THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Horticulture)

DEGREE

Horticulture	Horticulture
FIELD	**DEPARTMENT**

TITLE: Characterization of MYB Transcription Factors and Anthocyanin Biosynthesis Genes of Mangosteen Fruit during Red Colouration

NAME: Mr. Yossapol Palapol

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Professor Saichol Ketsa, Ph.D.)

COMMITTEE MEMBER

(Assistant Professor Lop Phavaphutanon, Ph.D.)

COMMITTEE MEMBER

(Mrs. Parichart Burns, Ph.D.)

COMMITTEE MEMBER

(Professor Ian B. Ferguson, Ph.D.)

DEPARTMENT HEAD

(Associate Professor Poonpipop Kasemsap, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

CHARACTERIZATION OF MYB TRANSCRIPTION FACTORS AND ANTHOCYANIN BIOSYNTHESIS GENES OF MANGOSTEEN FRUIT DURING RED COLOURATION

YOSSAPOL PALAPOL

A Thesis Submitted in Partial Fulfilment of
the Requirements for the Degree of
Doctor of Philosophy (Horticulture)
Graduate School, Kasetsart University
2009

Yossapol Palapol 2009: Characterization of MYB Transcription Factors and Anthocyanin Biosynthesis Genes of Mangosteen Fruit during Red Colouration. Doctor of Philosophy (Horticulture), Major Field: Horticulture, Department of Horticulture. Thesis Advisor: Professor Saichol Ketsa, Ph.D. 240 pages.

The colour of mangosteen (*Garcinia mangostana* L.) fruit changes from green to purple black after harvest as the fruit ripening advances. The relationships between anthocyanin composition and content during colour development and fruit maturity and postharvest quality were determined. Fruit at different stages of maturity (light greenish-yellow with 5% scattered pink spots to purple black) were harvested and kept at 25°C (85-90% RH). Fruit from each maturity stage all developed to the final purple black stage. During the postharvest period, hue values and pericarp firmness decreased significantly, while soluble solids content increased concomitant with a decrease in titratable acidity (stage 4-6). Anthocyanin content in the outer pericarp was higher than in the inner pericarp and increased to a maximum at the final stage. Sensory evaluation and fruit quality (hue values, soluble solids and titratable acidity) of fruit harvested at the different stages did not differ once the fruit had finally developed to the purple black stage. Anthocyanins in the outer pericarp mainly consisted of five compounds, identified by HPLC/MS as cyanidin-sophoroside, cyanidin-glucoside, cyanidin-glucoside-pentoside, cyanidin-glucoside-X, cyanidin-X_2 and cyanidin-X, where X denotes an unidentified residue of *m/z* 190, a mass which did not correspond to any common sugar residue. Cyanidin-3-sophoroside and cyanidin-3-glucoside were the major compounds that increased with fruit colour development. In all plant species previously studied, the anthocyanin pathway is co-ordinatively regulated by transcription factors of the MYB-super family. Three full-length mangosteen MYB transcription factors (GmMYB1, GmMYB7 and GmMYB10) and all the anthocyanin biosynthesis genes (GmPal to GmUFGT) were characterized. Sequence analysis at the protein level of the R2R3-MYB transcription factor family showed GmMYB10 had a high degree of similarity with production of anthocyanin pigment1 (PAP1) in *Arabidopsis* and as well as sequences from other plant species related to the elevation of anthocyanin pigmentation. In transient transactivation assays, *GmMYB10*, co-expressed with *AtbHLH2*, strongly activated the *GmDFR* and *AtDFR* promoters. Transcript levels of *GmMYB10* and *GmUFGT* were highly abundant with onset of pigmentation and subsequently during red colouration. Our results suggest that *GmMYB10* plays an important role in regulating anthocyanin biosynthesis both on the tree and after harvest, while *GmUFGT* may be a key biosynthetic gene in mangosteen pigmentation. The expression patterns of *GmMYB10* and *GmUFGT* correlated with ethylene production that increased linearly until stage 5 (dark purple) and decreased thereafter. 1-Methycyclopropene (1-MCP) and low temperature storage (15°C) clearly delayed red colouration with resulting down-regulation of *GmMYB10.* These results suggest that the effect of ethylene on anthocyanin biosynthesis may be via the regulation of *GmMYB10* expression.

_____ _____ ___ / ___ / ___

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor, Professor Dr. Saichol Ketsa for valuable advice and supervision, critically reviewing the manuscript and his encouragement throughout the course of my Ph.D. studies. I also wish to express my sincere appreciation to Professor Dr. Ian B. Ferguson, Dr. Andrew C. Allan, Assistant Professor Dr. Lop Phavaphutanon and Dr. Parichart Burns for their kindly suggestions, comments and assistance which were essential for the completion of this thesis. I greatly appreciate their advice, insight and perspectives.

I also express my gratitude to Kui Lin-Wang and all members in the Gene Discovery and Function team and everyone in the Fruit Genomic Group, particularly Sarah Johnston for her experience in real-time PCR, David Stevenson and Janine Cooney for anthocyanin analysis, and the staff of Plant & Food Research, New Zealand for friendship and kind assistance and support throughout the year.

I would like to thank the staff of Postharvest Technology Center, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand for their hospitality and facilitating the instruments and materials during the research.

I am indebted to the Thailand Research Fund grant number PHD/00210/2547 and Commission on Higher Education, Ministry of Education for awarding me a scholarship to undertake this study and for the financial support by Postharvest Technology Innovation Center, which is part of a collaborative programme with Plant & Food Research, Mt Albert Research Centre, Auckland, New Zealand.

Finally, I express my special appreciation to my family, particularly my parents and my grandparent whose constant encouragement and support contributed enormously throughout my study.

Yossapol Palapol

May 2009

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

1-MCP	=	1-Methylcyclopropene
ANS	=	Anthocyanidin synthase
bHLH	=	Basic helix-loop-helix
BLAST	=	Basic local alignment search tool
bp	=	Base pairs
CaMV	=	Cauliflower mosaic virus
cDNA	=	Complementary DNA
cds	=	Coding sequence
CHI	=	Chalcone isomerase
CHS	=	Chalcone synthase
Cp	=	Crossing point
Ct	=	Cycle threshold
DEPC	=	Diethyl pyrocarbonate
DFR	=	Dihydroflavonol-4-reductase
DNA	=	Deoxyribonucleic acid
DNase	=	Deoxyribonuclease
dNTPs	=	Deoxynucleotide triphosphate (s)
EDTA	=	Ethylene diamine tetraacetic acid
ELF	=	Elongation factor 1 alpha
F3H	=	Flavanone-3-hydrogenase
F3'H	=	Flavonoid 3'-hydroxylase
GT/UFGT	=	UDP glucose-flavonoid 3-*o*-glucosyl transferase.
HPLC	=	High-performance liquid chromatography
LB medium	=	Luria-Bertani medium
LC-MS	=	Liquid chromatography-mass spectrometry
LDOX	=	Leucoanthocyanidin dioxygenase
NCBI	=	National Center for Biotechnological Information
PAL	=	Phenylalanine ammonia lyase
PCR	=	Polymerase chain reaction
PERE	=	Primary ethylene response element

LIST OF ABBREVIATIONS (Continued)

PVPP	=	Polyvinyl polypyrolidone
RACE	=	Rapid amplification of cDNA ends
RH	=	Relative humidity
RNA	=	Ribonucleic acid
RT-PCR	=	Reverse transcriptase polymerase chain reaction
SDS	=	Sodium dodecyl sulfate
TF	=	Transcription factor
UTR	=	Untranslated Region
X-Gal	=	5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside

CHARACTERIZATION OF MYB TRANSCRIPTION FACTORS AND ANTHOCYANIN BIOSYNTHESIS GENES OF MANGOSTEEN FRUIT DURING RED COLOURATION

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is well known as the 'Queen of Fruit' due to its quality in terms of colour, shape and pleasant flavor. It is an important economic fruit and grown widely in the east and south of Thailand. In 2008, the amount of export was 44,271 tons with value of 744 million baht (Office of Agriculture Economics, 2009). However, the export of mangosteen fruit is limited as a result of many problems associated with low yield and low fruit quality, including internal disorders, flesh translucence, gamboges, wrinkled stylar end and pericarp hardening.

For mangosteen, fruit colour is a primary attribute used for harvesting as well as marketing. It strongly influences consumer acceptance and sale. Fruit colour is also a major criterion for fruit maturity (Paull and Ketsa, 2004; Palapol *et al*., 2009a). The fruit have a light greenish-yellow skin with scattered pinkish spots which develop to dark purple but do not ripen to full flavor if harvested. It is generally accepted that the earliest harvest stage for high quality fruit is when the skin has distinct irregular, pink-red spots over the whole of the fruit surface. Fruit are at the edible ripe stage, when the skin has darkened to reddish-purple, when no latex remains in the skin and when the flesh segments separate easily from the skin (Tongdee and Suwanagul, 1989). In contrast, Noichinda (1992) reported ripe fruit having a pale yellow to reddish purple colour, as the best eating quality fruit, while a light green colour implied inferior flavor. In addition, Tongdee and Suwanagul (1989) suggested that fruit at stage 2 to 4 (light pinkish yellow with distinct irregular pink-red spots covering the entire fruit to red to reddish brown) are suitable for export. However, fruit at these stages rapidly turn to black and are considered

unattractive and undesirable. In order to fulfill consumer demand, these problems should be solved.

The development of pericarp colour of mangosteen fruit is involved in anthocyanin pigmentation (Du and Francis, 1977). Anthocyanins are water-soluble flavonoid pigments that are found in higher plants and are responsible for red, blue and purple colours of many fruit, vegetables, flowers and seeds. Anthocyanin biosynthesis involves a common branch of the flavonoid pathway and has been intensively investigated in many plants including *Arabidopsis*, petunia, maize and grape. The regulation of anthocyanin biosynthesis is mainly at the level of transcriptional regulation of the structural genes and transcription factors (TFs) (Winkel-Shirley, 2001). The MYB transcription factor and anthocyanin biosynthetic genes have been characterized from many fruit. In grape, *VvMYBA1* and *VvMYBA2* regulate the expression of the UFGT gene (Kobayashi *et al*., 2002, 2004; Walker *et al*., 2007). In a red fleshed apple variety, Espley *et al*. (2007) found that *MdMYB10* controlled apple anthocyanin production in fruit and leaves. Analysis of transcript levels in apple fruit, indicated that most of the genes in the biosynthetic pathway (*CHS*, *F3H*, *DFR*, *ANS* and *UFGT*) are co-ordinately expressed during anthocyanin accumulation in apple skin (Honda *et al*., 2002). The expression pattern in apple differs from that in grape (*Vitis vinifera* and *V. labruscana*), in which *UFGT* induction during anthocyanin accumulation is a key regulatory step in colouration (Boss *et al*., 1996; Kobayashi *et al*., 2001). Anthocyanin accumulation can be influenced by many factors such as light, temperature and ethylene (Saure, 1990). Ratanamarno *et al.* (2005) studied the effects of light and temperature on anthocyanin content in mangosteen fruit. They found that sunlight had no significant effect on phenylalanine ammonia lyase (PAL) activity and anthocyanin content in all stages whereas the PAL activity was affected by temperature. Ethylene is a plant hormone that regulates many mechanisms in fruit ripening (Abeles *et al*., 1992). Studies in grapes and apple suggested that the ethylene induces colour development through accelerating anthocyanin biosynthesis (Boss *et al.,* 1996; Kim *et al.,* 2000; El-Kereamy *et al.,* 2003; Jeong *et al*., 2004). However, there is little information

regarding the colour development after harvest and anthocyanin biosynthesis in mangosteen.

Most reports on the molecular control of fruit colour have focused on fruit crops that develop red colour on the tree. Mangosteen fruit can develop full pigmentation both on the tree and after harvest (off-tree) within seven days. The fruit is a typical climacteric fruit in that ethylene affects fruit ripening (Palapol *et al*. 2009a). Therefore this fruit provides a system for investigating the control of anthocyanin synthesis in relation to ethylene. The ethylene action inhibitor, 1-methycyclopropene (1-MCP), and low temperature storage were used to control anthocyanin biosynthesis during colour development of mangosteen fruit. This thesis focuses on fruit quality and anthocyanin pigmentation. It examines anthocyanin-related genes, both those associated with the biochemical pathway and R2R3 MYB transcription factors during fruit colour development.

OBJECTIVES

1. To study colour development, quality and postharvest changes of mangosteen fruit harvested at different maturity stages.

2. To study factors controlling colour development in mangosteen fruit.

3. To study anthocyanin biosynthesis of mangosteen pericarp.

4. To clone and characterize the controlling MYB transcription factors and anthocyanin biosynthesis genes of mangosteen fruit during red colouration.

LITERATURE REVIEW

1. Mangosteen

Mangosteen (*Garcinia mangostana* Linn.) is one of the most admired tropical fruits and has been known as 'Queen of Fruits' (Popenoe, 1974) due to its beautiful purple colour and delicious flavor. The edible aril of mangosteen fruit is white, soft and juicy with a sweet, slightly acid taste and pleasant aroma (Martin, 1980). Apart from the aril being consumed as a dessert fruit, the dried fruit rind, which contains tannin and xanthones, is used as a native anti-inflammatory and anti-diarrhea medicine and for treatment of dysentery (Yapwattanaphun *et al*., 2002). Mangosteen is an important economic fruit in Thailand and its production and export tend to increase every year, although this is very limited due to slow growth and internal disorder. In addition, Yapwattanaphun *et al*.(2002) reported that several *Garcinia* species in Thailand such as *Garcinia schomburgkiana* (Madan), *Garcinia dulcis* (Maphuut), *Garcinia cowa* (Chamuang), *Garcinia atroviridis* (Som Khaek), *Garcinia hanburyi* (Rong), *Garcinia bancana* (Chamuang paa), *Garcinia xanthochymus* (Mada Luang), *Garcinia thorelii* (Mada Kheenon), *Garcinia hombroniana* (Waa), *Garcinia speciosa* (Phawaa).have potential to be improved as horticultural crops for commercial use as timber, gum, and various other natural products

Mangosteen fruit is at the edible or ripe stage when the pericarp has darkened to reddish-purple and when no latex remains in the pericarp and the aril segments separate easily from the pericarp. The globe-shaped and smooth berry is 4 to 7 cm (1.6 to 2.8 inches) across, with a persistent calyx. The pericarp is 6 to 10 mm (0.24 to 0.4 inches) thick and turns purple during ripening. It contains a bitter, yellowish latex and purple-staining juice. The edible white aril has 4 to 8 segments with 1 or 2 larger segments containing apomictic seeds. There is no true seed. Fruit development takes 100-120 days from anthesis and up to 180 days in cooler areas or at high elevations (Nakasone and Paull, 1998; Paull and Ketsa, 2004).

Skin colour is the major criterion for fruit maturity. A fully mature fruit is identified by the occurrence of red lines appearing on the outer pericarp. The number of lines, so called 'bloodlines', increase as the fruit becomes mature. Alternatively, the maturity can be gauged by the way in which the fruit separates from the peduncle stalk. Fully mature fruit, when picked, will detach easily and clearly from the point of stalk attachment. After picking, metabolic changes continue within the fruit. (Kanchanapoom and Kanchanapoom, 1998).

At present, there is no universal maturity index. Malaysia, Thailand and Australia, all have developed their own indices for harvesting. In Thailand, the changes in skin colour are divided into seven stages (Tongdee and Suwanagul, 1989) as follows:

Stage 0: Pericarp uniformly yellowish white or yellowish white with light green tinge or grayish spotting. Yellowish latex in pericarp very severe. Pericarp and aril not separable.

Stage 1: Pericarp light greenish yellow with scattered pinkish spots. Latex in pericarp severe. Pericarp and aril not separable.

Stage 2: Pericarp light pinkish yellow with distinct irregular pink-red spots covering the entire fruit. Latex in pericarp moderate. Pericarp and aril separation difficult to moderate.

Stage 3: Pericarp background uniformly pinkish, spottings not as distinct as in stage 2. Latex in pericarp slight to very slight. Pericarp and aril separation moderate.

Stage 4: Pericarp red to reddish brown, some with purple tinge. Latex in pericarp very slight to none. Pericarp and aril separate readily.

Stage 5: Pericarp darkened to reddish purple. No latex remain in pericarp. Complete and easy separation of pericarp and aril.

Stage 6: Pericarp purple, dark purple or black, with or without purple colouration remain. No latex in pericarp. Complete and easy separation of pericarp and aril.

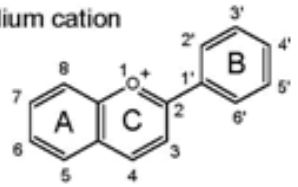
2. Anthocyanins

Most of the bright-red and blue colours found in higher plants are anthocyanins. Anthocyanins (from the Greek anthos, a flower, and kyanos, dark blue) are flavonoids commonly found in nature. Their structure is based on a C15 skeleton comprised of two phenyl rings (called the A- and B-ring) connected by a three-carbon bridge that usually forms a third ring (called C-ring) (Figure 1A). The basic chemical 'backbones' that make up the anthocyanins are called anthocyanidins or aglycones, because they have no sugars attached to aromatic rings (Boss and Davies, 2001). Anthocyanins are highly diverse in nature and produced by the chemical combination of the basic C6-C3-C6 anthocyanin structure with sugars and/or acyl groups. The most abundant anthocyanin group has 17 carbons. Their differences are in the number and position of the hydroxyl and/or methyl ether groups. In nature, the most common anthocyanidins are pelargonidin, cyanidin, peonidin, delphinidin, malvidin and petunidin (Figure 1B and 1C). These aglycones differ in the number of hydroxyl and methoxyl groups in the B-ring of the flavylium cation (Delgado-Vargas and Paredes-lopez, 2002; Davies, 2004).

The stability of anthocyanins is increased considerably by the addition of a glycoside. Anthocyanins with a β-glucosidic linkage are mostly formed in fruit. Each anthocyanidin can vary in the number and type of sugar substitution. In monoglycosides, glycosylation generally occurs at the 3 position on the C-ring, and the most common substitutes are glucose, arabinose or galactose. In diglycosides, either disaccharide can attach at position 3 e.g. 3-glucoside, or two monosaccharides can be linked to different hydroxyls e.g. 3, 5-diglucosides. Triglycosides are rare in fruit. Anthocyanins can be acylated with either organic or phenolic acids, which often contribute to their stability (Macheix *et al*., 1990).

Anthocyanins are water-soluble pigments and accumulate in the vacuoles of plant cells. Their structures produce a great range of colours from scarlet to blue that are clearly represented in fruit, flowers, leaves and storage organs. The main anthocyanins in fruit are glycosides of six anthocyanidins. Cyanidin is the most common anthocyanidin (Macheix *et al*., 1990). The composition of flavonoids in different fruit species varies greatly. In berries, the anthocyanin concentration correlates well with the darkness of the berry colour and hue (Rein, 2005). Du and Francis (1977) reported that mangosteen pericarp contains a substantial amount of red pigment. The major pigment is identified as cyanidin-3-sophoroside, while a minor pigment is identified as cyanidin-3-glucoside. Total anthocyanin content in the pericarp at stage 6 ranged from 84 to 105 mg/100 g fresh weight (Ratanamarno, 1999). Anthocyanin content increased accordingly and had the highest content at stage 6 (Ratanamarno *et al*., 2005).

A



B

Name	Hydroxyl group in	Colour
Apigeninidin	5, 7, 4'	Orange
Aurantinidin	3, 5, 6, 7, 4'	Orange
Cyanidin	3, 5, 7, 3', 4'	Magenta and crimson
Delphynidin	3, 5, 7, 3', 4', 5'	Purple, mauve and blue
8-Hydroxycyanidin	3, 5, 6, 7, 3', 4'	Red
Luteolinidin	5, 7, 3', 4'	Orange
Pelargonidin	3, 5, 7, 4'	Orange, salmon
Triacetidin	5, 7, 3', 4', 5'	Red

C

Name	Hydroxyl group in	Colour
Capensinidin	5, 3', 5'	Bluish red
Europenidin	5, 3'	Bluish red
Hirsutidin	7, 3', 5'	Bluish red
Malvidin	3, 5'	Purple
5-Methylcyanidin	5	Orange-red
Peonidin	3'	Magenta
Petunidin	3'	Purple
Pulchellidin	5	Bluish red
Risinidin	7	Red

Figure 1 Basic structure of anthocyanin pigments in which R_x could be H (A), OH (B) or OCH_3 (C) depending on the pigment considered. The most commonly accepted nomenclature numbering carbons is indicated inside the structure. The most important naturally-occurring anthocyanidins are underlined.

Source: Delgado-Vargas and Paredes-lopez (2002)

3. Anthocyanin biosynthesis

The anthocyanin branch of the flavonoid biosynthetic pathway has been extensively studied in horticultural and cultivated plant species. The precursors of anthocyanins are produced by the glycolytic pathway (phosphoenolpyruvate) and the pentose-phosphate pathway/Calvin cycle. Two main parts constitute the anthocyanin biosynthesis pathway: (1) precursors of general phenylpropanoid metabolism (Figure 2A and 2B) and (2) specific steps toward flavonoid biosynthesis (Figure 2B). Phenylalanine is converted to *p*-coumaryl-CoA in a process involving three enzymes: phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumaryl-CoA-ligase (4CL). *p*-Coumaryl-CoA is the main precursor of flavonoid, lignin and other phenylpropanoids (Figure 2A). *p*-Coumaric acid is used to construct the C-6-C-3 (B aromatic rings and carbons corresponding to the C-ring) portion of the basic flavonoid structure (Figure 2B). PAL activity increases concomitantly with accumulation of anthocyanin in many plants, including apple (Lister *et al*., 1996, Macheix *et al.*, 1990). However, the activity of PAL increases despite the absence of anthocyanin. It has been concluded that PAL is not a regulatory enzyme of anthocyanin formation (Ju *et al.*, 1995, Wang *et al*., 2000). Sparvoli *et al*. (1994) isolated a partial cDNA encoding *PAL* from grapevine using an *Antirrhinum* PAL clone as a probe. Kumar and Ellis (2001) found two PAL genes in raspberry and *RiPAL1* was associated with early fruit ripening events, whereas expression of *RiPAL2* was correlated in later stages of flower and fruit development. The second part of the pathway flavonoids is involved in generating (Figure 2B).

3.1 Chalcone synthase (CHS)

Chalcone synthase is considered to be the key enzyme in flavonoid biosynthesis and catalyzes the condensation of three molecules of malonyl-CoA with 4-coumaryl-CoA to form the C_{15} compound; 2', 4', 6', 4 tetrahydroxychalcone (naringenin chalcone). Chalcones are the first flavonoids and direct precursors for other flavonoids. As can be deduced, acetyl-CoA provides ring A and the oxygen of the central pyran ring throughout malonyl-CoA (Heller and Forkmann, 1994; Holton

and Cornish, 1995). The first CHS gene was isolated from parsley (Kreuzaler *et al.*, 1983). Sparvoli *et al*. (1994) isolated a *CHS* homologue from grape seedling and it is a small gene family. Goto-Yamamoto *et al.* (2002) found 3 genomic clones of chalcone synthase in grape (*CHS1, CHS2* and *CHS3*) which are expressed in different tissues and fruit development. Tsuda *et al.* (2004) examined the gene expression of six genes in the anthocyanin biosynthesis pathway of white and red peaches and a deep-red nectarine. The results suggest that *CHS* and *DFR* are the key regulatory genes in mature red peach and nectarine.

3.2 Chalcone isomerase (CHI)

After formation of the chalcone, many plants do not accumulate chalcones. Naringenin chalcone is rapidly isomerized by chalcone isomerase (CHI) to form the flavanone naringenin. Even in the absence of CHI, naringenin chalcone may spontaneously isomerise to form naringenin (Holton and Cornish, 1995). CHI mutants of aster and carnation cause their petals to be yellow in colour. For *Arabidopsis*, the CHI mutant causes change in seed coat colour (Forkmann and Heller, 1999), and also the fruit peel of tomato accumulates the yellow-coloured naringenin chalcone (Muir *et al*., 2001). Almieda *et al*. (2007) reported that the *CHI* transcript level of ‘Queen Elisa’ strawberry during fruit development was highest in the turning stage and decreased at the red stage.

3.3 Flavonoid hydroxylation (F3H/F3'H/F3'5'H)

The subsequent hydroxylation in position C-3 of flavanones to dihydroflavonols has been demonstrated for a wide variety of plant species including *Petunia*, snapdragon, tomato and maize. The reaction is carried out by flavanone-3-hydroxylase (F3H), a member of the 2-oxoglutarate-dependent dioxygenase family which is highly conserved among widely divergent plant species as shown by sequence comparison (Britsch *et al*., 1993). F3H and F3'5'H belong to the cytochrome P450 super family and catalyze hydroxylation at the 3' and 3'5' positions of the B-ring of the flavonoid, respectively. The products are precursors for cyanidin-

based anthocyanins (red) and delphinidin-based anthocyanins (blue to purple). cDNA clones of the F3'H and F3'5'H genes were first isolated from petunia. A mutation of F3H causes loss of activity and prevents the progression along the anthocyanin pathway. The mutants of *Petunia* and *Antirrhinum* have white flowers (Martin *et al*., 1991; Britsch *et al*., 1992). Using the snapdragon F3H clone to isolate grape homologues, Sparvoli *et al*. (1994) found the *F3'H* gene and the *F3'5'H* gene. In flowers, stems, tendrils and seeds of grape, they accumulated at a higher level of mRNA for *F3'H* than *F3'5'H*. The berry skin at the harvest stage accumulated a high transcript level of *F3'5'H* and a high level of delphinidin-based anthocyanins but small leaf accumulated prodelphinidin (Jeong *et al.*, 2006).

3.4 Dihydroflavonol 4-reductase (DFR)

Dihydroflavonol-4-reductase catalyzes the conversion of dihydrokaempferol, dihydroquercetin and dihydromyricetin into leucoanthocyanidins using NADPH as a co-factor (Kristiansen and Rohde, 1991; Delgado-Vargas and Paredes-lopez, 2002). These leucoanthocyanidins are the immediate precursors for anthocyanin synthesis. DFR genes have been isolated from many plants such as grape (Sparvoli *et al*., 1994; Gollop *et al*., 2002) and apple (Honda *et al*., 2002). For strawberry, the DFR gene is a mainly involved in anthocyanin biosynthesis during colour development at the late stages of fruit ripening (Moyano *et al.*, 1998; Almieda *et al*., 2007). Tsuda *et al.* (2004) reported that *DFR* is the key regulatory gene of anthocyanin biosynthesis in mature red peach and nectarine.

3.5 Anthocyanin synthase (ANS)/ Leucoanthocyanidin dioxygenase (LDOX)

The leucoanthocyanidins are converted to anthocyanidins by anthocyanidin synthase, which is also called ‘leucoanthocyanidin dioxygenase’, another member of the 2-oxoglutarate-dependent dioxygenase family. ANS shows large homology to F3H and FLS (Martin *et al*., 1991; Tanaka *et al*., 1998). Genomic or cDNA sequences encoding ANS have been obtained from several plant species including *Arabidopsis, Antirrhinum, Petunia*, *Vitis vinifera* and maize. ANS mutants,

as well as mutations in regulatory genes affecting ANS gene expression, have been studied in these plants (Martin *et al*., 1991; Jackson *et al.*, 1992; Pelletier *et al*., 1999; Bradley *et al*., 1998). Almieda *et al*. (2007) found that the transcript levels in 'Queen Elisa' strawberry of *ANS* increased during fruit development and were highest at the red stage.

3.6 Flavonoid 3-*O*-glucosyltransferase (UFGT, 3GT, UFGluT)

In general, flavonoids and anthocyanidins with a free hydroxyl group at the 3 position of the heterocyclic ring are unstable under physiological conditions and are not found in nature (Forkmann and Heller, 1999). The enzyme UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT, 3GT) is responsible for the transfer of the glucose from UDP-glucose to the hydroxyl group in position 3 of the C-ring. Since this is an essential final step required to stabilize anthocyanidins so that they can accumulate as water soluble pigments in the vacuoles, 3GT is regarded as an indispensable enzyme of the main biosynthetic pathway to anthocyanins, rather than simply as a modifying enzyme. It is interesting to note that mutants with decreased DFR and ANS activity also show decreased 3GT activity, suggesting that the late genes of the anthocyanin pathway are co-regulated or may exist as a functional complex (Hrazdina and Wagner, 1985; Hrazdina and Jensen, 1992). Depending on the B-ring hydroxylation pattern, three major types of anthocyanins can finally be distinguished. Each type has a characteristic colour, since the visible absorption maximum becomes longer as the number of hydroxyl groups in the B-ring increases: pelargonidin-derived pigments are responsible for orange, pink or red colours; cyanidin-derived pigments are responsible for red or magenta and delphinidin-derived pigments are responsible for purple or blue (Zuker *et al*., 2002). Boss *et al*. (1996) reported the expression of the UFGT gene as the key regulatory gene to control red colour skin in grape. This activity is also strongly correlated to anthocyanin accumulation in apple. Kobayashi *et al*. (2001) found that there are no differences in either coding or promoter sequences of *UFGluT* between white and red skin grape cultivars. They concluded that the mutation of the regulatory gene controlling UFGluT gene expression causes the change from white to red skin.

Castellarin and Gaspero (2007) reported that the transcript abundance of *UFGT* peaked at the 50% veraison red and decreased thereafter. The transcript level showed strong correlation ($R^2 = 0.80$) with the final anthocyanin content and the transcript level of structure genes explained *per se* the final phenotype for anthocyanin content, anthocyanin composition, colour intensity and colour hue of grapes at berry maturity. In 'Queen Elisa' strawberry, Almieda *et al*. (2007) found that the transcript level of *UFGT* was expressed increasingly during fruit development. Griesser *et al.* (2008) also reports the function of *UFGT* in strawberry fruit in down-regulating an anthocyanidin-3-*O*-glycosyltransferase gene by RNA interference. The average *FaGT1* transcript levels of transgenic fruit were about 15% of the levels in control fruit and the pelargonidin 3-*O* glucoside content in the fruit was reduced to 7.5% of control levels.

Further flavonoid modification by acylation, additional glycosylation to flavonoid disaccharides or trisaccharides, methylation or hydroxylation, may occur within each flavonoid class. However, modifications like phenylation, sulfation and C-glycosylation are restricted to particular flavonoid groups. Most modifications are performed by the end products including anthocyanin-3-glucosides, flavonols, flavones and (iso)flavones, while intermediates of the pathway can be used as substrates (Heller and Forkmann, 1994).

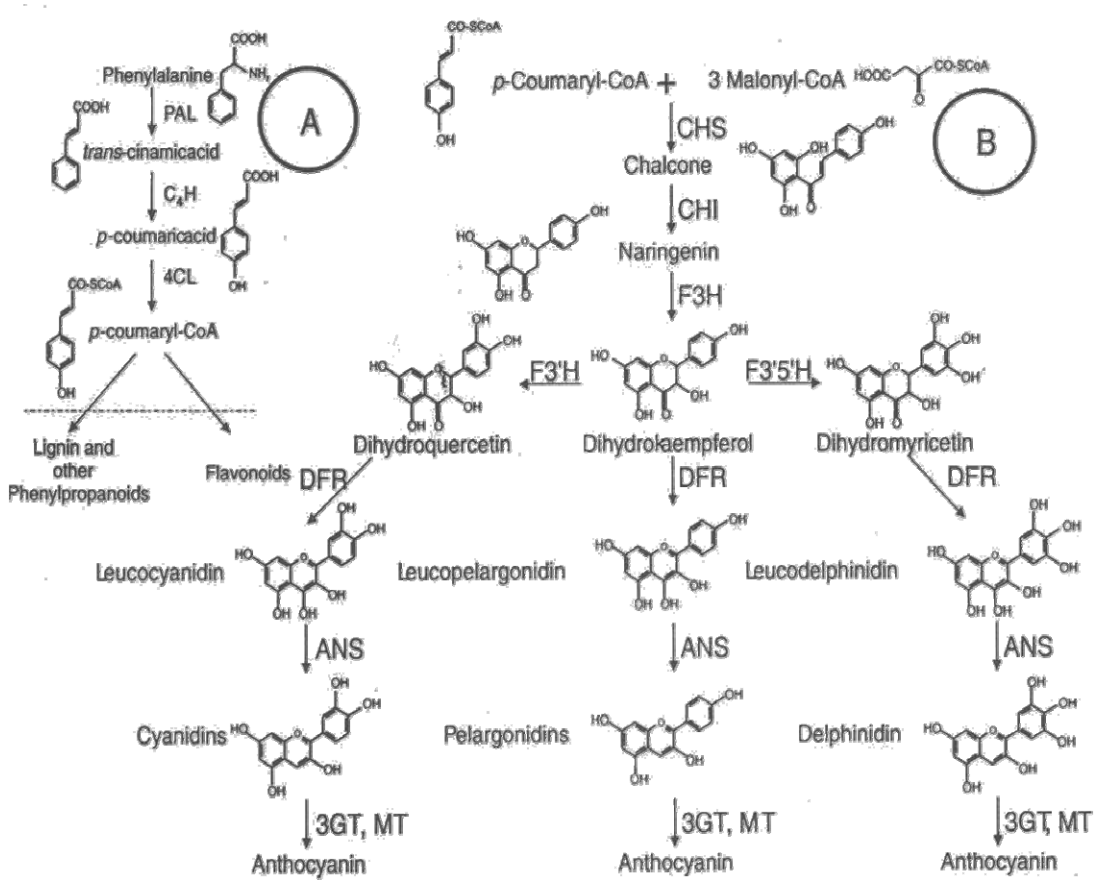


Figure 2 Anthocyanin biosynthesis pathway. (A) General phenylpropanoid metabolism. Enzymes involved: PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl:CoA ligase. (B) Specific steps of anthocyanin biosynthesis. Enzymes involved: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, F3'H, F3'5'H, flavanol hydroxylase; DFR, dihydroflavonol-4-reductase; ANS, anthocyanidin synthase; 3GT, UFGT, UDP glucose-flavonoid 3-*o*-glucosyl transferase.

Source: Delgado-Vargas and Paredes-lopez (2002)

4. Regulators controlling anthocyanin biosynthesis

Regulation of gene expression at the transcriptional level controls many crucial biological processes including anthocyanin biosynthesis (Table 1). A number of different factors are required for the process of transcription. These include factors required for chromatin remodelling and DNA unwinding, as well as proteins of the pre-initiation complex and the RNA polymerase II complex. In addition to this general inventory, other factors control promoter strength. These factors are referred to as 'transcription factors', a term usually used to describe proteins that recognize DNA in a sequence-specific manner and that regulate the frequency of initiation of transcription upon binding to specific sites in the promoter of target genes. Transcription factors (TFs) can be activators, repressors or both (Pabo and Suaer, 1992). The regulation of the pathway is largely at the transcription level of the regulators and of the corresponding biosynthetic genes. Regulatory genes controlling expression of the biosynthetic genes have been identified in many plants. These genes influence the intensity and pattern of anthocyanin biosynthesis and generally control expression of many different structural genes. Evidence for this regulation can be obtained by either enzyme assays or mRNA assays of structural gene activity (Holton and Cornish, 1995). Advanced methods are being used to understand the regulation of flavonoid biosynthesis, particularly molecular genetic approaches such as transposon tagging and positional cloning. This has led to the identification of a number of novel regulatory proteins that are beginning to fill the void between signals that induce the pathway and well known flavonoid regulators such as the MYB domain and basic helix-loop-helix (bHLH) transcription factors (Winkel-Shirley, 2001).

The first identification of a MYB gene was the 'oncogene' *v-Myb* derived from the avian myeloblastosis virus (Klempnauer *et al*., 1982). The sequence comparisons indicate that *v-Myb* may have originated from a vertebrate gene, which mutated once it became part of the virus. Many vertebrates contain three genes related to *v-Myb* that are *c-Myb, A-Myb* and *B-Myb* (Weston, 1998) and other similar genes have been identified in insects, plants, fungi and slime moulds (Lipsick, 1996).

The so-called MYB domain consists of three imperfect repeats, each forming a helix-turn-helix structure of about 53 amino acids. Three regularly spaced tryptophan residues, which form a tryptophan cluster in the three-dimensional helix-turn-helix structure, are characteristic of a MYB repeat (Ogata *et al*., 1992; Konig *et al*., 1998). The three repeats in c-Myb are referred to as R1, R2 and R3. The DNA binding domain encoded by most of the plant MYB genes including MYBs controlling anthocyanin biosynthesis, is formed by two repeats, which are most similar to repeats R2 and R3 of c-MYB proteins (Jackson *et al*., 1991; Avila *et al*., 1993; Quaedvlieg *et al*., 1996; Martin and Paz-Ares, 1997; Stracke *et al*., 2001; Allan *et al*., 2008).

The bHLH proteins belong to a large family of transcriptional regulators present in both animals and plants. The bHLH was initially identified in the animal transcriptional regulators MyoD and Myc (Murre *et al*., 1989). The family of TFs is characterized by the signature bHLH domain comprising approximately 60 amino acids that encode two functionally distinctive regions, the basic region and the HLH region. The basic region is located at the N terminus of the bHLH domain and functions as a DNA-binding motif. It consists of approximately 15 amino acids, which typically include six basic residues. The basic region of the domain binds to DNA containing the canonical E-box (CANNTG) sequence. The HLH region is comprised of hydrophobic residues that form two α-helices separated by a loop region of variable sequence, length and functions via the homo- and heterodimerization (Atchley *et al*., 1999; Bailey *et al*. 2003; Heim *et al*., 2003; Toledo-Ortiz *et al*., 2003). The plant Myc-like bHLH TFs contain an N-terminal interaction domain as Myb-interacting region (MIR) that has been shown to interact with an R2R3-MYB domain protein and affect transcription of anthocyanin biosynthetic pathway genes (Goff *et al*., 1990, 1992; Grotewold *et al*., 2000; Hernandez *et al*., 2004). Another group of regulatory factors, WD40 repeat proteins, is also important for the transcription of genes for anthocyanin biosynthesis. WD40 repeat proteins comprise a family in the β-propeller protein group, which is characterized by the presence of a 40 residue core region delineated by a glycine–histidine (GH) dipeptide and a tryptophan–aspartate (WD) dipeptide (Smith, 1999). This motif is tandemly repeated four to 16 times in the same protein. The most

extensively studied WD40 repeat protein is the Gβ subunit of heterotrimeric G proteins involved in signal transduction, which forms a seven-bladed β-propeller structure containing seven WD40 repeats. In addition to signal transduction, these proteins are involved in many functions. A common function of WD40 repeat units is that they facilitate protein–protein interactions and have no intrinsic enzymatic function. Several WD40 repeat containing proteins affecting epidermal cell structure and anthocyanins have been identified from petunia (*anthocyanin11*, *AN11*) and *Arabidopsis* (*TRANSPARENT TESTA GLABRA1*, *TTG1*) (de Vatten *et al*., 1997; Walker *et al*., 1999; Carey *et al*., 2004; Ramsey and Glover, 2005). The transcription complex composed of MYB, bHLH, and WD40 regulates the expression of multiple distinct target genes in a range of plant species generating plant epidermal cellular diversity including anthocyanin production (Broun, 2005; Koes *et al*., 2005; Ramsey and Glover, 2005).

Recently, molecular approaches have proven to be very useful for isolating flavonoid regulatory genes for which no previous information existed concerning their gene sequence, function or final products. There are numerous reports of the regulation of genes in the anthocyanin pathway by transcription factors (TFs), controlling anthocyanin biosynthesis in all higher plants (Holton and Cornish, 1995). There are 126 R2R3 MYB genes in *Arabidopsis* (Stracke *et al*., 2001) and 108 in grape (Matus *et al*., 2008). On the basis of the R2R3 motif in *Arabidopsis*, the MYBs have been classified into 22 subgroups (Stracke *et al*., 2001). The Production of Anthocyanin Pigment 1 (*AtPAP1* or *AtMYB75*) MYB of *Arabidopsis* falls into a subgroup that has been termed subgroup 10 (Allan *et al*., 2008). The protein sequence of AtPAP1 has a high degree of amino acid conservation with other known MYB regulators of anthocyanin production in other plant species (Allan *et al*., 2008). Overexpression of *AtPAP1* and *AtPAP2* strongly induced anthocyanin accumulation in *Arabidopsis* as the result of the upregulation of all the genes in the anthocyanin biosynthesis pathway (Borevitz *et al*., 2000; Tohge *et al*., 2005). The partnerships between colour-related MYBs and bHLHs such as the maize ZmC1 MYB and ZmB bHLH, the petunia AN2 MYB and AN1 and JAF13 bHLHs, and the *Antirrhinum* Ros1, Ros2 and Ve MYBs and the Mut and Del bHLHs, have been reported (Goff *et*

al., 1992; Goodrich *et al*., 1992; Mol *et al*., 1998; Schwinn *et al*., 2006). Over-expressing *Arabidopsis* lines of PAP1 has elevated transcript levels of the *TT8* that encodes a bHLH protein involved in regulating condensed tannin and anthocyanin biosynthesis (Nesi *et al*., 2001; Tohge *et al*., 2005).

In fruit, MYBs controlling anthocyanin biosynthesis have been characterized from apple, grape and strawberry. In grape, *VvMYBA1* and *VvMYBA2* regulate the expression of the UFGT gene (Kobayashi *et al*., 2002, 2004; Walker *et al*., 2007). In strawberry, *FaMYB1* plays a key role in anthocyanin and flavonol biosynthesis. However, results indicate that *FaMYB1* represses transcription of anthocyanin-related genes late in fruit maturation (Aharoni *et al*., 2001). In apple, several MYBs have been isolated which control anthocyanin biosynthesis. *MdMYBA* was isolated from a pale skinned cultivar 'Tsugaru' and deep-red skinned 'Jonathan' fruit (Ban *et al*. 2007), whereas *MdMYB1* was isolated from the red skinned apple 'Cripps Pink' (Takos *et al*., 2006). *MdMYB1* and *MdMYBA* expression correlated with anthocyanin synthesis in the fruit skin. When the fruit were exposed to sunlight after bagging, *MdMYB1* transcript levels increased over several days, correlating with skin anthocyanin accumulation (Takos *et al*., 2006). In a red fleshed apple variety, Espley *et al*. (2007) found that *MdMYB10* controlled apple anthocyanin production in fruit and leaves. The apple MYB needs a bHLH (MdbHLH3 or MdbHLH33) partner to achieve full functionality (Allan *et al*., 2008).

Table 1 Anthocyanin/flavonoid pigmentation biosynthetic and regulatory genes characterized in different plant species.

Gene products	Gene name	Symbols
Maize		
CHS	*colorless2*	*c2*
	white pollen	*whp*
CHI	*chalcone isomerase1*	*chi1*
F3'H	*red aleurone*	*pr1*
DFR	*anthocyaninless1*	*a1*
ANS	*anthocyaninless2*	*a2*
UF3GT	*bronze1*	*bz1*
GST	*bronze2*	*bz2*
MYB	*colorless1*	*c1*
	purple plant	*pl*
	pericarp color	*p*
bHLH	*red*	*r*
	booster	*b*
	intensifier	*in1*
WD40	*pale aleurone color1*	*pac1*
Snapdragon		
CHS	*nivea*	*niv*
F3H	*incolorata*	*inc*
DFR	*pallida*	*pal*
ANS	*candi*	*candi*
MYB	*rosea*	*ros*
	venosa	*ven*
bHLH	*dellila*	*del*
Petunia		
CHS		*chsA*
		chsJ

Table 1 (Continued).

Gene products	Gene name	Symbols
CHI		*po*
F3H	*anthocyanin3*	*an3*
F3'H		*ht1*
F3'5'H		*hf1, 2*
DFR	*anthocyanin6*	*an6*
UF3GRT		*rt*
GST	*anthocyanin9*	*an9*
MYB	*anthocyanin2*	*an2*
bHLH	*anthocyanin1*	*an1*
		jaf13
WD40	*anthocyanin11*	*an11*
Arabidopsis		
CHS	*TRANSPARENT TESTA4*	*TT4*
CHI	*TRANSPARENT TESTA5*	*TT5*
F3H	*TRANSPARENT TESTA6*	*TT6*
F3'H	*TRANSPARENT TESTA7*	*TT7*
DFR	*TRANSPARENT TESTA3*	*TT3*
ANS	*TRANSPARENT TESTA18*	*TT18*
	TANNIN DEFICENT SEED4	*TDS4*
ANR	*BANYULS*	*BAN*
UF3GT	*UGT78D2*	
UF5GT	*UGT75C1*	
GST	*TRANSPARENT TESTA19*	*TT19*
MATE	*TRANSPARENT TESTA12*	*TT12*
MYB	*TRANSPARENT TESTA2*	*TT2*
	PRODUCTION OF ANTHOCYANIN PIGMENT1	*PAP1*

Table 1 (Continued).

Gene products	Gene name	Symbols
bHLH	*TRANSPARENT TESTA8*	*TT8*
	GLABRA3	*GL3*
	ENHANCER OF GLABRA3	*EGL3*
WD40	*TRANSPARENT TESTA GLABRA1*	*TTG1*
Morning Glory		
CHS	*R1* (N)	*R1*
	Anthocyanin (P)	*A*
CHI	*Speckled* or *Cream* (N)	*Sp*, *Cr*
F3'H	*Magenta* (N)	*Mg*
	Pink (P)	*P*
	Fuchsia (T)	*Fuchsia*
DFR	*A3* (N)	*A3*
ANS	*Pearly* (T)	*Pearly*
UF3GT	*R3* (N)	*R3*
UF3GGT	*Duskish* (N)	*Dk*
	Dusky (N)	*Dy*
MYB	*C1* (N)	*C1*
	W (P)	*W*
bHLH	*Ivory seed* (P)	*IVS*
	Ivory seed (T)	*IVS*
WD40	*Ca* (N)	*Ca*

(N), (P), and (T) under Morning Glory indicate *I. nil*, *I. purpurea*, and *I. tricolor*, respectively.

Source: Adapted from Chopra *et al*. (2006)

5. Factors affecting fruit colouration

There are many factors affecting fruit colour as discussed below:

5.1 Light

The most important influence on anthocyanin biosynthesis is light. Many researchers have studied the effect of light on anthocyanin biosynthesis in fruit such as apple (Saure, 1990; Ju *et al*., 1995, 1998) and grape (Spayd *et al*., 2002; Jeong *et al*., 2004). In apple, the formation of anthocyanin is absolutely light dependent. Bagging affected anthocyanin formation and reduced red colour development but did not affect fruit maturity. When bagged fruit was exposed to light, they started to accumulate anthocyanin rapidly and reached a maximum after 3 days of light exposure. Chalcone synthase activity and flavonoid contents were relatively high and constant from the fruitlet to the maturation stage and suggested that CHS does not play a regulatory role in anthocyanin synthesis in apples exposed to light (Saure, 1990; Ju *et al*, 1995; Ju, 1998). The effect of light on anthocyanin biosynthesis is expressed in the activation of different enzymes (Gross, 1987). In contrast, Ratanamarno *et al*. (2005) reported that the sunlight had no effect to phenylalanine ammonia lyase (PAL) activity and anthocyanin content in every stage of mangosteen development. For grape, Jeong *et al.* (2004) found that shading suppressed anthocyanin accumulation and gene expression of anthocyanin biosynthesis. Takos *et al*. (2006) also reported that *MdMYBB1* transcript levels increased over several days, when the bagged fruit were exposed to light, and correlated with anthocyanin content.

5.2 Temperature

Most anthocyanin-containing fruit develop higher colouration in cooler regions. High temperatures in autumn inhibit anthocyanin formation whereas low temperature promotes it. The optimal temperature regimes changed with maturity. High temperature (12^{o}C day and night) had more effects on mature fruit (Gross,

1987). Sun light exposure affected total skin monomeric anthocyanins in grape clusters (Spayd, 2002). Ubi *et al*. (2006) found that UV-B and low temperature were important factors for anthocyanin accumulation in apple fruit skin by inducing the expression of the anthocyanin biosynthetic genes, especially CHS, ANS and UFGluT.

5.3 Plant growth regulators

Ethylene is a ripening hormone that increases the rate of anthocyanin accumulation as these pigments are associated with fruit maturation. Many researchers have reported effects of ethylene treatments on inducing anthocyanin accumulation (Gomez-Cordoves *et al*., 1996; El-Kereamy *et al*., 2003). For apple, chalcone synthase (CHS) and UDPGal:flavonoid-3-*O* -glycosyltransferase (UFGalT) activities correlated with anthocyanin accumulation during fruit maturation in both 'Delicious' and 'Ralls' apples. Ethephon treatment increased UFGalT activity and also promoted anthocyanin formation (Ju *et al*., 1995). Ethylene induced internal ethylene, anthocyanin accumulation and gene expression of anthocyanin biosynthesis in grape (El-Kereamy *et al*., 2003). 1-Methycyclopropene (1-MCP) is an ethylene action inhibitor and has been found to delay ripening of many climacteric fruit. 1-MCP delayed red colouration and anthocyanin content in grapes (Chervin *et al*., 2004) and strawberries (Jiang *et al*., 2001) and also delayed colour development in apples by inhibiting phenylalanine ammonia lyase activity (MacLean *et al.*, 2006).

However, other hormones can have an effect on anthocyanin biosynthesis. Awad and Jager (2002) treated apple with (S)-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride (ABG-3168), gibberellins (GA4+7 and GA3), Alar, cycocel (CCC to inhibit gibberellins). ABG and GA3 applications significantly retarded anthocyanin accumulation but had no effect on flavonoid compounds or chlorogenic acid. ABG delayed the transition to rapid anthocyanin accumulation but none of the other chemicals had an influence on the formation of anthocyanin, total flavonoids and chlorogenic acid in fruit skin.

5.4 Storage conditions

Differing storage conditions can have effects on anthocyanin accumulation in many fruit. Holcroft *et al*. (1998) reported the effects of carbon dioxide on anthocyanin content in pomegranate and found that the aril of pomegranates stored in air at 10^{o}C displayed a deeper red compared to the initial control and carbon dioxide-enriched atmospheres. Mangosteen fruit at stage 1 stored at low temperatures (15^{o}C) changed to stage 6 later than fruit stored at high temperatures (35^{o}C) and fruit stored at room temperature (30^{o}C) (Ratanamarno *et al.,* 2004).

6. Analysis of gene expression

In all organisms, gene expression can be divided into two main phases; transcription and translation. Quantitative determination of gene expression at the mRNA level is a powerful approach for comparative analysis. Several methods have been developed to study the expression of specific RNA levels or proteins (Dale and von Schantz, 2003).

6.1 Northern blotting

Analysis of gene expression patterns is essential for understanding and elucidating gene functions. Northern blotting, known as northern hybridization, is a technique used for detection and quantification of specific RNA levels. The steady-state level of RNA transcripts is one of the most convenient parameters used to monitor the activity of an endogenous or introduced gene in cell lines and tissues. Northern blotting involves several steps that include the isolation of RNA, transfer to a membrane, detection by nucleic acid hybridization and autoradiography. Transcription patterns of genes are often complex and multiple RNA species can be generated from the same gene. Northern analysis provides information on the relative number, size and abundance of RNAs derived from a gene. This technique generates a record of the RNA that is stored on the membrane that can be used many times. Therefore, the expression of several genes can be analyzed on the same RNA

samples by using multiple probes to rehybridize the filter. The major limitations of northern blot analysis are RNA degradation and low sensitivity. The RNA amount must be high quality and not degraded, which can be difficult in some tissues or for inexperienced workers (Krumlauf, 1994; Streit *et al*., 2009).

6.2 Reverse transcription polymerase chain reaction (RT-PCR)

The polymerase chain reaction (PCR) amplification is an extremely powerful technique for obtaining readily detectable, and manipulation of DNA or RNA. Reverse transcription polymerase chain reaction (RT-PCR) is a common, sensitive and powerful tool for RNA expression analysis. RT-PCR permits analysis of gene expression from very small amounts of RNA. It is possible to detect a specific mRNA in a single cell or to analyze gene expression in cells that are difficult to obtain in large amounts (Dale and von Schantz, 2003; Auta *et al*., 2007). Furthermore, this method can be conducted on a large number of samples and/or many different genes in the same experiments (Freeman *et al*., 1999). The relative RT-PCR method involves determination of the levels of both the target mRNA and an internal control mRNA (generally, a housekeeping gene). Comparison of housekeeping mRNA levels in different samples is used to standardize samples such that the each sample contains the same amount of the housekeeping genes. Based on the assumption that the level of housekeeping genes is constant, the relative levels of target mRNA in each sample can be determined (Giambernardi and Klebe, 2000).

6.3 Real-time PCR

In essence, real-time PCR refers to the ability to monitor DNA amplification at each cycle in the PCR reaction. Real-time qRT-PCR has advantages compared with conventionally performed ‘semi-quantitative end point’ RT-PCR or other methods, because of its high sensitivity, high specificity, good reproducibility and wide dynamic quantification range (Bustin, 2000; Gachon *et al*., 2004). The duration of a whole real-time PCR allow rapid production of data. It ranges from 20 min to 2 h. Real-time PCR provides high sensitivity for the detection of DNA or

RNA due to a combination of the amplification performed by the PCR step and the system of detection (Bustin, 2000). Basically, real-time quantitative PCR may be used for quantifying DNA or RNA abundance, leading to some applications such as detection and quantification of foreign DNA (e.g. micro-organisms contamination, transgenic plants and gene expression studies). Real-time qPCR has become a routine and robust approach for measuring the expression of genes of interest, validating microarray experiments, and monitoring biomarkers (VanGuilder *et al*., 2008).

Real-time reactions are carried out in a thermocycler that permits measurement of a fluorescent detector molecule, which decreases post-processing steps and minimizes experimental error. The most commonly used systems involve use of fluorescence-based technologies to probe sequences that fluoresce upon hydrolysis (TaqMan; Applied Biosystems, Foster City, CA, USA) or hybridization (LightCycler; Roche, USA), fluorescent hairpins and intercalating dyes (SYBR Green). SYBR Green is an example of an intercalating dye binding to the product and emits a strong fluorescent signal that is easily detected. Intercalating dyes are not sequence-specific, and are inexpensive and simple to use compared to sequence probes and can be used for any reaction. However, because they do not discriminate between gene sequences, they cannot be used for multiplexed analyses (Zipper *et al* 2004; VanGuilder *et al*., 2008). Analysis of real-time qPCR data has also reached a mature stage of development. Analysis can be either of absolute levels (numbers of copies of a specific RNA per sample) or relative levels (e.g. sample 1 has twice as much mRNA of a specific gene as sample 2). The majority of analysis is relative quantification that is easier to measure and is of primary interest to researchers. The most common method for relative quantification is the $2^{-\Delta\Delta CT}$ method. This method relies on two assumptions. The first is that the reaction is occurring with 100% efficiency. In other words, the amount of product doubles with each cycle of PCR. The relative expression of a gene of interest in relation to another gene, mostly to an appropriate reference gene, can be calculated on the basis of ‘delta crossing point’ (ΔC_p) or ‘delta delta cycle threshold’ ($\Delta\Delta C_t$) values (Livak and Schmittgen, 2001). Recently, various mathematical models have been established to calculate the relative expression ratio, based on the comparison of the distinct cycle differences. The

relative expression can be determined by various methods including efficiency corrected calculation (Pfaffl, 2001).

7. Gene function testing using dual-luciferase assays

Reporter assay systems are widely used for studying interactions between promoter and transcription factors, promoters, signal transduction, and other cellular activities, and are also applicable to target screening both *in vitro* and *vivo* (Bronstein *et al*., 1994; Wilson and Hastings, 1998). Luciferases are most frequently employed because the sensitivity and range of linear response are superior to those of other typical reporters including β-galactosidase, chloramphenicol acetyltransferase, β-glucuronidase and green fluorescent protein. Therefore, luciferases are the most suitable reporter genes for the quantification of gene expression (Nalor, 1999; Nakajima *et al*., 2005). Luciferases are a general term for enzymes catalyzing visible light emission by living organisms (bioluminescence). Research on this subject led to the discovery of an enzyme-substrate system, the former called luciferase and the latter luciferin (from the Latin Lucifer, 'Light-bringer') (Marques and Esteves da Silva, 2009). Dual reporters are used to make relational or ratiometric measurements within an experimental system. Typically, one reporter is used as an internal control to which measurement of the other reporter is normalized. In measurements of gene expression, dual reporters are generally used in transient transfections of cultured cells, where one vector containing the experimental reporter gene is co-transfected with a second vector containing a distinct reporter gene serving as the control. Usually, the experimental reporter is coupled to a regulated promoter to study the structural or physiological basis of regulated gene expression. Relative changes in the expression of reporter activity correlate to changes in the transcriptional activity of the coupled regulated promoter. To provide an internal control for transcriptional activity, the second reporter gene is coupled to a constitutive promoter that is unperturbed by the various experimental conditions. By this method, it is possible to minimize experimental variability that can undermine experimental accuracy such as differences in the number and health of the cultured cells, and the efficiencies of cell transfection and lysis. Dual-reporter applications utilizing firefly luciferase in

combination with either chloramphenicol acetyltransferase (CAT), beta-galactosidase (β-Gal) or beta-glucuronidase (GUS) have become popular in recent years. However, these co-reporter combinations diminish the performance advantages of luciferase. The ideal dual-reporter method would allow the user to assay both reporters in a single sample with speed, sensitivity, and linearity. Promega's Dual-Luciferase™ Reporter (DLR) Assay System meets these demanding criteria by combining the assays of two luciferase reporter systems, those of the firefly *(Photinus pyralis)* and the sea pansy *(Renilla reniformis)*, in an integrated, single-tube assay format (Sherf *et al*., 1996).

Firefly luciferase is a 61 kDa monomeric protein that does not require post-translational processing for enzymatic activity (Wood *et al*., 1984; de Wet *et al*., 1985). Thus, it functions as a genetic reporter immediately upon translation. Photon emission occurs via oxidation of beetle luciferin in a reaction that requires ATP, Mg^{2+} and O_2 (Figure 3). Under conventional reaction conditions, the oxidation of luciferin occurs through a luciferylAMP intermediate that turns over very slowly. As a result, this assay chemistry generates a 'flash' of light that rapidly decays after the substrate and enzyme are mixed. However, the coenzyme A (CoA) enhances reaction kinetics by promoting rapid enzyme turnover resulting in an extended 'glow' luminescence signal. The *Renilla* luciferase, a 36 kDa monomeric protein, is composed of 3% carbohydrate when purified from its natural source (Matthews *et al*., 1977; Lorenz *et al*., 1991). However, like firefly luciferase, post-translational modification is not required for activity and the enzyme may function as a genetic reporter immediately following translation. The luminescent reaction catalyzed by *Renilla* luciferase utilizes O_2 and coelenterate luciferin (coelenterazine) (Figure 3). Quantification of the luminescent signal from the two luciferase reporter enzymes may be performed immediately following lysate preparation and completed in a short time. Hellens *et al*. (2005) use the dual luciferase method to characterize the enzymatic activity of plant genes cloned into these *Agrabacterium* vectors, and screened relative transcriptional activities of transcription factor genes.

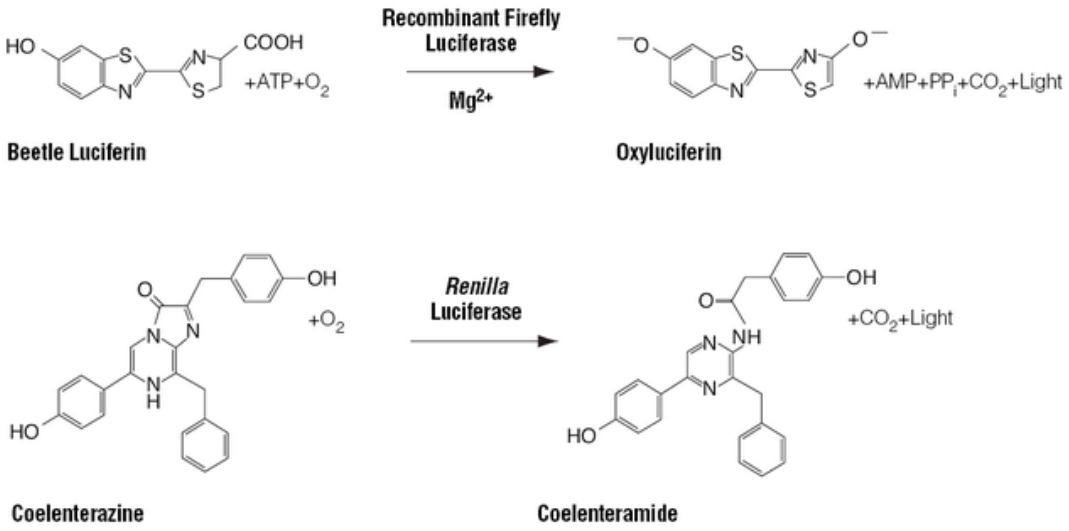


Figure 3 Bioluminescent reaction catalyzed by firely and *Renilla* luceferases.

Source: Sherf *et al*. (1996).

MATERIALS AND METHODS

Mangosteen fruit were obtained from a commercial orchard in Chanthaburi province. Fruit were carefully harvested, packed into 10 kg per plastic boxes, and transported within 6 h by a refrigerated truck (15^{o}C) to the Postharvest Research Unit, Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom. Fruit were selected by their uniformity of color and size (75-90 g). The fruit were separated by skin colour into 7 stages, using a colour index slightly modified from that of Tongdee and Suwanagul (1989): yellowish white or yellowish white with light green (stage 0), light greenish yellow with 5-50% scattered pink spots (stage 1), light greenish yellow with 51-100% scattered pink spots (stage 2), spots not as distinct as in stage 2 or reddish pink (stage 3), red to reddish purple (stage 4), dark purple (stage 5) and purple black (stage 6) (Figure 4).

1. Study of colour development and fruit quality of mangosteen fruit harvested at different stages of maturity

In this experiment mangosteen fruit were separated into two groups. To study fruit colour development (A), fruit were harvested at stage 1 and analyzed immediately for quality and anthocyanin content. The remaining fruit were then kept at 25^{o}C (85-90% RH). Quality and anthocyanin assessments were made at regular intervals until the fruit reached stage 6. To study fruit maturity relationships (B), fruit were harvested at stages 1-6 and stored at 25^{o}C (85-90% RH) and when fruit reached stage 6, the fruit were transferred to 15^{o}C (85-90% RH) to preserve fruit quality. When all fruit reached stage 6, they were analyzed for quality and anthocyanin contents with three replicates of 7 fruit in each replicate.

1.1 Quality assessment and sensory evaluation

Fruit colouration was evaluated using a colour index. Colour development was based on a scale from 1 to 6; 1 = light greenish yellow with 5-50%

scattered pink spots; 2 = light greenish yellow with 51-100% scattered pink spots; 3 = spots not as distinct as in stage 2 or reddish pink; 4 = red to reddish purple; 5 = dark purple; 6 = purple black (Figure 4). Fruit colour was measured using a Minolta CR-300 chromameter (Minolta, Osaka, Japan) as L^*, a^*, b^* values (CIE L a b) and converted to hue angle by using the formula: h^o = arctan (b/a) (colour wheel, with red-purple at an angle of 0º, yellow at 90º, bluish-green at 180º). The colour reading was taken twice at the equatorial region of each fruit and averaged to give a value for each fruit. After fruit colour measurement, the fruit pericarp was separated into outer (0.1 mm thickness) and inner pericarps (Figure 5). The pericarp was cut, and immediately frozen in liquid nitrogen and kept at -80^{o}C for anthocyanin and RNA analysis. Replicate pericarp tissue samples were pooled for RNA extraction.

Pericarp firmness was measured using a hand-held fruit firmness tester (Effegi, Alfonsine, Italy) equipped with a cylindrical plunger 0.5 cm in diameter. The plunger was inserted to a depth of 0.5 cm and the force recorded in newtons (multiply by 9.807).

To measure soluble solids content (SSC) and titratable acidity (TA) on the flesh juice, the white flesh of the arils, with seeds, was wrapped in cheesecloth, and squeezed by hand to separate juice from seeds. SSC was measured with a hand-held refractometer (Atago, Tokyo, Japan) and calibrated with distilled water. TA was determined from a 5 mL aliquot by titration with 0.1 mol L^{-1} NaOH with 1% phenolphthalein as an indicator and results are given as grams of citric acid per 100 mL. The SSC/TA ratio was calculated.

To study of effect of different stages of fruit maturity on fruit quality. Ten judges conducted a sensory panel evaluation of mangosteen flavor for all fruit from different harvest maturities (stages 1-6) that had reached stage 6. The stems and calyxes of the fruit were removed to reduce possible bias due to visible appearance. The judges rated the overall flavor and acceptability of mangosteen aril on a scale of 1-5, where 5 = excellent, 4 = very good, 3 = good, 2 = poor, and 1 = very poor.

