, kaedah NSTEP merupakan kaedah pemencilan keseluruhan RNA yang paling sesuai untuk tisu-tisu daripada manggis. Satu perpustakaan



cDNA tertolak (subtracted cDNA library) telah dibina untuk memudahkan pemencilan transkript-transkript yang dinaik-aturkan daripada bunga manggis. Penyaringan northern 'berbalik' dan analisa jujukan mendapati 28.5 % (149/522) dari transkript-transkript tersebut dinaik-aturkan dalam bunga manggis. Di antara transkript-transkript ini, 82 daripada mereka terkumpul dalam 30 'contigs' manakala 67 adalah 'singletons'. Sejumlah 63.9 % unigen-unigen ini mempunyai padanan yang tidak sah dengan jujukan-jujukan dalam pangkalan protein tidak bertindan di 'GenBank', 19.6 % mempunyai padanan yang sah dengan protein yang tidak diketahui dan 16.5 % yang lain yang mempunyai fungsi ramalan telah dikelaskan selanjutnya kepada enam kategori mengikut fungsi biologikal mereka. Tiga daripada transkript-transkript ini telah dipilih untuk pencirian selanjutnya dengan menggunakan 'real-time reverse transcription polymerase chain reaction' dan analisa penghibridan 'southern'. Mereka ialah GmAGmbp (protein dengan domain jejari zink jenis GATA), GmHsa32 (protein yang berkaitan dengan phosphosulfolactate synthase) dan GmbZIP (faktor transkripsi bZIP). 3' 'untranslated region' (UTR) untuk tiga transkript ini telah dipencilkan daripada perpustakaan cDNA yang dibina dengan menggunakan bunga bersaiz 0.5-1.0 sm. Semua transkript ini telah disahkan adalah predomnan di dalam tisu bunga manggis. GmAGmbp dan GmHsa32 didapati mungkin adalah gen-gen yang mempunyai salinan tunggal dalam genom manggis. Semua cDNA tertolak yang dipencilkan dalam kajian ini mungkin boleh digunakan sebagai penanda ekspresi untuk memajukan tanaman pada masa akan datang. Walaubagaimanapun, pencirian corak penzahiran dan analisa fungsi selanjutnya diperlukan untuk mengumpul maklumat yang lebih bermakna tentang bagaimana transkript-transkript tersebut berfungsi dalam proses pembungaan.

## ACKNOWLEDGEMENTS

Firstly, I would like to thank Dr. Ho Chai Ling for giving me the opportunity to pursue my studies in the field of plant molecular biology and for her guidance and advice throughout this project. Besides that, I would like to express my appreciation to Dr. Suhaimi Napis and Dr. Parameswari for being my supervisory committee members and for their guidance and advice. I would also like to thank the Ministry of Science and Technology for funding this project. My sincere thanks to Mr. Adrian (MARDI) for his kindness and funding support.

I would like to acknowledge Faculty of Agriculture and Faculty of Design and Architecture for allowing me to collect samples for this project in ‘Ladang 10’ and ‘Nursery Bahan Bakar Buah-buahan’, respectively.

My deepest appreciation also goes to all the members of Genetic Lab (UPM) and special thanks are extended to Choong, Ky, Thuc, Sock Hwa and Siti. I would not have completed my project without their help and guidance.

Lastly, my deepest gratitude to my parents for their endless love and support.



I certify that an Examination Committee met on 11<sup>th</sup> July 2008 to conduct the final examination of Chan Kam Lock on his Master of Science thesis entitled "Isolation and Characterization of Upregulated Floral Transcripts From Mangosteen (*Garcinia mangostana* L.)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.

Members of the Examination Committee are as follows:

**Norihan Mohd. Saleh, PhD**

Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Janna Ong Abdullah, PhD**

Lecturer  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Siti Nor Akmar Abdullah, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Rofina Yasmin Othman, PhD**

Professor  
Faculty of Science  
University of Malaya  
Malaysia  
(External Examiner)

---

**HASANAH MOHD. GHAZALI, Ph.D.**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:





This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Ho Chai Ling, PhD**

Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Suhaimi b. Napis, PhD**

Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

**Parameswari a/p Namasivayam, PhD**

Lecturer  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

---

**AINI IDERIS, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 11 September 2008



## **DECLARATION**

I hereby declare that the thesis is based on my original work except for equations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

