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FACULTY OF SCIENCE AND MATHEMATICS
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ACKNOWLEDGEMENTS

Alhamdulillah, I uttered the highest gratitude to Allah, for His mercy and blessings and for giving me the strength of knowledge, skills, and opportunity to dedicate myself to this study and to persevere and complete it successfully. Without His blessings, this achievement would not have been possible. My highest appreciation goes to Dr Remmy Keong Bun Poh as my supervisor and Dr Florence C. Ginibun as my co-supervisor for trust and give me chance to fulfill this great work. A really big thank you for all guidance, encouragement and constructive suggestions. I am also grateful to the Malaysian Agriculture and Research Development Institute (MARDI) and the Fundamental Research Grant Scheme (FRGS) [FRGS/1/2020/STG03/UPSI/02/2] for sponsoring my study. Special thanks go to Department of Agriculture (DOA) Serdang, for allowing me to perform morphological observation on durian samples collected on the workspace provided. Thanks to Dr Abdul Rasip, Mr Adib, Mr Zul, Mr Syawal, Mr Fazlisyam and Mr Azizul and DOA staffs for assisting me during the morphological examination. I would also like to express thanks to Dr Faridah Salam, my Director of Biotechnology and Nanotechnology Research Centre (BN) to allow me to use my room for workspace and stored samples at Centre For Marker Discovery and Validation (CMDV) laboratory. I am also thankful to Madame Siti Nadhrah, Madame Nor Helwa Ezzah, Mr Zulfadhli and Mr Taufiq for assisting me in lab equipment and materials and Mr Hj Burhan for teach me on how to identify matured durian fruit stage. Miss Azrin and Mr. Fairuz were helpful to me during my thesis correction, especially on bioinformatics analysis. I feel blessed thank you for Dr Roket and 2M world's team for making my master a wonderful and enjoyable journey through coaching session, strategies, and supportive post-graduates circle group, Pesona 6.3 and Geng Study especially Cheq su. Thank you for inspiring me in life, motivating me to pursue my academic goals and making me a better person. My deepest appreciation goes to my husband, Mr. Muhammad Hanif Azhari Noor, for endlessly supporting and assisting me in conducting my research throughout these years, cheering me up, and being my first place to discuss when challenges come. A lot of thanks to my mother, Rosnah Othman for her love, support, guidance, as well as continuously dua' throughout my journey as a student. She was excited to attend my convocation, and this became a strong motivator for me to finish this study as soon as possible. An endless love for my late father, who raised me to be who I am. To my baby, Halwatul Zahra, you are my big why to finish thesis correction as soon as possible. Indeed, I appreciate all the support my family and in-laws provided in any capacity they could. May Allah grant them a reward for each word written in my thesis. Lastly, I would acknowledge and present many thanks to everyone who knows me directly or indirectly for their help and support. Without their participation in my life, my goals would not be possible, and this thesis could not be completed properly. May His blessing always be with us and grant us highest paradise.

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ABSTRACT

This study aimed to characterise D24, D99, D160, D168, D197 and D200 varieties at matured, riped, and dehisced stages through morphological observation and molecular characterisation using transcriptomic analysis. Twenty-eight morphological traits were evaluated according to durian descriptor 'National Guidelines for The Conduct of Tests for Distinctness, Uniformity and Stability'. Separately, a total of 21 durian samples were submitted to transcriptome analysis to access biosynthesis pathways during fruit developmental stages. The results of the study showed that these six Malaysian durian varieties demonstrated a considerable level of morphological variation. Statistical ANOVA and canonical discriminant analysis identified pedicel length trait as the most significant quantitative trait to differentiate the six varieties. Qualitative traits dendrogram clustered the varieties into two main clades includes Clade A (mostly of D160, D168, D197 and D200) and Clade B (mostly of D24 and D99). The transcriptomic analysis identified 49,601 genes, with 26,212 genes were annotated by the GO database and 7,205 genes were annotated by the KEGG database. The most significant GO term is cell wall and the most common KEGG pathways are carotenoid biosynthesis, fatty acid biosynthesis, starch and sucrose metabolism, phenylpropanoid biosynthesis and galactose metabolism. In conclusion, this discovery could serve as references for morphological identification of D24, D99, D160, D168, D197, and D200 and disclosure of significant functional genes. The implication of this study could lead to breeding strategies for variety improvement in the future.

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PEMERHATIAN MORFOLOGI DAN PENCIRIAN MOLEKUL TERHADAP PERINGKAT PERKEMBANGAN BUAH BAGI VARIETI DURIAN MALAYSIA TERPILIH

ABSTRAK

Kajian ini bertujuan untuk mencirikan varieti D24, D99, D160, D168, D197 dan D200 pada peringkat matang, masak, dan merekah melalui pemerhatian morfologi dan pencirian molekul menggunakan analisis transkriptom. Dua puluh lapan ciri morfologi telah dinilai mengikut deskriptor durian ‘Garis Panduan Kebangsaan Untuk Menjalankan Ujian Kelainan, Keseragaman Dan Kestabilan'. Sejumlah 21 sampel durian telah dijalankan untuk analisis transkriptom secara berasingan bagi mengakses laluan biosintesis semasa peringkat perkembangan buah. Hasil kajian mendapati enam varieti durian Malaysia ini menunjukkan tahap variasi morfologi yang ketara. Statistik ANOVA dan analisis diskriminasi kanonik mengenal pasti ciri panjang pedisel sebagai ciri kuantitatif yang paling ketara untuk membezakan enam varieti. Dendrogram sifat kualitatif mengelompokkan varieti kepada dua klad utama iaitu Klad A (kebanyakannya D160, D168, D197 dan D200) dan Klad B (kebanyakannya D24 dan D99). Analisis transkriptom mengenal pasti 49,601 gen, dengan 26,212 gen telah dianotasi dalam pangkalan data GO dan 7,205 gen telah dianotasi dalam pangkalan data KEGG. Terma GO yang paling signifikan ialah dinding sel dan laluan KEGG yang paling biasa ialah biosintesis karotenoid, biosintesis asid lemak, metabolisme kanji dan sukrosa, biosintesis fenilpropanoid dan metabolisme galaktosa. Kesimpulannya, penemuan ini boleh menjadi rujukan untuk pengenalpastian morfologi D24, D99, D160, D168, D197, dan D200 dan pendedahan gen berfungsi penting. Implikasi kajian ini boleh mendorong kepada strategi pembaikbakaan untuk penambahbaikan varieti pada masa hadapan.

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LIST OF ABBREVIATIONS

ºC	Degree Celsius
ANOVA	Analysis of variance
BK	Raub Mining & Development Co. Sdn Bhd (durian orchard in Bukit Koman, Raub, Pahang)
BN	Biotechnology and Nanotechnology Research Centre
bp	Base pair
BP	Biological process
CC	Cellular component
cDNA	Complementary DNA
CMDV	Centre for Marker Discovery and Validation
D. zibethinus	*Durio zibethinus*
D123	Chanee
D145	Beserah
D158	Kan Yau
D159	Mon Thong
D160	Buluh Bawah/Tekka
D168	Hajjah Hasmah/IOI
D169	Tok Litok
D175	Udang Merah
D188	MDUR 78
D189	MDUR 79
D190	MDUR 88
D197	Raja Kunyit/ Musang King
D200	Ochee/Black Thorn
D96	Bangkok A

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D98	Katoi
D99	Kop Kecil
DOA	Department of Agriculture
DSLR	Digital Single-Lens Reflex
dTTP	deoxythymidine triphosphate
dUTP	deoxyuridine triphosphate
e-nose	electronic nose
e-tongue	electronic tongue
FPKM	Fragments Per Kilobase of transcript per Million mapped reads
fq	FASTQ
FRVM	Fuzzy Relevance Vector Machin
GC-MS	Gas chromatography-mass spectrometry
GO	Gene Ontology
HPLC	High-performance liquid chromatography
KEGG	Kyoto Encyclopedia of Genes and Genomes
lncRNA	Long non-coding RNA
MARDI	Malaysian Agricultural Research and Development Institute
MF	Molecular function
MT	Metric tonnes
N/A	Information is not available
NGS	Next-Generation Sequencing
p	P-value
padj	Adjusted p-value
PCR	Polymerase chain reaction
PQ	Pseudo-qualitative
PVP	Plant Variety Protection Malaysia
QC	Quality control
QL	Qualitative

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QN	Quantitative
R^2	Pearson correlation coefficient
RIN	RNA integrity
RNA	Ribonucleic acid
RNA-Seq	RNA sequencing
SAC	Serdang Agricultural Complex
SD	Standard deviation
spp	Species (plural)
tRNA	Transfer RNA
UPGMA	Unweighted pair group method with an arithmetic mean

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CHAPTER 1

INTRODUCTION

1.1 Introduction

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Durian (*Durio zibethinus* Murray) belongs to Malvaceae family and was included in the Helicteroideae subfamily, and the genus is *Durio*. Durian has crowned as 'King of Fruits' because of its sweet and unique taste, pungent aroma and popular among Malaysian. The famed naturalist Alfred Russel Wallace once remarked on durian: "the more you eat of it, the less you feel inclined to stop" (Teh et al., 2017).

The durian name derives from Malay word 'duri' meaning thorns, so the fruit is named as durian because presence of sharp pines in its outer skin (Ramawas & Mohamad, 2008). There is other name for durian which differ according to country. For example in Indonesia, this fruit is known as duren, duyin (Burmese), thureen (Cambodian), thurian (Thai), saurieng (Vietnamese), dulian (Phillipines), stinkvrucht (Dutch) and kadu (Sudan) (Paull & Ketsa, 2014).

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Although it is pungent in aroma, the durian has become extremely popular in Asian countries due to its high nutritional value and the widespread acceptance of its processed products. Recently, durian gained acceptance by westerners, even though the aril's strong odour and unique flavour were not appealing to the general western population.

In Malaysia, durian farming begun locally by farmers as a complement plant together with other commercial crops such as oil palm, rubber, and cocoa. Durian cultivation used to be a small-scale operation by smallholdings or “dusun”, usually within the plan area of half or one hectare each, and usually intercropped with other fruit trees. However, with decreasing price for oil palm, rubber, and cocoa, interest in durian farming has increased over time (Zakaria, 2021). Some durian growers, such as Top Fruit Sdn Bhd and Zamri Agro Farm, began planting commercial-valued durian in larger farms and estates. The two main varieties exported to China are the D24 and D197 varieties (Safari, Razali, Wan Ibrahim, & Abdul Rahim, 2021).

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In Malaysia, it has been reported that the durian plantations area has covers approximately 66 038 ha (about 35% of the total number of fruit crops). Percentage of hectare area by varieties; D24 (0.73%), D160 (11.37%), D168 (1.91%), D197 (36.11%), D200 (1.03%), durian kampung (38.85%) and other varieties (10.01%) (Figure 1.1) (Ministry of Agriculture and Food Security [MAFS], n.d.). The states of Pahang, Johor and Sarawak are the three main contributing regions for national durian production (Figure 1.2). Raub state in Pahang is the main durian production centre for D197 variety. In 2021, durian became the most important fruit commodity, with the country producing 448,272 metric tonnes (MT) and exporting 24,684MT in a single year (Hazim, 2023).

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Figure 1.1

Percentage of hectare area occupied for durian plantation by variety

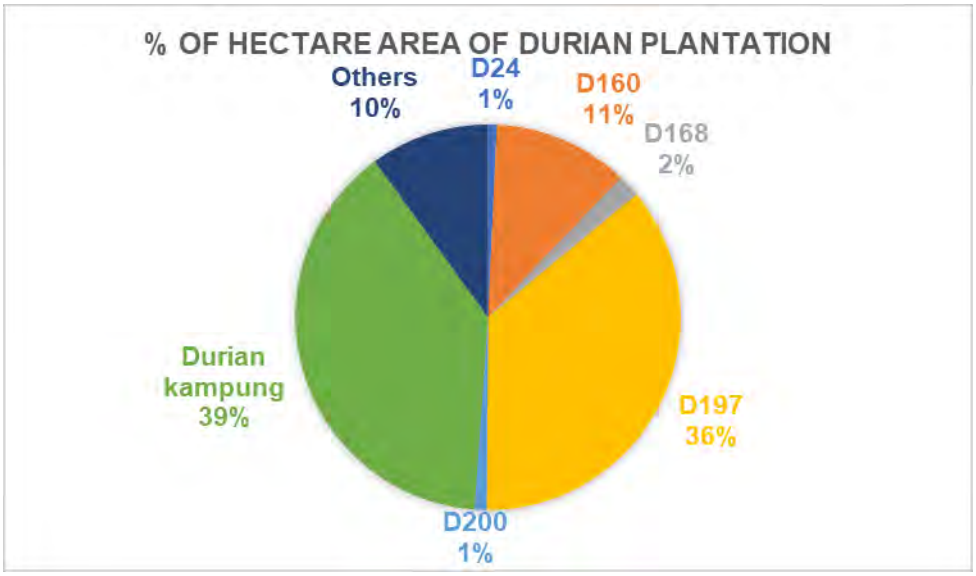
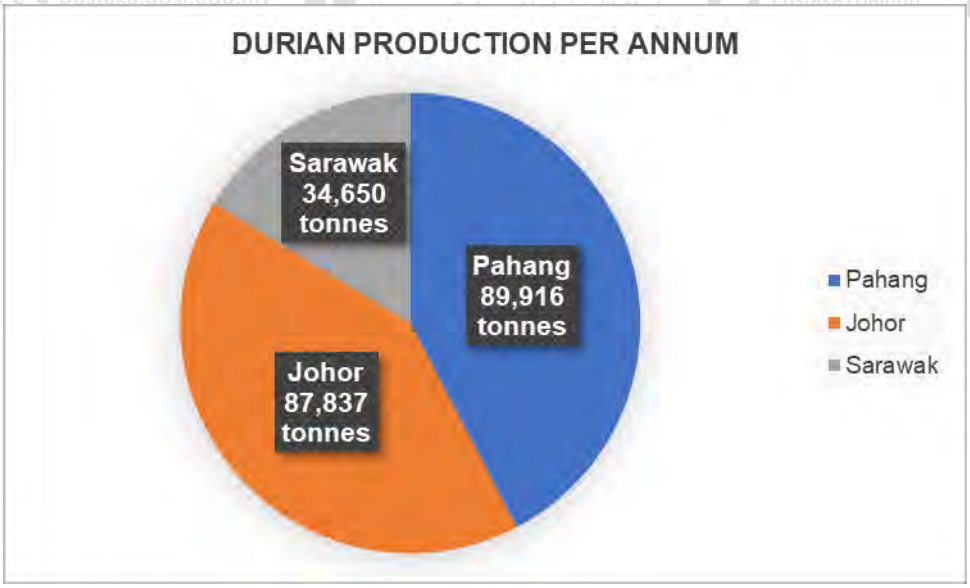


Figure 1.2

Durian production per annum by states



Local folk classification of morphological characteristics is solely dependent on trained experts and descriptions frequently differ from one person to another, making them inconsistent and controversial whereas durian research at molecular level in Malaysia is still lacking. The first genomic resource available for durian is

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sequenced by Teh et al. (2017) and revealed transcriptomic analysis for regulation of fruit-related processes in durian. Recently, Thailand also re-sequenced the genomes of three popular durian varieties; Kradumthong, Monthong, and Puangmanee (Nawae et al., 2023). Thus, to understand durian fruit developmental process, both morphological observation and molecular characterisation approach are complementary. Therefore, this study was conducted with two approaches. First, unique morphological traits were identified on six commercially popular durian varieties based on flower and fruit traits. Second, transcriptomic analysis was performed using NGS technology on durian fruit flesh from three different fruit developmental stages.

1.2 Research Background

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Malaysia has more than 139 different durian varieties native to this country, each with a unique flesh colour, flavour, and aroma (Department of Agriculture [DOA], n.d.). Department of Agriculture (DOA) had made intensive effort to rank Malaysian durian varieties since 2012 and had selected 14 varieties for large scale planting due to their trait's superiority. The varieties are D24, D99, D123 (Chanee), D145 (Beserah), D158 (Kan Yau), D159 (Mon Thong), D168 (Hajjah Hasmah/IOI), D169 (Tok Litok), D175 (Udang Merah), D188 (MDUR 78), D189 (MDUR 79), D190 (MDUR 88), D197 (Musang King) and D200 (Ochee) (Department of Agriculture [DOA], 2012; Department of Agriculture [DOA], 2014).

For the 14 durian varieties aforementioned, each has its' own uniqueness in aroma, taste and texture. Despite this, all durians undergo similar fruit development stages. In general, durian fruit development could be divided into two main stages: the flowering (anthesis and pollination) and fruit developmental stages (maturity and

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ripening). In other words, fruit development started during the onset of fertilization, followed by fruit maturation, ripening and ends with dehiscence.

Durian's flowers are hermaphrodites, having both stamen and pistil on the same flower (Nasrika & Retnoningsih, 2021). Moreover, flower bud shape, shape of apex and stigma position in relation to anther in the flower were unique to each variety. For example, D24 variety has ovate flower bud shape whereas D123 variety has circular flower bud shape (Department of Agriculture [DOA], 2017). Generally, flowers in durian were genetically inherited and flower traits were not likely to be influenced by environment changes (Idris, 1996). Thus, these characteristics could be used as an indicator to differentiate between durian varieties.

Very often, morphology characters are used for variety identifications in plants. For example, Chanee variety (D123) has creamy, sweet taste and strong aroma, Beserah (D145) has medium thick aril and creamy, sweet taste, and Kan Yau variety (D158) has a long pedicel and sweet taste (Hean et al., 2009). However, a comprehensive characterization and evaluation of durian varieties merely depending on morphological characters is very often insufficient, could be deceiving and quite controversial. Morphological identification is highly dependent on the observer, and sentiment could differ from one person to another. Moreover, morphological traits differences within the same variety were highly likely as they can be influenced by environment factors (Masri, 1999), pollinations (Ketsa, Wisutiamonkul, Palapol, & Paull, 2020) and the age of durian plants (Siew et al., 2018). For instance, studies had shown that environmental factors such as climate, nutrient and moisture content, and soil type have the potential to affect durian flowering, fruit set and fruitlet drop (Rushidah & Razak, 2001; Masri, 1999). In addition, water deficits significantly affected durian vegetative growth leading to a decline in leaf growth and photosynthetic activity (Masri, 1999). Incomplete pollination also causes variation in

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fruit shape, resulting in deformed fruits. If a locule contains an unfertilized ovule, that locule fails to develop and the fruit becomes irregular in shape (Ketsa et al., 2020). Therefore, other means of characterization for variety identification such as using molecular method is essential.

Fruit developmental period in durian could differ according to variety. For example, the D24 variety takes approximately 105-110 days after anthesis for the fruit to become mature, whereas the D99 variety matures early (90–100 days after anthesis) and the D197 variety, the maturity time is relatively longer (100-110 days after anthesis) (DOA, 2012). During ripening stage, the fruit become sweet, creamy, and aromatic and is at the optimal condition for consumption. Finally, the durian fruit enters the dehiscence stage which it starts to deteriorate within 36 to 72 hours from fruit drop (Pascua & Cantila, 1992). Many biochemical processes and changes occur during these stages. For instance, releasing of ethylene (Gao, Zhang, Li & Liu, 2020) and carotenoid accumulations (Wisutiamonkul, Ampomah-Dwamena, Allan, & Ketsa, 2017). In other fruits, anthocyanin accumulation during fruit ripening contributes to the red coloration of pear fruits (Ni et al., 2019). In durian fruit, significant changes could be observed, especially during ripening, where the fruit's arils change colour and have a sulphury and onion-like aroma, sweetness as well as creaminess were remarkably enhanced (Ketsa et al., 2020). However, not much effort had been done to understand and dissect the molecular mechanism during durian fruit development.

Nowadays, the advent of Next-Generation Sequencing (NGS) technology has substantially aided molecular approach in obtaining mass sequence data for gene discovery, transcriptional analysis and molecular marker development (Parchman, Geist, Grahnen, Benkman, & Buerkle, 2010). This technology enables identification of potential genes that affect fruit quality and understanding the biological functions and pathway mechanism that underlying fruit developmental stages. This approach is

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easy to implement, more accurate, cannot be influenced by external factors, and accurately reflects the mechanism behind fruit developmental stages (Satam et al., 2023).

Since comprehensive characterization should be done on minimum number, six varieties were selected to focus on this study (Table 1.1). These varieties are recommended by DOA and commercially cultivated throughout Peninsular Malaysia (MAFS, n.d.). The selected durian varieties are D24, D99 (Kop Kecil), D160 (Buluh Bawah/Tekka), D168 (Hajjah Hasmah/IOI), D197 (Raja Kunyit/Musang King) and D200 (Ochee) varieties. Even though D160 was not on the list of DOA recommendations in 2014 (DOA, 2014), it was still chosen for this study because this variety has a great potential in recent years. In 2017, this variety was rebranded as Musang Queen and was listed as one of the top ten varieties in Malaysia (Anem, 2019). The states of origin clone studied are mostly originated from Malaysia except D99 (Table 1.1). The D99 variety was also chosen in this study because it has adapted well in Malaysia and has become a popular local variety nowadays.

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Table 1.1

Registered durian varieties (Department of Agriculture [DOA], 2021)

Variety Registration Number	Variety Name	Registration Year	State of origin
D24	Bukit Merah	1937	Perak
D99	Kop Kecil	1970	Thailand
D160	Buluh Bawah/Tekka	1987	Selangor
D168	Hajjah Hasmah/IOI	1989	Johor
D197	Raja Kunyit/Musang King	1993	Kelantan
D200	Ochee/Black Thorn	2016	Pulau Pinang

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1.3 Problem Statement

Durian is a highly valued fruit in Malaysia and is grown in many different regions of the country, with each region producing durians with distinct flavours, textures, and aromas (DOA, n.d.). However, the large number of different durian varieties, combined with the fact that many of them have overlapping characteristics, can make it difficult to identify them accurately (Idris, 1996). This can be a problem for farmers, traders, and consumers, who may be unsure about the specific variety of durian they are buying or selling.

Very little work has been done on the characterization of all the durian varieties and information related to variety identification were either partial or incomplete (Idris, 1996). This may be due to the limitation of seasonal availability and difficult to measure and evaluate (Songnuan et al., 2019). Moreover, most people find it difficult to differentiate amongst durian varieties as morphological traits in durian are usually overlapping and not distinct. According to Songnuan et al. (2019), folk classification based on morphological traits are only discernible by trained experts where it is frequently describable but at the same time debatable. Furthermore, morphology characterization is only available for certain varieties and was recorded in the “National Guidelines for The Conduct of Tests for Distinctness, Uniformity and Stability”, initiated by DOA (DOA, 2017).

The durian fruit undergoes several developmental stages before it becomes fully mature and ready to be consumed. Briefly, these stages are the flowering (anthesis and pollination) and fruit developmental stages (maturity and ripening). Many biochemical processes and changes occur during these stages especially after the onset of maturity to ripening (Ketsa et al., 2020). In Malaysia, common durian harvesting practice is via natural fruit drop. The dropped fruit is usually ripened. After

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the fruit fell from trees, ripening progresses into dehiscence stage, where durian fruit husk starts to crack at the apex, revealing its' arils. Prolong exposure of durian arils to the air will invite pest or fungi which eventually causes it to rot (Razali, Rosly, Hamzah, Abdullah, & Hussin, 2020). At this stage, the fruit is considered over-riped and unsuitable for consumption. Usually the duration for ripening to dehiscence stage takes 36 to 72 hours (Pascua & Cantila, 1992), depending on variety and environmental factors. Due to the short transition time between the two stages, it will be a problem for durian seller, as the fruit might crack before it could be sold. Understanding the relevant biochemical and biomolecular pathways could better assist in durian breeding strategies to enhance durian fruit qualities.

Even though durian is an important tropical fruit crop, molecular research on durian is also nearly non-existent in Malaysia. The first durian genome publicly available was sequenced by Teh et al. (2017). Recently, three popular durian varieties, Kradumthong, Monthong, and Puangmanee, have been re-sequenced by Thailand (Nawae et al., 2023).

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1.4 Research Objectives

The objectives of this study are:

1) To characterize six commercial durian varieties in Malaysia using morphological observation.
2) To determine the biological functions and molecular pathway underlying fruit developmental stages.

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1.5 Research Questions

The research questions for this study are:

1) What are the morphological traits that could be used characterize six commercial durian varieties in Malaysia?
2) What are the biological functions and molecular pathway underlying fruit developmental stages?

1.6 Research Limitations

Although our study provides valuable insights into morphological traits, biological functions and molecular pathway mechanism underlying fruit developmental stages, there are several limitations that should be acknowledged. For morphological study, samples sample size was relatively small, and limited to observation on flowers, fruit, and fruit arils, which may have limited the generalizability of our findings. Methodology used were observations (colour, shape), taste (sweetness, bitterness, texture, creaminess) and measurements (length and weight). For molecular work, our study is only focused on biological functions and molecular pathway mechanism underlying fruit developmental stages utilizing transcriptomic analysis employing RNA-seq analysis. Finally, our study only took into considerations of six commercially grown durian varieties in Malaysia, which were sampled in two localities namely, Serdang Agricultural Complex, Selangor, and Bukit Koman, Raub, Pahang. Future research should consider addressing these limitations by using larger sample sizes from other sampling sites, provide replicates (i.e., in morphology study), examining morphological traits using more reliable methods (i.e., gas chromatography – mass spectrometry for volatile compound analysis), and controlling for potential confounding variables.

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1.7 Research Significant

The morphology observation information in this study act as initial findings that cover the gap in the partial available information in earlier records and studies. This information can be a good starting point to develop durian morphology database that can store the information systematically and initiating a good documentation especially on these six durian varieties. This is very helpful for durian growers, inspecting officer and public people to identify and differentiate between these six durian varieties clearly.

In addition, molecular characterization by transcriptome analyses will provide insights into the regulation of biological functions and pathways that are highly expressed throughout fruit developmental stages. This information will help and support the morphological information about the changes in durian fruit characteristics throughout fruit developmental stages more clearly. Interestingly, mass information on differential expressed genes obtained provides wide platform for future genetic and functional genomic research.

Detailed durian information on each of the three developmental stages will be helpful, especially for durian growers and durian sellers, to strategize post-harvest handling systems to avoid fruit cracking and fungal infection, keep durians fresh, and estimate the time for durians to be sold before they dehisce. If the fruit can be maintained to its excellent quality, this will increase durian production to meet local and global supply demand.

Thus, combination on both morphological observation and molecular characterization has filled the research gap information, aided in durian variety identification, and provides a comprehensive reference of complicated morphological

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and biological changes that happen throughout durian fruit developmental stages. This information obtained could be further explored to enhance the durian quality through genetic breeding strategies in the future.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Durian (*Durio zibethinus* Murr.) is one of Southeast Asia's most important fruit crops. Consumers prefer the taste, colour, and aroma of this durian species, increasing its economic value (Nasrika & Retnoningsih, 2021). The aril of the durian fruit is enclosed in a thick rind with a spike-like spine. Durian aril is a creamy yellow fruit with pungent odour. Despite its pungent odour, many people in Southeast Asia enjoy its flavour. Durian is now enjoyed by people all over the world, including those in the Western world. Its economic significance is growing all across the world (Honsho, Yonemori, Somsri, Subhadrabandhu, & Sugiura, 2004).

Malaysia has rich diversity of durian varieties that are cultivated throughout Peninsular Malaysia. D24, D99, D160, D168, D197 and D200 are some of the durian varieties that are commercially cultivated. Unfortunately, the potential of these durian commercial is still not well studied and documented. Many people believe that morphological observation is unimportant and trivial. In fact, morphological traits information serves as the foundation for the development of other disciplines such as plant systematics, ecology, genetics and physiology (Kaplan, 2001) (Figure 2.1).

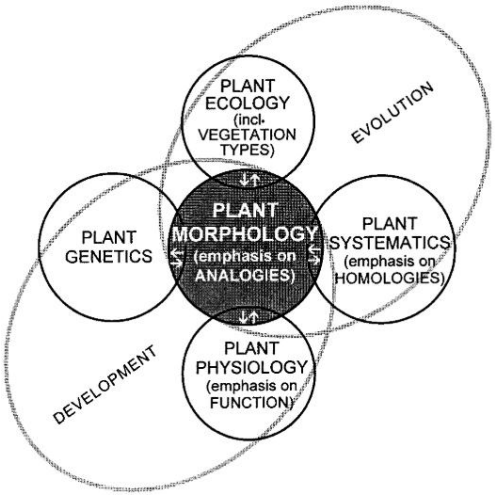
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There hasn't been much proper research done in Malaysia to observe the durian itself first in order to recognize and value the commercial trait that they represent.