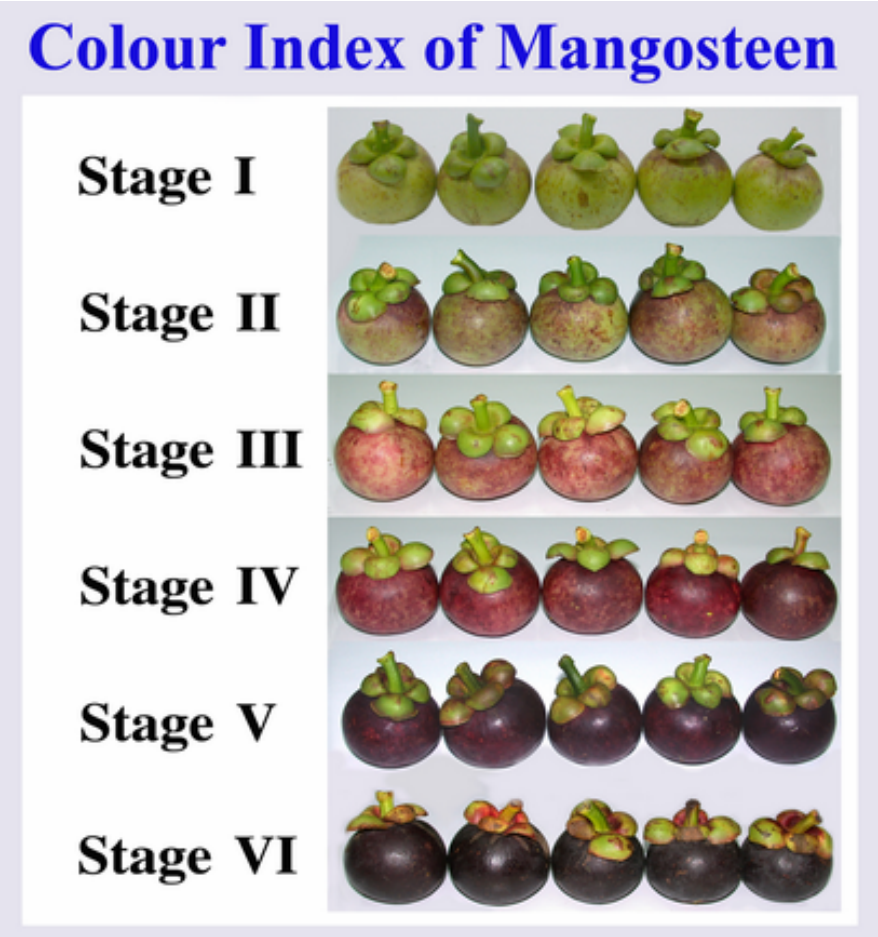


Figure 4 Colour development of mangosteen fruit.

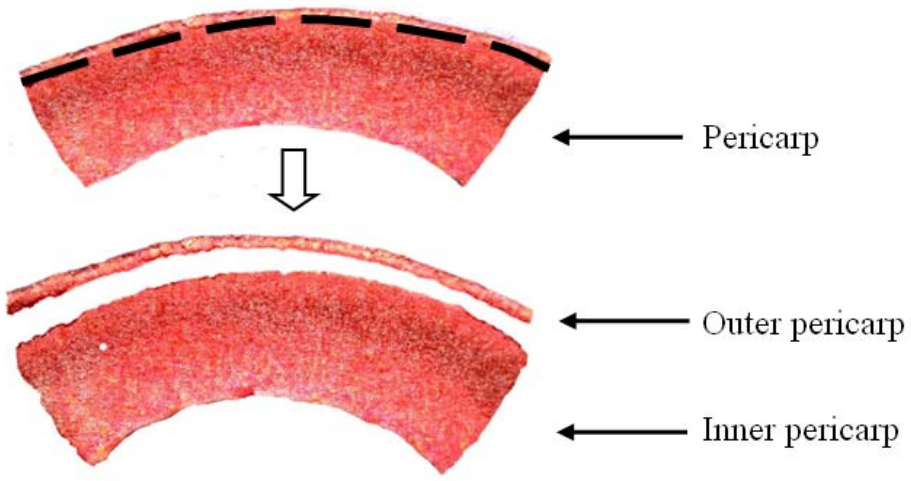


Figure 5 Pericarp separation of mangosteen fruit.

1.2 Ethylene production

Ten fruit at stage 1 were individually weighed and placed into separate 0.8 L plastic containers at 25°C (85-90% RH). The air was flushed before closing the container. After 30 min, air samples were taken from the headspace using a syringe. Concentrations of ethylene within containers were measured by sampling through a sampling port with a syringe and measured with a gas chromatograph (Shimadzu GC 8A, Kyoto, Japan), equipped with a flame ionization detector and a 2.1 m x 2.4 mm stainless steel column filled with activated alumina of 177-149 μm. The column temperature was 80°C. Injector and detector temperatures were 150°C. The data were the average values from 10 fruit.

1.3 Anthocyanin analysis

Total anthocyanins were extracted using methanol-HCl (Piccaglia *et al*., 2002). One gram of outer pericarp or two grams of inner pericarp were homogenized with 20 mL of methanol:HCl (99:1 v/v), and the homogenates then shaken for 6 h at 4 °C. The aqueous phase was removed and the pellets were re-extracted 4 times within 24 h and then adjusted to a final volume of 100 mL with methanol:HCl. The combined aqueous extracts were centrifuged at 8,000 x *g* for 10 min (4°C) and anthocyanin contents then measured at an absorbance of 530 nm. The anthocyanin contents were calculated and expressed as cyanidin equivalents. The samples were kept at -80°C until the individual anthocyanins were analyzed.

$$\text{Total anthocyanin (mg kg}^{-1}) = \frac{\text{A x MW X DF x 1000}}{\varepsilon L \text{ x Wt}}$$

A = Absorbance

ε = Cyd-3-glu molar absorbance (34,300)

MW = anthocyanin molecular weight (287)

DF = dilution factor

Wt = sample weight (mg)

L = cell path length (usually 1cm)

Individual anthocyanin compounds were analyzed by HPLC and LC-MS as described by Stevenson *et al*. (2006). Aliquots of 500 μL (A) were dried down in a Labconco Centrivap Concentrator (Labconco, Kansas City, MO, USA). Samples were resuspended in 20% methanol (250 μL). Samples were then kindly analysed by David Stevenson and Janine Cooney (Plant & Food Research, Ruakura, New Zealand) using the following steps. Forty microlitres of the sample were analyzed using a Shimadzu analytical HPLC with a column oven, auto-sampler, vacuum solvent degas module and diode-array detector. (Shimadzu, Kyoto, Japan). Separations were achieved on a 250 mm × 4.6 mm column, Synergi®, 4 μm particle size, Polar-RP, 8 nm pore size (Phenomenex, Auckland, NZ), using (A) acetonitrile + 0.1% formic acid, and (B) acetonitrile/water/formic acid (5:92:3). Flow rate was 25 μL s^{-1} at a column temperature of 45°C. The content of solvent A was 0% at zero time and ramped linearly to 20% at 20 min, 30% at 26 min, 50% at 28.5 min, 50% at 28.5 min, 95% between 32-35 min and back to 0% between 36-42 min).

LC-MS analysis of the outer pericarp (purple black) sample was carried out to confirm compound identify. Identification was based on both mass (M+) of molecular ions and characteristic fragments, and comparison of retention times and fragmentation with authentic standards; cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside (Polyphenols, Norway). Quantification was achieved by reference to standards of anthocyanin compounds at 520 nm.

1.4 Light microscopy

Fruit pericarps at different stages of colour development were hand-sectioned using a razor blade and mounted with a drop of distilled water. The slides were examined in bright field using a light microscope (Carl Zeiss, Gottingen, Germany) equipped with a digital camera.

2. Study of the effects of ethylene on colour development of mangosteen fruit

Fruit at stage 1 were harvested and randomly separated into four groups for the following treatments: 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-methylcyclopropene (1-MCP) for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M). All treatments were fumigated at 25°C and kept at 25°C (85-90% RH). 1-MCP was generated by adding water to EthylBloc® (Floralife Inc., Walterboro, SC, USA) powder placed in a vial in a chamber (0.174 m^3). Introducing water into the vial with Ethylbloc® powder resulted in a final concentration of 1 μg L^{-1} of 1-MCP gas in the chamber. Fans were used in the chambers to maintain air circulation. All fruit were treated at 25°C for 12 h and stored at 25°C (85-90% RH). After the treatments, 21 fruit per treatment were randomly assessed for fruit colour and pericarp firmness (same as study 1). After fruit colour measurement, the outer pericarp of the fruit (0.1 mm thickness) was cut, and immediately frozen in liquid nitrogen and kept at -80°C for total anthocyanin and RNA analysis. Replicate pericarp tissue samples were pooled for RNA extraction.

For ethylene production, five fruit from each treatment in the ethylene experiment were individually weighed and each fruit placed into separate 0.8 L plastic containers at 25°C (85-90% RH). The air was flushed before closing the container. After 30 min, 1 mL gas samples were taken from the headspace using a syringe. Concentrations of ethylene were measured using a gas chromatograph (Shimadzu GC 8A, Kyoto, Japan) as for ethylene production in study 1.

3. Study of the effects of storage temperature on colour development of mangosteen fruit

Fruit at stage 1 were harvested and stored at 25°C (85-90% RH) (control), and 15°C (85-90% RH) for seven days. After seven days, fruit stored at 15°C were transferred to 25°C (85-90% RH). After the treatments, 21 fruit per treatment were randomly assessed for fruit colour and fruit firmness (same as for study 1). After fruit colour measurement, the outer pericarp of the fruit (0.1 mm thickness) was cut, and

immediately frozen in liquid nitrogen and kept at -80^{o}C for total anthocyanin and RNA analysis. Replicate pericarp tissue samples were pooled for RNA extraction.

For ethylene production, five fruit from each treatment in the experiment were individually weighed and each fruit placed into separate 0.8 L plastic containers at 25^{o}C (85-90% RH). The air was flushed before closing the container. After 30 min, 1 mL gas samples were taken from the headspace using a syringe. Concentrations of ethylene were measured using a gas chromatograph (Shimadzu GC 8A, Kyoto, Japan) as for ethylene production in study 1.

4. Cloning and characterization of MYB transcription factor and anthocyanin biosynthesis genes of mangosteen fruit during red colouration

The mangosteen MYB transcription factor (*GmMYBs*) and anthocyanin biosynthesis genes were isolated using degenerate primers or 3' race. Complete fragments were obtained using RACE PCR strategy. Promoters of anthocyanin biosynthesis genes (DFR, LDOX and UFGT) were isolated using ligation-mediated PCR. The *cis*-element of DFR, LDOX and UFGT promoters were analyzed using database assisted bioinformatics. The transcriptional activities of GmMYBs were determined using dual luciferase. Gene expression was analyzed using real-time and end-point PCR.

4.1 Isolation and cloning of MYB transcription factors and anthocyanin biosynthesis genes of mangosteen fruit

4.1.1 Total RNA extraction

Approximately 10 g of frozen samples were ground using a Mixer Mill MM 301 (Retsch, Germany) under liquid N_2, kept in the RNAse-free Falcon tubes and then stored at -80^{o}C until use (for 1-2 weeks). Total RNA was isolated from 2 g of outer pericarp tissue as modified from Lopez-Gomez and Gomez-Lim (1992) to make it suitable for extracting RNA from the pericarp of mangosteen fruit.

Ground tissue was added to 15 mL of extraction buffer containing 150 mM Tris base (pH 7.5), 2% SDS, 2% β-mercaptoethanol, 50 mM EDTA and 0.5 g of polyvinylpolypyrrolidone (PVPP). The mixture was shaken vigorously for 1 min by vortexing and 1.5 mL of 5 M potassium acetate and 4.0 mL of absolute ethanol added thereafter. The mixture was shaken vigorously for 1 min by vortexing, then 15 mL of chloroform: isoamyl alcohol (24:1 v/v) was added, shaken vigorously for 5 min and centrifuging at 7,000 x *g* for 20 min. The supernatant was collected and extracted by phenol: chloroform: isoamyl alcohol (25:24:1). The mixture was shaken vigorously for 5 min by vortexing and centrifuged at 7,000 x *g* for 20 min. The supernatant was collected and re-extracted with 15 mL of chloroform: isoamyl alcohol. The supernatant was collected and precipitated with 8 M LiCl to get 3 M final concentration at 4^{o}C overnight. After centrifugation at 10,000 x *g* for 20 min, the RNA pellet was washed in 5 mL of 3 M LiCl. The RNA pellet was re-suspended in 350 μL DEPC-water and transferred to microcentrifuge tube and then 35 mL of 3 M potassium acetate and 962.5 mL of absolute alcohol were added. The RNA was precipitated at -80ºC for 1 h. The mixture was centrifuged in a microcentrifuge at 10,000 x *g* for 30 min and washed once with 500 mL of 70% ethanol and re-suspended in 50 μL of chilled DEPC-water. RNA quantification was determined using a spectrophotometer at wavelengths of 230, 260, and 280 nm. The quality was confirmed by gel electrophoresis on 1% agarose gels. One absorbance unit at 260 nm corresponded to approximately 40 μg mL^{-1} (Sambrook and Russel, 2001). The RNA concentration of each sample was estimate in μg mL^{-1} by the following equation: RNA concentration = A260 x dilution factor x 40 μg mL^{-1}. Quality of the purified total RNA was determined by calculating the A260/A280 ratio. A ratio of between 1.8-2.0 indicated good quality.

4.1.2 cDNA synthesis (Reverse Transcription Reaction)

The first strand cDNA was synthesized from 2 μg of the RNA using Omniscript RT kit (Qiagen, Germany) for anthocyanin biosynthesis genes. In quantitative real-time PCR, RNA samples were DNase treated (DNA-free, Ambion) for eliminating DNA contamination. cDNA was synthesized from 4 μg of the RNA

following the protocol of SuperscriptIII kit (Invitrogen, USA). Fifty-fold diluted cDNA was used for quantitative real-time RT-PCR and semi-quantitative RT-PCR. The synthesized cDNA was used as a template to amplify the targeted genes by PCR. The first strand cDNA was further used as a template in PCR reaction or stored at -20°C until use.

4.1.3 PCR amplification of mangosteen cDNA fragments

Mangosteen MYB transcription factors (*GmMYB*s) and anthocyanin biosynthesis genes were isolated using degenerate primers and 3' race. Degenerate primers with designs based on two highly conserved amino acid sequences found in several plants, which are MYB transcription factors regulating anthocyanin biosynthesis and all enzymes in the anthocyanin biosynthesis pathway, were used as forward and reverse primers. The sequences of the primers are described in Table 2. The reaction mixture (50 μL) consisted of 10x PCR buffer (5 μL), 50 mM $MgCl_2$ (2 μL), 10 mM dNTPs (1 μL), 10 μM of each primer (5 μL), cDNA (1 μL), Platinum Taq (0.2 μL) and water (30.8 μL) (Invitrogen, USA). The reaction conditions displayed in Table 3.

Table 2 Degenerate primers for fragment amplification of MYB transcription factors and anthocyanin biosynthesis genes.

Gene name	Sequence	Annealing temperature (ºC)	Expected size (bp)
GmPAL (FJ197127)	F- GYDATYTTYGCWGARGTBATG R- AGATTNGAHGGYAABCCRTTGTTG	48	467
GmCHS (FJ197128)	F-CAGCCCAARTCCAARATCAC R-ATCCAGAARAKBGARTTCCA	55	548
GmCHI (FJ197129)	F-AAGTTCACRGSSATMGGMGTRTAC TTGG and 3' race	55	700
GmF3H (FJ197131)	F-G TCCVAAGGTKGCYTAYAAYG R-CYTTGCTCATCTTCYTCYTGTAC	50	912
GmF3'H (FJ197132)	F-TAYAAYTAYCARGAYYTBGT R- CTDGATGWBGTRTCWGTYCC	48	570
GmDFR (FJ197130)	F-YTCWTGGCT SGTCATGAGRC R-SCAGWDATGAGGCTYGGHG	55	572
GmLDOX (FJ197133)	F-ARAARGAGAAGTATGCHAAYGASC R-CCAYGARATYCTMACCTTYTCC	54	578
GmUFGT (FJ197134)	F-CAGGARGAYATHGAGYTSTTCATG ARKGC and 3' race	59	1430
GmMYB1 (FJ197135)	F- TGYATIRAIAWIYAHGGAGAR GGMAARTGG and 3' race	59	1240
GmMYB7 (FJ197136)	F-AAAGTTGCAGRYTTAGRTGGT TGAATTATYTGARGCC and 3' race	59	1020
GmMYB10 (FJ197137)	F- TGYATIRAIAWIYAHGGAGAR GGMAARTGG and 3' race	61	930

Table 3 PCR conditions for fragment amplification of MYB transcription factors and anthocyanin biosynthesis genes.

Temperature (°C)	Time	No. of cycles
94	3 min	1
94	30 s	
55	30 s	35
72	1 min	
72	10 min	1

4.1.4 Rapid amplification of cDNA ends (RACE)

To amplify mangosteen *MYBs* and anthocyanin biosynthesis full-length cDNAs, both 5′ and 3′ RACE were performed to generate full-length cDNAs following the protocol of GeneRacer (Invitrogen, USA) (Appendix Figure 1). The cDNA fragments of *GmCHI*, *GmUFGT* and *GmMYBs* were obtained using the degenerate primer and 3' RACE primer as 3' RACE strategy. The 5' and 3' cDNA fragments were isolated using gene specific primers (real-time primers and GeneRacer primer) (Table 4). All specific primers were designed with Tm 60$\pm$1^{o}C using Vector NTI 10.0 (Invitrogen, USA) and GeneRacer primers were shown as below.

GeneRacer 5' primer 5'-CGACTGGAGCACGAGGACACTGA-3'
GeneRacer 5' Nested primer 5'GGACACTGACATGGACTGAAGGAGTA-3' GeneRacer 5' primer 5'-GCTGTCAACGATACGCTACGTAACG-3'
GeneRacer 5' Nested primer 5'-CGCTACGTAACGGCATGACAGTG-3'

The first amplification reactions were carried out in 50 µL volumes (Table 5) and assessed using the reaction conditions displayed in Table 6.

Table 4 Specific primers for 5’ and 3’ RACE and real-time PCR.

Gene name	Sequence	Expected size (bp)
GmPAL (FJ197127)	F- ATGGCTCGGCCCACCTATTGA R- CTGGAAGTTGCCACCGTGTAATG	130
GmCHS (FJ197128)	F- GGCCTTCTGATACCCACTTGGACT R- GGATGGTTTGGGCCGCAGATA	141
GmCHI (FJ197129)	F- GAAGAGGAGGAAGAGGCGTTGGA R- GGCAGTGGGTGAAGTTACTGGGAA	108
GmF3H (FJ197131)	F- TTGTTGAGGCTTGTGAGGAATGG R- CGGACATATCGAATCGGAGCTTT	141
GmF3'H (FJ197132)	F-GCACATTCGAGAGGCAGAGTTGG R-TGAATACCCTCCTCCCCACCATT	140
GmDFR (FJ197130)	F- GCGTCTGCTGGTGCATTGGA R- GCTCTCTCCGCCTTTGTCTTGG	140
GmLDOX (FJ197133)	F- ACCAGCGAGTACGCAAGGCAA R- TGGAGGGCCAGTTCTTCAATGC	128
GmUFGT (FJ197134)	F-GCGAGTTTGTGATTTGCCTGAAGG R-ACCGCATCCGCTTTAGGTAACG	105
GmMYB1 (FJ197135)	F- GCTGTGGCAAGAGTTGTAGGCTAAGA R- TCTCCCAGCAATCAATGACCACCTA	143
GmMYB7 (FJ197136)	F- CTTGCCTGGAAGAACGGACAATG R- CGCAACGCATCGTGTCTGTGA	119
GmMYB10 (FJ197137)	F-TGGAGAAATAACTCAAGGGACAACGG R-GCCATTTGTTCTGGGCTGAATAGACT	120

Table 5 PCR reactions for primary condition of 5’ and 3’ RACE.

Reagent	Volume (μL)	Volume (μL)
GeneRacer 5’ or 3’ primer	3	3
Degenerate primer	10	
Specific primer		1
cDNA (GeneRacer)	1	1
10x PCR Buffer	5	5
10 mM dNTPs	1	1
50 mM MgCl2	1.5	1.5
Platinum Taq	0.5	0.5
Water	28	37

Table 6 PCR conditions for primary condition of 5’ and 3’ RACE.

Temperature (°C)	Time	No. of cycles
94	3 min	1
94	30 s	5
72	1.5 min	
94	30 s	5
70	1.5 min	
94	30 s	
57, 61	30 s	32
72	1.5 min	
72	10 min	1

After the first PCR, the quality of PCR product was analyzed using gel electrophoresis on 1% agarose then a positive reaction was selected that had the expected band for secondary PCR (Nested PCR). The second amplification reactions were carried out in 50 µL volumes (Table 7) and assessed using the reaction conditions displayed in Table 8.

Table 7 PCR reactions for secondary reaction of 5’ and 3’ RACE.

Reagent	Volume (µL)	Volume (µL)
GeneRacer 5’ or 3’ nested primer	1	1
Degenerate primer	10	
Specific primer		1
Initial PCR product	1	1
10x PCR Buffer	5	5
10 mM dNTPs	1	1
50 mM MgCl2	1.5	1.5
Platinum Taq	0.5	0.5
Water	30	39

Table 8 PCR conditions for secondary reaction of 5' and 3' RACE.

Temperature (°C)	Time	cycles
94	3 min	1
94	30 s	
55, 57, 59, 61, 63	30 s	30
68	1.5 min	
68	10 min	1

The full-length cDNA clones of *GmMYBs* were obtained using the gene specific primers designed to the 5' and 3' UTR regions (Table 9). The reaction mixture (50 μL) consisted of 10x PCR buffer (5 μL), 50 mM $MgCl_2$ (2 μL), 10 mM dNTPs (1 μL), 10 μM of each primer (1 μL), cDNA (1 μL), Platinum Taq (0.2 μL) and water (38.8 μL) (Invitrogen, USA). The reaction conditions displayed in Table 10.

Table 9 Specific primers for full-length amplification of MYB transcription factor genes.

Gene name	Sequence	Expected size (bp)
GmMYB1 (FJ197135)	F- GAAAATGAGGAGACCTTGTTGTG R- TTGTCTCATCTAAAGAGAAGTAACGTG	738
GmMYB7 (FJ197136)	F- ATGGAATCCCAAATAACCTCTACTTT R- TTTATCCAAAGCCTCTCTATTTCTGA	815
GmMYB10 (FJ197137)	F- GAAGCTAAATGGAGAGAAGTTCAGG R- GGATTCATCGAAAAACTACATTTGC	878

Table 10 PCR conditions for full-length amplification of MYB transcription factor genes.

Temperature (°C)	Time	No. of cycles
94	3 min	1
94	30 s	
55	30 s	35
72	1 min	
72	10 min	1

4.1.5 PCR products cloning and DNA sequencing

After visualizing the PCR product by gel electrophoresis on 1% agarose, the amplified PCR fragments from each gene were purified using QIAquick Gel Extraction Kit (Qiagen, Germany) or PureLink PCR Purification Kit (Invitrogen, USA), and cloned into pGEM®-T Easy Vector (Promega, USA) (Appendix Figure 2). as described in the manufacturer's instructions.

1) Ligations using the pGEM®-T Easy vectors

The ligation reactions were set up using 5 μL of 2x Rapid Ligation Buffer, 1 μL of pGEM®-T Easy Vector (50 ng), 3 μL of PCR product and 1 μL of T4 DNA Ligase to a final volume of 10 μL The reactions were mixed gently by pipetting and then incubated overnight at 4°C.

2) Transformations using the pGEM®-T Easy vector ligation Reactions

DH5-α competent cells were used for the transformations. Two LB/ampicillin//X-Gal plates for each ligation reaction were prepared, and equilibrated at room temperature prior to plating. The 50 μL frozen DH5-α tube(s) were removed from -80°C and placed in an ice bath until just thawed (about 10 min). The ligation reaction (10 μL) was carefully put into each competent cell. The tubes

were gently flicked to mix and then placed on ice for 30 min. The cells were heat-shocked for 40 s in a water bath or heat block at exactly 42^{o}C without shaking and immediately returned to ice for 2 min. Then 450 μL SOC medium was added to the tubes and incubated for 1.5 h at 37°C with shaking (~150 rpm). Each transformation culture (50 and 100 mL) was plated onto duplicate LB/ampicillin//X-Gal plates and incubated overnight (16-24 h) at 37°C. White colonies would be selected for the next step.

3) Plasmid DNA Purification using Purelink Quick Plasmid Miniprep Kit (Invitrogen, USA)

Single white colonies containing the inserted genes were cultured in 4 mL LB medium overnight. Cells were centrifuged at 7,000 x *g* for 10 min and only the pellet cells collected. The pellet cells were re-suspended in 250 μL of re-suspension buffer and transferred to a 1.5 mL microtube. Then, 250 μL of lysis buffer was added and the tube gently inverted to mix. Three hundred and fifty μL of neutralization buffer were added and the tube inverted immediately and then centrifuged at 12,000 x *g* for 10 min. The mixture was transferred to the spin column by pipetting. The tube was then centrifuged for 1 min and the flow-through discarded. The spin column was washed by adding 700 μL of washing buffer and centrifuged for 1 min, the flow-through discarded, and centrifuged again to remove any residual. DNA was eluted by adding 50 μL of elution buffer, incubated 1 min in the room temperature and centrifuged for 1 min. DNA was kept at -20^{o}C until used for cutting with the *Eco*RI restriction enzyme (New England Biolabs, Inc., USA) and the insert size was analyzed by gel electrophoresis before sequencing.

4) DNA sequencing and analysis

The sequence analysis of the clone was conducted by automatic sequencing using the ABI PRISM® 3730 DNA sequencer (Applied Biosystems, USA). Comparison and analysis of the *GmMYB* transcription factors and anthocyanin biosynthesis sequences were performed with the advanced basic local alignment

search tool (BLAST) at the National Center for Biotechnological Information (NCBI) (http://www.ncbi.nlm.nih.gov/). The DNA and protein sequences were analyzed by the Vector NTI 10.0 program (Invitrogen, USA) and submitted to GenBank (NCBI). Full-length sequences were aligned using Vector NTI AlignX (opening = 15, extension = 0.3). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 (Kumar *et al*., 2004) using a minimum evolution phylogeny test and 1,000 bootstrap replicates

4.2 Promoter isolation and analysis of *GmDFR*, *GmLDOX* and *GmUFGT* genes

The 5' flanking region of DFR, LDOX and UFGT were isolated using GenomeWalker Kit (Clontech, USA) (Appendix Figure 3). The genomic DNA (gDNA) was isolated from young mangosteen leaves using DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions. The libraries were prepared by separate digestion of 2.5 µg of genomic DNA with 80 units of *Dra*I, *Eco*RV, *Hpa*I, *Msc*I, *Sca*I, *Ssp*I and *Stu*I (New England Biolabs, Inc., USA). The DNA mixtures were incubated at 37ºC overnight to create blunt-end fragments. All DNA sample were purified using QIAquick Gel Extraction Kit (Qiagen, Germany) following the manufacturer's instructions, then the DNA libraries were ligated with GonomeWalker adapter using Rapid DNA Ligation Kit (Roche, USA) and incubated overnight (16–24 h) at 4°C. All ligated mixtures were purified using QIAquick Gel Extraction Kit (Qiagen, Germany). The 5′ flanking region of DFR, LDOX and UFGT were amplified following the protocol of GenomeWalker Kit (Clontech, USA). The gene-specific primers were designed in the 5′ end of *GmDFR*, *GmLDOX* and *GmUFGT* and used for each genomic-walking PCR. The adaptor primers are shown as below.

AP1 5′-GTAATACGACTCACTATAGGGC-3′ (primary PCR)
AP2 5′-ACTATAGGGCACGCGTGGT-3′ (nested PCR).

The gene-specific primers 5′-ATCAGGATCACGGACAGTGGCTCTAACC -3′ (primary PCR) and 5′-

AGCCATGACCCGATGAACCCTGATG -3′ (nested PCR) were used for the mangosteen DFR promoter. The gene-specific primers 5′-GATGTTTCCTAAGTTGGTTAGCTCCTCTTGTGG -3′ (primary PCR) and 5′-GGGCGGATGTACTCTTTTGGGATGCA -3′ (nested PCR) were used for the mangosteen LDOX promoter. Finally, the gene-specific primers 5′-GGAAAGTGCGGCTAAGTGGTGTGTTATGG -3′ (primary PCR) and 5′-AGGAGTGGGGCTGCATGTGTTCCA -3′ (nested PCR) were used for the mangosteen UFGT promoter. The first amplification reactions were carried out in 50 µL volumes, with the same conditions as the full-length cDNA amplification, and the reaction conditions displayed in Table 11. After the first PCR, the second amplification reactions, same as under condition for the full-length cDNA amplification, were carried out in 50 µL volumes using the first PCR product as DNA template, and the second reaction conditions displayed in Table 12. PCR products were analyzed by gel electrophoresis on a 1% agarose gel, the longest of amplified PCR fragments from each gene were purified using QIAquick Gel Extraction Kit (Qiagen, Germany) or PureLink PCR Purification Kit (Invitrogen, USA), and cloned into pGEM®-T Easy Vector (Promega, USA) as described as above. Promoter fragments were analyzed for *cis*-elements using PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html) and PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Table 11 PCR conditions for primary reaction of Genome walking.

Temperature (°C)	Time	No. of cycles
94	3 min	1
94	30 s	7
72	3 min	
94	30 s	32
67	3 min	
67	10 min	1

Table 12 PCR conditions for secondary reaction of Genome walking.

Temperature (°C)	Time	No. of cycles
94	3 min	1
94	30 s	5
72	3 min	
94	30 s	30
67	3 min	
67	10 min	1

4.3 Functional testing of GmMYBs using transient tobacco assays

The dual luciferase assay of transiently transformed *Nicotiana benthamiana* leaves were used to screen the activities of GmMYB transcription factors. A transient dual luciferase assay was used as previously reported (Hellens *et al*., 2005; Espley *et al*., 2007). The promoters of *AtDFR*-LUC fusion in pGreenII 0800-LUC (*TT3*, AT5g42800), *AtPAP1* in pGreen II 62-SK 0029 (*AtMYB75*, At5g56650) and *AtbHLH2* (*AtEGL3*) gene in pHEX2 were obtained from Dr. Roger P. Hellen (personal communication). The methods are described in more detail below and illustrated in Figure 6.

4.3.1 Preparation the construct

The mangosteen promoter of *GmDFR* (FJ197138) was amplified using the pimers (Forward 5'- ACCAGCCCATCCACATGGTCA-3' and Reverse 5'-TTTCATTTTGGGAA**CCCATGG**AT-3' and cloned into pGEM®-T Easy Vector (Promega, USA) as described above. The DFR promoter was digested out of pGEM®-T easy using *Nco*I and *Not*I (New England Biolabs, Inc., USA), and ligated into *Nco*I and *Not*I digested pGreenII 0800-LUC (Appendix Figure 4). The modification of the 3' end of the sequence was introduced the *Nco*I site using reverse primer (bold letters), allowing the promoter to be cloned as a transcriptional fusion with the firefly luciferase gene (LUC). Thus, TFs that bind to the DFR promoter and increase the rate of transcription could be identified as an increase in luminescence

activity. In the same construct, a luciferase gene from *Renilla* (REN) under the control of a 35S promoter provided an estimate of the extent of transient expression. The LUC activity relative to REN was expressed as a ratio to show activation of the promoter by a transcription factor included in another plasmid.

The full-length cDNA clone of *GmMYBs* were digested out of pGEM®-T easy using double digestion (GmMYB1; *Sac*I and *Apa*I, GmMYB6 and GmMYB10; *Not*I and *Spe*I) and ligated into the double digested pGreen II 62-SK 0029 binary vectors (Appendix Figure 5). All double digestions were digested following the manufacturer's instructions (New England Biolabs, Inc., USA). The constructs were transformed into *E. coli* (DH5-α), as described as above, with 50 μg mL^{-1} kanamycin. The positive clones were confirmed by sequencing. (New England Biolabs, Inc., USA).

4.3.2 Agrobacterium Transformation

Agrobacterium cells (*A. tumefaciens* GV3101 (MP90)) were used for all transformations. The LB plates were prepared, and equilibrated at room temperature prior to plating. The 40 μL frozen *Agrobacterium* tube(s) were removed from -80°C and placed in an ice bath until just thawed (about 10 min). One microliter of recombinant plasmid containing GmDFR promoter and GmMYBs were added to *Agrobacterium* cells. The mixture was added into a chilled cuvette then transformed immediately using electroporation (Bio-Rad) at 2.5 kV, 25 μF, 400 Ω and time constant 8-9 ms. After pulsing, the cells were resuspended in 1 mL of SOC medium and incubated in a shaking incubator at 28^{o}C for 60 min. The dilutions were plated out on LB agar containing antibiotics such as kanamycin, gentamycin rifampicin and spectinomycin (Table 13) incubated 48 h at 28^{o}C then kept the *Agrobacterium* culture in 15% glycerol and stored in -80^{o}C.

Table 13 Antibiotics for *Agrobacterium* culture.

Insert	Vector	Antibiotics (μg mL^{-1})
AtDFR-LUC	pGreenII 0800	Kan 50
GmDFR -LUC	pGreenII 0800	Kan 50
AtPAP1	pGreen II 0029 62-sk	Kan 50
GmMYB1	pGreen II 0029 62-sk	Kan 50
GmMYB7	pGreen II 0029 62-sk	Kan 50
GmMYB10	pGreen II 0029 62-sk	Kan 50
AtbHLH2	pHex2	Rif 25 / Gent 10 / Spec 50
Empty vector	pHex2	Rif 25 / Gent 10 / Spec 50

.

4.3.3 Agrobacterium Suspension Preparation

Before plant transformation (3 days), all *Agrobacterium* cultures were plated and prepared freshly. A 10 μL loop of confluent bacterium was re-suspended in 10 mL of infiltration buffer (10 mM $MgCl_2$, 0.5 μM acetosyringone) to an OD_{600} of 0.2, and incubated at room temperature without shaking for 2 h before infiltration. *Agrobacterium* mixtures were comprised 450 mL effector (35S-TFs) with or without 450 mL 35:AtbHLH2 (partner) plus 100 μL DFR-LUC and adjusted to a final volume of 1 mL with infiltration buffer.

4.3.4 Agrobacterium-Mediated Transient Expression

Nicotiana benthamiana plants were grown under glasshouse conditions in full potting mix, under natural light with daylight extension to 16 h, until the plants had at least 6 leaves. Approximately 150 μL of the *Agrobacterium* mixture were syringe-infiltrated at four points into a young leaf of *N. benthamiana*. Three days after inoculation, 3 mm leaf discs (4 technical replicates from each plant) were cut with a hole-puncher, placed into wells of a 96-well-plate containing 50 μL of 1x PBS (phosphate buffered saline) in each well, and gently crushed with the hole-puncher. Dual-luciferase assays were performed using Dual-Glo™ Luciferase Assay (Promega, USA) according to the manufacturer's instructions. The analysis was

carried out using Orion Microplate Luminometer (Berthold Detection System). The cycle was involved adding 50 µL of firefly luciferase, shaking for 10 s, waiting for 10 min, reading the LUC signal, adding 50 µL of renilla luciferase, shaking for 10 s, waiting for 10 min and reading the REN signal. The LUC/REN ratio was calculated.

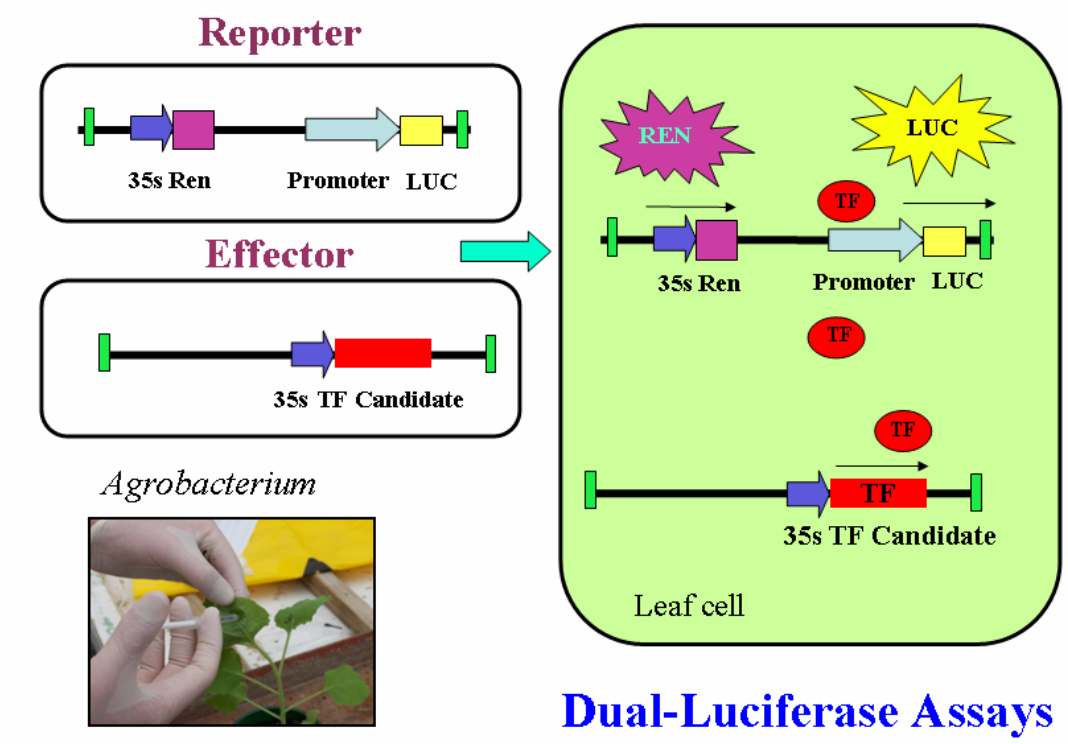


Figure 6 Functional testing using dual -luciferase assays

Source: Adapted from Andrew P. Dare (personal communication).

4.4 Expression analysis of *GmMYB*s and anthocyanin biosynthesis genes during colour development of mangosteen fruit

Fifty-fold diluted cDNA samples were used for quantitative real-time RT-PCR. Gene-specific primers (Table 4) were tested for specificity using plasmid amplification and the product analyzed on a 1% agarose gel stained with ethidium bromide. qPCR was performed on the Light Cycler 480 system (384-well plates) and the Light Cycler® 480 SYBR Green I Master kit (Roche Diagnostics, Germany) following the manufacturer's instructions. All reactions were performed in triplicate (technical replication) using 3 µL of the diluted template (50x), 1 µL of each primer (2.5 µM), and 5 µL of 2x Master mix to a final volume of 10 µL. PCR was initiated by 5 min at 95°C, followed by 40 cycles of 95°C for 5 s, 60°C for 5 s, 72°C for 10 s and completed by a melting curve analysis program. The negative water control and melting curve were included in every run. The melting peak, dissociation curve and sequencing were analyzed to confirm that there was no primer dimer and an expected product. The data were analysed and normalized to mangosteen elongation factor 1 alpha (*GmELF*, EU274578) to minimize variation in cDNA template levels. The primers of GmELF primers were designed with Vector NTI 10.0 (Invitrogen, CA, USA) as described above (Forward 5'-GCCCAAAAGACCATCAGACAAGC-3' and Reverse 5'- CGGAAGGACCAAAAGTGACAACC-3'). The size of qPCR products ranged from 100-150 bp. *GmELF* was selected for normalization because of its consistent transcript level throughout the fruit samples with crossing point (Cp) values changing by < 2. The standard curve was generated for each gene by using a cDNA serial dilution (at least 5 dilutions), and the resultant PCR efficiency calculations (ranging between 1.893 and 1.999) were imported into relative expression data analysis.

The expression levels were shown as a ratio relative to the fruit at stage 0 for colour development studies and the fruit after harvest (day 0) for the ethylene and temperature experiment. The ratio of the calibrator was set to the nominal value of 1. The relative expression was analyzed as transcript abundance ratio of target gene to reference gene following equation as below (Roche, USA).

$$\text{ratio} = \frac{(E_{\text{Ref}})^{C_P \text{ sample}}}{(E_{\text{target}})^{C_P \text{ sample}}} \div \frac{(E_{\text{Ref}})^{C_P \text{ calibrator}}}{(E_{\text{target}})^{C_P \text{ calibrator}}}$$

The E_{ref} and E_{target} are the efficiencies of the primers for the reference and target gene, respectively, and Cp_{ref} and Cp_{target} are the mean Cp value of reference and target genes, respectively.

The transcript level of *GmMYB10* was confirmed using semi-quantitative RT-PCR. To find the suitable cycle, PCR reactions were tested using a number of cycles ranging from 24 to 32. The PCR mixture was amplified under the following conditions: 94°C for 30 s, 60°C for 30 s with 28 cycles (*GmMYB10*) and 26 cycles (*GmELF*), and 72°C for 30 s with a final extension at 72°C for 10 min. The amplification reactions were carried out in 50 μL volumes, under the same conditions as for the full-length cDNA amplification. The PCR product was analyzed using gel electrophoresis on a 1% agarose gel.

5. Statistical analysis

Using a SAS package, variance analysis using *t*-tests and *F*-tests were performed to determine differences between means of the treatments, at $P \leq 0.05$ and the significance of the differences between means was estimated by Duncan's new multiple range test (DMRT). Data were the mean $\pm$ standard error (SE). All experiments were at least repeated once.

THE EXPERIMENTAL TIME AND PLACES

The experiments were carried out during June 2005-Dec 2008 and the research was conducted at the places as described below:

1. Postharvest Technology center, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand.

2. Plant & Food Research, (Mt Albert Research Centre), Auckland, New Zealand (formerly Hortresearch)

RESULTS

1. Study of colour development and fruit quality of mangosteen harvested at different stages of maturity

1.1 Fruit colour development and quality

During ripening of fruit harvested at stage 1(group A), the L* and b* values decreased sharply during colour development. The a* value increased rapidly with colour development from stage 0 to 4 then decreased sharply to stage 6 (Figure 7b, Appendix Table 1). The hue value decreased significantly and correlated closely with red colouration from stage 0 to 6 ($P \leq 0.001$) (Figure 7d, Appendix Table 1). The red colouration developed rapidly to the purple black (stage 6) within 9 days, with colour development from stage 5 to 6 being slower than between the other stages (Table 14).

Pericarp firmness decreased sharply from stage 0 to 6, whereas SSC and SSC/TA ratio increased slightly, and TA decreased slightly during colour development from stage 3 to 6 (Table 14, A columns).

When fruit at the six different stages of maturity (group B) were harvested and kept at 25^{o}C, each stage completely developed to the purple black stage (Figure 8). No matter at what stage the fruit were harvested, they all ripened such that there were no significant differences in colour especially L*, b* and hue value (Figure 8, Appendix Table 2), fruit in sensory evaluation and fruit quality, including hue values, firmness, SSC and TA, when the fruit were assessed at stage 6 ($P>0.05$) (Table 14, B columns).

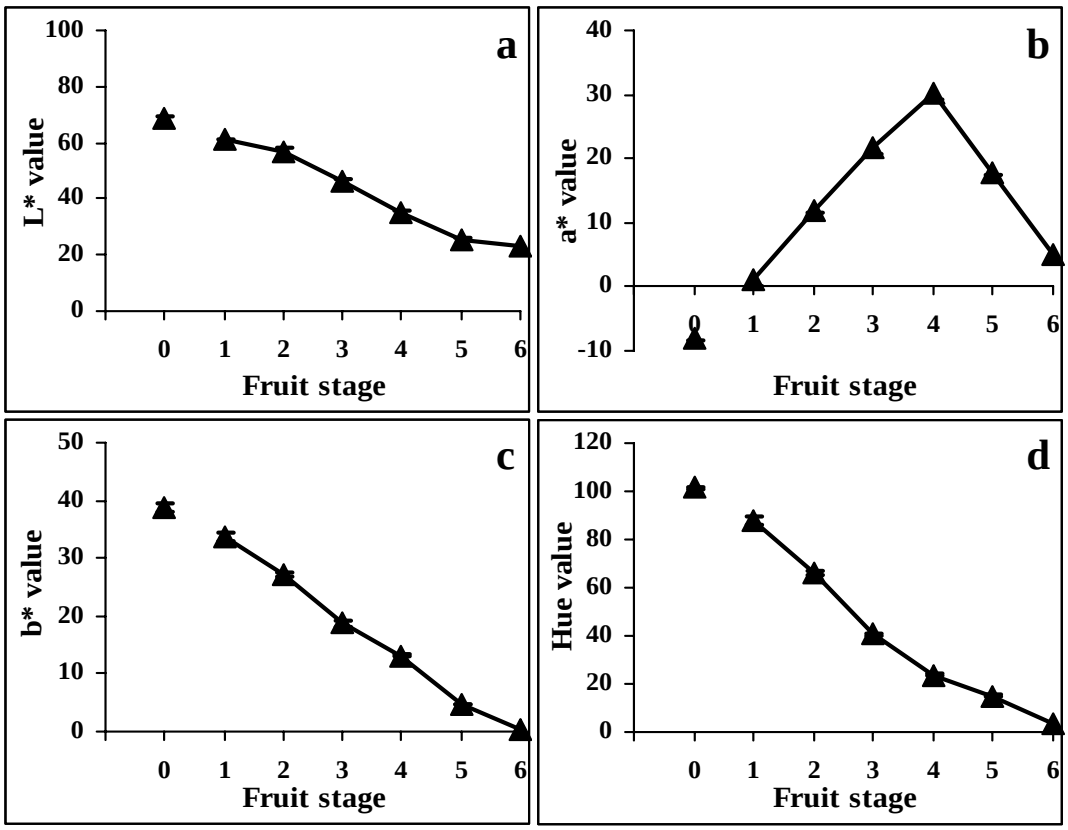


Figure 7 Colour development (L*, a*, b* and hue value) of mangosteen fruit harvested at stage 1 compared to stage 0. Data are means $\pm$ SE of three replications.

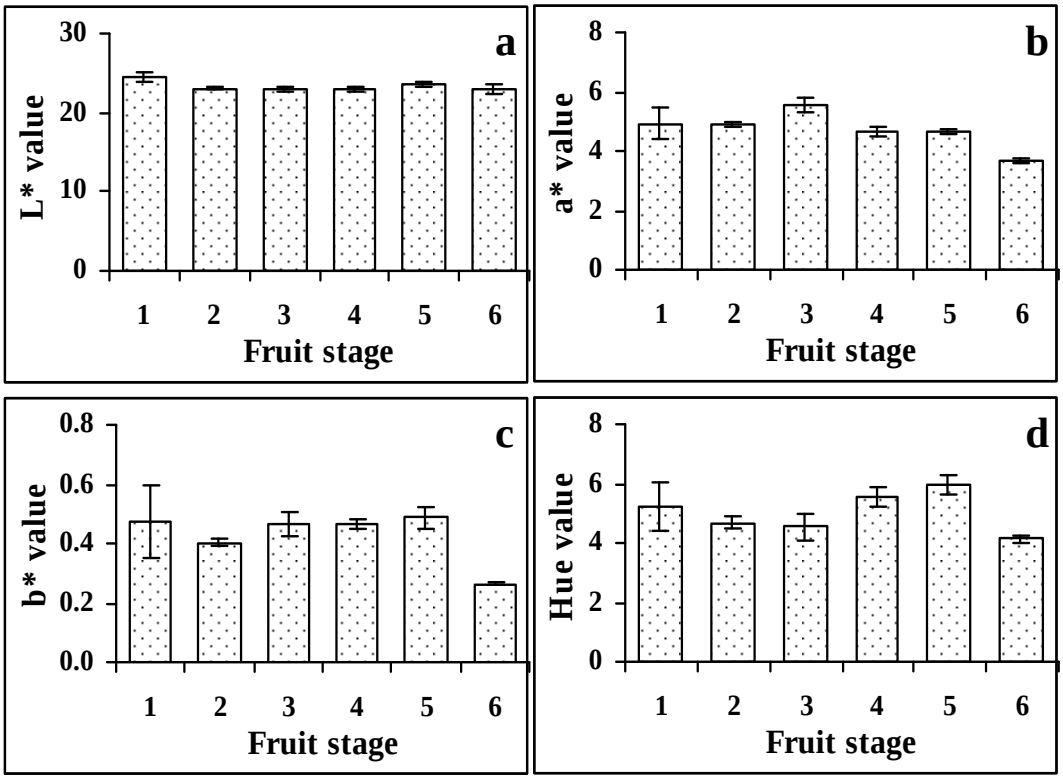


Figure 8 Fruit colour (L*, a*, b* and hue value) of mangosteen fruit at stage 6 developed from 6 different maturity stages. Data are means ± SE of three replications.

Table 14 Time, quality and sensory evaluation of mangosteen fruit harvested at different stage.

Fruit stage		Time (d)	Firmness (N)[1]		% SSC[1]		% TA[1]		SSC/TA[1]		Sensory[1]
			A	B	A	B	A	B	A	B	B
0		-	898.3a	-	13.33c	-	0.68d	-	19.6bc	-	-
1		0	779.3b	44.8	15.2b	17.2	0.77bc	0.81	19.8bc	21.2b	4.2
2		1	201.3c	43.6	15.3b	17.3	0.78abc	0.81	19.6bc	21.3b	3.7
3		2	136.0d	46.0	16.3a	17.5	0.84a	0.80	19.3c	21.9ab	3.8
4		3	98.4e	42.9	16.6a	17.9	0.80ab	0.78	20.8bc	23.0ab	4.0
5		5	66.5f	45.0	17.1a	17.5	0.79abc	0.75	21.7ab	23.5a	3.7
6		9	46.5g	47.9	17.2a	17.4	0.73dc	0.74	23.7a	23.7a	4.1
F-test			***	ns	***	ns	**	ns	**	*	ns

Table 14 (Continued).

Fruit were either harvested at stage 1 (A) and allowed to ripen at 25^{o}C , or harvested at the 6 different maturity stages and measurements made when the fruit of each maturity had reached stage 6(B). The firmness in A column were log (In) transformed data and untransformed values were presented. [1]Means within any column followed by the same letter are not significantly different ($P > 0.05$). ns = non-significantly different, *= significantly different at $P \leq 0.05$, ** = significantly different at $P \leq 0.01$, *** = significantly different at $P \leq 0.001$.

1.2 Ethylene production of mangosteen fruit harvested at stage 1

During ripening of fruit harvested at stage 1(group A), ethylene production of mangosteen fruit increased linearly until stage 5 (dark purple) by 5 days, then decreased slightly thereafter (Figure 9). Ethylene production decreased significantly with red colouration from stage 5 to 6 ($P \leq 0.001$) (Appendix Table 3).

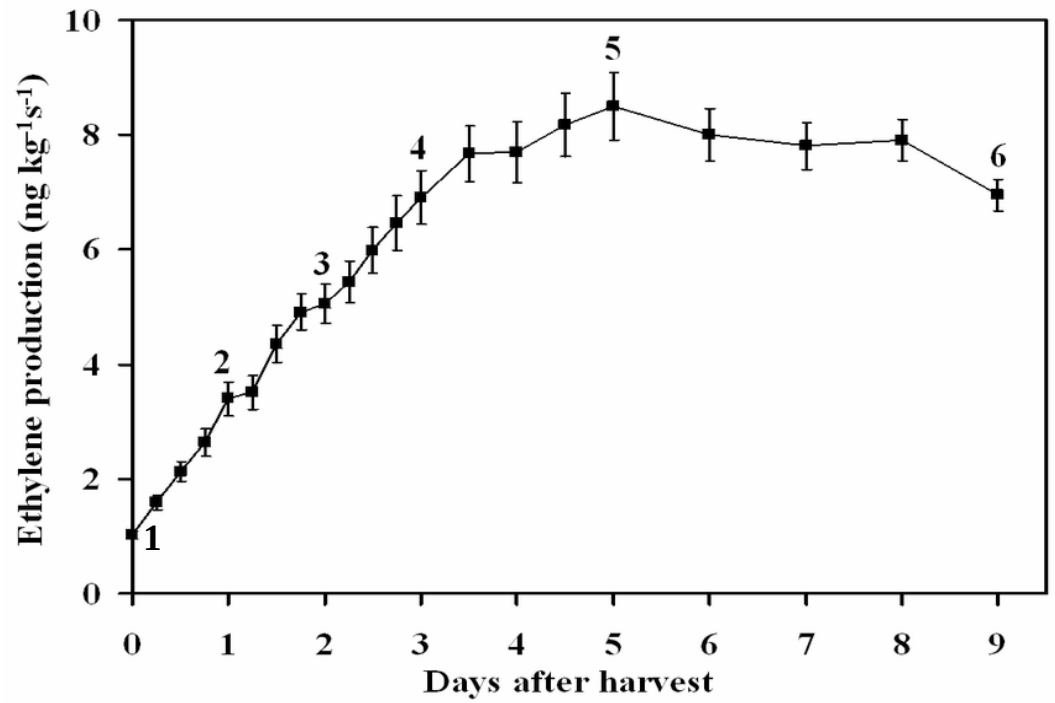


Figure 9 Ethylene production of mangosteen fruit harvested at stage 1 during storage at 25°C. The numbers of 1 to 6 in the graph represent maturity stages of mangosteen fruit at stages 1 to 6. Data are means of 10 fruit ± SE.

1.3 Anthocyanins in mangosteen pericarp

During fruit colour development, the total anthocyanin contents in the outer and inner pericarp increased more than 70- and 18-fold from stage 1 to stage 6, respectively. The total anthocyanin contents were significantly different in outer and inner pericarps ($P \leq 0.001$), and hue angle values decreased sharply (Figure 10). The total anthocyanin contents in the inner pericarp tissue increased following the same trend, although the contents at all stages were less than those in the outer pericarp (Figure 10). Hue angle value and total anthocyanin content were closely associated with fruit colour development.

When fruit at the six different stages of maturity (group B) were harvested and kept at 25^{o}C, each stage completely developed to the purple black stage (Figure 8). The total anthocyanin contents in outer and inner pericarp were significant difference with $P \leq 0.001$ and $P \leq 0.05$, respectively (Figure 11, Appendix Table 4). However, no matter at what stage the fruit were harvested, they all ripened and developed to stage 6 (purple black) (Table 14, B columns).

The anthocyanins in the outer pericarp mainly consisted of 5 compounds (Table 15, Figure 12). These compounds were identified by HPLC/MS as cyanidin-sophoroside (M+ 611, major fragment, *m/z* 287), cyanidin-glucoside (M+ 449, *m/z* 287), cyanidin-glucoside-pentoside (M+ 581, *m/z* 287), cyanidin-glucoside-X (M+ 639, *m/z* 287), cyanidin-X_2 (M+ 667, *m/z* 287) and cyanidin-X (M+ 477, *m/z* 287) (Appendix Figure 6). X denotes an unidentified residue of *m/z* 190, a mass which does not correspond to any common sugar residue. The two major compounds from HPLC and LC-MS analyses corresponded to those of authentic standards of cyanidin-3-sophoroside and cyanidin-3-glucoside. The concentration of these two compounds increased significantly during fruit colour development, approximately doubling between each stage (Table 15). In addition, cyanidin-glucoside-pentoside was found at low levels, with patterns similar to those of the two major anthocyanins (Table 15). The other anthocyanins had initial low concentrations and decreased further by stage 6. The inner pericarp contained essentially the same compounds, but

at much lower concentrations and increased significantly during colour development (Table 16).

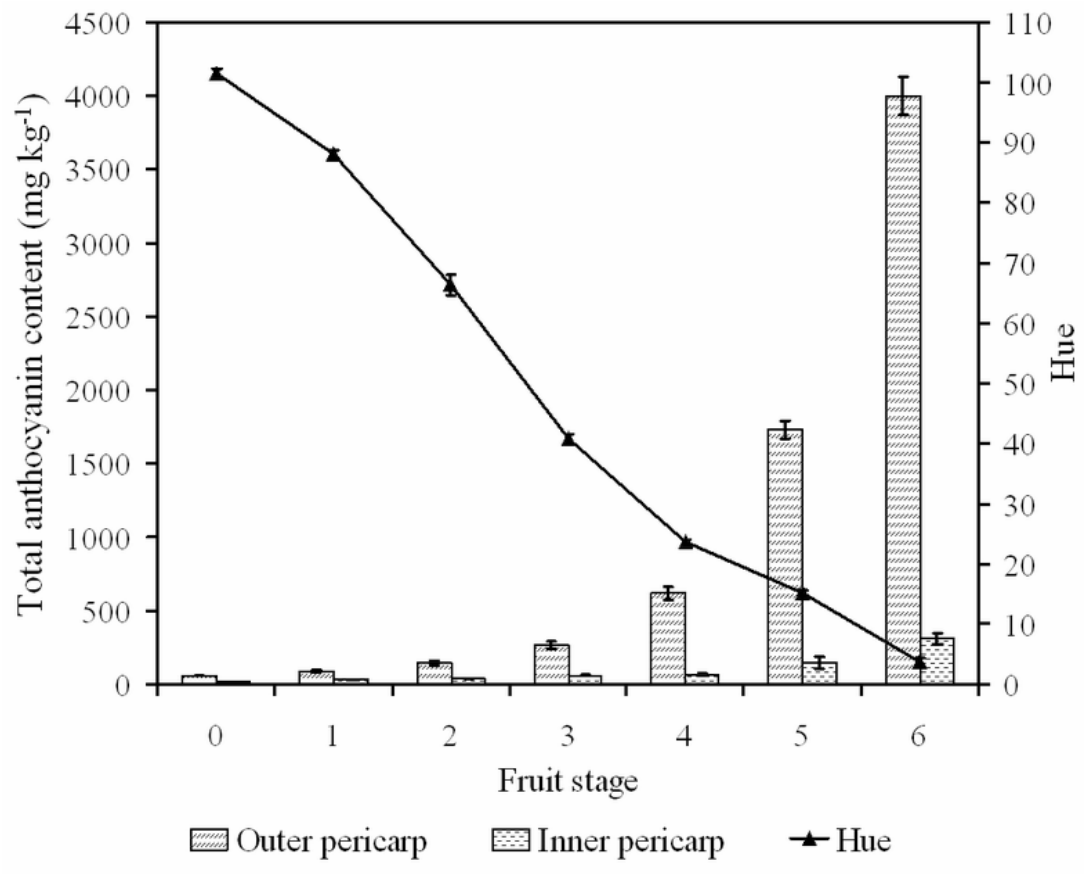


Figure 10 Total anthocyanin content and hue value of mangosteen fruit harvested at stage 1 compared to stage 0. Data are means ± SE of three replications.

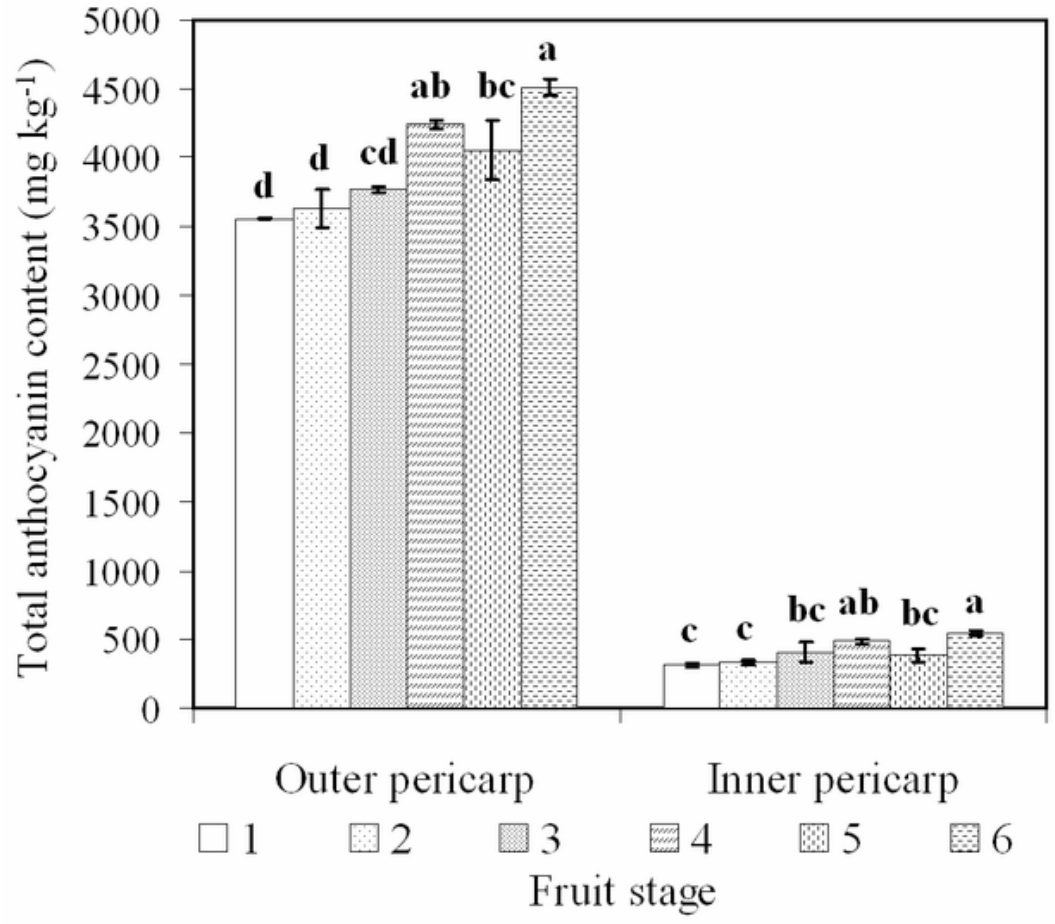


Figure 11 Total anthocyanin content of mangosteen at stage 6 developed from 6 different maturity stages. Data are means ± SE of three replications.

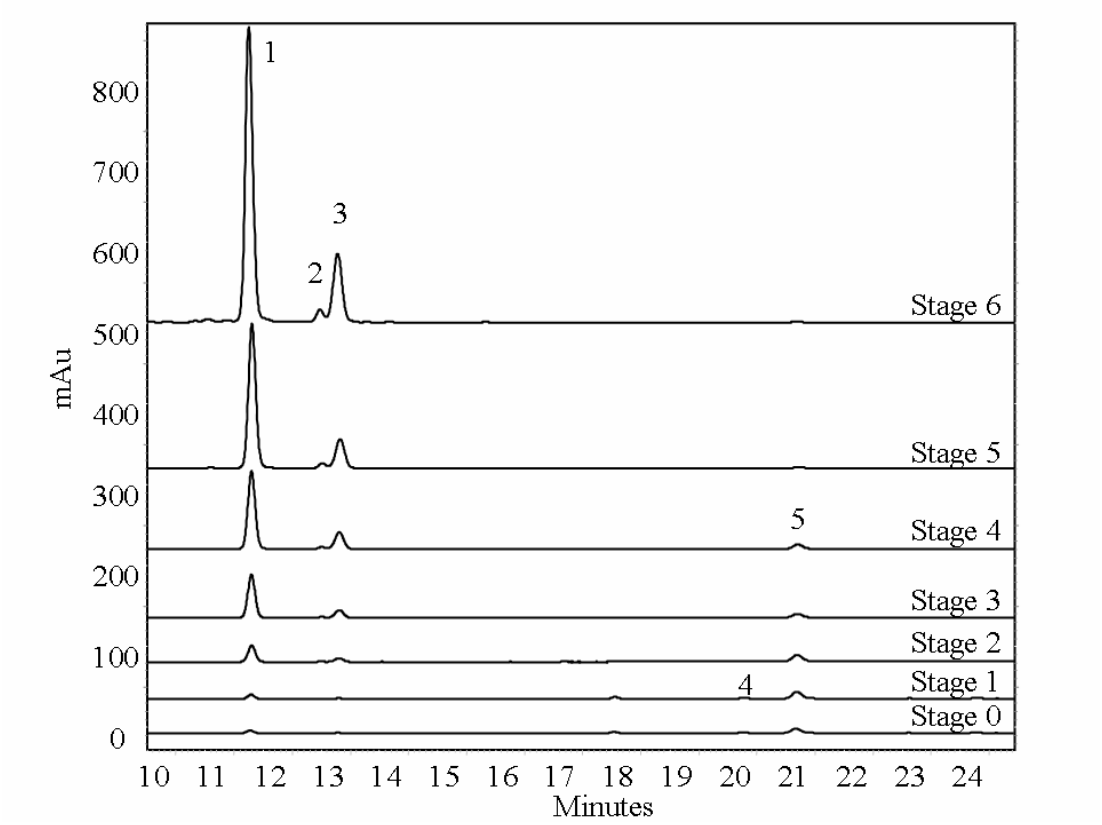


Figure 12 Anthocyanin profiles in outer pericarp of mangosteen fruit during colour development after harvest and compared to stage 0. Peak identity was as follow: 1) cyanidin-sophoroside, 2) cyanidin-glucoside-pentoside, 3) cyanidin-glucoside and cyanidin-glucoside-X (overlapping peak), 4) cyanidin-X_2, and 5) cyanidin-X. X denotes a residue of *m/z* 190 which is unified atomic mass units.