---

**CHAN KAM LOCK**

Date: 31 July 2008



## TABLE OF CONTENT

	<b>Page</b>
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	iv
<b>ACKNOWLEDGEMENTS</b>	vi
<b>APPROVAL</b>	vii
<b>DECLARATION</b>	ix
<b>LIST OF TABLES</b>	xiii
<b>LIST OF FIGURES</b>	xiv
<b>LIST OF ABBREVIATIONS</b>	xvii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>4</b>
2.1 Mangosteen	4
2.1.1 Morphology of Mangosteen Flower	6
2.1.2 Uses of Mangosteen	10
2.1.3 Problems Encountered in Mangosteen Plantation and Its Solution	12
2.2 Flowering	14
2.2.1 Internal and External Control On Flowering	16
2.2.2 The Status of Research on Mangosteen Flowering Mechanisms	26
2.3 The Molecular Cloning of Plant Genes	27
2.3.1 Isolation of Differentially Expressed Genes	28
2.3.2 The Principle of SSH	33
<b>3 MATERIALS AND METHODS</b>	<b>37</b>
3.1 Sample Collection	37
3.2 Isolation of Total RNA	37
3.2.1 Method 1: Jill-Winter Method with Modifications (Rochester <i>et al.</i> , 1986)	37
3.2.2 Method 2: Phenol-SDS Method	38
3.2.3 Method 3: NTES Method (Matsumura <i>et al.</i> , 1999)	39
3.2.4 Method 4: NSTEP Method (NTES Method with Modifications)	40
3.2.5 RNA Quantification	40
3.2.6 Formaldehyde Denaturing Agarose Gel Electrophoresis	41
3.3 Isolation of mRNA from Total RNA	41
3.3.1 Isolation of mRNA	41
3.3.2 Quantification of mRNA	43
3.4 Construction of cDNA Libraries	43



3.4.1	Construction of Mangosteen Floral cDNA Library	43
3.4.2	Construction of Subtracted cDNA Library	52
3.5	Reverse Northern Analysis	58
3.6	DNA Sequencing and Sequence Analysis	60
3.7	Isolation of Housekeeping Gene	61
3.7.1	RT-PCR	61
3.7.2	Cloning of PCR Product	62
3.8	Molecular Characterization of Selected Differentially Expressed Sequences	63
3.8.1	Real-time RT-PCR	63
3.8.2	Southern Blot Analysis	71
3.9	Full-Length Isolation	77
3.9.1	Isolation of the 3'-end cDNA	77
3.9.2	Isolation of the 5'-end cDNA	80
3.9.3	Sequence Analysis of the Full-length cDNA	80
<b>4</b>	<b>RESULTS</b>	<b>82</b>
4.1	Sample Collection and Total RNA Isolation	82
4.2	Construction of cDNA library	88
4.3	Analysis of Subtracted cDNA Library	90
4.3.1	Construction of Subtracted cDNA Library	90
4.3.2	Reverse Northern Analysis	92
4.3.3	Sequence Analysis of the Upregulated cDNAs from Mangosteen Flower	97
4.4	Full-Length Isolation of the Three Selected Subtracted cDNA Clones	103
4.4.1	Sequence Analysis of Contig 17	103
4.4.2	Sequence Analysis of Clone P5G11	108
4.4.3	Sequence Analysis of Clone P6C4	112
4.5	Isolation of Housekeeping Gene from <i>G. mangostana</i>	116
4.6	Analysis of Selected Clones by Real-time RT-PCR	118
4.6.1	PCR Optimization and Endogenous Control Selection with RT-PCR	118
4.6.2	Validation of Amplification Efficiencies for the Comparative C <sub>T</sub> Method	124
4.6.3	Relative Quantification of Gene Expression by Relative Standard Curve Method	126
4.7	Southern Blot Hybridization of GmAGmbp and GmHsa32	131
<b>5</b>	<b>DISCUSSION</b>	<b>134</b>
5.1	Total RNA Isolation	134
5.2	Screening for Upregulated Transcripts From Mangosteen Flower	136
5.3	Sequence Analysis and Categorization of Subtracted Clones	138
5.4	Real-time RT-PCR Analysis of GmAGmbp, GmHsa32 and GmbZIP	142
5.5	Southern Blot Analysis of GmAGmbp and GmHsa32	143