Figure 2.1

Plant morphology connects other disciplines such as plant systematics, ecology, genetics and physiology



Adaptation from Kaplan, 2001

The main characterization problem with the durian varieties is due to the earlier description of the varieties which were based on incomplete material and insufficient field notes. Flower and fruit characters have been found to be important in the identification of the durian varieties, but unfortunately complete information on flowers or fruits are still lacking in some varieties. Characters such as the position of the flowers, colour of the fruit and aril and time of dehiscence (whether the fruit splits on the tree or drop unsplit) are important diagnostic characters, which are often not recorded by collectors (Idris, 1996).

Another difficulty is that durian varieties that have been cultivated show great variation in terms of quality, such as taste and colour of the aril. The wide variation within the varieties has not yet been fully studied. Since Malaysia is rich in genetic

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diversity of durian varieties, a comprehensive study on these varieties of great commercial value is very important.

As we know, morphological observation itself is not enough to classify durian varieties due to possibility occurrence of morphological variation. Morphological variation may suffer from phenotypic plasticity by environmental factors such as climate, soil type and nutrient and moisture content (Siew et al., 2018). Age of tree is also may contribute to morphological variation. Thus, there is a need to support this morphological information with molecular characterization. Both assessments are very important for continuous effort for genetic resource management, improvement, and durian production in the future.

2.2 Botanical Characteristics of Durian

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2.2.1 Morphology

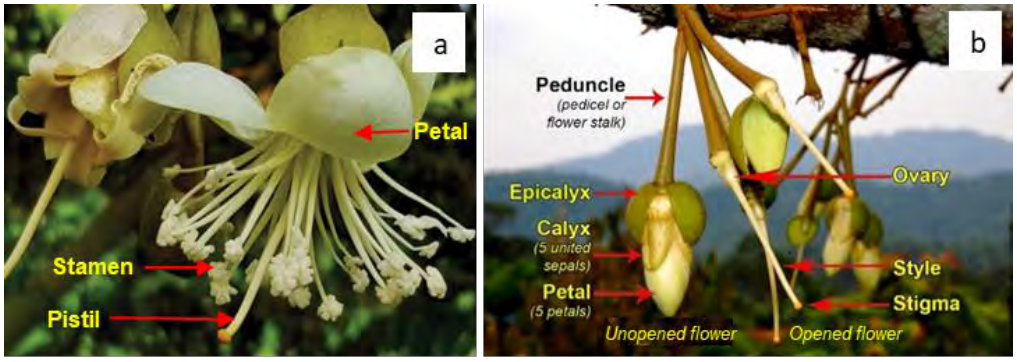
Flowers and fruit are important morphology characteristics while discussing on fruit development stages. Durian flowers are large, have five petals and contain both stamens and pistil in the same flower (Figure 2.2a). Each flower cluster has eight to 20 or more separate flowers, but only one to two fruits are set and collected from each cluster. Individual flowers from the same flush do not develop at the same rate, hence the size of the fruit in the same cluster can vary (Ketsa et al., 2020).

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Figure 2.2

Durian flower morphology. (a) Complete flower of durian contains both stamens and pistil (Ketsa et al., 2020) and (b) unopened and opened durian flower



Adaptation from *Botany of The Common Durian*, n.d.

Durian fruit is recognized by its large size, pungent odour, and hard thorny husk, which generally takes 95 to 130 days to mature. The fruit grows on a peduncle that grows from the underside of the branches, with an abscission zone slightly above the fruit (Figure 2.3). The fruit is round to oblong in shape, ranging 13 to 16 cm wide and 15 to 25 cm long on average, but can reach up to 35 cm long (Wisutiamonkul et al., 2017). The fruit has 3-5 longitudinal sutures that stretch from apical to basal and are known to open after the fruit has fully ripened (Husin, Rahman, Karunakaran, & Bhore, 2018).

Figure 2.3

Abscission zone on durian peduncle



Adaptation from *Botany of The Common Durian*, n.d.

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The fruit is often olive green to yellow in colour and covered with broadly pyramidal, and sharp spines that may weigh over than 3 kg. The edible part of the fruit is the fleshy aril. After successful pollination, the aril begins to form after about four weeks. The colour, texture, and thickness of the aril vary depending on the varieties (Ketsa et al., 2020). The colour of the aril may vary from white to yellow to red (Voon, Sheikh Abdul Hamid, Rusul, Osman, & Quek, 2007). The taste of the aril ranges from highly sweet, sweet, and pleasant to bland. The arils of cultivated varieties are noticeably thicker, fleshier, juicy, and tasty when compared to wild species (Ketsa et al., 2020).

Variations in fruit morphology can help differentiate between varieties to some extent. The form of the spines, for example, has been described for various Thai cultivars (Hiranpradit, Jantrajoo, Lee-Ungulasatian, & Polprasid, 1992). Fruit shape and spine morphology, according to Ketsa et al. (2020), are very helpful for distinguishing durians into distinct groups, and both are highly heritable traits. The presence of seeds affects the shape of durian fruit. If an unfertilized ovule is present in a locule, that locule won't develop, and the fruit will be irregular in shape (Ketsa et al., 2020). Fruit shape has a significant impact on marketability, therefore understanding pollination and its impact on durian fruit development is critical for durian improvement.

Tree height in *Durio* species varies from small (height less than 15 m) in *D. perakensis* to medium trees (16-30 m tall) such as *D. kutejensis* to very large trees (taller than 45 m) as in *D. zibethinus* (Idris, 1996). After seed germination, mature durian trees usually yield fruits after five to seven years. Yields in the early stages of production are inconsistent and low (10 - 60 fruits per tree) and can increase to 50 - 100 fruits/tree at the age of 10 years. Durian trees that reach the age of 20 years or more can produce up to 100 - 200 fruits per tree per season. However, yields may

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vary from year to year depending on weather conditions (Department of Agriculture Pulau Pinang, n.d.).

In Malaysia, durian is a climacteric seasonal fruit that is only available between the months of May and August. Durian trees have a biennial fruiting season, with a main season followed by a small season the following year. The season in Kedah, Perlis, Penang, Terengganu, and Kelantan occurs from May to August, whereas the season in Negeri Sembilan, Melaka, Johor, Selangor, Perak, Pahang, and Sabah occur from May to August, as well as November to January. In Sarawak, the season is only from November to January (Idris, 1996).

Early durian harvesting may also be available between the middles of March and October. The durian seasons are divided into three stages: early harvest, peak harvest, and late harvest. The best time to harvest durians for the domestic and global markets is during peak season. Durian's peak season lasts three to four months, depending on the condition of the fruit (Mohd Ali, Hashim, Abd Aziz, & Lasekan, 2020).

Durian quality parameters to consider include durian with a sweet flavour and nice pulp texture, few or small seeds, a large aril percentage, and a marketable weight of 1.5 to 3.5 kg, elongated to round shape, good shelf-life, good rind colour and thickness, and being free of disease and insects. However, country preferences for high-quality durian varies. Singaporeans prefer durians that are entirely ripe with no splitting, whilst Thais prefer durians that are less ripe with firmer pulp and less volatiles (Paull & Ketsa, 2014). Malaysians, on the other hand, love durian, which has a strong aroma, creamy flavour, and vibrant yellow pulp.

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2.2.2 Physiology

Information on physiology part is very crucial to understand the fruit developmental stages in durian. The stages of fruit development include flowering (anthesis and pollination) and fruit growth stages (maturity and ripening). Soil moisture is important in flower induction. Flowering is also triggered by a period of dry weather (three to four weeks). Durian flowers appear in the last month of the driest month or shortly after a long period of drought (Hoe & Palaniappan, 2013). A prolonged period of drought, on the other hand, does not induce trees to produce more flowers, and rainy, humid circumstances reduce flower numbers. These findings indicate that floral bud development need some drought, with subsequent bud growth and development required moderate irrigation (Masri, 1999).

The first sign of flowering is the appearance of a little "pimple" on the branches. Usually, the duration from flowering to fruit harvest is about five months (Idris, 1996). These buds enlarge, develop, and differentiate into the inflorescence throughout the course of a month, ending in an open flower stage (Ketsa et al., 2020). Just one to two weeks before flower opening, floral bud growth accelerates dramatically, with a substantial increase in flower bud size the night before anthesis (Honsho, Yonemori, Sugiura, Somsri, & Subhadrabandhu, 2004). When the flowers initial open at anthesis, nectar containing sucrose, fructose, and glucose is produced in large quantities. The receptive stigma, which is covered with stigmatic fluid, protrudes from the petals around 13.00 h before flower petal opening in the late afternoon. Anther dehiscence takes place between 18.00 and 19.00 h or later, depending on the variety (Ketsa et al., 2020).

Pollination is the transfer of pollen from an anther to the stigma to allow fertilization. Pollen is released between 18.00 to 19.00 h. Pollen germinated once

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attached to the stigma and grew down the style, with 40% entering the ovule and forming fruit (Lo, Chen, & Chang, 2007; Honsho, Yonemori, Somsri, et al., 2004). The delay in time between stigma receptivity and pollen release has an impact on pollination effectivity as the pollen grain's ability to germinate decreases later in the evening. After pollination, all floral parts including stamens, except for the ovary and pistil, abscise spontaneously and fall to the ground by 24.00 h (Figure 2.2b). Then, the filament dries up as the ovary grows bigger. Fruitlets are generated from the ovary if pollination is successful. The ovary declines seven to 10 days after anthesis if pollination is not occurring (Ketsa et al., 2020).

Self-incompatibility is common among durian varieties and thus cross pollination is required (Jutamanee & Sirisuntornlak, 2017). Durian rarely self-pollinates because the stigma is no longer receptive after the petals open, and the pollen is released. Cross-pollination of the D24 variety resulted in a fruit set rate of 54–60%, while self-pollination yielded just 5%. As a result, crosspollination significantly boosts fruit set in durian (Ketsa et al., 2020).

The abundant production of nectar attracts all pollinators. Fruit bats consume nectar from durian flowers as a food source. Wind dispersal is unlikely since pollen grains disperse in clumps. The effectiveness of various pollinators, on the other hand, is debatable. Although the giant honeybee (*Apis dorsata Fabricius*) is a major pollinator, honeybees (*Apis mellifera Linnaeus*) perform a minor role in pollination since they forage during the day. Some moths, ants, and large beetles are involved, as well as the nectar bat (*Eonycteris spelaea Dobson*) and lesser dog-faced fruit bat (*Cynopterus brachyotis* (Muller) (Bumrungsri, Sripaoraya, Chongsiri, Sridith, & Racey, 2009).

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Fruit growth on durian follows a single sigmoidal curve, with a comparable pattern of development for fruit weight, pulp weight, fruit width, fruit length, and pulp dry weight (Subhadrabandhu & Shoda, 1997). There is little difference in fruit length and diameter two weeks following pollination. After the fourth week, the length of the fruit increases more rapidly than the diameter. For example, the length of the Monthong variety increases rapidly than the width, resulting in an oblong shape. Next, fruit development is fast until the 13th week, then slows until the 16th week, when the fruit has matured. Then, the fruit will abscise when fully ripe. Depending on the varieties, the time from fruit set to maturity ranges from 95 to 135 days (Thongkum et al., 2018).

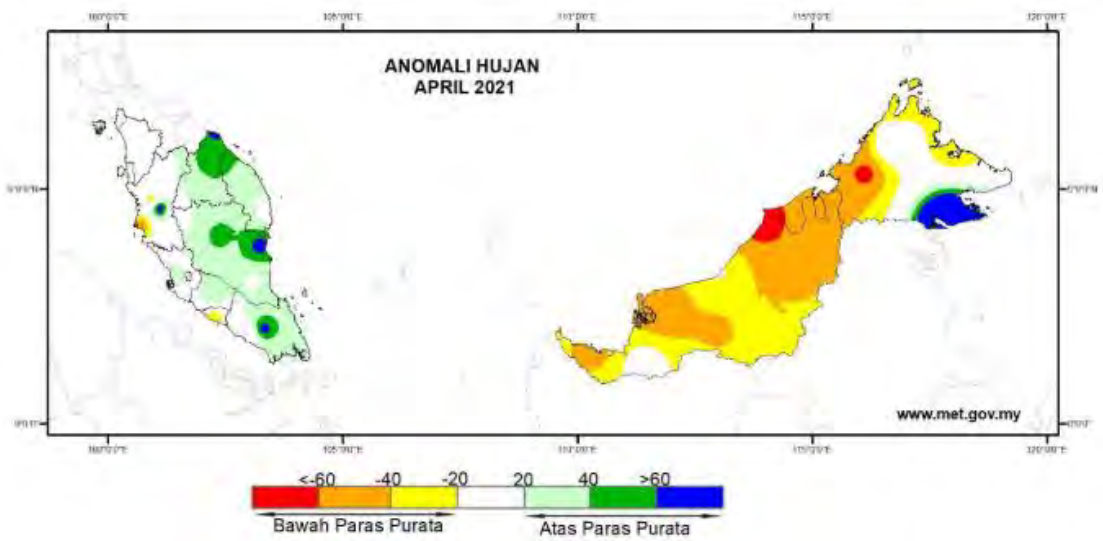
In durian, the rainfall factors play important roles and have impact on durian productivity. The fruits begin to mature during the rainy season, which ranges from April to September depending on the type of monsoon (Figure 2.4-2.5). In Malaysia, dry period with adequate rainfall can induce flowering in durian. However, excessive rainfall were not good for durian flowering and can be detrimental for flower pollination and fruit set (Rushidah & Razak, 2001). In order to survive, durian trees must be in a warm, humid climate with a best suited temperature range of 25 to 30°C and a rainfall distribution of 1.5 to 2 m per year (Foo & Hameed, 2011).

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Figure 2.4

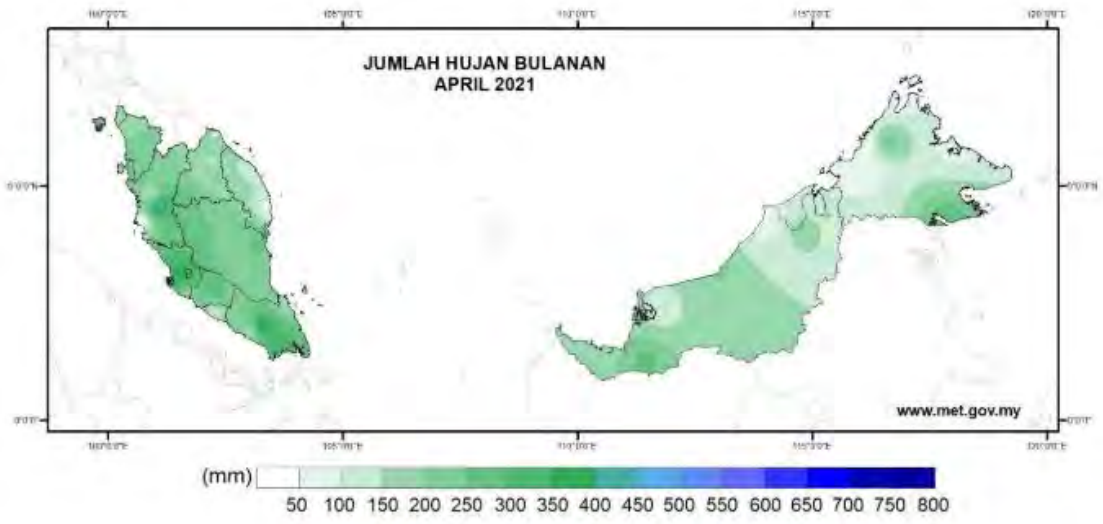
Rainfall Anomaly (April 2021)



Adaptation from Malaysian Meteorological Department [MET], 2021

Figure 2.5

Monthly amount of rainfall (April 2021)



Adaptation from MET, 2021

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2.3 Chemical composition and nutritional values

The commercial market for durian consumption, marketing, and manufacturing has grown dramatically (Paull & Ketsa, 2014). This interest has gradually shifted toward investigating the fruit's chemical composition and nutritional value. Durians are also high in minerals and vitamins, as well as essential macronutrients and micronutrients (Li, Schieberle, & Steinhaus, 2012). The agricultural and food processing sectors are interested in these nutrients. The benefits of durian as a valuable resource can also be related to the fruit's volatile components and phytochemical properties.

According to Wisutiamonkul, Ampomah-Dwamena, Allan, and Ketsa (2017), the volatile compounds varied depending on the durian varieties and concentration. The onion-like and strong odour produced by sulfuric chemicals, as well as the fruity odour produced by alcohols and esters, are the main components of durian odour (Phuangsombut, Phuangsombut, Talabnark, & Terdwongworakul, 2018). Table 2.1 shows a summary of the primary volatile elements in durians from various research. According to the findings reported in various research works, different varieties and analytical procedures produced varying results in identifying the volatile components of durian. Furthermore, the chemical contents of different durian varieties are mostly determined by the fruit's maturity level. As a result, studies focusing on durian volatile compounds may be required to determine the fruit's key aroma volatile (Mohd Ali et al., 2020).

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Table 2.1

Identification of selective volatiles compound in durian from various research studies

Compound Types	Compound	Relative amount (ng/g)	References
Aldehydes	Acetaldehyde	38.0-289.5	Voon et al. (2007)
		19.2-93.9	Belgis, Wijaya, Apriyantono, Kusbiantoro, and Yuliana (2017)
	Hexanol	2.6-18.0	Voon et al. (2007)
		39.0-131	Belgis et al. (2017)
Sulphur compounds	Diethyl disulphide	12.42-18.76	Chin et al. (2007)
		175.5-500.0	Terada et al. (2014)
		2.48-4.69	Aschariyaphotha, Wongs-Aree, Bodhipadma, and Noichinda (2021)
	Bis(ethylthio)methane	49.3-246.1	Belgis et al. (2017)
	Diethyl trisulphide	2.52-14.68	Chin et al. (2007)
Esters	Ethyl acetate	1.53-38.6	Voon et al. (2007)
			Belgis et al. (2017)
		9.5-52.4	Aschariyaphotha et al. (2021)
		8.81	
	Methyl propanoate	0.71-0.97	Chin et al. (2007)
		7.2-202.0	Voon et al. (2007)
			Belgis et al. (2017)
		6.8-52.2	
	Ethyl propanoate	1.85-3.11	Chin et al. (2007)
		3.8-1570.5	Voon et al. (2007)
			Terada et al. (2014)
		74.2-109.0	Belgis et al. (2017)
		87.5-742.3	Aschariyaphotha et al. (2021)
		3.39-5.7	
	Ethyl butanoate	0.04-2.22	Chin et al. (2007)
		76.6	Voon et al. (2007)
			Belgis et al. (2017)
		73.2-252.3	Aschariyaphotha et al. (2021)
		1.08-2.69	
	Propyl-2-methylbutanoate	4.77-12.67	Chin et al. (2007)
		16.0-327.9	Voon et al. (2007)
			Terada et al. (2014)
		30.4-91.6	Belgis et al. (2017)
		11.8-1047.8	Aschariyaphotha et al.

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Compound Types	Compound	Relative amount (ng/g)	References
		45.52	(2021)
	Ethyl hexanoate	1.17-5.52	Chin et al. (2007)
		12.2-405.8	Belgis et al. (2017)
		10.35-162.79	Aschariyaphotha et al. (2021)
Esters	Ethyl heptanoate	0.15-0.25	Chin et al. (2007)
		42.4-116.3	Belgis et al. (2017)
		8.20-15.5	Aschariyaphotha et al. (2021)
Alcohols	Ethanol	0.56-1.09	Chin et al. (2007)
		393.7-839.8	Voon et al. (2007)
			Belgis et al. (2017)
		27.1-1091.3	
Ketones	3-Pentanone	2.6	Belgis et al. (2017)
	3-Hydroxy-2-butanone	10.31-58.0	Voon et al. (2007)
		22.3-125.4	Belgis et al. (2017)
Acids	2-Methyl butanoic acid	4.5	Belgis et al. (2017)
	Octanoic acid	4.1	Belgis et al. (2017)
		2.86-12.57	Aschariyaphotha et al. (2021)

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A wide range of foods have been recognised as producing volatile chemicals that contribute significantly to the overall scent and odour (Mohd Ali et al., 2020). The durian aril can be made into traditional Malay cakes such lempuk, bubur, dodol, and pengat durian, or fermented into tempoyak, as well as used to the cooking of a variety of recipes. It can also be processed into a puree for ice cream flavour or a paste by boiling the pulp in sugar (Idris, 1996). Durian chips, dessert, biscuit, cake and juice are examples of modern durian products in recent years (Mohd Ali et al., 2020).

Fruit odour is made up of a variety of volatile compounds, but the major volatile element is the benchmark by which the odour matter is determined (Aschariyaphotha, Wongs-Aree, Bodhipadma, & Noichinda, 2021) (Table 2.2). β-oxidation of fatty acids, amino acid metabolism and alcohol acyltransferase could be

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essentially involved in the production of esters in most durians (Aschariyaphotha et al., 2021).

Table 2.2

Odour description of volatile compounds in durian (Aschariyaphotha et al., 2021)

Compound Types	Compound	Odour Description
Ester	Methyl-2-methylbutanoate	Sweet, fruity, apple-like odour
	Ethyl acetate	Fruity, sweet, grape- and rum-like odour
	Ethyl propanoate	Green, fruity apple-like odour
	Ethyl-2-methylpropanoate	Fruity
	Ethyl butanoate	Fruity, pineapple
	Propyl propanoate	Sharp, chemical, pungent, sweet, fruity
	Ethyl-2-methylbutanoate	Fruity
	Methyl-2-methyl-2-butenoate	Caramel note, ethereal rum
	Methyl hexanoate	Fruity, pineapple, ethereal
	Propyl-2-methylbutanoate	Winey
	Ethyl-2-methyl-2-butenoate	Sweet fruity, green notes
	Methyl-2-hexenoate	Fruity green banana honey
	Ethyl hexanoate	Apple-like, fruity, aniseed-like, sweet
	Methyl heptanoate	Sweet, fruity and green, with a waxy apple-like note
	Ethyl-2-methyl pentanoate	Fruity, green, melon and waxy with a fatty nuance
	Ethyl-2-hexenoate	Fruity, green, pulpy pineapple and apple
	Propyl hexanoate	Sweet, fruity, juicy, pineapple, green and tropical
	Ethyl heptanoate	Fruity pineapple cognac rum wine
	Methyl octanoate	Waxy, green, sweet, orange, aldehydic, vegetable, herbal
	Ethyl octanoate	Pleasant, fruity, floral odour, wine apricot note
	Ethyl-(E)-2-octenoate	Fruity, green with a fatty waxy note
	2-Methylbutyl hexanoate	Ethereal
	Methyl decanoate	Oily, winey, fruity, floral
	Ethyl decanoate	Fruity, grape-, cognac-, and brandy-like odour
	Methyl dodecanoate	Waxy, soapy nutty, coconut, mushroom
	Ethyl dodecanoate	Waxy, soapy, rummy, nutty, floral

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Compound Types	Compound	Odour Description
	Ethyl noanoate	Slightly fatty, oily, fruity, nutty, reminiscent of cognac with a rosy fruity note
Sulphur Compound	Diethyl disulfide	Onion, garlic
	3,5-Dimethyl-1,2,4-trithiolane	Sulphury, onion, meaty
Acid	Propanoic acid	Pungent, acidic, dairy
	Hexanoic acid	Sour, fatty, sweaty, cheesy
	Octanoic acid	Fatty, waxy, rancid oily, vegetable cheesy
Aldehyde	Trans-2-Methyl-2-butenal	Strong green fruit

Previous research has shown that durian fruit has a high potential as a therapeutic indicator for individuals suffering from various diseases (Ho & Bhat, 2015). Table 2.3 shows the proximate composition of durian based on several studies. It should be emphasised that different durian varieties, as well as different analytical methods, resulted in differences in identifying the fruit's phytochemical contents. The biological properties of producing a food-related product from durian have aroused curiosity due to the fruit's diversity in phytochemical components. Thus, phytochemical property investigations are useful for evaluating the nutrient composition and validating the major chemical components of a wide range of durian food products (Mohd Ali et al., 2020).

Table 2.3

Durian proximate composition (µg/mg/g/100 g fresh sample) from several research

Nutritional Composition	Charoenkiatkul, Thiyajai, and Judprasong (2016)	Aziz and Jalil (2019)	Thiyajai et al. (2020)
Carbohydrate	62.9-70.7	15.65-34.65	18.1-37.3
Protein	7.6-9.7	1.4-3.5	2.2-3.8
Moisture	58.3-69.3	56.1-69.3	54.2-73.3
Fat	10.1-14.0	1.59-5.39	2.6-6.1
Total sugar	47.9-56.4	3.1-19.97	12.6-21.8
Dietary Fiber	7.5-9.1	0.9-3.8	1.0-3.7
Ash	2.9-4.3	N/A	0.9-1.5

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Nutritional Composition	Charoenkiatkul, Thiyajai, and Judprasong (2016)	Aziz and Jalil (2019)	Thiyajai et al. (2020)
Carotenoid	265-2248	5.13-6000	N/A
Phenolic content	67-289	21.44-374.3	N/A
Anthocyanins	N/A	0.32-633.44	N/A
Flavonoids	N/A	1.9-93.9	N/A
Fatty acid	0.01-7.8	0.11-2543.70	N/A
Calcium	9-17	3.21-40.00	1-6
Phosphorus	84-117	25.79-44.00	31-49
Sodium	37-67	1.0-40.0	10-21
Potassium	1057-1685	70.0-601.0	406-676
Magnesium	56-81	19.28-30.00	15-23
Iron	0.75-1.42	0.18-1.9	0.21-0.47
Copper	0.41-0.83	0.12-0.27	0.07-0.25
Vitamin A	N/A	2.0	N/A
Vitamin B1/Thiamine	N/A	100-374	N/A
Vitamin B2/Riboflavin	N/A	100-200	N/A
Vitamin B3/Niacin	N/A	1074-13650	N/A
Vitamin B6/Pyridoxine	N/A	316	N/A

Note, N/A = not available.

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2.4 Germplasm Resources and Improvement of Durian in Malaysia

Malaysia currently has over 139 durian varieties as a result of cross-pollination, providing a broad spectrum for the selection of desirable variety (DOA, n.d.) (Figure 2.6). These varieties have been selected and registered by the DOA since 1934 (Figure 2.7). The years 1970-1990 demonstrate that durian variety registration was quite active, with the highest durian variety registration of 21 varieties in 1970. The main contributors to the registration of durian varieties are the states of Perak and Selangor, with each registering 33 and 29 varieties, respectively (Figure 2.8). Two varieties, from Thailand, have also been registered: Katoi (D98) and Kop Kecil (D99). There are even more varieties that originated from Thailand, such as D123 (Chanee),

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D158 (Kan Yau) and D159 (Mon Thong), but they are registered under local addresses.

However, certain information is not properly documented and missing from the variety registration document. For example, the variety registration number is D1 until D215 (Figure 2.6). There should be 215 varieties in the record, but there are only 139 varieties listed. There are some missing sequence numbers in the registration. Furthermore, there are 13 varieties with missing variety name information in the list. Missing information also happens in the registration year (three varieties) (Figure 2.7) and variety origin (six varieties) (Figure 2.8).

The DOA has recommended fourteen superior varieties among the varieties registered: D24, D99, D123 (Chanee), D145 (Beserah), D158 (Kan Yau), D159 (Mon Thong), D168 (Hajjah Hasmah/IOI), D169 (Tok Litok), D175 (Udang Merah), D188 (MDUR 78), D189 (MDUR 79), D190 (MDUR 88), D197 (Musang King) and D200 (Ochee) (DOA, 2012, 2014). Aside from local varieties, introduced varieties from Thailand, such as D99, D123, D158, and D159, generally adapt well to the local environment and then become popular local varieties. D7 (no variety name), D16 (no variety name), and D96 (Bangkok A) are no longer recommended varieties because they are less superior (Idris, 1996). There are also some varieties that are believed to be extinct due to natural disasters, the World War II as well as attacks from pests and diseases (Anem, 2013).