Table 15 Anthocyanin contents (mg kg^{-1}) in outer pericarp of mangosteen during colour development after harvest (stage 1 to 6).

Fruit stage	Unknown[1] 1	Unknown[1] 2	Unknown[1] 3	Cy-sop[1]	Cy-glu-pent[1]	Cy-glu + Cy-glu-X[1]	Unknown[1] 4	Unknown[1] 5	Cy[1]	Cy-$(190)_2$[1]	Cy-(190)[2]	Total[2]
0	0c	0c	0c	0e	0d	0e	0b	2b	21a	18a	94a	135e
1	0c	0c	0c	52de	0d	11e	0b	0b	11ab	11b	83a	167e
2	0c	0c	0c	143de	0d	36de	0b	0b	3b	10b	87a	278de
3	0c	0c	0c	359d	9d	81d	0b	0b	0b	2c	58b	509d
4	0c	6c	3c	823c	27c	191c	0b	0b	0b	6bc	56b	1111c
5	10b	20b	14b	1403b	62b	290b	0b	0b	0b	0c	26c	1824b
6	15a	44a	30a	3126a	125a	842a	9a	16a	0b	0c	27c	4235a
F-test	***	***	***	***	***	***	***	***	*	***	***	***

Total values represent the sum of the individual compounds. Cy-sop: cyanidin-3-sophoroside, Cy-glu-pent: cyanidin-glucoside-pentoside, Cy-glu: cyanidin-3-glucoside, Cy-gluc-X: cyaniding-glucosideX Cy-X: cyanidin-X (X denotes a residue of *m/z* 190 which has not been identified. The [1]values were x+1 transformed data and untransformed values were presented. [1, 2]Means within any column followed by the same letter are not significantly different ($P > 0.05$) using DMRT. ns = non-significantly different, * = significantly different at $P \leq 0.05$, *** = significantly different at $P \leq 0.001$

Table 16 Anthocyanin contents (mg kg^{-1}) in inner pericarp of mangosteen during colour development after harvest (stage 1 to 6).

Fruit stage	Unknown[1] 1	Unknown[1] 2	Unknown[1] 3	Cy-sop[1]	Cy-glu-pent[1]	Cy-glu + Cy-glu-X[1]	Unknown[1] 4	Unknown[1] 5	Cy[1]	Cy-$(190)_2$[2]	Cy-(190)[2]	Total[2]
0	0	0	0	0.0e	0b	0.0b	0	0.0b	7.0b	9.7	50.8	67.5e
1	0	0	0	24.2de	0b	5.7b	0	9.8a	6.0b	11.5	52.0	109.2de
2	0	0	0	41.7cde	0b	11.3b	0	3.3b	15.0a	10.3	49.6	131.3cde
3	0	0	0	68.2cd	0b	13.8b	0	1.0b	15.8a	10.0	52.5	161.3cd
4	0	6	0	89.2c	0b	17.8b	0	0.0b	7.2b	10.7	56.7	181.5c
5	0	1.2	0	223.3b	1.8b	66.0a	0	0.0b	0.0c	5.5	44.2	350.7b
6	0	1.2	0	345.2a	7.0a	72.2a	0	0.0b	1.2bc	6.2	41.2	474.0a
F-test	-	ns	-	***	***	***	-	*	***	ns	ns	***

Total values represent the sum of the individual compounds. Cy-sop: cyanidin-3-sophoroside, Cy-glu-pent: cyanidin-glucoside-pentoside, Cy-glu: cyanidin-3-glucoside, Cy-glu-X: cyanidin-glucoside-X, Cy-X: cyanidin-X (X denotes a residue of *m/z* 190 which has not been identified. The [1]values were x+1 transformed data and untransformed values were presented. [1, 2]Means within any column followed by the same letter are not significantly different ($P > 0.05$) using DMRT. ns = non-significantly different, * = significantly different at $P \leq 0.05$, *** = significantly different at $P \leq 0.001$

1.4 Anatomy of mangosteen skin

The outer pericarp (fruit skin) anatomy from fruit at stages 1 to 6 was examined (Figure 13). The red pigment released from the sectioned outer pericarp was more intense in the purple black (stage 6) than other stages due to breakdown of cells while cutting. The density of red pigment was higher in cells of purple black outer pericarp (stage 6) than for other stages.

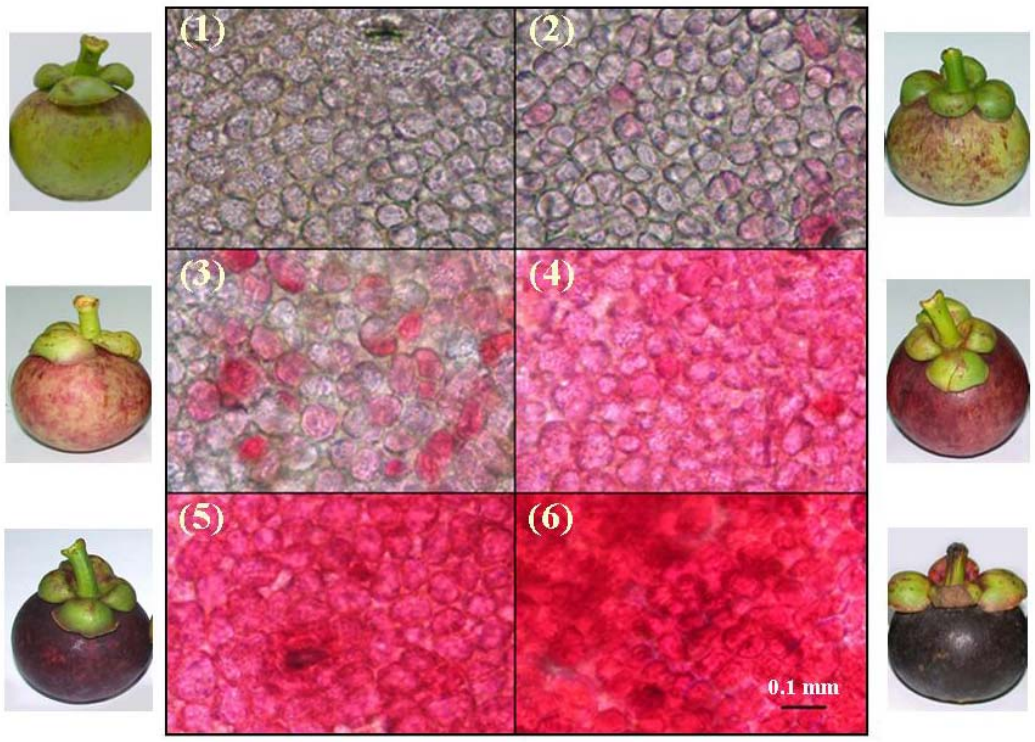


Figure 13 Light microscopy of outer pericarp (skin) of mangosteen fruit. The bar in the outer pericarp section shows 0.1 mm. Numbers refer the maturity stage of the fruit.

2. Study of the effects of ethylene on colour development of mangosteen fruit

Mangosteen fruit at stage 1 (light greenish yellow with 5% scattered pink spots) were treated with ethylene and the ethylene reception inhibitor (1-MCP) in the following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-methylcyclopropene (1-MCP) for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M). After treatment, the ethylene- and air-treated fruit developed their colour faster than the 1-MCP treated fruit (Figure 14). In air and ethylene treatments, fruit colour (hue value) decreased sharply, whereas the fruit colour of 1-MCP-treated fruit did not change greatly during fumigation. In air and ethylene treatment, the fruit colours were increased continuously and developed to stage 6 (purple black) within 7 and 9 days, respectively (Figure 15a). Both 1-MCP and E+M treatments, 1-MCP significantly delayed fruit colour (hue value and colour index) (Figure 14 and 15, Appendix Table 5 and 6). In 1-MCP treatment, the fruit colour did not change greatly during storage at 25^{o}C (Figure 15).

Pericarp firmness decreased sharply during fumigation (Figure 16). In air- and ethylene-treated fruit, pericarp firmness decreased faster than the 1-MCP- and E+M-treated fruit. 1-MCP significantly delayed pericarp firmness (Figure 16, Appendix Table 7).

After fruit were treated, the ethylene production increased continuously for 5 days and decreased thereafter with the pattern of an ethylene climacteric. The ethylene production of 1-MCP and E+M treatments peaked more slowly than for air and ethylene treatments (Figure 17a). The total anthocyanin content of both treatments was increased continuously (Figure 17b) and correlated closely with ethylene production (Figure 17a). The total anthocyanin content of ethylene-treated fruit increased to a higher level than other treatments (Figure 17b, Appendix Table 8). 1-MCP application significantly slowed down the increase of anthocyanin content in 1-MCP- and ethylene +1-MCP-treated fruit that correlated closely with ethylene production and fruit colour (Figure 17, Appendix Table 8).

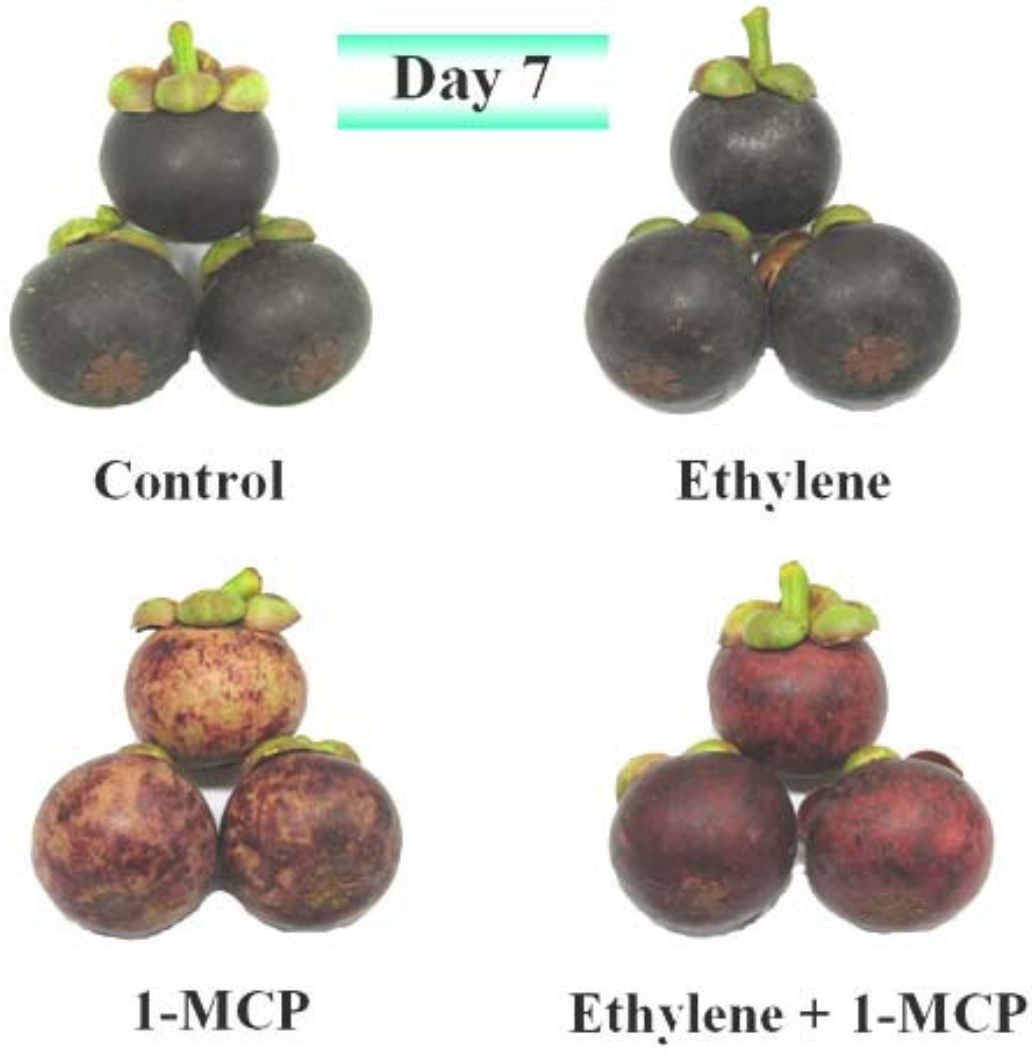


Figure 14 Mangosteen fruit at day 7 treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

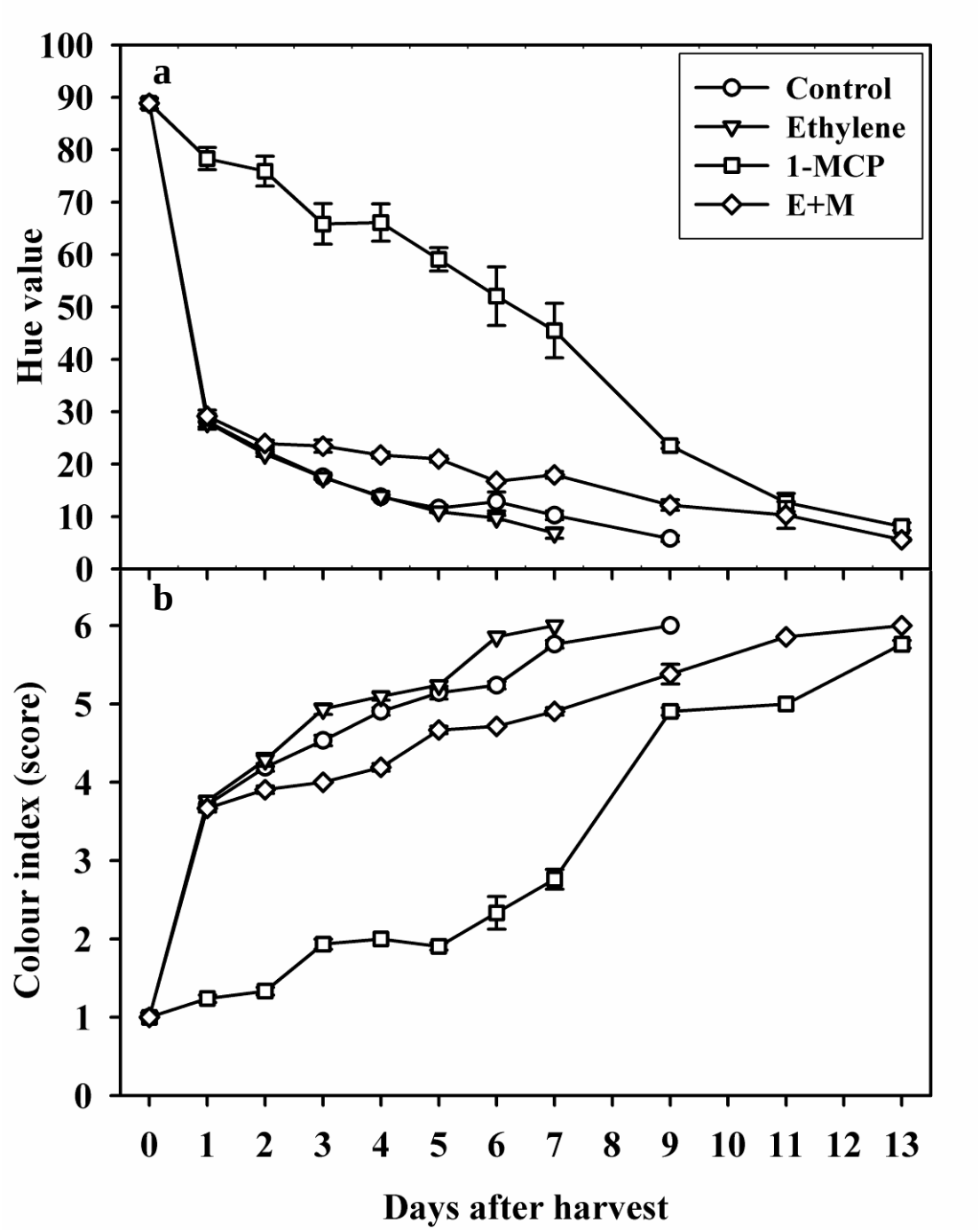


Figure 15 Change in fruit colour (hue value) and colour index (score) of fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M). Data are means $\pm$ SE of three replications.

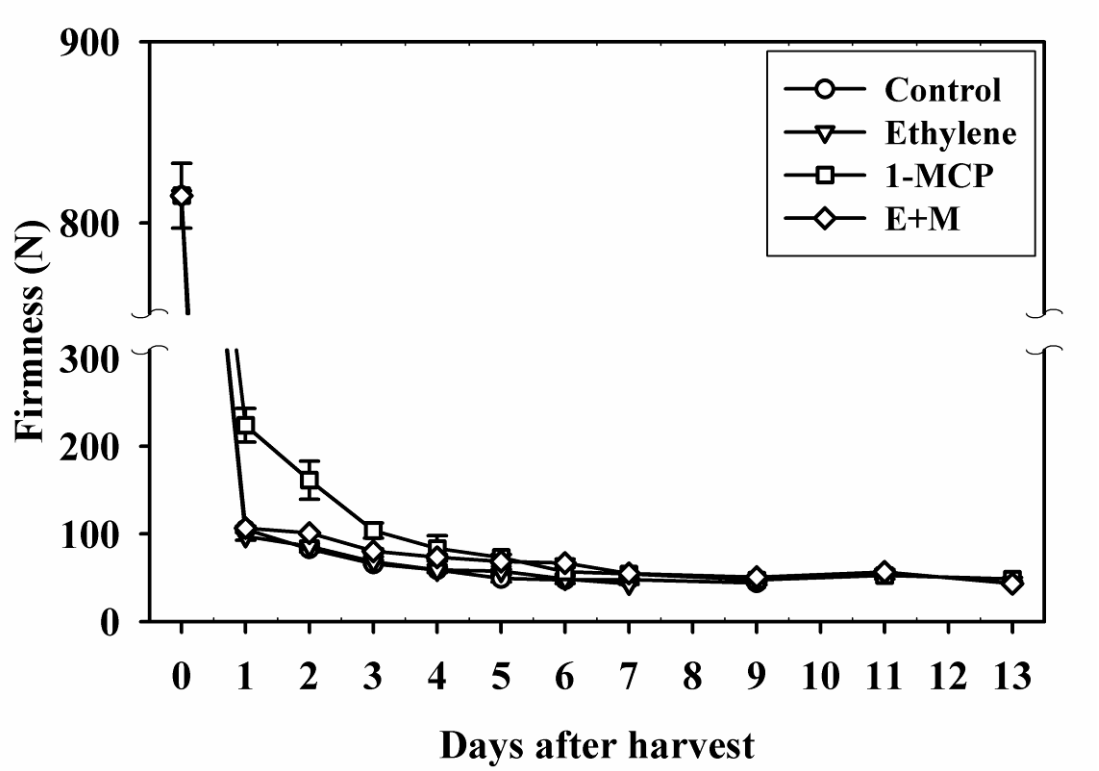


Figure 16 Change in pericarp firmness treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M). Data are means ± SE of three replications.

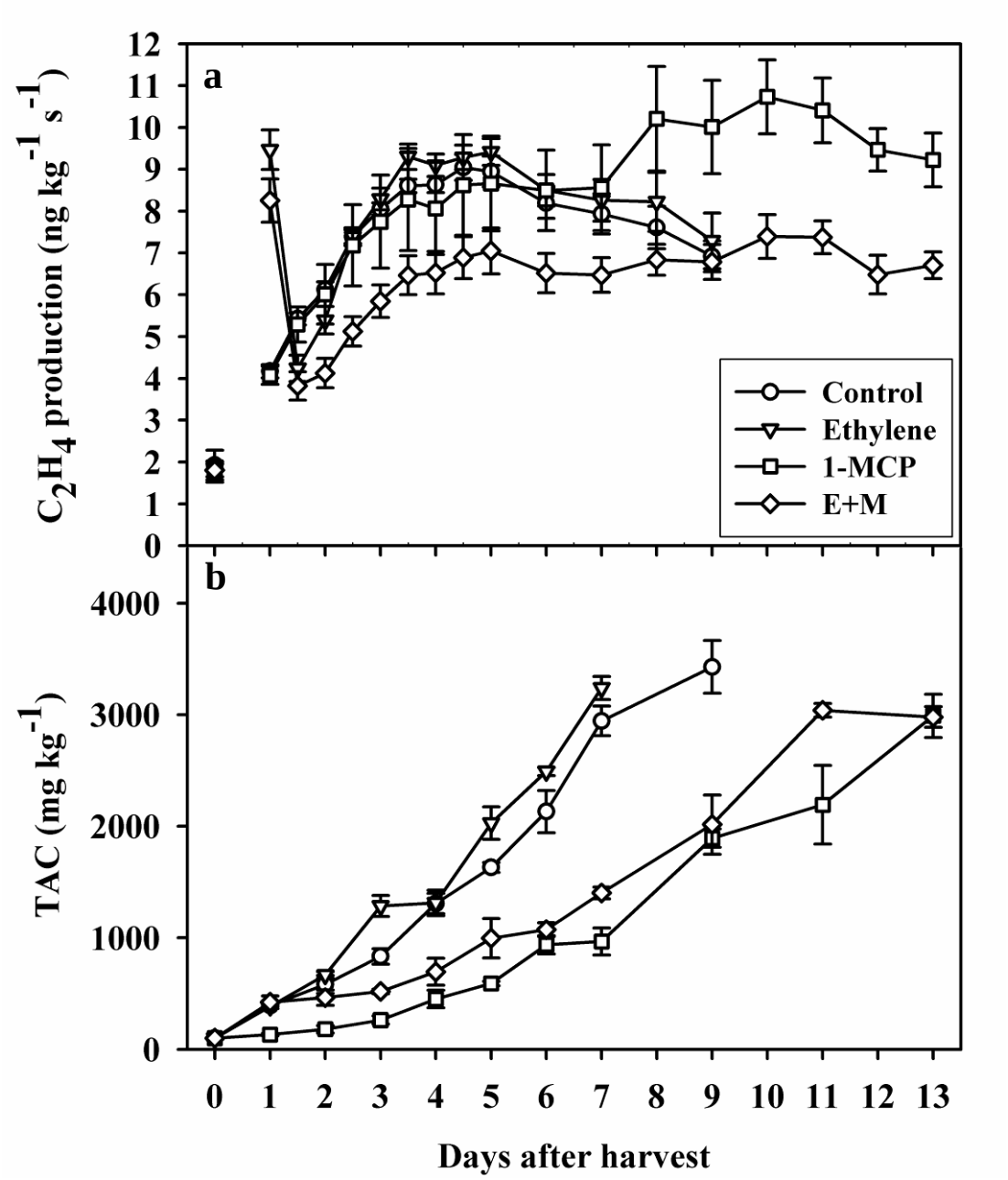


Figure 17 Change in ethylene production of fruit and total anthocyanin content of mangosteen pericarp (TAC) treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M). Data are means $\pm$ SE of five replications for ethylene production and three replications for total anthocyanin content.

3. Study of the effects of temperature storage on colour development of mangosteen fruit

Mangosteen fruit at stage 1 (light greenish yellow with 5% scattered pink spots) were stored at 25°C (control) and 15°C for 7 days then transferred to 25°C. The fruit stored at 25°C developed fruit colour faster than the fruit stored at 15°C (Figure 18, Appendix Table 9 and 10) and developed to purple black (stage 6) within 9 days, while the fruit stored at 15°C developed within 13 days (Figure 19). Hue value of fruit stored at 25°C decreased sharply (Figure 19a) and correlated closely with increase of colour index (Figure 19b). The colour of fruit stored at 15°C (hue value and colour index) developed more rapidly when fruit were transferred to 25°C (Figure 19).

Pericarp firmness decreased sharply during strorage but the fruit stored at 25°C lost firmness more rapidly than those stored at 15°C (Figure 20, Appendix Table 11).

After storage, ethylene production increased continuously for 5 days and decreased thereafter with an ethylene climacteric pattern. The total anthocyanin content of both treatments increased continuously (Figure 21b) and correlated closely with ethylene production (Figure 21a). The total anthcyanin content of fruit stored at 25°C significantly increased more than for those stored at 15°C (Figure 21b, Appendix Table 12). The ethylene production and total anthocyanin content increased rapidly when fruit were transferred to 25°C (Figure 21). Fruit stored at 15°C had significantly delayed ethylene production and anthocyanin accumulation (Figure 21, Appendix Table 12).

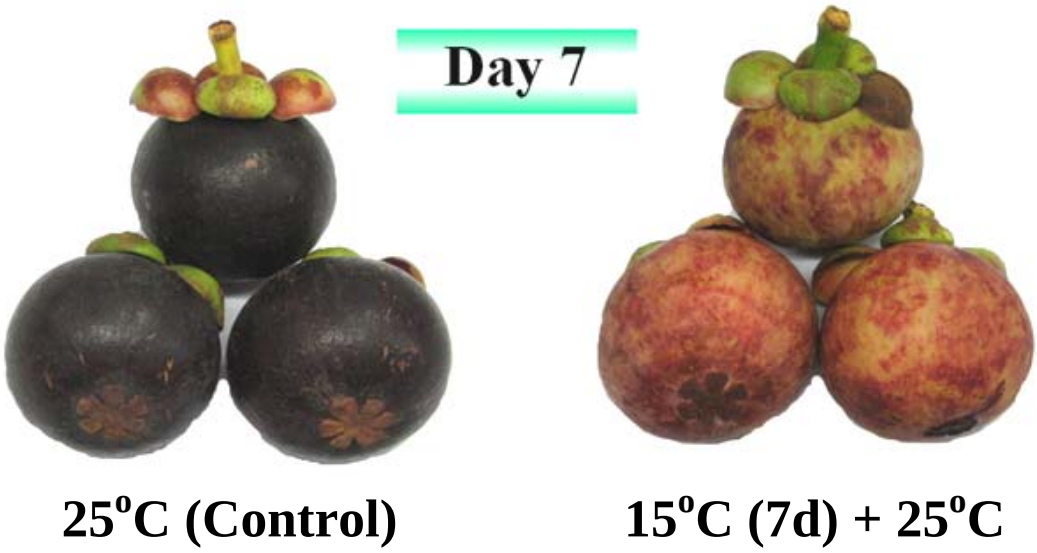


Figure 18 Mangosteen fruit at day 7 stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

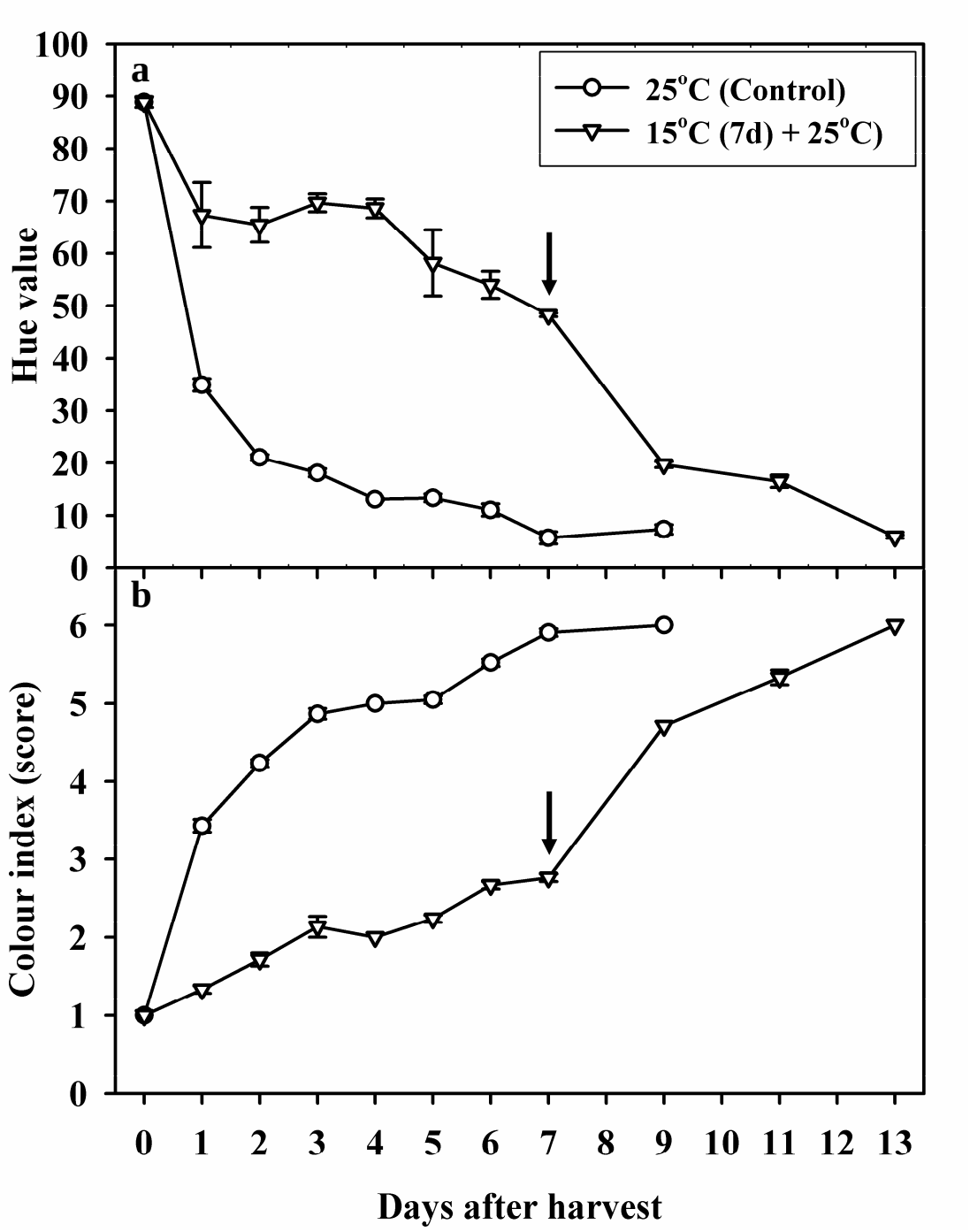


Figure 19 Change in fruit colour (hue value) and colour index (score) of fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C (arrows). Data are means ± SE of three replications.

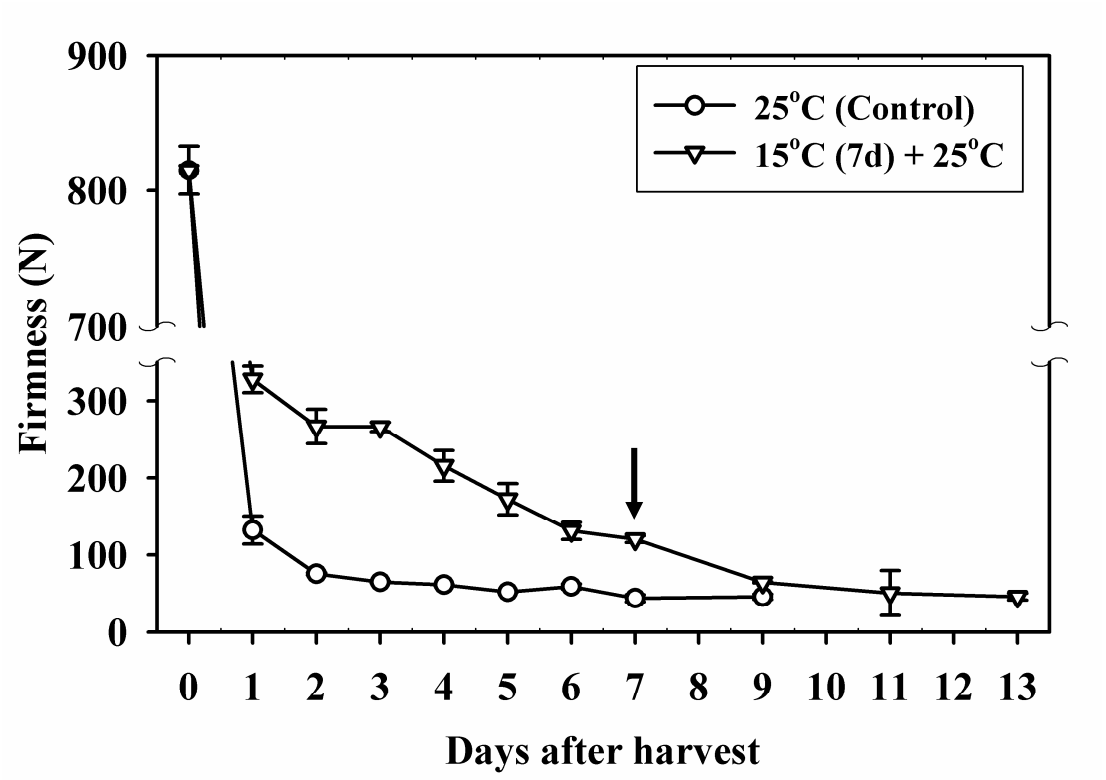


Figure 20 Change in pericarp firmness of fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C (arrow). Data are means ± SE of three replications.

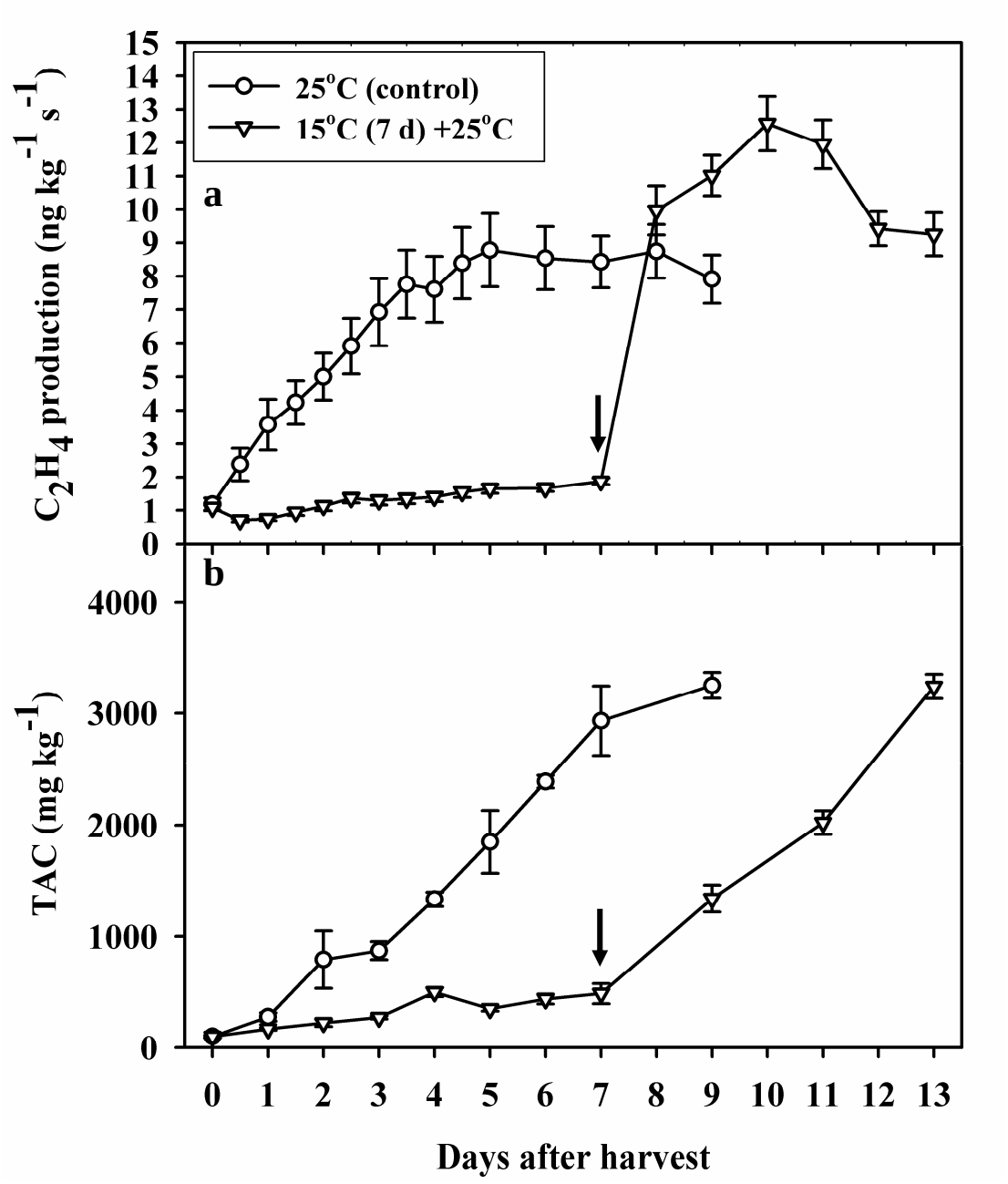


Figure 21 Change in ethylene production of fruit and total anthocyanin content of mangosteen pericarp (TAC) stored at 25°C (control) and 15°C for 7 days then transferred to 25°C (arrows). Data are means ± SE of three replications

4. Cloning and characterization of MYB transcription factors and anthocyanin biosynthesis genes of mangosteen fruit during red colouration

4.1 Isolation and cloning of MYB transcription factors and anthocyanin biosynthesis genes of mangosteen fruit

4.1.1 MYB transcription factor

In order to isolate and characterize the mangosteen MYB transcription factors that play a role in anthocyanin biosynthesis, two degenerate primers were designed in the R2R3 domain. Three putative R2R3 MYB genes were isolated by 3' race and full-length sequences obtained by 5' race. The sequences of *GmMYBs* were translated to deduce amino acid and alignment using Vector NTI 10.0 program (Invitrogen, USA). The first cloned full-length cDNA was termed *GmMYB1* (FJ197135) and was a 1,433 bp transcript encoding a predicted protein of 242 amino acids which shared high homology to strawberry *FaMYB1* (Figure 22-24). The *GmMYB1* sequence showed the longest 3' UTR (649 bp) compared to its predicted ORF (726 bp). In a circular phylogenic tree of amino acid sequences with *Arabidopsis* MYB transcription factors (generated using MEGA version 3.1), *GmMYB1* clustered in the same group as *FaMYB1* of strawberry (Figure 24). After alignment of amino acid with other plants, *GmMYB1* shared 57-63% homology with MYB1 of *Fragaria ananassa*, C2 repressor of *Vitis vinifera* and MYB6 of *Gossypium hirsutum*, respectively. GmMYB1 consisted of a motif pdLNL$^{D}/_{E}$Lxi$^{G}/_{S}$ also present in *FaMYB1*(Aharoni *et al*., 2001).

A second full-length cDNA was termed *GmMYB7* (FJ197136) which was 1,367 bp in length and encoded a predicted protein of 284 amino acids (Figure 25). A bootstrapped circular phylogenic tree generated using MEGA version 3.1 shows that *GmMYB7* shared high homology with several *Arabidopsis* MYB sequences in subgroup 7 (Figure 24). After alignment of amino acid with other plants, *GmMYB7* shared 52-74% homology with MYB7 of *Malus domestica*, MYB2 of *Arabidopsis thaliana* and MYB108 of *Vitis vinifera,* respectively. In the R3 domain,

GmMYB7 shows residues that differ from the amino acid of bHLH interaction motif [DE]Lx_2[RK]$x_3Lx_6Lx_3R$ (arrows shown on Figure 23) (Zimmermann *et al*., 2004).

A third full-length cDNA called *GmMYB10* (FJ197137) encoded a 995 bp transcript with a predicted protein of 284 amino acids (Figure 26). This cDNA shared high homology with other plant MYB sequences in the anthocyanin-regulating subgroup (Figure 23 and 24). *GmMYB10* is closely related to the *Arabidopsis* MYBs in subgroup 10, especially *AtPAP1* with 80% amino acid identity in the R2R3 DNA-binding domain and 66% identity over the whole protein. After alignment of amino acid with other plants, *GmMYB10* shared 45-66% homology with MYB10 of *Malus domestica*, MYBA1 of *Vitis vinifera,* ROSEA1 of *Antirrhinum majus* and MYB10 of *Prunus domestica*, respectively. A bootstrapped circular phylogenic tree generated using MEGA version 3.1 shows that *GmMYB10* clustered in subgroup 10 (Figure 24).

```
   1  TACTACCTAAACCTTCTCTCTTCTATTCTACATTCTATCATTTTGAAACAAAAAGAAAAT  60
                                                                M

  61  GAGGAGACCTTGTTGTGATAAACAAGGAAACAATAAAGGTGCATGGTCTAAGCAAGAAGA  120
        R  R  P  C  C  D  K  Q  G  N  N  K  G  A  W  S  K  Q  E  D

 121  CCAGAAACTAATTGACTATATTAGGGCTCATGGTGAAGGTTGTTGGCGTTCCTTACCCAA  180
        Q  K  L  I  D  Y  I  R  A  H  G  E  G  C  W  R  S  L  P  K

 181  GGCTGCAGGGTTGCACCGCTGTGGCAAGAGTTGTAGGCTAAGATGGATAAATTATCTAAG  240
        A  A  G  L  H  R  C  G  K  S  C  R  L  R  W  I  N  Y  L  R

 241  GCCGGACATTAAACGAGGTAACTTTGCTCAAGATGAAGAAGATCTCATAATCAAGCTTCA  300
        P  D  I  K  R  G  N  F  A  Q  D  E  E  D  L  I  I  K  L  H

 301  TGCGCTCCTCGGCAATAGGTGGTCTTTGATTGCCGGGAGGTTGCCAGGAAGAACTGACAA  360
        A  L  L  G  N  R  W  S  L  I  A  G  R  L  P  G  R  T  D  N

 361  TGAGGTGAAGAACTACTGGAATTCTCACATAAAAAAAAAGTTGATAAACATGGGAATAGA  420
        E  V  K  N  Y  W  N  S  H  I  K  K  K  L  I  N  M  G  I  D

 421  CCCCAACAATCATAAGCTTAACCAAGTCCTCCAACGTCCCCAACTGGGCCACAATTCCCC  480
        P  N  N  H  K  L  N  Q  V  L  Q  R  P  Q  L  G  H  N  S  P

 481  CAATATTAATGCAACCACCAGCCCATCTTTAGAAGCTTCCTTGAACCATTCAACCAAACC  540
        N  I  N  A  T  T  S  P  S  L  E  A  S  L  N  H  S  T  K  P

 541  AAAGAAACCAAAGGTTGAGAATGGTGGAGTCTCAGATTCTGGAAGCTGTCTAGATGATGA  600
        K  K  P  K  V  E  N  G  G  V  S  D  S  G  S  C  L  D  D  D

 601  TGATGATGATGATGATGATGAATCCAATTCAGACTCATCTTATCTTAATCTTGACCTAAC  660
        D  D  D  D  D  D  E  S  N  S  D  S  S  Y  L  N  L  D  L  T

 661  TATTGGTGTTCCTTCTACTTCATTTAATAGTTCGGAAAATAAGCAAGTTGTGGAGATTGA  720
        I  G  V  P  S  T  S  F  N  S  S  E  N  K  Q  V  V  E  I  D

 721  CCAGCAGGTGATAACTAGTGGTCAACTTGGCAGTGAACCATTGCCCACGTTACTTCTCTT  780
        Q  Q  V  I  T  S  G  Q  L  G  S  E  P  L  P  T  L  L  L  F

 781  TAGATGAGACAAATTGTTAGCCATATGAACTTGTTCATTCATGCCTTTCATGGAAGGCAT  840
        R

 841  GAGGAGGAATGAGAAAGGGTCAACTCAACATGCATGGGTTGATTAGATCTTTAATCGTAA  900

 901  AGAGTTAGCTCCAATTCTATTATGAGATTTTAGACCTGGTCCACTATATAGAAAATGAAT  960

 961  AGATGAACTCTGGGTTCCATAAATACTCAGTCAAAGAGTTTTGAGTCTAATCAGTAAGTC 1020

1021  CCAAAAATTGAAGTATAGCCCAAACTATTTTACTGGGCGTTTATAGTGCCCAAAGTTCAC 1080

1081  CGATTTGGATGGTTCATAATTTACATGGAAGAACACATGTATTATATATGTATGTTCGGG 1140

1141  CTAGAGTGGAAGATTAAGAAGTTTCATATTGCATTATGATTATGATATTGTTAGAAATGA 1200

1201  CTTTTGAGAGCTAACTCAAGTTCGTTAAAGGGTTCGGGTTGAACTTTACTTACATTATCC 1260

1261  AATTTGTAATATGATCGGGGAAGAGATCAATTCTTGTTGTTCTTTTCAGCTTGATATAAG 1320

1321  CTATAGTGTATTAGGGCAATTTGGTTTTATCCATGTGTAATCTCTTATTACTGGCTATAA 1380

1381  TTATGATTATATTAAAGAAAATTCTAGATGGCTCTTTAAAAAAAAAAAAAAAA  1433
```

Figure 22 Nucleotide sequence of the gene encoding MYB1 from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

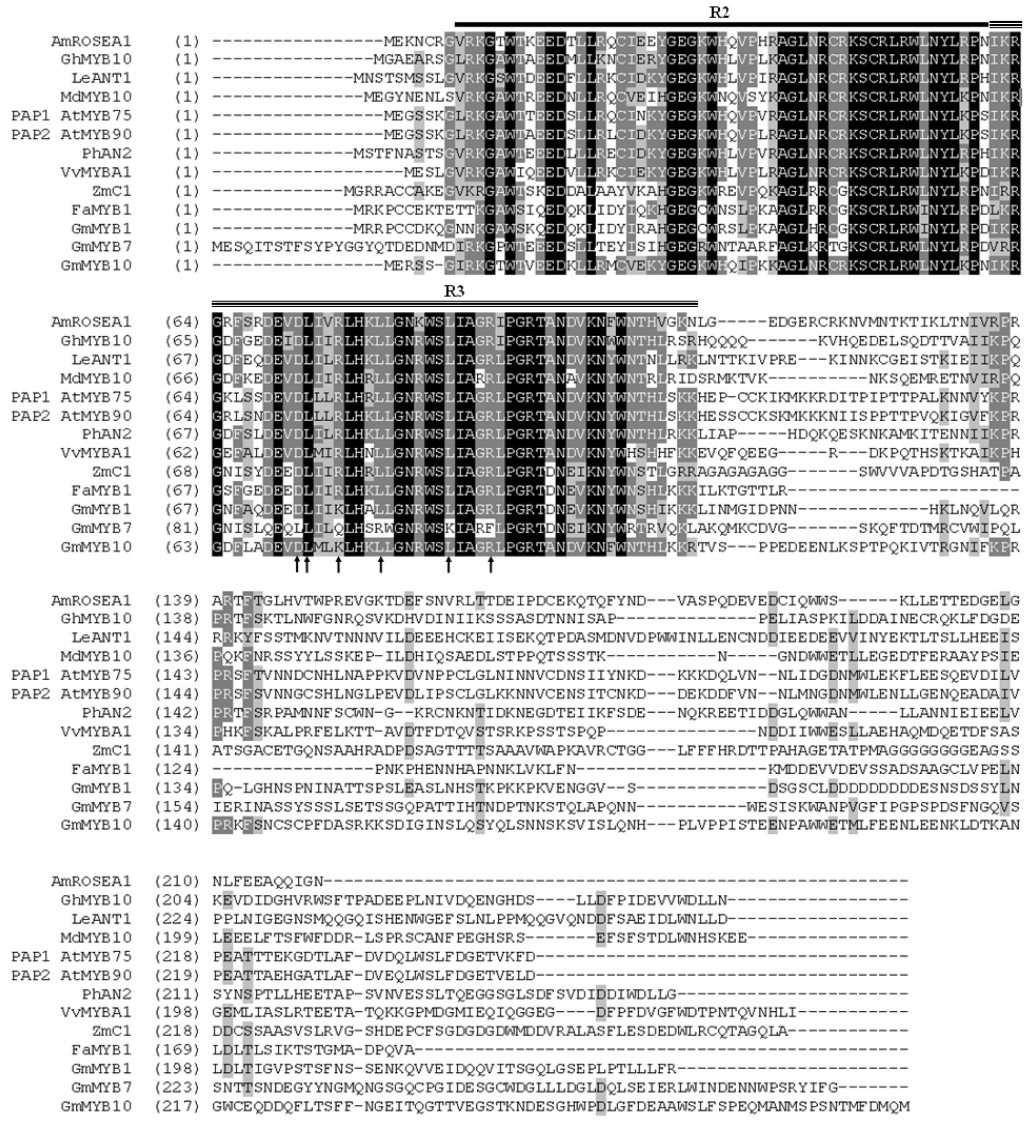


Figure 23 Protein sequence alignment of GmMYBs with anthocyanin MYB regulators from other plants. Arrows indicate the specific residues that contribute to a motif implicated in bHLH co-factor interaction in *Arabidopsis* (Zimmermann *et al*., 2004). Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; AmROSEA1, ABB83826; GhMYB10, CAD87010; LeANT1, AAQ55181; MdMYB10, DQ267896; AtPAP1, CAB09230; AtPAP2, NP176813; PhAN2, AAF66727; VvMYBA1, AB242302; ZmC1, P10290; FaMYB1, AF401220; GmMYB1, FJ197135; GmMYB7, FJ197136; GmMYB10, FJ19713.

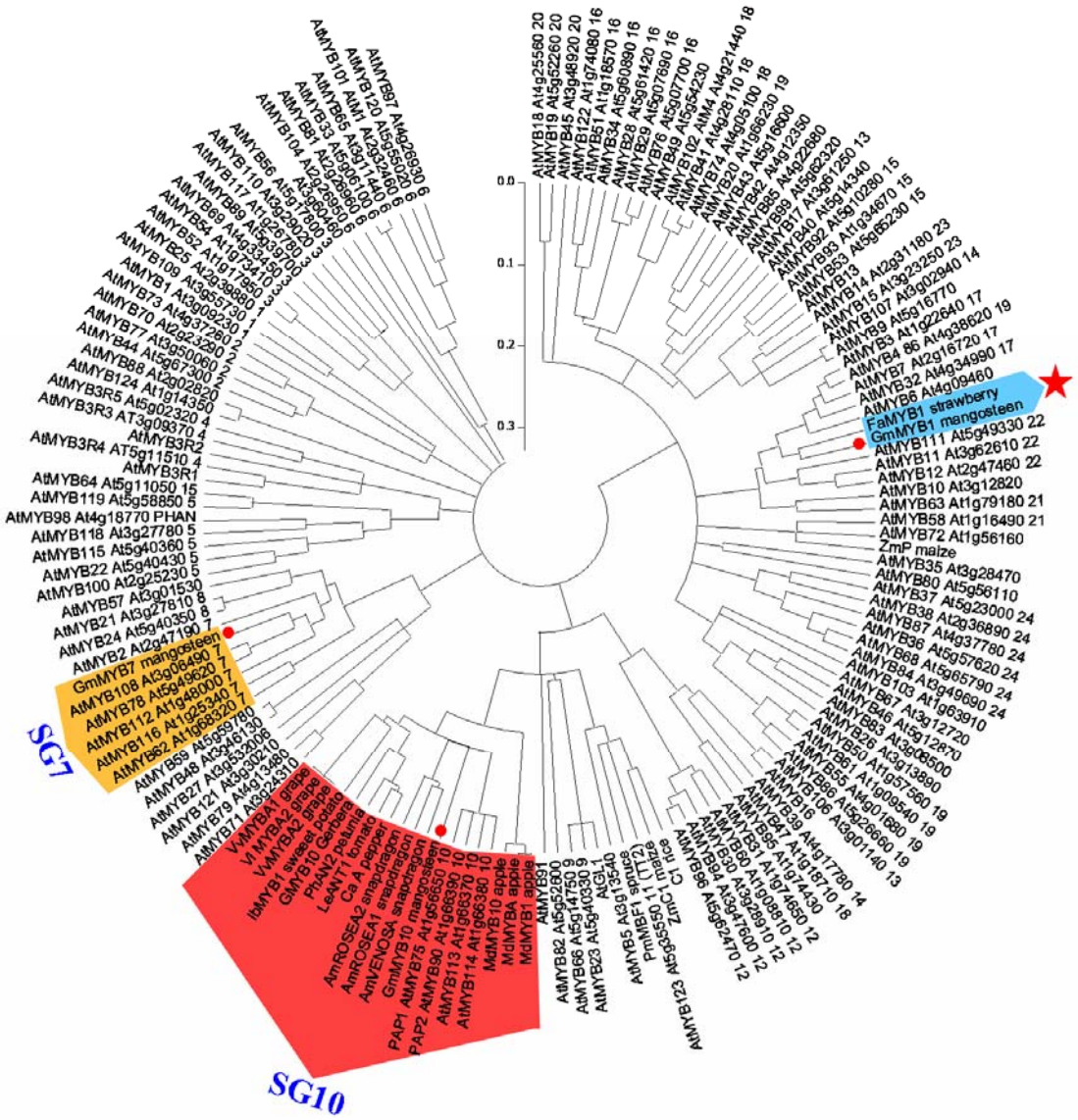


Figure 24 Phylogenetic relationship of *Arabidopsis* MYB transcription factors and anthocyanin-related MYBs from other plants with mangosteen MYBs. The amino acid sequence of *GmMYBs* marked with the red dot were clustered and showed the subgroup member as the label. Subgroup numbers are those described by Stracke *et al*. (2001) and are shown as a suffix after most MYB descriptors. Sequences were aligned using AlignX (opening=15, extension=0.3) in Vector NTI 10.0. The tree was constructed using MEGA 3.1 with minimum evolution phylogeny test and 1,000 bootstrap replicates.

```
   1  CAAAATAAAAGATGAAGTAAACAAAGGAATAAACAATAACTCCTGCGGAAACTTTATATA  60

  61  CTTTGACTCTAAATGTTCTTGAAGTGGCTCTTAACAGTCACCCTTTGTAGCTTTAACCCA  120

 121  CTATATATAAGTACCTCCCCTTCTGACTTGTCTACGAATAACCAAACAATCTTCTCCACC  180

 181  TTCTCCCTCTATATAGATACTACCCTTTTTGTTTCTTTCTTTCTTAGTTAATATTAGAGA  240

 241  TGGAATCCCAAATAACCTCTACTTTTAGCTACCCTTATGGAGGGTATCAAACTGATGAAG  300
      M  E  S  Q  I  T  S  T  F  S  Y  P  Y  G  G  Y  Q  T  D  E

 301  ATAATATGGACATAAGGAAGGGCCCATGGACTGAGGAAGAAGACTCCTTACTCACTGAGT  360
      D  N  M  D  I  R  K  G  P  W  T  E  E  E  D  S  L  L  T  E

 361  ACATATCCATCCATGGTGAAGGTCGTTGGAATACGGCTGCTCGTTTTGCAGGATTGAAGC  420
      Y  I  S  I  H  G  E  G  R  W  N  T  A  A  R  F  A  G  L  K

 421  GGACTGGTAAAAGCTGCAGGCTAAGATGGTTGAATTATTTGAGGCCAGATGTTCGAAGAG  480
      R  T  G  K  S  C  R  L  R  W  L  N  Y  L  R  P  D  V  R  R

 481  GGAACATTTCCCTCCAAGAACAACTCTTAATTCTTCAACTCCATTCTCGCTGGGGTAACA  540
      G  N  I  S  L  Q  E  Q  L  L  I  L  Q  L  H  S  R  W  G  N

 541  GGTGGTCAAAAATAGCGCGATTCTTGCCTGGAAGAACGGACAATGAGATTAAGAATTACT  600
      R  W  S  K  I  A  R  F  L  P  G  R  T  D  N  E  I  K  N  Y

 601  GGAGGACACGTGTTCAGAAGCTGGCAAAGCAGATGAAATGTGACGTCGGTAGCAAACAAT  660
      W  R  T  R  V  Q  K  L  A  K  Q  M  K  C  D  V  G  S  K  Q

 661  TCACAGACACGATGCGTTGCGTCTGGATACCCCAATTAATTGAACGTATCAATGCTTCCT  720
      F  T  D  T  M  R  C  V  W  I  P  Q  L  I  E  R  I  N  A  S

 721  CTTATTCTTCTTCTTTGTCCGAAACCTCGTCGGGTCAACCCGCCACCACCATCCACACTA  780
      S  Y  S  S  S  L  S  E  T  S  S  G  Q  P  A  T  T  I  H  T

 781  ATGATCCTACCAACAAAAGTACTCAACTTGCTCCTCAAAACAATTGGGAGTCAATTTCTA  840
      N  D  P  T  N  K  S  T  Q  L  A  P  Q  N  N  W  E  S  I  S

 841  AATGGGCCAACCCGGTGGGCTTTATCCCGGGCCCATCACCAGACTCTTTTAACGGTCAAG  900
      K  W  A  N  P  V  G  F  I  P  G  P  S  P  D  S  F  N  G  Q

 901  TATCCTCCAACACTACATCAAATGACGAAGGCTATTACAATGGCATGCAAAATGGGTCGG  960
      V  S  S  N  T  T  S  N  D  E  G  Y  Y  N  G  M  Q  N  G  S

 961  GTCAATGCCCGGGAATTGACGAATCGGGTTGTTGGGATGGACTATTATTGGATGGTTTGG  1020
      G  Q  C  P  G  I  D  E  S  G  C  W  D  G  L  L  L  D  G  L

1021  ATCAGTTATCAGAAATAGAGAGGCTTTGGATAAATGATGAGAATAATTGGCCCTCTAGAT  1080
      D  Q  L  S  E  I  E  R  L  W  I  N  D  E  N  N  W  P  S  R

1081  ACATTTTTGGCTAATAATAATATTTTACGTGTTGGCTTTGTTAAGTTAGAATTTTAATCC  1140
      Y  I  F  G

1141  GTTACGCATTGAATCATTTGAGATTCTGTCGTGTGATGTGATTAGAATTTTGAATTTTTG  1200

1201  TTGTGGCTAATTCTTTCTGACTTTTATGTACCTTATTTACACTTACGGGATGTATAAAGG  1260

1261  TAGAACATGAATTTAATAAATTGAAGATAACATATTGTTTTGAATATCATAGTTATTTGG  1320

1321   CTTAAATGAGAAGTTTAATGCGCTGATGGTTGCTAAAAAAAAAAAAA  1367
```

Figure 25 Nucleotide sequence of the gene encoding MYB7 from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

```
  1  GAAGCTAAATGGAGAGAAGTTCAGGAATTAGGAAAGGAACATGGACCGTAGAGGAAGACA  60
                M  E  R  S  S  G  I  R  K  G  T  W  T  V  E  E  D

 61  AACTTCTGAGAATGTGCGTTGAGAAATATGGAGAAGGAAAATGGCACCAAATTCCTAAAA  120
     K  L  L  R  M  C  V  E  K  Y  G  E  G  K  W  H  Q  I  P  K

121  AAGCTGGGCTAAATAGGTGTCGAAAAAGCTGTAGATTGAGGTGGTTGAATTATCTTAAAC  180
     K  A  G  L  N  R  C  R  K  S  C  R  L  R  W  L  N  Y  L  K

181  CAAATATCAAGAGGGGAGACTTTCTTGCAGATGAAGTGGATTTAATGCTCAAGTTACACA  240
     P  N  I  K  R  G  D  F  L  A  D  E  V  D  L  M  L  K  L  H

241  AGCTGCTTGGTAACAGATGGTCATTAATCGCTGGTAGACTGCCGGGGAGAACAGCCAACG  300
     K  L  L  G  N  R  W  S  L  I  A  G  R  L  P  G  R  T  A  N

301  ACGTGAAAAACTTTTGGAATACCCACCTGAAGAAGAGGACAGTAAGTCCTCCAGAGGATG  360
     D  V  K  N  F  W  N  T  H  L  K  K  R  T  V  S  P  P  E  D

361  AAGAAAATTTAAAATCTCCAACTCCACAGAAAATTGTGACAAGAGGCAATATATTCAAGC  420
     E  E  N  L  K  S  P  T  P  Q  K  I  V  T  R  G  N  I  F  K

421  CTCGACCTCGGAAATTCTCCAATTGTTCATGTCCCTTCGATGCAAGCCGGAAAAAATCAG  480
     P  R  P  R  K  F  S  N  C  S  C  P  F  D  A  S  R  K  K  S

481  ATATTGGTATAAACTCACTCCAGTCCTATCAACTATCAAATAATTCAAAATCAGTTATTA  540
     D  I  G  I  N  S  L  Q  S  Y  Q  L  S  N  N  S  K  S  V  I

541  GCCTACAAAACCACCCATTGGTCCCTCCTATCTCTACAGAAGAAAATCCAGCATGGTGGG  600
     S  L  Q  N  H  P  L  V  P  P  I  S  T  E  E  N  P  A  W  W

601  AGACCATGTTATTCGAAGAAAACTTAGAAGAAAATAAATTGGACACTAAAGCCAATGGCT  660
     E  T  M  L  F  E  E  N  L  E  E  N  K  L  D  T  K  A  N  G

661  GGTGTGAGCAAGATGATCAATTTCTCACAAGCTTTTTCAATGGAGAAATAACTCAAGGGA  720
     W  C  E  Q  D  D  Q  F  L  T  S  F  F  N  G  E  I  T  Q  G

721  CAACGGTAGAGGGCTCCACAAAAAATGATGAGAGTGGTCATTGGCCTGACCTTGGTTTTG  780
     T  T  V  E  G  S  T  K  N  D  E  S  G  H  W  P  D  L  G  F

781  ACGAGGCTGCATGGAGTCTATTCAGCCCAGAACAAATGGCAAATATGTCACCATCCAATA  840
     D  E  A  A  W  S  L  F  S  P  E  Q  M  A  N  M  S  P  S  N

841  CGATGTTTGATATGCAAATGTAGTTTTTCGATGAATCCTAGCAGTATTGGTGTTTTTTAC  900
     T  M  F  D  M  Q  M

901  AATAATATTGATTATGGACACAATATGTTTCAATGTAACGAAGTTGATAAAATAATAAAT  960

961  AAAGTATAATTATCTTTTTATGTTTAAAAAAAAAA  995
```

Figure 26 Nucleotide sequence of the gene encoding MYB10 from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

4.1.1 Anthocyanin biosynthesis genes

All mangosteen anthocyanin biosynthesis genes were isolated and characterized using two degenerate primers or 3' race primers. The full-length cDNA sequences obtained by 5' and 3' race. All genes showed high homology to other plant anthocyanin biosynthetic genes (GenBank accessions FJ197127-34).

1) Phenylalanine ammonia lyase (PAL)

The sequence of mangosteen PAL was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmPAL* (FJ197127) and was a 2,449 bp transcript encoding a predicted protein of 718 amino acids (Figure 27). After alignment of amino acids with other plants, *GmPAL* was found to share 82-84% homology with PAL2 of *Prunus avium*, *Rubus idaeus*, *Vitis vinifera*, *Daucus carota*, *Pyrus communis* and *Arabidopsis thaliana,* respectively. In addition, the putative amino acid sequence had all the PAL protein conserved motifs, particularly the active site consensus sequence: G-[STG]-[LIVM]-[STG]-[AC]-S-G-[DH]-L-x-P-L-[SA]-x(2)-[SAV] (underlined in Figure 28) (Mahesh *et al.*, 2006).

2) Chalcone synthase (CHS)

The sequence of mangosteen CHS was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmCHS* (FJ197128) and was a 1,373 bp transcript encoding a predicted protein of 394 amino acids (Figure 29). After alignment of amino acids with other plants, *GmCHS* was found to share 87-89% homology with *Vitis vinifera*, *Petunia hybrida*, *Fragaria ananassa,* and *Malus domestica*, respectively. The putative amino acid sequence had all the CHS protein conserved motifs, particularly the active site consensus sequence: GY[FY][GA]GGTX(2)R (underlined in Figure 30) (Liew *et al.*, 1998).

3) Chalcone isomerase (CHI)

The sequence of mangosteen CHI was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmCHI* (FJ197129) and was a 826 bp transcript encoding a predicted protein of 209 amino acids (Figure 31). After alignment of amino acids with other plants, *GmCHI* was found to share 31-82% homology with *Petunia hybrida, Pyrus communis*, *Arabidopsis thaliana* and *Populus trichocarpa*, respectively. The putative amino acid sequence had the CHI protein conserved motifs, particularly the active site consensus sequence (arrows in Figure 32) (Ralston *et al.*, 2005).