5.6	Molecular Analyses Three Selected Subtracted Clones	145
5.6.1	GmAGmbp Encodes for a Protein with GATA-type Zinc Finger Domain	145
5.6.2	GmHsa32 Encodes for a Truncated PSL	147
5.6.3	GmbZIP Encodes for a Truncated bZIP Transcription Factor	148
5.7	Future Studies	149
<b>6</b>	<b>CONCLUSION</b>	<b>151</b>
	<b>BIBLIOGRAPHY</b>	<b>153</b>
	<b>APPENDIX A</b>	<b>174</b>
	<b>APPENDIX B</b>	<b>178</b>
	<b>APPENDIX C</b>	<b>179</b>
	<b>APPENDIX D</b>	<b>185</b>
	<b>BIODATA OF STUDENT</b>	<b>198</b>
	<b>LIST OF PUBLICATIONS</b>	<b>199</b>



## LIST OF TABLES

<b>Tables</b>		<b>Page</b>
1	The primer sequences and expected size of the amplicons for each pair of primer for real time RT-PCR.	66
2	The primer sequences for each pair of primer for probe preparation of southern blot.	74
3	The primer sequences for the isolation of 3'-end cDNA.	79
4	The primer sequences for the isolation of 5'-end cDNA.	81
5	Absorbance and yield of RNA extracted by using different methods from mangosteen.	86
6	Yield and quality of RNA extracted by using NSTEP method.	86
7	BLASTX results of the 97 unigenes from flower specific SSH library.	179
8	Putative functions of 16 unigenes from mangosteen subtracted cDNA library that were classified into six functional categories.	101
9	The relationship between $\Delta C_T$ and template dilutions.	184
10	Average gene quantities of target genes in the respective tissues.	130



## LIST OF FIGURES

<b>Figures</b>		<b>Page</b>
1	Mangosteen trees.	7
2	Mangosteen flowers.	8
3	Flower development pathways.	17
4	Scheme of SSH method.	34
5	Developmental stages of mangosteen flower.	83
6	Floral buds of different sizes (A – E) and fully open flower (F).	83
7	Comparison of total RNA isolated by four different methods.	84
8	Comparison of total RNA isolated from different tissues by using Method 4.	87
9	Quantification of mRNA concentration by using EtBr plate assay.	89
10	Size distribution of synthesized second strand cDNA.	89
11	PCR amplification of cDNA inserts from randomly chosen clones of mangosteen flower cDNA library using M13R and M13F primers.	91
12	Optimization of secondary PCR cycle using the PCR-select cDNA subtraction system.	93
13	The secondary PCR product of the forward subtracted cDNA.	94
14	PCR amplification of cDNA inserts of 32 subtracted clones from the mangosteen flower SSH library.	94
15	Hybridization pattern of forward subtracted clones identified by reverse northern analysis	96
16	Reverse northern analysis of 32 subtracted clones from the mangosteen flower SSH library.	96



17	Classification of contigs according to the number of sequences per contig.	98
18	Nucleotide sequence of the 97 unigenes in fasta format.	197
19	The score distribution of unigenes based on BLASTX algorithm.	98
20	The classification of 97 unigenes from mangosteen subtracted cDNA library based on their putative functions.	100
21	The 35 unigenes that have significant matches showed high similarity (97.1 %) to plant genes and only a small portion (2.9 %) matched with virus sequence.	102
22	The nucleotide and deduced amino acids sequence of Contig 17.	105
23	Multiple sequence alignment of GmAGmbp with examples of other members from GATA zinc finger domain family.	107
24	A neighbor-joining tree of GmAGmbp and other members from GATA zinc finger family.	109
25	The nucleotide and deduced amino acids sequence of Clone P5G11.	111
26	Multiple sequence alignment of GmHsa32 with examples of other members from PSL family.	113
27	The nucleotide and deduced amino acids sequence of Clone P6C4.	114
28	Multiple sequence alignment of GmbZIP with examples of other members from bZIP family.	115
29	PCR amplification and cloning of <i>Cyp</i> .	117
30	The nucleotide and deduced amino acids sequence of Clone P6C4.	117
31	Multiple sequence alignment of GmCyp with examples of other members from <i>Cyp</i> family.	119
32	Optimized PCR product for all specific primers at 55 °C.	120
33	Product melting curve analysis for each real-time RT-PCR primer pairs.	122
34	Plots for amplification detected during real-time RT-PCR amplification.	123





35	Relative efficiency plots of $\Delta C_T$ versus log cDNA dilution.	125
36	Standard curves for quantification of various target gene quantities in the two sample organs in two separate run.	129
37	Relative expression level of DEGs in flower tissue as compared to the calibrator (young shoot).	130
38	Genomic DNA isolated from mangosteen leaf.	132
39	Southern blot analysis.	133