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Figure 2.6

Durian variety name registered under Plant Variety Protection Malaysia (PVP), DOA

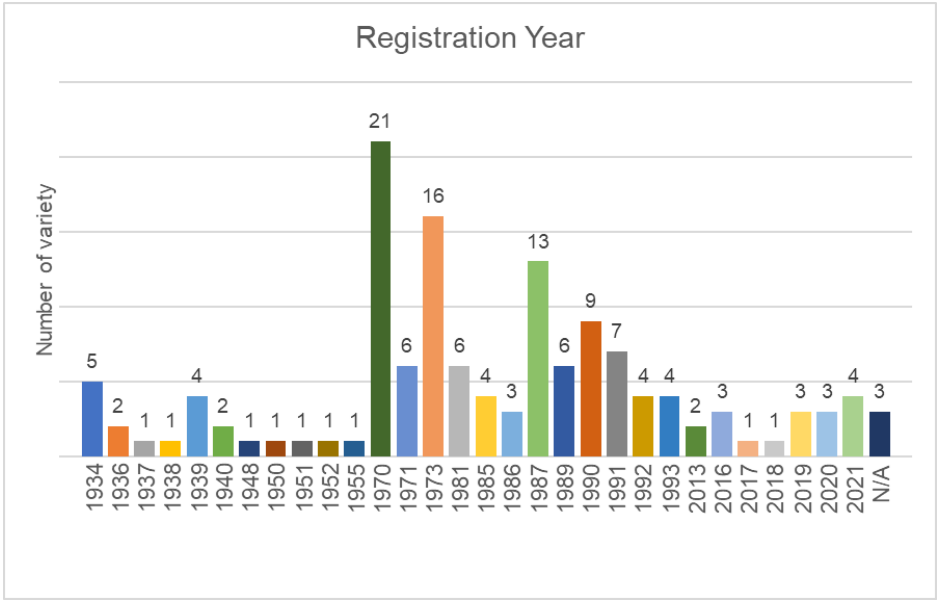
Variety Registration	D1-D215
Total Variety	139

1.Ang Bak/ Isi merah	36.Eddie Special	71.Kop Besar	106.Penang Bintang
2.Apian	37.Emas Enggang	72.Kop Jantung	107.Pikat
3.BalikPulau 604	38.Emas Pahang II	73.Kop Kecil (D99)	108.PK110
4.Bangkok 8	39.Emas Perak	74.Kop T.24	109.PK285
5.Bangkok A	40.Empring Emas	75.Kop T.25	110.Raja Hutan
6.Bangkok Tree 16	41.Foo	76.Kuala Paya Merah P089	111.Raja Kunyit/Musang King
7.Bangkok Tree 17	42.Foo Fatt	77.Lempur Mas	112.Raja Patani
8.Bangkok Tree 28	43.Gabai	78.Mas Pahang1	113.Repok B2
9.Biancheng	44.Ghani	79.MDUR78	114.RimD1
10.Bintang	45.Hj. Abu	80.MDUR79	115.RimD5
11.Bola 828	46.Ho Keun No. 1	81.MDUR88	116.S17
12.Buaya	47.Hor Lor/ Labu	82.Merah	117.Saddam
13.Bukit Merah (D24)	48.Hybrid 7 (D24xD2)	83.Mon Thong/ Bantal Mas	118.Sakai No. 1
14.Buluh Bawah/Tekka	49.Hybrid T.No.30 (D101xD2)	84.N/A	119.Seberang
15.Chanee	50.Hybrid T.No.34 (D66xD2)	85.N/A	120.Seberang Manong 4
16.Chanee T. 41	51.Hybrid T.No.57 (D2xD7)	86.N/A	121.Seberang Manong 5
17.Cheh Chee	52.Jantung	87.N/A	122.Senggarang 1
18.Dato Nina (D2)	53.Juara 90 Penang	88.N/A	123.Senggarang 2
19.Durian Batu	54.Jurong 3	89.N/A	124.Senggarang 3
20.Durian Botak	55.K.Kangsar 2	90.N/A	125.Senggarang 4
21.Durian Ganja/ Taiping 3	56.Kampan	91.N/A	126.Simpang Permata
22.Durian Ganjil	57.Kan Yau T.63	92.N/A	127.Sri Kaya
23.Durian Gombak	58.Kan Yau/ Tangkai Panjang	93.N/A	128.Taiping 2
24.Durian Haji Sani	59.Kancong Darat	94.N/A	129.Taiping 5
25.Durian Hijau	60.Kang Hai	95.N/A	130.Tawa
26.Durian Kuning	61.Kappai	96.N/A	131.Tepi Sungai
27.Durian Lipur	62.Katak	97.Nasi Kunyit	132.Titi Kerawang
28.Durian Mas Hjh.Hasmah/IOI	63.Katoi	98.Nasi Kunyit Terengganu	133.Tok Litok
29.Durian Raja	64.Katoi T.9	99.Ochee/Black Thorn	134.Tok Merah
30.Durian Rambutan	65.Kepala Babi	100.Paduka	135.Tuan Mek Hijau/ Beserah
31.Durian Sg.Sut	66.Kg. Perak	101.Pak Awang V22	136.TUNGKU 3
32.Durian Siew	67.Khaw	102.Pakta T.66	137.TUPAI 226
33.Durian Tembaga	68.Kim Hong/Golden Phoenix	103.Paya Lintah Kuning	138.Udang Merah
34.Durian Ular Kekek	69.Kim Luang	104.Penang 88	139.WM
35.DX/ Rouge D24	70.KK5/ Manong	105.Penang 99	

Note, N/A = information is not available.

Figure 2.7

Registration year of durian varieties under Plant Variety Protection Malaysia, DOA



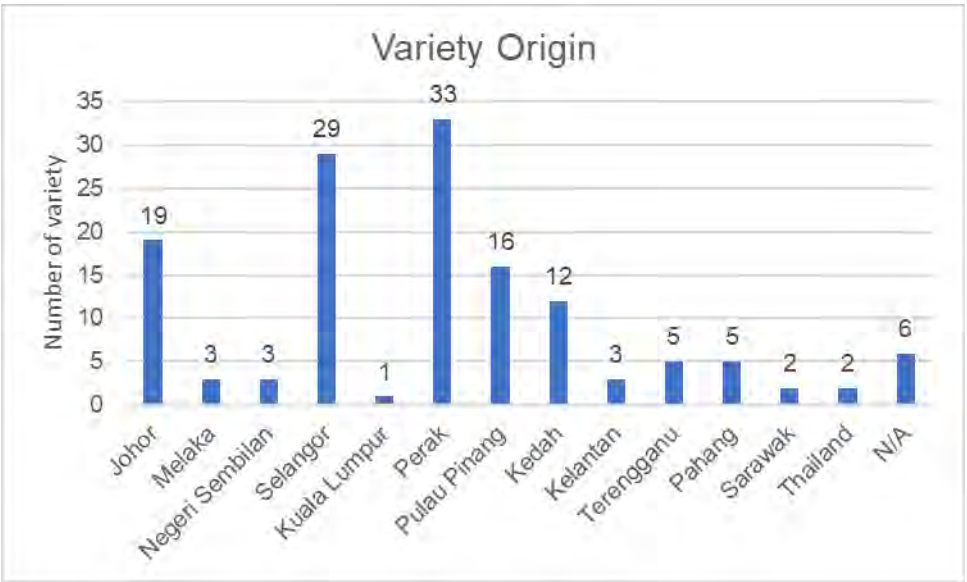
Note, N/A = information is not available.

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Figure 2.8

Durian variety origin



Note, N/A = information is not available.

A quick survey of recent online searches on popular Malaysian durian varieties on five websites was carried out to discover the most favourite variety among the public (Figure 2.9). The durian rating has been scored based on the durian varieties mentioned on the websites (Figure 2.10). The top rated and most popular among the public are D24, Ochee/Black Thorn, Raja Kunyit/Musang King, and Udang Merah. The varieties marked in green are all registered under PVP. However, there are three more popular varieties (Black pearl, Golden Bun (D13) and XO) that are marked in red but not yet registered under PVP. Even though the Golden Bun variety (highlight in red) has a PVP registration code, this variety is not listed in the PVP. Since all these varieties have gained popularity among the public, they must be cross-checked with the PVP list to determine whether they are a new variety or an identical variety. There is a possibility that the public may refer to similar varieties by different names in different places. For example, the XO variety is said to be a higher-quality variant of D24 (*XO Durian*, n.d.).

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Figure 2.9

Popular durian varieties based on public people opinion

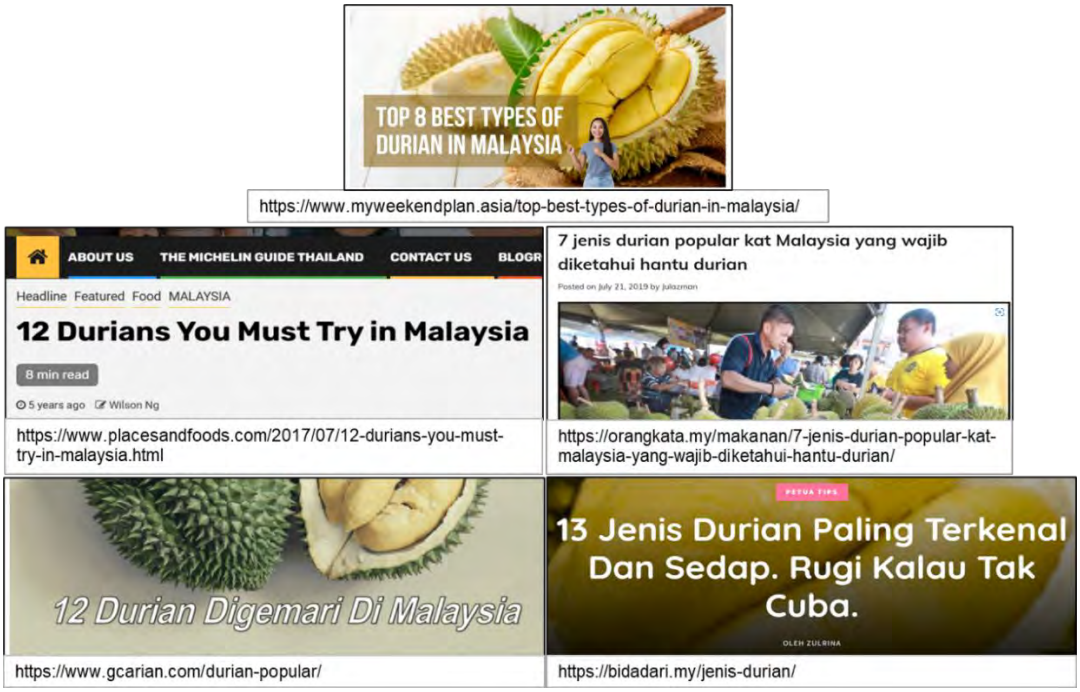


Figure 2.10

Durian rating based on five different websites on popular durian varieties



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To remain competitive and viable in the market, new varieties of durian are constantly needed. Furthermore, breeding strategies aimed at increasing yield and resistance to pests and diseases, as well as quality attributes relevant to customer preferences such as taste, flavour, and appearance, are crucial. These qualities have a significant impact on market acceptability and pricing. Malaysian Agricultural Research and Development Institute (MARDI) has made some key efforts in durian breeding, such as improving propagation and hybridization among popular local varieties. Malaysia is the first country to produce commercial durian varieties developed through F1 hybrid breeding and selection programme. The programme began in the late 1960s with D10 and D24 varieties as parents and culminated in the early 1990s with the release of hybrid F1 varieties, namely MDUR 78, MDUR 79, and MDUR 88. The advantage of these hybrids is that they are able to provide yields as early as five to six years, have high yield potential and quality, and most importantly, are tolerant to patch canker attacks (Sani, Abbas, Buniamin, Nordin, & Abdul Rashed, 2015). The evaluation of more durian hybrid trees is still currently in progress to determine which are the best to introduce and commercialize later.

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2.5 Durian Morphological Characterization Research

Morphological characterization usually includes the description of variation for polymorphic and highly heritable morphological traits that are of direct interest to users. Morphological characteristics are often straightforward, fast, and inexpensive to score, even from preserved specimens, because morphological evaluations do not require complicated equipment or preparatory procedures (Bretting & Widrlechner, 1995). However, the evaluation should have specific guidelines to avoid misidentification, confusion, and to ensure the use of the same parameters to reach an equivalent standard.

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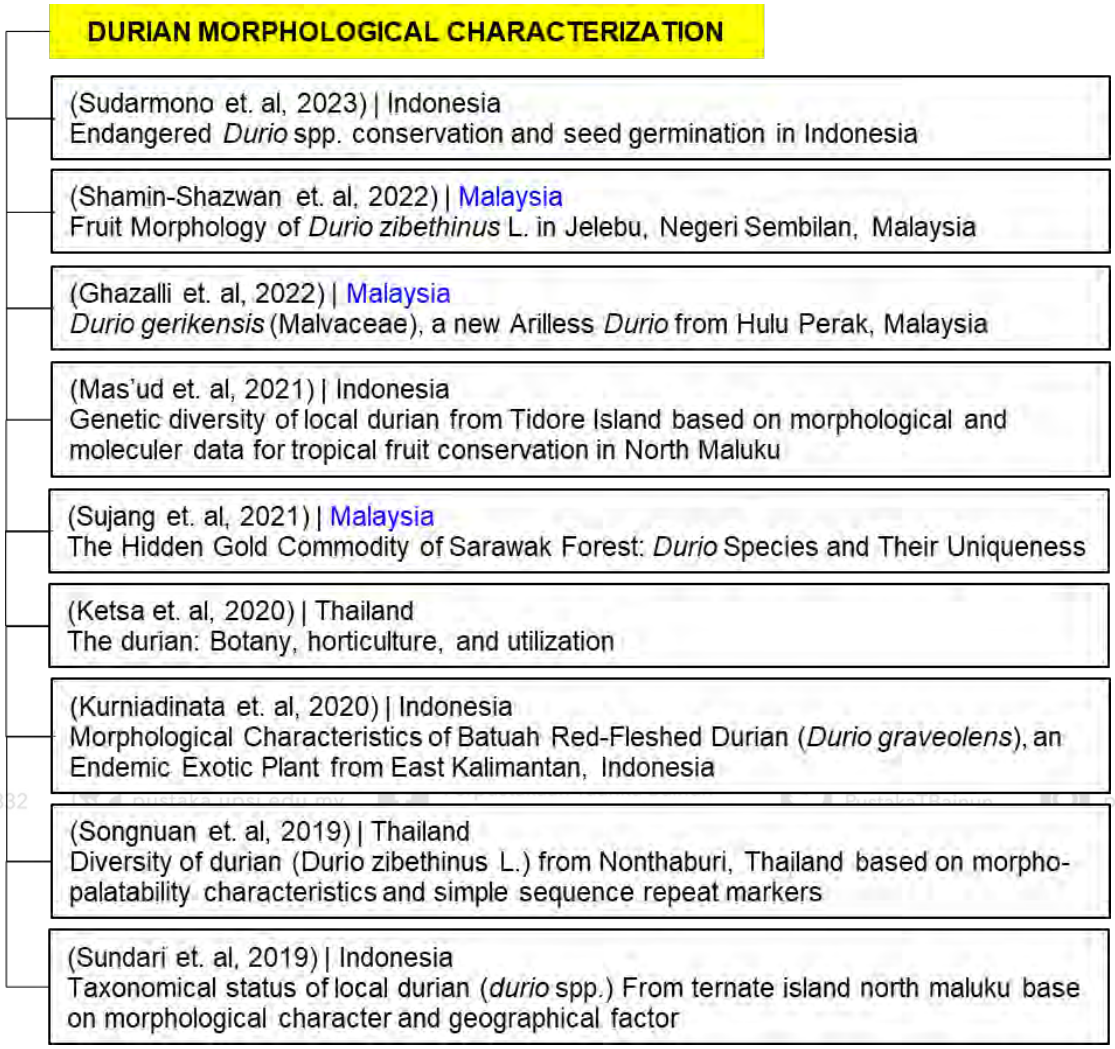
In the past five years, some durian morphological characterization research has been carried out in Malaysia, Indonesia, and Thailand (Figure 2.11). There are three study in Malaysia has been done by Ghazalli et al. (2022), Shamin-Shazwan, Razali, Shahari, Amri, and Go (2022) and Sujang, Ramaiya, and Saupi (2021). It is a good exploration as they study wild *Durio* species and Durian Kampung, which have diverse genetic variation and may be beneficial for breeding programmes. Sujang et al. (2021) has studied six wild *Durio* species that are in Sarawak. The *Durio* species are *D. dulcis* (Kulit merah), *D. graveolens* (Isu), *D. testudinarum* (Durian kura-kura), *D. kutejensis* (Nyekak), *D. oxleyanus* (Durian daun) and *D. zibethinus*. Then, Ghazalli et al. (2022) discovered a new *Durio* species, *Durio gerikensis*, with distinctive reddish-pink flowers. Next, Shamin-Shazwan et al. (2022) focused on the identification and classification of *D. zibethinus* accessions in Jelebu germplasm, which is known as the top hotspot area for people looking for genuine Durian Kampung.

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Figure 2.11

Durian morphological characterization research from 2019-2023



However, morphological information for the commercialized durian variety is still lacking and partially characterized. Many of the available information is based on general characteristics rather than scientific research. A search of the literature regarding commercialized durian discovered only two studies, done in 1996 and 2004. Idris (1996) studied and compared 24 *Durio* species from herbarium specimen and 45 varieties of *D. zibethinus* by obtained data from morphological, anatomical and palynological analysis. Later, Santoso (2004) described fruit size, fruit shape, fruit skin colour, flesh colour, flesh taste, flesh thickness, seed and thorn

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characteristics for 125 clones which data obtained from the Register Book of Durian, at DOA, Serdang, Selangor.

A comparison has been made between the findings of Idris (1996) and Santoso (2004) and the information available in the National Guidelines for The Conduct of Tests For Distinctness, Uniformity and Stability (DOA, 2017) (Table 2.4). The morphological traits evaluation has used different descriptors (i.e., Fruit weight and Fruit size descriptors), the findings are not in line (i.e., D197 fruit shape: Santoso-elongate; DOA-elliptic), and the information available is not complete for the six durian varieties under study.

Table 2.4

Morphological traits comparison between DOA (2017), Idris (1996) and Santoso (2004) for six varieties under study

(Idris, 1996)	(Santoso, 2004)	(DOA, 2017)
Fruit Weight D24: (1.1-)1.6(-2.2) D99: (1.1-)1.8(-3.3)	Fruit size D24: 2=medium D99: 2=medium D160: 3= large D168: 2=medium D197: 2=medium	Fruit Weight No variety information
No descriptor	Fruit shape D24: 3 = elongate D99: 2 = obtuse D160: 4 = others D168: 3 = elongate D197: 3 = elongate	Fruit: shape D197: 3 = elliptic D200: 4 = circular
Shape of Base of Fruit D24: round D99: cordate	No descriptor	Fruit: shape of base D99: 3 = cordate D168: 2 = rounded

Only a brief description of the morphology for some varieties were recorded in the National Guidelines for The Conduct of Tests for Distinctness, Uniformity and Stability, DOA. The flower and fruit of the durian are only partially characterized for some varieties and characteristics (Table 2.5). Therefore, it is necessary to revise, to

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complete the characterization, and to establish proper documentation in order to define the six durian varieties.

Table 2.5

Morphological description on durian flower and fruit information recorded in the National Guidelines For The Conduct Of Tests For Distinctness, Uniformity and Stability, DOA for D24, D99, D160, D168 and D200 varieties (DOA, 2017)

No.	Characteristic	D24	D99	D160	D168	D197	D200
1	Flower bud: shape	√					
2	Flower bud: shape of apex	√					
3	Fruit: depth of groove		√		√		
4	Fruit: shape					√	√
5	Fruit: shape of base		√		√		
6	Fruit: shape of the stylar end				√		
7	Fruit: colour of skin		√		√		
8	Fruit: spines around the base of the pedicel		√			√	
9	Fruit: curvature of the spine apex at the base of the pedicel				√		
10	Fruit: spines at the stylar end					√	
11	Fruit: ridges along the line dividing sections					√	
12	Flesh: main colour	√				√	√
13	Flesh: bitterness						√

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Morphological characterization study has also been done for durian producing countries such as Thailand and Indonesia. Thailand has been reported durian diversity in Nonthaburi province using morphological characteristics and molecular marker analysis. Morphological variation in 31 durian varieties was analysed using a modified durian descriptor that measured 22 qualitative traits and 33 quantitative traits from the leaves, flowers, and fruits. Nine from 33 quantitative traits are commonly used by local gardeners to identify specific varieties or are of interest to consumers or both (Songnuan et al., 2019).

In Kalimantan, Indonesia, morphological characterization study has been done for Batuah Red-Fleshed Durian (*Durio graveolens*). Kalimantan has rich in genetic resources especially for *Durio spp.* diversity. They study on *Durio graveolens* because this species is unique due to its red flesh. They want to explore if this species has superior potential that will be benefit for their durian industry. Based on this study, they figured out that this species is differ from other *Durio spp.*, it has red, dark colour flesh, unique and strong aroma with soft texture aril. Thus, this species has a great potential economic value to be improve later as Superior Commodities from East Kalimantan Province, Indonesia (Kurniadinata, Wenpei, & Rusdiansyah, 2020).

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2.6 Durian Molecular Characterization Research

As Teh et al. (2017) was announced the first draft of the durian genome in 2017, the discovery has gained a lot of interest, and researchers have begun to focus on durian molecular research (Figure 2.12). The durian genome was sequenced from the Musang King variety (D197) from Malaysia. In the following years, the molecular study of durian using the sequencing approach has risen, and Thailand is ahead in

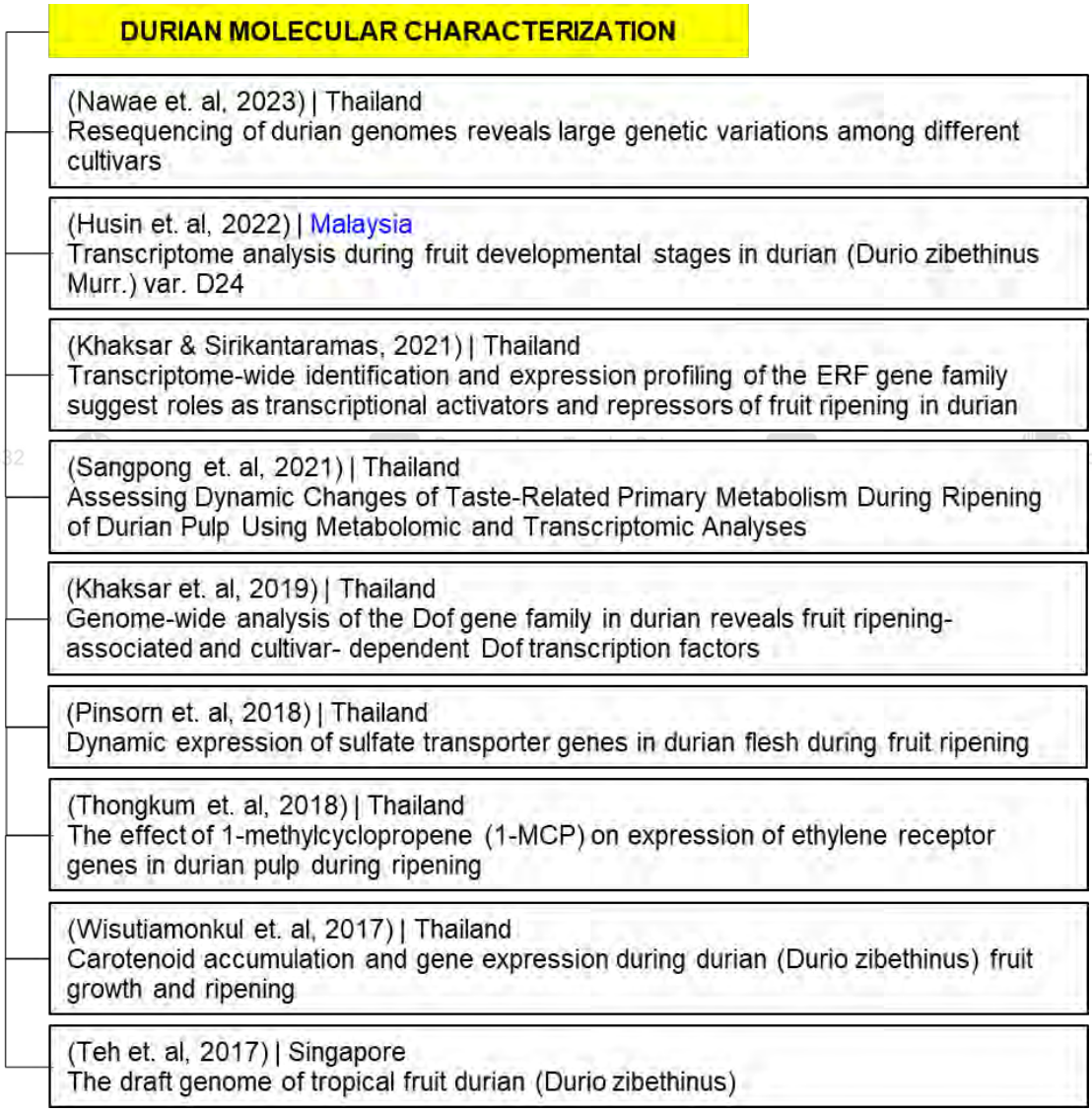
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gene expression studies. Recently, Thailand has also re-sequenced its three popular durian varieties (Kradumthong, Monthong, and Puangmanee) (Nawae et al., 2023). A literature search just found one study from Malaysia that has been done by Husin, Rahman, Karunakaran, and Bhore (2022).

Figure 2.12

Durian molecular characterization research from 2017-2023



Even Teh et al. (2017) and Husin et al. (2022) conducted research on Malaysian durian, their study just focusing on a single variety. Teh et al. (2017) have performed genome sequencing on Musang King (D197) and determined

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phylogenetic relationships with other Malvaceae members. They also compare transcriptome data from different plant organs (fruit aril, leaf, stem, and root) as well as between different fruits (banana, mango, avocado, tomato, and blueberry) and compare Musang King aril with Puang Manee aril (Thailand variety). The findings of genomic and transcriptomic analysis revealed information about the evolution and regulation of fruit-related processes in durian, such as ripening, flavonoid synthesis, and sulphur metabolism.

Husin et al. (2022) then investigated the stages of fruit development in the D24 variety. They obtained 11,394–24,351 differentially expressed genes during 90–127 days post-anthesis. KEGG enrichment analysis found that metabolism of cofactors and vitamins, nucleotide metabolism, and carbohydrate metabolism were upregulated at the ripening stage. Therefore, the durian information currently available is for the D24 and D197 varieties. To gain a deeper understanding of how gene expression expresses durian characteristics towards fruit developmental stages, the other four durian varieties (D99, D160, D168, and D200) need to have their molecular genetics explored as they take a different fruit developmental period.

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2.7 Summary

The presence of various durian varieties from various regions in Malaysia offers a wide genetic variation and potential traits that need to be explored. However, proper durian identification is required to differentiate the varieties and avoid redundancy. Therefore, it is necessary to understand the traits and select the desirable traits that are beneficial for the durian industry. This is critical for identifying, differentiating, and establishing durian groupings, which is important especially to durian breeders for durian quality trait enhancement in the future. Thus, this study is critical for advancing

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durian research in Malaysia by providing information on both morphological and molecular characterization. Since durian Malaysia has been getting attention worldwide recently, it has been the responsibility of researchers to explore more durian potential with the latest discoveries in the fields of science and technology.

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CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter discussed about sample size, sampling sites, parameters used for morphological observation, and molecular characterization approaches. The research design carried out for this study is presented in Section 3.2. Methodology presented here was divided into two sections based on objectives. Section 3.3 discussed methodology related to characterization of six commercial durian varieties using morphological observation, while section 3.4 described methodology regarding transcriptomic analysis of six commercial durian varieties based on three fruit developmental stages (matured, riped, and dehisced).

3.2 General Research Design for Morphology and Molecular Work

Firstly, in morphological study, a total of 28 morphological traits were recorded, measured and photographed as listed in Table 3.1. In summary, eight quantitative (QN), two qualitative (QL) and 18 pseudo-qualitative (PQ) traits were taken on flower buds, flowers, fruits, durian's flesh and seeds. Quantitative traits (QN) were

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measured in centimetres (cm) and kilogrammes (kg), while qualitative traits (QL) and pseudo-qualitative (PQ) traits were assessed using standard descriptors outlined in the national guidelines (DOA, 2017).