4) Flavonone 3-hydroxylase (F3H)

The sequence of mangosteen F3H was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmF3H* (FJ197131) and was a 1,318 bp transcript encoding a predicted protein of 365 amino acids (Figure 33). After alignment of amino acids with other plants, *GmF3H* was found to share 81-85% homology with *Arabidopsis thaliana, Actinidia chinensis*, *Rubus coreanus*, *Citrus sinensis*, *Fragaria ananassa* and *Vitis vinifera*, respectively. The putative amino acid sequence had five motifs with high overall similarity (underlined in Figure 34) (Britsch *et al.*, 1993).

5) Flavonone 3'-hydroxylase (F3'H)

The sequence of mangosteen F3'H was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmF3'H* (FJ197132) and was a 1,711 bp transcript encoding a predicted protein of 507 amino acids (Figure 35). The most highly conserved region was the heme-binding domain centered around a cysteine (C) residue that binds heme in the active site. The characteristic proline-rich region (PPxP), which forms a hinge between the membrane-anchored N-terminal helix and other parts of the protein, as well as the (A/G)Gx(D/E)T(T/S) consensus, which is involved in oxygen activation

and the transfer of protons to the active site (underlined in Figure 36) (Mori *et al.*, 2004). After alignment of amino acids with other plants, *GmF3'H* was found to share 66-75% homology with *Arabidopsis thaliana*, *Antirrhinum majus, Pelargonium hortorum*, *Petunia hybrida* and *Vitis vinifera*, respectively.

6) Dihydroflavonol 4-reductase (DFR)

The sequence of mangosteen DFR was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmDFR* (FJ197130) and was a 1,229 bp transcript encoding a predicted protein of 334 amino acids (Figure 37). A putative NADP-binding region at the N-terminal, which is likely part of the co-factor binding site, was conserved in mangosteen DFR (Lacombe *et al.*, 1997). The putative amino acid sequence had the substrate specificity domain (underlined in Figure 38) (Johnson *et al.*, 2001). After alignment of amino acids with other plants, *GmDFR* was found to share 69-76% homology with *Petunia hybrida*, *Arabidopsis thaliana*, *Fragaria ananassa*, *Pyrus communis, Vitis vinifera* and *Malus domestica,* respectively.

7) Leucoanthocyanidin dioxygenase (LDOX)

The sequence of mangosteen DFR was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmLDOX* (FJ197133) and was a 1,285 bp transcript encoding a predicted protein of 354 amino acids (Figure 39). The His and Asp residues as required for ferrous-iron coordination, and Arg residue of putative binding site of 2-oxoglutarate were found in mangosteen LDOX (arrows in Figure 40) (Saito *et al.*, 1999). After alignment of amino acids with other plants, *GmLDOX* was found to share 77-83% homology with *Arabidopsis thaliana*, *Malus domestica*, *Citrus sinensis*, *Fragaria ananassa*, *Prunus persica* and *Vitis vinifera*, respectively.

6) UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UFGT)

The sequence of mangosteen UFGT was translated to deduced amino acid using Vector NTI 10.0 program (Invitrogen, USA). The full-length cDNA was termed *GmDFR* (FJ197134) and was a 1,680 bp transcript encoding a predicted protein of 457 amino acids (Figure 41). The two domains of C-terminal region were conserved among all the glucosyltransferases, which are the common motifs found in the family of UDP-glucose-dependent glucosyltransferases (underlined in Figure 42) (Yamazaki *et al.*, 2002). After alignment of amino acids with other plants, *GmDFR* was found to share 48-59% homology with *Petunia hybrida*, *Malus domestica*, *Citrus sinensis*, *Arabidopsis thaliana*, *Fragaria ananassa* and *Vitis vinifera*, respectively.

```
   1  TCTACCACTCACCCTTTAAGCTTTCCTTTAAGCTTTTTTCTCCACACCTTCTCTCATCAG  60

  61  AAGCCTCTTAGCTCACCAATAGTATTCTTTGTGAAGGAAATGGAAACAACAATCACACAG  120
                                           M  E  T  T  I  T  Q

 121  AACGGCCACCACATGAATGGCTTGTGCATGAACGGCTCGGCTCACGTGAATAGTGACCCA  180
       N  G  H  H  M  N  G  L  C  M  N  G  S  A  H  V  N  S  D  P

 181  TTGAACTGGGGATACTTAGCTGAGTCGTTGAAGGGGAGCCATTTGGAGGAAGTGAAGAGG  240
       L  N  W  G  Y  L  A  E  S  L  K  G  S  H  L  E  E  V  K  R

 241  ATGGTGGAGGAGTACAGGAAGCCTCTAGTGAAGTTGGGTGGGGAGACTTTGACCATAGCT  300
       M  V  E  E  Y  R  K  P  L  V  K  L  G  G  E  T  L  T  I  A

 301  CAAGTGGCAGCTGTGGCTGGCGGCTTCGAAGCTGGGGTCAAAGTGGAGCTGGCTGAGTCG  360
       Q  V  A  A  V  A  G  G  F  E  A  G  V  K  V  E  L  A  E  S

 361  GCTAGAGCCGGCGTTAAGGCTAGTAGCGATTGGGTCATGGATAGTATGAACAATGGAACC  420
       A  R  A  G  V  K  A  S  S  D  W  V  M  D  S  M  N  N  G  T

 421  GATAGTTACGGGGTTACTACTGGTTTTGGTGCTACTTCTCATAGGAGAACCAAACAGGGC  480
       D  S  Y  G  V  T  T  G  F  G  A  T  S  H  R  R  T  K  Q  G

 481  GCTGCCCTTCAAAAAGAGCTCATCAGATTCTTGAACGCTGGTATTTTCGGCAACGGGACA  540
       A  A  L  Q  K  E  L  I  R  F  L  N  A  G  I  F  G  N  G  T

 541  GAGACAAGCCACACGCTGCCTCATTCTGCAACCAGAGCAGCCATGCTAGTGAGGATAAAC  600
       E  T  S  H  T  L  P  H  S  A  T  R  A  A  M  L  V  R  I  N

 601  ACACTACTCCAAGGCTACTCCGGCATTAGATTTGAGATCTTGGAAGCCCTCACCAAGTTT  660
       T  L  L  Q  G  Y  S  G  I  R  F  E  I  L  E  A  L  T  K  F

 661  CTCAACTACAACATTACTCCTTGCCTGCCTCTTCGCGGCACCATTACAGCTTCCGGTGAT  720
       L  N  Y  N  I  T  P  C  L  P  L  R  G  T  I  T  A  S  G  D

 721  TTAGTCCCTCTTTCCTACATTGCTGGTTTATTGACAGGCAGGTCTAATGCTAAAGCTGTT  780
       L  V  P  L  S  Y  I  A  G  L  L  T  G  R  S  N  A  K  A  V

 781  GGTCCCAATGGCCAAACCTTGAATGCCGAGGAAGCGTTTTCGCTGGCTGGAGTTGGATAT  840
       G  P  N  G  Q  T  L  N  A  E  E  A  F  S  L  A  G  V  G  Y

 841  CAATTTTTTGAGTTGCAGCCTAAGGAAGGGCTTGCTCTTGTGAATGGAACCGCGGTGGGG  900
       Q  F  F  E  L  Q  P  K  E  G  L  A  L  V  N  G  T  A  V  G

 901  TCTGGCTTGGCTTCTATGGTCCTTTTCGAGGCCAACATTCTTGGTGTTTTAGCAGAACTC  960
       S  G  L  A  S  M  V  L  F  E  A  N  I  L  G  V  L  A  E  L

 961  TTGTCTGCAATTTTTGCAGAAGTTATGAATGGTAAAGCAGAGTTCACTGACCACTTGACT 1020
       L  S  A  I  F  A  E  V  M  N  G  K  A  E  F  T  D  H  L  T

1021  CACAAATTGAAGCACCATCCAGGCCAGATTGAGGCTGCAGCTATAATGGAGCACATTCTT 1080
       H  K  L  K  H  H  P  G  Q  I  E  A  A  A  I  M  E  H  I  L

1081  GATGGAAGCTCTTATATGAAAGAAGCCAAGAGATTGCATGAGATGGATCCCTTGCAGAAG 1140
       D  G  S  S  Y  M  K  E  A  K  R  L  H  E  M  D  P  L  Q  K

1141  CCTAAACAAGATCGATACGCTCTCAGGACTTCACCTCAATGGCTCGGCCCACCTATTGAA 1200
       P  K  Q  D  R  Y  A  L  R  T  S  P  Q  W  L  G  P  P  I  E

1201  GTCATCCGTTTCGCTACAAAAATGATTGAAAGGGAGATTAACTCTGTGAATGACAATCCT 1260
       V  I  R  F  A  T  K  M  I  E  R  E  I  N  S  V  N  D  N  P
```

Figure 27 Nucleotide sequence of the gene encoding PAL from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG)

```
1261  TTGATTGATGTTTCCAGGAATAAGGCATTACACGGTGGCAACTTCCAGGGAACCCCAATT  1320
       L  I  D  V  S  R  N  K  A  L  H  G  G  N  F  Q  G  T  P  I

1321  GGAATGTCAATGGACAATGCAAGATTGGCTATTGCTGCTATTGGAAAACTCATGTTTGCT  1380
       G  M  S  M  D  N  A  R  L  A  I  A  A  I  G  K  L  M  F  A

1381  CAATTCAGTGAGCTTGTTAATGACTACTACAATAATGGGTTGCCATCAAATCTCACAGCC  1440
       Q  F  S  E  L  V  N  D  Y  Y  N  N  G  L  P  S  N  L  T  A

1441  AGCAGGAATCCCAGTCTAGACTATGGCTTCAAGGGAGCTGAAATTGCAATGGCTTCTTAT  1500
       S  R  N  P  S  L  D  Y  G  F  K  G  A  E  I  A  M  A  S  Y

1501  TGTTCCGAGCTGCAGTACCTTGCAAGCCCCGTCACTACCCACGTGCAAAGCGCAGAGCAG  1560
       C  S  E  L  Q  Y  L  A  S  P  V  T  T  H  V  Q  S  A  E  Q

1561  CATAACCAAGATGTGAACTCTTTGGGACTCATCTCTTCAAGAAAGACCCAAGAAGCTATA  1620
       H  N  Q  D  V  N  S  L  G  L  I  S  S  R  K  T  Q  E  A  I

1621  GACATTCTGAAGCTCATGTCCTCAACTTTCTTGGTGGCAATCTGCCAGGCTGTTGACTTG  1680
       D  I  L  K  L  M  S  S  T  F  L  V  A  I  C  Q  A  V  D  L

1681  AGGCATTTGGAGGAGAACCTGAAGAGCACTGTCAAGAACACAGTGAGTCAAGTTGCCAAG  1740
       R  H  L  E  E  N  L  K  S  T  V  K  N  T  V  S  Q  V  A  K

1741  AGGGTCTTAACCACAGGAGCCAATGGAGAGCTTCATCCATCCAGGTTTTGCGAAAAGGAC  1800
       R  V  L  T  T  G  A  N  G  E  L  H  P  S  R  F  C  E  K  D

1801  TTGCTCAAAGTGGTTGATCGCGAGTATGTTTTCGCCTACGCTGATGATCCTTGCAGCGCA  1860
       L  L  K  V  V  D  R  E  Y  V  F  A  Y  A  D  D  P  C  S  A

1861  ACGTATCCACTGATGCAAAAGCTGAGGCAAGTTCTGGTGGATCACGCGCTGGCTAATGGT  1920
       T  Y  P  L  M  Q  K  L  R  Q  V  L  V  D  H  A  L  A  N  G

1921  GAAGGTGAGAGGAATCCAAACACATCAGTCTTCCAAAAGATTGCAGCATTTGAGGAGGAA  1980
       E  G  E  R  N  P  N  T  S  V  F  Q  K  I  A  A  F  E  E  E

1981  TTGAAGGACCTTTTGCCAAAGGAAATTGAGGGTGTGAGACTTGCTTATGAGAGTGGAAAC  2040
       L  K  D  L  L  P  K  E  I  E  G  V  R  L  A  Y  E  S  G  N

2041  ACAGCAATTCCTAACAGGATTAAGGAGTGCAGATCTTACCCTCTTTATAAGTTTGTTAGG  2100
       T  A  I  P  N  R  I  K  E  C  R  S  Y  P  L  Y  K  F  V  R

2101  GAGGTAGCAGGCACTTCGTTGCTTACAGGGGAAAAGGTCACTTCTCCAGGGGAGGAGCTT  2160
       E  V  A  G  T  S  L  L  T  G  E  K  V  T  S  P  G  E  E  L

2161  GACAAGGTTTTTACTGCAATTTGCCAAGGCAAAATCATTGATCCCATCCTGGATTGCCTT  2220
       D  K  V  F  T  A  I  C  Q  G  K  I  I  D  P  I  L  D  C  L

2221  GAGGAATGGGATGGAACCCCACTTCCTATCTGTTAGAGAATCAACAACCCTTTTTCTTTC  2280
       E  E  W  D  G  T  P  L  P  I  C

2281  CTTCTTTTTTTTTTTCTGTTTTGTTCAGTGCTTCAATTAGTATGGTTTCTTTTGTGTTAT  2340

2341  ACCCAACGAAATGTACGGACGTCCCTTGTTTCTTTTATTATTTGTCAACTTGTTCAATGA  2400

2401  AGGAAATAATAACTTCCAAATCAACAATTAAAAAAAAAAAAAAAAAAAA  2449
```

Figure 27 (Continued).

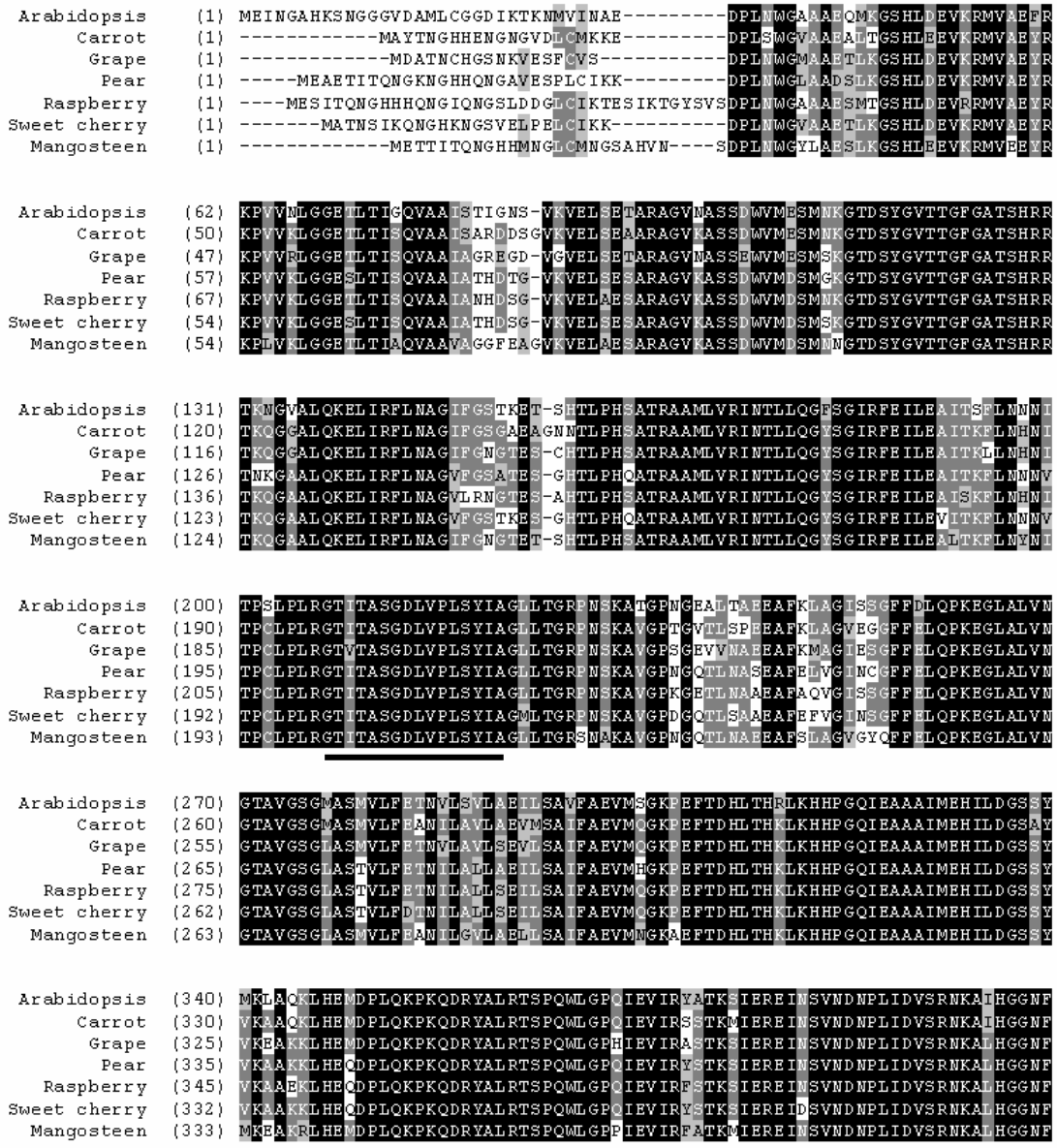


Figure 28 Protein sequence alignment of mangosteen PAL with other PAL proteins. The active site consensus sequence is underlined. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; *Arabidopsis*, AAP59438; carrot, BAG31930; grape, ABM67591; pear, ABB70117; sweet cherry, AAF40224; mangosteen, ACM62741.

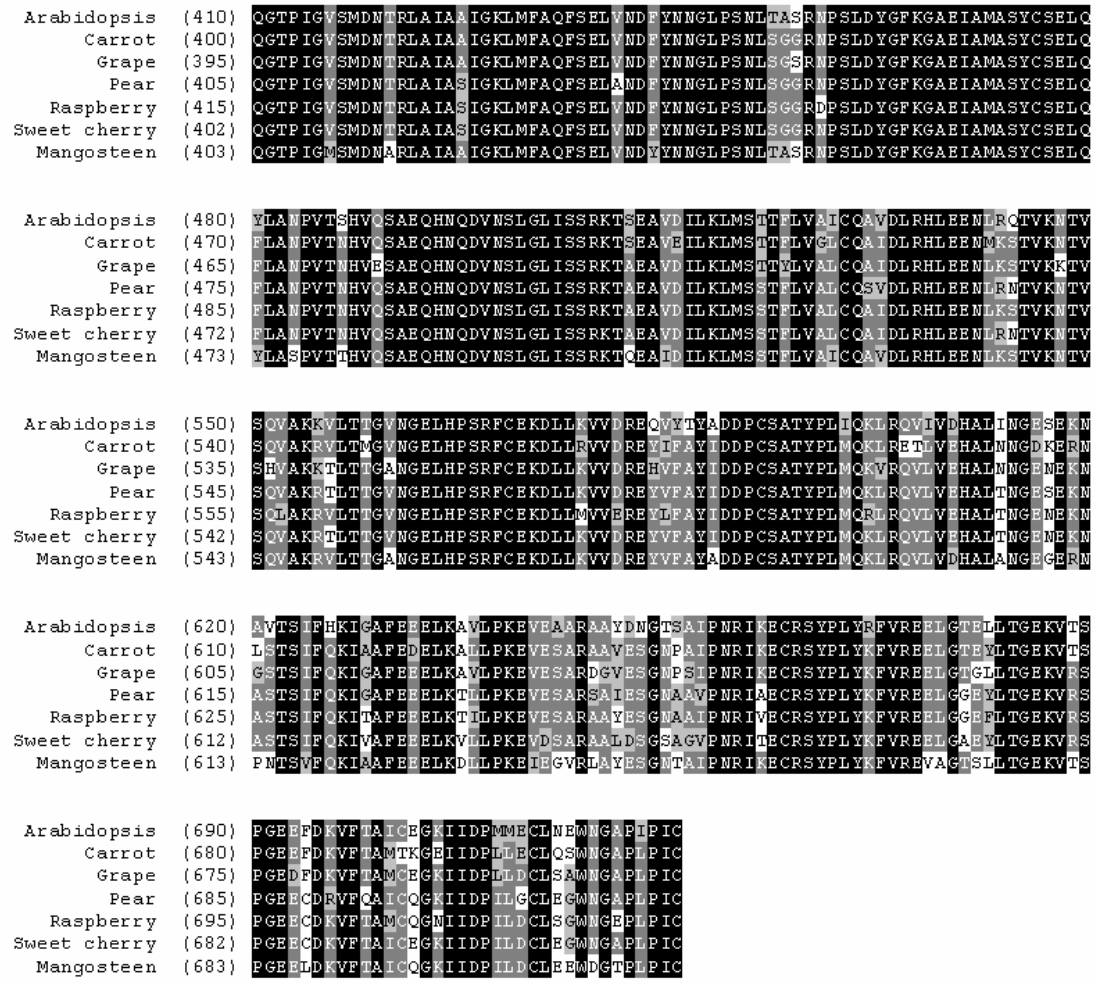


Figure 28 (Continued).

```
   1  GACTCTTAAGTGTAGACATCAAAAAAGAAACAATCGAAATGGCACCAACGGTTGAGGAGG   60
                                            M  A  P  T  V  E  E

  61  TTAGGAATGCACAGAGAGCACAAGGGCCAGCCACGGTGCTAGCCATTGGCACTGCTACTC  120
      V  R  N  A  Q  R  A  Q  G  P  A  T  V  L  A  I  G  T  A  T

 121  CATCGAACTGTGTGCTCCAGGCTGAGTATCCTGACTACTATTTCCGTATCACTAATAGCG  180
      P  S  N  C  V  L  Q  A  E  Y  P  D  Y  Y  F  R  I  T  N  S

 181  AACACAAGACCGAGCTCAAGGAGAAATTCAGGCGCATGTGCGAAAAATCAATGATCAAGA  240
      E  H  K  T  E  L  K  E  K  F  R  R  M  C  E  K  S  M  I  K

 241  AGCGTTACATGCACCTAACCGAGGAAATCCTCAAGGAAAATCCAAAGATGTGTGACTATT  300
      K  R  Y  M  H  L  T  E  E  I  L  K  E  N  P  K  M  C  D  Y

 301  GGTCACCATCCCTAGACGCCCGCCAAGACATAGTGGTAGTGGAAATTCCAAAGCTCGGGA  360
      W  S  P  S  L  D  A  R  Q  D  I  V  V  V  E  I  P  K  L  G

 361  AAGAAGCCGCAGTCAAAGCCATCAAAGAGTGGGGTCAACCCAAGTCCAAGATCACCCACC  420
      K  E  A  A  V  K  A  I  K  E  W  G  Q  P  K  S  K  I  T  H

 421  TCGTTTTTTGCACCACTTCAGGCGTTGACATGCCCGGAGCTGACTACCAGCTCACTAAGC  480
      L  V  F  C  T  T  S  G  V  D  M  P  G  A  D  Y  Q  L  T  K

 481  TTCTCGGTCTCCGCCCCCACGTCAAACGTTTGATGATGTATCAACAGGGTTGCTTTGCGG  540
      L  L  G  L  R  P  H  V  K  R  L  M  M  Y  Q  Q  G  C  F  A

 541  GTGGCACCGTTCTCCGCCTAGCAAAAGACTTGGCGGAGAACAACAAAGGTGCTCGTGTGC  600
      G  G  T  V  L  R  L  A  K  D  L  A  E  N  N  K  G  A  R  V

 601  TTGTGATTTGCTCCGAAATTACTGCTGTTACCTTCCGTGGGCCTTCTGATACCCACTTGG  660
      L  V  I  C  S  E  I  T  A  V  T  F  R  G  P  S  D  T  H  L

 661  ACTCTCTAGTGGGCCAGGCCCTTTTCGGTGATGGGGCCGCTGCTATTATTGTTGGGTCCG  720
      D  S  L  V  G  Q  A  L  F  G  D  G  A  A  A  I  I  V  G  S

 721  ACCCTGATCCAGCTATTGAGCGCCCATTATTCCAAATTGTATCTGCGGCCCAAACCATCC  780
      D  P  D  P  A  I  E  R  P  L  F  Q  I  V  S  A  A  Q  T  I

 781  TTCCTGACTCGGATGGGGCCATTGATGGACACTTGCGTGAAGTGGGCCTCACTTTCCATT  840
      L  P  D  S  D  G  A  I  D  G  H  L  R  E  V  G  L  T  F  H

 841  TGTTAAAGGACGTTCCTGGGCTTATCTCCAAGAATATTGAGAAAAGCCTTGTTGAGGCTT  900
      L  L  K  D  V  P  G  L  I  S  K  N  I  E  K  S  L  V  E  A

 901  TTACACCTATTGGTATTAGTGATTGGAACTCTCTTTTCTGGATTGCTCACCCTGGTGGGC  960
      F  T  P  I  G  I  S  D  W  N  S  L  F  W  I  A  H  P  G  G

 961  CTGCTATCTTGGACCAAGTTGAGGTTAAGTTGGGCCTTAAAGAAGAGAAGTTGAGAGCTA 1020
      P  A  I  L  D  Q  V  E  V  K  L  G  L  K  E  E  K  L  R  A

1021  CTAGGCATGTGTTAAGTGAGTTTGGGAATATGTCCAGTGCATGTGTGCTGTTTATTTTGG 1080
      T  R  H  V  L  S  E  F  G  N  M  S  S  A  C  V  L  F  I  L

1081  ATGAGATGAGGAAAAAGGCCTTGGAAGAAGGAAAGCCCACTACTGGAGAAGGCCTTGACT 1140
      D  E  M  R  K  K  A  L  E  E  G  K  P  T  T  G  E  G  L  D

1141  GGGGAGTCCTCTTTGGATTTGGGCCTGGTCTAACCGTTGAGACTGTTGTTTTGCACAGTG 1200
      W  G  V  L  F  G  F  G  P  G  L  T  V  E  T  V  V  L  H  S

1201  TCCCAACGGAAACGAGAGCATAGGCCCAACTAAATAAATTCTAATAGTGGTCGTATGTGG 1260
      V  P  T  E  T  R  A

1261  AAATTGGAATGTGGGATAAAACCTCGTATCGATTTCACAGTTACTTTGCTGCATTGAAAT 1320

1321  TTGTAATAAAACGTGATTTCTTATATGGTATTCCTTTTCTTCTTTAAAAAAAA  1373
```

Figure 29 Nucleotide sequence of the gene encoding CHS from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

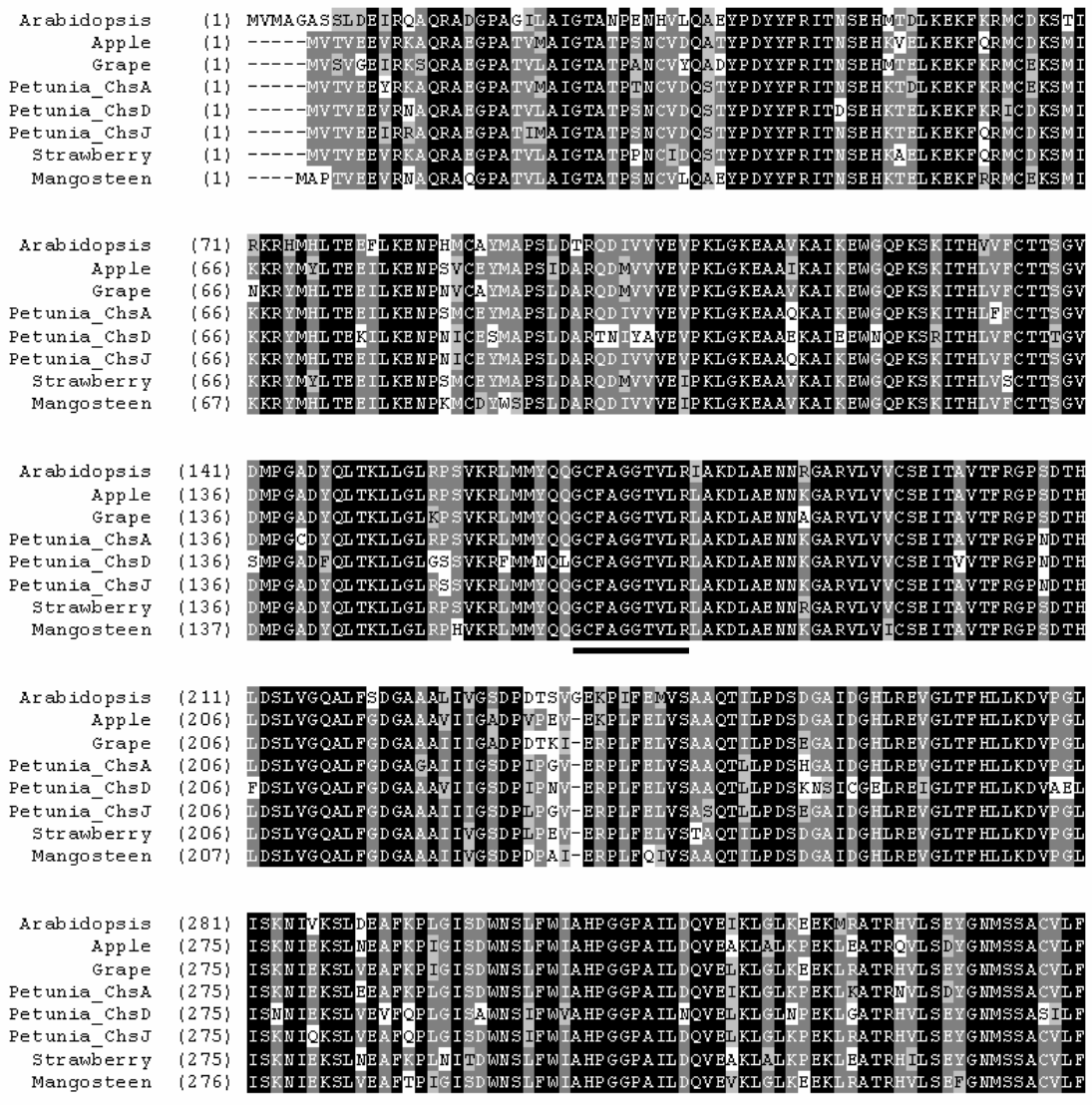


Figure 30 Protein sequence alignment of mangosteen CHS with other CHS proteins. The active site consensus sequence is underlined. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; *Arabidopsis*, NP_196897; apple, BAB92996; grape, BAB84112; petunia (chsA), CAA32731; petunia (chsD), CAA32733; petunia, (chsJ), CAA32737; strawberry, AAX99413; mangosteen, ACM62742.

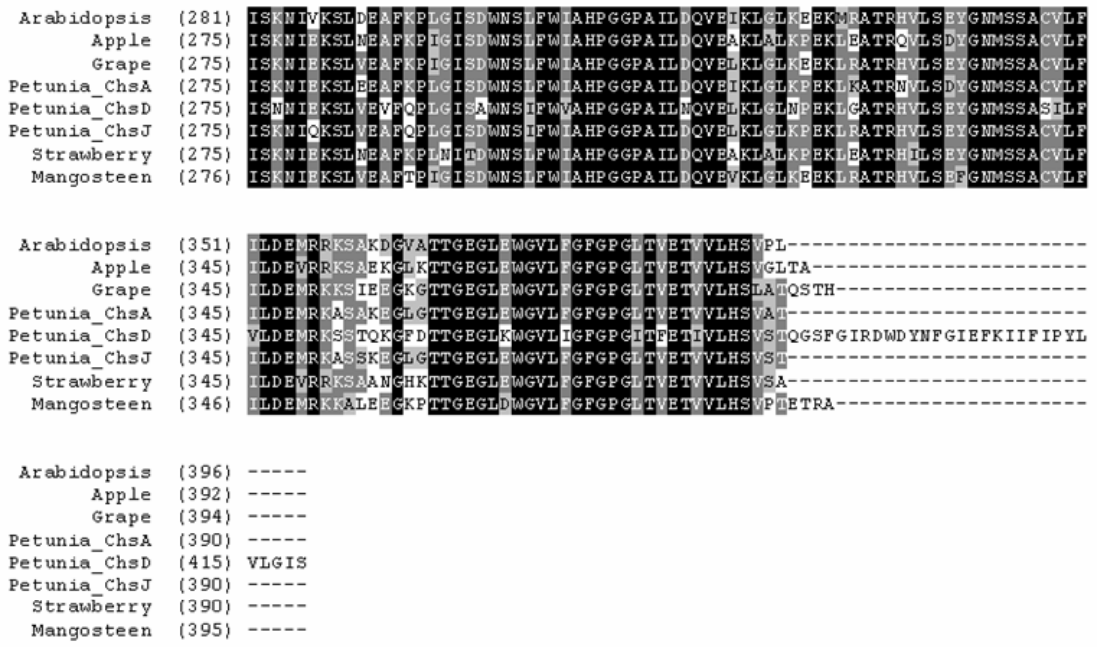


Figure 30 (Continued).

```
  1  ATATTTTATCCTTTGGTTATCTCCAGCAACGTGCAGGGAAACATGGCTACTGAAGTGGTG  60
                                                 M  A  T  E  V  V

 61  ATGGTGGATGAAGTGTCATTCCCACCCCAGATTACTACCACCAAGCCTTTATCTCTCCTT  120
      M  V  D  E  V  S  F  P  P  Q  I  T  T  T  K  P  L  S  L  L

121  GGCCATGGAATGACGGACATCGAGATACACTTTCTCCAGATTAAGCTCACAGCAATAGGA  180
      G  H  G  M  T  D  I  E  I  H  F  L  Q  I  K  L  T  A  I  G

181  GTGTACTTGGAGCCCGAAGTGCTGAGCCATTTGCAGAAATGGAAGGGCAAACCCGGAAAT  240
      V  Y  L  E  P  E  V  L  S  H  L  Q  K  W  K  G  K  P  G  N

241  GAACTTGCTGAGAATGACGAATTCTTTGATGCTCTCATTGCAGCTCCTGTTGAGAAGTTC  300
      E  L  A  E  N  D  E  F  F  D  A  L  I  A  A  P  V  E  K  F

301  CTGAGGGTTGTGATTATAAAGGAGATAAAAGGTTCACAATATGGGGTGCAGCTAGAGAGC  360
      L  R  V  V  I  I  K  E  I  K  G  S  Q  Y  G  V  Q  L  E  S

361  TCCGTGAGGGATAGACTCGCAGAAGAGGATAAGTACGAGGAAGAGGAGGAAGAGGCGTTG  420
      S  V  R  D  R  L  A  E  E  D  K  Y  E  E  E  E  E  E  A  L

421  GAGAAAATTGTCGAGTTCTTCCAATCCAAGTACTTAAAGAAACACTCTGTCATCACCTTC  480
      E  K  I  V  E  F  F  Q  S  K  Y  L  K  K  H  S  V  I  T  F

481  CATTTCCCAGTAACTTCACCCACTGCCGAGATTGTGGTTTCCACAGAAGGGAAAGAGGAT  540
      H  F  P  V  T  S  P  T  A  E  I  V  V  S  T  E  G  K  E  D

541  AGCAAGATTTTGGTGGAGAATGCAAATGTGGTGGAGATGATCAAGAGGTGGTATTTAGGT  600
      S  K  I  L  V  E  N  A  N  V  V  E  M  I  K  R  W  Y  L  G

601  GGAACCAGGGGGGGTGTCCCCTTCAACCATTTCTTGCCTGGCTAATGCACTCTCTGCTGAG  660
      G  T  R  G  V  S  P  S  T  I  S  C  L  A  N  A  L  S  A  E

661  TTGGCCAAATGAACGCTATCAGGGTGTTCTTGGGTTGTTTGCTGCTGATTTATGTACGAT  720
      L  A  K

721  GATGGCAGTTTGATTTTAGTCATTATTAGAGACTTGGTTTTGTTGCTTGGGTTCTCTCTT  780

781  GCTACTATGGATATGAATTACTCTTGAGTGTTTTTAAAAAAAAAAA  826
```

Figure 31 Nucleotide sequence of the gene encoding CHI from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

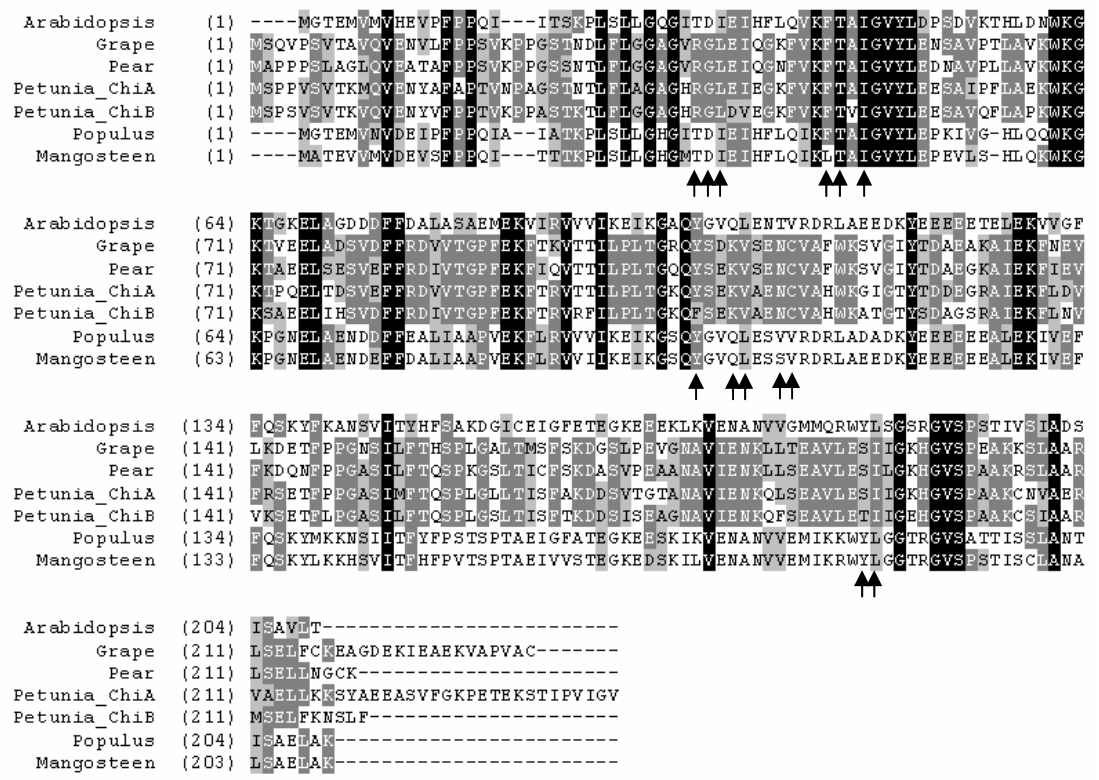


Figure 32 Protein sequence alignment of mangosteen CHI with other CHI proteins. The active site consensus sequences are indicated by arrows. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; *Arabidopsis*, NP_568154; grape, CAA53577; pear, ABQ08639; petunia (chiA), AAF60296; petunia (chiB), CAA32730; Populus, XP_002325926; mangosteen, ACM62743.

```
   1  CCATAAGAGAGATAACCAAACTATATGGCTCCTACCCCAACAACACTTACCGCTCTTGAA  60
                             M  A  P  T  P  T  T  L  T  A  L  E

  61  GGAGAGACAATCCTTCGAGCCAGCTTTGTAAGGGATGAAGATGAACGTCCCAAAGTGGCA  120
       G  E  T  I  L  R  A  S  F  V  R  D  E  D  E  R  P  K  V  A

 121  TATAACCAATTTAGCAATGACGTTCCTGTGATTTCACTTGAAGGGATTGATGAAGGTGGC  180
       Y  N  Q  F  S  N  D  V  P  V  I  S  L  E  G  I  D  E  G  G

 181  CAAAAAAGGGCTGAGATTTGTAAGAAAATTGTTGAGGCTTGTGAGGAATGGGGGATTTTC  240
       Q  K  R  A  E  I  C  K  K  I  V  E  A  C  E  E  W  G  I  F

 241  CAAGTGGTTGACCATGGTGTTGATACTAAGCTTGTTTCTGAAATGACACGTTTGGCTAGG  300
       Q  V  V  D  H  G  V  D  T  K  L  V  S  E  M  T  R  L  A  R

 301  GCATTCTTTGCCTTGCCACCAGAGGAAAAGCTCCGATTCGATATGTCCGGTGGTAAAAAG  360
       A  F  F  A  L  P  P  E  E  K  L  R  F  D  M  S  G  G  K  K

 361  GGTGGTTTCATTGTCTCCAGTCATTTGCAGGGAGAGGCAGTGCAAGATTGGCGTGAGATA  420
       G  G  F  I  V  S  S  H  L  Q  G  E  A  V  Q  D  W  R  E  I

 421  GTGACATATTTCTCATACCCAACGAGGACCCGTGACTACTCAAGGTGGCCCGATAAACCC  480
       V  T  Y  F  S  Y  P  T  R  T  R  D  Y  S  R  W  P  D  K  P

 481  GATGGCTGGGTGGACGTCACCAAGGACTATGGTGACCAGCTCATGGGCCTGGCCTGCAAA  540
       D  G  W  V  D  V  T  K  D  Y  G  D  Q  L  M  G  L  A  C  K

 541  CTCCTGGAGGTCCTATCTGAGGCCATGGGATTGGAAAAGGAGGCCTTGACTAAGGCCTGT  600
       L  L  E  V  L  S  E  A  M  G  L  E  K  E  A  L  T  K  A  C

 601  GTGGACATGGACCAGAAGATTGTGGTCAATTATTATCCCAAGTGTCCACAACCAGACCTC  660
       V  D  M  D  Q  K  I  V  V  N  Y  Y  P  K  C  P  Q  P  D  L

 661  ACTCTTGGGCTGAAGAGGCACACTGACCCAGGCACTATTACTCTATTGCTTCAGGACCAA  720
       T  L  G  L  K  R  H  T  D  P  G  T  I  T  L  L  L  Q  D  Q

 721  GTTGGTGGGCTTCAGGCCACTAGAGATAATGGAGAGACTTGGATTACTGTTCAGCCCATT  780
       V  G  G  L  Q  A  T  R  D  N  G  E  T  W  I  T  V  Q  P  I

 781  GAAGGTGCTTTTGTTGTCAATCTTGGTGATCATGGCCATTTCCTTAGCAATGGAAGGTTT  840
       E  G  A  F  V  V  N  L  G  D  H  G  H  F  L  S  N  G  R  F

 841  AGAAATGCTGACCATCAAGCAGTGGTCAACTCAAATTGTAGCCGATTGTCCATAGCAACA  900
       R  N  A  D  H  Q  A  V  V  N  S  N  C  S  R  L  S  I  A  T

 901  TTCCAGAACCCAGCCCCAGATGCAATTGTTTATCCACTAAAGATAAGGGAAGGAGAGAAA  960
       F  Q  N  P  A  P  D  A  I  V  Y  P  L  K  I  R  E  G  E  K

 961  TCAATTCTTGAGGAGCCAATCACATTCTCTGAGATGTACAGGAGGAAAATGGCCAAGGAC 1020
       S  I  L  E  E  P  I  T  F  S  E  M  Y  R  R  K  M  A  K  D

1021  TTGGAATTAGCCAGGCTTAAGAAGCTTGCTAAGGAGCAGCAATTGACGGACGTTGAGAAA 1080
       L  E  L  A  R  L  K  K  L  A  K  E  Q  Q  L  T  D  V  E  K

1081  GCTAAGTTGGAGGCCAAGCCCATCGAAAAGATCCTTGCTTAAATGCTATAAAACTACAAC 1140
       A  K  L  E  A  K  P  I  E  K  I  L  A

1141  ATTTTATAGTGTATCTACTTGGATCCTATATTTGCACGCTTCACTGTAATTTTTCATGTT 1200

1201  ATTGTAATGCTTTTCGGTAAGGAAATGCGTTTCCGTGTGCTTGTGCTGGGAAAAATGAGA 1260

1261  TATGGGAACCGTTTTTCTAAATAAAAGAATTTTATTCGTTACCTTAAAAAAAAAAAAA 1310
```

Figure 33 Nucleotide sequence of the gene encoding F3H from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

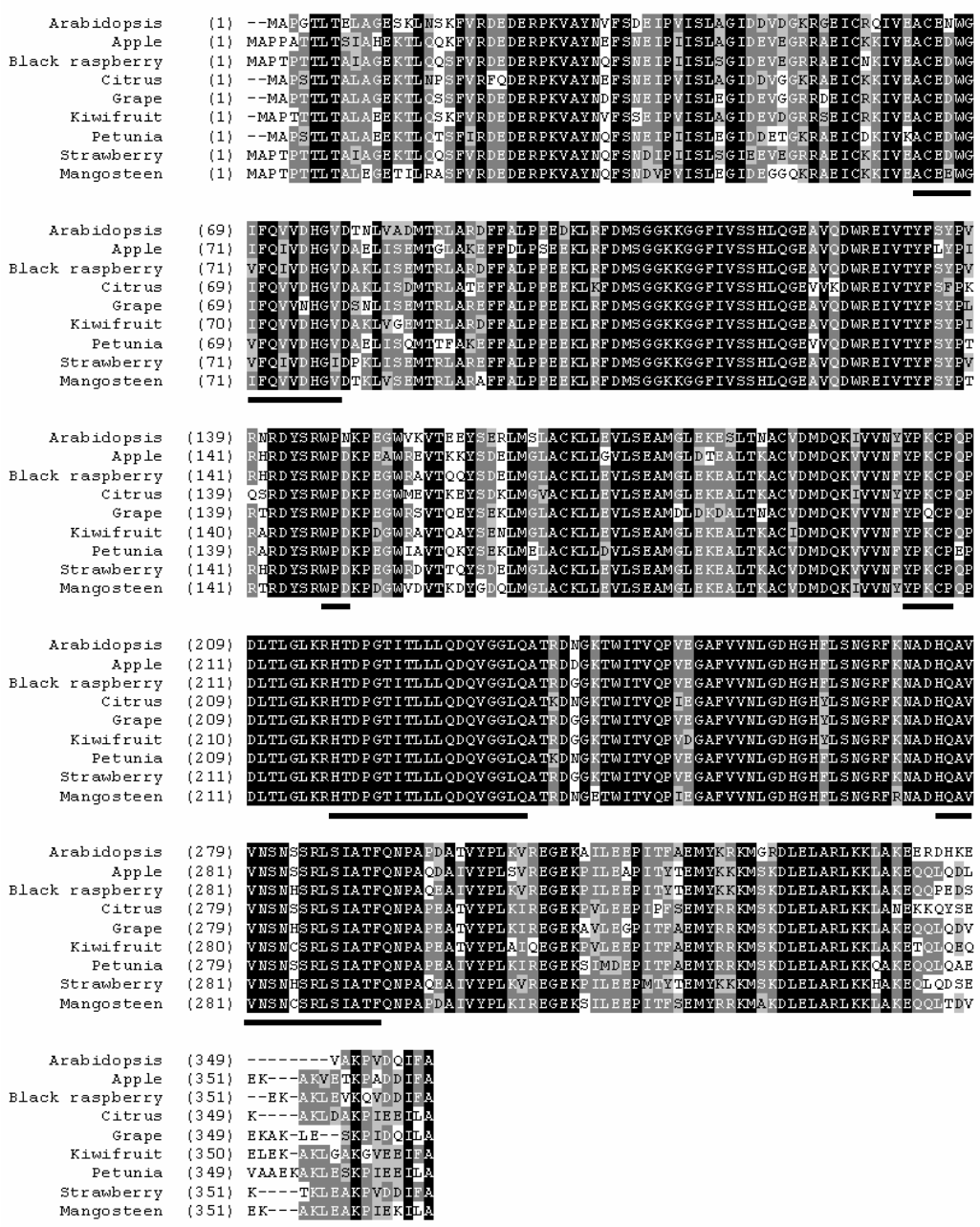


Figure 34 Protein sequence alignment of mangosteen F3H with other F3H proteins. Five motifs conserved in flavanone 3-hydroxylases are underlined. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; Arabidopsis, AAC49176; apple, BAB92997; black raspberry, ABY84868; citrus, BAA36553; grape, ABM67589; kiwifruit, ACL54955; petunia, AAC49929; strawberry, AAU04792; mangosteen, ACM62745.

```
   1  ATCCTGTGCTTCTCCTTCAAAAGTCTCTATCCTGTACTTCTCCTTCAATATAAAATCAAA  60

  61  AATAGGAAGTTTACTAAACAAAAGGAGGAAACACAATGTCTCCTTTTATTCTCTACTCCA  120
                                          M  S  P  F  I  L  Y  S

 121  TTCTAATAGCAATACTCGTCTACGTTCTCATAAAGTTGGGTTCTCTCAGTAGTGGTCGTC  180
      I  L  I  A  I  L  V  Y  V  L  I  K  L  G  S  L  S  S  G  R

 181  GCCTACCACCAGGCCCAAGACCCTTACCTCTTGTGGGGAACTTACCGCACTTGGGCTCAA  240
      R  L  P  P  G  P  R  P  L  P  L  V  G  N  L  P  H  L  G  S

 241  TGCCTCACCAGTCCATTGCCTCATTAGTCAAGAAATATGGGCCTCTAATGTACCTCAGGC  300
      M  P  H  Q  S  I  A  S  L  V  K  K  Y  G  P  L  M  Y  L  R

 301  TAGGCTATGTGGACGTTGTAGTGGCGGCCTCTGCGTCGGTCGCGGCCCAAATTTTTAAAA  360
      L  G  Y  V  D  V  V  V  A  A  S  A  S  V  A  A  Q  I  F  K

 361  ACCATGACGCCAATTTTTCCAGCCGCCCCCCCAATTCCGGTGCCAAATACGTAGCGTACA  420
      N  H  D  A  N  F  S  S  R  P  P  N  S  G  A  K  Y  V  A  Y

 421  ATTACCATGACCTTGTTTTTGCACCGTACGGGCCAAGGTGGCGCATGCTTAGGAAGATCA  480
      N  Y  H  D  L  V  F  A  P  Y  G  P  R  W  R  M  L  R  K  I

 481  GTTCCGTCCACCTCTTCTCCAACAAGGCATTGGATGACTTTAGGCACATTCGAGAGGCAG  540
      S  S  V  H  L  F  S  N  K  A  L  D  D  F  R  H  I  R  E  A

 541  AGTTGGCAGTGCTGACACAAACACTAGCAAGTGCGGGCAAAGCACCTGTAAACTTGGGGC  600
      E  L  A  V  L  T  Q  T  L  A  S  A  G  K  A  P  V  N  L  G

 601  AACTACTAAATGTGTGCACCACCAACGCCCTAGGCCGGGTAATGGTGGGGAGGAGGGTAT  660
      Q  L  L  N  V  C  T  T  N  A  L  G  R  V  M  V  G  R  R  V

 661  TCAACGACGGCGTTGATCCAAAGGCAAGTGATTTCAAGGACATGACATTGGAGCTAATGC  720
      F  N  D  G  V  D  P  K  A  S  D  F  K  D  M  T  L  E  L  M

 721  AATTGGCGGGTGTGTTTAACATTGGTGATTTTGTTCCTGCATTGGAGTGGCTGGACTTAC  780
      Q  L  A  G  V  F  N  I  G  D  F  V  P  A  L  E  W  L  D  L

 781  AAGGAGTGGCATCTAAAATGAAAAGGCTACACAAGAGGTTTGATGATTTTTTGACTACCA  840
      Q  G  V  A  S  K  M  K  R  L  H  K  R  F  D  D  F  L  T  T

 841  TCGTGGAAGAGCACAGGAACGGGGGTCAAGAAAAGCATGTGGACTTGTTGAGCACGTTGA  900
      I  V  E  E  H  R  N  G  G  Q  E  K  H  V  D  L  L  S  T  L

 901  TTTCGTTAAAAGATAATGCTGATGGTGACGGTGGAAAGCTCACAGACACCGAAATTAAAG  960
      I  S  L  K  D  N  A  D  G  D  G  G  K  L  T  D  T  E  I  K

 961  CATTGCTTTTGAATTTTTTTACTGCTGGGACCGACACATCATCAAGCACAGTGGAATGGG  1020
      A  L  L  L  N  F  F  T  A  G  T  D  T  S  S  S  T  V  E  W

1021  CTATTGCAGAACTTCTTAGGCATCCAAAAATCTTGACCCAGGTCCAAAGAGAGCTGGATT  1080
      A  I  A  E  L  L  R  H  P  K  I  L  T  Q  V  Q  R  E  L  D

1081  CTGTTGTGGGCCGAGATCGTCTCGTTAGCGACTTGGACCTACCCCAACTCACTTACCTTA  1140
      S  V  V  G  R  D  R  L  V  S  D  L  D  L  P  Q  L  T  Y  L

1141  GTGCTGTTATTAAAGAGACATTTCGGCTCCACCCATCGACGCCCCTATCACTGCCTCGAA  1200
      S  A  V  I  K  E  T  F  R  L  H  P  S  T  P  L  S  L  P  R
```

Figure 35 Nucleotide sequence of the gene encoding F3'H from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

```
1201  TGGCGGCTGAGAGTTGCGAAATCGACGGCTATCATATTCCGAAAGGTGCAACCCTTTTGG  1260
      M  A  A  E  S  C  E  I  D  G  Y  H  I  P  K  G  A  T  L  L

1261  TTAATGTATGGGCCATAGCACGTGACCCAGATGTGTGGGCCGAGCCTTTGGTGTTCATGC  1320
      V  N  V  W  A  I  A  R  D  P  D  V  W  A  E  P  L  V  F  M

1321  CCGAAAGGTTTCTACCTGGTGGAGAAAAGGCCAAAGTTGATGTGAGGGGCAATGACTTTG  1380
      P  E  R  F  L  P  G  G  E  K  A  K  V  D  V  R  G  N  D  F

1381  AGCTTATTCCATTCGGTGGTGGTAGGAGAATTTGTGCCGGTTTGAGTTACGGATTGCGTG  1440
      E  L  I  P  F  G  G  G  R  R  I  C  A  G  L  S  Y  G  L  R

1441  TAGTTTATTTAATGGCTGCCACGTTACTCCATGCATTTGATTGGGAACTGGCCAATGGAT  1500
      V  V  Y  L  M  A  A  T  L  L  H  A  F  D  W  E  L  A  N  G

1501  TGATTCCTGAAAAGTTAAACATGGATGAAGCCTATGGATTGACCCTTCAGCGAGCTGCTC  1560
      L  I  P  E  K  L  N  M  D  E  A  Y  G  L  T  L  Q  R  A  A

1561  CCTTAATGGTGCACCCTAAGCCAAGGTTAAGCCCTCAGGCTTACAAAGCAAAAAATTGAA  1620
      P  L  M  V  H  P  K  P  R  L  S  P  Q  A  Y  K  A  K  N

1621  GAAATACATGTGATAAGCATTGTCTTTATTTAAGCTTATGAATTACGTTAATTAATAAAT  1680

1681  GTCTTTTATTTCAAAAAAAAAAAAAAAAAAA  1711
```

Figure 35 (Continued).