## LIST OF ABBREVIATIONS

AFLP	-	Amplified Fragment Length Polymorphism
AIMS	-	Amplification of Insertion Mutagenised Sites
AMV	-	Avian Myeloblastosis Virus
<i>API</i>	-	<i>APETALA 1</i>
BAS	-	Bureau of Agricultural Statistic
BLAST	-	Basic Local Alignment Search Tool
bp	-	base pair
bZIP	-	basic leucine zipper
CaMV 35S	-	Cauliflower Mosaic Virus 35S
<i>CCA1</i>	-	<i>CIRCADIAN CLOCK ASSOCIATED 1</i>
CDS	-	coding region
CI	-	chloroform: isoamyl alcohol
<i>CO</i>	-	<i>CONSTANS</i>
CoM	-	Coenzyme M
ComA	-	(2R)-phospho-3- sulfolactate synthase
COX 2	-	Cyclooxygenase 2
<i>CRY</i>	-	Cryptochromes
CsCl	-	caesium chloride
$C_T$	-	threshold cycle
CTAB	-	hexadecyl (or cetyl) trimethyl ammonium bromide
<i>Cyp</i>	-	<i>Cyclophilin</i>



DDRT-PCR	-	Differential Display Reverse Transcription Polymerase Chain Reaction
DEPC	-	diethylpyrocarbonate
DMSO	-	dimethyl sulphoxide
Dnase I	-	deoxyribonuclease I
EDTA	-	ethylene diamine tetracetate
<i>ELF3</i>	-	<i>EARLY FLOWERING3</i>
EST	-	Expressed Sequence Tag
EtBr	-	ethidium bromide
<i>FLC</i>	-	<i>FLOWERING LOCUS C</i>
<i>FLO</i>	-	<i>FLORICAULA</i>
FMs	-	floral meristems
<i>FPF1</i>	-	<i>FLOWERING PROMOTIVE FACTOR 1</i>
<i>FRI</i>	-	<i>FRIGIDA</i>
<i>FT</i>	-	<i>FLOWERING LOCUS T</i>
<i>FWA</i>	-	a late flowering gene
GA	-	gibberellin
<i>GI</i>	-	<i>GIGANTEA</i>
GmAGmbp	-	<i>Garcinia mangostana</i> AG motif binding protein
GmbZIP	-	<i>Garcinia mangostana</i> basic leucine zipper
GmCyp	-	<i>Garcinia mangostana</i> cyclophilin
GmHsa32	-	<i>Garcinia mangostana</i> heat stress associated 32
Gm $\alpha$ tubulin	-	<i>Garcinia mangostana</i> $\alpha$ -tubulin
GTC	-	guanidium thiocyanate



HIV	-	Human Immunodeficiency Virus
HS	-	heat shock
Hsa32	-	heat stress associated 32
HSP	-	heat shock protein
IBPGR	-	International Board of Plant Genetic Resources
IMs	-	inflorescence meristems
IPGRI	-	International Plant Genetic Resources Institute
LB	-	Luria-Bertani
LD	-	Long-day
<i>LFY</i>	-	<i>LEAFY</i>
<i>LHY</i>	-	<i>LATE ELONGATED HYPOCOTYL</i>
LiCl	-	lithium chloride
MADS	-	MCM1-AGAMOUS-DEFICIENS-SRF
MgSO <sub>4</sub>	-	magnesium sulphate
MOPS	-	3-(N-morpholino)propanesulfonic acid
MRSA	-	Methicillin-Resistant <i>Staphylococcus aureus</i>
NaCl	-	sodium chloride
NaOAc	-	sodium acetate
NaOH	-	sodium hydroxide
NCBI	-	National Center for Biotechnology Information
NH <sub>4</sub> OAc	-	ammonium acetate
NLS	-	nuclear localization signal
OD	-	optical density



ORFs	-	open reading frames
PBZ	-	paclobutrazol
PCI	-	phenol: chloroform: isoamyl alcohol
PCR	-	polymerase chain reaction
Pfr	-	Phytochromes of far red light-absorbing form
Pfu	-	plaque forming unit
PGE2	-	Prostaglandin E <sub>2</sub>
<i>PHY</i>	-	Phytochromes
pI	-	isoelectric point
Poly (A)	-	polyadenylated (mRNA)
ppm	-	parts per million
PPO	-	polyphenol oxidase
Pr	-	Phytochromes of red light-absorbing form
PSL	-	phosphosulfolactate synthase-related protein
PVP	-	polyvinylpyrrolidone
R <sup>2</sup>	-	correlation coefficient
RAPDs	-	randomly amplified polymorphic DNA markers
RFLP	-	restriction fragment length polymorphism
RT-PCR	-	Reverse transcription – PCR
SAGE	-	serial analysis of gene expression
SAM	-	shoot apical meristem
SD	-	Short-day
SDS	-	sodium dodecyl sulfate