Table 3.1

Twenty-eight morphological trait observations in six varieties of Malaysian durian

Trait	Legend	Characteristic	State
1	PQ	Flower bud: shape	ovate (1), elliptic (2), circular (3)
2	PQ	Flower bud: shape of apex	acute (1), obtuse (2), rounded (3)
3	PQ	Flower: stigma position in relation to anther	below (1), level/equal (2), above (3)
4	QN	Pedicel: length	centimetres (cm)
5	QN	Fruit: weight	kilogrammes (kg)
6	QN	Fruit: length	centimetres (cm)
7	QN	Fruit: width	centimetres (cm)
8	PQ	Fruit: depth of groove	absent or weak (1), medium (2), strong (3)
9	PQ	Fruit: shape	ovate (1), oblong (2), elliptic (3), circular (4), oblate (5), obovate (6)
10	PQ	Fruit: symmetry	symmetric (1), slightly asymmetric (2) strongly asymmetric (3)
11	PQ	Fruit: shape of base	acute (1), rounded (2), cordate (3)
12	PQ	Fruit: shape of the stylar end	pointed (1), rounded (2), retuse (3)
13	PQ	Fruit: color of skin	RHS colour chart
14	PQ	Fruit: presence of spines	absent (1), present (9)
15	PQ	Fruit: type of spine	Type I (1), Type II (2), Type III (3), Type IV (4), Type V (5), Type VI (6)
16	QL	Fruit: spines around the	absent (1), present (9)

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Trait	Legend	Characteristic	State
		base of the pedicel	
17	QL	Fruit: spines at the stylar end	absent (1), present (9)
18	QN	Fruit: weight of peel	kilogrammes (kg)
19	QN	Fruit: weight of seeds	kilogrammes (kg)
20	QN	Fruit: number of seeds per fruit	number of seeds
21	QN	Fruit: number of flattened seeds per fruit	number of flattened seeds
22	PQ	Flesh: main colour	white (1), light yellow (2), medium yellow (3), dark yellow (4), yellow orange (5), pink yellow (6), pink (7), red orange (8), red (9)
23	PQ	Flesh: aroma	weak (3), medium (5), strong (7)
24	PQ	Flesh: sweetness	weak (3), medium (5), strong (7)
25	PQ	Flesh: bitterness	weak (3), medium (5), strong (7)
26	PQ	Flesh: texture	fine (3), medium (5), coarse (7)
27	PQ	Flesh: creaminess	weak (3), medium (5), strong (7)
28	PQ	Seed: shape	oblong (1), circular (2), elliptic (3), ovate (4), obovate (5)

Secondly, study was undertaken to investigate the molecular aspect of durian by using transcriptome sequencing analysis. RNA extraction and library preparation were performed on the flesh durian samples collected. The transcriptome sequencing was run through Next Generation Sequencing on the Illumina Novaseq 6000 platform. After that, a bioinformatic analysis pipeline was performed on the raw data obtained and biological interpretation was studied in detail. Detailed workflow for RNA sequencing and bioinformatic analysis pipeline carried out is displayed in Figure 3.1 and Figure 3.2. In Figure 3.3, the whole research approach is summarized.

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Figure 3.1

RNA sequencing workflow

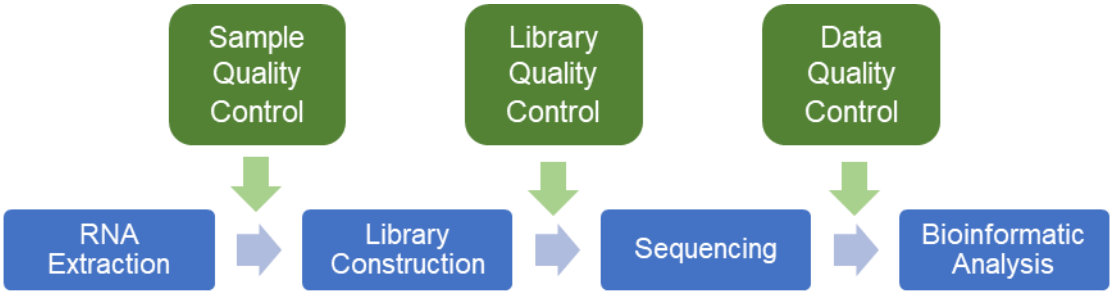
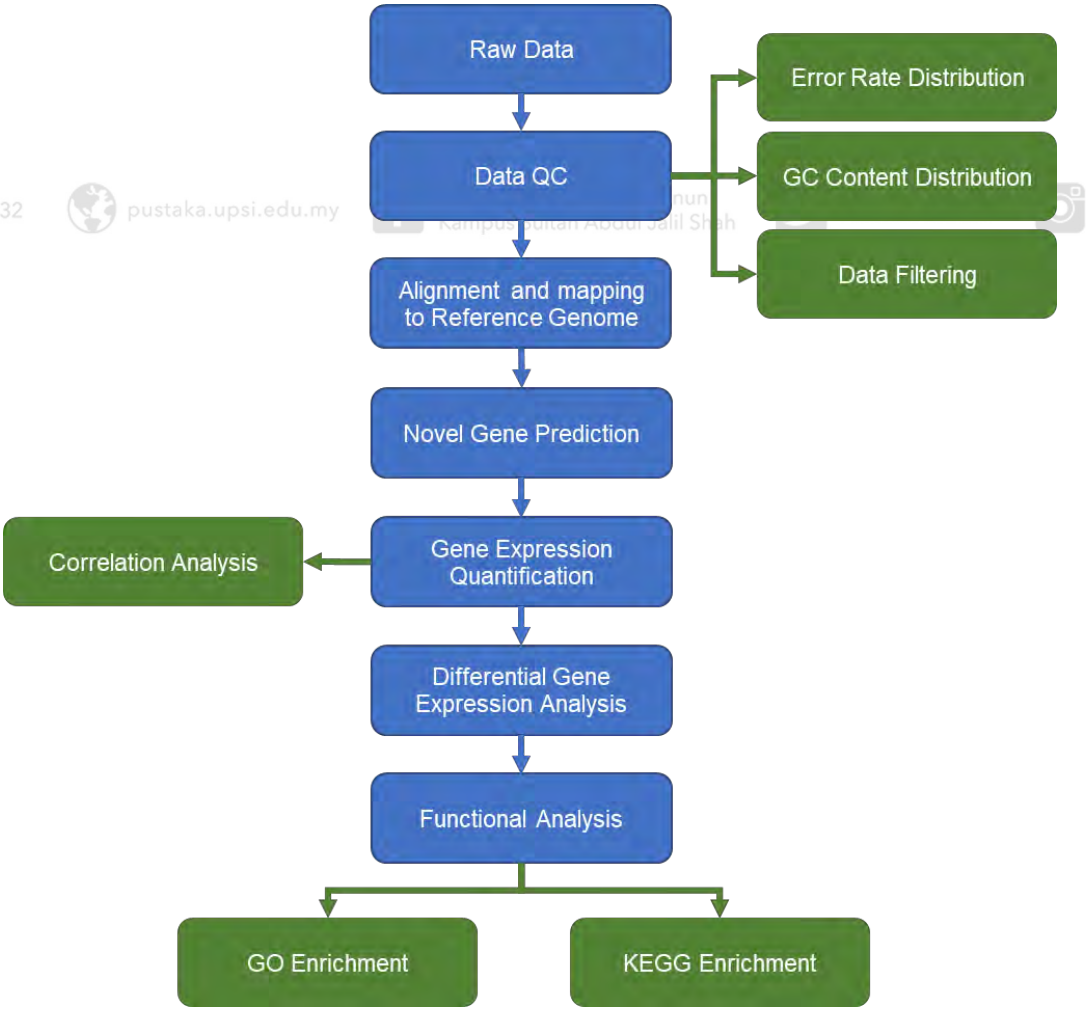


Figure 3.2

Bioinformatic analysis pipeline for mRNA sequencing data with a reference genome

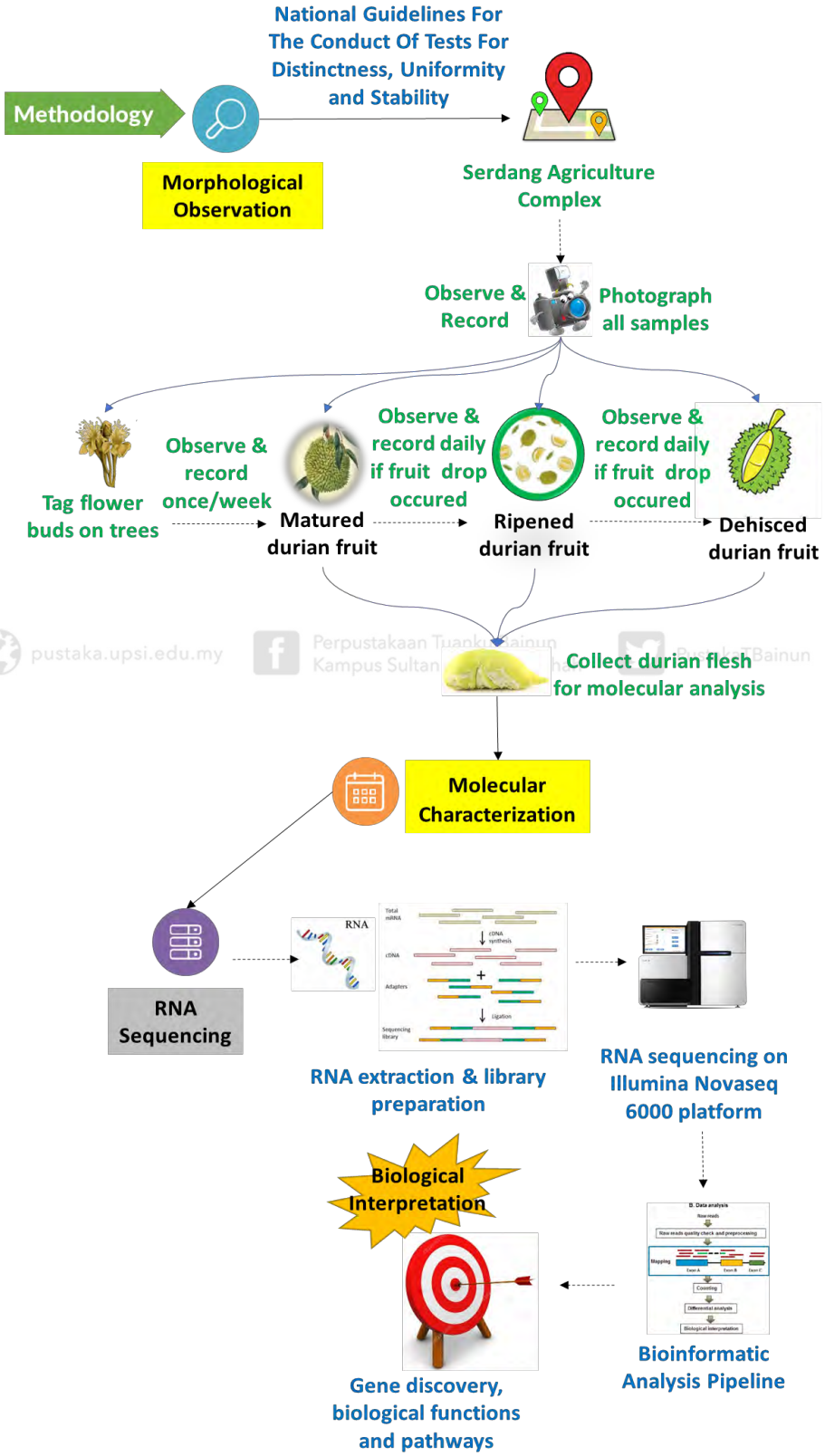


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Figure 3.3

Summary of research approach for both morphological observation and molecular characterization



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3.3 Characterization of Six Commercial Durian Varieties in Malaysia Using Morphological Observation

3.3.1 Plant Material and Sampling Sites

There were two sampling sites used, namely, Serdang Agriculture Complex (germplasm collection sites) at DOA Serdang, Selangor, and a privately owned durian orchard in Bukit Koman, Raub, Pahang (Raub Mining & Development Co. Sdn. Bhd.) (Figure 3.4). Since Pahang durian orchard is a privately owned orchard, comprehensive observation is only accessible to the Serdang Agriculture Complex. Furthermore, most of the varieties studied (D24, D99, D168, and D197) were available at the Serdang Agriculture Complex. In the Pahang durian orchard, the D160 and D200 varieties samples were available, and the evaluation authorised was to verify the trees with the orchard owner, choose suitable trees, take pictures, and collect the fruits.

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Six varieties were collected from these two localities (Table 3.2). For morphology observation, two plant structures were studied, flower and fruit. The observation of flowers started during the flowering season, which begins in March 2021 and ends in April 2021. Riped fruits were harvested from trees beginning in June 2021 and ended in August 2021. Due to the heavy rainy season between April and May 2021 (explained in more detail in Chapter 2.2.2), the D197 durian variety trees located at Serdang Agriculture Complex were unable to bear any fruits, hence D197 fruit samples were also collected from the Pahang durian orchard together with the D160 and D200 variety fruits.

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Figure 3.4

(a) Durian orchards located at Serdang Agriculture Complex, Serdang, Selangor and (b) Bukit Koman, Raub, Pahang



Table 3.2

Durian sampling sites and varieties used in this study

No.	Variety registration number	Local name	Location
1	D24	Bukit Merah	Serdang, Selangor
2	D99	Kop Kecil	Serdang, Selangor
3	D160	Buluh Bawah / Tekka	Raub, Pahang
4	D168	Durian Mas Hjh. Hasmah / IOI	Serdang, Selangor
5	D197	Raja Kunyit / Musang King	Serdang, Selangor (Flower)* Raub, Pahang (Fruit)*
6	D200	Ochee / Black Thorn	Raub, Pahang

Note, * = D197's flower bud and flower were obtained from Serdang, Selangor while D197's fruits were obtained from Raub, Pahang.

The Serdang Agriculture Complex has about 268 durian trees within 3.7 hectares of planting area (Figure 3.5). The durian trees were planted in specialized arranged plots (Figure 3.6) while observation of flowers and harvesting of fruits were carried out for twenty-two durian trees of D24, D99, D160, D168, D197 and D200 varieties as specified in Table 3.3.

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The trees in the Serdang Agriculture Complex were selected based on the maturity of the trees. The durian tree is said to reach maturity when it bears fruit for the first time. It started bearing fruits at the age of 5 years after being cultivated. The orchard owner has confirmed with the trees the year they were planted and their history of fruiting seasons. Upon the onset of the durian season, every tree on the farm was scrutinised, and only the trees that were flowering were tagged. However, the trees in the Pahang durian orchard were chosen with the supervision of the orchard owner.

Figure 3.5

Durian tree observation was made on C Block, located in the durian orchard at Serdang Agriculture Complex



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Figure 3.6

Plot layout of durian orchard at Serdang Agriculture Complex. The green highlighted box are the durian trees that has been sampled in this study

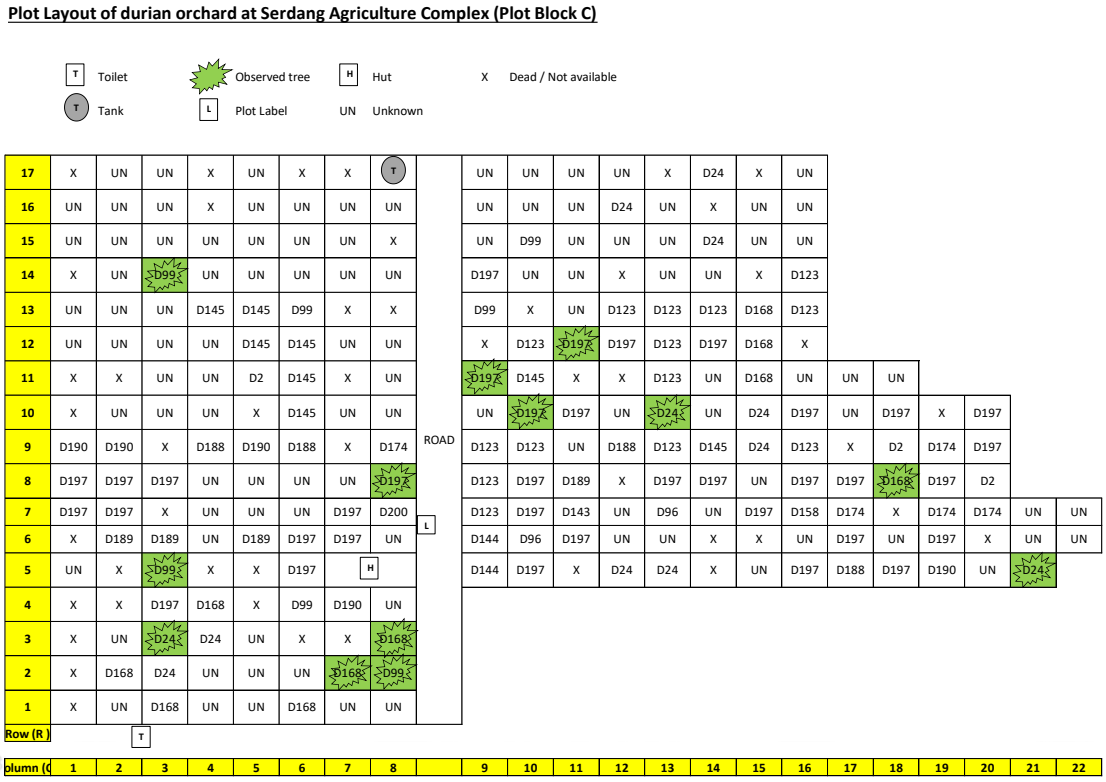


Table 3.3

Durian trees in the Serdang Agriculture Complex and Pahang Durian orchard

No.	Variety	Tree No.	Plot No.	Location
1	D24	D24_1	R10, C13	SAC
2		D24_2	R3, C3	SAC
3		D24_3	R5, C21	SAC
4	D99	D99_1	R5, C3	SAC
5		D99_2	R2, C8	SAC
6		D99_3	R14, C3	SAC
7	D160	D160_1	N/A	BK
8		D160_2	N/A	BK
9		D160_3	N/A	BK
10	D168	D168_1	R2, C7	SAC
11		D168_2	R3, C8	SAC
12		D168_3	R8, C18	SAC

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No.	Variety	Tree No.	Plot No.	Location
13	D197	D197_1	R11, C9	SAC
14		D197_2	R8, C8	SAC
15		D197_3	R12, C11	SAC
16		D197_4	R10, C10	SAC
17	D197	D197_1	N/A	BK
18		D197_2	N/A	BK
19		D197_3	N/A	BK
20	D200	D200_1	N/A	BK
21		D200_2	N/A	BK
22		D200_3	N/A	BK

Note,
R = Plot row based on Figure 3.6;
C = Plot column based on Figure 3.6;
SAC = Serdang Agricultural Complex;
BK = Raub Mining & Development Co. Sdn. Bhd. (durian orchard in Bukit Koman, Raub, Pahang);
N/A = Information is not available.

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3.3.2 Flower Morphological Observation

Briefly, flower development could be detailed and grouped into three main stages namely: inflorescence buds, pre-anthesis, and anthesis (Alagna et al., 2016). Initially, the flower buds on durian trees were first tagged in the early stages of the durian season in March 2021. For D24, D99, D168 and D197 varieties, tagging was done when small "pimple" protuberances were observed on the branches (locally known as "mata ketam") (Figure 3.7). However, D160 and D200 varieties were only observed through photographs taken by camera due to being difficult to reach as the tree is quite high. When the flower bud had reached the size of a pea, the flower bud shape has been compared with the standard descriptors listed in Table 3.1 on several branch regions and a representative flower bud was selected, cut off and photographed to record the flower bud shape. The durian trees were then examined

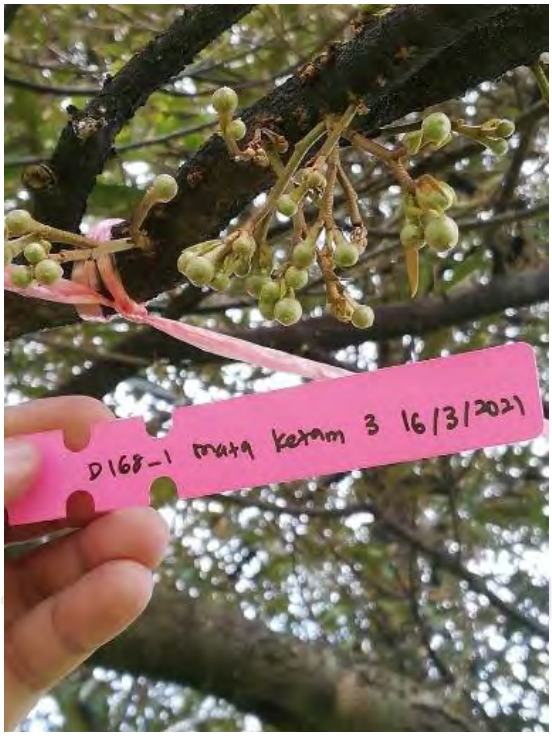
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once a week (for a duration of seven weeks) to see if other flower buds had successfully developed into full blossom flowers, immature or already withered.

Figure 3.7

Flower buds on durian trees were first tagged in the early durian season



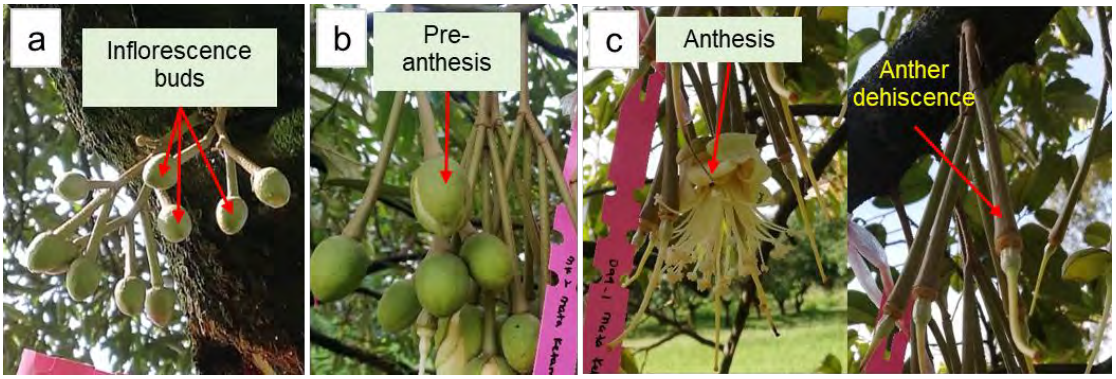
Next, those flower buds that were left un-excised would then continue to grow (Figure 3.8a) and develop until they entered the pre-anthesis stage (Figure 3.8b), usually happened at late evening. The flower's open blossom stage started when several bee species began foraging on the flower and continued to visit throughout the night. Representative sample of flower blossom stage was collected and photographed early in the morning (Figure 3.8c). Approaching 24 hours, anther dehiscence occurred in which all flower parts including stamens (except the pistil) were spontaneously abscised and fall to the ground (Figure 3.8c).

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Figure 3.8

Flower bud stages of anthesis. (a-b) Flower bud enlarged until ready to enter pre-anthesis stage in the late of evening. (c) Anther dehiscence occurred and flower fall to the ground



All pictures were taken using a Canon DSLR (Digital Single-Lens Reflex) camera with a tripod against a grey background, aligned with a ruler as a scale and controlled lighting in a mini studio (Figure 3.9). The flower bud shape, shape of apex and stigma position (Trait 1-3) were evaluated according to pre-determined shape standard descriptors listed in Table 3.1. Characteristics of durian flower were recorded to the closest most resembled descriptor. Then, the durian flowers that were successfully pollinated were observed continuously every once a week (for a duration of sixteen weeks) to see the fruit development stages (matured, ripened or dropped before fully developed) (Figure 3.10).

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Figure 3.9

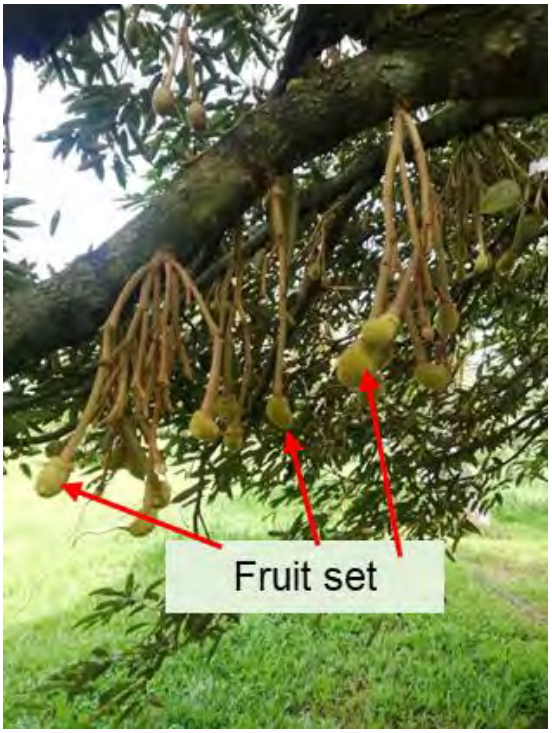
Professional pictures were taken using a mini studio set-up



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Figure 3.10

Successful pollinated flowers developed into fruit sets



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3.3.3 Fruit Morphological Observation

Overall, fruit morphological data were collected and observed for the six durian varieties based on three fruit developmental stages; matured, riped, and dehisced stages. In this study, one matured durian fruit from each tree was sampled while the fruits were still on the tree (Figure 3.11 a-b). The mature fruit, usually produces a hollow sound when tapped with a wood, rattan or stick, was cut using a knife from the tree and cut above the abscission zone. Two ripened durian fruits from each tree were collected after it falls to the ground naturally (Figure 3.12 a-b). One fruit was observed on the day it fell, and the other were stored at room temperature until it dehisced (Figure 3.12c).

Figure 3.11

Matured durian fruit's observation. (a) Fruits were observed until they reach maturity. (b) Durian fruit were cut off to be observed, photographed, and sampled for durian flesh

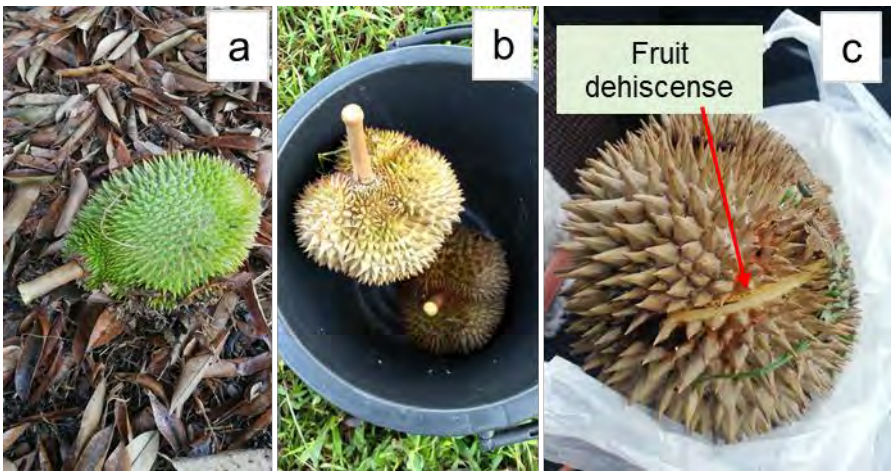


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Figure 3.12

Ripened durian fruit's observation. (a) Ripened durian fruit (D24 variety) that falls to the ground. (b)Two ripened fruit (D99 variety) collected and one of them stored at room temperature. (c) Fruit dehiscence (D168 variety) occurred, making a cracked line



The sampled fruits were examined for the traits as listed in Table 3.1 and photographed (Figure 3.13a). The length of the pedicel was measured using a paper ruler (Trait 4 in Table 3.1; Figure 3.13b). The fruit's length and width were measured using a ruler held in place by two book stand holders (Trait 6-7, Figure 3.13c). The fruit weight was measured using a CAS weighing balance (Trait 5, Figure 3.13d). The colour of the fruit skin was determined by comparing it with the RHS colour scale chart (Trait 13, Figure 3.13e-g). Other fruit morphology traits (Traits 8-12 and 14-17) were evaluated according to pre-determined standard descriptors listed in Table 3.1. Characteristics of durian fruit were recorded to the closest most resembled descriptor. The weight of the peel (Trait 18, Figure 3.13h) and seeds (Trait 19, Figure 3.13i) were measured using the CAS weighing balance.