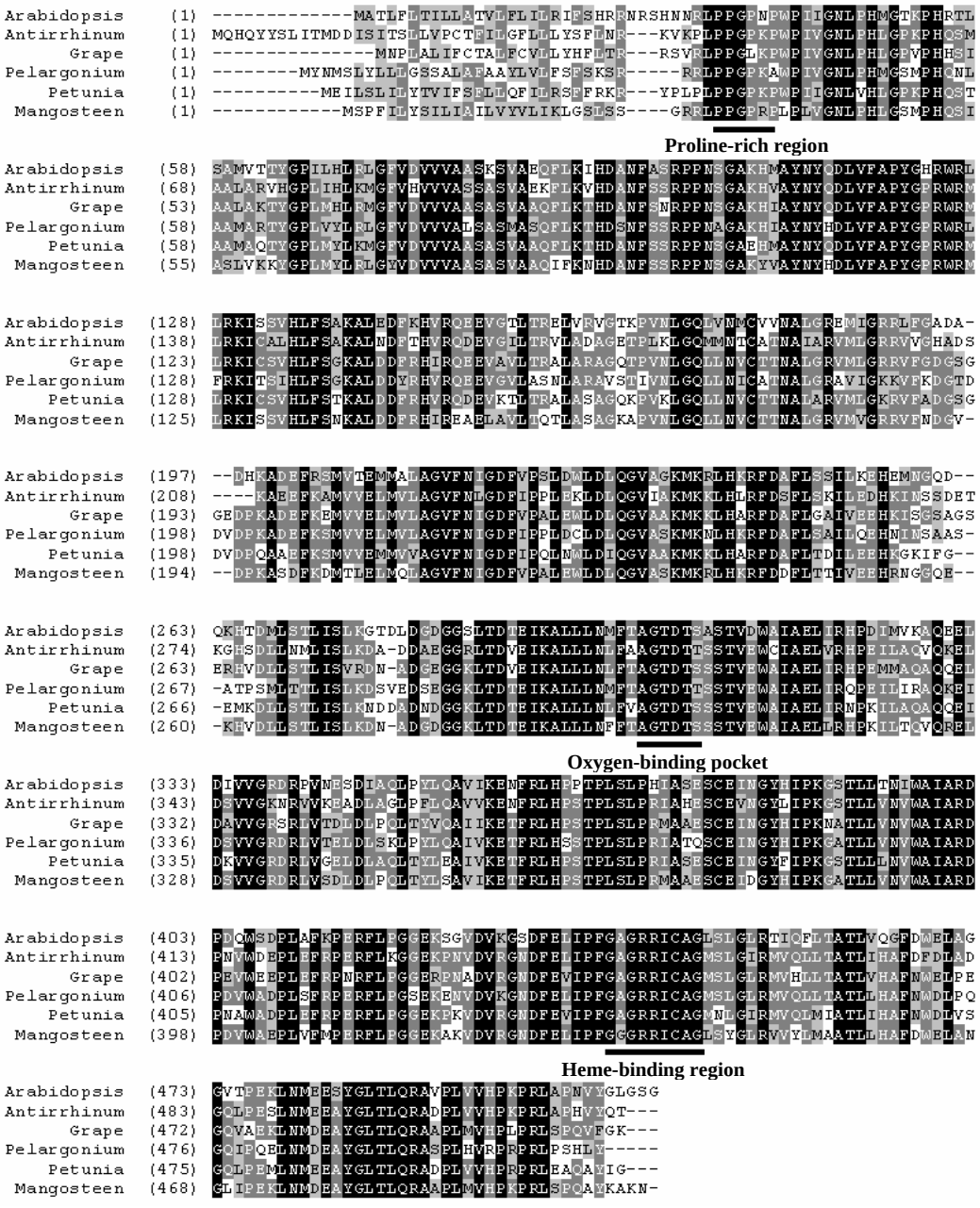


Figure 36 Protein sequence alignment of mangosteen F3'H with other F3'H proteins. The proline-rich region, oxygen-binding pocket and heme-binding region are underlined. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; *Arabidopsis*, AAG16746; antirrhinum, ABB53383; grape, CAI54278; pelargonium AAG49315; petunia AAD56282; mangosteen, ACM62746.

```
   1  GCTCTCTGCCTAGCTGTATTTCTTGTTGACCATATTTTGTCACCCAATTATTCATCTTTA  60

  61  AAAATTCATGGGTTCCCAAAATGAAATTGTATGTGTCACCGGGGCATCAGGGTTCATCGG  120
             M  G  S  Q  N  E  I  V  C  V  T  G  A  S  G  F  I  G

 121  GTCATGGCTCGTCATGAGACTTCTTGAAAGGGGGTATACGGTTAGAGCCACTGTCCGTGA  180
       S  W  L  V  M  R  L  L  E  R  G  Y  T  V  R  A  T  V  R  D

 181  TCCTGATAACGCAAAGAAGGTGCAACATTTGTTGGAGTTACCTAAAGCCAAGACGCACTT  240
       P  D  N  A  K  K  V  Q  H  L  L  E  L  P  K  A  K  T  H  L

 241  GACACTGTGGAAAGCTGAACTTGGAATTGAAGGAAGCTTTGATGAAGCGATTCAAGGGTG  300
       T  L  W  K  A  E  L  G  I  E  G  S  F  D  E  A  I  Q  G  C

 301  CTCCGGTGTGTTCCATGTTGCCACCCCTATGGACTTTGAGTCCAAGGACCCGGAGAATGA  360
       S  G  V  F  H  V  A  T  P  M  D  F  E  S  K  D  P  E  N  E

 361  AGTGATAAAGCCAACTATTGATGGGATGATTGACATATTGAAATCATGTGCCAAAGCCAA  420
       V  I  K  P  T  I  D  G  M  I  D  I  L  K  S  C  A  K  A  K

 421  GGTGCGTAGGATAGTGTTCACTGCGTCTGCTGGTGCATTGGACGTGGAAGAGCATCGGAG  480
       V  R  R  I  V  F  T  A  S  A  G  A  L  D  V  E  E  H  R  R

 481  GCCTGTCTATGATGAGAATTGTTGGAGTGACTTGGAATTTATCAACTCCGTCAAAATGAC  540
       P  V  Y  D  E  N  C  W  S  D  L  E  F  I  N  S  V  K  M  T

 541  AGGATGGATGTATTTCGTCTCCAAGACAAAGGCGGAGAGAGCAGCATGGAAGTTTGCCAA  600
       G  W  M  Y  F  V  S  K  T  K  A  E  R  A  A  W  K  F  A  K

 601  AGAGAACAACCTTGATTTCATTAGTATAATCCCATCTCTTGTTGTTGGTCCTTTCATCAT  660
       E  N  N  L  D  F  I  S  I  I  P  S  L  V  V  G  P  F  I  M

 661  GCAATCAATGCCACCTAGCCTTATCAGTGCCCTCGCTCTAATTACTGGAAATGAAGGTCA  720
       Q  S  M  P  P  S  L  I  S  A  L  A  L  I  T  G  N  E  G  H

 721  CTACACAATTCTGAAGCAAGGCCACTACGTGCACTTGGATGACCTAGTCGAGTCACATAT  780
       Y  T  I  L  K  Q  G  H  Y  V  H  L  D  D  L  V  E  S  H  I

 781  TTACCTGTACGAGAATCCAAAGGCAGAGGGAAGGTACATTTGCTCTAATTACGACGTCAA  840
       Y  L  Y  E  N  P  K  A  E  G  R  Y  I  C  S  N  Y  D  V  N

 841  CATTTTTGAACTTGCCAATATGCTCAACAAGAAGTATCCAGAGTACAACATCCCCACCAC  900
       I  F  E  L  A  N  M  L  N  K  K  Y  P  E  Y  N  I  P  T  T

 901  GTTCAAGGGGATTGAGGAGAACTTGCCAAGTGTGATTTTCTCTTCCAAGAAATTGTTGGA  960
       F  K  G  I  E  E  N  L  P  S  V  I  F  S  S  K  K  L  L  D

 961  CCATGGATTTGAATTCAAGTACACCCTGGATGACATGTTTCAGGGAGCCGTTGAAACCTG 1020
       H  G  F  E  F  K  Y  T  L  D  D  M  F  Q  G  A  V  E  T  C

1021  TCGGAAAAAGGGATTGATTCCACTTTCTCATTTTAATAATGATGCAAAATAAATGGATGG 1080
       R  K  K  G  L  I  P  L  S  H  F  N  N  D  A  K

1081  AGTACAAAACATCGCAAGCATAAATATTTGTTTCGATGTTTTATCCTTGGAGGATGTGAC 1140

1141  CAGTGAGACAGTCAGTAATAAGTAACATTGTTTATGCCAGATTTTATAAAATAAAAATGT 1200

1201   CCACGGTACTTCCTTGGATGAAAAAAAAA  1229
```

Figure 37 Nucleotide sequence of the gene encoding DFR from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

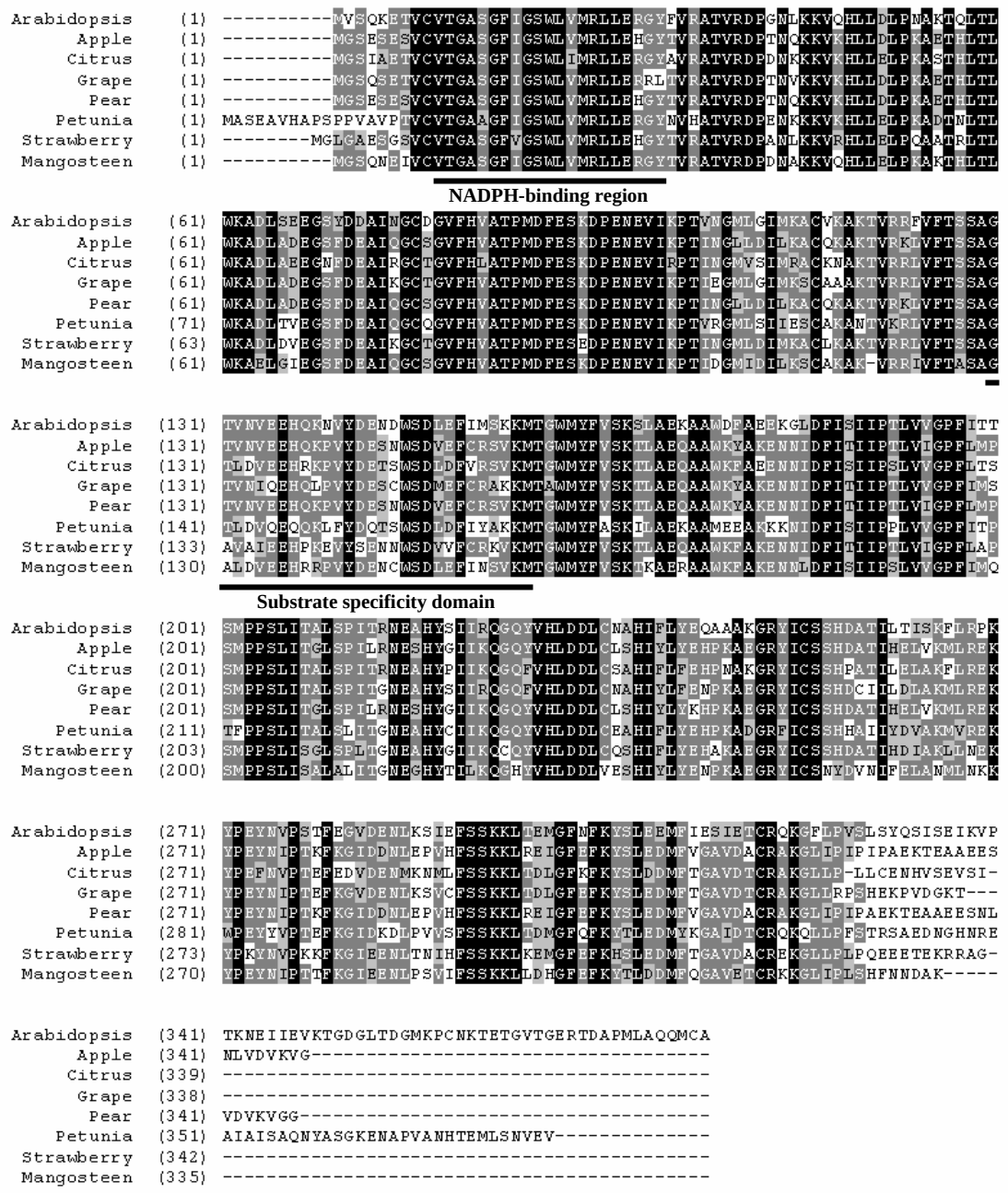


Figure 38 Protein sequence alignment of mangosteen DFR with other DFR proteins. The putative NADP-binding domain and substrate specificity domain are underlined. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; *Arabidopsis*, AAA32783; apple, AAD26204; citrus, AAS00611; grape, CAA53578; pear, AAO39819; petunia, CAA56160; strawberry, AAC25960; mangosteen, ACM62744.

```
   1  AAAAGAAGGCCAAGATGGTGACCTCAGTGGCTCCAAGAGTAGAGACATTAGCAAGCAGCG   60
                 M  V  T  S  V  A  P  R  V  E  T  L  A  S  S

  61  GGATCCAATGCATCCCAAAAGAGTACATCCGCCCACAAGAGGAGCTAACCAACTTAGGAA  120
      G  I  Q  C  I  P  K  E  Y  I  R  P  Q  E  E  L  T  N  L  G

 121  ACATCTTTGAGCAAGAGAAGAAAGAAGGCCCCCAGGTGCCAACCATTGATTTAGAAGGCA  180
      N  I  F  E  Q  E  K  K  E  G  P  Q  V  P  T  I  D  L  E  G

 181  TAGTTTCTGAAGACAAGGAAGTGAGGGACAAATGTTGGGATGAACTAATGAAGGCTGCCA  240
      I  V  S  E  D  K  E  V  R  D  K  C  W  D  E  L  M  K  A  A

 241  AGGAATGGGGGGTAATGCACTTGGTTAACCATGGAATTTCCAATGAACTCACTGAGAAGG  300
      K  E  W  G  V  M  H  L  V  N  H  G  I  S  N  E  L  T  E  K

 301  TGAAGATTGCTGGAGAGGCTTTCTTTCAACTTCCCATAGAGGAGAAGGAGAAGTATGCTA  360
      V  K  I  A  G  E  A  F  F  Q  L  P  I  E  E  K  E  K  Y  A

 361  ATGATCAAGGGTCTGGGATGATCCAAGGGTATGGAAGCAAGTTGGCTAATAATGCTAGTG  420
      N  D  Q  G  S  G  M  I  Q  G  Y  G  S  K  L  A  N  N  A  S

 421  GGCGGCTTGAGTGGGAGGATTACTTCTTTCACTTGGTGTTCCCTGAGGAGAAGAGGGACT  480
      G  R  L  E  W  E  D  Y  F  F  H  L  V  F  P  E  E  K  R  D

 481  TGTCCATTTGGCCTAAGACACCTAGTGACTATATTGAGGTAACCAGCGAGTACGCAAGGC  540
      L  S  I  W  P  K  T  P  S  D  Y  I  E  V  T  S  E  Y  A  R

 541  AACTGAGAGCTCTTGCAACAAAGGTCCTATCAGCACTATCACTGTGCTTGGGATTAGAAG  600
      Q  L  R  A  L  A  T  K  V  L  S  A  L  S  L  C  L  G  L  E

 601  AAGGAAGACTAGAGAAAGAAGTTGGAGGCATTGAAGAACTGGCCCTCCAAATGAAGATCA  660
      E  G  R  L  E  K  E  V  G  G  I  E  E  L  A  L  Q  M  K  I

 661  ACTACTACCCCAAGTGTCCTCAACCCGAGCTAGCCCTTGGTGTGGAGGCTCACACCGACG  720
      N  Y  Y  P  K  C  P  Q  P  E  L  A  L  G  V  E  A  H  T  D

 721  TGAGTGCACTAACCTTCATCCTCCACAACATGGTCCCTGGCCTCCAACTCTTCTACGAGG  780
      V  S  A  L  T  F  I  L  H  N  M  V  P  G  L  Q  L  F  Y  E

 781  GCGAATGGGTCACAGCCAAATGTGTCCCTAACTCAATTATCATGCACATTGGTGACACAT  840
      G  E  W  V  T  A  K  C  V  P  N  S  I  I  M  H  I  G  D  T

 841  TGGAGATTTTGAGCAATGGGAAGCTTAAGAGTATTCTTCATAGGGGAGTTGTTAATAAGG  900
      L  E  I  L  S  N  G  K  L  K  S  I  L  H  R  G  V  V  N  K

 901  AGAAAGTGAGGATTTCTTGGGCTGTTTTTTGTGAGCCTCCTAAAGATAAGATCATTCTTA  960
      E  K  V  R  I  S  W  A  V  F  C  E  P  P  K  D  K  I  I  L

 961  AGCCTTTGCCTGAGCTTGTGAGTGAGACTGAGCCACCTATGTTTCCGCCTCGCACGTTTT 1020
      K  P  L  P  E  L  V  S  E  T  E  P  P  M  F  P  P  R  T  F

1021  CACAGCATATTCAGCACAAGCTCTTCAGGAAGAACCAAGATGATGTTGGTCCCAACTGAT 1080
      S  Q  H  I  Q  H  K  L  F  R  K  N  Q  D  D  V  G  P  N

1081  TACTTGATGTTATTGTGTTTAAATAAAATGTGTGGTCTTATTATTGTTAACTGCATTTAA 1140

1141  TAAGTTCGTGACGTTCAACGTACTTTTATTAGTTAAGTAGTTTTATATTCATGTGTGACA 1200

1201  TTAATGGGTTCATTAAAAGAATATATCCAAAACTACTATGTAAGATGTAATGAAATGAGT 1260

1261  TTTACATGCTTTCCTTCAAAAAAAA 1285
```

Figure 39 Nucleotide sequence of the gene encoding LDOX from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

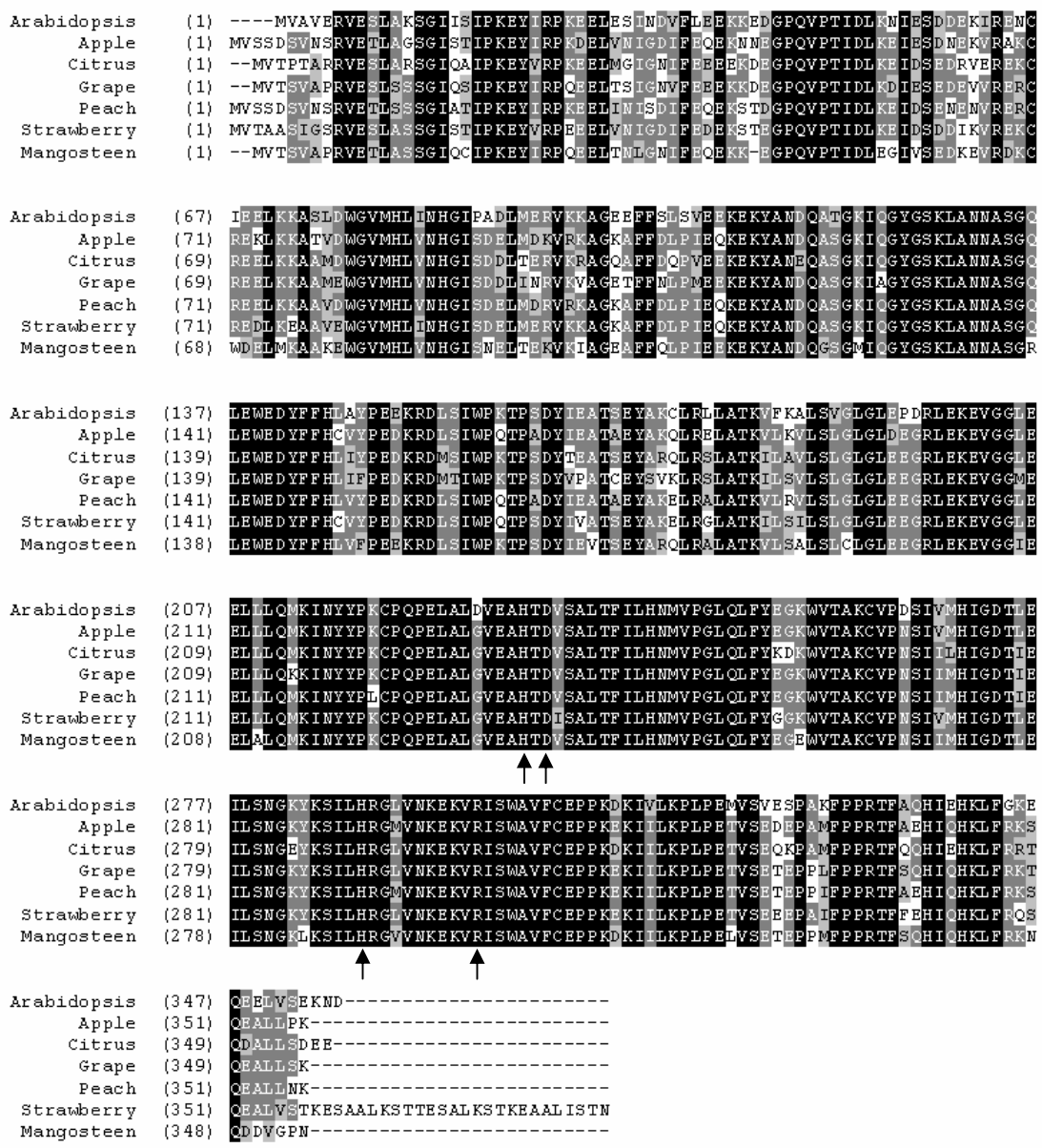


Figure 40 Protein sequence alignment of mangosteen LDOX with other LDOX proteins. Arrows indicate conserved His and Asp residues as required for ferrous-iron coordination, and Arg residue of putative binding site of 2-oxoglutarate. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; *Arabidopsis*, CAD91994; apple, BAB92998; citrus, AAT02642; grape, BAC07545; peach, ABX89943; strawberry, AAU12368 ; mangosteen, ACM62747.

```
   1  ACTCTAATCACACACTCACTTCCAAGCTTCTGCTCTTTTCACAAAGCCAATGACCAAACC  60
                                                      M  T  K  P

  61  CACCACCGATGATCATCCCCACGTGGCAGTGCTAGCCTTCCCATTTGGAACACATGCAGC  120
        T  T  D  D  H  P  H  V  A  V  L  A  F  P  F  G  T  H  A  A

 121  CCCACTCCTCTCCATAACACACCACTTAGCCGCACTTTCCCCCTCCACTCACTTCTCCTT  180
        P  L  L  S  I  T  H  H  L  A  A  L  S  P  S  T  H  F  S  F

 181  CTTTGGCACCCCATCTTCCAACTCCTTCATCCTCTCGTCCAACACCAACTTGCCACCCAA  240
        F  G  T  P  S  S  N  S  F  I  L  S  S  N  T  N  L  P  P  N

 241  CGTCAAGCCCTACGACGTCTGGGATGGCACCCCTGATGGTTATGCCTATACAGGTGACGT  300
        V  K  P  Y  D  V  W  D  G  T  P  D  G  Y  A  Y  T  G  D  V

 301  ACAAGAGGAAATGGGGTTGTTTATAAGTGCGGCTCATGAAAGCTTTAGGAAGGGGGTGGA  360
        Q  E  E  M  G  L  F  I  S  A  A  H  E  S  F  R  K  G  V  D

 361  TAGGGCTGTGGAGGAGAGTGGAAGAAGGGTTAGTTGTTTGATGAGTGATGCGTTTTTTTG  420
        R  A  V  E  E  S  G  R  R  V  S  C  L  M  S  D  A  F  F  W

 421  GTTTGGGAAGGAGATGGCTGAGGAGATTGGTGGTGGTGTTATGTGGGTACCCTTTTGGAC  480
        F  G  K  E  M  A  E  E  I  G  G  G  V  M  W  V  P  F  W  T

 481  TGCTGGCCCTCATGCGCTTTCTAGTCATCTTTATACTGATTTTATCAGGGAGAGTTTTGC  540
        A  G  P  H  A  L  S  S  H  L  Y  T  D  F  I  R  E  S  F  A

 541  TGGAGATGTGACGCAGCGTGAAGATGAGCTACTAAGCTCAATCCCAGGAATGTCTAGAGT  600
        G  D  V  T  Q  R  E  D  E  L  L  S  S  I  P  G  M  S  R  V

 601  GCGAGTTTGTGATTTGCCTGAAGGAGTGGTCTTTGGAAGATTGGATTCACTCTTCTCTCA  660
        R  V  C  D  L  P  E  G  V  V  F  G  R  L  D  S  L  F  S  Q

 661  AATGCTACACAAAATGGGACAAGCGTTACCTAAAGCGGATGCGGTCTTCATAAACTCATT  720
        M  L  H  K  M  G  Q  A  L  P  K  A  D  A  V  F  I  N  S  F

 721  TGAAGAACTGGACCCGACGTTTACAAACGACCTCAAGTCCAAGCTCAAATGCTGTCTCAA  780
        E  E  L  D  P  T  F  T  N  D  L  K  S  K  L  K  C  C  L  N

 781  CATTGGACCATTCAACTTGATCTCGCCACCGGCACAAGTACCAGATACATATGGCTGCAT  840
        I  G  P  F  N  L  I  S  P  P  A  Q  V  P  D  T  Y  G  C  I

 841  ACCCTGGCTTGACAAGCAACAATTAGCCTCCGTGGCTTATGTAAGTTTTGGATCGGCAAC  900
        P  W  L  D  K  Q  Q  L  A  S  V  A  Y  V  S  F  G  S  A  T

 901  GATACCACTGCCTCATGAGCTCGTGGCACTGGCCGAGGCCTTGGAGGATAGGAAGGTTCC  960
        I  P  L  P  H  E  L  V  A  L  A  E  A  L  E  D  R  K  V  P

 961  TTTCATATGGTCACTAAAGGACAACGCAAAAGTACATTTGCCAGATGGGTTCTTGGAGAC  1020
        F  I  W  S  L  K  D  N  A  K  V  H  L  P  D  G  F  L  E  T

1021  GACAAAGTTTCAAGGGATTGTGATACCTTGGGCTCCCCAAGCAAAGGTTCTAGGACATAA  1080
        T  K  F  Q  G  I  V  I  P  W  A  P  Q  A  K  V  L  G  H  K

1081  AGCAGTTGGGGTGTTTATTACCCACTGTGGGTGGAACTCACTTTTAGAAACTATAGTTGG  1140
        A  V  G  V  F  I  T  H  C  G  W  N  S  L  L  E  T  I  V  G

1141  AGGGGTGCCCGTGATCTGTAGGCCTTTTTATGGTGATCAAAGACTTAATGCGAGAATGAT  1200
        G  V  P  V  I  C  R  P  F  Y  G  D  Q  R  L  N  A  R  M  I
```

Figure 41 Nucleotide sequence of the gene encoding UFGT from mangosteen with its deduced amino acid sequence. The underlined letters indicate the translation start site (ATG).

```
1201  AGGGGACGTTTGGAAAATTGGTGTTATTGTTAACGGTGGAGTTCTTGCAAAGGAGGCAAT  1260
        G  D  V  W  K  I  G  V  I  V  N  G  G  V  L  A  K  E  A  M

1261  GATTGATTGCTTTGATAAGATACTGTTGCAAGAGGATGGGAAACAGATGAGGGGAAGAAT  1320
        I  D  C  F  D  K  I  L  L  Q  E  D  G  K  Q  M  R  G  R  I

1321  AAAATCCCTAAAAGATCTTGCACTTGCGGCTACTGCTTACAAAGGAAGCTCTAGCGACAA  1380
        K  S  L  K  D  L  A  L  A  A  T  A  Y  K  G  S  S  S  D  N

1381  TATGAGAGAATTGTCTCGGCTAGTATCGAGCCCCTGCAAGTAATAACTGCTGAGAGCCGA  1440
        M  R  E  L  S  R  L  V  S  S  P  C  K

1441  ATCCTTCGTTTTTCTGTGGTGCATGTAGGTTTATCATGTCAAATGATCAGAACATTTCAC  1500

1501  TTCAAATAACAACAAAGACACGGTGCAAAATAACGTAGGCTTTCCAAAGTAGCACATGAA  1560

1561  CCCAAGCTTGCTCTTTAAAATAATGGCCATTGAGTTTTGAATGAACATTAGGCCATATTC  1620

1621  ACCTATTTCAACTTCCATCTAATTTAATTATTCAACCGTTTTTTCTTTAGATCAAAAAAA  1680
```

Figure 41 (Continued).

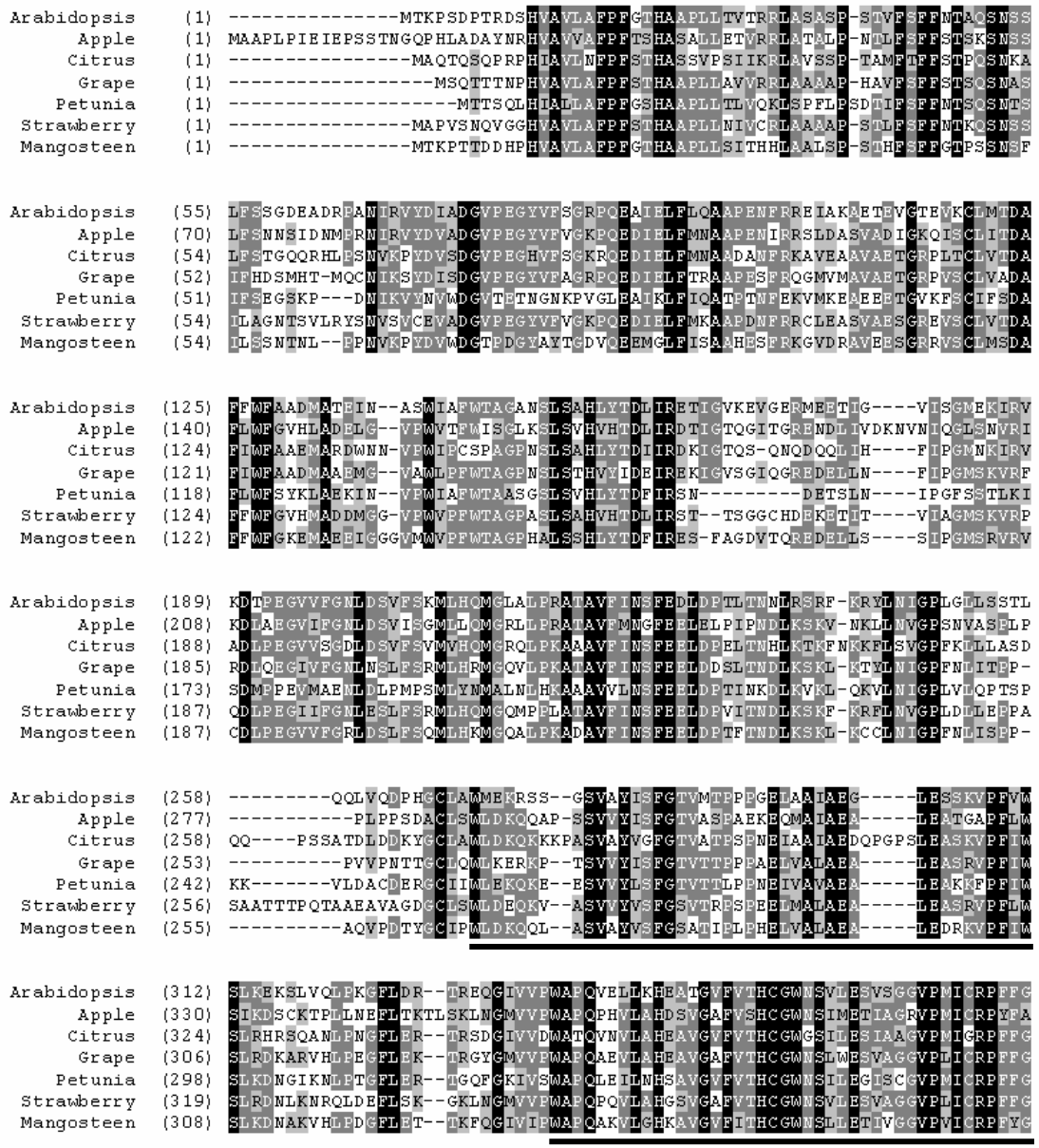


Figure 42 Protein sequence alignment of mangosteen UFGT with other UFGT proteins. The underline indicates the common motif found in glycosyltransferases. Identical residues are shown in black, conserved residues in dark grey and similar residues in light grey. The accession numbers of these proteins in the GenBank database are as follows; *Arabidopsis*, NP_197207; apple, AAD26203; citrus, AAS00612; grape, BAB41021; petunia BAA89008; strawberry, AAU09442; mangosteen, ACM62748.

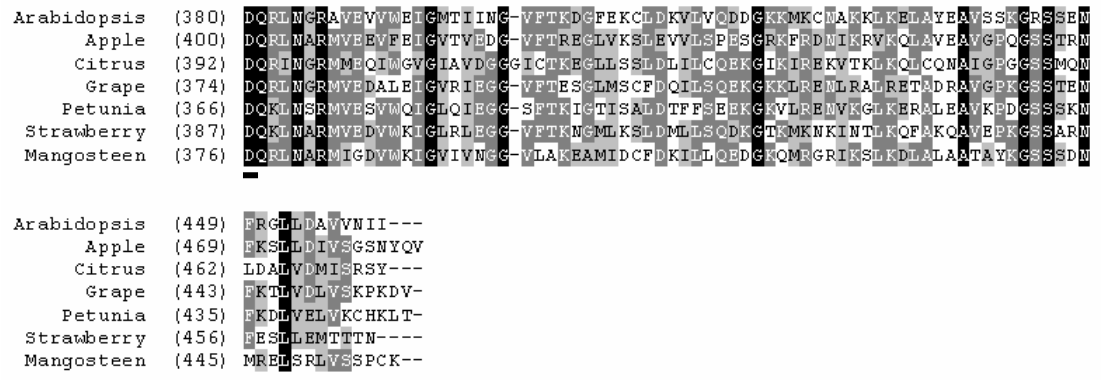


Figure 42 (Continued).

4.2 Promoter isolation and analysis of *GmDFR*, *GmLDOX* and *GmUFGT* genes

PCR-based DNA walking of seven libaries (*Dra*I, *Eco*RV, *Hpa*I, *Msc*I, *Sca*I, *Ssp*I and *Stu*I) was used to isolate 5'-flanking regions of *GmDFR* (FJ197138), *GmLDOX* (FJ197139) and *GmUFGT* (FJ197140) of sizes 1,973, 513 and 1,507 bp, respectively (Figure 43-45). The regulatory regions of all sequences were analyzed by PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html) (Appendix Table 13-15) and confirmed the result using PlantCARE database (Appendix Table 16-18) (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). These showed several putative *cis*-elements for regulatory motifs involved in plant development. The ethylene responsive element (E4, AWTTCAAA) was found in the *GmDFR* promoter (+430 and -1311) (Figure 43). A motif similar to an ethylene response element (ATTTNAAA) was also found in the *GmDFR* (-728 and -399), *GmLDOX* (-346) and *GmUFGT* promoter (-829) (Figure 43-45) that is similar to the UFGT promoter of grape (El-Kereamy *et al*., 2003; Tira-Umphon *et al*., 2007). Several MYB binding sites, such as MYBPLANT (MACCWAMC) and MYBCORE (CNGTTR), were presented in all promoters (Appendix Table 13-18).

```
   1  ATCCACATGGTCATTCCTTTCTAGCTCAAACAGTTCACGTGTTAGTTCGATTGAGGATGC  60
  61  CCAGTTAGTTTGTATGGCCTATGTGGCATGTTTTATTTCAGATACTTAGTTGCAAAGTGA  120
 121  CTATAAGGCTCGTATTGACCAACTAGATCACTTTGCTTCATAAACAATTCATAACTGAGG  180
 181  TAAATGAATTTGGTCTAGGGCATTCACCTCAGTAAGGCCCATTTCCTTGTTATCCAAGTC  240
 241  AAAATAAACCCTTTCGCATCTCTGAATCGAGATGAAACACTCTTATCAAGTTGTGTATGG  300
 301  TACTAAACTGCACGAGCTCACCTCGTTCAGTTTCACCTACCATTCATAATTCTTCGGATG  360
 361  TTAAACACAAATTCCAAGACCTTGTAATCATAAGCATAATTTGTACTAGCATATATTACC  420
 421  TCATTTCAAGAACATAAAGGCTAGACAGGTTAAAGTAAACAGTTAATAGTCTCCAAGCAA  480
 481  GCCAATCAGATGACTCCAATTGGCCACCATCATCAGCTCGGTAGAATTCTCAAGGACTCT  540
 541  TAACATGAAAATTTGTCACATCAATCAATTTTCAACAACCACCATCAATTCTTGTTCACT  600
 601  ATAAATAGCAAGGAATCTTCTTCAATAATTCATTCATGCAAGTCCATAAGCTCAAATTCA  660
 661  AAACTTAATGAAGGCAAACTCTTTGACCCTTATTCCTCTTTCATTTACCACTTTGGTAAT  720
 721  TACTAAAAGCTAAGCTCTGAGCTCGCACTTCTTTCAAAAACAGAAAACTAATTTGACTTT  780
 781  CAGAGAGTTTCTAAAGACAAATCTTTTGAAACCTTTTAGTAACGTATGCTCATCTATTTG  840
 841  AAGTGTCCAAACAGACTTGGCAGGGACTCTTTTGCCTCTCTTTTTTTTTTTTGAGAACGT  900
 901  ATTTCGTGTACAATTGTAACTCTACAAAATCTTATAACATCAAATATCAATACAAACATA  960
 961  AATTAATTAAATGTTAACACCACCCAAATTTAAACCTTTTTTTATTATAGTATAGATACG  1020
1021  TATGTTACTATGATGAAGTTATTTGTATCGTTTTTGTCATTATTAATTTGCCTATGTTTT  1080
1081  ATCATGTAGAATTAGCGAAATAAAAGGATTCATAAATGAATTATTTGTCATGAAAAAGTC  1140
1141  GATTAAAAAAAAAATCTATTTAATCTTTTTTTTTTTTCCAAATATAATTATATACATAATAA  1200
1201  ACATGTCATTATTAAATCAATGTGGAAACAATAGGAGATTTGAAAATGGGTCATAGTCTT  1260
1261  TCGTATATTAGAAAAAATCTCTTAGATATATGGGTAATTTAGAGAGAATTTTCGTTTAAT  1320
1321  GTATTTGATCATCACCATAGTAAATCTTCTGGAAAATTTATATAATTCTTTCAAAATTAA  1380
1381  TCATGTATAAGTTTAATTTTAAGCAAGATAATAAATATTACACATTGACCACCCCTAACT  1440
1441  GAAAAATATCGCCACTAACGACCCCTAACTAACAAGTATCGCTATTGCTTTGAGGCTTGT  1500
1501  TCATTTCTAATGTTGTTTATAAACACAAATTATTGTTTGAAATTGAGTAGTGGTTAATGA  1560
1561  CTATAAATTTAAAACATTGTCTATATTTGGCCATGTATTTTATAAAAATTTTAAGAAAAT  1620
1621  ATTTGTGTTTACAATCAAAGTCAAAAGGAACAATGCCATAGTATGATTTAAATTCTCTCT  1680
```

Figure 43 Nucleotide sequence of *GmDFR* 5' flanking region. The basal promoter elements (TATA and CAAT boxes) are highlighted in gray. The underline letters indicate ethylene responsive element (E4, AWTTCAAA and ATTTNAAA) and bold letters indicate the translation start site (ATG).

```
1681  AAATTATAATGAACAGTAGTAATGTAGAAGCAGCCCATTGGCGGTTGCTGTAACTGAAAA  1740
1741  TTTGGAAGCTCCATCTACTTTCCACGAGTCGTCTGTTAAGTAAACACGTGATGGGCCATA  1800
1801  ACGTCAAAGCCCAAGATAGACTGAACGCTGTAACATACTTCTTGCGCCACTCATATAATA  1860
1861  TATAAAATGGTCAAGTTGCAGACGTTATTAGTCTCAAGTTCATTTGGCTCTCTGCCTAGC  1920
1921  TGTATTTCTTGTTGACCATATTTTGTCACCCAATTATTCATCTTTAAAAATTCATG  1976
```

Figure 43 (Continued).

```
  1  ATTCCTCTCAATGTGCACTTTCATATGACAGGCGTCTTGGCTATATTTTTTCTTATAACG  60
 61  AAAAAGATATTAGTAAAATACCGAAATTATCTCTAAAAATCAAAAAAATTATGAAATTAG  120
121  TATTTTTTTGTCTTTTAGTGGGTACTATAGCACTTTAAAATTTTAAAGTTCTTTTTTATC  180
181  TGAAAACCAAACGGACCCTTAGTAATGGTTAGATGAGATCGAGCCAACGTGCAACTAATT  240
241  GTCAACGCTTAATTTGATCCTTAACCTTAGAAACAACCAGGCCTACTCGAGTCTGGTGAA  300
301  GTCACCCATCACGTGTAAATCTGACCCCCCAACCTTCCCATCATAAGTAGACCCTCACCT  360
361  TCCTCTAGAAACTCGTTGGAGTTAAACCACTGAGCACTACGCTTATCATCTTCCCTATAA  420
421  AAGCGCGACAGAACATTAACCTTCCAACCATCATCTTCCATTAAATATACCACCCACCCC  480
481  CCTCCCACCCCCAAAAAAAAAAAGAAGGCCAAGATG  516
```

Figure 44 Nucleotide sequence of *GmLDOX* 5' flanking region. The basal promoter elements (TATA and CAAT boxes) are highlighted in gray. The underline letters indicate ethylene responsive element (ATTTNAAA) and bold letters indicate the translation start site (ATG).

```
   1  CCATCACCATAAACCACAAAAAGCTCGTTTTGCTAAAAAATAGCTCGATTACCGAGCTAA  60
  61  ACCAAGAAAACTAAGGCTAAAATCAAGATAAACCGAGTTAAAACTGAGCTAAACCCAACT  120
 121  AAAACTGAGCTAAACTGAGCTAGAAGGCCACCAACCGATCTCTCAATGAAAACTAAGCTA  180
 181  AGTTAGCTCAGCTCGTCCAAGCGTGCTGACACTCACAGCTTCTAAAGCCATTAAATGTAG  240
 241  CATTAAATGACCTGAACCTTACAAGGTCATAGGTAGTCAACATCAGCATTAAATAGCTAC  300
 301  AAGGTCAACAAATGAGAGGCCCCCACTTGGCTCAGCAATTTAGCTCGACACAAGTCCTAA  360
 361  GGTTCCTAAATTTGATGCAAGTCACATCAATCAAGTCTCACCAACTTTTACGAACTAGGC  420
 421  CTCAAGTATAAATACCCACAAATCCTTCCAATTTGAGGATCCTAATACTCCATAGTACTC  480
 481  AAAGCTCTTATTTAGCTTGGTATACACATTCTAACTCATTTGCTCACCTTTAACCTTTCA  540
 541  CACATTGCCTTATTAGCTCGGCTAACCTAACATTTAGTTCGGTTGGACCTCAAAACAGCT  600
 601  TAAAACTAGCTTAACACACCTTGATTTAGCTCGGCAACATCCATTTGTTGCCCTCCGAGC  660
 661  TCTTTTATATATTTTAAACACCTCAAATACTCTATTAACTTGATCGTCGGAAGATCTTCA  720
 721  AAGACCAAAACCTTTGAAGGCTTCTTAGTGATGTTTGCTCATTGTTGCAAGCTTAGAGCA  780
 781  GCTTCTAGCCAGTTTCTTTACTCGACTTCCATCTTGGAGAACGTGCCCAGAACATAGTTG  840
 841  TCACCCTGTGCAATTTGGTAGCATCATAACTATTATTTATAAGGTAAGTTCTAGGTTCTT  900
 901  GAGTAAAAACTCTCCCACCCTGAAATTGTTCACTCCTATTTCATATGTAGACTTTACCAA  960
 961  TAAAATAGATAAGGTGTGAATCACGTTAAGAAACAACGTGGCTAGTCAAATTGTGGTACA  1020
1021  CAATCTTATTATGTGGTTTTTTTTTTTTTTTTTTGTTTCCCCCCTTATTTGTAATAGTT  1080
1081  ATGACTTTACAGATCAAACAGATGCATTTCCTCCTCCTCATTGTAATGTATTAGCGGATT  1140
1141  GATATCAACCATAATCAGAATGCAAGAGCCCACAATACTTCACAAGCGGGGGTTGCCACA  1200
1201  TGCTTCCTACTCCAATTTGGACTTTTCATCAACCAAAATCCAGCTTCACGACTTTTGACT  1260
1261  GGTAAAATGAGTGGCTCCAACCCCCGACTTTTTATTTTAGTCATTTTTGACTTCACATCA  1320
1321  TATAACAGGTCAATAAAAATTTATGACTTACGAGTCCTCCGTAATCTTTTATTTGTCAGA  1380
1381  ATTTGACAAATTTTTATTACCACTCAACTCCTAAGCATACAATATATATTCCTCACACTT  1440
1441  GGCACTCAACTCACCACCACTCTAATCACACACTCACTTCCAAGCTTCTGCTCTTTTCAC  1500
1501  AAAGCCAATG  1510
```

Figure 45 Nucleotide sequence of *GmUFGT* 5' flanking region. The basal promoter elements (TATA and CAAT boxes) are highlighted in gray. The underline letters indicate ethylene responsive element (ATTTNAAA) and bold letters indicate the translation start site (ATG).

4.3 Functional testing of GmMYBs using transient tobacco assays

The function of the three mangosteen MYBs was investigated using the dual luciferase assay in *Nicotiana benthamiana* as previously reported (Hellens *et al*., 2005; Espley *et al*., 2007). The *GmDFR* promoter was fused to luciferase as was the previously tested *AtDFR* promoter from *Arabidopsis* (Zimmermann *et al*., 2004). All the *GmMYBs* and *AtPAP1* were driven by the cauliflower mosaic virus 35S promoter and were co-transformed with and without *AtbHLH2* construct. The LUC activity relative to REN was expressed as a ratio to show activation of DFR promoter by a transcription factor included in another plasmid (Figure 46). The activities showed similar patterns with both *AtDFR* and *GmDFR* promoters. The activities of the two promoter assays increased significantly when *GmMYB10* or *AtPAP1* was co-transformed with *Arabidopsis AtbHLH2* (Figure 46). The activity of GmMYB10 and AtPAP1 was higher than that of *GmMYB1* and *GmMYB7* (Figure 46). The results show that *GmMYB10* can activate the DFR biosynthesis gene promoter to a similar extent as *AtPAP1* which regulates *Arabidopsis* pigmentation (Zimmermann *et al*., 2004).

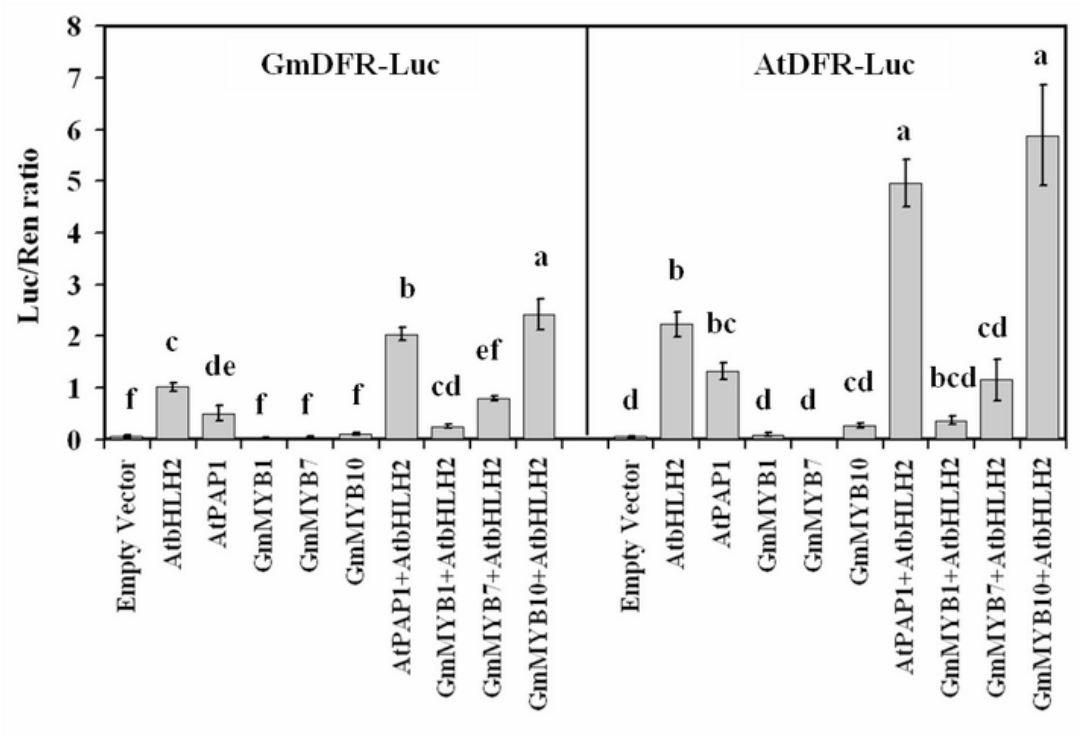


Figure 46 Transient activation of the mangosteen and *Arabidopsis* DFR promoter by *GmMYB*s, *AtPAP1*, and *AtbHLH2* transcription factors. All TFs were co-infiltrated with DFR-Luc promoter in transient tobacco transformation assays. The dual luciferase assay shows promoter activity expressed as a ratio of DFR promoter luciferase (LUC) to 35S Renilla (REN), where an increase in activity equates to an increase in LUC relative to REN. Data are mean $\pm$ SE of four replicate reactions. The bars with the same letters are not significantly different ($P > 0.05$) using DMRT.

4.4 Expression analysis of *GmMYB*s and anthocyanin biosynthetic genes during colour development of mangosteen fruit

4.4.1 Rapid colouration of mangosteen fruit correlates with changes in gene expression

The red colouration of mangosteen fruit was measured in 7 stages. The fruit can develop their colour both on tree and after harvest. Transcript levels of the isolated anthocyanin biosynthetic genes and MYB transcription factors were determined by real-time PCR during fruit colouration. Transcript levels of *GmMYB10* were confirmed by semi-quantitative RT-PCR. For most of the genes, transcript levels of the on-tree group were highest in the late colour development at stage 5 (Figure 47a-e, g-h) then declined at the final stage (Figure 47). All these genes showed large fold changes in comparison to the light green stage 0, with at least a 12-fold change for PAL. The transcript abundance of all genes in the postharvest fruit at stages 3-6 was higher than the on-tree fruit of the same colour stage (Figure 47, Appendix Table 19 and 20). The transcript levels of *GmUFGT* increased with the greatest fold-change among all the anthocyanin biosynthetic genes, being 571-fold and 763-fold at stage 5 of the on-tree and off-tree fruit, respectively (Figure 47).

The expression of GmMYB genes showed that the transcript levels of *GmMYBs* increased during fruit colouration and decreased thereafter (Figure 47i-k). *GmMYB10* showed the highest fold changes of the three MYB transcription factors, with more than a 500-fold change at stage 5 (off-tree) and decreased thereafter. The transcript level of *GmMYB10* was confirmed with RT-PCR result (Figure 48). This correlated with the highest ethylene production during colour development (Figure 9 and 10 in study 1). All genes showed high abundance with onset of fruit colour at stage 1 (Figure 47). *GmUFGT* and *GmMYB10* transcript levels both peaked at stage 5 and showed clear upregulation in highly pigmented stages.

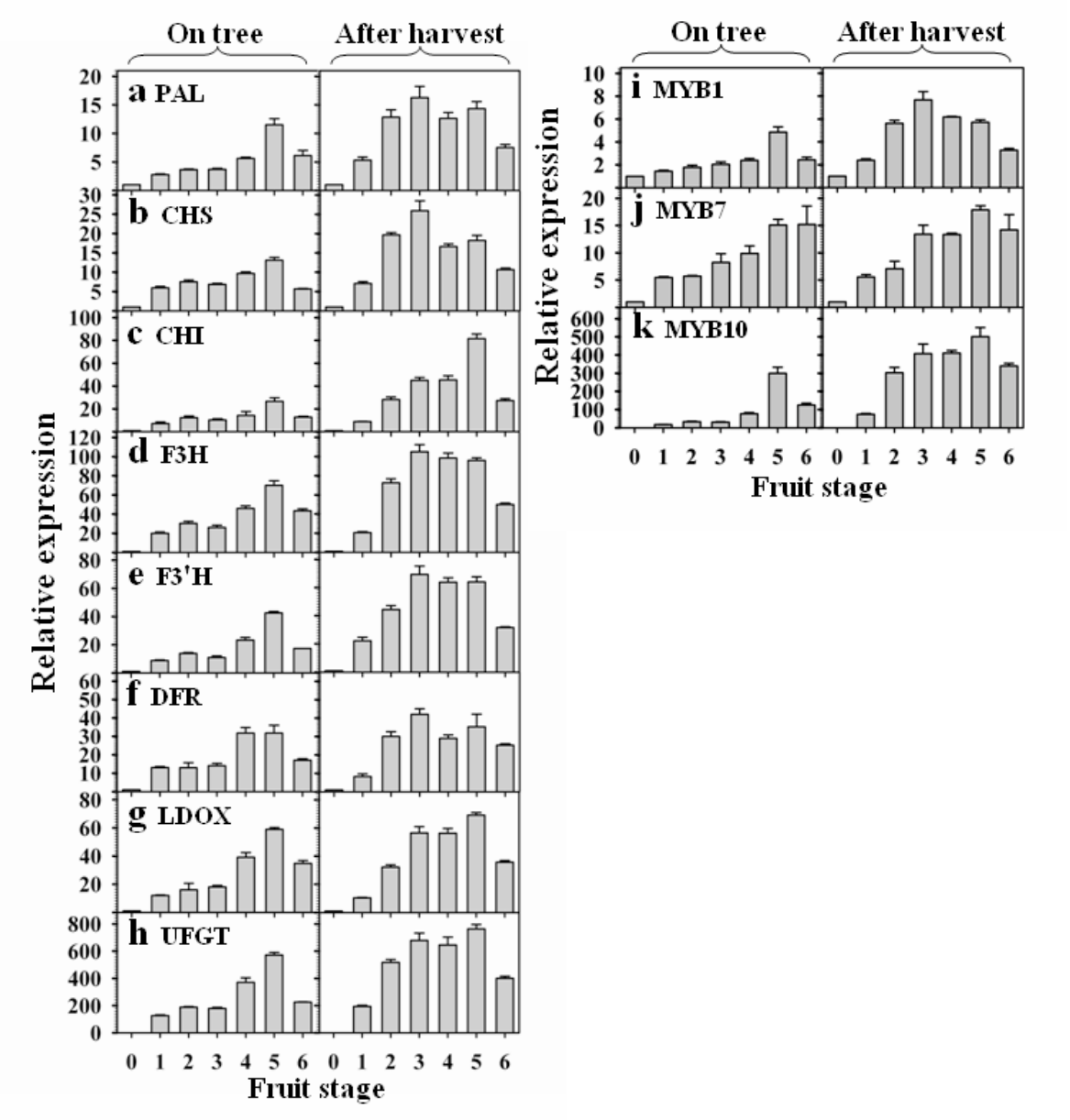


Figure 47 Expression profiling of mangosteen anthocyanin biosynthetic and MYB transcription factor genes during colour development. Real-time PCR was used to analyze *GmPAL* (a), *GmCHS* (b), *GmCHI* (c), *GmF3H*(d), *GmF3'H* (e) , *GmDFR* (f), *GmLDOX* (g), *GmUFGT* (h), *GmMYB1* (i), *GmMYB7* (j) and *GmMYB10* (k) expression patterns. Each column height indicates relative mRNA abundance of mature green fruit (stage 0) which was set to 1. All real time-PCR reactions were normalized using the Cp value corresponding to a mangosteen ELF gene. Data are mean ± SE of three replicate reactions.

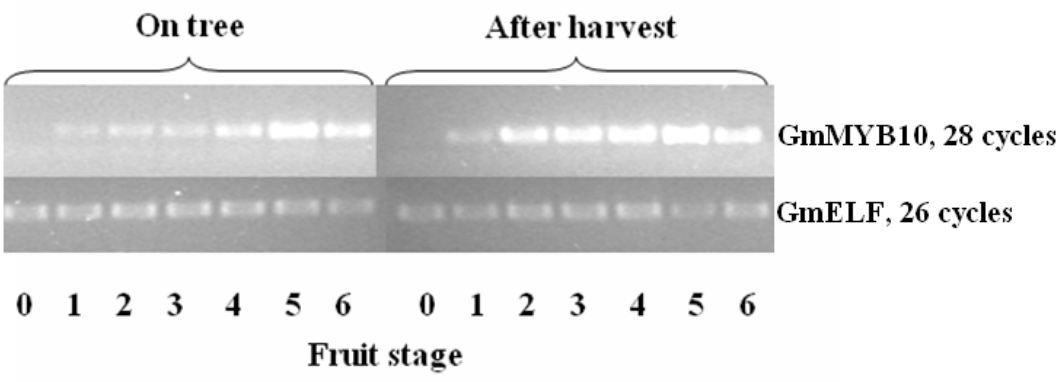


Figure 48 Semi-quantitative RT-PCR of MYB10 transcription factor genes during colour development.

4.4.2 Ethylene regulates red colouration of mangosteen fruit and correlates with *GmMYB* expression.

To study of the effect of ethylene on anthocyanin biosynthesis in mangosteen fruit, the fruit were treated with ethylene and 1-MCP. In fruit treated with air, ethylene and ethylene + 1-MCP (E+M), transcript levels of all anthocyanin biosynthetic genes and *GmMYBs* were similar, with a relatively high abundance within 1 day, whereas the transcript level of 1-MCP treated fruit was constant until day 3 (Figure 49-51). For most of biosynthetic genes, except *GmDFR*, transcript levels increased until day 7 and decreased thereafter (Figure 49). In contrast, 1-MCP inhibited significantly the increases in all anthocyanin biosynthetic genes and *GmMYB* transcript levels after being treated (day 1-3) (Figure 49-51, Appendix Table 21-31). The transcript levels of all genes were transiently down-regulated by 1-MCP application, especially E+M at day 2 (Figure 49-51).

The transcript abundance of *GmMYB10* showed fold changes and increased more than 10-fold within 7 days (Figure 51c). Gene expression associated with ethylene in the air- and ethylene-treated fruit was confirmed by the 1-MCP treatment, which inhibited the transcript levels of all anthocyanin biosynthetic genes and *GmMYBs* (Figure 49-51, Appendix Table 21-31).

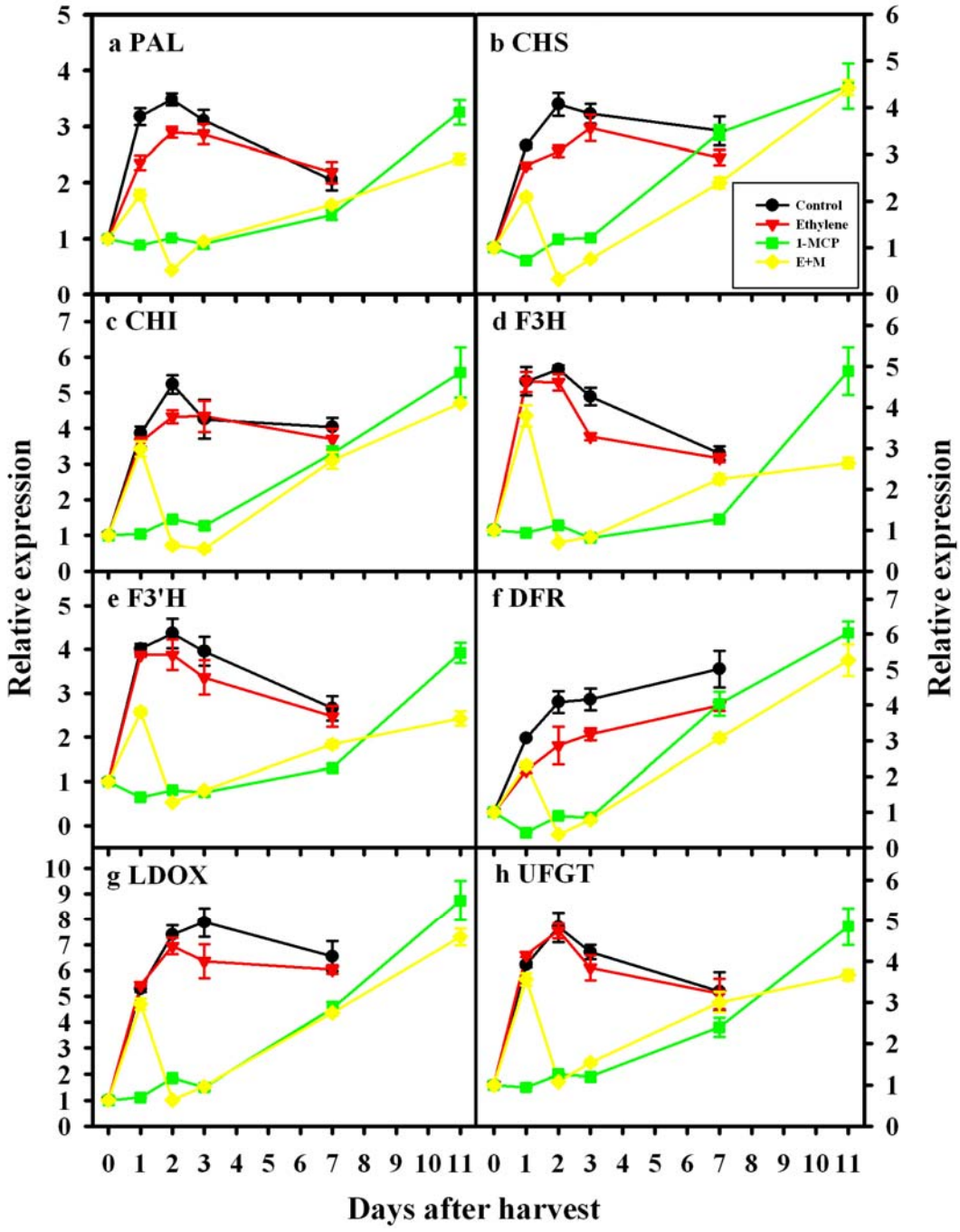


Figure 49 Expression analysis of anthocyanin biosynthetic genes in mangosteen fruit. Fruit were treated with air (control), 200 µL L^{-1} ethylene for 24 h (ethylene), 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M). The sample of air (control) at harvest time (day 0) was set at 1. Data are mean ± SE from three replications.

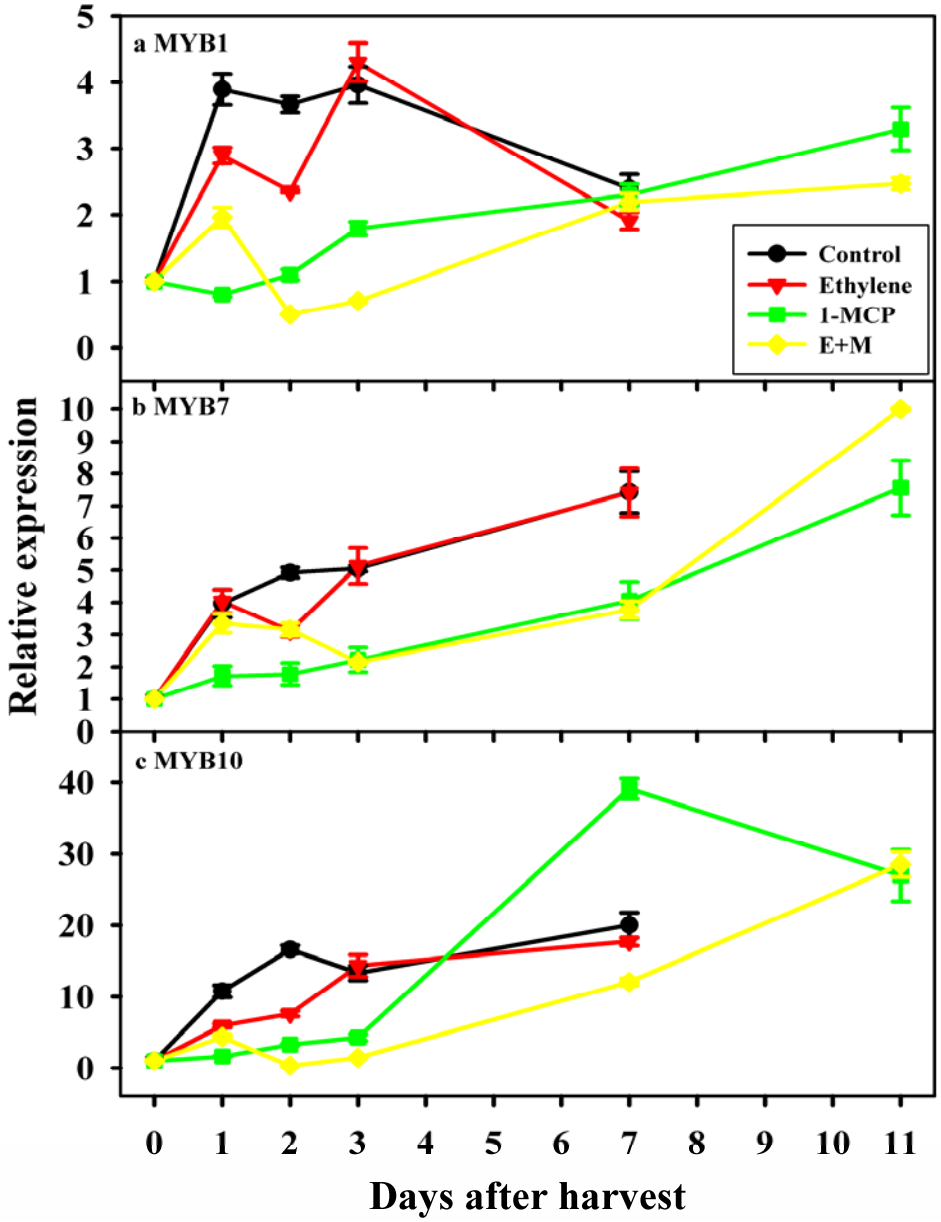


Figure 50 Expression analysis of MYB transcription factor genes in mangosteen fruit. Fruit were treated with air (control), 200 µL L^{-1} ethylene for 24 h (ethylene), 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M). The sample of air (control) at harvest time (day 0) was set at 1. Data are mean ± SE from three replications.

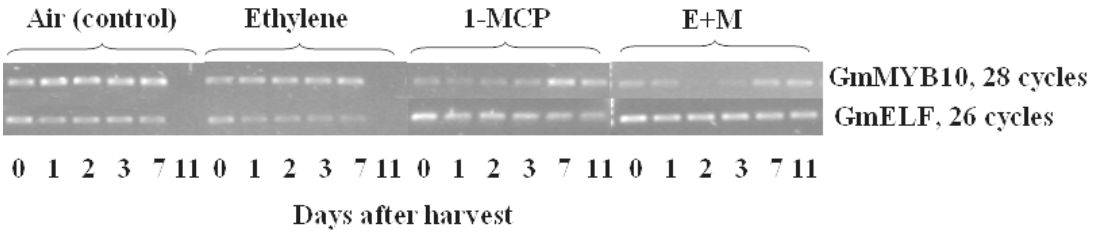


Figure 51 Semi-quantitative RT-PCR of MYB10 transcription factor genes during colour development. Fruit were treated with air (control), 200 µL L^{-1} ethylene for 24 h (ethylene), 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

4.4.3 Low temperature regulates red colouration of mangosteen fruit and correlates with *GmMYB* expression.

To study the effect of temperature on anthocyanin biosynthesis in mangosteen fruit, the fruit were stored at 25°C (control) and 15°C for 7 days then the later transferred to 25°C. In fruit stored at 25°C (control), transcript levels of all anthocyanin biosynthetic genes and *GmMYBs* increased greatly within 1 day, whereas the transcript levels in the fruit stored at 15°C were constitutive during storage at 15°C (Figure 52-54). For most of biosynthetic genes, except *GmDFR*, transcript levels increased until day 2 and decreased thereafter (Figure 52). After transfer of the fruit from 15°C to 25°C, the levels of all genes increased sharply (Figure 52-54). Low temperature storage at 15°C inhibited significantly transcript levels of all anthocyanin biosynthetic genes and *GmMYBs* (Appendix Table 32-42).

Of all the genes analyzed, the transcript abundance of *GmMYB10* showed the highest levels and increased continuously during storage (Figure 53c). Low temperature inhibited the transcript levels of all anthocyanin biosynthetic genes and *GmMYBs* (Figure 52-54).

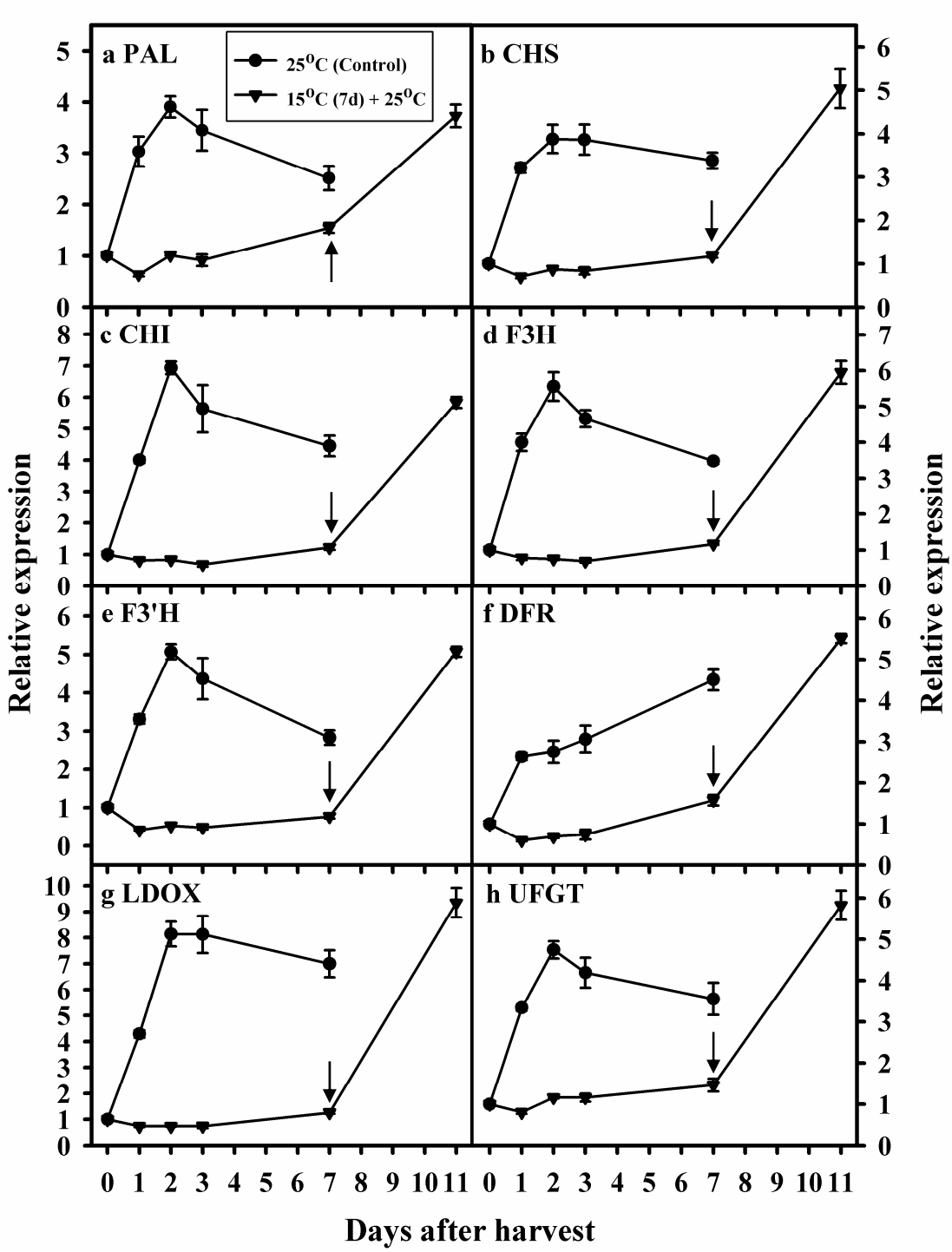


Figure 52 Expression analysis of anthocyanin biosynthetic genes in mangosteen fruit. Fruit were stored at 25°C (control) and 15°C for 7 days then transferred to 25°C (arrows). The sample of 25°C at harvest time (day 0) was set at 1. Data are means ± SE of three replications.