<i>SOCI</i>	-	<i>SUPPRESSOR OF OVEREXPRESSION OF CO1</i>
<i>spy</i>	-	<i>spindly</i>
SQDG	-	sulfoquinovosyl diacylglycerol
<i>SQUA</i>	-	<i>SQUAMOSA</i>
SSC	-	standard saline citrate
SSH	-	suppression subtractive hybridization
TAE	-	Tris-acetate-EDTA
T-DNA	-	Transferred-DNA
TE	-	Tris-EDTA
T <sub>m</sub>	-	melting temperature
<i>TOCI</i>	-	<i>TIME OF CHLOROPHYLL A/B BINDING PROTEIN1</i>
Tris	-	tris[hydroxymethyl]aminomethane
Tris-HCl	-	tris-hydrochloride
U	-	unit
USD	-	U.S. Dollars
UTR	-	untranslated region
UV	-	ultraviolet
V	-	volt
<i>VRN2</i>	-	<i>VERNALISATION2</i>
YAC	-	yeast artificial chromosome
<i>ZIM</i>	-	Zinc-finger protein expressed in Inflorescence Meristem



# CHAPTER 1

## INTRODUCTION

The mangosteen (*Garcinia mangostana* L.) is thought to have originated from Peninsular Malaysia and early cultivation of this crop was limited to Southeast Asia. The mangosteen spread to other tropical regions during the past few centuries. Mangosteen trees grow naturally as understorey plants in forest communities and are usually propagated by apomictic seeds. Mangosteen is one of the slowest-growing and longest living tropical fruit tree. It has been considered as the most delicious fruit of the tropics and has been named the ‘queen of fruits’. In Southeast Asia, the fruit pericarp has been used traditionally as medicine for inflammation, diarrhoea, dysentery, wounds and skin infections. The mangosteen pulp contains high amounts of energy, vitamins and minerals, hence it can greatly improve food quality of low-income rural households especially children. Aside from being a source of fresh and processed food, the fruit rind contains 7-14 % catechin tannin and is used for tanning of leather and it also produces a natural black dye (<http://www.civil.soton.ac.uk/icuc/factsheets.html>).

The demand for mangosteen fruits usually exceeds the supply as mangosteen trees are rarely planted in commercial quantities. However, in recent years, the mangosteen has been subjected to renewed interest and it has gained increased recognition in the international markets. Thailand and Malaysia are the major commercial producers and suppliers of mangosteen to United Kingdom, Hong Kong, Singapore, Taiwan and Japan.



In 1990, the export quantity of mangosteen in Malaysia was 1, 544 tons valued at USD 456, 000, and about a decade later, the export quantity has increased to 1, 961 tons valued at USD 1, 127, 000 (Mohamad and Abd Rahman, 2006). In 2002, the total cultivation area for mangosteen in Thailand was 48, 000 hectares which yielded 160, 000-190, 000 tons fruits. The export value of mangosteen was USD 10 millions with overseas sales growing at an average rate of 102 % (Office of Agricultural Economics, 2003). It is expected that Thailand and Malaysia will maintain to be the major suppliers of mangosteen in the world market as both countries are still expanding their mangosteen production areas.

Nevertheless, mangosteen is still not cultivated on large scale despite tremendous consumer acceptance, good transport infrastructure and long shelf life. It is because of its long pre-bearing period resulting from the extremely slow growth of the developing seedlings, unusually long juvenile phase, low fruit yield, biennial bearing and short viability of seeds. Much efforts are needed to solve these problems in order to expand the mangosteen fruit industry to be one of the major ones in Malaysia. Research and development activities must be carried out intensively in order to solve the problems and to fulfill the increasing demand. Flowering is a fundamental process in plant development that leads to fruit formation. The molecular mechanisms underlying flowering, development and maturation of flower in mangosteen are poorly understood. Therefore, it is of paramount importance to study the flowering process in mangosteen to solve problems such as its long juvenile phase by genetic controlling of its flowering time.



The objectives of this study are:

1. To isolate, identify and sequence the mangosteen transcripts that are upregulated in the floral tissues with the aim to further understand the flowering process in this fruit tree.
2. To study the gene expression of the selected upregulated floral transcripts in floral bud and young shoot of mangosteen.
3. To determine the gene copy number of the selected upregulated floral transcripts in mangosteen.