Lastly, data on fruit arils was excised from dehiscence fruit sample, mainly focusing on the flesh and seed. On each sample, the number of seeds and flattened seeds (Trait 20-21 in Table 3.1) were counted. Data for flesh colour, aroma, taste,

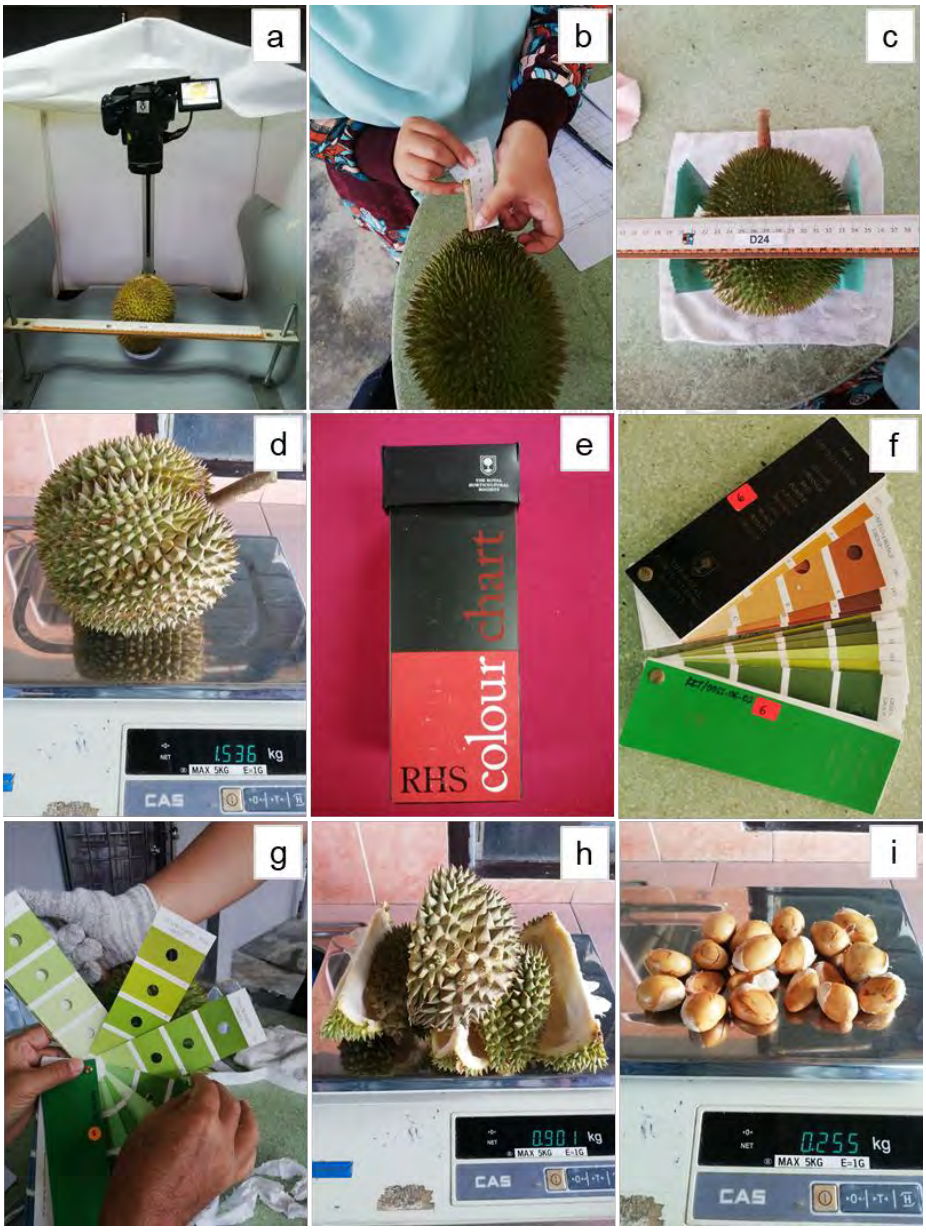
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texture, and shape of seed (Trait 22-28) were evaluated by five observers according to pre-determined standard descriptors as listed in Table 3.1.

Figure 3.13

Durian fruit's observation. (a) Fruit characteristics were photographed in a mini studio. (b) The length of pedicel was measured by using ruler paper. (c) The fruit's length and width were measured by a ruler placed on top of two book stands. (d) Fruit was weighed on a CAS weighing scale. (e-g) The RHS colour chart was used to determine fruit skin colour. (h-i) Fruit peel and seeds were weighed on a CAS weighing scale



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3.3.4 Morphological Data Analysis

Prior to analysis, values and scores recorded for each QN, QL and PQ traits were recorded in Microsoft Excel. Firstly, the values for the QN traits were utilized to generate column charts using Microsoft Excel. Then, the mean and standard deviation for each of the QN traits were calculated. The significance difference (at the 95% confidence level, $p<0.05$) among the samples was determined by using one-way analysis of variance (ANOVA) and Tukey's test using the SPSS® version 25 software. ANOVA was performed under the assumption that the sample population is normally distributed by normality tests such as Shapiro-Wilk test and Kolmogorov-Smirnov test. The SPSS software was further utilized to perform the canonical discriminant analysis to build a scatter plot of cluster distribution based on two canonical discriminant functions.

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Secondly, QL and PQ traits were scored based on the standard descriptors for each trait that were listed in Table 3.1. These numbers are treated as ranks (like non-parametric tests) prior to analysis. Subsequently, scored QL and PQ data were then converted into a PowerMarker data input file (Liu & Muse, 2005) to calculate the genetic distance formulated by Nei and Chesser (1983). Nei and Chesser (1983) employed F-statistics to determine differences or similarities between samples. Then, the genetic distance values were used as input to generate a UPGMA dendrogram using NTSYSpc 2.21s software.

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3.4 Transcriptomic Analysis on Biological Functions and Molecular Pathway Mechanism Underlying Fruit Developmental Stages

3.4.1 Plant Material and Sampling Sites

The first purpose of this study was to perform RNA-seq analysis to investigate the gene set expressed in durian fruit aril throughout different fruit developmental stages (matured, riped and dehisced) for D24 durian variety. Since the D24 variety was the first to bear fruits, the fruits were collected at five-day intervals for the matured stage to evaluate whether there was significant gene expression for the fruit to be mature. Towards to the dehiscent stage, a total of 7 fruits of D24 variety has been collected. Secondly, similar transcriptomic analysis is to be performed focusing on the same aspect aforementioned but between different durian varieties which are D99, D160, D168, D197 and D200 on three different fruit aril developmental stages (matured, riped and dehisced). Towards to the dehiscent stage, a total of 15 fruits of D99, D160, D168, D197 and D200 varieties have been collected. Overall, a total of 22 durian fruit samples with three replicates each from six durian varieties were used for transcriptomic analysis (Table 3.4).

These samples were obtained from Serdang Agriculture Complex (germplasm collection sites, the DOA), Serdang, Selangor and Raub Mining & Development Co. Sdn. Bhd. (durian orchard in Bukit Koman, Raub, Pahang). These samples were collected for three fruit developmental stages (matured, riped, and dehisced stage). Briefly, matured fruit was excised from tree and ripened fruit was collected after it naturally fell from the tree. Separately, dehisced fruit was obtained by leaving a ripened fruit at room temperature (23-28^{o}C) until the fruit cracks naturally.

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Table 3.4

Sample collection details

No.	Variety	Sample Name	Location	Harvest Day	Harvest Method	Stage	Fruit cracking period
1	D24	D24_M_105	SAC	105th day	Picked	Matured	N/A
2		D24_M_110	SAC	110th day	Picked	Matured	N/A
3		D24_M_115	SAC	115th day	Picked	Matured	N/A
4		D24_M_120	SAC	120th day	Picked	Matured	N/A
5		D24_R_125	SAC	125th day	Natural drop	Riped	N/A
6		D24_R_127	SAC	127th day	Natural drop	Riped	N/A
7		D24_D_143	SAC	143rd day	Natural drop	Leave to dehisce	5th day
8	D99	D99_M	SAC	90th day	Picked	Matured	N/A
9		D99_R	SAC	85th day	Natural drop	Riped	N/A
10		D99_D	SAC	85th day	Natural drop	Leave to dehisce	6th day
11	D160	D160_M	BK	N/A	Picked	Matured	N/A
12		D160_R	BK	N/A	Natural drop	Riped	N/A
13		D160_D	BK	N/A	Natural drop	Leave to dehisce	5th day
14	D168	D168_M	SAC	100th day	Picked	Matured	N/A
15		D168_R	SAC	103rd day	Natural drop	Riped	N/A
16		D168_D	SAC	108th day	Natural drop	Dehisced	1st day
17	D197	D197_M	BK	N/A	Picked	Matured	N/A
18		D197_R	BK	N/A	Natural drop	Riped	N/A
19		D197_D	BK	N/A	Natural drop	Leave to dehisce	3rd day
20	D200	D200_M	BK	N/A	Picked	Matured	N/A
21		D200_R	BK	N/A	Natural drop	Riped	N/A
22		D200_D	BK	N/A	Natural drop	Leave to dehisce	4th day

Note,
SAC = Serdang Agricultural Complex;
BK = Raub Mining & Development Co. Sdn. Bhd. (durian orchard in Bukit Koman, Raub, Pahang);
Harvest day = Count from Day 1 after flower bud protuberances on the branches and being tagged;
Fruit cracking period = Count from harvest day until the day of the fruit has been cracked itself;
N/A = Information is not available.

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The durian flesh was taken using a spatula and put into a cryo tube (Figure 3.14a). Then, the cryo tube was dipped in a tank containing liquid nitrogen (Figure 3.14b). After completing this process on all the collected fruit samples, the tank was brought to the Biotechnology and Nanotechnology Research Centre (BN Centre) located in the Malaysian Agriculture Research and Development Institute (MARDI) headquarters, Serdang, Selangor. The cryo tubes were taken out of the tank, put in a zip lock bag and stored in a -80^{o}C fridge in the Centre for Marker Discovery and Validation (CMDV) laboratory before transporting to Apical Sdn. Bhd. for RNA extraction.

Figure 3.14

Durian flesh sampling. (a) Durian flesh was taken by spatula and put into a cryo tube and (b) dipped into a tank containing liquid nitrogen



3.4.2 RNA Extraction and Quality Control

RNA-seq experiments were performed to determine the RNA profiles on the durian flesh samples collected. Total RNA was extracted using the SEPa Plant RNA Isolation Reagent Kit (1st BASE) according to the manufacturer's instructions and the

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RNA quality was monitored on a 1% agarose gel in 1x TAE buffer at 100 V for 60 mins. The RNA amount, concentration, and purity were then measured using a Nanodrop Spectrophotometer (ThermoFisher Scientific). The minimum quality control (QC) requirements were set for RNA amount (≥ 400 ng), RNA concentration (≥ 20ng/µl), and RNA purity (OD 260/280 ≥ 2.0 and OD 260/230 ≥ 2.0). Then, the Agilent 2100 Bioanalyzer (Agilent) were used to assess the RNA integrity (RIN). After comparing the minimal value of the quantification results from each stage of fruit development, the RIN threshold values were set to ≥ 7.0 for the matured stage, ≥ 5.0 for the riped stage, and ≥ 2.0 for the dehisced stage.

3.4.3 Library Construction and RNA-Seq

After that, NEBNext Ultra RNA Library Prep Kit for Illumina (Cat No. 7530) was used for RNA library preparation. The messenger RNA (mRNA) was purified from total RNA using poly-T oligo-attached magnetic beads. After fragmentation, the first strand cDNA was synthesized using random hexamer primers, followed by the second strand cDNA synthesis using either deoxythymidine triphosphate (dTTP) for non-directional library (Figure 3.15a) or deoxyuridine triphosphate (dUTP) for directional library (Figure 3.15b). For the non-directional library, the library was constructed after end repair, A-tailing, adapter ligation, size selection, PCR amplification and purification. For the directional library, the library was constructed after end repair, A-tailing, adapter ligation, size selection, USER® Enzyme (NEB) digestion, PCR amplification, and purification.

Then, the library was checked with Qubit and real-time PCR for quantification and Bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on Illumina platforms, according to effective library concentration (0.5-2.5

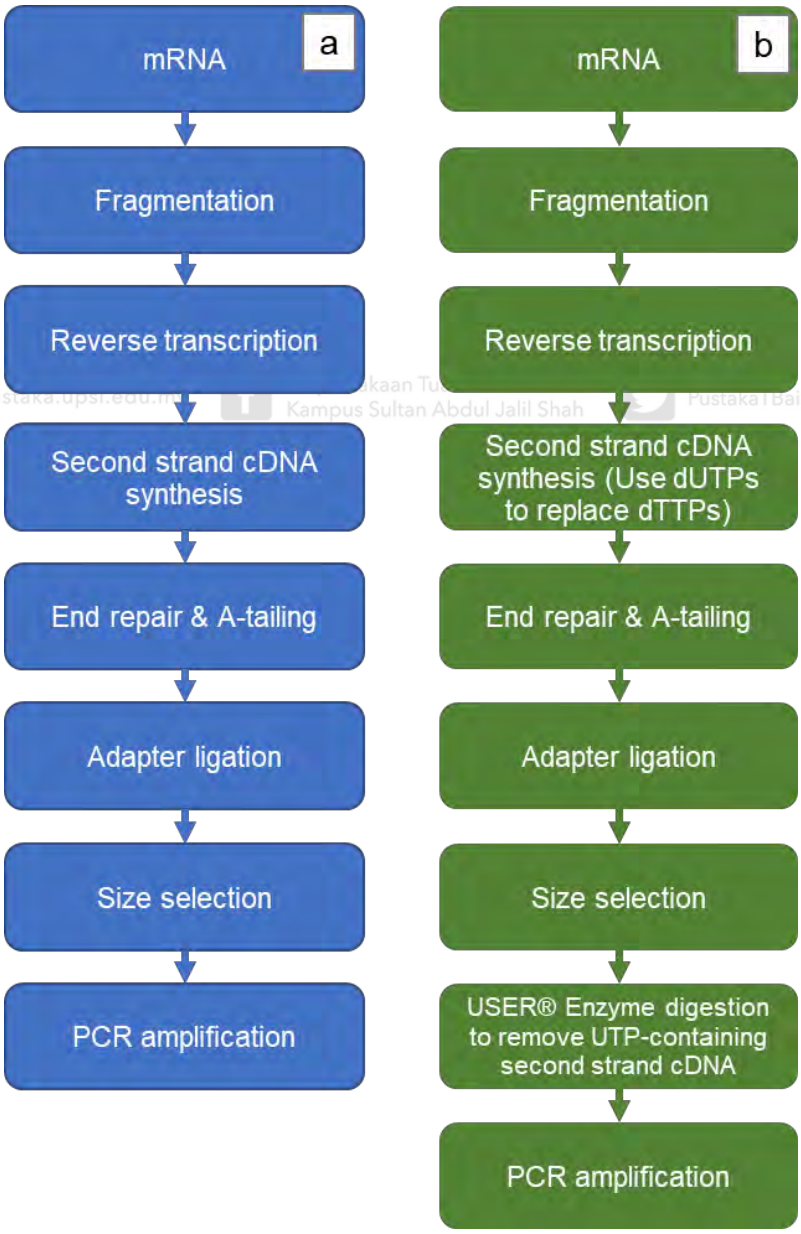
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nM) and data amount. The clustering of the index-coded samples was performed according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Novaseq 6000 and paired-end reads were generated.

Figure 3.15

Workflow of library construction. (a) Workflow of non-directional library construction. (b) Workflow of directional library construction



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3.4.4 Bioinformatics Analysis Pipeline

3.4.4.1 Data Quality Control

Raw data generated from the Illumina platform was transformed using CASAVA base recognition (Base Calling). Raw data was stored in FASTQ (fq) format files, which contain read sequences and base quality. Each read contains four descriptive lines, as shown in Figure 3.16. The details of Illumina sequencing identifier were described in Table 3.5.

Figure 3.16

Descriptive line for raw reads

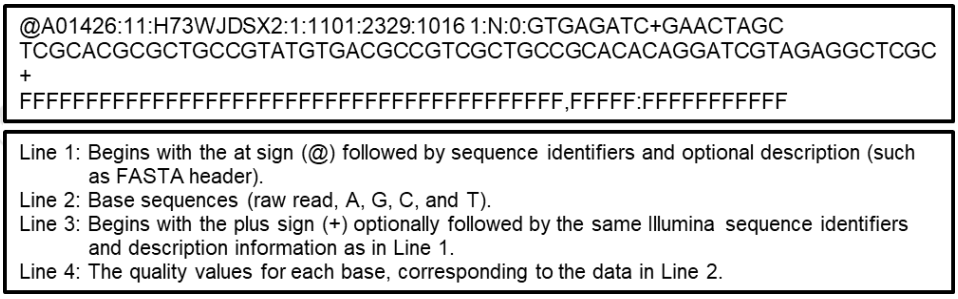


Table 3.5

Details of Illumina sequencing identifier

Identifier	Meaning
A01426	Instrument – unique identifier of the sequencer
11	run number – Run number on instrument
H73WJDSX2	Flowcell ID - ID of flowcell
1	LaneNumber - positive integer
1101	TileNumber - positive integer
2329	X - x coordinate of the spot. Integer which can be negative
1016	Y - y coordinate of the spot. Integer which can be negative
1	Read Number - 1 can be single read or Read 2 of paired-end
N	Y if the read is filtered out (did not pass), N otherwise

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Identifier	Meaning
0	control number - 0 when none of the control bits are on, otherwise it is an even number
GTGAGATC+GAACTAGC	Illumina index sequences

3.4.4.1.1 Error Rate Distribution

The error rate for each base was transformed using the Phred score, as shown in Figure 3.17. Table 3.6 demonstrated a concise correspondence between base recognition and Phred score. Due to the incomplete binding of random hexamers utilized in priming cDNA synthesis, the first six bases have a quite high error rate (Jiang et al., 2011). In general, the single base error rate should be less than 1%.

Figure 3.17

Qphred equation formula

$$Qphred = -10 \log_{10} (e)$$

e= sequencing error rate
Qphred= base quality values of Illumina platforms

Table 3.6

Illumina Casava 1.8 version base recognition and Phred score

Phred.score	Base.Calling.error.rate	Base.Calling.correct.rate	Q. score
10	1/10	90%	Q10
20	1/100	99%	Q20
30	1/1000	99.9%	Q30
40	1/10000	99.99%	Q40

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3.4.4.1.2 GC Content Distribution

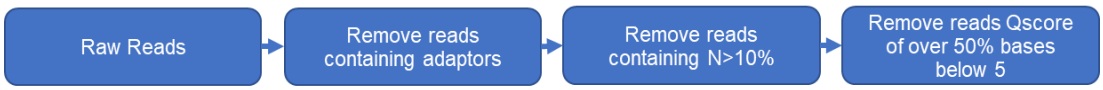
The distribution of GC content suggests the possibility of AT/GC separation, which influences the overall gene expression measurement. According to random fragmentation and the biological law of G/C-A/T content, G and C, A and T should be equal, and the content should be stable throughout the entire sequencing process for the non-stranded library (If the library is strand-specific, AT separation or GC separation may occur). Since random primers were used in library construction, large variations in sequencing error in the first 6-7 bases are allowed.

3.4.4.1.3 Data Filtering

Filtering raw readings was done by following the steps described in Figure 3.18. First, reads with adapter contamination were eliminated. Then, the reads with uncertain nucleotides comprising for more than 10% of either read (N > 10%) were discarded. Finally, readings in which low-quality nucleotides (Base Quality less than 5) constitute for more than 50% of the read were eliminated. Table 3.7 listed the adapter sequences used.

Figure 3.18

Raw readings filtering



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Table 3.7

Adapter Sequences

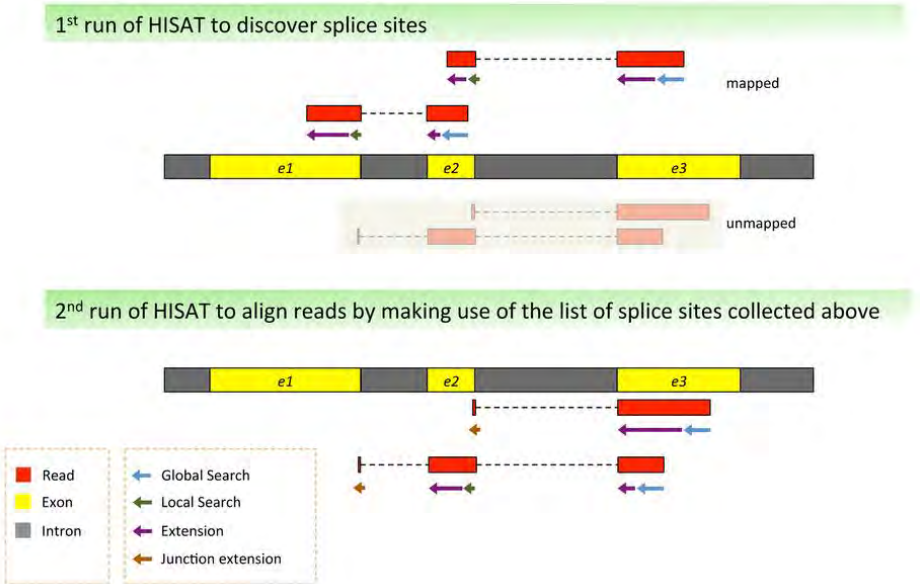
Adapter	Sequence
P5 Adapter	P5-AATGATACGGCGACCACCGAGA (5'-3') P5'-TTACTATGCCGCTGGTGGCTCT (3'-5')
P7 Adapter	CGTATGCCGTCTTCTGCTTG-P7' (5'-3') GCATACGGCAGAAGACGAAC-P7 (3'-5')

3.4.4.2 Alignment and Mapping

HISAT2 was used to perform alignments to the *D. zibethnius* reference genome (https://www.ncbi.nlm.nih.gov/genome/?term=durio) and act as mapping tool using the gene model annotation file (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Durio_zibethinus/100/) .This reference genome was used to identify the genes that are expressed, quantify their expression levels, and detect novel transcripts. This reference genome was also sequenced from D197 (Musang King) variety from Malaysia (Teh et al., 2017). Figure 3.19 shows the algorithm of split reads comparison by HISAT2.

Figure 3.19

The algorithm of split reads comparison by HISAT2



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The Hisat's algorithm is divided into three sections:

1) A single exon was used to align the whole sequence.
2) Two exons of the genome were piecewise aligned with the sequencing sequence.
3) More than three (including three) exons of the genome were segmented and aligned to the sequencing sequence.

The total map, calculated based on equation; “total mapping rate: (mapped reads)/(total reads)*100”, should be higher than 70%. After that, the mapped reads were assembled by StringTie (v1.3.3b) in a reference-based approach (Pertea et al., 2015).

3.4.4.3 Novel Gene Prediction

Mapping data from all samples was compiled and added as input to the standard Cufflinks assembler into transfrags. After that, the assembled transfrags were matched with the reference transcripts to see if they differ enough to qualify as novel. The start and end information of known transcripts were optimized. Following that, databases such as Pfam, Swiss-Prot, GO, and KEGG were used to annotate the novel gene using the clusterProfiler software with threshold value $P > 0.05$.

3.4.4.4 Gene Expression Quantification

FeatureCounts v1.5.0-p3 was used to count the read numbers mapped to each gene to quantify gene expression levels. Then, FPKM (short for the expected number of Fragments Per Kilobase of Transcript Sequence per Millions Base Pairs Sequenced) of each gene was calculated based on the length of the gene and the read count

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mapped to the gene. The number of genes that were uniquely expressed within each group/sample was displayed in the co-expression Venn diagram, with the overlapping regions indicating the number of genes that were co-expressed in two or more groups/samples.

3.4.4.4.1 Correlation Analysis

The Pearson correlation coefficient (Figure 3.20) was used in the analysis to measure the strength of linear association between two variables (Sedgwick, 2012). In this study, correlation analysis was carried out between samples to evaluate the expression level between them. The samples are more similar when the correlation coefficient is close to 1. The square of Pearson correlation coefficient (R2) acceptable threshold should be greater than 0.8 and 0.92 (under ideal experiment conditions). The correlation coefficient of samples between groups is computed and displayed as heat maps in accordance with all gene expression levels (FPKM) of each sample.

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Figure 3.20

Pearson correlation coefficient

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

n : sample size

x_i, y_i : the individual sample points indexed with *i*

$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$: (the sample mean); and analogously for $\bar{y}$

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3.4.4.5 Differential Gene Expression Analysis

Following the quantification of gene expression, statistical analysis of the expression data was required to identify genes with significantly variable expression levels in different conditions. The differential analysis was separated into three major steps:

1) Normalization of the raw read count was done primarily to correct the sequencing depth.
2) The hypothesis test's probability (p value) is calculated using a statistical model.
3) FDR (false discovery rate) values are calculated using multiple hypothesis test corrections.

The software for gene expression differential analysis was selected as indicated in Table 3.8. Prior to differential gene expression analysis, for each sequenced library, the read counts were adjusted by the edgeR programme package through one scaling normalized factor. The DESeq2R package (1.20.0) was then used to perform differential expression analysis based on the differential gene screening thresholds of |log2(FoldChange)| >= 1 and padj <= 0.05. If the number of different genes screened according to the above threshold is too small (less than 100), the functional enrichment analysis will most likely yield no significant results. Therefore, the threshold for screening different genes based on the specific parameters will be reduced. In contrast, if the number of differential genes obtained is too large, at this point, screening could be done using stricter threshold criteria.

Table 3.8

Software for differential analysis and differential gene screening criteria

Type	Software	Normalized Method	Pvalue Calculation Model	FDR Calculation Method	Differential Gene Screening Threshold
No Biological Replicates	edgeR (Robinson, McCarthy, & Smyth, 2010)	TMM	The Negative Binomial Distribution	BH	|log2(FoldChange)| >= 1 & padj <= 0.05
Biological Replicates	DESeq2 (Love, Huber, & Anders, 2014)	DESeq	The Negative Binomial Distribution	BH	|log2(FoldChange)| >= 1 & padj <= 0.05

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Genes were considered differentially expressed if their expression differs by more than twice as much in both sets of samples. If log2(FoldChange) is positive, it indicates upregulation (higher expression in Treatment Group). If log2(FoldChange) is negative, it indicates downregulation (lower expression in Treatment Group).

Table 3.9 listed the sample groups that were being analysed in the differential gene analysis. The control groups serve as a baseline for comparison. The treatment group is compared to a control group, whose samples further undergo fruit development. Specific for the D24 variety, the control group for the earliest matured stage serves as a baseline for comparison between other matured samples to measure differences in gene expression during the maturation stage.

A hypothesis test was performed on the expression data of all genes between these two samples to determine whether the difference in expression between the two samples is due to various errors or essential differences. The transcriptome analysis was performed on thousands of genes, resulting numerous false positives. Therefore, the incorporation of padj to the hypothesis test P-value was calibrated to control the proportion of false positives since the number of genes increases the cumulative degree of false positives in the hypothesis test (Young, Wakefield, Smyth, & Oshlack, 2010).

Table 3.9

Samples group for differential gene analysis

Comparison Group	Treatment Group	Control Group
1	D24_M_110	D24_M_105
2	D24_M_115	D24_M_105
3	D24_M_120	D24_M_105
4	D24_R_125	D24_M_105

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Comparison Group	Treatment Group	Control Group
5	D24_R_127	D24_M_105
6	D24_D_143	D24_R_125
7	D24_D_143	D24_R_127
8	D99_R	D99_M
9	D99_D	D99_R
10	D160_R	D160_M
11	D160_D	D160_R
12	D168_R	D168_M
13	D168_D	D168_R
14	D197_R	D197_M
15	D197_D	D197_R
16	D200_R	D200_M

The differential gene set was formed by combining all the differentially expressed genes in the comparison group. Cluster analysis was performed on different gene sets and genes with comparable expression patterns that can be clustered together. The classes of genes that are over-represented in a large set of genes may have involvement in the same biological pathway. The fpkm values of genes were clustered using mainstream hierarchical clustering, which homogenized the row (Z-score). Genes or samples with similar expression patterns were grouped together in the heat map.

3.4.4.6 Functional Annotation

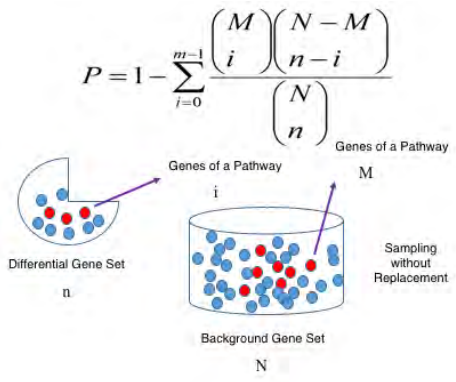
For enrichment analysis, the clusterProfiler software (Yu, Wang, Han, & He , 2012) was used to perform, particularly for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment (Figure 3.21).

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Figure 3.21

Gene Enrichment Analysis Schematic



3.4.4.6.1 GO Enrichment

The Gene Ontology (GO) (http://www.geneontology.org/) is a comprehensive bioinformatics classification system that integrates the presentation of gene properties across all species. It is divided into three categories: cellular component, molecular function, and biological process. GO terms with padj < 0.05 indicate significant enrichment.

All the GO annotation results were downloaded in the specified folder according to the differential sample groups. The GO annotations were listed in the Excel file, including information such as the GO ID, description, gene ratio, padj value, gene ID, and list of up- and down-regulated genes (Figure 3.22). Based on this information, the GO terms were arranged and compared for each differential sample group according to the top significant enrichment and GO categories (Figure 3.23). Then, the number of differential sample groups related to specified GO terms was counted to determine the top 15 most common GO terms among the six durian

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varieties (Figure 3.24). The top 15 most common GO terms were then displayed in a clustered column chart.