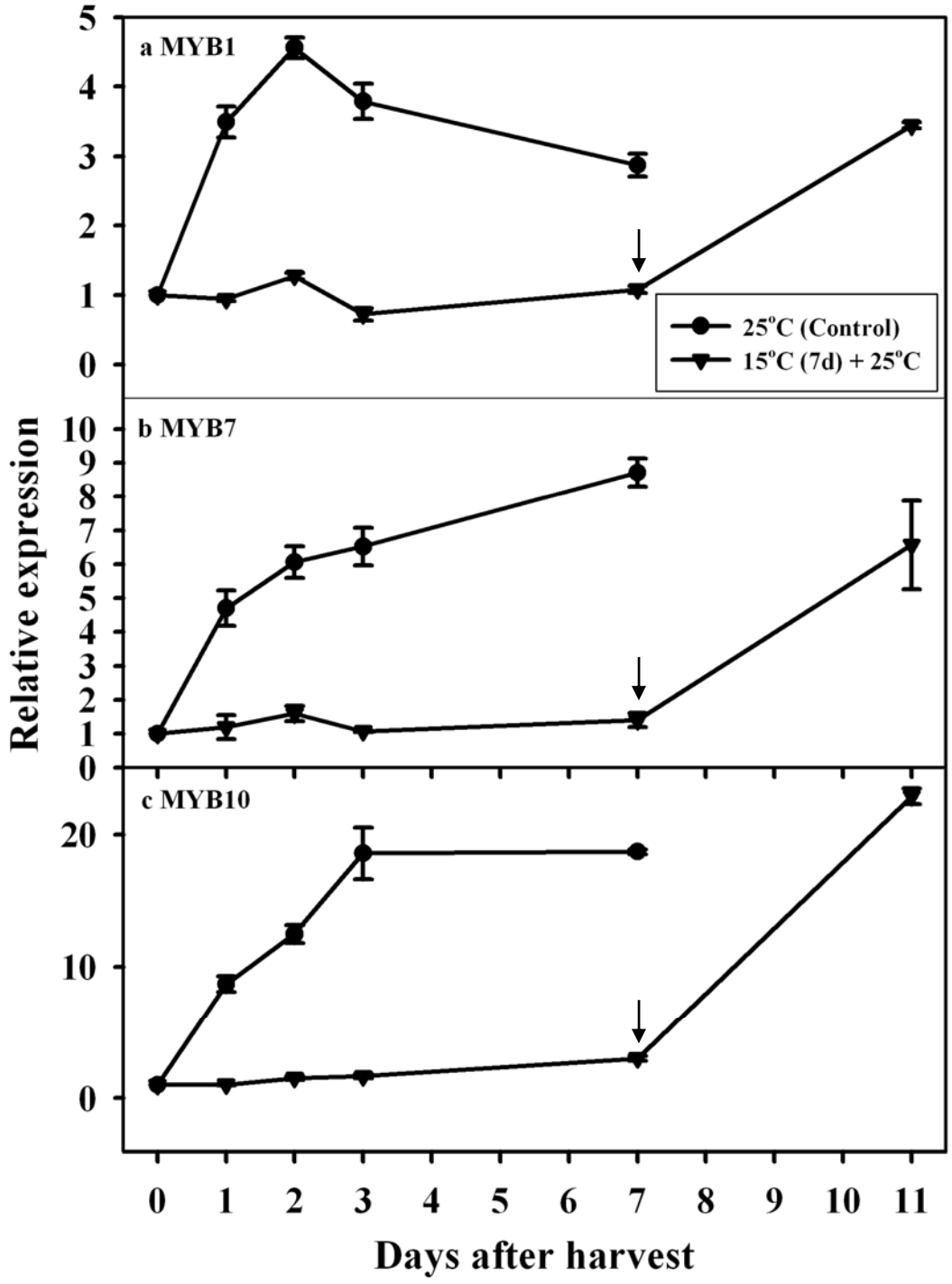


Figure 53 Expression analysis of MYBs transcription factor genes in mangosteen fruit. Fruit were stored at 25°C (control) and 15°C for 7 days then transferred to 25°C (arrows). The sample of 25°C at harvest time (day 0) was set at 1. Data are means ± SE of three replications.

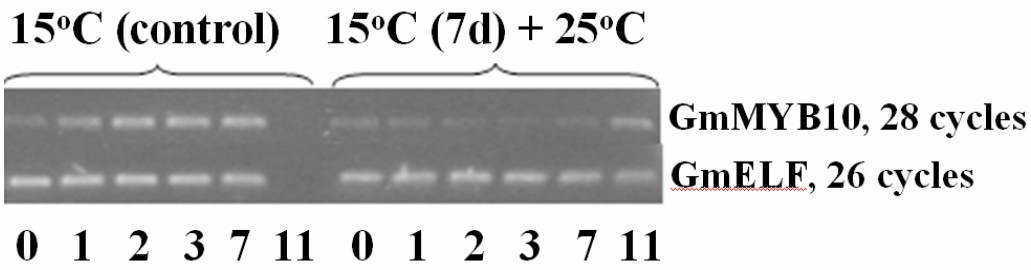


Figure 54 Semi-quantitative RT-PCR of MYB10 transcription factor genes during colour development. Fruit were stored at 25°C (control) and 15°C for 7 days then transferred to 25°C. Data are means ± SE of three replications.

DISCUSSION

1. Colour development and fruit quality of mangosteen fruit during ripening and after harvest

Mangosteen is one of the few species of fruit that develops red colour after harvest, similar to the dark purple changes observed in saskatoon (Rogiers and Knowles, 1999),'Hass' avocado (Cox *et al*., 2004) and Chinese bayberry (Zhang *et al*., 2005). While most of the mangosteen fruit in the current study had started development of red colour on the tree, colour development proceeded quickly during the postharvest period at 25^{o}C. The red colouration of mangosteen fruit harvested at stage 1 (light greenish yellow with 5% scattered pink spots) developed rapidly to dark purple (stage 5) within 5 days (Table 14). Hue values decreased rapidly from 88.1 to 3.7 correlating directly with red colouration. This result indicates that hue value can be used to judge fruit maturity (Figure 7) as has been shown in the fruit. Zhang *et al.* (2005) reported that the fruit colour of bayberry was useful to separate fruit into maturity categories. Mercado-Silva *et al.* (1998) also reported that fruit colour was the best maturity index of guava. The colour changes in mangosteen correlated well with ethylene production (Figure 9), as has also been found in grapes (El-Kereamy *et al*., 2003) and saskatoon (Rogiers *et al.*, 1998), suggesting that a useful study could be made on the specific regulatory role of ethylene in stimulating the anthocyanin biosynthetic pathway in fruit such as mangosteen, grape or avocado. The pattern of ethylene production in mangosteen fruit was similar to that in previous reports (Kanchanapoom and Kachanapoom, 1998; Paull and Ketsa, 2004; Noichinda *et al*., 2007). Both hue values and pericarp firmness decreased rapidly and along with the increase in SSC during fruit colour development, these changes are major phenomena of the mangosteen ripening processes (Noichinda, 1992; Wills *et al*., 2007).

Postharvest quality of fruit is generally dependent on the stage of maturity at harvest. We found that fruit harvested at any of the defined maturity stages, 1 to 6, ripened such that at stage 6 (purple black) for each of them, there were no significant

differences in fruit qualities (pericarp firmness, SSC and TA) and sensory evaluation (Table 14, B columns). In Malaysia, a guideline for exporting mangosteen recommends harvesting fruit when showing a colour of reddish-yellow with patches of red, which is equivalent to stage 1 for Thailand fruit (Osman and Milan, 2006). This suggests that ripening development was already stimulated and underway at harvest for all stages. There is a practical advantage from this. Current grower practice in Thailand is to harvest fruit at stage 1 (light greenish yellow with 5% scattered pink spots) for export. These results confirm that this has no detrimental effect on final fruit quality, with the advantage of a slightly longer shelf-life over fruit harvested at later stages.

The major anthocyanins found in the pericarp were cyanidin-3-sophoroside and cyanidin-3-glucoside, confirming the brief report of Du and Francis (1977). LC-MS data show at least three cyanidin-3-glycosides, including the pentoside and a further cyanidin with an unidentified residue. The pentoside derivative and unidentified residue (*m/z* 190) are the first reports in mangosteen. The identification of the residue (*m/z* 190) may require NMR technique in the future. Small amounts of the other cyanidin derivatives were detected. Colour development in mangosteen pericarp was closely correlated with the strongly increasing concentrations of total anthocyanin, cyanidin-3-sophoroside and the cyanidin-3-glucosides (Table 15 and 16, Figure 10 and 12). The rapid elevation of anthocyanic colour suggests that precursor polyphenolics are readily available for conversion to cyanidins. In the absence of other pigments, the dramatic increase in levels of anthocyanin pigments alone explains the final appearance. Results on outer pericarp (skin) section confirmed the general result of increasing red pigment level during colour development (Figure 13). Cox *et al*. (2004) reported a similar increase in cyanidin-3-glucoside which correlated closely with skin colouration of ‘Hass’ avocado.

2. Cloning and characterization of MYB transcription factors and anthocyanin biosynthesis genes of mangosteen fruit during red colouration

2.1 MYB transcription factors

The regulation of enzymes and genes associated with the anthocyanin pathway has been studied in a number of plant species. In *Arabidopsis*, petunia, grape and apple, both MYB transcription factors and biosynthetic genes have been identified (Borevitz *et al*., 2000; Kobayashi *et al*., 2002; Koes *et al*., 2005; Espley *et al*., 2007). In this research, MYB transcription factors and anthocyanin biosynthesis genes of mangosteen fruit during red colouration were characterized.

Based on sequence similarity, expression profile and functional testing, we hypothesize that principal *GmMYB10* is the MYB transcription factor regulating anthocyanin biosynthesis in mangosteen fruit. We found three MYB genes encoding putative R2R3-MYB proteins. Stracke *et al*. (2001) clustered the 133 *Arabidopsis* R2R3 MYBs into 24 subgroups and *AtPAP1* (*AtMYB75)* regulating anthocyanin biosynthesis was classified into subgroup 10 (Allan *et al*., 2008). Phylogenetic analysis clearly placed the GmMYBs in clades with the *Arabidopsis* MYB transcription factors in subgroups 1, 7 and 10 (Figure 24). *GmMYB1* had sequence similarity to *FaMYB1* of strawberry containing a putative repressor domain (Figure 23 and 24). The peptide sequence LNLDLTIG was found to be similar to the pdLNL$^{D}/_{E}$Lxi$^{G}/_{S}$ motif that was proposed to act as a transcriptional repressor of the late flavonoid biosynthesis genes in strawberry *FaMYB1* (Aharoni *et al.*, 2001) and the grape C2 repressor (Matus *et al.*, 2008). This result suggests that *GmMYB1* may function as a repressor in mangosteen. *GmMYB7* clustered into subgroup 7, close to *AtMYB78* and *AtMYB108* which are involved in stress signaling (Mengiste *et al.*, 2003; Matus *et al.*, 2008). *AtMYB108* encodes BOS1 (BOtrytis-Susceptible1) which is responsible for the activation of a subset of defense pathways (Mengiste *et al.*, 2003) and also acts with *AtMYB24* to regulate jasmonate-mediated stamen development (Mandaokar and Browse, 2008). *GmMYB7* has the peptide sequence LLILQLHSRWGNRWSKIARF that differs from the amino acid motif

[DE]Lx_2[RK]$x_3Lx_6Lx_3$R predicted to allow interaction with bHLHs, suggesting that it does not interact with bHLH proteins (Figure 23) (Zimmermann *et al.*, 2004). In *GmMYB10,* sequence analysis of the *GmMYB10*-encoded protein shows high homology with *Arabidopsis* PAP1, *Antirrhinum* ROSEA1, apple MYB10 and other anthocyanin-related MYBs (Figure 23 and 24) (Mol *et al.*, 1998; Schwinn *et al.*, 2006; Allan *et al.*, 2008). Thus phylogeny suggests that *GmMYB10* is a new member of the MYB anthocyanin activators and *GmMYB1* could be a repressor of the flavonoid pathway. A signature motif [DE]Lx_2[RK]$x_3Lx_6Lx_3$R for the interaction between MYB and bHLH proteins was found in the R2R3 DNA-binding domain of the GmMYB partner (Zimmermann *et al.*, 2004).

2.2 Anthocyanin biosynthesis genes

In anthocyanin biosynthesis pathway, there are several steps. The mangosteen anthocyanin biosynthesis genes have now been completely characterized. All the biosynthetic genes had sequence similarity to anthocyanin-related genes in other plants especially grape, citrus and strawberry. In the hydroxylation step, we found only flavanone 3'-hydroxylase (F3'H). This enzyme catalyzes the hydroxylation of flavonoids at the 3' positions of the B-ring leading to the respective cyanidin derivatives (Winkel-Shirley, 2001). However, identification of the flavonoid 3', 5'-hydroxylase (F3'5'H) using both nested and race PCR strategy was not successful. Based on anthocyanin identification, these results correlated with cyanidin pigments in mangosteen fruit. The results suggest that mangosteen fruit does not have a functional flavonoid 3', 5'-hydroxylase enzyme similar to *Arabidopsis,* apple and rose (Forkmann, 1991). It may be concluded that mangosteen cannot produce blue pigment.

2.3 Promoter isolation and analysis of *GmDFR*, *GmLDOX* and *GmUFGT* genes

The 5' flanking regions of *GmDFR*, *GmLDOX* and *GmUFGT* were obtained using ligation –mediated PCR (Genome Walker Kit, Clontech). *Cis*-acting

elements present in *GmDFR*, *GmLDOX* and *GmUFGT* 5'flanking regions were predicted using PLACE database and confirmed using PlantCARE and manual searching. We found several MYB binding sites, such as MYBPLANT (MACCWAMC) and MYBCORE (CNGTTR) and many ethylene response elements (ATTTNAAA, AWTTCAAA) in all 5' flanking regions (Montgomery *et al*., 1993; Solano *et al.*, 1995; Tamagnone *et al.*, 1998). Espley *et al*. (2009) reported that MYB10 protein autoregulated transcript levels by binding to a putative MYB binding domain. Our result suggested that *GmMYB* may activate the anthocyanin biosynthetic genes via the MYB binding sites that were found in both promoters as MYBPLANT (MACCWAMC) and MYBCORE (CNGTTR). Furthermore, ethylene may regulate gene activity by activating ethylene response elements in the promoter of ethylene inducible genes such as E4 and E8 (Montgomery *et al*., 1993; Deikman *et al*., 1998). Solano *et al.* (1998) showed that EIN3/EILs, ethylene-related transcription factors, regulated gene expression by binding directly to a primary ethylene response element (PERE) related to the tomato E4-element. This motif was found in the *GmDFR*, *GmLDOX* and *GmUFGT* 5'flanking regions, similar to the UFGT promoter of grape that is up-regulated transcript level. (El-Kereamy *et al.*, 2003; Tira-Umphon *et al.*, 2007).

2.4 Functional testing of GmMYBs using transient tobacco assays

In transient expression assays, we demonstated that *GmMYB10* can function as an anthocyanin regulator by infiltrating the GmMYBs with and without *AtbHLH2* (*AtEGL3*) which activated *GmDFR* and *AtDFR* promoters. The results were confirmed using *AtPAP1* infiltration as a positive control. *GmMYB10* display was more specific to both *GmDFR* and *AtDFR* promoters than GmMYB1 and GmMYB7 (Figure 46). Both *GmMYB10* and *AtPAP1* showed a high dependence on *AtbHLH2* (*AtEGL3*) as a partner, similar to other anthocyanin-related MYBs (Bogs *et al.*, 2007; Walker *et al.*, 2007). The results suggest that *GmMYB10* can activate the expression of genes encoding enzymes of anthocyanin biosynthesis. Furthermore, *GmMYB10* could activate the anthocyanin biosynthetic genes including *GmLDOX* and *GmUFGT* promoter via the MYB binding sites that were found in both

promoters as MYBPLANT (MACCWAMC) and MYBCORE (CNGTTR) (Solano *et al*., 1995; Tamagnone *et al*., 1998).

In summary, the results of sequence analysis and functional testing indicate that *GmMYB10* is a MYB transcription factor playing a key role in regulating anthocyanin biosynthesis in mangosteen fruit.

3. Rapid colouration of mangosteen fruit correlates with changes in gene expression

Mangosteen fruit clearly develop intense red colour both on the tree and after harvest with high anthocyanin pigmentation. The transcript levels of the three mangosteen MYBs, *GmMYB1, GmMYB7* and *GmMYB10*, increased markedly with onset of red colouration both on-tree and after harvest (Figure 47i-j). However, *GmMYB10* was the most up-regulated of *GmMYB* transcription factors and the most abundant (up to 299-fold in on-tree fruit and 501-fold in postharvest fruit at stage 5 - dark purple), and declined at the final stage (black purple). This expression pattern was similar to that of *MdMYB10* in apple (Espley *et al.*, 2007) and that of *VvMYBPA1* in grape berry skins (Bogs *et al.*, 2007). Expression patterns therefore give further support to the suggestion that *GmMYB10* is a candidate to regulate anthocyanin biosynthesis of mangosteen fruit. The expression patterns of all anthocyanin biosynthesis genes correlated with those of *GmMYB10,* in that fold changes were substantial with onset of colour development and declined at the final stages. Almieda *et al*. (2007) found that the transcript level of anthocyanin biosynthesis pathway genes in 'Queen Elisa' strawberry increased during fruit development and decreased at the red stage. This expression pattern was similar to that found in apple after light exposure with an increase in colour development and anthocyanin content (Kim *et al*., 2003). Of the anthocyanin biosynthesis genes, transcript levels of *GmUFGT* showed the greatest fold change suggesting *GmUFGT* may be the key biosynthetic gene similar to the results in grape (Boss *et al.*, 1996; Kobayashi *et al.*, 2001) and may controlled by *GmMYB10*. In grape, *VvMYBA1* and *VvMYBA2* transcription factors regulate specifically the expression of the UFGT

gene (Kobayashi *et al.*, 2002; Walker *et al.*, 2007). Regulation of anthocyanin biosynthesis has been shown to be at the level of transcription in pigmented organs in other species (Mol *et al.*, 1998; Schwinn *et al.*, 2006), and transcript levels indicate final phenotype including colour, anthocyanin content and composition (Castellarin and Gaspero 2007).

4. Ethylene regulates red colouration of mangosteen fruit correlated with *GmMYB* expression.

From results on tree and after harvest, the red colouration of mangosteen fruit is controlled by ethylene and the ethylene inhibitor (1-MCP) application. 1-MCP clearly delayed red colouration (hue value), pericarp firmness, ethylene production and anthocyanin accumulation (Figure 14-17). The application of 1-MCP in delaying red colouration and anthocyanin content has been found previously in fruit such as grape (Chervin *et al.*, 2004) and strawberry (Jiang *et al.*, 2001). Jiang *et al.* (2001) reported that 1-MCP delayed fruit colour (hue value), firmness, ethylene production and anthocyanins in strawberry fruit. 1-MCP also inhibited PAL activity which is the first enzyme in anthocyanin biosynthesis pathway. However, ethylene application could not induce red colouration and ethylene production in mangosteen fruit (Figure 15 and 17a). Piriyavinit (2008) also reported that exogenous ethylene did not affect colour development and ethylene production in mangosteen fruit when compared with the control. This ineffectiveness of ethylene application in inducing red colouration agrees with low transcript levels of all biosynthetic and MYB transcription factor genes when compared with the control (air) (Figure 49 and 50). This result may be caused by the time of ethylene application during the pre-climateric period (Noichinda, unpublished data). We also found that ethylene production of mangosteen fruit at stage 1 increased sharply (Figure 9). These results indicated that ethylene treatment did not induce red colouration of mangosteen fruit when the fruit at stage 1 (light greenish yellow with 5% scattered pink spots) were treated.

1-MCP application in both 1-MCP and ethylene+1-MCP treatments down-regulated *GmMYBs*, especially *GmMYB10*, and all anthocyanin biosynthesis gene expression. These results indicate that ethylene and *GmMYB10* expression work closely to control the anthocyanin biosynthesis in mangosteen pigmentation. Ethylene may directly regulate *GmMYB10* and all anthocyanin biosynthesis genes at the transcription level. In grape, ethylene induced both anthocyanins and internal ethylene concentrations when externally applied by up-regulating anthocyanin biosynthetic genes (El-Kereamy *et al.*, 2003). Tira-Umphon et al. (2007) showed that ethylene-induced anthocyanin in grape berries and cell suspensions were dependent on *UFGT* expression and independent of *MYBA* expression. The report showed little change in the expression of the MYB transcription factor, with different responses to ethylene. Mangosteen, a climacteric fruit, is more sensitive to ethylene than grape (a non-climateric fruit) and red colouration of mangosteen fruit changed rapidly (Palapol *et al.*, 2009). In addition, we found two ethylene response elements (E4, AWTTCAAA) in the *GmDFR* promoter. We also found a motif similar to an ethylene response element (ATTTNAAA) in the *GmDFR*, *GmLDOX* and *GmUFGT* promoters that is similar to the UFGT promoter of grape (El-Kereamy *et al.*, 2003; Tira-Umphon *et al.*, 2007). Solano *et al*. (1998) showed that EIN3/EILs, ethylene-related transcription factors, regulate gene expression by binding directly to a primary ethylene response element (PERE) related to the tomato E4-element. This data indicates that *GmMYB10* and anthocyanin structural genes may be co-ordinately regulated for anthocyanin synthesis in mangosteen fruit in response to ethylene.

5. Low temperature storage regulates red colouration of mangosteen fruit correlated with *GmMYB* expression.

In this study, colour of fruit stored at 15^{o}C developed much slower than in fruit stored at 25^{o}C (Figure 17). Ratanamarno *et al.* (2004) reported that mangosteen fruit at stage 1 stored at 15^{o}C changed to stage 6 later than fruit stored at high temperatures (30-35^{o}C). Similarly low temperature storage of strawberry maintained fruit quality and delayed an increase in anthocyanin content (Shin *et al*., 2008). After transfer of the fruit from 15^{o}C to 25^{o}C, the increase in red colouration and

anthocyanin content, and the decrease in pericarp firmness changed markedly and correlated closely with an increase in ethylene production (Figure 19-21). This data support that the ethylene plays an important role in ripening of mangosteen fruit. Kim *et al*. (2003) reported that the transcript level of anthocyanin biosynthesis genes correlated with anthocyanin content. However, the study of low temperature storage regulated anthocyanin biosynthesis is still underway. All transcript levels in mangosteen fruit stored at 25^{o}C in this study were firstly reported to increase clearly with red colouration. Low temperature storage at 15^{o}C inhibited the transcript levels of all anthocyanin biosynthetic genes and then increased abundance when the fruit were transferred to 25^{o}C (Figure 52). This was concomitant with an increase in ethylene production after transfer of fruit to 25^{o}C (Figure 21). El-Kereamy *et al.* (2003) also reported that ethylene induced both anthocyanins and internal ethylene concentration up-regulating anthocyanin biosynthetic genes. Low ethylene production in fruit stored at 15^{o}C and an increase in ethylene production with transfer from 15^{o}C to 25^{o}C, indicates that ethylene may directly regulate fruit colouration during low temperature storage via up-regulation of transcript level of anthocyanin-related genes.

In summary, we concluded that anthocyanin pigmentations were synthesized from PAL to UFGT as illustrated in Figure 55. *GmMYB10* plays a role in the regulation of anthocyanin biosynthesis genes in mangosteen fruit including *GmDFR*, *GmLDOX* and *GmUFGT* (red arrows). Anthocyanin biosynthesis in mangosteen fruit is dependent on ethylene which may directly (green arrows), or via other signal transduction intermediates (blue arrow), modulate transcript levels of *GmMYB10* and anthocyanin biosynthetic genes especially *GmUFGT*. Ethylene may regulate gene activity by activating the ethylene response element (PERE) within the promoters of anthocyanin-related genes. The role of ethylene in red colouration of mangosteen fruit can be also illustrated in Figure 55.

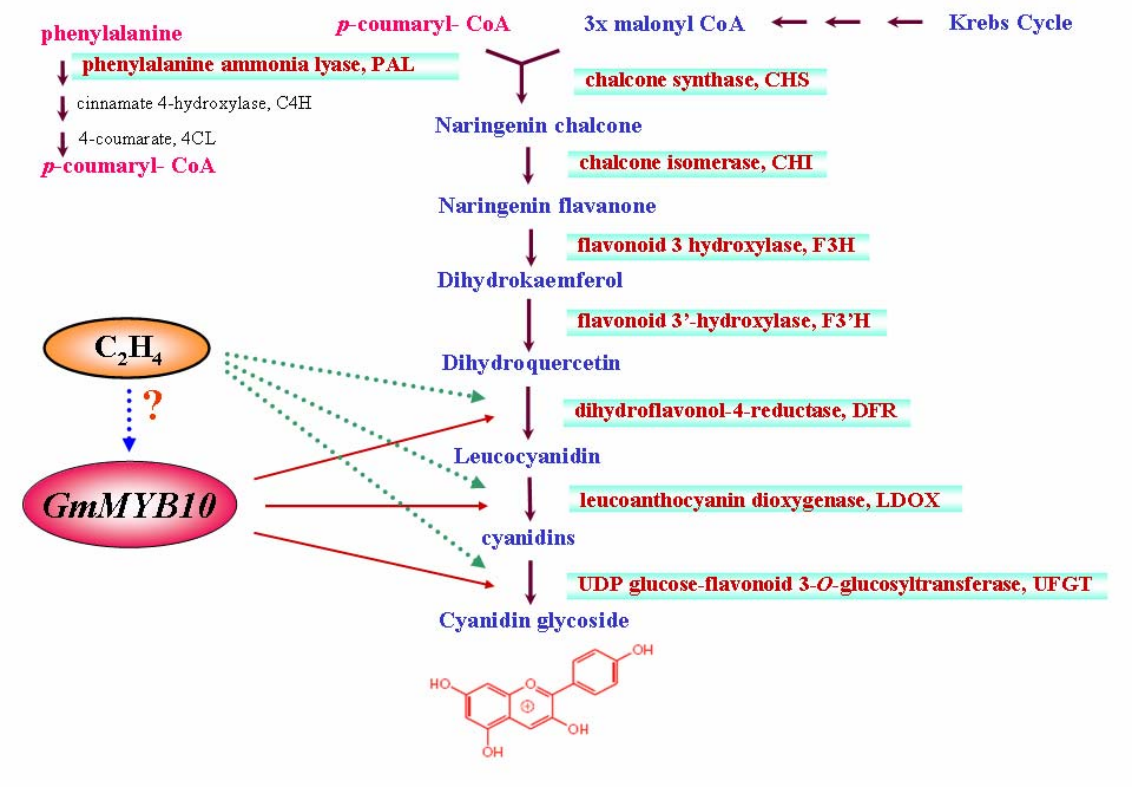


Figure 55 Model of anthocyanin biosynthesis in mangosteen fruit.

CONCLUSIONS

Characterization of MYB transcription factors and anthocyanin biosynthesis genes of mangosteen fruit during red colouration can be summarized as following:

1. Mangosteen fruit harvested from stage 1 (light greenish yellow with 5-50% scattered pink spots) can develop colour to the final stage (purple black).

2. The minimum harvest stage for high quality fruit was when the fruit skin is light greenish yellow with 5-50% scattered pink spots.

3. Anthocyanin content in the outer pericarp is higher than in the inner pericarp.

4. Cyanidin-3-sophoroside and cyanidin-3-glucoside are the major compounds and increased with fruit colour development.

5. Anthocyanin biosynthesis in mangosteen fruit is dependent on ethylene.

6. 1-Methycyclopropene (ethylene inhibitor) and low temperature storage (15 ^{o}C) can delay colour development and anthocyanin pigmentation with resulting down-regulation of the *GmMYB* transcription factor and all anthocyanin biosynthesis genes.

7. *GmUFGT* is a key biosynthetic gene in mangosteen pigmentation.

8. *GmMYB10* plays a role in the specific regulation of anthocyanin biosynthesis both on- and off-tree in mangosteen fruit.

9. The ethylene response elements (E4, AWTTCAAA and ATTTNAAA) were found in all promoters.

LITERATURE CITED

Abeles, F.B., P.W. Morgan and M.E. Saltveit. 1992. **Ethylene in Plant Biology**, 2nd eds. Academic Press, NY.

Aharoni, A., C.H.R.D. Vos, M. Wein, Z. Sun, R. Greco, A. Kroon, J.N.M. Mol and A.P. O'Connell. 2001. The strawberry *FaMYB1* transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. **Plant J.** 28:319-332.

Ali, M.B., N. Singh, A.M. Shohael, E.J. Hahn, K.Y. Paek. 2006. Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress. **Plant Sci**. 171: 147-154.

Allan, A.C., R.P. Hellens and W.A. Laing. 2008. MYB transcription factors that colour our fruit. **Trends Plant Sci.** 13:99-102.

Almeida, J.R.M., E. D'Amico, A. Preuss, F. Carbone, C.H.R. de Vos, B. Deiml, F. Mourgues, G. Perrotta, T.C. Fischer, A.G. Bovy, S. Martens and C. Rosati. 2007. Characterization of major enzymes and genes involved in flavonoid and proanthocyanidin biosynthesis during fruit development in strawberry (*Fragaria x ananassa*). **Arch. Biochem. Biophys.** 465:61-71.

Atchley, W.R., W. Terhalle and A. Dress. 1999. Positional dependence, cliques, and predictive motifs in the bHLH protein domain. **J. Mol. Evol.** 48:501-516.

Auta, J., Y. Chen, W. Ruzicka and D. Grayson. 2007. Nucleic acid quantitation using the competitive polymerase chain reaction. pp. 341-361. *In* **Handbook of Neurochemistry and Molecular Neurobiology**. Kluwer Academic Publishers, New York.

Avila, J., C. Nieto, L. Canas, M.J. Benito and J. Paz-Ares. 1993. *Petunia hybrida* genes related to the maize regulatory C1 gene and to animal myb proto-oncogenes. **Plant J.** 3:553-562.

Awad, M.A. and A. de Jager. 2002. Relationships between fruit nutrients and concentrations of flavonoids and chlorogenic acid in 'Elstar' apple skin. **Sci. Hortic.** 92: 265–276.

Bailey, B.A., H. Bae, M.D. Strem, G.A. de Mayolo, M.J. Guiltinan, J.A. Verica, S.N. Maximova and J.H. Bowers. 2005. Developmental expression of stress response genes in *Theobroma cacao* leaves and their response to Nep1 treatment and a compatible infection by *Phytophthora megakarya*. **Plant Physiol. Biochem.** 43:611-622.

Ban, Y., C. Honda, Y. Hatsuyama, M. Igarashi, H. Bessho and T. Moriguchi. 2007. Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin. **Plant Cell Physiol.** 48:958-970.

Bogs, J., F.W. Jaffe, A.M. Takos, A.R. Walker and S.P. Robinson. 2007. The grapevine transcription factor VvMYBPA1 regulates proanthocyanidin synthesis during fruit development. **Plant Physiol.** 143:1347-1361.

Borevitz, J.O., Y. Xia, J. Blount, R.A. Dixon and C. Lamb. 2000. Activation tagging Identifies a conserved MYB regulator of phenylpropanoid biosynthesis. **Plant Cell** 12:2383-2394.

Boss, P.K., C. Davies and S. Robinsson. 1996. Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv. Shiraz grape berries and the implications for pathway regulation. **Plant Physiol.** 111: 1059-1066.

_____ and C. Davies. 2001. Molecular biology of sugar and anthocyanin accumulation in grape berries, pp. 1-34. *In* Roubelakis-Angelakis, K.A., ed. **Molecular Biology and Biotechnology of the Grapevine**. Kluwer Academic Press, The Netherlands.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal. Biochem.** 72: 248-254.

Bradley, J.M., K.M. Davies, S.C. Deroles, S.J. Bloor and D.H. Lewis. 1998. The maize LC regulatory gene up-regulates the flavonoid biosynthetic pathway of Petunia. **Plant J.** 13: 381–392.

Britsch, L., B. Ruhnau-Brich and G. Forkmann. 1992. Molecular cloning, sequence analysis, and *in vitro* expression of flavanone 3 beta-hydroxylase from *Petunia hybrida*. **J. Biol. Chem.** 267: 5380– 5387.

_____ , J. Dedio, H. Saedler and G. Forkmann. 1993. Molecular characterization of flavanone 3 beta-hydroxylases consensus sequence, comparison with related enzymes and the role of conserved histidine residues. **Eur. J. Biochem.** 217, 745–754.

Bronstein, I., J. Fortin, P.E. Stanley, G.S.A.B. Stewart and L.J. Kricka. 1994. Chemiluminescent and bioluminescent reporter gene assays. **Anal. Biochem.** 219:169-181.

Broun, P. 2005. Transcriptional control of flavonoid biosynthesis: a complex network of conserved regulators involved in multiple aspects of differentiation in *Arabidopsis*. **Curr. Opin. Plant Biol.** 8:272-279.

Bustin, S. A. 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. **J. Mol. Endocrinol.** 25: 169-193.

Carey, C.C., J.T. Strahle, D.A. Selinger and V.L. Chandler. 2004. Mutations in the pale aleurone color1 regulatory gene of the *Zea mays* anthocyanin pathway have distinct phenotypes relative to the functionally similar TRANSPARENT TESTA GLABRA1 gene in *Arabidopsis thaliana*. **Plant Cell** 16:450-464.

Castellarin, S. and G. Di Gaspero. 2007. Transcriptional control of anthocyanin biosynthetic genes in extreme phenotypes for berry pigmentation of naturally occurring grapevines. **BMC Plant Biol.** 7:46.

Chervin, C., A. El-Kereamy, J.P. Roustan, A. Latch, J. Lamon and M. Bouzayen. 2004. Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. **Plant Sci.** 167:1301-1305.

Chopra, S., Hoshino, A., Boddu, J. and Iida, S. (2006) Flavonoid pigments as tools in molecular genetics, pp. 147–173. *In* Glotewold, E., ed. **The Science of Flavonoids**. Springer Press, New York.

Cox, K.A., T.K. McGhie, A. White and A.B. Woolf. 2004. Skin colour and pigment changes during ripening of 'Hass' avocado fruit. **Postharvest Biol. Technol.** 31:287-294.

Dale, J. W. and M. von Schantz. 2003. **From Genes to Genomes Concepts and Applications of DNA Technology.** John Wiley & Sons, Ltd., West Sussex.

Davies, K. 2004. **Plant Pigments and Their Manipulation.** Blackwell Publishing Ltd., Oxford.

de Vetten, N., F. Quattrocchio, J. Mol and R. Koes. 1997. The an11 locus controlling flower pigmentation in petunia encodes a novel WD-repeat protein conserved in yeast, plants, and animals. **Genes Dev**. 11:1422-1434.

de Wet, J.R., K.V. Wood, D.R. Helinski and M. DeLuca. 1985. Cloning of firefly luciferase cDNA and the expression of active luciferase in *Escherichia coli*. **PNAS** 82:7870-7873.

Deikman, J., R. Xu, M.L. Kneissl, J.A. Ciardi, K.-N. Kim and D. Pelah. 1998. Separation of cis elements responsive to ethylene, fruit development, and ripening in the 5′-flanking region of the ripening-related E8 gene. **Plant Mol. Biol.** 37:1001-1011.

Delgado-Vargas, F. and O. Paredes-Lopez 2002. **Natural Colorants for Food and Nutraceutical Uses.** CRC Press, USA.

Du, C.T. and F.J. Francis. 1977. Anthocyanin of mangosteen (*Garcinia mangostana* L). **J. Food Sci.** 42: 1667-1668.

El-Kereamy A., C. Chervin, J.P. Roustan, V. Cheynier, J.M. Souquet, M. Moutounet, J. Raynal, C. Ford, A. Latche, J.C. Pech and M. Bouzayen. 2003. Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. **Physiol. Plant.** 119(2): 175-182.

Espley, R.V., R.P. Hellens, J. Putterill, D.E. Stevenson, S. Kutty-Amma and A.C. Allan. 2007. Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. **Plant J.** 49: 414-427.

Forkmann, G. 1991. Flavonoids as Flower Pigments: The formation of the natural spectrum and its extension by genetic engineering. **Plant Breeding**. 106: 1-26.

_____ and W. Heller. 1999. Biosynthesis of flavonoids. pp. 713–748. *In* Sankawa, U., ed. **Comprehensive Natural Products Chemistry**. Amsterdam, Elsevier.

Freeman, W. M., S. J. Walker and K. E. Vrana. 1999. Quantitative RT-PCR: pitfalls and potential. **BioTechniques** 26: 112-125.

Gachon, C., A. Mingam and B. Charrier. 2004. Real-time PCR: what relevance to plant studies? **J. Exp. Bot.** 55(402): 1445-1454.

Giambernardi, T.A. and R.J. Klebe. 2000. Relative reverse transcription-polymerase chain reaction. pp. 51-58. *In* **Developmental Biology Protocols.** Humana Press, NJ

Goff, S.A., K.C. Cone and V.L. Chandler. 1992. Functional analysis of the transcriptional activator encoded by the maize B gene: Evidence for a direct functional interaction between two classes of regulatory proteins. **Genes Dev**. 6: 864-875.

_____ , T.M. Klein, B.A. Roth, M.E. Fromm, K.C. Cone, J.P. Radicella and V.L. Chandler. 1990. Transactivation of anthocyanin biosynthetic genes following transfer of *B* regulatory genes into maize tissues. **EMBO J.** 9: 2517-2522.

Gollop, R., S. Even, V. Colova-Tsolova and A. Perl. 2002. Expression of the grape dihydroflavonol reductase gene and analysis of its promoter region. **J. Exp. Bot.** 53(373): 1397-1409.

Gomez-Cordoves, M.C., F. Varela, C. Larrigaudiere and M. Vendrell. 1996. Effect of ethephon and seniphos treatments on the anthocyanin composition of starking apples. **J. Agric. Food Chem.** 44: 3449-3452.

Goodrich, J., R. Carpenter and E.S. Coen. 1992. A common gene regulates pigmentation pattern in diverse plant species. **Cell** 68:955-964.

Goto-Yamamoto, N., G.H. Wan, K. Masaki and S. Kobayashi. 2002. Structure and transcription of three chalcone synthase genes of grapevine (*Vitis vinifera*). **Plant Sci.** 162: 867-872.

Griesser, M., T. Hoffmann, M.L. Bellido, C. Rosati, B. Fink, R. Kurtzer, A. Aharoni, J. Munoz-Blanco and W. Schwab. 2008. Redirection of flavonoid biosynthesis through the down-regulation of an anthocyanidin glucosyltransferase in ripening strawberry fruit. **Plant Physiol.** 146: 1528-1539.

Gross, J. 1987. **Pigments in Fruits.** Academic Press, London.

Grotewold, E., M.B. Sainz, L. Tagliani, J.M. Hernandez, B. Bowen and V.L. Chandler. 2000. Identification of the residues in the Myb domain of maize C1 that specify the interaction with the bHLH cofactor R. **PNAS** 97: 13579-13584.

Heim, M.A., M. Jakoby, M. Werber, C. Martin, B. Weisshaar and P.C. Bailey. 2003. The basic helix-loop-helix transcription factor family in plants: A genome-wide study of protein structure and functional diversity. **Mol. Biol. Evol.** 20: 735-747.

Heller, W. and G. Forkmann. 1994. Biosynthesis of flavonoids, pp. 499-535. *In* Harborne, J.B., ed. **The Flavonoids- Advances in Research since 1986.** Chapman and Hall, London.

Hernandez, J.M., G.F. Heine, N.G. Irani, A. Feller, M.G. Kim, T. Matulnik, V.L. Chandler and E. Grotewold. 2004. Different mechanisms participate in the R-dependent activity of the R2R3 MYB transcription factor C1. **J. Biol. Chem.** 279: 48205-48213.

Holcroft, D.M., M.I. Gil and A.A. Kader. 1998. Effect of carbon dioxide on anthocyanins, phenylalanine ammonia lyase and glucosyltransferase in the arils of stored pomegranates. **J. Amer. Soc. Hort. Sci.** 123(1): 136-140.

Holton, T.A. and E.C. Cornish. 1995. Genetics and biochemistry of anthocyanin biosynthesis. **Plant Cell** 7: 1071-1083.

Honda, C., K. Nobuhiro, W. Masato, K. Satoru, K. Shozo, S. Junichi, Z. Zilian, T. Tomomi and M. Takaya, 2002. Anthocyanin biosynthetic genes are coordinately expressed during red coloration in apple skin. **Plant Physiol. Biochem.** 40: 955-962.

Hrazdina, G. and G.J. Wagner. 1985. Metabolic pathways as enzyme complexes: evidence for the synthesis of phenylpropanoids and flavonoids on membrane associated enzyme complexes. **Arch. Biochem. Biophys.** 237: 88-100.

_____ and R.A. Jensen. 1992. Spatial organization of enzymes in plant metabolic pathways. **Annu. Rev. Plant. Physiol. Plant Mol. Biol.** 43: 241-267.

Jaakola, L., K. Maatta, A.M. Pirttila, R. Torrenen, S. Karenlampi, and A. Hohtola 2002. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. **Plant Physiol.** 130: 729-739.

Jackson, D., F. Culianez-Macia, A.G. Prescott, K. Roberts and C. Martin. 1991. Expression patterns of myb genes from Antirrhinum flowers. **Plant Cell** 3: 115-125.

_____ , K. Roberts, and C. Martin. 1992. Temporal and spatial control of expression of anthocyanin biosynthetic genes in developing flowers of *Antirrhinum majus*. **Plant J.** 2: 425-434.

Jeong, S.T., N. Goto-Yamamoto, K. Hashizume and M. Esaka. 2006. Expression of the flavonoid 3′-hydroxylase and flavonoid 3′,5′-hydroxylase genes and flavonoid composition in grape (*Vitis vinifera*). **Plant Sci.** 170(1): 61-69.

_____ , _____ , S. Kobayashi and M. Esaka. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. **Plant Sci.** 167: 247-252.

Jiang, Y., D.C. Joyce and L.A. Terry. 2001. 1-Methycyclopropene treatment affects strawberry fruit decay. **Postharvest Biol. Technol.** 23: 227-232.

Johnson, E.T., S. Ryu, H. Yi, B. Shin, H. Cheong and G. Choi. 2001. Alteration of a single amino acid changes the substrate specificity of dihydroflavonol 4-reductase. **Plant J.** 25: 325-333.

Ju Z.G., Y. Yuan, C. Liu and S. Xin. 1995. Relationships among phenylalanine ammonia-lyase activity, simple phenol concentrations and anthocyanin accumulation in apple. **Sci. Hortic.** 61: 215-266.

Ju, Z. 1998. Fruit bagging, a useful method for studying anthocyanin synthesis and gene expression in apples. **Sci. Hortic.** 77: 155-164.

Kanchanapoom, K. and M. Kanchanapoom. 1998. Mangosteen, pp. 191-216. *In* Shaw P.E., Jr., H.T. Chan and S. Nagi, eds. **Tropical and Subtropical Fruits**. AgScience Inc., USA.

Kim, S., L. Jae-Rin, H. Sung-Tae, Y. Yung-Keun, A. Gynheung and K. Seong-Ryong. 2003. Molecular cloning and analysis of anthocyanin biosynthesis genes preferentially expressed in apple skin. **Plant Sci.** 165: 403-413.

Klempnauer, K.H., T.J. Gonda and J. Michael Bishop. 1982. Nucleotide sequence of the retroviral leukemia gene v-myb and its cellular progenitor c-myb: The architecture of a transduced oncogene. **Cell** 31: 453-463.

Kobayashi, S., M. Ishimaru, C.K. Ding, H. Yakushiji, and N. Goto. 2001. Comparison of UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) gene sequences between white grapes (*Vitis vinifera*) and their sports with red skin. **Plant Sci.** 160: 543-550.

_____ , _____ , K. Hiraoka and C. Honda. 2002. Myb-related genes of the Kyoho grape (Vitis labruscana) regulate anthocyanin biosynthesis. **Planta** 215: 924-933.

_____ , N. Goto-Yamamoto and H. Hirochika. 2004. Retrotransposon-induced mutations in grape skin color. **Science** 304: 982.

Koes, R., W. Verweij and F. Quattrocchio. 2005. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. **Trends Plant Sci.** 10: 236-242.

Kondo, S., K. Hiraoka, S. Kobayashi, C. Honda and N. Terahara. 2002. Changes in the expression of anthocyanin biosynthetic genes during apple development. **J. Amer. Soc. Hort. Sci.** 127: 971-976.

Konig, P., R. Giraldo, L. Chapman and D. Rhodes. 1996. The crystal structure of the DNA-binding domain of yeast RAP1 in complex with telomeric DNA. **Cell** 85: 125-136.

Kosiyachinda, S. and Tansiriyakul, S. 1988. Respiration rate and ethylene production of fresh fruits, vegetables and cut flowers. **J. Food.** 18: 1-10 (In Thai).

Kreuzaler, F., Ragg, H., Fauz, E., Kuhn, D.N. and K. Hahlbrock. 1983. UV-induction of chalcone synthase mRNA in cell suspension cultures of *Petroselinum hortense*. **PNAS** 80: 2591-2593.

Kristiansen, K.N. and W. Rohde. 1991. Structure of the *Hordeum vulgare* gene encoding dihydroflavonol-4-reductase and molecular analysis of ANT18 mutants blocked in flavonoid synthesis. **Mol. Gen. Genet.** 230: 49-59.

Krumlauf, R. 1994. Analysis of gene expression by Northern blot. **Mol. Biotechnol.** 2: 227-242.

Kumar, A. and B.E. Ellis. 2001. The phenylalanine ammonia-lyase gene family in raspberry: structure, expression, and evolution. **Plant Physiol.** 127: 230-239.

Kumar, S., K. Tamura and M. Nei. 2004. MEGA3: Integrated software for olecular evolutionary genetics analysis and sequence alignment. **Brief Bioinform**. 5: 150-163.

Lacombe, E., S. Hawkins, J.V. Doorsselaere, J. Piquemal, D. Goffner, O. Poeydomenge, A.M. Boudet and J. Grima-Pettenati. 1997. Cinnamoyl CoA reductase, the first committed enzyme of the lignin branch biosynthetic pathway: cloning, expression and phylogenetic relationships. **Plant J.** 11: 429-441.

Liew, C.F., C.J. Goh, C.S. Loh and S.H. Lim. 1998. Cloning and characterization of full-length cDNA clones encoding chalcone synthase from the orchid *Bromheadia finlaysoniana*. **Plant Physiol. Biochem.** 36:647-656.

Lipsick, J.S. 1996. One billion years of myb. **Oncogene** 13: 223-235.

Lister, C.E., J.E. Lancaster and J.R.L. Walker. 1996. Developmental changes in enzymes of flavonoid biosynthesis in the skins of red and green apple cultivars. **J. Sci. Food Agric.** 71: 313-330.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $^{\Delta\Delta Ct}$ method. **Methods**. 25: 402-408.

Lopez-Gomez, R. and M.A. Gomez-Lim. 1992. A method for extracting intact RNA from fruits rich in polysaccharides using ripe mango mesocarp. **HortScience** 27: 440–442.

Lorenz, W.W., R.O. McCann, M. Longiaru and M.J. Cormier. 1991. Isolation and expression of a cDNA encoding *Renilla reniformis* luciferase. **PNAS** 88: 4438-4442.

Macheix, J.J., A. Fleuriet and J. Billot. 1990. **Fruit Phenolics.** CRC Press, Inc., Boca Raton, Florida.

MacLean, D.D., D.P. Murr, J.R. DeEll and C.R. Horvath. 2006. Postharvest variation in apple (*Malus domestica* Borkh.) flavonoids following harvest, storage, and 1-MCP treatment. **J. Agr. Food Chem.** 4(3): 870-8.

Mahesh, V., J. Rakotomalala, L. Le Gal, H. Vigne, A. de Kochko, S. Hamon, M. Noirot and C. Campa. 2006. Isolation and genetic mapping of a *Coffea canephora* phenylalanine ammonia-lyase gene (CcPAL1) and its involvement in the accumulation of caffeoyl quinic acids. **Plant Cell Rep.** 25: 986-992.

Mandaokar, A. and J. Browse. 2009. MYB108 acts together with MYB24 to regulate jasmonate-mediated stamen maturation in *Arabidopsis*. **Plant Physiol.** 149: 851-862.

Marques, S.M. and J.C.G. Esteves da Silva. 2009. Firefly bioluminescence: A mechanistic approach of luciferase catalyzed reactions. **IUBMB Life**. 61: 6-17.

Martin, C. and J. Paz-Ares. 1997. MYB transcription factors in plants. **Trends Genet.** 13: 67-73.

_____, A. Prescott, S. Mackay, J. Bartlett and E. Vrijlandt. 1991. Control of anthocyanin biosynthesis in flowers of *Antirrhinum majus*. **Plant J.** 1: 37-49.

Martin, F.W. 1980. Durian and mangosteen, pp. 407-414. *In* S. Nagy and P.E. Shawx, eds. **Tropical and Subtropical Fruits : Composition, Properties and Uses.** The AVI Publishing, Westport, CN.

Matthews, J.C., K. Hori and M.J. Cormier. 1977. Purification and properties of Renilla reniformis luciferase. **Biochemistry** 16: 85-91.

Matus, J. T., F. Aquea and P. Arce-Johnson. 2008. Analysis of the grape MYB R2R3 subfamily reveals expanded wine quality-related clades and conserved gene structure organization across Vitis and *Arabidopsis* genomes. **BMC Plant Biol.** 8: 83.

Mengiste, T., X. Chen, J. Salmeron and R. Dietrich. 2003. The BOTRYTIS SUSCEPTIBLE1 gene encodes an R2R3MYB transcription factor protein that is required for biotic and abiotic stress responses in *Arabidopsis*. **Plant Cell** 15: 2551-2565.

Mercado-Silva, E., P. Benito-Bautista and M.A. Garcia-Velasco. 1998. Fruit development, harvest index and ripening changes of guavas produced in central Mexico. **Postharvest Biol. Technol.** 13: 143-150.

Mol, J., E. Grotewold and R. Koes. 1998. How genes paint flowers and seeds. **Trends Plant Sci.** 3: 212-217.

Montgomery, J., V. Pollard, J. Deikman and R.L. Fischer. 1993. Positive and negative regulatory regions control the spatial distribution of polygalacturonase transcription in tomato fruit pericarp. **Plant Cell** 5: 1049-1062.

Mori, S., H. Kobayashi, Y. Hoshi, M. Kondo and M. Nakano. 2004. Heterologous expression of the flavonoid 3',5'-hydroxylase gene of *Vinca major* alters flower color in transgenic *Petunia hybrida*. **Plant Cell Rep.** 22: 415-421.

Moyano, E., I. Portero-Robles, N. Medina-Esco-bar, V. Valpuesta, J. Muñoz-Blanco and J.L. Caballero. 1998. A fruit specific putative dihydroflavonol 4-reductase gene is differentially expressed in strawberry during the ripening process. **Plant Physiol.** 117: 711-716.

Muir, S.R., G.J. Collins, S. Robinson, S. Hughes, S. Bovy, C.H. De Vos, A.J. van Tunen and M.E. Verhoeyen. 2001. Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. **Nature Biotechnol.** 19: 470-474.

Murre, C., P.S. McCaw and D. Baltimore. 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. **Cell** 56: 777-783.

Nakajima, Y., T. Kimura, K. Sugata, T. Enomoto, A. Asakawa, H. Kubota, M. Ikeda and Y. Ohmiya. 2005. Multicolor luciferase assay system: one-step monitoring of multiple gene expressions with a single substrate. **BioTechniques** 38: 891-894.

Nakasone, H.Y. and R.E. Paull. 1998. **Tropical Fruits.** CAB International, Wallingford.

Naylor, L.H. 1999. Reporter gene technology: the future looks bright. **Biochem. Pharmacol.** 58: 749-757.

Nesi, N., C. Jond, I. Debeaujon, M. Caboche and L. Lepiniec. 2001. The *Arabidopsis* TT2 gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. **Plant Cell** 13: 2099-2114.

Noichinda, S. 1992. **Effect of modified atmosphere on quality and storage life of mangosteen (*Garcinia mangostana* L.) fruit.** M.S. thesis. Kasetsart University (In Thai).

_____, K. Bodhipadma, S. Singkhornart and S. Ketsa. 2007. Changes in pectic substances and cell wall hydrolase enzymes of mangosteen (*Garcinia mangostana*) fruit during storage. **NZ J. Crop Hort. Sci**. 35: 229-233.

Office of Agriculture Economics. 2009. **Export statistics: Mangosteen**. Available source: http://www.oae.go.th//export/13statistic01Mug.xls , February 10, 2009.

Ogata, K., H. Hojo, S. Aimoto, T. Nakai, H. Nakamura, A. Sarai, S. Ishii and Y. Nishimura. 1992. Solution structure of a DNA-binding unit of Myb: a helix-turn-helix-related motif with conserved tryptophans forming a hydrophobic core. **PNAS** 89: 6428-6432.

Osman, M.B.and A.R. Milan. 2006. **Mangosteen: *Garcinia mangostana* L.** Southampton Centre for Underutilised Crops, University of Southampton, Southampton, UK.

Pabo, C.O. and R.T. Sauer. 1992. Transcription factors: structural families and principles of DNA recognition. **Annu. Rev. Biochem.** 61: 1053-1095.

Palapol, Y., S. Ketsa, D. Stevenson, J.M. Cooney, A.C. Allan and I.B. Ferguson. 2009. Colour development and quality of mangosteen (*Garcinia mangostana* L.) fruit during ripening and after harvest. **Postharvest Biol. Technol.** 51: 349-353.

Paull, R.E. and S. Ketsa. 2004. Mangosteen. *In* Gross, K.C., C.Y. Wang and M.E. Salveit, eds. **The Commercial Storage of Fruits Vegetables and florist and Nursery Stocks**. USDA Agric. Handb. No. 66 (revised). Available source http://usna.usda.gov/hb66/092mangosteen.pdf, May 10, 2006.

Pelletier, M.K., I.E. Burbulis and B. Winkel-Shirley. 1999. Disruption of specific flavonoid genes enhances the accumulation of flavonoid enzymes and end-products in *Arabidopsis* seedlings. **Plant Mol. Biol.** 40: 45-54.

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. **Nucl. Acids Res.** 29: e45.

Piccaglia, R., M. Marotti and G. Baldoni. 2002. Factors influencing anthocyanin content in red cabbage (*Brassica oleracea* var. *capitata* L. f. rubra (L.) Thell.). **J. Sci. Food Agric**. 82: 1504-1509.

Piriyavinit, P. 2008. **Control of Ripening in Mangosteen (*Garcinia mangostana* L.) Fruit after Harvest**. M. S. Thesis. Kasetsart University (In Thai).

Popenoe, W. 1974. **Manual of Tropical and Subtropical Fruits.** Hafner Press, New York.

Quaedvlieg, N., J. Dockx, G. Keultjes, P. Kock, J. Wilmering, P. Weisbeek and S. Smeekens. 1996. Identification of a light-regulated MYB gene from an *Arabidopsis* transcription factor gene collection. **Plant Mol. Biol.** 32: 987-993.

Ralston, L., S. Subramanian, M. Matsuno and O. Yu. 2005. Partial reconstruction of flavonoid and isoflavonoid biosynthesis in yeast using soybean type I and type II chalcone isomerases. **Plant Physiol.** 137: 1375-1388.

Ramsay, N.A. and B.J. Glover. 2005. MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. **Trends Plant Sci.** 10: 63-70.

Ratanamarno, S. 1999. **Effects of light and temperature on pigment contents and phenylalanine ammonia-lyase (PAL) activity in mangosteen pericarp**. Ph.D. Thesis, Chiangmai University (in Thai).

_____, U. Chamnong and S. Kobkiat. 2005. Effects of bagging and storage temperature on anthocyanin content and phenylalanine ammonia-lyase (PAL) activity in mangosteen (*Garcinia mangostana* L.) fruit pericarp during maturing. **Songklanakarin J. Sci. Technol.** 27(4): 711-717.

Rein, M. J. 2005. **Copigmentation reactions and color stability of berry anthocyanins.** Available source: http://ethesis.helsinki.fi/julkaisut/maa/skemi/vk/rein. March 15, 2006.

Rogiers, S.Y. and N.R. Knowles. 1999. A comparison of preharvest and postharvest ethylene production and respiration rates of saskatoon (*Amelanchier alnifolia* Nutt.) fruit during development. **Can. J. Bot.** 77: 323-332.

_____ , G.N.M. Kumar and N.R. Knowles. 1998. Regulation of ethylene production and ripening by saskatoon (*Amelanchier alnifolia* Nutt.) fruit. **Can. J. Bot.** 76: 1743-1754.

Saito, K., M. Kobayashi, Z. Gong, Y. Tanaka and M. Yamazaki. 1999. Direct evidence for anthocyanidin synthase as a 2-oxoglutarate-dependent oxygenase: Molecular cloning and functional expression of cDNA from a red forma of *Perilla frutescens*. **Plant J.** 17: 181-189.

Sambrook, J. and D.W. Russel 2001. **Molecular Cloning: A Laboratory Manual.** 3rd eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Saure, M.C. 1990. External control of anthocyanin formation in apple. **Sci. Hortic.** 42: 181-218.

Schwinn, K., J. Venail, Y. Shang, S. Mackay, V. Alm, E. Butelli, R. Oyama, P. Bailey, K. Davies and C. Martin. 2006. A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus Antirrhinum. **Plant Cell** 18: 831-851.

Shin, Y., J.A. Ryu, R.H. Liu, J.F. Nock and C.B. Watkins. 2008. Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. **Postharvest Biol. Technol.** 49: 201-209.

Sherf B.A., S.L. Navarro, R.R. Hannah and K.V. Wood. 1996 Dual-luciferase reporter assay: an advanced co-reporter technology integrating firefly and *Renilla* luciferase assay. **Promega Notes** 57: 2.

Smith, T.F., C. Gaitatzes, K. Saxena and E.J. Neer. 1999. The WD repeat: a common architecture for diverse functions. **Trends Biochem. Sci.** 24: 181-185.

Solano, R., A. Stepanova, Q. Chao and J.R. Ecker. 1998. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. **Genes Dev.** 12: 3703-3714.

Solano, R., C. Nieto, J. Avila, L. Canas, I. Diaz and J. Paz-Ares. 1995. Dual DNA binding specificity of a petal epidermis-specific MYB transcription factor (MYB.Ph3) from Petunia hybrida. **EMBO J.** 14: 1773-1784.

Sparvoli, F., C. Martin, A. Scienza, G. Gavazzi and C. Tonelli. 1994. Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). **Plant Mol. Biol.** 24: 743-755.

Spayd, S.E., J.M. Tarara, D.L. Mee, and J.C. Ferguson. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. **Amer. J. Enol. Vitic.** 53: 171-182.

Stevenson, D.E., R. Wibisono, D.J. Jensen, R.A. Stanley and J.M. Cooney. 2006. Direct acylation of flavonoid glycosides with phenolic acids catalysed by *Candida antarctica* lipase B (Novozym 435®). **Enzyme Microb. Technol.** 39: 1236-1241.

Stracke, R., M. Werber and B. Weisshaar. 2001. The R2R3-MYB gene family in *Arabidopsis thaliana*. **Curr. Opin. Plant Biol.** 4: 447-456.

Streit, S., C.W. Michalski, M. Erkan, J. Kleeff and H. Friess. 2009. Northern blot analysis for detection and quantification of RNA in pancreatic cancer cells and tissues. **Nat. Protocols** 4: 37-43.

Takos, A.M., F.W. Jaffe, S.R. Jacob, J. Bogs, S.P. Robinson and A.R. Walker. 2006. Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples. **Plant Physiol.** 142: 1216-1232.

Tamagnone, L., A. Merida, A. Parr, S. Mackay, F.A. Culianez-Macia, K. Roberts and C. Martin. 1998. The AmMYB308 and AmMYB330 transcription factors from Antirrhinum regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. **Plant Cell** 10: 135-154.

Tanaka, Y., S. Tsuda and T. Kusumi. 1998. Metabolic engineering to modify flower color. **Plant Cell Physiol.** 39: 1119-1126.

Tira-Umphon, A., J. P. Roustan and C. Chervin. 2007. The stimulation by ethylene of the UDP glucose-flavonoid 3-O-glucosyltransferase (UFGT) in grape tissues is independent from the MybA transcription factors. **Vitis** 46: 210-211.

Tohge, T., Y. Nishiyama, M.Y. Hirai, M. Yano, J.I. Nakajima, M. Awazuhara, E. Inoue, H. Takahashi, D.B. Goodenowe, M. Kitayama, M. Noji, M. Yamazaki and K. Saito. 2005. Functional genomics by integrated analysis of metabolome and transcriptome of *Arabidopsis* plants over-expressing an MYB transcription factor. **Plant J.** 42: 218-235.

Toledo-Ortiz, G., E. Huq and P.H. Quail. 2003. The *Arabidopsis* Basic/Helix-Loop-Helix transcription factor family. **Plant Cell** 15: 1749-1770.

Tongdee, S.C. and A. Suwanagul. 1989. Postharvest mechanical damage in mangosteen. **ASEAN Food J**. 4(4): 151-155.

Tsuda, T., M. Yamaguchi, C. Honda and T. Moriguchi. 2004. Expression of anthocyanin biosynthesis genes in the skin of peach and nectarine fruit. **J. Amer. Soc. Hort. Sci.** 129: 857–862.

Ubi, B.E., C. Honda, H. Bessho, S. Kondo. M. Wada, S. Kobayashi, T. Moriguchi. 2006. Expression analysis of anthocyanin biosynthesis genes in apple skin: Effect of UV-B and temperature. **Plant Sci.** 170: 571-578.

VanGuilder, H.D., K.E. Vrana and W.M. Freeman. 2008. Twenty-five years of quantitative PCR for gene expression analysis. **BioTechniques** 44: 619-626.

Walker, A.R., E. Lee, J. Bogs, D.A.J. McDavid, M.R. Thomas and S.P. Robinson. 2007. White grapes arose through the mutation of two similar and adjacent regulatory genes. **Plant J.** 49: 772-785.

_____ , P.A. Davison, A.C. Bolognesi-Winfield, C.M. James, N. Srinivasan, T.L. Blundell, J.J. Esch, M.D. Marks and J.C. Gray. 1999. The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in *Arabidopsis*, encodes a WD40 repeat protein. **Plant Cell** 11: 1337-1349.

Wang, H., O. Arakawa and Y. Motomura. 2000. Influence of maturity and bagging on the relationship between anthocyanin accumulation and phenylalanine ammonia-lyase (PAL) activity in 'Jonathan' apples. **Postharvest Biol. Technol.** 19: 123-128.

Weston, K. 1998. Myb proteins in life, death and differentiation. **Curr. Opin. Genet. Dev.** 8: 76-81.

Wills, R., B. McGlasson, D. Graham and D. Joyce. 2007. **Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals** (5th ed.). UNSW Press. Sydney.

Wilson, T. and J.W. Hastings. 1998. BIOLUMINESCENCE. **Annu. Rev. Cell. Dev. Biol.** 14: 197-230.

Winkel-Shirley, B. 2001. Flavonoid biosynthesis. a colorful model for genetics, biochemistry, cell biology, and biotechnology. **Plant Physiol.** 126: 485-493.

Wood, K.V., J.R. de Wet, N. Dewji and M. DeLuca. 1984. Synthesis of active firefly luciferase by *in vitro* translation of RNA obtained from adult lanterns. **Biochem. Biophys. Res. Commun.** 124: 592-596.

Yamazaki, M., E. Yamagishi, Z. Gong, M. Fukuchi-Mizutani, Y. Fukui, Y. Tanaka, T. Kusumi, M. Yamaguchi and K. Saito. 2002. Two flavonoid glucosyltransferases from *Petunia hybrida*: molecular cloning, biochemical properties and developmentally regulated expression. **Plant Mol. Biol.** 48: 401-411.

Yapwattanaphum, C., Subhadrabanhu, S. Sugiura, A., Yonemori, K. and Utsunomiya, N. 2002. Utilisation of some *Garcinia* species in Thailand. **Acta Hort.** 575(2): 563-570.

Zhang, W., K. Chen, B. Zhang, C. Sun, C. Cai, C. Zhou, W. Xu, W. Zhang and I.B. Ferguson. 2005. Postharvest responses of Chinese bayberry fruit. **Postharvest Biol. Technol.** 37: 241-251.

Zimmermann, I.M., M.A. Heim, B. Weisshaar and J.F. Uhrig. 2004. Comprehensive identification of *Arabidopsis thaliana* MYB transcription factors interacting with R/B-like BHLH proteins. **Plant J.** 40: 22-34.

Zipper, H., H. Brunner, J. Bernhagen and F. Vitzthum. 2004. Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications. **Nucl. Acids Res.** 32: e103.

Zuker, A., T. Tzfira, H. Ben-Meir, M. Ovadis, E. Shklarman, H. Itzhaki, G. Forkmann, S. Martens, I. Neta-Sharir, D. Weiss and A. Vainstein. 2002. Modification of flower color and fragrance by antisense suppression of the flavanone 3-hydroxylase gene. **Mol. Breeding** 9: 33-41.

APPENDIX

Appendix Table 1 Colour development (L*, a*, b* and hue value) of mangosteen fruit harvested at stage 1 compared to stage 0.

Stage	L* value[1]	a* value[1,2]	b* value[1]	hue value[1]
0	68.8a	-7.9f	38.8a	101.5a
1	61.1b	1.0e	33.8b	88.1b
2	56.8c	11.8c	27.1c	66.4c
3	46.3d	21.7b	18.7d	40.8d
4	35.0e	30.1a	13.2e	23.7e
5	25.6f	17.8b	4.8f	15.1f
6	23.0f	4.9d	0.3g	3.7g
F-test	***	***	***	***

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

[2] The [1]values were x+100 transformed data and untransformed values were presented

***= significantly different at $P \leq 0.01$

Appendix Table 2 Fruit colour (L*, a*, b* and hue value) of mangosteen at stage 6 developed from 6 different maturity stages.

Stage	L* value[1]	a* value[1]	b* value[1]	hue value[1]
1	24.5	4.9ab	0.47	5.23
2	23.6	4.9ab	0.40	4.69
3	22.9	5.6a	0.47	4.54
4	23.1	4.6b	0..46	5.57
5	23.6	4.7b	0.49	5.94
6	23.0	3.7c	0.26	4.13
F-test	ns	**	ns	ns

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

** = significantly different at $P \leq 0.01$

Appendix Table 3 Ethylene production (mg kg^{-1} s^{-1}) of mangosteen fruit at stage 6 developed from 6 different maturity stages.

Days after harvest (days)	Ethylene production (mg kg^{-1} s^{-1})[1]
0	1.02l
0.25	1.59kl
0.5	2.13kl
0.75	2.63jk
1.0	3.39ij
1.25	3.51ij
1.5	4.36hi
1.75	4.91gh
2.0	5.05gh
2.25	5.44fgh
2.5	5.99efg
2.75	6.46def
3.0	6.91cde
3.5	7.68abc
4.0	7.70abc
4.5	8.17ab
5.0	8.49a
6	8.00abc
7	7.81abc
8	7.90abc
9	7.17bcd
F-test	***

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

***= significantly different at $P \leq 0.001$

Appendix Table 4 Total anthocyanin content (mg kg^{-1}) of mangosteen at stage 6 developed from 6 different maturity stages.

Stage	Outer pericarp[1]	Inner pericarp[1]
1	3550.3d	312.5c
2	3629.4d	331.9c
3	3765.2cd	407.4bc
4	4239.9ab	488.8ab
5	4050.2bc	385.8bc
6	4509.9a	544.8a
F-test	***	**

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 5 Fruit colour (hue value) of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	88.9	28.1b	22.5b	17.6b	13.8c	11.6c	12.9b	10.2bc	5.8c		
ethylene	88.9	28.0b	21.9b	17.5b	13.9c	10.9c	9.7b	6.8c			
1-MCP	88.9	78.3a	75.9a	65.8a	66.1a	59.1a	52.1a	45.5a	23.5a	12.7	8.1
E+M	88.9	29.2b	23.9b	23.4b	21.7d	21.0b	16.7b	17.9b	12.2b	10.3	5.6
F-test	ns	***	***	***	***	***	***	***	***	na	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 6 Colour index (score) of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	1.0	3.7a	4.2a	4.5b	4.9b	5.1a	5.2a	5.8b	6.0a		
ethylene	1.0	3.8a	4.3a	4.9a	5.1a	5.2a	5.9a	6.0a			
1-MCP	1.0	1.2b	1.3c	1.9d	2.0c	1.9c	2.3d	2.8d	4.9b	5.0	5.8
E+M	1.0	3.7a	3.9b	4.0c	4.2c	4.7b	4.7c	4.9c	5.4b	5.9	6.0
F-test	ns	***	***	***	***	***	***	***	***	na	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 7 Pericarp firmness (N) of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	815.0	105.3b	82.7b	65.8b	58.9a	49.1b	47.4b	47.9b	44.0a		
ethylene	815.0	97.2b	85.8b	68.4b	58.9a	57.4b	48.4b	42.9b			
1-MCP	815.0	224.0a	161.3a	103.9a	83.4a	73.1a	56.1ab	54.3a	47.4a	52.7	48.4
E+M	815.0	106.7b	101.0b	80.3b	73.4a	68.6a	66.7a	54.8a	50.7a	56.5	43.4
F-test	ns	***	**	**	ns	**	*	**	ns	na	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 8 Total anthocyanin content (mg kg^{-1}) of mangosteen fruit treated with the ethylene inhibitor 1-MCP following 4 treatments;1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	98.8	391.2a	582.3ab	832.6b	1307.0a	1630.5b	2131.2b	2944.8a	3428.8a		
ethylene	98.8	384.9a	667.0a	1284.4a	1312.4a	2028.7a	2491.1a	3239.8a			
1-MCP	98.8	131.5b	180.0c	262.0d	451.3b	590.5d	937.1c	967.7c	1893.3b	2192.0	2990.3
E+M	98.8	422.3a	463.0b	518.5c	695.1b	997.2c	1073.6c	1402.1b	2015.3b	3039.7	2979.4
F-test	ns	***	**	***	***	***	***	***	**	na	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 9 Fruit colour (hue value) of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	88.9	34.9	21.1	18.1	13.1	13.3	11.1	5.7	7.4		
15°C (7 d) + 25°C	88.9	67.3	65.5	69.7	68.6	58.2	54.0	48.3	19.7	16.5	5.9
t-test	ns	**	***	***	***	*	***	***	***		

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

*** = significantly different at $P \leq 0.001$

Appendix Table 10 Colour index (score) of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	1.0	3.4	4.2	4.9	5.0	5.0	5.5	5.9	6.0		
15°C (7 d) + 25°C	1.0	1.3	1.7	2.1	2.0	2.2	2.7	2.8	4.7	5.3	6.0
t-test	ns	***	***	***	***	***	***	***	***		

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

***= significantly different at $P \leq 0.001$

Appendix Table 11 Pericarp firmness (N) of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	815.0	132.2	75.3	65.0	61.2	52.2	59.1	43.8	45.7		
15°C (7 d) + 25°C	815.0	327.9	266.9	266.1	215.6	172.3	131.0	120.3	64.4	50.5	45.7
t-test	ns	**	*	***	*	*	**	***	**		

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

*** = significantly different at $P \leq 0.001$

Appendix Table 12 Total anthocyanin content (mg kg^{-1}) of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]										
	0	1	2	3	4	5	6	7	9	11	13
Air (control)	98.8	275.2	790.7	871.9	1334.0	1849.3	2386.6	2931.7	3250.7		
15°C (7 d) + 25°C	98.8	167.2	222.6	270.5	497.7	347.1	435.2	484.0	1340.5	2023.2	3240.6
F-test	ns	ns	ns	**	***	*	***	**	***		

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 13 *Cis*-acting element in *GmDFR* 5' flanking region using PLACE database.

Factor or sitename	Location	(Strand)	Signal sequence	SITE
-300ELEMENT	111	(+)	TGHAAARK	S000122
-300ELEMENT	1131	(+)	TGHAAARK	S000122
-300ELEMENT	1440	(+)	TGHAAARK	S000122
ABRELATERD1	37	(+)	ACGTG	S000414
ABRELATERD1	1786	(+)	ACGTG	S000414
ABRELATERD1	36	(-)	ACGTG	S000414
ABRELATERD1	1785	(-)	ACGTG	S000414
ABRERATCAL	36	(+)	MACGYGB	S000507
ABRERATCAL	1784	(-)	MACGYGB	S000507
ACGTABOX	1017	(+)	TACGTA	S000130
ACGTABOX	1017	(-)	TACGTA	S000130
ACGTATERD1	37	(+)	ACGT	S000415
ACGTATERD1	822	(+)	ACGT	S000415
ACGTATERD1	897	(+)	ACGT	S000415
ACGTATERD1	1018	(+)	ACGT	S000415
ACGTATERD1	1786	(+)	ACGT	S000415
ACGTATERD1	1801	(+)	ACGT	S000415
ACGTATERD1	1882	(+)	ACGT	S000415
ACGTATERD1	37	(-)	ACGT	S000415
ACGTATERD1	822	(-)	ACGT	S000415
ACGTATERD1	897	(-)	ACGT	S000415
ACGTATERD1	1018	(-)	ACGT	S000415
ACGTATERD1	1786	(-)	ACGT	S000415
ACGTATERD1	1801	(-)	ACGT	S000415
ACGTATERD1	1882	(-)	ACGT	S000415
AMYBOX1	1772	(-)	TAACARA	S000020
ANAERO2CONSENSUS	1709	(+)	AGCAGC	S000478
ANAERO3CONSENSUS	1329	(+)	TCATCAC	S000479
ARR1AT	1236	(+)	NGATT	S000454
ARR1AT	48	(+)	NGATT	S000454
ARR1AT	1140	(+)	NGATT	S000454
ARR1AT	1106	(+)	NGATT	S000454
ARR1AT	1664	(+)	NGATT	S000454
ARR1AT	265	(-)	NGATT	S000454
ARR1AT	386	(-)	NGATT	S000454
ARR1AT	484	(-)	NGATT	S000454
ARR1AT	563	(-)	NGATT	S000454
ARR1AT	614	(-)	NGATT	S000454
ARR1AT	800	(-)	NGATT	S000454
ARR1AT	928	(-)	NGATT	S000454
ARR1AT	1152	(-)	NGATT	S000454
ARR1AT	1215	(-)	NGATT	S000454
ARR1AT	1276	(-)	NGATT	S000454
ARR1AT	1343	(-)	NGATT	S000454
ARR1AT	1379	(-)	NGATT	S000454
ARR1AT	1633	(-)	NGATT	S000454
ASF1MOTIFCAMV	1802	(-)	TGACG	S000024
BIHD1OS	554	(+)	TGTCA	S000498
BIHD1OS	1055	(+)	TGTCA	S000498

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
BIHD1OS	1204	(+)	TGTCA	S000498
BIHD1OS	1944	(+)	TGTCA	S000498
BOXLCOREDCPAL	335	(+)	ACCWWCC	S000492
CAATBOX1	165	(+)	CAAT	S000028
CAATBOX1	483	(+)	CAAT	S000028
CAATBOX1	497	(+)	CAAT	S000028
CAATBOX1	562	(+)	CAAT	S000028
CAATBOX1	566	(+)	CAAT	S000028
CAATBOX1	586	(+)	CAAT	S000028
CAATBOX1	623	(+)	CAAT	S000028
CAATBOX1	911	(+)	CAAT	S000028
CAATBOX1	948	(+)	CAAT	S000028
CAATBOX1	1218	(+)	CAAT	S000028
CAATBOX1	1229	(+)	CAAT	S000028
CAATBOX1	1632	(+)	CAAT	S000028
CAATBOX1	1651	(+)	CAAT	S000028
CAATBOX1	1951	(+)	CAAT	S000028
CAATBOX1	50	(-)	CAAT	S000028
CAATBOX1	134	(-)	CAAT	S000028
CAATBOX1	499	(-)	CAAT	S000028
CAATBOX1	913	(-)	CAAT	S000028
CAATBOX1	1424	(-)	CAAT	S000028
CAATBOX1	1484	(-)	CAAT	S000028
CAATBOX1	1532	(-)	CAAT	S000028
CAATBOX1	1542	(-)	CAAT	S000028
CAATBOX1	1576	(-)	CAAT	S000028
CAATBOX1	1717	(-)	CAAT	S000028
CACGTGMOTIF	36	(+)	CACGTG	S000042
CACGTGMOTIF	1785	(+)	CACGTG	S000042
CACGTGMOTIF	36	(-)	CACGTG	S000042
CACGTGMOTIF	1785	(-)	CACGTG	S000042
CACTFTPPCA1	149	(+)	YACT	S000449
CACTFTPPCA1	278	(+)	YACT	S000449
CACTFTPPCA1	597	(+)	YACT	S000449
CACTFTPPCA1	709	(+)	YACT	S000449
CACTFTPPCA1	746	(+)	YACT	S000449
CACTFTPPCA1	1453	(+)	YACT	S000449
CACTFTPPCA1	1848	(+)	YACT	S000449
CACTFTPPCA1	103	(+)	YACT	S000449
CACTFTPPCA1	301	(+)	YACT	S000449
CACTFTPPCA1	404	(+)	YACT	S000449
CACTFTPPCA1	721	(+)	YACT	S000449
CACTFTPPCA1	1026	(+)	YACT	S000449
CACTFTPPCA1	1756	(+)	YACT	S000449
CACTFTPPCA1	1836	(+)	YACT	S000449
CACTFTPPCA1	116	(-)	YACT	S000449
CACTFTPPCA1	211	(-)	YACT	S000449
CACTFTPPCA1	454	(-)	YACT	S000449
CACTFTPPCA1	818	(-)	YACT	S000449
CACTFTPPCA1	842	(-)	YACT	S000449

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
CACTFTPPCA1	1009	(-)	YACT	S000449
CACTFTPPCA1	1339	(-)	YACT	S000449
CACTFTPPCA1	1475	(-)	YACT	S000449
CACTFTPPCA1	1546	(-)	YACT	S000449
CACTFTPPCA1	1549	(-)	YACT	S000449
CACTFTPPCA1	1660	(-)	YACT	S000449
CACTFTPPCA1	1695	(-)	YACT	S000449
CACTFTPPCA1	1698	(-)	YACT	S000449
CACTFTPPCA1	1779	(-)	YACT	S000449
CANBNNAPA	39	(-)	CNAACAC	S000148
CARGCW8GAT	599	(+)	CWWWWWWWWG	S000431
CARGCW8GAT	1526	(+)	CWWWWWWWWG	S000431
CARGCW8GAT	599	(-)	CWWWWWWWWG	S000431
CARGCW8GAT	1526	(-)	CWWWWWWWWG	S000431
CBFHV	1138	(-)	RYCGAC	S000497
CCA1ATLHCB1	1149	(+)	AAMAATCT	S000149
CCA1ATLHCB1	1273	(+)	AAMAATCT	S000149
CCAATBOX1	482	(+)	CCAAT	S000030
CCAATBOX1	496	(+)	CCAAT	S000030
CCAATBOX1	1950	(+)	CCAAT	S000030
CCAATBOX1	499	(-)	CCAAT	S000030
CCAATBOX1	1717	(-)	CCAAT	S000030
CIACADIANLELHC	140	(+)	CAANNNNATC	S000252
CIACADIANLELHC	478	(+)	CAANNNNATC	S000252
CIACADIANLELHC	146	(-)	CAANNNNATC	S000252
CPBCSPOR	1266	(+)	TATTAG	S000491
CPBCSPOR	1886	(+)	TATTAG	S000491
CURECORECR	300	(+)	GTAC	S000493
CURECORECR	403	(+)	GTAC	S000493
CURECORECR	908	(+)	GTAC	S000493
CURECORECR	300	(-)	GTAC	S000493
CURECORECR	403	(-)	GTAC	S000493
CURECORECR	908	(-)	GTAC	S000493
DOFCOREZM	114	(+)	AAAG	S000265
DOFCOREZM	436	(+)	AAAG	S000265
DOFCOREZM	452	(+)	AAAG	S000265
DOFCOREZM	726	(+)	AAAG	S000265
DOFCOREZM	793	(+)	AAAG	S000265
DOFCOREZM	1103	(+)	AAAG	S000265
DOFCOREZM	1135	(+)	AAAG	S000265
DOFCOREZM	1637	(+)	AAAG	S000265
DOFCOREZM	1644	(+)	AAAG	S000265
DOFCOREZM	1806	(+)	AAAG	S000265
DOFCOREZM	17	(-)	AAAG	S000265
DOFCOREZM	151	(-)	AAAG	S000265
DOFCOREZM	251	(-)	AAAG	S000265
DOFCOREZM	681	(-)	AAAG	S000265
DOFCOREZM	698	(-)	AAAG	S000265
DOFCOREZM	711	(-)	AAAG	S000265
DOFCOREZM	751	(-)	AAAG	S000265

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
DOFCOREZM	777	(-)	AAAG	S000265
DOFCOREZM	803	(-)	AAAG	S000265
DOFCOREZM	813	(-)	AAAG	S000265
DOFCOREZM	869	(-)	AAAG	S000265
DOFCOREZM	880	(-)	AAAG	S000265
DOFCOREZM	996	(-)	AAAG	S000265
DOFCOREZM	1164	(-)	AAAG	S000265
DOFCOREZM	1258	(-)	AAAG	S000265
DOFCOREZM	1368	(-)	AAAG	S000265
DOFCOREZM	1488	(-)	AAAG	S000265
DOFCOREZM	1758	(-)	AAAG	S000265
DOFCOREZM	1962	(-)	AAAG	S000265
DPBFCOREDCDC3	1784	(+)	ACACNNG	S000292
DPBFCOREDCDC3	36	(-)	ACACNNG	S000292
E2FCONSENSUS	1717	(+)	WTTSSCSS	S000476
EBOXBNNAPA	4	(+)	CANNTG	S000144
EBOXBNNAPA	36	(+)	CANNTG	S000144
EBOXBNNAPA	487	(+)	CANNTG	S000144
EBOXBNNAPA	497	(+)	CANNTG	S000144
EBOXBNNAPA	911	(+)	CANNTG	S000144
EBOXBNNAPA	1785	(+)	CANNTG	S000144
EBOXBNNAPA	1901	(+)	CANNTG	S000144
EBOXBNNAPA	4	(-)	CANNTG	S000144
EBOXBNNAPA	36	(-)	CANNTG	S000144
EBOXBNNAPA	487	(-)	CANNTG	S000144
EBOXBNNAPA	497	(-)	CANNTG	S000144
EBOXBNNAPA	911	(-)	CANNTG	S000144
EBOXBNNAPA	1785	(-)	CANNTG	S000144
EBOXBNNAPA	1901	(-)	CANNTG	S000144
EECCRCAH1	775	(+)	GANTTNC	S000494
EECCRCAH1	785	(+)	GANTTNC	S000494
EECCRCAH1	638	(-)	GANTTNC	S000494
EECCRCAH1	916	(-)	GANTTNC	S000494
EECCRCAH1	1340	(-)	GANTTNC	S000494
ELRECOREPCRP1	135	(+)	TTGACC	S000142
ELRECOREPCRP1	683	(+)	TTGACC	S000142
ELRECOREPCRP1	1425	(+)	TTGACC	S000142
ELRECOREPCRP1	1932	(+)	TTGACC	S000142
ELRECOREPCRP1	1869	(-)	TTGACC	S000142
ERELEE4	655	(+)	AWTTCAAA	S000037
ERELEE4	1536	(-)	AWTTCAAA	S000037
GARE1OSREP1	1772	(-)	TAACAGA	S000419
GARE2OSREP1	820	(+)	TAACGTA	S000420
GAREAT	1470	(+)	TAACAAR	S000439
GAREAT	226	(-)	TAACAAR	S000439
GATABOX	101	(+)	GATA	S000039
GATABOX	1015	(+)	GATA	S000039
GATABOX	1285	(+)	GATA	S000039
GATABOX	1407	(+)	GATA	S000039
GATABOX	1815	(+)	GATA	S000039

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
GATABOX	231	(-)	GATA	S000039
GATABOX	284	(-)	GATA	S000039
GATABOX	945	(-)	GATA	S000039
GATABOX	1046	(-)	GATA	S000039
GATABOX	1080	(-)	GATA	S000039
GATABOX	1447	(-)	GATA	S000039
GATABOX	1477	(-)	GATA	S000039
GT1CONSENSUS	179	(+)	GRWAAW	S000198
GT1CONSENSUS	547	(+)	GRWAAW	S000198
GT1CONSENSUS	715	(+)	GRWAAW	S000198
GT1CONSENSUS	1132	(+)	GRWAAW	S000198
GT1CONSENSUS	1242	(+)	GRWAAW	S000198
GT1CONSENSUS	1271	(+)	GRWAAW	S000198
GT1CONSENSUS	1293	(+)	GRWAAW	S000198
GT1CONSENSUS	1351	(+)	GRWAAW	S000198
GT1CONSENSUS	1352	(+)	GRWAAW	S000198
GT1CONSENSUS	1407	(+)	GRWAAW	S000198
GT1CONSENSUS	1441	(+)	GRWAAW	S000198
GT1CONSENSUS	1615	(+)	GRWAAW	S000198
GT1CONSENSUS	1736	(+)	GRWAAW	S000198
GT1CONSENSUS	221	(-)	GRWAAW	S000198
GT1CONSENSUS	415	(-)	GRWAAW	S000198
GT1CONSENSUS	568	(-)	GRWAAW	S000198
GT1CONSENSUS	1308	(-)	GRWAAW	S000198
GT1CONSENSUS	704	(-)	GRWAAW	S000198
GT1CONSENSUS	1078	(-)	GRWAAW	S000198
GT1CONSENSUS	1171	(-)	GRWAAW	S000198
GT1CONSENSUS	1172	(-)	GRWAAW	S000198
GT1CORE	448	(+)	GGTTAA	S000125
GT1CORE	1552	(+)	GGTTAA	S000125
GT1GMSCAM4	1132	(+)	GAAAAA	S000453
GT1GMSCAM4	1271	(+)	GAAAAA	S000453
GT1GMSCAM4	1441	(+)	GAAAAA	S000453
GT1GMSCAM4	1171	(-)	GAAAAA	S000453
GTGANTG10	117	(+)	GTGA	S000378
GTGANTG10	1788	(+)	GTGA	S000378
GTGANTG10	35	(-)	GTGA	S000378
GTGANTG10	148	(-)	GTGA	S000378
GTGANTG10	204	(-)	GTGA	S000378
GTGANTG10	318	(-)	GTGA	S000378
GTGANTG10	333	(-)	GTGA	S000378
GTGANTG10	556	(-)	GTGA	S000378
GTGANTG10	596	(-)	GTGA	S000378
GTGANTG10	1332	(-)	GTGA	S000378
GTGANTG10	1946	(-)	GTGA	S000378
HEXMOTIFTAH3H4	1801	(+)	ACGTCA	S000053
IBOX	282	(-)	GATAAG	S000124
IBOXCORE	1407	(+)	GATAA	S000199
IBOXCORE	230	(-)	GATAA	S000199
IBOXCORE	283	(-)	GATAA	S000199

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
IBOXCORE	1079	(-)	GATAA	S000199
IBOXCORENT	281	(-)	GATAAGR	S000424
INRNTPSADB	420	(+)	YTCANTYY	S000395
INRNTPSADB	326	(+)	YTCANTYY	S000395
INRNTPSADB	1500	(+)	YTCANTYY	S000395
INRNTPSADB	1540	(-)	YTCANTYY	S000395
INRNTPSADB	1818	(-)	YTCANTYY	S000395
MARTBOX	881	(+)	TTWTWTTWTT	S000067
MARTBOX	882	(+)	TTWTWTTWTT	S000067
MARTBOX	883	(+)	TTWTWTTWTT	S000067
MARTBOX	997	(+)	TTWTWTTWTT	S000067
MARTBOX	1165	(+)	TTWTWTTWTT	S000067
MARTBOX	1166	(+)	TTWTWTTWTT	S000067
MYB1AT	1551	(-)	WAACCA	S000408
MYB1LEPR	41	(+)	GTTAGTT	S000443
MYB1LEPR	64	(+)	GTTAGTT	S000443
MYB1LEPR	1467	(-)	GTTAGTT	S000443
MYB2AT	172	(+)	TAACTG	S000177
MYB2AT	1436	(+)	TAACTG	S000177
MYB2AT	1731	(+)	TAACTG	S000177
MYB2AT	62	(-)	TAACTG	S000177
MYB2AT	460	(-)	TAACTG	S000177
MYB2CONSENSUSAT	172	(+)	YAACKG	S000409
MYB2CONSENSUSAT	1436	(+)	YAACKG	S000409
MYB2CONSENSUSAT	1731	(+)	YAACKG	S000409
MYB2CONSENSUSAT	62	(-)	YAACKG	S000409
MYB2CONSENSUSAT	460	(-)	YAACKG	S000409
MYBCORE	62	(+)	CNGTTR	S000176
MYBCORE	460	(+)	CNGTTR	S000176
MYBCORE	1722	(+)	CNGTTR	S000176
MYBCORE	1773	(+)	CNGTTR	S000176
MYBCORE	172	(-)	CNGTTR	S000176
MYBCORE	1436	(-)	CNGTTR	S000176
MYBCORE	1731	(-)	CNGTTR	S000176
MYBPLANT	334	(+)	MACCWAMC	S000167
MYBPZM	336	(+)	CCWACC	S000179
MYBST1	231	(-)	GGATA	S000180
MYCATERD1	4	(-)	CATGTG	S000413
MYCATRD22	4	(+)	CACATG	S000174
MYCCONSENSUSAT	4	(+)	CANNTG	S000407
MYCCONSENSUSAT	36	(+)	CANNTG	S000407
MYCCONSENSUSAT	487	(+)	CANNTG	S000407
MYCCONSENSUSAT	497	(+)	CANNTG	S000407
MYCCONSENSUSAT	911	(+)	CANNTG	S000407
MYCCONSENSUSAT	1785	(+)	CANNTG	S000407
MYCCONSENSUSAT	1901	(+)	CANNTG	S000407
MYCCONSENSUSAT	4	(-)	CANNTG	S000407
MYCCONSENSUSAT	36	(-)	CANNTG	S000407
MYCCONSENSUSAT	487	(-)	CANNTG	S000407
MYCCONSENSUSAT	497	(-)	CANNTG	S000407

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
MYCCONSENSUSAT	911	(-)	CANNTG	S000407
MYCCONSENSUSAT	1785	(-)	CANNTG	S000407
MYCCONSENSUSAT	1901	(-)	CANNTG	S000407
NAPINMOTIFBN	1419	(+)	TACACAT	S000070
NODCON1GM	801	(-)	AAAGAT	S000461
NODCON1GM	1162	(-)	AAAGAT	S000461
NODCON1GM	1960	(-)	AAAGAT	S000461
NODCON2GM	280	(+)	CTCTT	S000462
NODCON2GM	537	(+)	CTCTT	S000462
NODCON2GM	679	(+)	CTCTT	S000462
NODCON2GM	696	(+)	CTCTT	S000462
NODCON2GM	867	(+)	CTCTT	S000462
NODCON2GM	878	(+)	CTCTT	S000462
NODCON2GM	1279	(+)	CTCTT	S000462
NTBBF1ARROLB	451	(-)	ACTTTA	S000273
OSE1ROOTNODULE	801	(-)	AAAGAT	S000467
OSE1ROOTNODULE	1162	(-)	AAAGAT	S000467
OSE1ROOTNODULE	1960	(-)	AAAGAT	S000467
OSE2ROOTNODULE	280	(+)	CTCTT	S000468
OSE2ROOTNODULE	537	(+)	CTCTT	S000468
OSE2ROOTNODULE	679	(+)	CTCTT	S000468
OSE2ROOTNODULE	696	(+)	CTCTT	S000468
OSE2ROOTNODULE	867	(+)	CTCTT	S000468
OSE2ROOTNODULE	878	(+)	CTCTT	S000468
OSE2ROOTNODULE	1279	(+)	CTCTT	S000468
PALBOXPPC	570	(+)	YTYYMMCMAMCMMC	S000136
POLASIG1	243	(+)	AATAAA	S000080
POLASIG1	1099	(+)	AATAAA	S000080
POLASIG1	1196	(+)	AATAAA	S000080
POLASIG1	1410	(+)	AATAAA	S000080
POLASIG1	92	(-)	AATAAA	S000080
POLASIG1	1001	(-)	AATAAA	S000080
POLASIG2	965	(+)	AATTAAA	S000081
POLASIG2	1392	(-)	AATTAAA	S000081
POLASIG3	624	(+)	AATAAT	S000088
POLASIG3	1059	(-)	AATAAT	S000088
POLASIG3	1120	(-)	AATAAT	S000088
POLASIG3	1208	(-)	AATAAT	S000088
POLASIG3	1529	(-)	AATAAT	S000088
POLASIG3	1953	(-)	AATAAT	S000088
POLLEN1LELAT52	762	(+)	AGAAA	S000245
POLLEN1LELAT52	1270	(+)	AGAAA	S000245
POLLEN1LELAT52	1614	(+)	AGAAA	S000245
POLLEN1LELAT52	18	(-)	AGAAA	S000245
POLLEN1LELAT52	788	(-)	AGAAA	S000245
POLLEN1LELAT52	1504	(-)	AGAAA	S000245
POLLEN1LELAT52	1925	(-)	AGAAA	S000245
PREATPRODH	1849	(+)	ACTCAT	S000450
PROLAMINBOXOSGLUB1	111	(+)	TGCAAAG	S000354
PYRIMIDINEBOXHVEPB1	1170	(+)	TTTTTTCC	S000298

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
PYRIMIDINEBOXOSRAMY1A	812	(+)	CCTTTT	S000259
PYRIMIDINEBOXOSRAMY1A	995	(+)	CCTTTT	S000259
PYRIMIDINEBOXOSRAMY1A	1102	(-)	CCTTTT	S000259
PYRIMIDINEBOXOSRAMY1A	1643	(-)	CCTTTT	S000259
RAV1AAT	573	(+)	CAACA	S000314
RAV1AAT	1511	(-)	CAACA	S000314
RAV1AAT	1930	(-)	CAACA	S000314
RHERPATEXPA7	310	(+)	KCACGW	S000512
RHERPATEXPA7	35	(+)	KCACGW	S000512
RHERPATEXPA7	1786	(-)	KCACGW	S000512
ROOTMOTIFTAPOX1	413	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	1265	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	1415	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	1583	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	1619	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	1938	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	943	(-)	ATATT	S000098
ROOTMOTIFTAPOX1	1179	(-)	ATATT	S000098
ROOTMOTIFTAPOX1	1414	(-)	ATATT	S000098
ROOTMOTIFTAPOX1	1445	(-)	ATATT	S000098
ROOTMOTIFTAPOX1	1618	(-)	ATATT	S000098
ROOTMOTIFTAPOX1	1857	(-)	ATATT	S000098
RYREPEATBNNAPA	635	(+)	CATGCA	S000264
S1FBOXSORPS1L21	297	(+)	ATGGTA	S000223
S1FBOXSORPS1L21	338	(-)	ATGGTA	S000223
SEBFCONSSTPR10A	553	(+)	YTGTCWC	S000391
SEBFCONSSTPR10A	1943	(+)	YTGTCWC	S000391
SEF1MOTIF	1410	(-)	ATATTTAWW	S000006
SEF4MOTIFGM7S	1050	(+)	RTTTTTR	S000103
SEF4MOTIFGM7S	755	(-)	RTTTTTR	S000103
SEF4MOTIFGM7S	1603	(-)	RTTTTTR	S000103
SEF4MOTIFGM7S	1965	(-)	RTTTTTR	S000103
IIATCYTC	1792	(+)	TGGGCY	S000474
IIATCYTC	1712	(-)	TGGGCY	S000474
IIATCYTC	1808	(-)	TGGGCY	S000474
IIATCYTC	216	(-)	TGGGCY	S000474
SORLIP1AT	503	(+)	GCCAC	S000482
SORLIP1AT	1451	(+)	GCCAC	S000482
SORLIP1AT	1846	(+)	GCCAC	S000482
SORLIP1AT	83	(-)	GCCAC	S000482
SORLIP2AT	1793	(+)	GGGCC	S000483
SORLIP2AT	216	(-)	GGGCC	S000483
SREATMSD	230	(+)	TTATCC	S000470
SURECOREATSULTR11	469	(-)	GAGAC	S000499
SURECOREATSULTR11	1891	(-)	GAGAC	S000499
SV40COREENHAN	1758	(-)	GTGGWWHG	S000123
TAAAGSTKST1	435	(+)	TAAAG	S000387
TAAAGSTKST1	451	(+)	TAAAG	S000387
TAAAGSTKST1	792	(+)	TAAAG	S000387

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
TAAAGSTKST1	1962	(-)	TAAAG	S000387
TATABOX2	600	(+)	TATAAAT	S000109
TATABOX2	1562	(+)	TATAAAT	S000109
TATABOX2	1356	(-)	TATAAAT	S000109
TATABOX3	1061	(+)	TATTAAT	S000110
TATABOX4	1359	(+)	TATATAA	S000111
TATABOX4	1859	(+)	TATATAA	S000111
TATABOX4	1186	(-)	TATATAA	S000111
TATABOX4	1358	(-)	TATATAA	S000111
TATABOX5	93	(+)	TTATTT	S000203
TATABOX5	1039	(+)	TTATTT	S000203
TATABOX5	1121	(+)	TTATTT	S000203
TATABOX5	242	(-)	TTATTT	S000203
TATABOX5	1098	(-)	TTATTT	S000203
TATABOXOSPAL	1156	(+)	TATTTAA	S000400
TATAPVTRNALEU	1357	(+)	TTTATATA	S000340
TATAPVTRNALEU	1859	(-)	TTTATATA	S000340
TATCCAOSAMY	231	(+)	TATCCA	S000403
TBOXATGAPB	150	(+)	ACTTTG	S000383
TBOXATGAPB	710	(+)	ACTTTG	S000383
TBOXATGAPB	113	(-)	ACTTTG	S000383
TBOXATGAPB	1636	(-)	ACTTTG	S000383
TGACGTVMAMY	1801	(-)	TGACGT	S000377
TGTCACACMCUCUMISIN	554	(+)	TGTCACA	S000422
TRANSINITDICOTS	1655	(-)	AMNAUGGC	S000201
TRANSINITMONOCOTS	1655	(-)	RMNAUGGC	S000202
UP1ATMSD	216	(+)	GGCCCAWWW	S000471
WBBOXPCWRKY1	682	(+)	TTTGACY	S000310
WBBOXPCWRKY1	772	(+)	TTTGACY	S000310
WBBOXPCWRKY1	237	(-)	TTTGACY	S000310
WBBOXPCWRKY1	1639	(-)	TTTGACY	S000310
WBOXATNPR1	135	(+)	TTGAC	S000390
WBOXATNPR1	683	(+)	TTGAC	S000390
WBOXATNPR1	773	(+)	TTGAC	S000390
WBOXATNPR1	1425	(+)	TTGAC	S000390
WBOXATNPR1	1932	(+)	TTGAC	S000390
WBOXATNPR1	238	(-)	TTGAC	S000390
WBOXATNPR1	1640	(-)	TTGAC	S000390
WBOXATNPR1	1803	(-)	TTGAC	S000390
WBOXATNPR1	1870	(-)	TTGAC	S000390
WBOXHVISO1	118	(+)	TGACT	S000442
WBOXHVISO1	491	(+)	TGACT	S000442
WBOXHVISO1	774	(+)	TGACT	S000442
WBOXHVISO1	1558	(+)	TGACT	S000442
WBOXHVISO1	237	(-)	TGACT	S000442
WBOXHVISO1	1639	(-)	TGACT	S000442
WBOXNTERF3	118	(+)	TGACY	S000457
WBOXNTERF3	136	(+)	TGACY	S000457
WBOXNTERF3	491	(+)	TGACY	S000457

Appendix Table 13 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
WBOXNTERF3	684	(+)	TGACY	S000457
WBOXNTERF3	774	(+)	TGACY	S000457
WBOXNTERF3	1426	(+)	TGACY	S000457
WBOXNTERF3	1558	(+)	TGACY	S000457
WBOXNTERF3	1933	(+)	TGACY	S000457
WBOXNTERF3	237	(-)	TGACY	S000457
WBOXNTERF3	1639	(-)	TGACY	S000457
WBOXNTERF3	9	(-)	TGACY	S000457
WBOXNTERF3	1249	(-)	TGACY	S000457
WBOXNTERF3	1869	(-)	TGACY	S000457
WRKY710S	118	(+)	TGAC	S000447
WRKY710S	136	(+)	TGAC	S000447
WRKY710S	491	(+)	TGAC	S000447
WRKY710S	684	(+)	TGAC	S000447
WRKY710S	774	(+)	TGAC	S000447
WRKY710S	1426	(+)	TGAC	S000447
WRKY710S	1558	(+)	TGAC	S000447
WRKY710S	1933	(+)	TGAC	S000447
WRKY710S	10	(-)	TGAC	S000447
WRKY710S	238	(-)	TGAC	S000447
WRKY710S	555	(-)	TGAC	S000447
WRKY710S	1056	(-)	TGAC	S000447
WRKY710S	1127	(-)	TGAC	S000447
WRKY710S	1205	(-)	TGAC	S000447
WRKY710S	1250	(-)	TGAC	S000447
WRKY710S	1640	(-)	TGAC	S000447
WRKY710S	1803	(-)	TGAC	S000447
WRKY710S	1870	(-)	TGAC	S000447
WRKY710S	1945	(-)	TGAC	S000447

Appendix Table 14 *Cis*-acting element in *GmLDOX* 5' flanking region using PLACE database.

Factor or sitename	Location	(Strand)	Signal sequence	SITE
ABRELATERD1	227	(+)	ACGTG	S000414
ABRELATERD1	311	(+)	ACGTG	S000414
ABRELATERD1	310	(-)	ACGTG	S000414
ABRERATCAL	226	(+)	MACGYGB	S000507
ABRERATCAL	310	(+)	MACGYGB	S000507
ACGTATERD1	227	(+)	ACGT	S000415
ACGTATERD1	311	(+)	ACGT	S000415
ACGTATERD1	227	(-)	ACGT	S000415
ACGTATERD1	311	(-)	ACGT	S000415
ARR1AT	98	(-)	NGATT	S000454
ARR1AT	318	(-)	NGATT	S000454
BIHD1OS	240	(+)	TGTCA	S000498
BIHD1OS	26	(-)	TGTCA	S000498
BOXCPSAS1	482	(+)	CTCCCAC	S000226
BOXLCOREDCPAL	332	(+)	ACCWWCC	S000492
BOXLCOREDCPAL	357	(+)	ACCWWCC	S000492
BOXLCOREDCPAL	439	(+)	ACCWWCC	S000492
BP5OSWX	225	(+)	CAACGTG	S000436
CAATBOX1	9	(+)	CAAT	S000028
CAATBOX1	238	(-)	CAAT	S000028
CACGTGMOTIF	310	(+)	CACGTG	S000042
CACGTGMOTIF	310	(-)	CACGTG	S000042
CACTFTPPCA1	16	(+)	YACT	S000449
CACTFTPPCA1	151	(+)	YACT	S000449
CACTFTPPCA1	388	(+)	YACT	S000449
CACTFTPPCA1	395	(+)	YACT	S000449
CACTFTPPCA1	143	(+)	YACT	S000449
CACTFTPPCA1	284	(+)	YACT	S000449
CACTFTPPCA1	72	(-)	YACT	S000449
CACTFTPPCA1	119	(-)	YACT	S000449
CACTFTPPCA1	137	(-)	YACT	S000449
CACTFTPPCA1	201	(-)	YACT	S000449
CACTFTPPCA1	346	(-)	YACT	S000449
CATATGGMSAUR	22	(+)	CATATG	S000370
CATATGGMSAUR	22	(-)	CATATG	S000370
CPBCSPOR	68	(+)	TATTAG	S000491
CURECORECR	142	(+)	GTAC	S000493
CURECORECR	142	(-)	GTAC	S000493
DOFCOREZM	63	(+)	AAAG	S000265
DOFCOREZM	165	(+)	AAAG	S000265
DOFCOREZM	420	(+)	AAAG	S000265
DOFCOREZM	501	(+)	AAAG	S000265
DOFCOREZM	18	(-)	AAAG	S000265
DOFCOREZM	132	(-)	AAAG	S000265
DOFCOREZM	153	(-)	AAAG	S000265
DOFCOREZM	171	(-)	AAAG	S000265
DPBFCOREDCDC3	310	(-)	ACACNNG	S000292
EBOXBNNAPA	22	(+)	CANNTG	S000144

Appendix Table 14 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
EBOXBNNAPA	310	(+)	CANNTG	S000144
EBOXBNNAPA	22	(-)	CANNTG	S000144
EBOXBNNAPA	310	(-)	CANNTG	S000144
EECCRCAH1	315	(-)	GANTTNC	S000494
EECCRCAH1	368	(-)	GANTTNC	S000494
GATABOX	66	(+)	GATA	S000039
GATABOX	88	(-)	GATA	S000039
GATABOX	177	(-)	GATA	S000039
GATABOX	404	(-)	GATA	S000039
GT1CONSENSUS	60	(+)	GRWAAW	S000198
GT1CONSENSUS	86	(-)	GRWAAW	S000198
GT1CONSENSUS	47	(-)	GRWAAW	S000198
GT1CONSENSUS	175	(-)	GRWAAW	S000198
GT1CORE	261	(-)	GGTTAA	S000125
GT1CORE	436	(-)	GGTTAA	S000125
GT1GMSCAM4	60	(+)	GAAAAA	S000453
GT1GMSCAM4	47	(-)	GAAAAA	S000453
GTGANTG10	296	(+)	GTGA	S000378
GTGANTG10	302	(-)	GTGA	S000378
GTGANTG10	309	(-)	GTGA	S000378
GTGANTG10	355	(-)	GTGA	S000378
IBOX	402	(-)	GATAAG	S000124
IBOXCORE	87	(-)	GATAA	S000199
IBOXCORE	176	(-)	GATAA	S000199
IBOXCORE	403	(-)	GATAA	S000199
LTRE1HVBLT49	81	(+)	CCGAAA	S000250
MARTBOX	493	(-)	TTWTWTTWTT	S000067
MARTBOX	494	(-)	TTWTWTTWTT	S000067
MYB1AT	184	(+)	WAACCA	S000408
MYB1AT	384	(+)	WAACCA	S000408
MYB1AT	206	(-)	WAACCA	S000408
MYBATRD22	206	(-)	CTAACCA	S000175
MYBCOREATCYCB1	190	(+)	AACGG	S000502
MYBPLANT	185	(+)	MACCWAMC	S000167
MYBPZM	329	(+)	CCWACC	S000179
MYBPZM	444	(+)	CCWACC	S000179
MYCCONSENSUSAT	22	(+)	CANNTG	S000407
MYCCONSENSUSAT	310	(+)	CANNTG	S000407
MYCCONSENSUSAT	22	(-)	CANNTG	S000407
MYCCONSENSUSAT	310	(-)	CANNTG	S000407
NODCON1GM	63	(+)	AAAGAT	S000461
NTBBF1ARROLB	152	(+)	ACTTTA	S000273
NTBBF1ARROLB	164	(-)	ACTTTA	S000273
OSE1ROOTNODULE	63	(+)	AAAGAT	S000467
POLLEN1LELAT52	269	(+)	AGAAA	S000245
POLLEN1LELAT52	367	(+)	AGAAA	S000245
POLLEN1LELAT52	49	(-)	AGAAA	S000245
REALPHALGLHCB21	185	(+)	AACCAA	S000362
RHERPATEXPA7	309	(+)	KCACGW	S000512
RHERPATEXPA7	227	(-)	KCACGW	S000512

Appendix Table 14 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
ROOTMOTIFTAPOX1	43	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	67	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	464	(-)	ATATT	S000098
SEF1MOTIF	460	(-)	ATATTTAWW	S000006
SEF4MOTIFGM7S	94	(-)	RTTTTTR	S000103
SURE2STPAT21	116	(-)	AATACTAAT	S000185
T/GBOXATPIN2	226	(+)	AACGTG	S000458
TAAAGSTKST1	164	(+)	TAAAG	S000387
TAAAGSTKST1	153	(-)	TAAAG	S000387
TATABOXOSPAL	461	(-)	TATTTAA	S000400
WBOXATNPR1	241	(-)	TTGAC	S000390
WBOXHVISO1	300	(-)	TGACT	S000442
WBOXNTCHN48	321	(+)	CTGACY	S000508
WBOXNTERF3	322	(+)	TGACY	S000457
WBOXNTERF3	300	(-)	TGACY	S000457
WRKY71OS	26	(+)	TGAC	S000447
WRKY71OS	322	(+)	TGAC	S000447
WRKY71OS	241	(-)	TGAC	S000447
WRKY71OS	301	(-)	TGAC	S000447
WUSATAg	458	(-)	TTAATGG	S000433

Appendix Table 15 *Cis*-acting element in *GmUFGT* 5' flanking region using PLACE database.

Factor or sitename	Location	(Strand)	Signal sequence	SITE
-300CORE	1085	(-)	TGTAAAG	S000001
-300ELEMENT	1084	(-)	TGHAAARK	S000122
-300ELEMENT	1221	(-)	TGHAAARK	S000122
ABRELATERD1	821	(+)	ACGTG	S000414
ABRELATERD1	996	(+)	ACGTG	S000414
ABRELATERD1	982	(-)	ACGTG	S000414
ABREOSRAB21	821	(+)	ACGTSSSC	S000012
ABRERATCAL	820	(+)	MACGYGB	S000507
ABRERATCAL	995	(+)	MACGYGB	S000507
ACGTABREMOTIFA2OSEM	996	(+)	ACGTGKC	S000394
ACGTATERD1	821	(+)	ACGT	S000415
ACGTATERD1	983	(+)	ACGT	S000415
ACGTATERD1	996	(+)	ACGT	S000415
ACGTATERD1	821	(-)	ACGT	S000415
ACGTATERD1	983	(-)	ACGT	S000415
ACGTATERD1	996	(-)	ACGT	S000415
ANAERO1CONSENSUS	1053	(-)	AAACAAA	S000477
ANAERO2CONSENSUS	777	(+)	AGCAGC	S000478
ARR1AT	46	(+)	NGATT	S000454
ARR1AT	1136	(+)	NGATT	S000454
ARR1AT	622	(+)	NGATT	S000454
ARR1AT	81	(-)	NGATT	S000454
ARR1AT	389	(-)	NGATT	S000454
ARR1AT	441	(-)	NGATT	S000454
ARR1AT	979	(-)	NGATT	S000454
ARR1AT	1022	(-)	NGATT	S000454
ARR1AT	1153	(-)	NGATT	S000454
ARR1AT	1237	(-)	NGATT	S000454
ARR1AT	1363	(-)	NGATT	S000454
ARR1AT	1464	(-)	NGATT	S000454
BIHD1OS	839	(+)	TGTCA	S000498
BIHD1OS	1374	(+)	TGTCA	S000498
BIHD1OS	207	(-)	TGTCA	S000498
BIHD1OS	1384	(-)	TGTCA	S000498
BOXCPSAS1	912	(+)	CTCCCAC	S000226
BOXIIPCCHS	996	(+)	ACGTGGC	S000229
BOXLCOREDCPAL	150	(+)	ACCWWCC	S000492
BP5OSWX	994	(+)	CAACGTG	S000436
BS1EGCCR	1185	(+)	AGCGGG	S000352
CAATBOX1	164	(+)	CAAT	S000028
CAATBOX1	336	(+)	CAAT	S000028
CAATBOX1	388	(+)	CAAT	S000028
CAATBOX1	449	(+)	CAAT	S000028
CAATBOX1	851	(+)	CAAT	S000028
CAATBOX1	958	(+)	CAAT	S000028
CAATBOX1	1021	(+)	CAAT	S000028
CAATBOX1	1173	(+)	CAAT	S000028
CAATBOX1	1213	(+)	CAAT	S000028
CAATBOX1	1331	(+)	CAAT	S000028

Appendix Table 15 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
CAATBOX1	1420	(+)	CAAT	S000028
CAATBOX1	544	(-)	CAAT	S000028
CAATBOX1	761	(-)	CAAT	S000028
CAATBOX1	925	(-)	CAAT	S000028
CAATBOX1	1010	(-)	CAAT	S000028
CAATBOX1	1120	(-)	CAAT	S000028
CAATBOX1	1138	(-)	CAAT	S000028
CACTFTPPCA1	210	(+)	YACT	S000449
CACTFTPPCA1	324	(+)	YACT	S000449
CACTFTPPCA1	931	(+)	YACT	S000449
CACTFTPPCA1	1401	(+)	YACT	S000449
CACTFTPPCA1	1436	(+)	YACT	S000449
CACTFTPPCA1	1443	(+)	YACT	S000449
CACTFTPPCA1	1458	(+)	YACT	S000449
CACTFTPPCA1	1471	(+)	YACT	S000449
CACTFTPPCA1	1475	(+)	YACT	S000449
CACTFTPPCA1	466	(+)	YACT	S000449
CACTFTPPCA1	476	(+)	YACT	S000449
CACTFTPPCA1	688	(+)	YACT	S000449
CACTFTPPCA1	799	(+)	YACT	S000449
CACTFTPPCA1	1176	(+)	YACT	S000449
CACTFTPPCA1	1208	(+)	YACT	S000449
CACTFTPPCA1	425	(-)	YACT	S000449
CACTFTPPCA1	474	(-)	YACT	S000449
CACTFTPPCA1	747	(-)	YACT	S000449
CACTFTPPCA1	902	(-)	YACT	S000449
CACTFTPPCA1	1270	(-)	YACT	S000449
CAREOSREP1	1405	(+)	CAACTC	S000421
CAREOSREP1	1447	(+)	CAACTC	S000421
CARGCW8GAT	287	(+)	CWWWWWWWWG	S000431
CARGCW8GAT	1366	(+)	CWWWWWWWWG	S000431
CARGCW8GAT	287	(-)	CWWWWWWWWG	S000431
CARGCW8GAT	1366	(-)	CWWWWWWWWG	S000431
CATATGGMSAUR	942	(+)	CATATG	S000370
CATATGGMSAUR	942	(-)	CATATG	S000370
CCAATBOX1	448	(+)	CCAAT	S000030
CCAATBOX1	957	(+)	CCAAT	S000030
CCAATBOX1	1212	(+)	CCAAT	S000030
CEREGLUBOX2PSLEGA	167	(+)	TGAAAACT	S000033
CGACGOSAMY3	705	(-)	CGACG	S000205
CPBCSPOR	551	(+)	TATTAG	S000491
CPBCSPOR	1129	(+)	TATTAG	S000491
CPBCSPOR	462	(-)	TATTAG	S000491
CURECORECR	475	(+)	GTAC	S000493
CURECORECR	1016	(+)	GTAC	S000493
CURECORECR	475	(-)	GTAC	S000493
CURECORECR	1016	(-)	GTAC	S000493
DOFCOREZM	20	(+)	AAAG	S000265
DOFCOREZM	224	(+)	AAAG	S000265
DOFCOREZM	481	(+)	AAAG	S000265

Appendix Table 15 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
DOFCOREZM	720	(+)	AAAG	S000265
DOFCOREZM	1501	(+)	AAAG	S000265
DOFCOREZM	405	(-)	AAAG	S000265
DOFCOREZM	528	(-)	AAAG	S000265
DOFCOREZM	535	(-)	AAAG	S000265
DOFCOREZM	662	(-)	AAAG	S000265
DOFCOREZM	732	(-)	AAAG	S000265
DOFCOREZM	796	(-)	AAAG	S000265
DOFCOREZM	952	(-)	AAAG	S000265
DOFCOREZM	1085	(-)	AAAG	S000265
DOFCOREZM	1222	(-)	AAAG	S000265
DOFCOREZM	1252	(-)	AAAG	S000265
DOFCOREZM	1288	(-)	AAAG	S000265
DOFCOREZM	1366	(-)	AAAG	S000265
DOFCOREZM	1493	(-)	AAAG	S000265
DPBFCOREDCDC3	348	(+)	ACACNNG	S000292
DPBFCOREDCDC3	1435	(+)	ACACNNG	S000292
E2FCONSENSUS	1057	(+)	WTTSSCSS	S000476
EBOXBNNAPA	309	(+)	CANNTG	S000144
EBOXBNNAPA	324	(+)	CANNTG	S000144
EBOXBNNAPA	517	(+)	CANNTG	S000144
EBOXBNNAPA	642	(+)	CANNTG	S000144
EBOXBNNAPA	942	(+)	CANNTG	S000144
EBOXBNNAPA	1099	(+)	CANNTG	S000144
EBOXBNNAPA	1197	(+)	CANNTG	S000144
EBOXBNNAPA	1436	(+)	CANNTG	S000144
EBOXBNNAPA	309	(-)	CANNTG	S000144
EBOXBNNAPA	324	(-)	CANNTG	S000144
EBOXBNNAPA	517	(-)	CANNTG	S000144
EBOXBNNAPA	642	(-)	CANNTG	S000144
EBOXBNNAPA	942	(-)	CANNTG	S000144
EBOXBNNAPA	1099	(-)	CANNTG	S000144
EBOXBNNAPA	1197	(-)	CANNTG	S000144
EBOXBNNAPA	1436	(-)	CANNTG	S000144
EECCRCAH1	804	(+)	GANTTNC	S000494
EECCRCAH1	1345	(+)	GANTTNC	S000494
EECCRCAH1	377	(-)	GANTTNC	S000494
ELRECOREPCRP1	303	(-)	TTGACC	S000142
ELRECOREPCRP1	1328	(-)	TTGACC	S000142
EMHVCHORD	1084	(-)	TGTAAAGT	S000452
GATABOX	87	(+)	GATA	S000039
GATABOX	968	(+)	GATA	S000039
GATABOX	1141	(+)	GATA	S000039
GATABOX	1143	(-)	GATA	S000039
GT1CONSENSUS	87	(+)	GRWAAW	S000198
GT1CONSENSUS	1261	(+)	GRWAAW	S000198
GT1CONSENSUS	48	(-)	GRWAAW	S000198
GT1CONSENSUS	1106	(-)	GRWAAW	S000198
GT1CONSENSUS	1396	(-)	GRWAAW	S000198
GT1CONSENSUS	953	(-)	GRWAAW	S000198

Appendix Table 15 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
GT1CORE	530	(-)	GGTTAA	S000125
GT1MOTIFPSRBCS	1394	(-)	KWGTGRWAAWRW	S000051
GTGANTG10	748	(+)	GTGA	S000378
GTGANTG10	976	(+)	GTGA	S000378
GTGANTG10	4	(-)	GTGA	S000378
GTGANTG10	213	(-)	GTGA	S000378
GTGANTG10	382	(-)	GTGA	S000378
GTGANTG10	398	(-)	GTGA	S000378
GTGANTG10	524	(-)	GTGA	S000378
GTGANTG10	538	(-)	GTGA	S000378
GTGANTG10	841	(-)	GTGA	S000378
GTGANTG10	930	(-)	GTGA	S000378
GTGANTG10	981	(-)	GTGA	S000378
GTGANTG10	1180	(-)	GTGA	S000378
GTGANTG10	1246	(-)	GTGA	S000378
GTGANTG10	1313	(-)	GTGA	S000378
GTGANTG10	1433	(-)	GTGA	S000378
GTGANTG10	1451	(-)	GTGA	S000378
GTGANTG10	1466	(-)	GTGA	S000378
GTGANTG10	1474	(-)	GTGA	S000378
GTGANTG10	1497	(-)	GTGA	S000378
IBOX	968	(+)	GATAAG	S000124
IBOXCORE	87	(+)	GATAA	S000199
IBOXCORE	968	(+)	GATAA	S000199
IBOXCORENT	968	(+)	GATAAGR	S000424
INRNTPSADB	1473	(+)	YTCANTYY	S000395
INRNTPSADB	929	(+)	YTCANTYY	S000395
INRNTPSADB	101	(-)	YTCANTYY	S000395
INRNTPSADB	122	(-)	YTCANTYY	S000395
INRNTPSADB	132	(-)	YTCANTYY	S000395
INRNTPSADB	1264	(-)	YTCANTYY	S000395
INTRONUPPER	881	(+)	MAGGTAAGT	S000085
L1BOXATPDF1	232	(+)	TAAATGYA	S000386
LECPLEACS2	669	(-)	TAAAATAT	S000465
LTRECOREATCOR15	1284	(+)	CCGAC	S000153
LTRECOREATCOR15	706	(-)	CCGAC	S000153
MARTBOX	1037	(+)	TTWTWTTWTT	S000067
MARTBOX	1038	(+)	TTWTWTTWTT	S000067
MARTBOX	1039	(+)	TTWTWTTWTT	S000067
MARTBOX	1040	(+)	TTWTWTTWTT	S000067
MARTBOX	1041	(+)	TTWTWTTWTT	S000067
MARTBOX	1042	(+)	TTWTWTTWTT	S000067
MARTBOX	1043	(+)	TTWTWTTWTT	S000067
MARTBOX	1044	(+)	TTWTWTTWTT	S000067
MARTBOX	1045	(+)	TTWTWTTWTT	S000067
MARTBOX	1046	(+)	TTWTWTTWTT	S000067
MYB1AT	11	(+)	WAACCA	S000408
MYB1AT	59	(+)	WAACCA	S000408
MYB1AT	1034	(-)	WAACCA	S000408
MYB26PS	564	(-)	GTTAGGTT	S000182

Appendix Table 15 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
MYBCORE	580	(+)	CNGTTR	S000176
MYBCORE	152	(-)	CNGTTR	S000176
MYBCORE	1323	(-)	CNGTTR	S000176
MYBPLANT	564	(+)	MACCWAMC	S000167
MYBPLANT	149	(+)	MACCWAMC	S000167
MYBPZM	151	(+)	CCWACC	S000179
MYBPZM	1277	(+)	CCWACC	S000179
MYBPZM	581	(-)	CCWACC	S000179
MYCATERD1	1197	(-)	CATGTG	S000413
MYCATRD22	1197	(+)	CACATG	S000174
MYCCONSENSUSAT	309	(+)	CANNTG	S000407
MYCCONSENSUSAT	324	(+)	CANNTG	S000407
MYCCONSENSUSAT	517	(+)	CANNTG	S000407
MYCCONSENSUSAT	642	(+)	CANNTG	S000407
MYCCONSENSUSAT	942	(+)	CANNTG	S000407
MYCCONSENSUSAT	1099	(+)	CANNTG	S000407
MYCCONSENSUSAT	1197	(+)	CANNTG	S000407
MYCCONSENSUSAT	1436	(+)	CANNTG	S000407
MYCCONSENSUSAT	309	(-)	CANNTG	S000407
MYCCONSENSUSAT	324	(-)	CANNTG	S000407
MYCCONSENSUSAT	517	(-)	CANNTG	S000407
MYCCONSENSUSAT	642	(-)	CANNTG	S000407
MYCCONSENSUSAT	942	(-)	CANNTG	S000407
MYCCONSENSUSAT	1099	(-)	CANNTG	S000407
MYCCONSENSUSAT	1197	(-)	CANNTG	S000407
MYCCONSENSUSAT	1436	(-)	CANNTG	S000407
NAPINMOTIFBN	503	(+)	TACACAT	S000070
NODCON1GM	1364	(-)	AAAGAT	S000461
NODCON2GM	485	(+)	CTCTT	S000462
NODCON2GM	660	(+)	CTCTT	S000462
NODCON2GM	1491	(+)	CTCTT	S000462
NODCON2GM	1164	(-)	CTCTT	S000462
NTBBF1ARROLB	951	(+)	ACTTTA	S000273
NTBBF1ARROLB	1084	(+)	ACTTTA	S000273
OSE1ROOTNODULE	1364	(-)	AAAGAT	S000467
OSE2ROOTNODULE	485	(+)	CTCTT	S000468
OSE2ROOTNODULE	660	(+)	CTCTT	S000468
OSE2ROOTNODULE	1491	(+)	CTCTT	S000468
OSE2ROOTNODULE	1164	(-)	CTCTT	S000468
POLASIG1	959	(+)	AATAAA	S000080
POLASIG1	1332	(+)	AATAAA	S000080
POLASIG1	1291	(-)	AATAAA	S000080
POLASIG1	1368	(-)	AATAAA	S000080
POLASIG1	1393	(-)	AATAAA	S000080
POLASIG3	872	(-)	AATAAT	S000088
POLLEN1LELAT52	65	(+)	AGAAA	S000245
POLLEN1LELAT52	989	(+)	AGAAA	S000245
POLLEN1LELAT52	793	(-)	AGAAA	S000245
PREATPRODH	514	(+)	ACTCAT	S000450
PREATPRODH	1267	(-)	ACTCAT	S000450

Appendix Table 15 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
PRECONSCRHSP70A	687	(-)	SCGAYNRNNNNNNNNNNNNNNHD	S000506
QELEMENTZMZM13	264	(+)	AGGTCA	S000254
QELEMENTZMZM13	302	(+)	AGGTCA	S000254
QELEMENTZMZM13	1327	(+)	AGGTCA	S000254
QELEMENTZMZM13	248	(-)	AGGTCA	S000254
RAV1AAT	278	(+)	CAACA	S000314
RAV1AAT	306	(+)	CAACA	S000314
RAV1AAT	635	(+)	CAACA	S000314
RAV1AAT	646	(-)	CAACA	S000314
RAV1AAT	763	(-)	CAACA	S000314
REALPHALGLHCB21	60	(+)	AACCAA	S000362
REALPHALGLHCB21	1231	(+)	AACCAA	S000362
RHERPATEXPA7	981	(+)	KCACGW	S000512
RHERPATEXPA7	1246	(+)	KCACGW	S000512
RHERPATEXPA7	821	(-)	KCACGW	S000512
ROOTMOTIFTAPOX1	669	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	1426	(+)	ATATT	S000098
ROOTMOTIFTAPOX1	1421	(-)	ATATT	S000098
SEBFCONSSTPR10A	838	(+)	YTGTCWC	S000391
SEF3MOTIFGM	112	(+)	AACCCA	S000115
SEF4MOTIFGM7S	1303	(+)	RTTTTTR	S000103
SEF4MOTIFGM7S	1390	(+)	RTTTTTR	S000103
SEF4MOTIFGM7S	904	(-)	RTTTTTR	S000103
SEF4MOTIFGM7S	1334	(-)	RTTTTTR	S000103
SITEIIATCYTC	1167	(-)	TGGGCY	S000474
SORLIP1AT	147	(+)	GCCAC	S000482
SORLIP1AT	1195	(+)	GCCAC	S000482
SORLIP1AT	998	(-)	GCCAC	S000482
SORLIP1AT	1271	(-)	GCCAC	S000482
SORLIP2AT	318	(-)	GGGCC	S000483
SURECOREATSULTR11	395	(-)	GAGAC	S000499
T/GBOXATPIN2	820	(+)	AACGTG	S000458
T/GBOXATPIN2	995	(+)	AACGTG	S000458
T/GBOXATPIN2	982	(-)	AACGTG	S000458
TAAAGSTKST1	223	(+)	TAAAG	S000387
TAAAGSTKST1	528	(-)	TAAAG	S000387
TAAAGSTKST1	796	(-)	TAAAG	S000387
TAAAGSTKST1	952	(-)	TAAAG	S000387
TAAAGSTKST1	1085	(-)	TAAAG	S000387
TATABOX2	427	(+)	TATAAAT	S000109
TATABOX2	875	(-)	TATAAAT	S000109
TATABOX4	665	(-)	TATATAA	S000111
TATABOX5	488	(+)	TTATTT	S000203
TATABOX5	873	(+)	TTATTT	S000203
TATABOX5	1066	(+)	TTATTT	S000203
TATABOX5	1292	(+)	TTATTT	S000203
TATABOX5	1369	(+)	TTATTT	S000203
TATABOXOSPAL	289	(-)	TATTTAA	S000400
TATAPVTRNALEU	664	(+)	TTTATATA	S000340
WBBOXPCWRKY1	1254	(+)	TTTGACY	S000310

Appendix Table 15 (Continued).

Factor or sitename	Location	(Strand)	Signal sequence	SITE
WBBOXPCWRKY1	1306	(+)	TTTGACY	S000310
WBBOXPCWRKY1	1004	(-)	TTTGACY	S000310
WBOXATNPR1	1255	(+)	TTGAC	S000390
WBOXATNPR1	1307	(+)	TTGAC	S000390
WBOXATNPR1	1383	(+)	TTGAC	S000390
WBOXATNPR1	276	(-)	TTGAC	S000390
WBOXATNPR1	304	(-)	TTGAC	S000390
WBOXATNPR1	1005	(-)	TTGAC	S000390
WBOXATNPR1	1329	(-)	TTGAC	S000390
WBOXHVISO1	1082	(+)	TGACT	S000442
WBOXHVISO1	1256	(+)	TGACT	S000442
WBOXHVISO1	1308	(+)	TGACT	S000442
WBOXHVISO1	1344	(+)	TGACT	S000442
WBOXHVISO1	275	(-)	TGACT	S000442
WBOXHVISO1	380	(-)	TGACT	S000442
WBOXHVISO1	1004	(-)	TGACT	S000442
WBOXHVISO1	1299	(-)	TGACT	S000442
WBOXNTERF3	248	(+)	TGACY	S000457
WBOXNTERF3	1082	(+)	TGACY	S000457
WBOXNTERF3	1256	(+)	TGACY	S000457
WBOXNTERF3	1308	(+)	TGACY	S000457
WBOXNTERF3	1344	(+)	TGACY	S000457
WBOXNTERF3	275	(-)	TGACY	S000457
WBOXNTERF3	380	(-)	TGACY	S000457
WBOXNTERF3	1004	(-)	TGACY	S000457
WBOXNTERF3	1299	(-)	TGACY	S000457
WBOXNTERF3	265	(-)	TGACY	S000457
WBOXNTERF3	303	(-)	TGACY	S000457
WBOXNTERF3	1328	(-)	TGACY	S000457
WRKY710S	207	(+)	TGAC	S000447
WRKY710S	248	(+)	TGAC	S000447
WRKY710S	1082	(+)	TGAC	S000447
WRKY710S	1256	(+)	TGAC	S000447
WRKY710S	1308	(+)	TGAC	S000447
WRKY710S	1344	(+)	TGAC	S000447
WRKY710S	1384	(+)	TGAC	S000447
WRKY710S	266	(-)	TGAC	S000447
WRKY710S	276	(-)	TGAC	S000447
WRKY710S	304	(-)	TGAC	S000447
WRKY710S	381	(-)	TGAC	S000447
WRKY710S	840	(-)	TGAC	S000447
WRKY710S	1005	(-)	TGAC	S000447
WRKY710S	1300	(-)	TGAC	S000447
WRKY710S	1329	(-)	TGAC	S000447
WRKY710S	1375	(-)	TGAC	S000447
WUSATAg	228	(-)	TTAATGG	S000433

Appendix Table 16 *Cis*-acting element in *GmDFR* 5' flanking region using PlantCARE database.