Figure 3.22

GO enrichment details for D24_M_110 vs D24_M_105

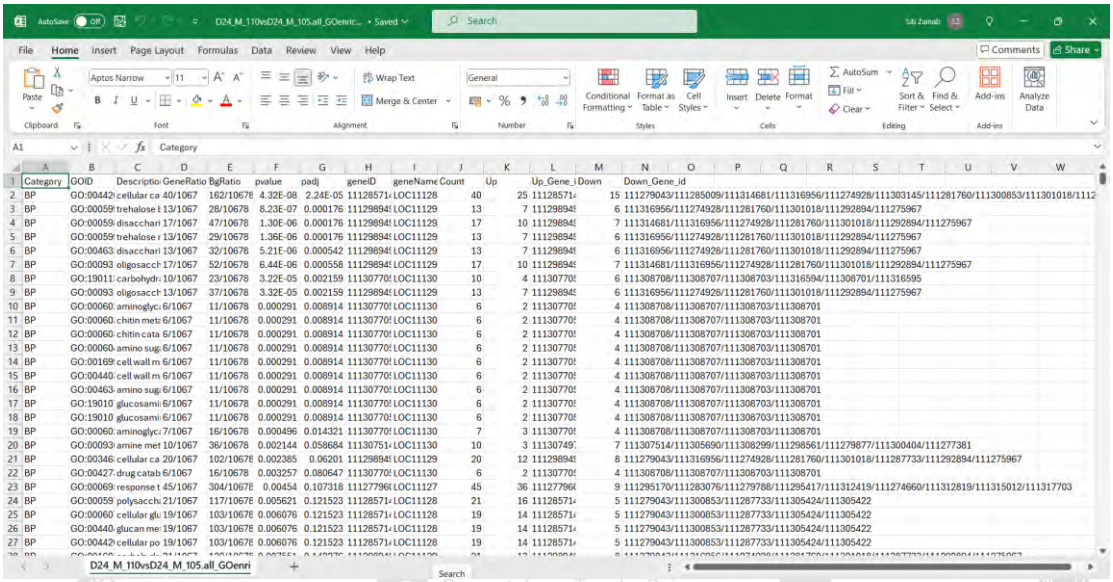
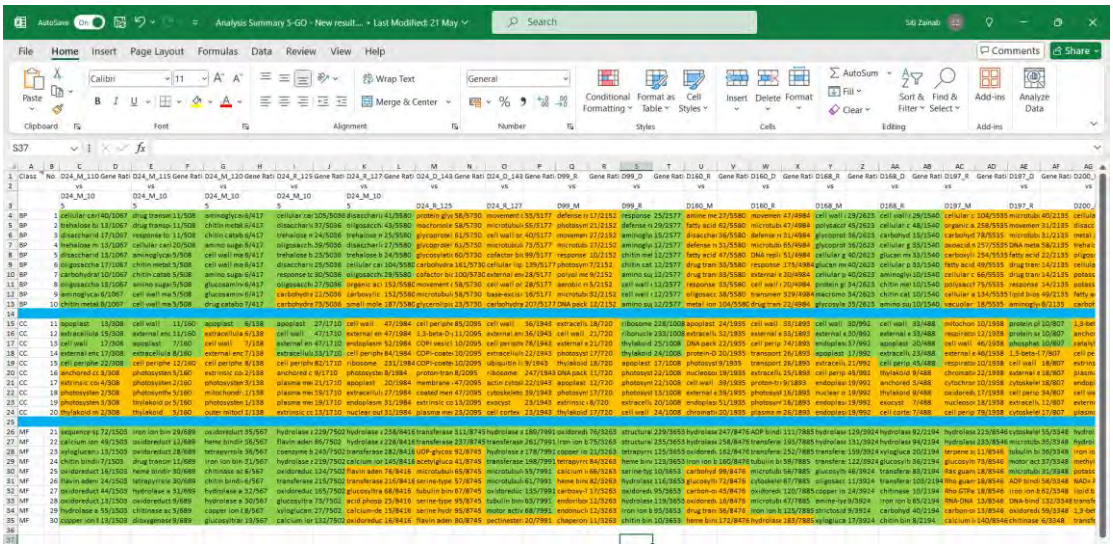


Figure 3.23

The top significant GO enrichment for each differential sample group

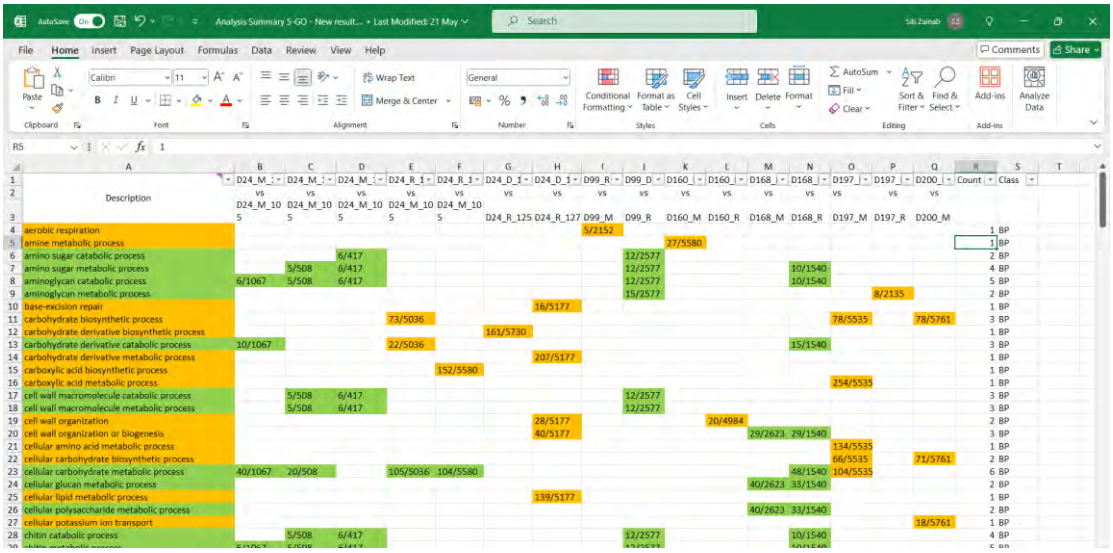


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Figure 3.24

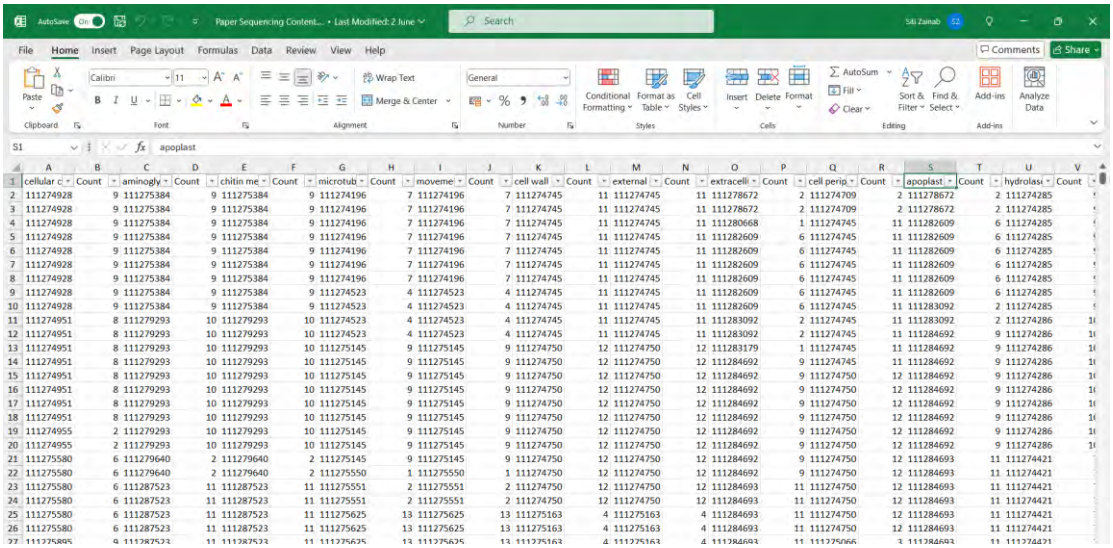
Number of differential sample groups related to specified GO terms



After that, the number of up- and down-regulated genes was listed based on the information available in Figure 3.22 for each differential sample group. Then, the top-hit up and down-regulated genes for each of the 15 most common GO terms were counted and identified (Figure 3.25).

Figure 3.25

List of up and down-regulated genes for the top 15 most common GO terms



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3.4.4.6.2 KEGG Enrichment

Multiple gene interactions may be involved in specific biological functions. The Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) is a collection of manually curated databases covering genomic, biological-pathway, and disease information (Kanehisa & Goto, 2000). Pathway enrichment analysis compares the whole genome background to identify significantly enriched metabolic or signal transduction pathways that associated with differentially expressed genes. Significant enrichment is found in KEGG pathways with padj < 0.05.

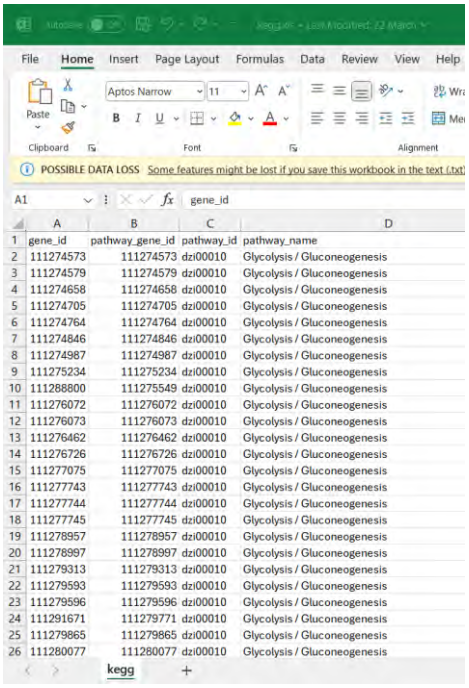
The KEGG pathways annotated were listed in the Excel file, including information such as gene ID, pathway ID, and pathway name (Figure 3.26). The total number of KEGG pathways annotated and the number of genes being expressed in the specified KEGG pathways were calculated. Then, the distribution of gene percentages annotated in the KEGG enrichment was displayed in a pie chart to show the KEGG pathways with the highest gene representation. Besides, all the KEGG pathways annotated could be viewed as interactive pathway maps under the pathway map menu in KEGG databases (Figure 3.27).

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Figure 3.26

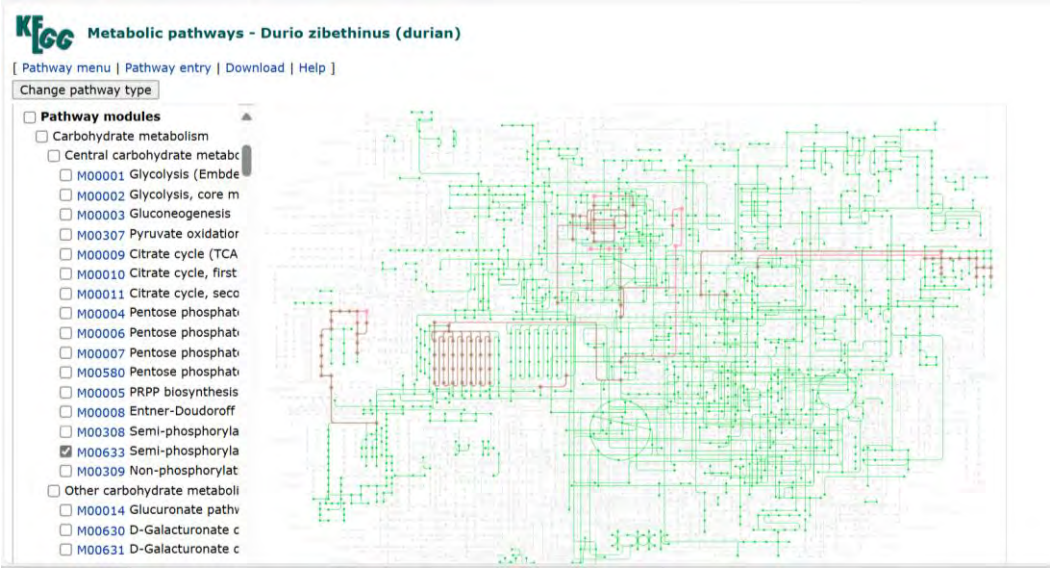
List of KEGG pathways annotated in the KEGG database



	A	B	C	D
1	gene_id	pathway_gene_id	pathway_id	pathway_name
2	111274573	111274573	dzi00010	Glycolysis / Gluconeogenesis
3	111274579	111274579	dzi00010	Glycolysis / Gluconeogenesis
4	111274658	111274658	dzi00010	Glycolysis / Gluconeogenesis
5	111274705	111274705	dzi00010	Glycolysis / Gluconeogenesis
6	111274764	111274764	dzi00010	Glycolysis / Gluconeogenesis
7	111274846	111274846	dzi00010	Glycolysis / Gluconeogenesis
8	111274987	111274987	dzi00010	Glycolysis / Gluconeogenesis
9	111275234	111275234	dzi00010	Glycolysis / Gluconeogenesis
10	111288800	111275549	dzi00010	Glycolysis / Gluconeogenesis
11	111276072	111276072	dzi00010	Glycolysis / Gluconeogenesis
12	111276073	111276073	dzi00010	Glycolysis / Gluconeogenesis
13	111276462	111276462	dzi00010	Glycolysis / Gluconeogenesis
14	111276726	111276726	dzi00010	Glycolysis / Gluconeogenesis
15	111277075	111277075	dzi00010	Glycolysis / Gluconeogenesis
16	111277743	111277743	dzi00010	Glycolysis / Gluconeogenesis
17	111277744	111277744	dzi00010	Glycolysis / Gluconeogenesis
18	111277745	111277745	dzi00010	Glycolysis / Gluconeogenesis
19	111278957	111278957	dzi00010	Glycolysis / Gluconeogenesis
20	111278997	111278997	dzi00010	Glycolysis / Gluconeogenesis
21	111279313	111279313	dzi00010	Glycolysis / Gluconeogenesis
22	111279593	111279593	dzi00010	Glycolysis / Gluconeogenesis
23	111279596	111279596	dzi00010	Glycolysis / Gluconeogenesis
24	111291671	111279771	dzi00010	Glycolysis / Gluconeogenesis
25	111279865	111279865	dzi00010	Glycolysis / Gluconeogenesis
26	111280077	111280077	dzi00010	Glycolysis / Gluconeogenesis

Figure 3.27

Interactive pathway map view for all KEGG pathways annotated in durian



Then, all the KEGG annotation results were downloaded in the specified folder according to the differential sample groups. The KEGG annotations were listed in the Excel file, including information such as the KEGG ID, description, gene ratio,

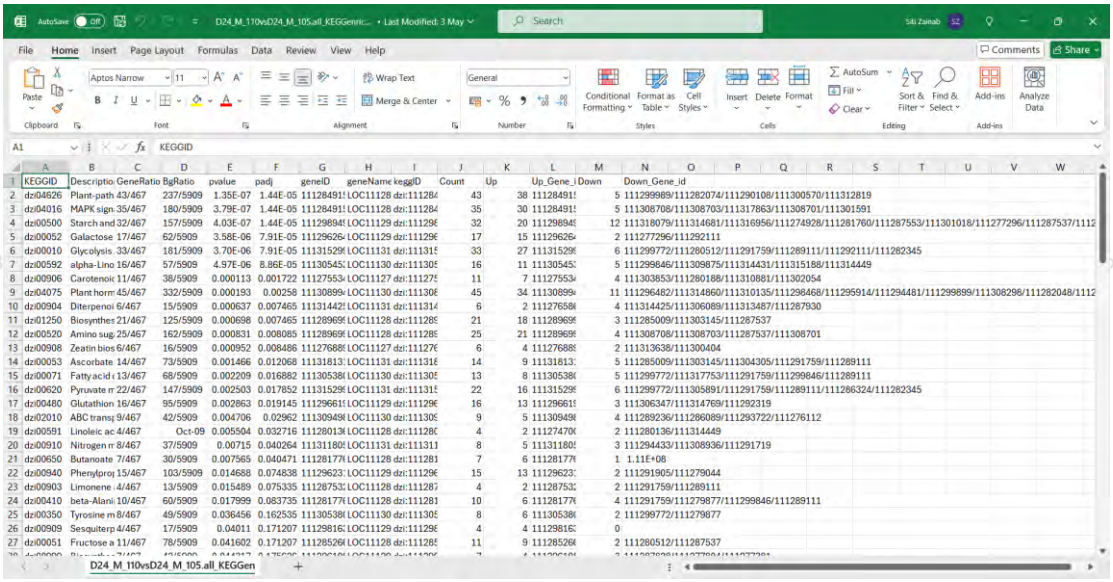
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padj value, gene ID, and list of up- and down-regulated genes (Figure 3.28). Based on this information, the KEGG terms were arranged and compared for each differential sample group according to the top significant enrichment (Figure 3.29). Then, the number of differential sample groups was counted related to specified KEGG pathways to determine the top five most common KEGG pathways among the six durian varieties (Figure 3.30). The top five most common KEGG pathways were then displayed in a clustered column chart.

Figure 3.28

KEGG enrichment details for D24_M_110 vs D24_M_105



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Figure 3.29

The top significant KEGG enrichment for each differential sample group

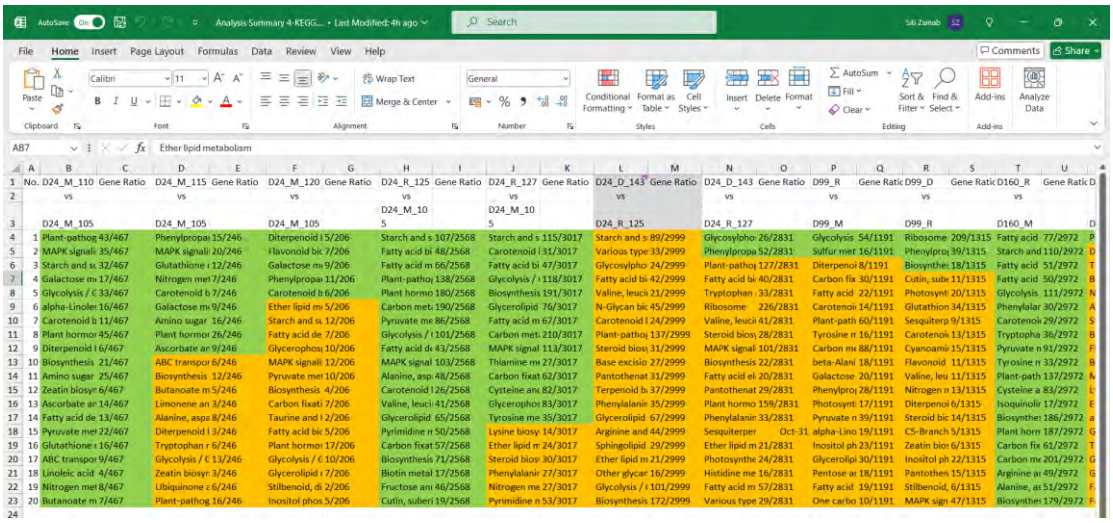
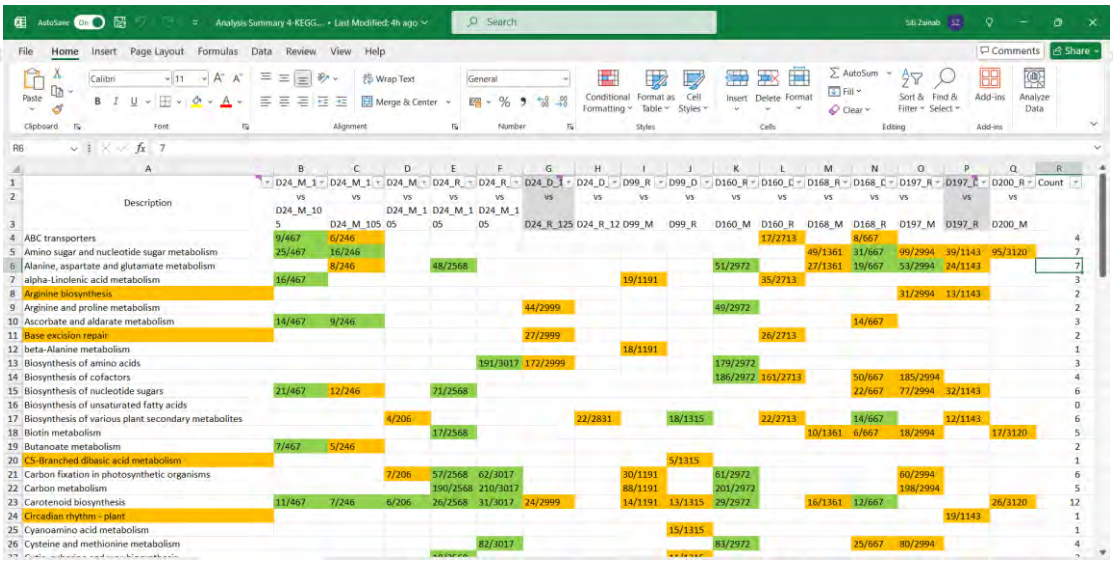


Figure 3.30

Number of differential sample groups related to specified KEGG pathways



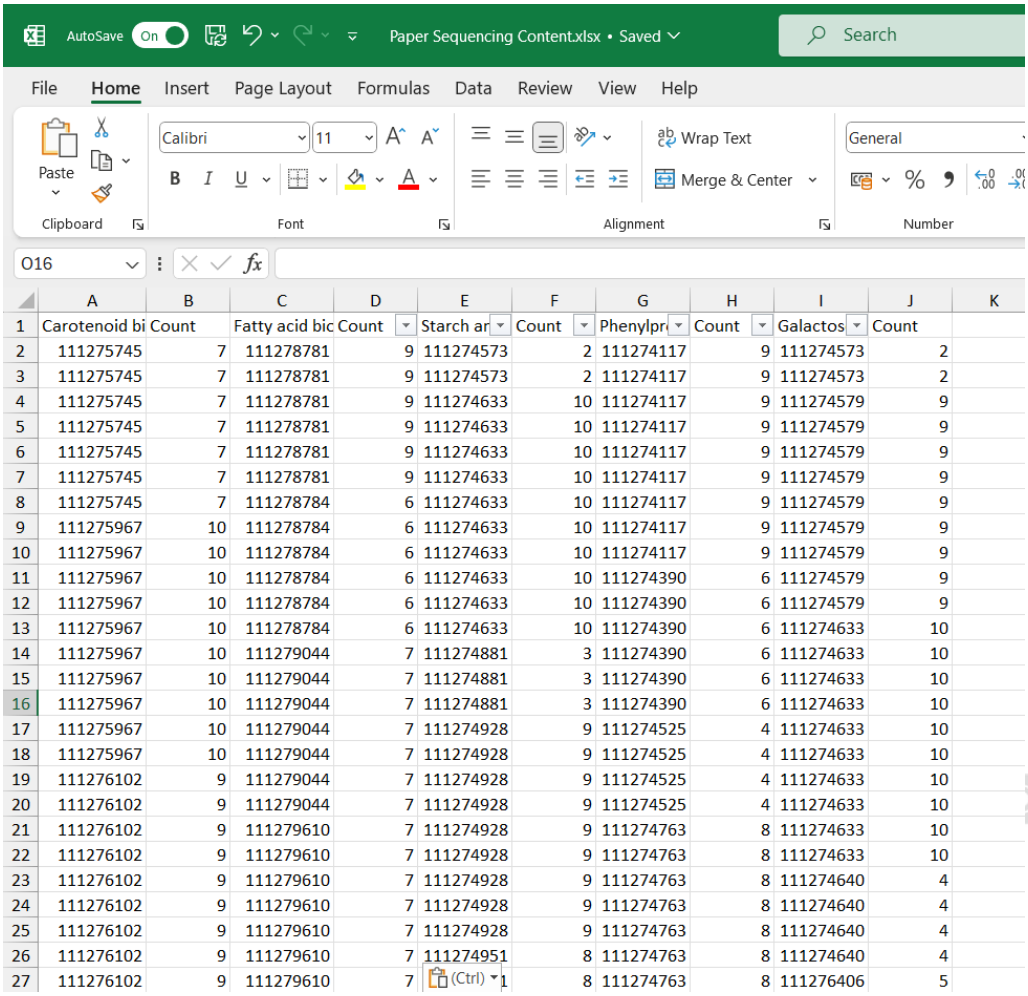
After that, the number of up- and down-regulated genes was listed based on the information available in Figure 3.28 for each differential sample group. Then, the top-hit up and down-regulated genes for each of the five most common KEGG pathways were counted and identified (Figure 3.31).

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Figure 3.31

List of up and down-regulated genes for the top five most common KEGG pathways



	A	B	C	D	E	F	G	H	I	J	K
1	Carotenoid bi	Count	Fatty acid bic	Count	Starch ar	Count	Phenylpr	Count	Galactos	Count	
2	111275745	7	111278781	9	111274573	2	111274117	9	111274573	2	
3	111275745	7	111278781	9	111274573	2	111274117	9	111274573	2	
4	111275745	7	111278781	9	111274633	10	111274117	9	111274579	9	
5	111275745	7	111278781	9	111274633	10	111274117	9	111274579	9	
6	111275745	7	111278781	9	111274633	10	111274117	9	111274579	9	
7	111275745	7	111278781	9	111274633	10	111274117	9	111274579	9	
8	111275745	7	111278784	6	111274633	10	111274117	9	111274579	9	
9	111275967	10	111278784	6	111274633	10	111274117	9	111274579	9	
10	111275967	10	111278784	6	111274633	10	111274117	9	111274579	9	
11	111275967	10	111278784	6	111274633	10	111274390	6	111274579	9	
12	111275967	10	111278784	6	111274633	10	111274390	6	111274579	9	
13	111275967	10	111278784	6	111274633	10	111274390	6	111274633	10	
14	111275967	10	111279044	7	111274881	3	111274390	6	111274633	10	
15	111275967	10	111279044	7	111274881	3	111274390	6	111274633	10	
16	111275967	10	111279044	7	111274881	3	111274390	6	111274633	10	
17	111275967	10	111279044	7	111274928	9	111274525	4	111274633	10	
18	111275967	10	111279044	7	111274928	9	111274525	4	111274633	10	
19	111276102	9	111279044	7	111274928	9	111274525	4	111274633	10	
20	111276102	9	111279044	7	111274928	9	111274525	4	111274633	10	
21	111276102	9	111279610	7	111274928	9	111274763	8	111274633	10	
22	111276102	9	111279610	7	111274928	9	111274763	8	111274633	10	
23	111276102	9	111279610	7	111274928	9	111274763	8	111274640	4	
24	111276102	9	111279610	7	111274928	9	111274763	8	111274640	4	
25	111276102	9	111279610	7	111274928	9	111274763	8	111274640	4	
26	111276102	9	111279610	7	111274951	8	111274763	8	111274640	4	
27	111276102	9	111279610	7	(Ctrl) 1	8	111274763	8	111276406	5	

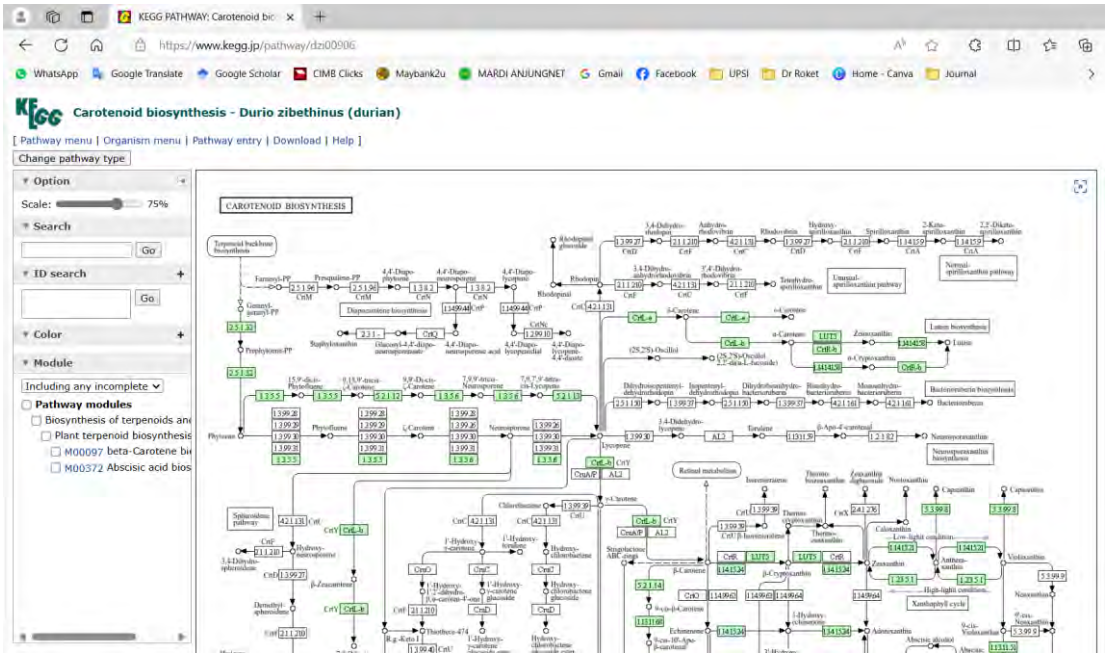
Next, the details pathway map could be viewed under the pathway map menu in KEGG databases according to KEGG ID (Figure 3.32). Then, the up and down-regulated genes can be viewed by moving the cursor to the specified genes in the KEGG pathway (Figure 3.33). Further, the KEGG modules could be viewed under the module menu in the KEGG database (Figure 3.34). The genes, compounds, and reactions involved in the KEGG module (Figure 3.35) were then displayed in the graphical diagrams. In addition, the list of genes involved in the KEGG pathway could also be viewed under the Brite menu (Figure 3.36).