3-AF1 binding site (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
3-AF1 binding site	*Solanum tuberosum*	1272	-	10	AAGAGATATTT
AAGAA-motif					
Site Name	Organism	Position	Strand	Matrix score.	sequence
AAGAA-motif	*Avena sativa*	748	-	7	GAAAGAA
AAGAA-motif	*Avena sativa*	1365	-	7	GAAAGAA
ABRE (function: *cis*-acting element involved in the abscisic acid responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ABRE	*Arabidopsis thaliana*	35	+	6	CACGTG
ABRE	*Arabidopsis thaliana*	1784	+	6	CACGTG
ABRE	*Hordeum vulgare*	77	+	9	CCTACGTGGC
AC-II					
Site Name	Organism	Position	Strand	Matrix score.	sequence
AC-II	*Phaseolus vulgaris*	569	+	9.5	(C/T)T(T/C)(C/T) (A/C)(A/C)C(A/C) A (A/C)C(C/A) (C/A)C
ACE (function: *cis*-acting element involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ACE	*Petroselinum crispum*	990	-	9	AAAACGTTTA
ACE	*Petroselinum crispum*	1014	+	9	GACACGTATG
ATGCAAAT motif (function: *cis*-acting regulatory element associated to the TGAGTCA motif)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ATGCAAAT motif	*Oryza sativa*	1040	-	8	ATACAAAT
Box 4 (function: part of a conserved DNA module involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Box 4	*Petroselinum crispum*	961	+	6	ATTAAT
Box 4	*Petroselinum crispum*	1375	+	6	ATTAAT
Box 4	Petroselinum crispum	1061	+	6	ATTAAT

Appendix Table 16 (Continued).

Box I (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Box I	*Pisum sativum*	751	+	7	TTTCAAA
Box I	*Pisum sativum*	1535	-	7	TTTCAAA
Box I	*Pisum sativum*	1238	-	7	TTTCAAA
Box I	*Pisum sativum*	804	-	7	TTTCAAA
Box I	*Pisum sativum*	1368	+	7	TTTCAAA
Box II (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Box II	*Pisum sativum*	412	-	11	GTGAGGTAATAT
Box-W1 (function: fungal elicitor responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Box-W1	*Petroselinum crispum*	134	+	6	TTGACC
Box-W1	*Petroselinum crispum*	1931	+	6	TTGACC
Box-W1	*Petroselinum crispum*	1424	+	6	TTGACC
Box-W1	*Petroselinum crispum*	682	+	6	TTGACC
Box-W1	*Petroselinum crispum*	1868	-	6	TTGACC
CAAT-box (function: common *cis*-acting element in promoter and enhancer regions)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CAAT-box	*Hordeum vulgare*	49	-	4	CAAT
CAAT-box	*Hordeum vulgare*	133	-	4	CAAT
CAAT-box	*Glycine max*	164	+	5	CAATT
CAAT-box	*Brassica rapa*	187	-	5	CAAAT
CAAT-box	*Brassica rapa*	367	+	5	CAAAT
CAAT-box	*Brassica rapa*	398	-	5	CAAAT
CAAT-box	*Arabidopsis thaliana*	481	+	5	CCAAT
CAAT-box	*Hordeum vulgare*	482	+	4	CAAT
CAAT-box	*Arabidopsis thaliana*	495	+	5	CCAAT
CAAT-box	*Glycine max*	496	+	5	CAATT
CAAT-box	*Glycine max*	497	-	5	CAATT
CAAT-box	*Arabidopsis thaliana*	498	-	5	CCAAT
CAAT-box	*Brassica rapa*	550	-	5	CAAAT
CAAT-box	*Hordeum vulgare*	561	+	4	CAAT
CAAT-box	*Glycine max*	565	+	5	CAATT
CAAT-box	*Glycine max*	585	+	5	CAATT

Appendix Table 16 (Continued).

CAAT-box (function: common *cis*-acting element in promoter and enhancer regions)

Site Name	Organism	Position	Strand	Matrix score.	sequence
CAAT-box	*Hordeum vulgare*	622	+	4	CAAT
CAAT-box	*Daucus carota*	648	+	11	AGCTCAATTTCA
CAAT-box	*Brassica rapa*	652	+	5	CAAAT
CAAT-box	*Brassica rapa*	770	-	5	CAAAT
CAAT-box	*Brassica rapa*	797	+	5	CAAAT
CAAT-box	*Brassica rapa*	835	-	5	CAAAT
CAAT-box	*Glycine max*	910	+	5	CAATT
CAAT-box	*Glycine max*	911	-	5	CAATT
CAAT-box	*Hordeum vulgare*	912	-	4	CAAT
CAAT-box	*Brassica rapa*	940	+	5	CAAAT
CAAT-box	*Hordeum vulgare*	947	+	4	CAAT
CAAT-box	*Brassica rapa*	984	+	5	CAAAT
CAAT-box	*Brassica rapa*	1040	-	5	CAAAT
CAAT-box	*Brassica rapa*	1065	-	5	CAAAT
CAAT-box	*Brassica rapa*	1122	-	5	CAAAT
CAAT-box	*Brassica rapa*	1176	+	5	CAAAT
CAAT-box	*Hordeum vulgare*	1217	+	4	CAAT
CAAT-box	*Hordeum vulgare*	1228	+	4	CAAT
CAAT-box	*Brassica rapa*	1237	-	5	CAAAT
CAAT-box	*Brassica rapa*	1322	-	5	CAAAT
CAAT-box	*Hordeum vulgare*	1423	-	4	CAAT
CAAT-box	*Hordeum vulgare*	1483	-	4	CAAT
CAAT-box	*Brassica rapa*	1525	+	5	CAAAT
CAAT-box	*Hordeum vulgare*	1531	-	4	CAAT
CAAT-box	*Glycine max*	1540	-	5	CAATT
CAAT-box	*Hordeum vulgare*	1541	-	4	CAAT
CAAT-box	*Hordeum vulgare*	1575	-	4	CAAT
CAAT-box	*Brassica rapa*	1584	-	5	CAAAT
CAAT-box	*Brassica rapa*	1620	-	5	CAAAT
CAAT-box	*Hordeum vulgare*	1650	+	4	CAAT
CAAT-box	*Arabidopsis thaliana*	1716	-	5	CCAAT
CAAT-box	*Brassica rapa*	1739	-	5	CAAAT
CAAT-box	*Brassica rapa*	1901	-	5	CAAAT
CAAT-box	*Arabidopsis thaliana*	1949	+	5	CCAAT
CAAT-box	*Glycine max*	1950	+	5	CAATT

Appendix Table 16 (Continued).

CAT-box (function: *cis*-acting regulatory element related to meristem expression)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CAT-box	*Arabidopsis thaliana*	1450	+	6	GCCACT
CAT-box	*Arabidopsis thaliana*	1845	+	6	GCCACT
CATT-motif (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CATT-motif	*Zea mays*	199	+	6	GCATTC
CGTCA-motif (function: *cis*-acting regulatory element involved in the MeJA-responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CGTCA-motif	*Hordeum vulgare*	1801	+	5	CGTCA
ERE (function: ethylene-responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ERE	*Dianthus caryophyllus*	1535	-	8	ATTTCAAA
G-Box (function: *cis*-acting regulatory element involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
G-Box	*Pisum sativum*	35	+	6	CACGTG
G-Box	*Pisum sativum*	1784	+	6	CACGTG
G-box (function: *cis*-acting regulatory element involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
G-box	*Solanum tuberosum*	3	+	7	CACATGG
G-box	*Nicotiana plumbaginifolia*	851	+	10	CAGACGTGGCA
G-box	*Arabidopsis thaliana*	35	+	6	CACGTG
G-box	*Arabidopsis thaliana*	1784	+	6	CACGTG
GA-motif (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GA-motif	*Helianthus annuus*	1957	-	8	AAAGATGA
GAG-motif (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GAG-motif	*Spinacia oleracea*	256	-	7	AGAGATG
GAG-motif	*Arabidopsis thaliana*	781	+	7	AGAGAGT

Appendix Table 16 (Continued).

GARE-motif (function: gibberellin-responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GARE-motif	*Brassica oleracea*	757	+	7	AAACAGA
GARE-motif	*Brassica oleracea*	848	+	7	AAACAGA
GCN4_motif (function: *cis*-regulatory element involved in endosperm expression)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GCN4_motif	*Oryza sativa*	477	+	7	CAAGCCA
GT1-motif (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GT1-motif	*Arabidopsis thaliana*	447	+	6	GGTTAA
GT1-motif	*Avena sativa*	1551	+	7	GGTTAAT
GT1-motif	*Solanum tuberosum*	574	-	10	ATGGTGGTTGG
HSE (function: element involved in heat stress responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
HSE	*Brassica oleracea*	1271	+	9	AAAAAATTTC
L-box (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
L-box	*Petroselinum crispum*	330	+	10	TCTCACCTACC
MBS (function: MYB binding site involved in drought-inducibility)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
MBS	*Arabidopsis thaliana*	61	-	6	TAACTG
MBS	*Arabidopsis thaliana*	1730	+	6	TAACTG
MBS	*Arabidopsis thaliana*	459	-	6	TAACTG
MBS	*Arabidopsis thaliana*	171	+	6	TAACTG
MBS	*Arabidopsis thaliana*	1435	+	6	TAACTG
P-box (function: gibberellin-responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
P-box	*Oryza sativa*	1641	-	7	CCTTTTG

Appendix Table 16 (Continued).

Skn-1_motif (function: *cis*-acting regulatory element required for endosperm expression)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Skn-1_motif	*Oryza sativa*	9	+	5	GTCAT
Skn-1_motif	*Oryza sativa*	1204	+	5	GTCAT
Skn-1_motif	*Oryza sativa*	1055	+	5	GTCAT
Skn-1_motif	*Oryza sativa*	1556	-	5	GTCAT
Skn-1_motif	*Oryza sativa*	489	-	5	GTCAT
Skn-1_motif	*Oryza sativa*	1249	+	5	GTCAT
Skn-1_motif	*Oryza sativa*	1126	+	5	GTCAT
Sp1 (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Sp1	*Zea mays*	979	+	5.5	CC(G/A)CCC
Sp1	*Zea mays*	1428	+	5.5	CC(G/A)CCC
TATA-box (function: core promoter element around -30 of transcription start)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Lycopersicon esculentum*	90	+	5	TTTTA
TATA-box	*Arabidopsis thaliana*	121	+	4	TATA
TATA-box	*Brassica napus*	410	+	6	ATATAT
TATA-box	*Arabidopsis thaliana*	411	+	4	TATA
TATA-box	*Glycine max*	413	-	5	TAATA
TATA-box	*Glycine max*	463	+	5	TAATA
TATA-box	*Nicotiana tabacum*	597	+	9	tcTATAAAta
TATA-box	*Ac*	599	+	7	TATAAAT
TATA-box	*Lycopersicon esculentum*	723	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	813	+	5	TTTTA
TATA-box	*Oryza sativa*	922	+	7	TACAAAA
TATA-box	*Arabidopsis thaliana*	931	-	5	TATAA
TATA-box	*Arabidopsis thaliana*	932	+	4	TATA
TATA-box	*Lycopersicon esculentum*	999	+	5	TTTTA
TATA-box	*Glycine max*	1002	-	5	TAATA
TATA-box	*Brassica napus*	1003	+	6	ATTATA
TATA-box	*Arabidopsis thaliana*	1004	-	5	TATAA
TATA-box	*Arabidopsis thaliana*	1010	+	4	TATA
TATA-box	*Glycine max*	1060	-	5	TAATA
TATA-box	*Lycopersicon esculentum*	1076	+	5	TTTTA

Appendix Table 16 (Continued).

TATA-box (function: core promoter element around -30 of transcription start)

Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Lycopersicon esculentum*	1100	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	1143	-	5	TTTTA
TATA-box	*Avena sativa*	1177	-	12	TATATTTATATTT
TATA-box	*Brassica oleracea*	1179	+	7	ATATAAT
TATA-box	*Arabidopsis thaliana*	1180	+	4	TATA
TATA-box	*Brassica napus*	1184	+	6	ATTATA
TATA-box	*Arabidopsis thaliana*	1185	-	7	TATATAA
TATA-box	*Arabidopsis thaliana*	1186	+	4	TATA
TATA-box	*Arabidopsis thaliana*	1188	+	4	TATA
TATA-box	*Glycine max*	1194	+	5	TAATA
TATA-box	*Glycine max*	1209	-	5	TAATA
TATA-box	*Arabidopsis thaliana*	1263	+	4	TATA
TATA-box	*Glycine max*	1265	-	5	TAATA
TATA-box	*Brassica napus*	1285	+	6	ATATAT
TATA-box	*Arabidopsis thaliana*	1286	+	4	TATA
TATA-box	*Antirrhinum majus*	1354	-	8	TATAAATT
TATA-box	*Ac*	1355	-	7	TATAAAT
TATA-box	*Arabidopsis thaliana*	1356	-	6	TATAAA
TATA-box	*Arabidopsis thaliana*	1357	-	7	TATATAA
TATA-box	*Arabidopsis thaliana*	1358	+	9	taTATAAAtc
TATA-box	*Brassica oleracea*	1359	+	7	ATATAAT
TATA-box	*Arabidopsis thaliana*	1360	+	4	TATA
TATA-box	*Helianthus annuus*	1383	-	6	TATACA
TATA-box	*Arabidopsis thaliana*	1385	+	4	TATA
TATA-box	*Lycopersicon esculentum*	1396	+	5	TTTTA
TATA-box	*Glycine max*	1408	+	5	TAATA
TATA-box	*Glycine max*	1415	-	5	TAATA
TATA-box	*Arabidopsis thaliana*	1515	-	6	TATAAA

Appendix Table 16 (Continued).

TATA-box (function: core promoter element around -30 of transcription start)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Arabidopsis thaliana*	1517	+	6	TATAAA
TATA-box	*Daucus carota*	1559	+	9	ccTATAAATT
TATA-box	*Ac*	1561	+	7	TATAAAT
TATA-box	*Lycopersicon esculentum*	1569	-	5	TTTTA
TATA-box	*Arabidopsis thaliana*	1581	+	4	TATA
TATA-box	*Pisum sativum*	1596	-	8	TATAAAAT
TATA-box	*Arabidopsis thaliana*	1597	-	7	TATAAAA
TATA-box	*Arabidopsis thaliana*	1598	-	6	TATAAA
TATA-box	*Arabidopsis thaliana*	1599	-	5	TATAA
TATA-box	*Arabidopsis thaliana*	1600	+	6	TATAAA
TATA-box	*Lycopersicon esculentum*	1602	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	1608	+	5	TTTTA
TATA-box	*Brassica napus*	1682	+	6	ATTATA
TATA-box	*Arabidopsis thaliana*	1683	-	5	TATAA
TATA-box	*Arabidopsis thaliana*	1684	+	4	TATA
TATA-box	*Brassica oleracea*	1852	+	7	ATATAAT
TATA-box	*Arabidopsis thaliana*	1853	+	4	TATA
TATA-box	*Glycine max*	1855	+	5	TAATA
TATA-box	*Arabidopsis thaliana*	1856	-	9	tcTATATAtt
TATA-box	*Brassica napus*	1857	+	6	ATATAT
TATA-box	*Arabidopsis thaliana*	1858	+	4	TATA
TATA-box	*Brassica oleracea*	1859	+	6	ATATAA
TATA-box	*Arabidopsis thaliana*	1860	+	6	TATAAA
TATA-box	*Lycopersicon esculentum*	1862	-	5	TTTTA
TATA-box	*Glycine max*	1885	-	5	TAATA
TATA-box	*Zea mays*	1962	+	8	TTTAAAAA
TATA-box	*Lycopersicon esculentum*	1964	-	5	TTTTA

Appendix Table 16 (Continued).

TC-rich repeats (function: *cis*-acting element involved in defense and stress responsiveness					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TC-rich repeats	*Nicotiana tabacum*	614	+	9	ATTTTCTTCA
TC-rich repeats	*Nicotiana tabacum*	1610	-	9	ATTTTCTTCA
TGA-element (function: auxin-responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TGA-element	*Brassica oleracea*	1456	+	6	AACGAC
TGACG-motif (function: *cis*-acting regulatory element involved in the MeJA-responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TGACG-motif	*Hordeum vulgare*	1801	-	5	TGACG
Unnamed__1					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__1	*Zea mays*	1761	-	5	CGTGG
Unnamed__11					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__11	*Zea mays*	1216	-	9	TCCACATAGA
Unnamed__13					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__13	*Zea mays*	1174	+	9	TCCAAGTATA
Unnamed__3					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__3	*Zea mays*	1761	-	5	CGTGG
Unnamed__4					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__4	*Petroselinum hortense*	470	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	1233	-	4	CTCC
Unnamed__4	*Petroselinum hortense*	493	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	1748	+	4	CTCC

Appendix Table 16 (Continued).

W box					
Site Name	Organism	Position	Strand	Matrix score.	sequence
W box	*Arabidopsis thaliana*	134	+	6	TTGACC
W box	*Arabidopsis thaliana*	1931	+	6	TTGACC
W box	*Arabidopsis thaliana*	1424	+	6	TTGACC
W box	*Arabidopsis thaliana*	682	+	6	TTGACC
W box	*Arabidopsis thaliana*	1868	-	6	TTGACC
chs-CMA1a (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
chs-CMA1a	*Daucus carota*	1775	-	8	TTACTTAA
circadian (function: *cis*-acting regulatory element involved in circadian control)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
circadian	*Lycopersicon esculentum*	139	+	6	CAANNNNATC
circadian	*Lycopersicon esculentum*	477	+	6	CAANNNNATC
circadian	*Lycopersicon esculentum*	145	-	6	CAANNNNATC

Appendix Table 17 *Cis*-acting element in *GmLDOX* 5' flanking region using PlantCARE database.

ABRE (function: *cis*-acting element involved in the abscisic acid responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ABRE	*Arabidopsis thaliana*	309	+	6	CACGTG
AC-II					
Site Name	Organism	Position	Strand	Matrix score.	sequence
AC-II	*Phaseolus vulgaris*	469	+	11	CCACCAACCCCC
AE-box (function: part of a module for light response)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
AE-box	*Arabidopsis thaliana*	268	+	8	AGAAACAA
ARE (function: *cis*-acting regulatory element essential for the anaerobic induction)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ARE	*Zea mays*	183	-	6	TGGTTT
ARE	*Zea mays*	383	-	6	TGGTTT
CAAT-box (function: common *cis*-acting element in promoter and enhancer regions)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CAAT-box	*Hordeum vulgare*	8	+	4	CAAT
CAAT-box	*Hordeum vulgare*	237	-	4	CAAT
CAAT-box	*Glycine max*	236	-	5	CAATT
CAAT-box	*Brassica rapa*	251	-	5	CAAAT
CE3 (function: *cis*-acting element involved in ABA and VP1 responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CE3	*Oryza sativa*	26	-	9	GACGCGTGTC
G-Box (function: *cis*-acting regulatory element involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
G-Box	*Pisum sativum*	225	-	6	CACGTT
G-Box	*Pisum sativum*	309	+	6	CACGTG
GATA-motif (function: part of a light responsive)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GATA-motif	*Solanum tuberosum*	258	-	9	AAGGATAAGG

Appendix Table 17 (Continued).

GT1-motif (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GT1-motif	*Arabidopsis thaliana*	260	-	6	GGTTAA
GT1-motif	*Arabidopsis thaliana*	435	-	6	GGTTAA
GT1-motif	*Avena sativa*	434	-	7	GGTTAAT
GT1-motif	*Solanum tuberosum*	443	-	10	ATGGTGGTTGG
LTR (function: *cis*-acting element involved in low-temperature responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
LTR	*Hordeum vulgare*	80	+	6	CCGAAA
Skn-1_motif (function: cis-acting regulatory element required for endosperm expression)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Skn-1_motif	*Oryza sativa*	24	-	5	GTCAT
Sp1 (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Sp1	*Zea mays*	324	+	5	CC(G/A)CCC
Sp1	*Zea mays*	480	+	5	CC(G/A)CCC
Sp1	*Zea mays*	473	+	5.5	CC(G/A)CCC
Sp1	*Zea mays*	484	+	5.5	CC(G/A)CCC
Sp1	*Zea mays*	469	+	5.5	CC(G/A)CCC
Sp1	*Zea mays*	476	+	5	CC(G/A)CCC
TATA-box (function: core promoter element around -30 of transcription start)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Arabidopsis thaliana*	41	+	4	TATA
TATA-box	*Arabidopsis thaliana*	145	+	4	TATA
TATA-box	*Glycine max*	67	-	5	TAATA
TATA-box	*Arabidopsis thaliana*	413	+	9	ccTATAAAaa
TATA-box	*Arabidopsis thaliana*	52	-	5	TATAA
TATA-box	*Lycopersicon esculentum*	160	+	5	TTTTA
TATA-box	*Lycopersicon esculentum*	93	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	417	-	5	TTTTA
TATA-box	*Oryza sativa*	49	-	8	TATAAGAA
TATA-box	*Lycopersicon esculentum*	155	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	73	-	5	TTTTA

Appendix Table 17 (Continued).

TATA-box (function: core promoter element around -30 of transcription start)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Arabidopsis thaliana*	41	+	4	TATA
TATA-box	*Arabidopsis thaliana*	415	+	6	TATAAA
TATA-box	*Arabidopsis thaliana*	53	+	4	TATA
TATA-box	*Lycopersicon esculentum*	173	+	5	TTTTA
TATA-box	*Lycopersicon esculentum*	132	+	5	TTTTA
TATA-box	*Arabidopsis thaliana*	465	+	4	TATA
TCA-element (function: cis-acting element involved in salicylic acid responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TCA-element	*Nicotiana tabacum*	498	-	9	CCATCTTTTT
Unnamed__4					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__4	*Petroselinum hortense*	377	-	4	CTCC
Unnamed__4	*Petroselinum hortense*	481	+	4	CTCC
box E					
Site Name	Organism	Position	Strand	Matrix score.	sequence
box E	*Petroselinum crispum*	303	+	9	ACCCATCAAG

Appendix Table 18 *Cis*-acting element in *GmUFGT* 5' flanking region using PlantCARE database.

4cl-CMA2b (function**:** light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
4cl-CMA2b	*Petroselinum crispum*	395	+	10	TCTCACCAACC
AAGAA-motif					
Site Name	Organism	Position	Strand	Matrix score.	sequence
AAGAA-motif	*Avena sativa*	792	-	9	gGTAAAGAAA
ABRE (function: cis-acting element involved in the abscisic acid responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ABRE	*Arabidopsis thaliana*	995	+	7	ACGTGGC
AC-I					
Site Name	Organism	Position	Strand	Matrix score.	sequence
AC-I	*Populus tremuloides*	395	+	10	TCTCACCAACC
AE-box (function: part of a module for light response)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
AE-box	*Arabidopsis thaliana*	988	+	8	AGAAACAA
ARE (function: cis-acting regulatory element essential for the anaerobic induction)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
ARE	*Zea mays*	10	-	6	TGGTTT
ARE	*Zea mays*	1033	+	6	TGGTTT
ARE	*Zea mays*	58	-	6	TGGTTT
Box II (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Box II	*Solanum tuberosum*	1393	-	9	TGGTAATAA
Box-W1 (function: fungal elicitor responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Box-W1	*Petroselinum crispum*	302	-	6	TTGACC
Box-W1	*Petroselinum crispum*	1327	-	6	TTGACC

Appendix Table 18 (Continued).

CAAT-box (function: common cis-acting element in promoter and enhancer regions)

Site Name	Organism	Position	Strand	Matrix score.	sequence
CAAT-box	*Hordeum vulgare*	163	+	4	CAAT
CAAT-box	*Brassica rapa*	308	+	5	CAAAT
CAAT-box	*Glycine max*	335	+	5	CAATT
CAAT-box	*Brassica rapa*	369	-	5	CAAAT
CAAT-box	*Hordeum vulgare*	387	+	4	CAAT
CAAT-box	*Brassica rapa*	438	+	5	CAAAT
CAAT-box	*Arabidopsis thaliana*	447	+	5	CCAAT
CAAT-box	*Glycine max*	448	+	5	CAATT
CAAT-box	*Brassica rapa*	450	-	5	CAAAT
CAAT-box	*Brassica rapa*	517	-	5	CAAAT
CAAT-box	*Arabidopsis thaliana*	543	-	6	gGCAAT
CAAT-box	*Brassica rapa*	642	-	5	CAAAT
CAAT-box	*Brassica rapa*	683	+	5	CAAAT
CAAT-box	*Hordeum vulgare*	760	-	4	CAAT
CAAT-box	*Glycine max*	850	+	5	CAATT
CAAT-box	*Brassica rapa*	852	-	5	CAAAT
CAAT-box	*Glycine max*	923	-	5	CAATT
CAAT-box	*Hordeum vulgare*	924	-	4	CAAT
CAAT-box	*Arabidopsis thaliana*	956	+	5	CCAAT
CAAT-box	*Hordeum vulgare*	957	+	4	CAAT
CAAT-box	*Brassica rapa*	1006	+	5	CAAAT
CAAT-box	*Glycine max*	1008	-	5	CAATT
CAAT-box	*Hordeum vulgare*	1009	-	4	CAAT
CAAT-box	*Hordeum vulgare*	1020	+	4	CAAT
CAAT-box	*Brassica rapa*	1067	-	5	CAAAT
CAAT-box	*Hordeum vulgare*	1119	-	4	CAAT
CAAT-box	*Hordeum vulgare*	1137	-	4	CAAT
CAAT-box	*Hordeum vulgare*	1172	+	4	CAAT
CAAT-box	*Arabidopsis thaliana*	1211	+	5	CCAAT
CAAT-box	*Glycine max*	1212	+	5	CAATT
CAAT-box	*Brassica rapa*	1214	-	5	CAAAT
CAAT-box	*Hordeum vulgare*	1330	+	4	CAAT
CAAT-box	*Brassica rapa*	1370	-	5	CAAAT

Appendix Table 18 (Continued).

CAAT-box (function: common cis-acting element in promoter and enhancer regions)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CAAT-box	*Brassica rapa*	1380	-	5	CAAAT
CAAT-box	*Brassica rapa*	1386	+	5	CAAAT
CAAT-box	*Hordeum vulgare*	1419	+	4	CAAT
CAT-box (function: cis-acting regulatory element related to meristem expression)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CAT-box	*Arabidopsis thaliana*	1269	-	6	GCCACT
CATT-motif (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
CATT-motif	*Zea mays*	1157	-	6	GCATTC
G-Box (function: cis-acting regulatory element involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
G-Box	*Pisum sativum*	819	-	6	CACGTT
G-Box	*Pisum sativum*	994	-	6	CACGTT
G-Box	*Pisum sativum*	981	+	6	CACGTT
G-box (function: cis-acting regulatory element involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
G-box	*Zea mays*	819	-	6	CACGTT
G-box	*Solanum tuberosum*	1432	+	10	TCACACGTGGC
G-box	*Zea mays*	994	-	6	CACGTT
G-box	*Zea mays*	981	+	6	CACGTT
G-box	*Zea mays*	1246	+	6	CACGAC
GA-motif (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GA-motif	*Arabidopsis thaliana*	964	+	8	ATAGATAA
GARE-motif (function: gibberellin-responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GARE-motif	*Brassica oleracea*	1095	+	7	AAACAGA
GC-motif (function: enhancer-like element involved in anoxic specific inducibility)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GC-motif	*Zea mays*	1186	-	6	CCCCCG
GC-motif	*Zea mays*	1280	+	6	CCCCCG
GT1-motif (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
GT1-motif	*Arabidopsis thaliana*	529	-	6	GGTTAA

Appendix Table 18 (Continued).

I-box (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
I-box	*Triticum aestivum*	966	+	8	AGATAAGG
L-box (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
L-box	*Petroselinum crispum*	395	+	10	TCTCACCAACC
MNF1 (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
MNF1	*Zea mays*	822	+	6.5	GTGCCC(A/T)(A/T)
MRE (function: MYB binding site involved in light responsiveness)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
MRE	*Petroselinum crispum*	563	+	7	AACCTAA
Skn-1_motif (function: *cis*-acting regulatory element required for endosperm expression)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Skn-1_motif	*Oryza sativa*	246	-	5	GTCAT
Skn-1_motif	*Oryza sativa*	1342	-	5	GTCAT
Skn-1_motif	*Oryza sativa*	1080	-	5	GTCAT
Skn-1_motif	*Oryza sativa*	265	+	5	GTCAT
Skn-1_motif	*Oryza sativa*	1299	+	5	GTCAT
Sp1 (function: light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Sp1	*Zea mays*	914	+	5.5	CC(G/A)CCC
Sp1	*Zea mays*	1059	+	5	CC(G/A)CCC
TATA-box (function: core promoter element around -30 of transcription start)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Lycopersicon esculentum*	33	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	77	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	98	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	119	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	405	+	5	TTTTA
TATA-box	*Ac*	426	+	7	TATAAAT
TATA-box	*Glycine max*	462	+	5	TAATA
TATA-box	*Arabidopsis thaliana*	500	+	4	TATA
TATA-box	*Glycine max*	550	-	5	TAATA
TATA-box	*Lycopersicon esculentum*	600	-	5	TTTTA

Appendix Table 18 (Continued).

TATA-box (function: core promoter element around -30 of transcription start)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Arabidopsis thaliana*	661	-	9	taTATAAAgg
TATA-box	*Arabidopsis thaliana*	662	-	7	TATAAAA
TATA-box	*Arabidopsis thaliana*	663	-	6	TATAAA
TATA-box	*Arabidopsis thaliana*	664	-	7	TATATAA
TATA-box	*Arabidopsis thaliana*	665	+	4	TATA
TATA-box	*Brassica napus*	666	+	6	ATATAT
TATA-box	*Arabidopsis thaliana*	667	+	4	TATA
TATA-box	*Lycopersicon esculentum*	671	+	5	TTTTA
TATA-box	*Glycine max*	692	-	5	TAATA
TATA-box	*Glycine max*	870	-	5	TAATA
TATA-box	*Daucus carota*	873	-	8	TATAAATA
TATA-box	*Ac*	874	-	7	TATAAAT
TATA-box	*Arabidopsis thaliana*	875	-	6	TATAAA
TATA-box	*Arabidopsis thaliana*	876	-	5	TATAA
TATA-box	*Arabidopsis thaliana*	877	+	4	TATA
TATA-box	*Lycopersicon esculentum*	903	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	960	-	5	TTTTA
TATA-box	*Glycine max*	1026	-	5	TAATA
TATA-box	*Glycine max*	1072	+	5	TAATA
TATA-box	*Glycine max*	1128	-	5	TAATA
TATA-box	*Lycopersicon esculentum*	1262	-	5	TTTTA
TATA-box	*Lycopersicon esculentum*	1289	+	5	TTTTA
TATA-box	*Lycopersicon esculentum*	1294	+	5	TTTTA
TATA-box	*Brassica oleracea*	1319	+	6	ATATAA
TATA-box	*Arabidopsis thaliana*	1320	+	4	TATA
TATA-box	*Lycopersicon esculentum*	1333	-	5	TTTTA

Appendix Table 18 (Continued).

TATA-box (function: core promoter element around -30 of transcription start)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TATA-box	*Arabidopsis thaliana*	661	-	9	taTATAAAgg
TATA-box	*Arabidopsis thaliana*	662	-	7	TATAAAA
TATA-box	*Lycopersicon esculentum*	1366	+	5	TTTTA
TATA-box	*Lycopersicon esculentum*	1391	+	5	TTTTA
TATA-box	*Glycine max*	1394	-	5	TAATA
TATA-box	*Brassica napus*	1421	+	6	ATATAT
TATA-box	*Arabidopsis thaliana*	1422	+	4	TATA
TATA-box	*Brassica napus*	1423	+	6	ATATAT
TATA-box	*Arabidopsis thaliana*	1424	+	4	TATA
TGG-motif (function: part of a light responsive element)					
Site Name	Organism	Position	Strand	Matrix score.	sequence
TGG-motif	*Gossypium hirsutum*	1190	+	8	GGTTGCCA
Unnamed__1					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__1	*Zea mays*	996	+	5	CGTGG
Unnamed__2					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__2	*Petroselinum hortense*	563	+	9	AACCTAACCT
Unnamed__3					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__3	*Zea mays*	996	+	5	CGTGG
Unnamed__4					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__4	*Petroselinum hortense*	467	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	1113	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	932	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	1407	+	4	CTCC

Appendix Table 18 (Continued).

Unnamed__4					
Site Name	Organism	Position	Strand	Matrix score.	sequence
Unnamed__4	*Petroselinum hortense*	815	-	4	CTCC
Unnamed__4	*Petroselinum hortense*	1274	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	1110	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	1209	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	652	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	1356	+	4	CTCC
Unnamed__4	*Petroselinum hortense*	911	+	4	CTCC
W box					
Site Name	Organism	Position	Strand	Matrix score.	sequence
W box	*Arabidopsis thaliana*	302	-	6	TTGACC
W box	*Arabidopsis thaliana*	1327	-	6	TTGACC

Appendix Table 19 The relative expression of mangosteen anthocyanin biosynthesis and MYB transcription factor genes during colour development harvested from tree.

Stage	Genes[1]										
	PAL	CHS	CHI	F3H	F3'H	DFR	LDOX	UFGT	MYB1	MYB7	MYB10
0	1.00d	1.00e	1.00d	1.00e	1.00f	1.00c	1.00d	1.00e	1.00d	1.00c	1.00d
1	2.78c	5.95d	7.11c	19.87d	8.87e	13.04b	11.97c	125.77d	1.44cd	5.47bc	17.37d
2	3.63c	7.51c	12.15bc	30.20c	13.82d	12.95bb	16.02c	187.15c	1.79bc	5.70bc	33.38d
3	3.70c	6.86	10.26bc	26.07cd	10.97de	14.03b	18.13c	178.68c	2.04bc	8.22b	31.08d
4	5.62b	9.69b	14.16b	45.98b	23.25b	31.83a	39.08b	370.74b	2.39b	9.89b	76.88c
5	11.51a	13.10a	26.65a	70.03a	42.58a	31.76a	58.95a	571.45a	4.87a	15.11a	299.26a
6	6.11b	5.70d	12.74bc	43.37b	17.28c	17.03b	34.79b	225.21c	2.43b	15.19a	124.99b
F-test	***	***	***	***	***	***	***	***	***	***	***

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

Appendix Table 20 The relative expression of mangosteen anthocyanin biosynthesis and MYB transcription factor genes during colour development after harvest.

Stage	Genes[1]										
	PAL	CHS	CHI	F3H	F3'H	DFR	LDOX	UFGT	MYB1	MYB7	MYB10
0	1.00c	1.00e	1.00e	1.00e	1.00d	1.00c	1.00e	1.00f	1.00d	1.00d	1.00d
1	5.30b	7.10d	8.80d	20.66d	22.10c	8.28c	10.34d	193.36e	2.38c	5.54c	73.50d
2	12.83a	19.68b	28.15c	72.62b	44.39b	30.07b	32.15c	517.39c	5.62b	7.07c	302.90c
3	16.26a	25.91a	44.91b	105.06a	69.34a	42.06a	56.49b	678.77ab	7.68a	13.39b	407.38
4	12.65a	16.65b	45.41b	98.36a	63.79a	29.02b	56.24b	645.10b	6.18b	13.35b	410.83ab
5	14.35a	18.24b	81.49a	95.92a	64.05a	35.33ab	69.10a	763.36a	5.73b	17.86a	500.96a
6	7.51b	10.67c	27.16c	49.80c	31.65c	25.26b	35.68c	400.87d	3.28c	14.21ab	340.13bc
F-test	***	***	***	***	***	***	***	***	***	***	***

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

Appendix Table 21 The relative expression of *PAL* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	3.18a	3.49a	3.12a	2.06ab	
ethylene	1.00	2.36b	2.90b	2.87a	2.18a	
1-MCP	1.00	0.88d	1.01c	0.91b	1.43c	3.26
E+M	1.00	1.79c	0.44d	0.96b	1.61bc	2.43
F-test	ns	***	***	***	*	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

* = significantly different at $P \leq 0.05$

*** = significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 22 The relative expression of *CHS* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	3.18a	4.07a	3.87a	3.50a	
ethylene	1.00	2.76b	3.06b	3.56a	2.93ab	
1-MCP	1.00	0.73d	1.18c	1.21b	3.46a	4.46
E+M	1.00	2.09c	0.32d	0.76b	2.39b	4.42
F-test	ns	***	***	***	*	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

* = significantly different at $P \leq 0.05$

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 23 The relative expression of *CHI* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	3.86a	5.24a	4.26a	4.04a	
ethylene	1.00	3.61a	4.33b	4.34a	3.70ab	
1-MCP	1.00	1.04b	1.45c	1.27b	3.30ab	5.57
E+M	1.00	3.42a	0.72d	0.62b	3.10b	4.71
F-test	ns	***	***	***	ns	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 24 The relative expression of *F3H* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	4.63a	4.94a	4.27a	2.88a	
ethylene	1.00	4.62a	4.62a	3.29b	2.76a	
1-MCP	1.00	0.94b	1.12b	0.81c	1.27c	4.89
E+M	1.00	3.79a	0.70c	0.84c	2.25b	2.64
F-test	ns	***	***	***	***	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 25 The relative expression of *F3'H* of mangosteen fruit treated with ethylene the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP) for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	4.01a	4.36a	3.95a	2.66a	
ethylene	1.00	3.87a	3.87a	3.36a	2.47ab	
1-MCP	1.00	0.65c	0.81b	0.75b	1.31c	3.92
E+M	1.00	2.58b	0.53b	0.80b	1.84bc	2.42
F-test	ns	***	***	***	**	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 26 The relative expression of *DFR* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	3.06a	4.09a	4.16a	5.01a	
ethylene	1.00	2.17b	2.87b	3.18b	3.99ab	
1-MCP	1.00	0.43c	0.89c	0.85c	4.04ab	6.03
E+M	1.00	2.31b	0.37c	0.78c	3.08b	5.26
F-test	ns	***	***	***	*	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

* = significantly different at $P \leq 0.05$

*** = significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 27 The relative expression of *LDOX* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	5.30a	7.38a	7.87a	6.55a	
ethylene	1.00	5.44a	6.94a	6.35b	6.04a	
1-MCP	1.00	1.11c	1.86b	1.50c	4.52b	8.74
E+M	1.00	4.70b	1.01c	1.54c	4.36b	7.30
F-test	ns	***	***	***	**	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 28 The relative expression of *UFGT* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	3.92a	4.84a	4.22a	3.26	
ethylene	1.00	4.12a	4.74a	3.84a	3.20	
1-MCP	1.00	0.95c	1.25b	1.19b	2.39	4.85
E+M	1.00	3.56b	1.07b	1.54b	3.00	3.66
F-test	ns	***	***	***	ns	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 29 The relative expression of *MYB1* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	3.89a	3.66a	3.96a	2.39	
ethylene	1.00	2.89b	2.35b	4.31a	1.91	
1-MCP	1.00	0.80d	1.10c	1.79b	2.30	3.29
E+M	1.00	1.96c	0.50d	0.71c	2.19	2.46
F-test	ns	***	***	***	ns	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 30 The relative expression of *MYB7* of mangosteen fruit treated with ethylene the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 µL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 µg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 µl L^{-1} ethylene for 24 h +1 µg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	3.97a	4.93a	5.05a	7.43a	
ethylene	1.00	4.04a	3.11b	5.13a	7.42a	
1-MCP	1.00	1.72b	1.78c	2.22b	4.06b	7.56
E+M	1.00	3.35a	3.16b	2.15b	3.78b	10.00
F-test	ns	**	***	***	**	ns

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 31 The relative expression of *MYB10* of mangosteen fruit treated with ethylene and the ethylene inhibitor 1-MCP following 4 treatments; 1) air (control), 2) 200 μL L^{-1} ethylene for 24 h (ethylene treatment), 3) 1 μg L^{-1} 1-MCP for 12 h (1-MCP treatment) and 4) 200 μl L^{-1} ethylene for 24 h +1 μg L^{-1} 1-MCP for 12 h (E+M).

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
Air (control)	1.00	10.73d	16.58a	13.30a	19.97b	
ethylene	1.00	5.93b	7.56b	14.31a	17.67b	
1-MCP	1.00	1.62d	3.26c	4.25b	39.10a	26.96
E+M	1.00	4.35c	0.31d	1.44b	12.04c	28.50
F-test	ns	**	***	***	***	na

[1] Mean values followed by different letters in the same column are significantly different at $P \leq 0.05$ using DMRT

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

na = no analysis

Appendix Table 32 The relative expression of *PAL* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	3.04	3.90	3.45	2.51	
15°C (7 d) + 25°C	1.00	0.63	1.01	0.91	1.54	3.73
t-test	ns	*	**	**	*	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

Appendix Table 33 The relative expression of *CHS* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	3.21	3.88	3.86	3.38	
15°C (7 d) + 25°C	1.00	0.70	0.87	0.83	1.18	5.04
t-test	ns	***	*	**	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 34 The relative expression of *CHI* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	4.00	6.93	5.63	4.45	
15°C (7 d) + 25°C	1.00	0.82	0.84	0.68	1.23	5.84
t-test	ns	***	***	*	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

***= significantly different at $P \leq 0.001$

Appendix Table 35 The relative expression of *F3H* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	4.00	5.56	4.66	3.48	
15°C (7 d) + 25°C	1.00	0.78	0.76	0.69	1.17	5.95
t-test	ns	***	**	***	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 36 The relative expression of *F3'H* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	3.31	5.07	4.37	2.83	
15°C (7 d) + 25°C	1.00	0.42	0.53	0.48	0.77	5.07
t-test	ns	**	**	*	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 37 The relative expression of *DFR* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	2.63	2.76	3.06	4.51	
15°C (7 d) + 25°C	1.00	0.60	0.69	0.74	1.58	5.51
t-test	ns	***	*	**	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 38 The relative expression of *LDOX* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	4.29	8.14	8.13	7.00	
15°C (7 d) + 25°C	1.00	0.74	0.73	0.74	1.26	9.35
t-test	ns	***	**	**	**	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 39 The relative expression of *UFGT* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	3.33	4.74	4.19	3.55	
15°C (7 d) + 25°C	1.00	0.80	1.16	1.16	1.46	5.83
t-test	ns	***	**	**	**	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 40 The relative expression of *MYB1* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	3.49	4.55	3.79	2.87	
15°C (7 d) + 25°C	1.00	0.95	1.27	0.72	1.08	3.44
t-test	ns	**	***	***	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 41 The relative expression of *MYB7* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	4.71	6.06	6.52	8.71	
15°C (7 d) + 25°C	1.00	1.19	1.60	1.07	1.41	6.57
t-test	ns	**	***	*	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

ns = non-significantly different

* = significantly different at $P \leq 0.05$

** = significantly different at $P \leq 0.01$

***= significantly different at $P \leq 0.001$

Appendix Table 42 The relative expression of *MYB10* of mangosteen fruit stored at 25°C (control) and 15°C for 7 days then transferred to 25°C.

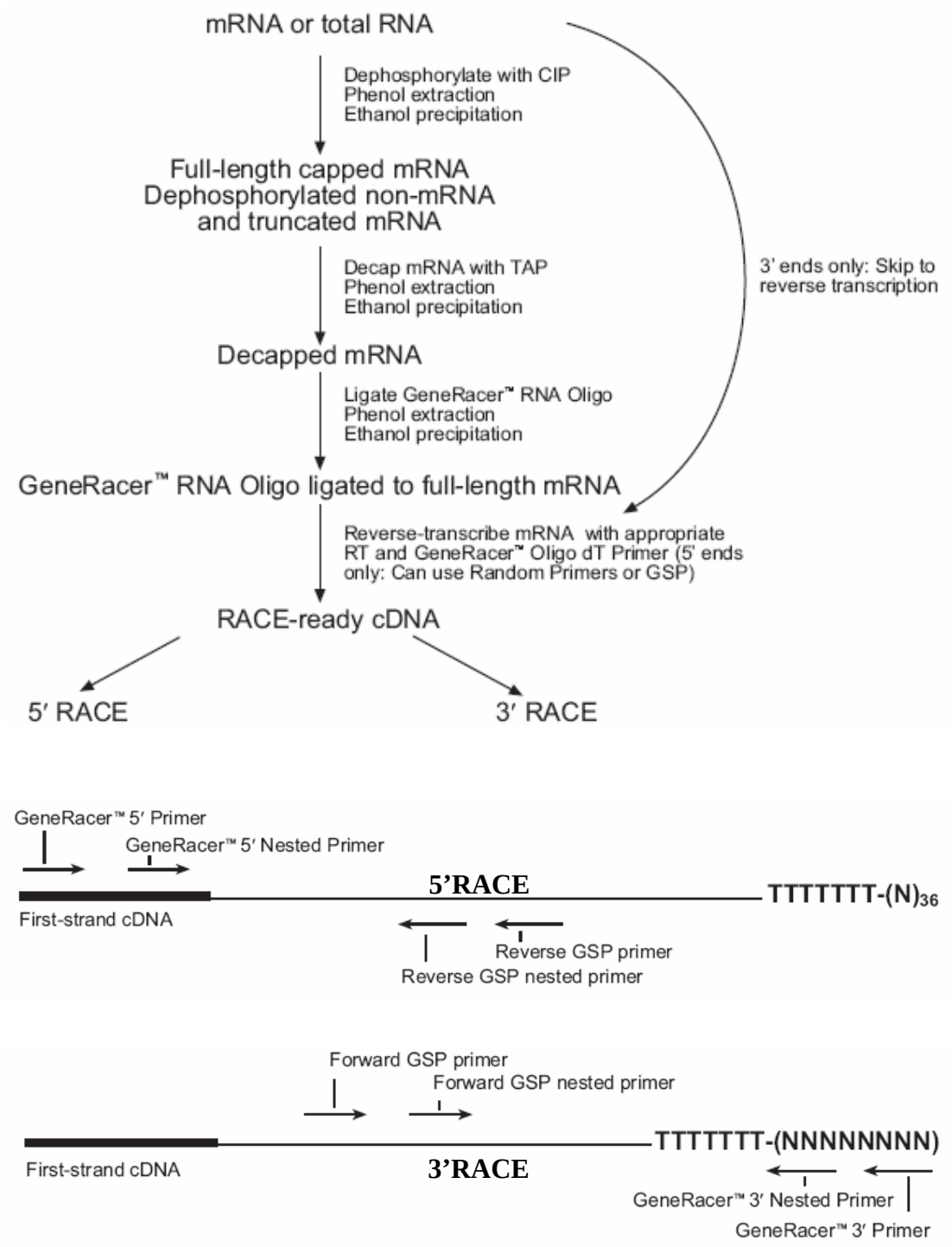
Treatment	Days after harvest[1]					
	0	1	2	3	7	11
25°C (control)	1.00	8.69	12.48	18.59	18.72	
15°C (7 d) + 25°C	1.00	1.02	1.52	1.67	3.00	22.90
t-test	ns	**	***	***	***	

[1] Asterisks indicate a significant difference between mean values according to Student *t*-test

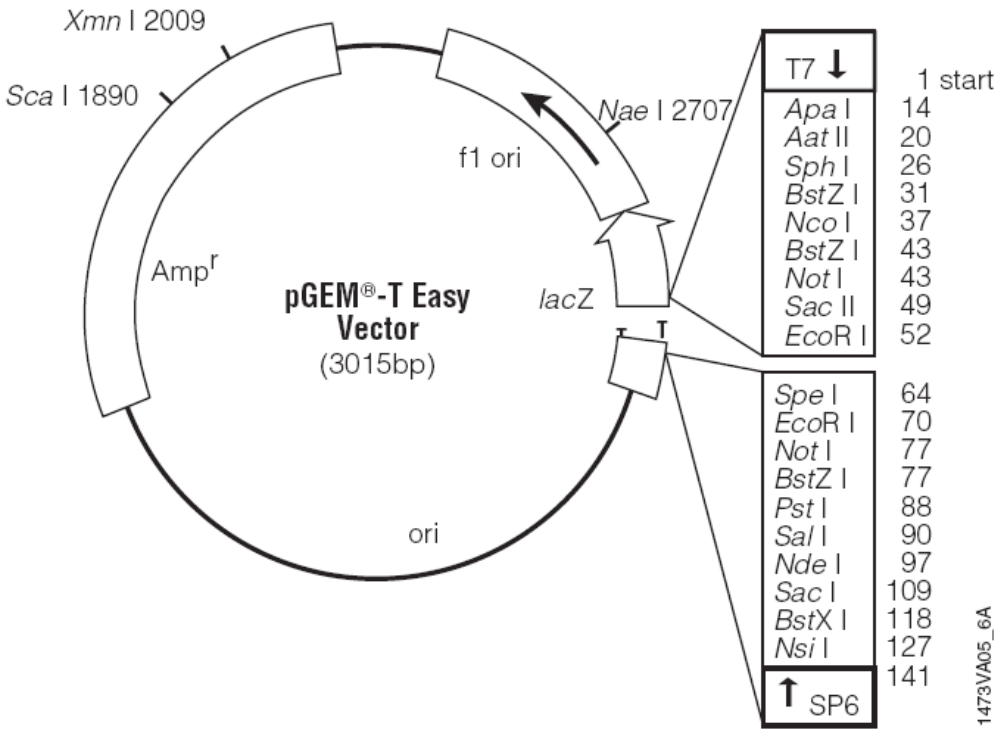
ns = non-significantly different

** = significantly different at $P \leq 0.01$

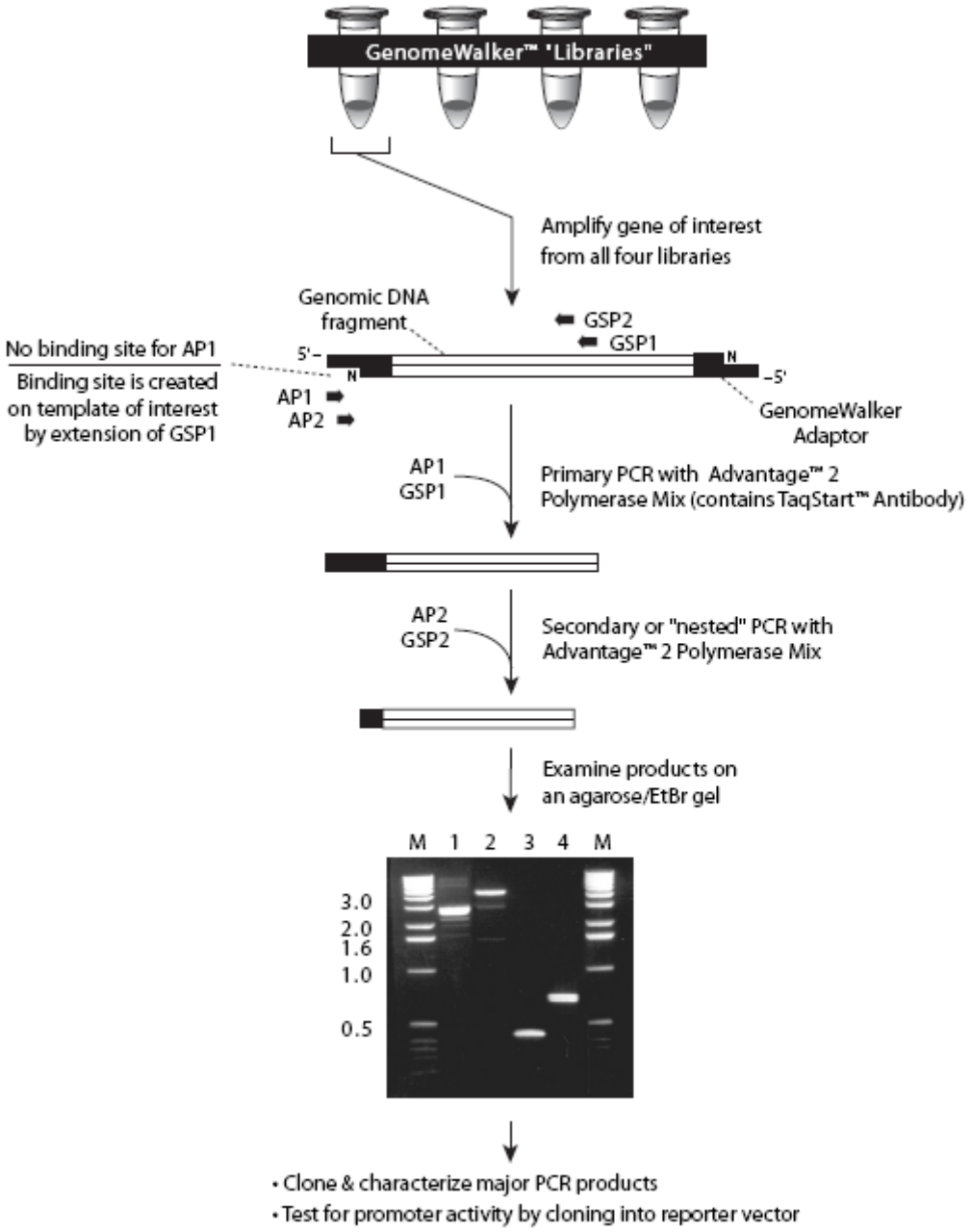
***= significantly different at $P \leq 0.001$



Appendix Figure 1 GeneRacer protocol (Invitrogen, USA).

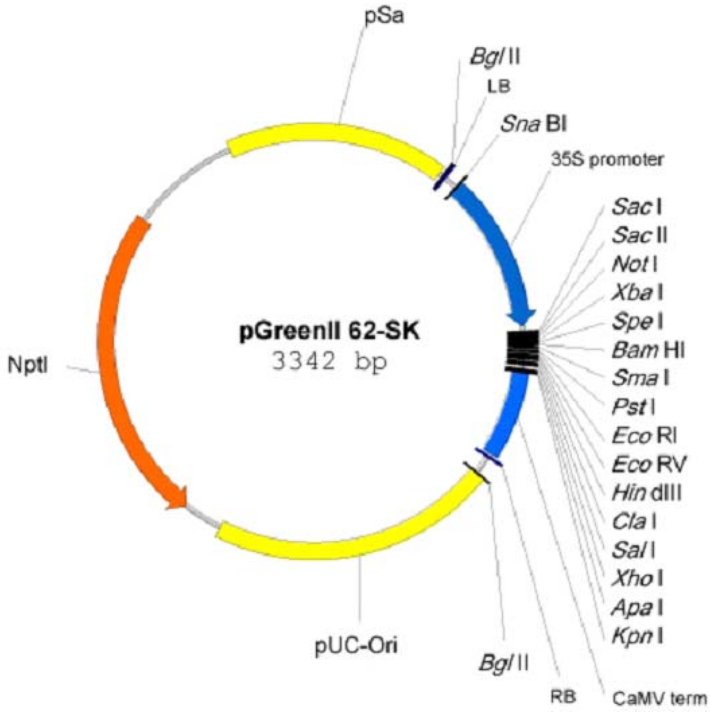


Appendix Figure 2 pGEM-T Easy vector (Promega, USA).



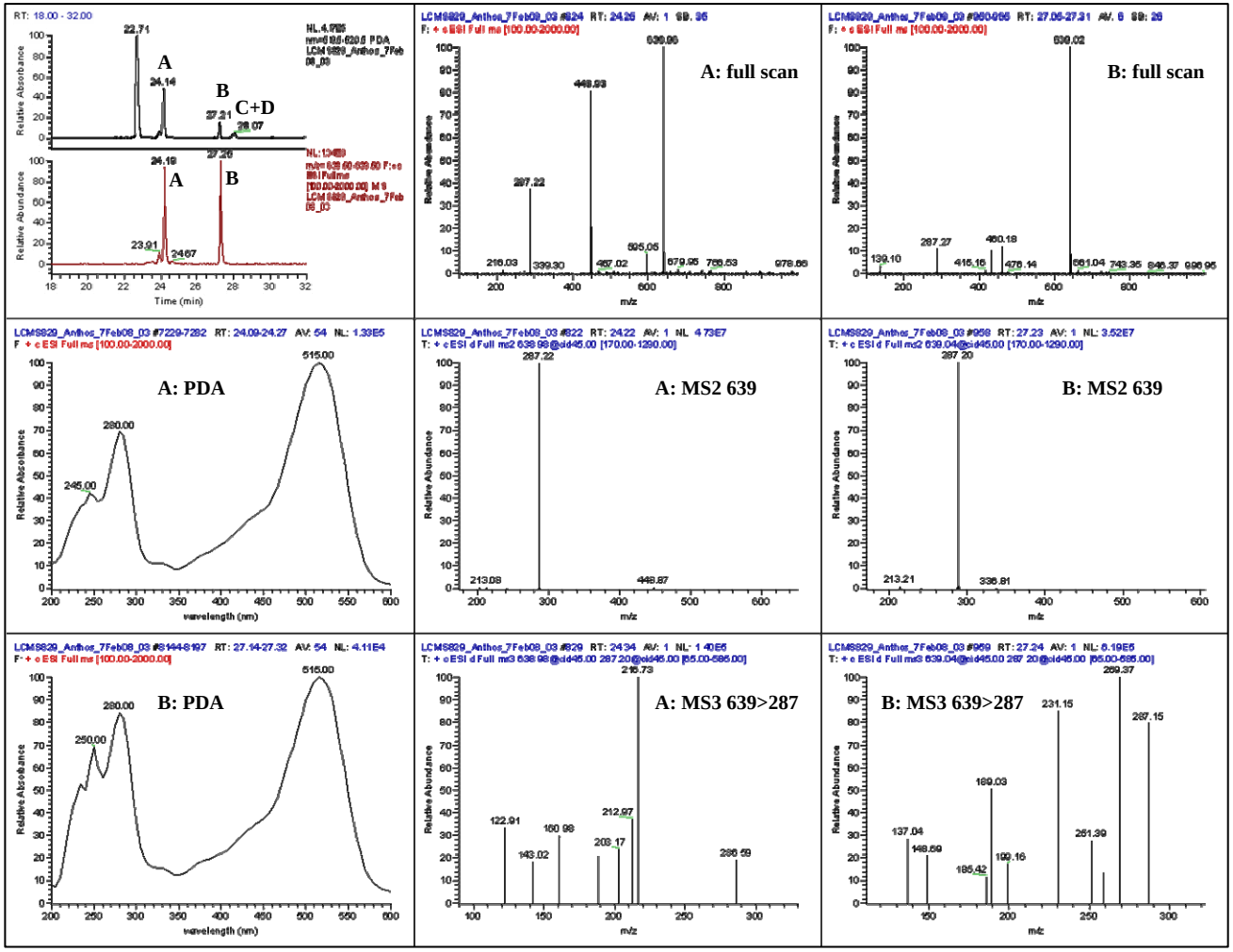
Appendix Figure 3 Genome Walker protocol (Clontech, USA).

Appendix Figure 4 T-DNA region of the transient expression vector pGreenII 0800-LUC.

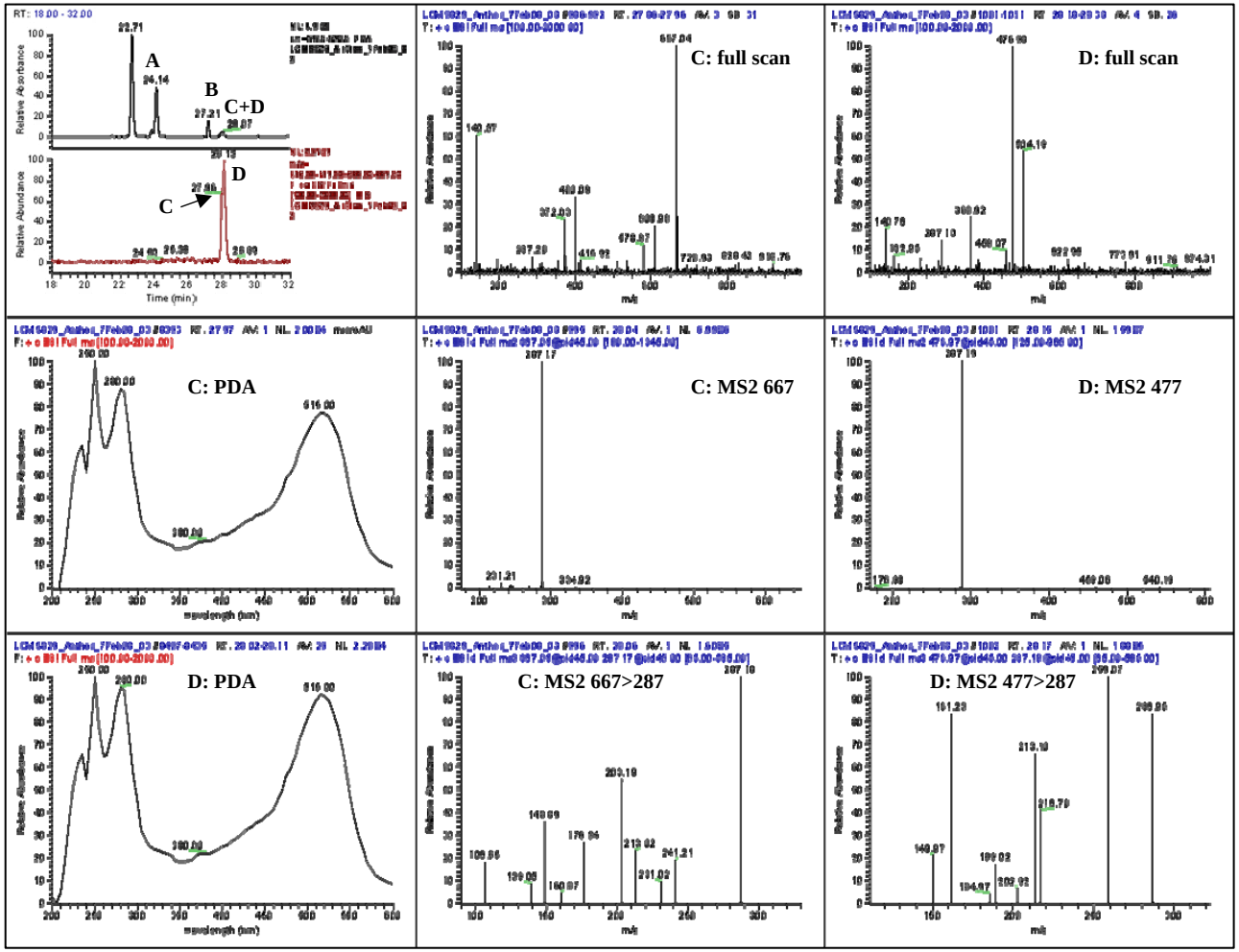


Appendix Figure 5 T-DNA region of the transient expression vector pGreenII 62-SK

Appendix Figure 6 LC-MS of anthocyanin in outer pericarp at stage 6 of mangosteen fruit.



Appendix Figure 6 (Continued).



CURRICULUM VITAE

NAME : Mr. Yossapol Palapol

BIRTH DATE : July 12, 1977

BIRTH PLACE : Chanthaburi, Thailand

EDUCATION :

YEAR	**INSTITUTE**	**DEGREE/DIPLOMA**
1999	KMITL.	B.Sc. (Agriculture)
2002	Kasetsart Univ.	M.S. (Horticulture)

POSITION/TITLE : Lecturer

WORK PLACE : Faculty of Science and Art, Burapha University Chanthaburi IT Campus

SCHORLARSHIP : Thailand Research Fund grant number PHD/00210/2547 and Commission on Higher Education, Ministry of Education