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Figure 3.32

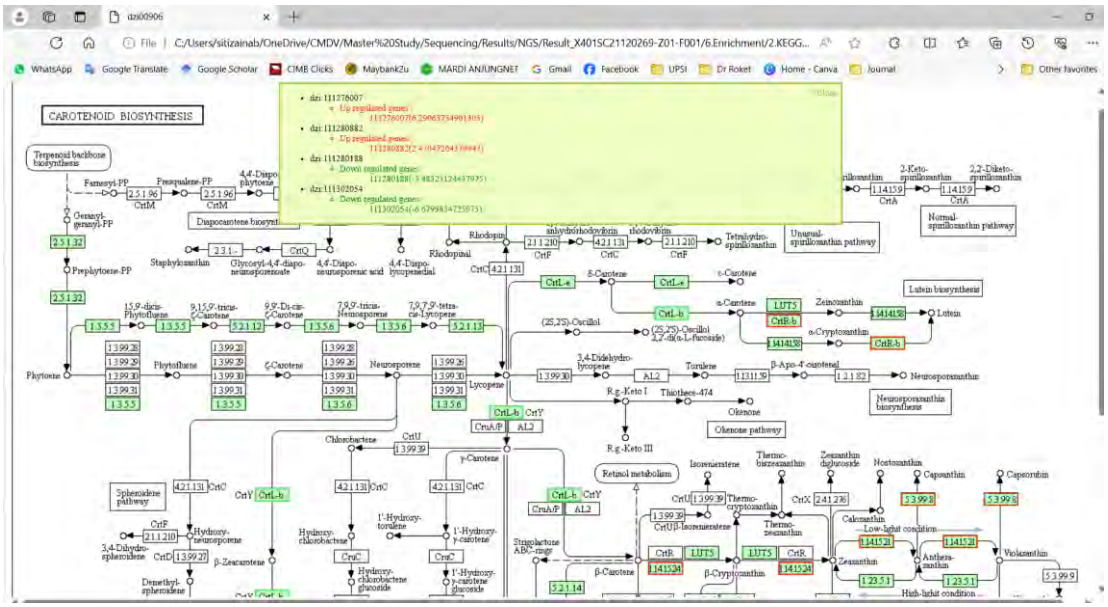
Detailed pathway map for Carotenoid biosynthesis



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Figure 3.33

Up and down-regulated genes in Carotenoid biosynthesis



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Figure 3.34

Module for Carotenoid biosynthesis

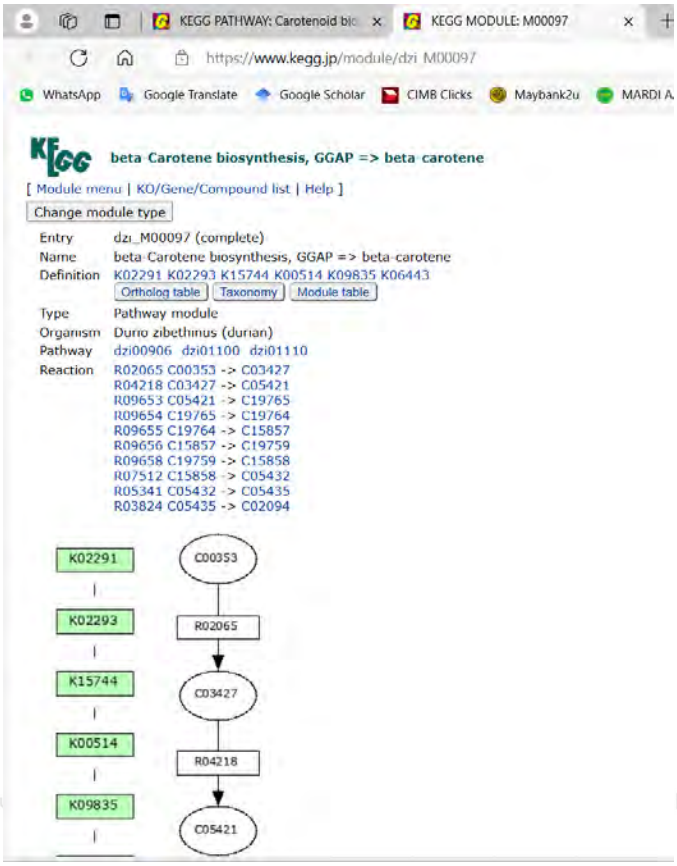
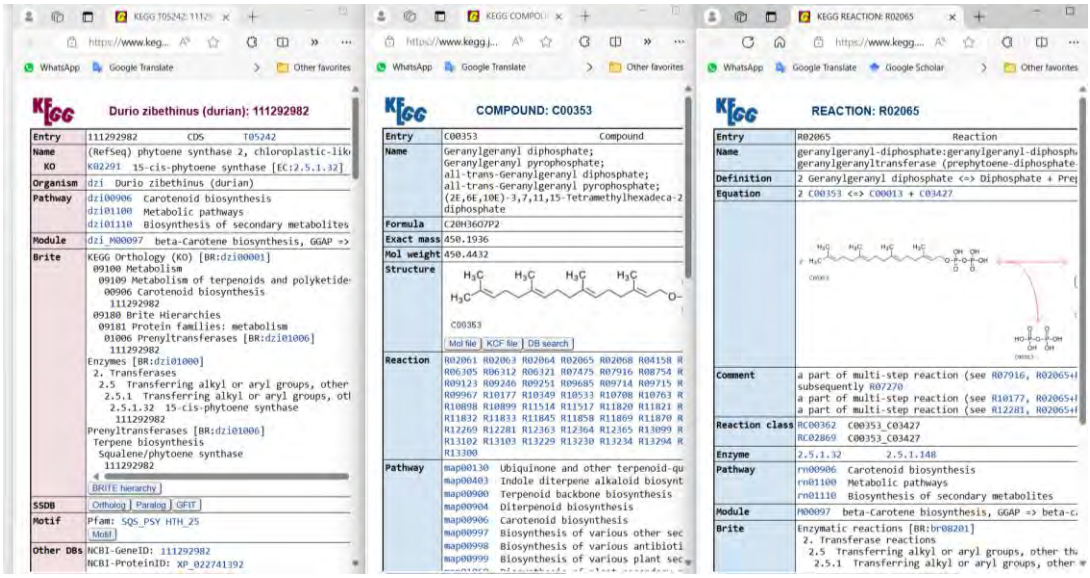


Figure 3.35

List of genes, compounds, and reactions involved in Carotenoid biosynthesis

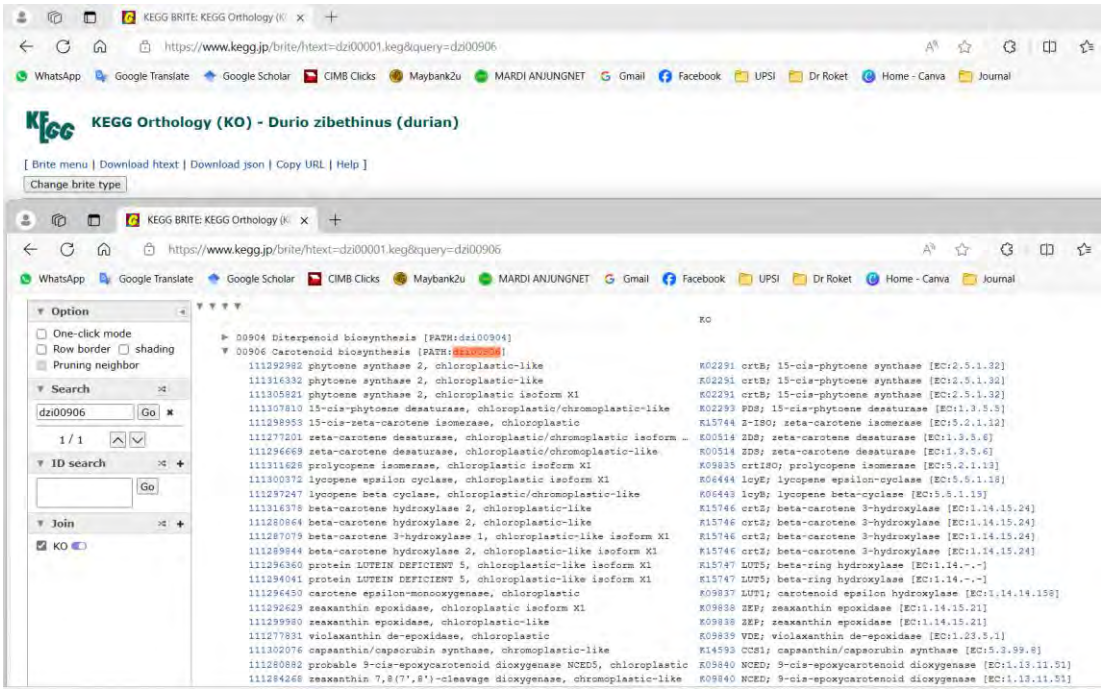


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Figure 3.36

List of genes involved in Carotenoid biosynthesis under the Brite menu of the KEGG database



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CHAPTER 4

RESEARCH FINDINGS

4.1 Introduction

A comprehensive morphology and molecular evaluation of durian fruit have been accomplished using the latest DOA morphology guidelines and sequencing technology. Research findings presented here was divided into two sections based on objectives. Section 4.2 represents findings related to characterization of the six commercial durian varieties using morphological observation, while section 4.3 described findings regarding transcriptomic analysis of six commercial durian varieties based on three fruit developmental stages (matured, riped, and dehisced). The data obtained allows for a better insight of the fruit developmental stages in each of the durian varieties evaluated.

4.2 Characterization of Six Commercial Durian Varieties in Malaysia Using Morphological Observation

In total, four samples (flower buds and flowers each of D24, D99, D168 and D197) and 22 durian fruits for the six durian varieties under study were collected throughout the durian season in 2021. However, flower buds and flowers for D160 and D200

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were examined through photographs taken by camera due to being difficult to reach as the tree is quite high and not suitable to prod as the flower is easy to dismantle. All samples collected were assessed for eight quantitative (QN), two qualitative (QL) and 18 pseudo-qualitative (PQ) traits. The morphological traits that had been observed were summarized in Table 4.1.

Table 4.1

Twenty-eight morphological trait observations in six varieties of Malaysian durian

Trait	State	D24	D99	D160	D168	D197	D200
Trait 1: Flower bud: shape (PQ)	1= ovate 2=elliptic 3=circular	elliptic	circular	elliptic	elliptic	elliptic	circular
Trait 2: Flower bud: shape of apex (PQ)	1= acute 2= obtuse 3=rounded	obtuse	rounded	obtuse	obtuse	obtuse	rounded
Trait 3: Flower: stigma position in relation to anther (PQ)	1=below; 2=level/ equal; 3=above	level/ equal	below	level/ equal	level/ equal	level/ equal	level/ equal
Trait 4: Pedicel: length (cm) (QN)	-	4.2 – 5.5	5.6 – 7.6	4.0 – 5.5	5.5 – 7.9	5.4 – 5.7	4.5 – 7.0
Trait 5: Fruit: weight (kg) (QN)	-	0.398 - 2.950	0.997 – 1.596	0.882 – 2.603	1.158 – 2.177	1.279 – 2.615	1.111 – 3.990
Trait 6: Fruit: length (cm) (QN)	-	10.0 - 27.0	15.0 – 15.5	17.4 – 24.0	19.3 – 23.4	17.3 – 25.0	15.9 – 21.5
Trait 7: Fruit: width (cm) (QN)	-	10.5 - 21.0	14.2 – 16.4	14.0 – 17.7	16.0 – 19.0	14.5 – 18.0	15.0 – 20.6
Trait 8: Fruit: depth of groove (PQ)	1=absent or weak 2= medium 3= strong	absent or weak	strong	absent or weak	absent or weak	absent or weak	medium
Trait 9: Fruit: shape (PQ)	1= ovate 2= oblong 3= elliptic 4= circular 5= oblate 6=obovate	elliptic	circular	ovate	oblong	obovate	circular
Trait 10: Fruit: symmetry (PQ)	1=symmetric 2= slightly asymmetric 3= strongly asymmetric	slightly asymmetric	strongly asymmetric	slightly asymmetric	slightly asymmetric	symmetric	slightly asymmetric
Trait 11: Fruit: shape of base (PQ)	1= acute 2= rounded 3= cordate	rounded	cordate	rounded	rounded	acute	rounded
Trait 12: Fruit: shape of the stylar end (PQ)	1=pointed 2= rounded 3= retuse	rounded	retuse	pointed	rounded	rounded	retuse
Trait 13: Fruit: color of skin (PQ)	RHS colour chart	146B: dark brown green	147C: medium brown green	153D: medium green brown	147C: medium brown green	147C: medium brown green	148D: light brown green
Trait 14: Fruit: presence of spines (PQ)	1= absent 9= present	present	present	present	present	present	present
Trait 15: Fruit: type of spine (PQ)	1= Type I 2= Type II	Type II	Type I	Type VI	Type IV	Type I	Type VI

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Trait	State	D24	D99	D160	D168	D197	D200
	3= Type III 4= Type IV 5= Type V 6= Type VI						
Trait 16: Fruit: spines around the base of the pedicel (QL)	1= absent 9= present	present	present	present	present	absent	present
Trait 17: Fruit: spines at the stylar end (QL)	1= absent 9= present	present	present	present	present	absent	absent
Trait 18: Fruit: weight of peel (kg) (QN)	-	0.235 – 1.845	0.455 – 0.997	0.431 – 1.467	0.826 – 1.533	0.883 – 1.663	0.518 – 2.413
Trait 19: Fruit: weight of seeds (kg) (QN)	-	0.034 – 0.332	0.158 – 0.231	0.046 – 0.194	0.096 – 0.134	0.027 – 0.204	0.183 – 0.441
Trait 20: Fruit: number of seeds per fruit (QN)	-	12 – 18	12 – 16	6 – 21	9 – 13	3 – 16	12 – 21
Trait 21: Fruit: number of flattened seeds per fruit (QN)	-	0 – 9	4 – 5	3 – 14	1 – 9	2 – 7	0 – 3
Trait 22: Flesh: main color (PQ)	1= white 2= light yellow 3= medium yellow 4= dark yellow 5= yellow orange 6= pink yellow 7= pink 8= red orange 9= red)	medium yellow	medium yellow	medium yellow	yellow orange	dark yellow	yellow orange
Trait 23: Flesh: aroma (PQ)	3= weak 5= medium 7= strong	medium	medium	medium	medium	medium	medium
Trait 24: Flesh: sweetness (PQ)	3= weak 5= medium 7= strong	medium	medium	strong	strong	strong	strong
Trait 25: Flesh: bitterness (PQ)	3= weak 5= medium 7= strong	weak	weak	medium	strong	strong	medium
Trait 26: Flesh: texture (PQ)	3= fine 5= medium 7= coarse	medium	fine	medium	fine	medium	medium
Trait 27: Flesh: creaminess (PQ)	3= weak 5= medium 7= strong	medium	weak	strong	strong	strong	strong
Trait 28: Seed: shape (PQ)	1= oblong 2= circular 3= elliptic 4= ovate 5= obovate	ovate	oblong	ovate	ovate	oblong	oblong

4.2.1 Quantitative Trait Evaluation (QN)

The quantitative traits (QN) consist of one pedicel trait and seven fruit traits. The QN trait values represent the average value for each fruit collected according to the fruit development stage. All eight quantitative trait values were displayed as column charts to ease comparison among varieties (Figure 4.1). Interestingly, some durian

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varieties had outstanding trait values that were above the average (indicated by arrow).

The D99, D168 and D200 have pedicel lengths above average (Figure 4.1a). The D200 has the highest values for fruit weight (more than 3.5 kg), weight of peel (more than 2.0 kg) and weight of seeds (more than 0.4 kg) (Figure 4.1b, 4.1e and 4.1f). On the other hand, D160 and D200 have the highest number of seeds per fruit (more than 20 seeds) (Figure 4.1g) and D160 has the most flattened seeds per fruit (more than 10 seeds) (Figure 4.1h).

Two quantitative traits have mediocre trait values among varieties which are fruit length and fruit width (Figure 4.1c and 4.1d). Despite being mediocre, these quantitative traits are worth mentioning as they could be used to identify specific varieties. For example, the fruit size of D99 was the smallest comparatively, as all D99 fruits recorded had lower values for fruit length and width than average (Figure 4.1 c and 4.1d).

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Analyses of variance (ANOVA) for QN traits results showed that only pedicel length (Trait 4) showed significant difference ($p<0.05$) between D168 variety with the other five varieties (Table 4.2). The Shapiro-Wilk test verifies that the pedicel length trait is normally distributed ($p=0.052$) (Appendix Table A1). Then, the canonical discriminant analysis simultaneously examines the differences in the morphological traits and indicates the relative contribution of each trait to varieties discrimination. The canonical discriminant analysis was done using eight QN traits further classified the variety into different groups identified similar pattern of clustering (Figure 4.2). Function 1 and 2 represents the interrelationships of the independent variables (traits) and the dependent variables (varieties).

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Figure 4.1

Eight quantitative traits chart of six durian varieties. (a) Pedicel: length (cm). (b) Fruit: weight (kg). (c) Fruit: length (cm). (d) Fruit: width (cm). (e) Fruit: weight of peel (kg). (f) Fruit: weight of seeds (kg). (g) Fruit: number of seeds per fruit. (h) Fruit: number of flattened seeds per fruit



Note, Dotted horizontal line indicates average trait values.

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Table 4.2

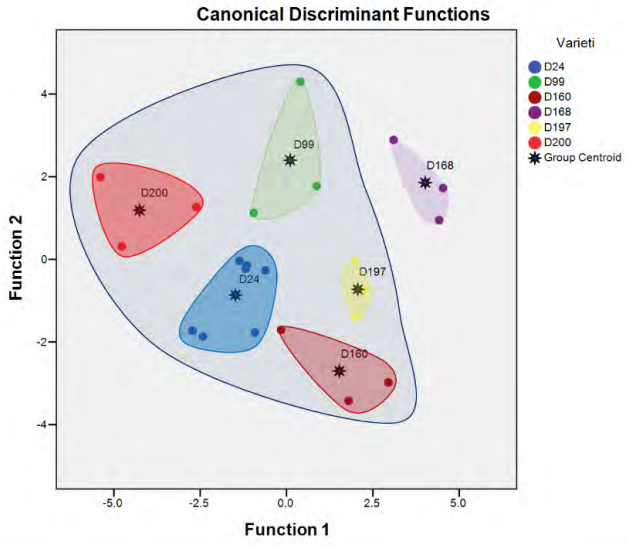
Mean and standard deviation (SD) of eight quantitative traits of six durian varieties

Trait	D24	D99	D160	D168	D197	D200	All
n	7	3	3	3	3	3	22
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Trait 4: Pedicel: length (cm)	4.94 [b] ± 0.55	6.60 [b] ± 1.00	4.70 [b] ± 0.75	6.83 [a] ± 1.22	5.53 [b] ± 0.15	5.50 [b] ± 1.32	5.55 + 1.08
Trait 5: Fruit: weight (kg)	1.90 ± 0.90	1.22 ± 0.33	1.73 ± 0.86	1.59 ± 0.53	1.78 ± 0.73	2.71 ± 1.47	1.84 + 0.88
Trait 6: Fruit: length (cm)	19.56 ± 5.22	15.23 ± 0.25	20.83 ± 3.31	20.73 ± 2.31	20.20 ± 4.19	18.80 ± 2.81	19.29 + 3.87
Trait 7: Fruit: width (cm)	16.59 ± 3.38	15.00 ± 1.22	15.77 ± 1.86	17.33 ± 1.53	16.67 ± 1.89	18.10 ± 2.85	16.58 + 2.44
Trait 18: Fruit: weight of peel (kg)	1.06 ± 0.56	0.70 ± 0.28	1.02 ± 0.53	1.14 ± 0.36	1.19 ± 0.41	1.65 ± 1.00	1.12 + 0.56
Trait 19: Fruit: weight of seeds (kg)	0.25 ± 0.10	0.20 ± 0.04	0.13 ± 0.08	0.11 ± 0.02	0.12 ± 0.09	0.29 ± 0.13	0.20 + 0.10
Trait 20: Fruit: number of seeds per fruit	14.43 ± 2.30	14.67 ± 2.31	13.00 ± 7.55	10.67 ± 2.08	9.33 ± 6.51	16.33 ± 4.51	13.32 + 4.37
Trait 21: Fruit: number of flattened seeds per fruit	2.14 ± 3.24	4.33 ± 0.58	6.67 ± 6.35	3.67 ± 4.62	4.00 ± 2.65	1.00 ± 1.73	3.36 + 3.59

Note, Letter [a, b] of statistical analysis ANOVA (Tukey's Test) at 95% confidence level with same letters indicating no significant difference ($p<0.05$).

Figure 4.2

Scatterplot of centroid values of the six durian varieties on the two canonical discriminant functions by Wilk's Lambda test



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4.2.2 Qualitative Trait Observation (QL and PQ)

A total of 20 qualitative and pseudo-qualitative traits have been observed for the six durian varieties under study. The qualitative traits consist of three flower traits (Trait 1-3), 10 fruit traits (Trait 8-17), six flesh traits (Trait 22-27) and one seed trait (Trait 28). Some of the qualitative traits were displayed in figures and charts to ease comparison among varieties.

The flower traits (Trait 1-3) were displayed in Figure 4.3 and Figure 4.4. The observation clearly revealed that the flower traits in D99 and D200 are very different from other varieties under study (D24, D160, D168 and D197). The D99 and D200 have a circular and rounded flower bud (Figure 4.3b and 4.3f) whereas D24, D160, D168 and D197 have elliptic and obtuse flower bud (Figure 4.3a, 4.3c, 4.3d and 4.3e). Moreover, only D99 flower has lower level of stigma position in relation to anther (Figure 4.4b). On the other hand, the D24, D160, D168, D197 and D200 varieties have equal level of stigma position in relation to anther (Figure 4.4a, 4.4c, 4.4d, 4.4e and 4.4f).

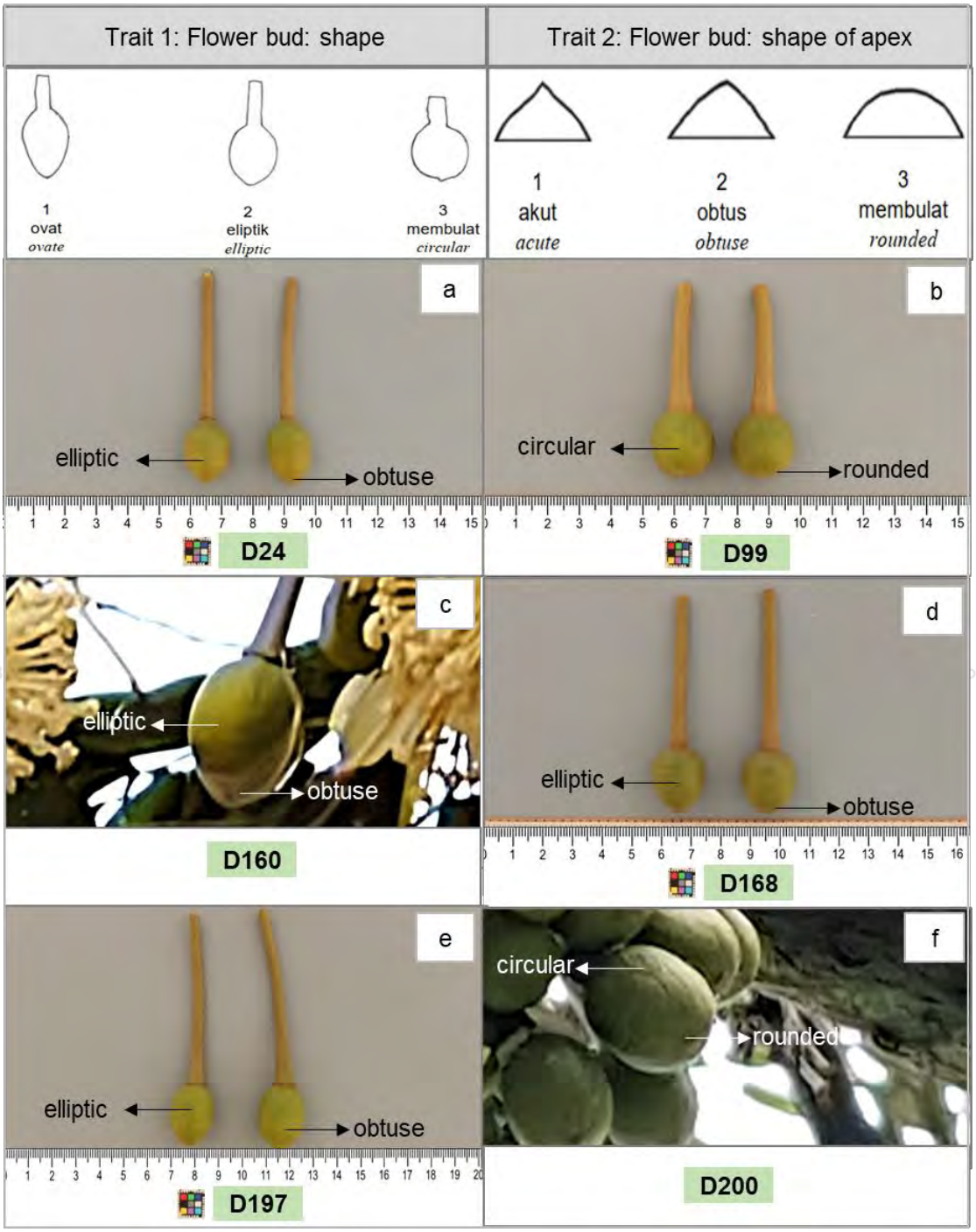
Fruit depth of groove (Trait 8) was displayed in Figure 4.5. The figures displayed represent the different types of grooves that the varieties have. Most of the varieties have absent or weak groove except D99 (strong groove) (Figure 4.5b) and D200 (medium groove) (Figure 4.5f).

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Figure 4.3

Flower bud shape and shape of apex. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200

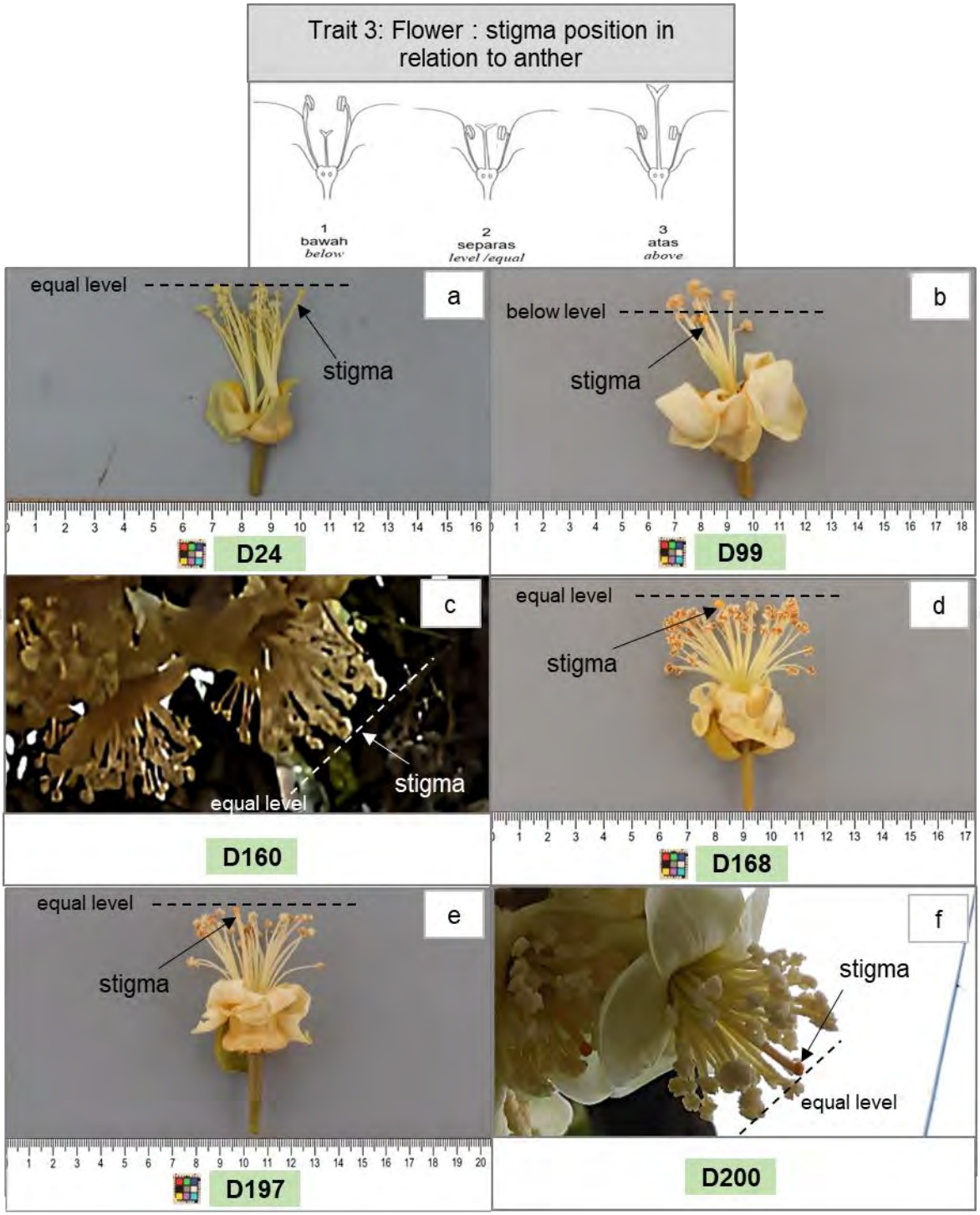


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Figure 4.4

Flower stigma position in relation to anther. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200

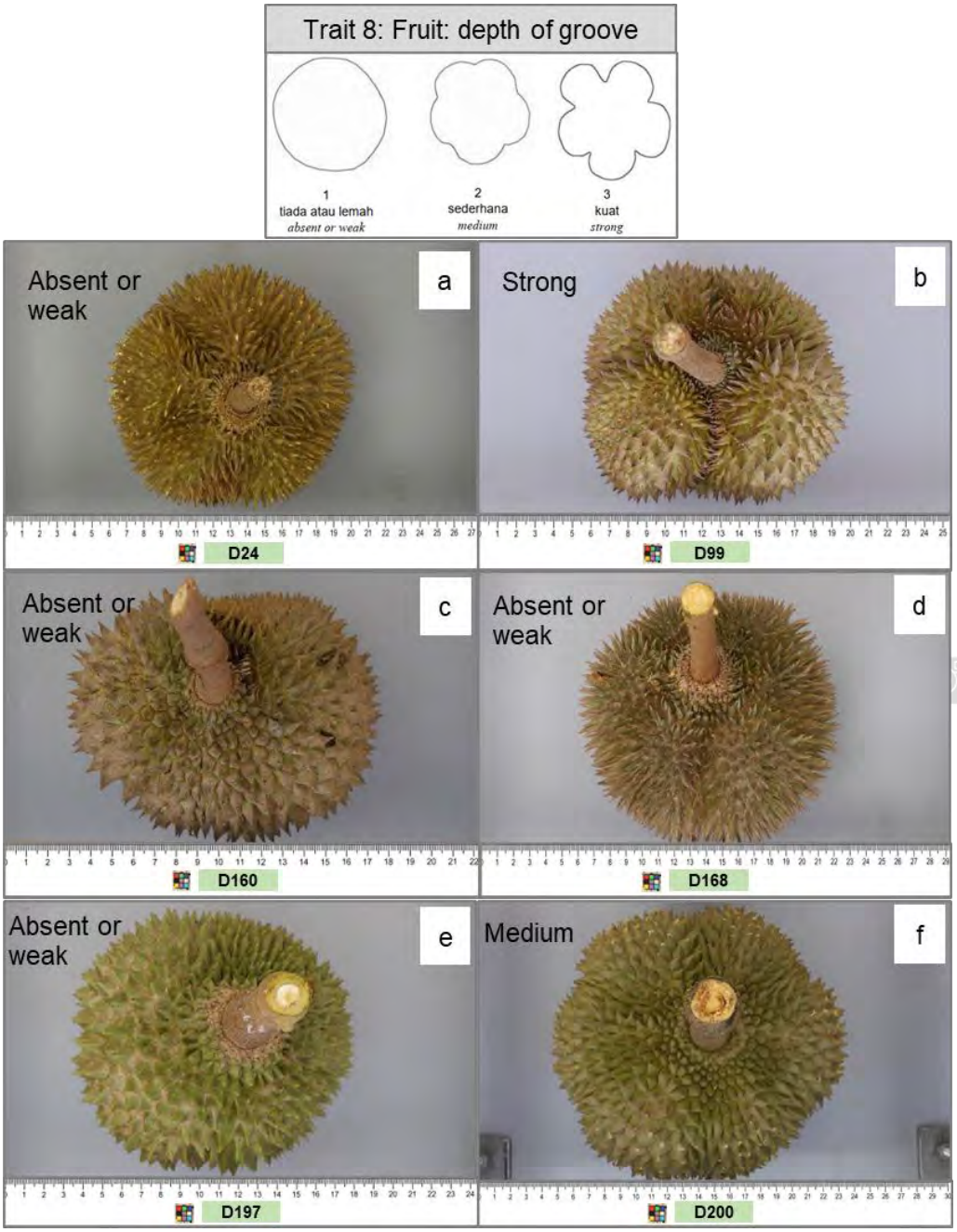


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Figure 4.5

Fruit depth of groove. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200



The fruit shape (Trait 9) and fruit symmetry (Trait 10) traits were displayed in Figure 4.6. The D24 fruit has an elliptic shape (Figure 4.6a), D99 and D200 have a circular fruit shape (Figure 4.6b and 4.6f), D160 has an ovate fruit shape (Figure 4.6c), D168 has an oblong fruit shape (Figure 4.6d) and D197 has an obovate fruit

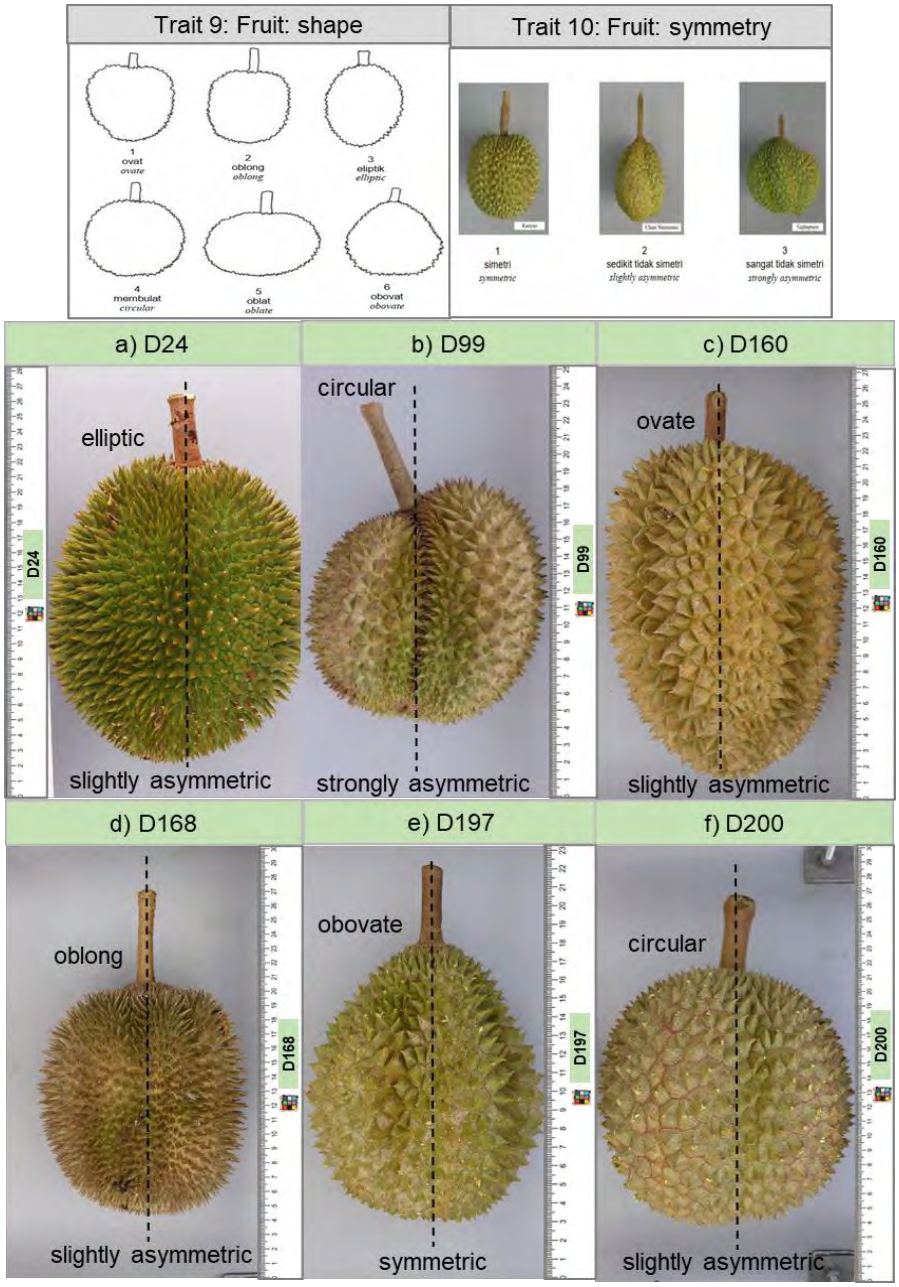
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shape (Figure 4.6e). Then, for the symmetric trait, the D24, D160, D168, and D200 were found to have slightly asymmetric fruit (Figure 4.6a, 4.6c, 4.6d and 4.6f), while D99 has strongly asymmetric fruit (Figure 4.6b). Only the D197 variety has symmetric fruit among the varieties under study (Figure 4.6e). Each variety can distinguish itself from the others based on these two traits.

Figure 4.6

Fruit shape and fruit symmetry. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200



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The shape of base (Trait 11) and shape of the stylar end (Trait 12) were displayed in Figure 4.7. The D24 and D168 fruits have a rounded base shape and a rounded stylar end shape (Figure 4.7a and 4.7d). The majority of D160 and D200 fruits also have a rounded base shape but have a pointed and retuse stylar end shape respectively (Figure 4.7c and 4.7f). Only D99 fruit has a cordate base shape and retuse stylar end shape while D197 fruit has an acute base shape and rounded stylar end shape (Figure 4.7b and 4.7e). In summary, both D24 and D168 varieties have similar traits of base shape and stylar end shape, while others are uniquely different.

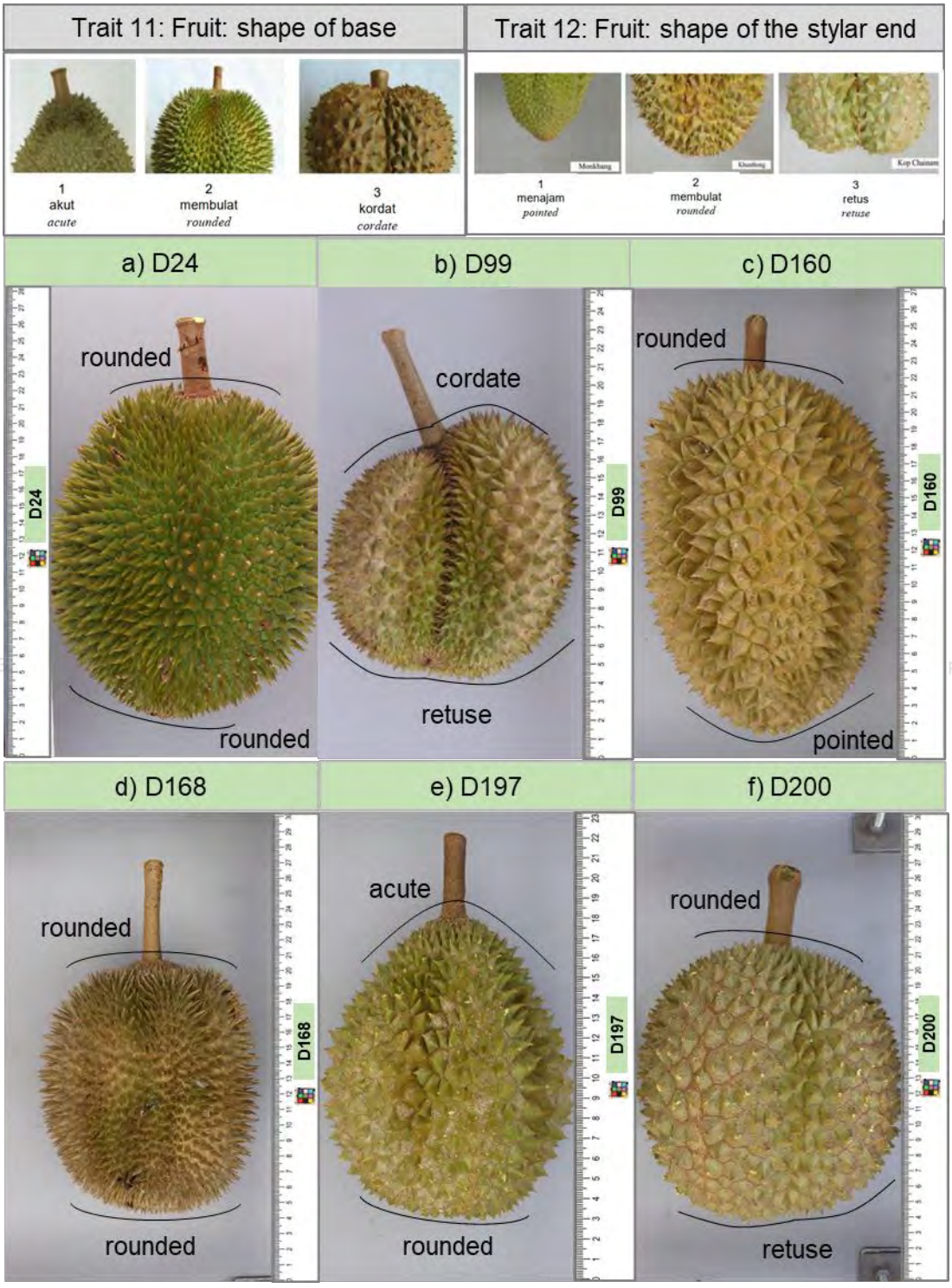
The colour of skin (Trait 13) of the six varieties under study was observed and compared with the RHS colour chart. A total of 11 types of colours have been detected for all six varieties under study (Figure 4.8). Due to the lighting, the figures displayed may have slightly different colours. The ranges of colour are light green (Figure 4.8a and 4.8b), dark brown green (Figure 4.8c), medium brown green (Figure 4.8d and 4.8e), medium brown (Figure 4.8f), light brown green (Figure 4.8g), medium green brown (Figure 4.8h and 4.8i), medium yellow brown (Figure 4.8j) and light brown (Figure 4.8k). The intensity of colour is represented by light, medium and dark. Then, in colour combinations, the second colour indicates the predominant colour with a blending of both colours resulting in what can look like a single colour. For example, in "brown green" the predominant colour is green and in "green brown" the predominant colour is brown. Based on Table 4.1, majority of the varieties have brown green colour type except D160 variety has a green brown colour type.

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Figure 4.7

Fruit shape of base and fruit shape of the stylar end. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200



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Figure 4.8

Types of fruit colour of skin for all varieties studied



Presence of spines (Trait 14) and type of spine (Trait 15) traits were displayed in Figure 4.9. All the samples that were collected had spines (Figure 4.9). D99 and

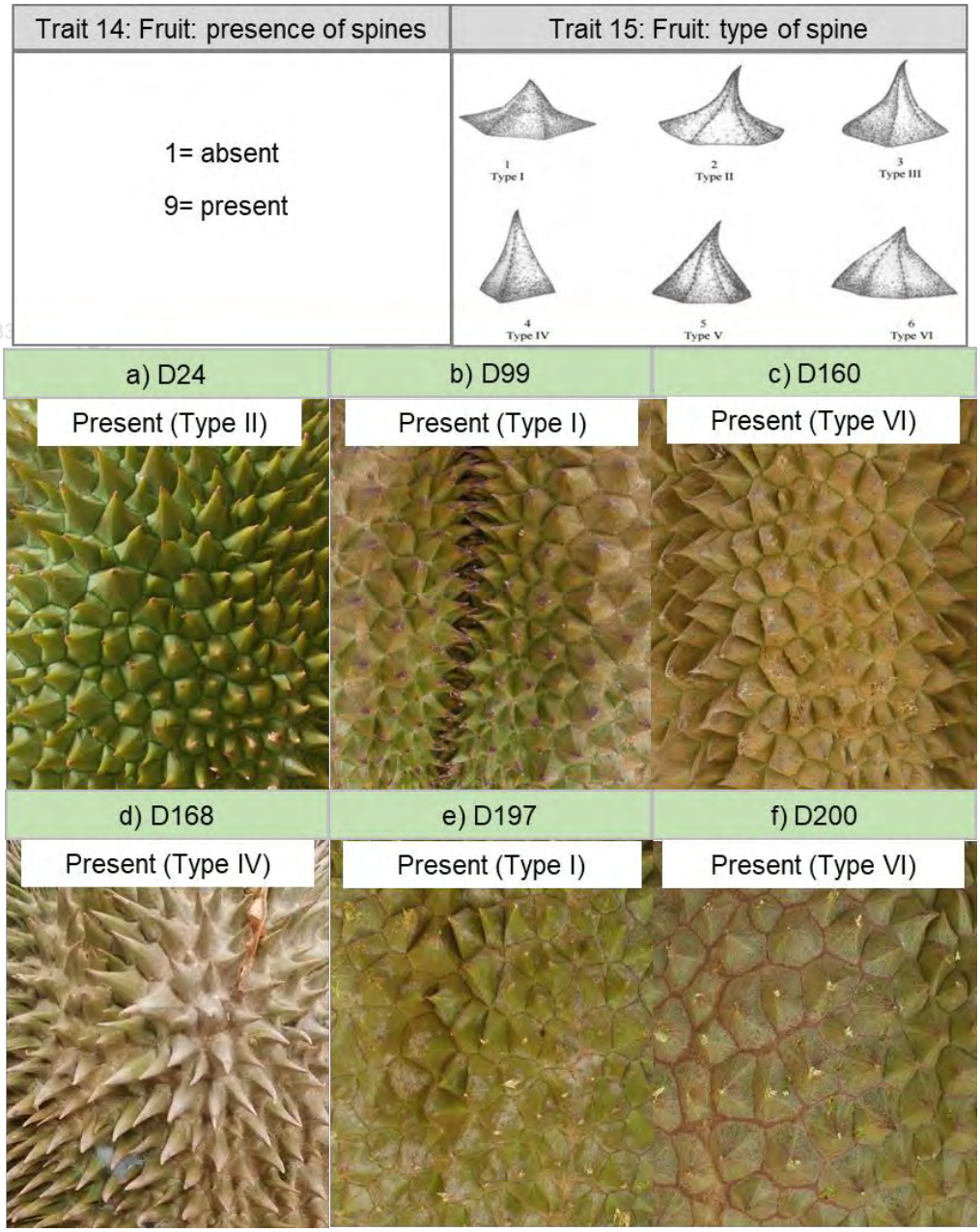
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D197 varieties have a similar spine type, which is Type I (Figure 4.9b and 4.9e), whereas the D160 and D200 have Type VI spines (Figure 4.9c and 4.9f). Only D24 variety has a Type II spines (Figure 4.9a) and D168 variety has a Type IV spine (Figure 4.9d). Thus, Type II and Type IV spines unique to D24 and D168 varieties respectively.

Figure 4.9

Presence of spines and type of spine. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200



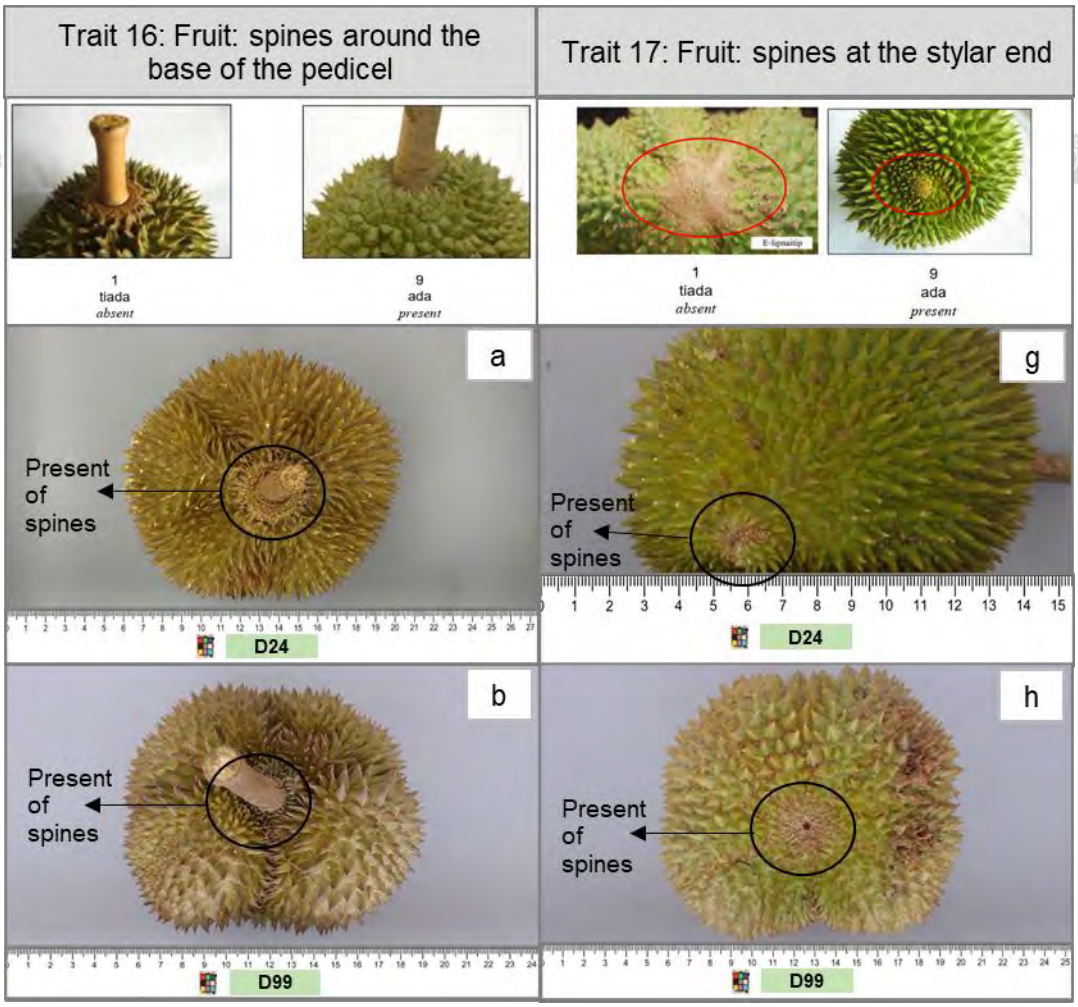
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The spines around the base of the pedicel (Trait 16) and spines at stylar end (Trait 17) were displayed in Figure 4.10. Four varieties (D24, D99, D160 and D168) have both present of spines around the base of the pedicel and at stylar end (Figure 4.10a-d and 4.10g-j). The D200 variety has also present of spines around the base of pedicel but absence of spines at stylar end (Figure 4.10f and 4.10l). Uniquely, D197 does not have spines at both around the base of the pedicel and at stylar end (Figure 4.10e and 4.10k). Thus, both traits (Trait 16 and Trait 17) can differentiate D197 and D200 varieties from other varieties under study.

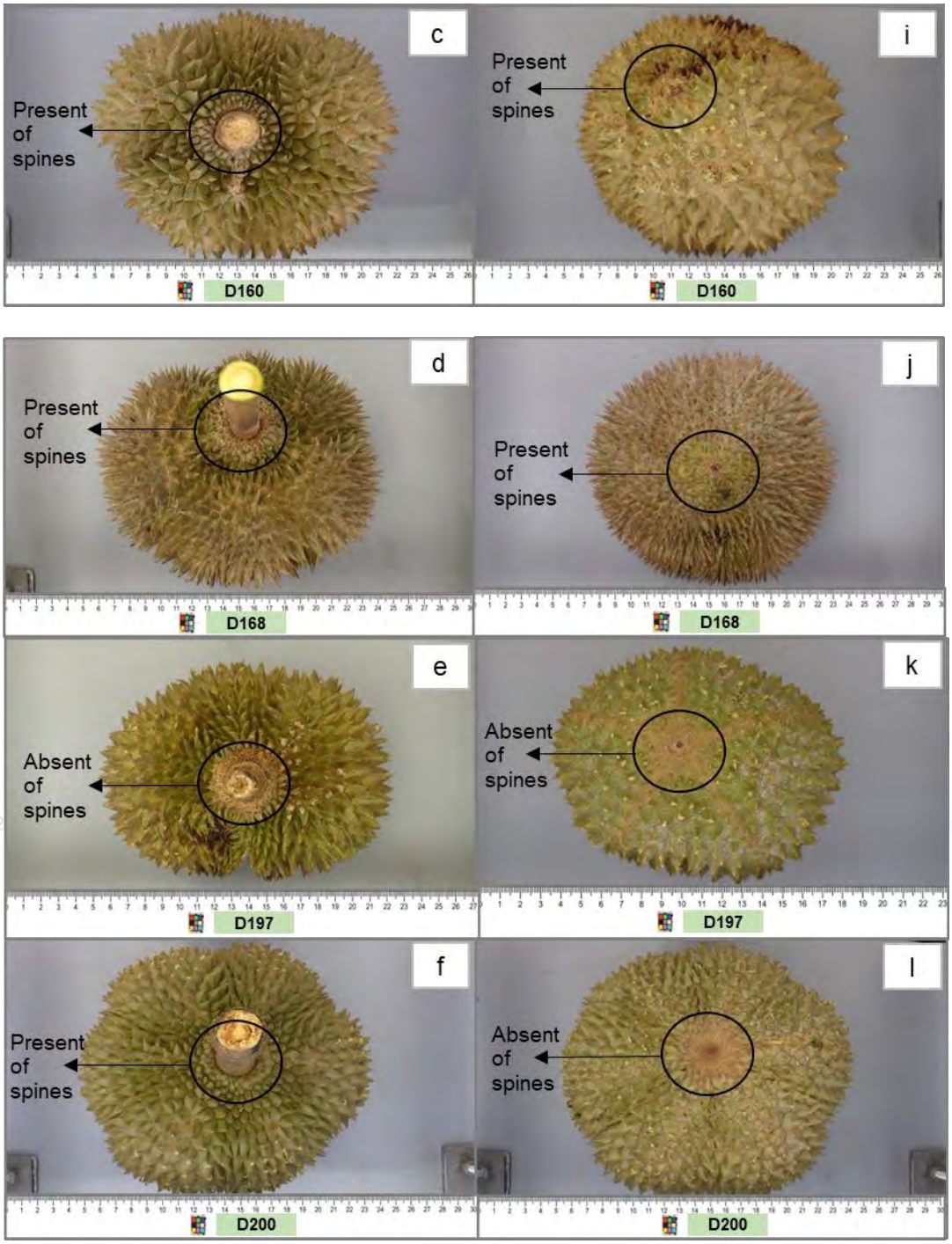
Figure 4.10

Fruit spines around the base of the pedicel and fruit spines at the stylar end. (a and g) D24. (b and h) D99. (c and i) D160. (d and j) D168. (e and k) D197. (f and l) D200



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The flesh main colour (Trait 22) was displayed in Figure 4.11. Due to the lighting, the figures displayed may also have slightly different colours. There are two varieties (D168 and D200) that stand out from the rest of the varieties for their exceptional colour (yellow orange) (Figure 4.11d and 4.11f). The yellow orange

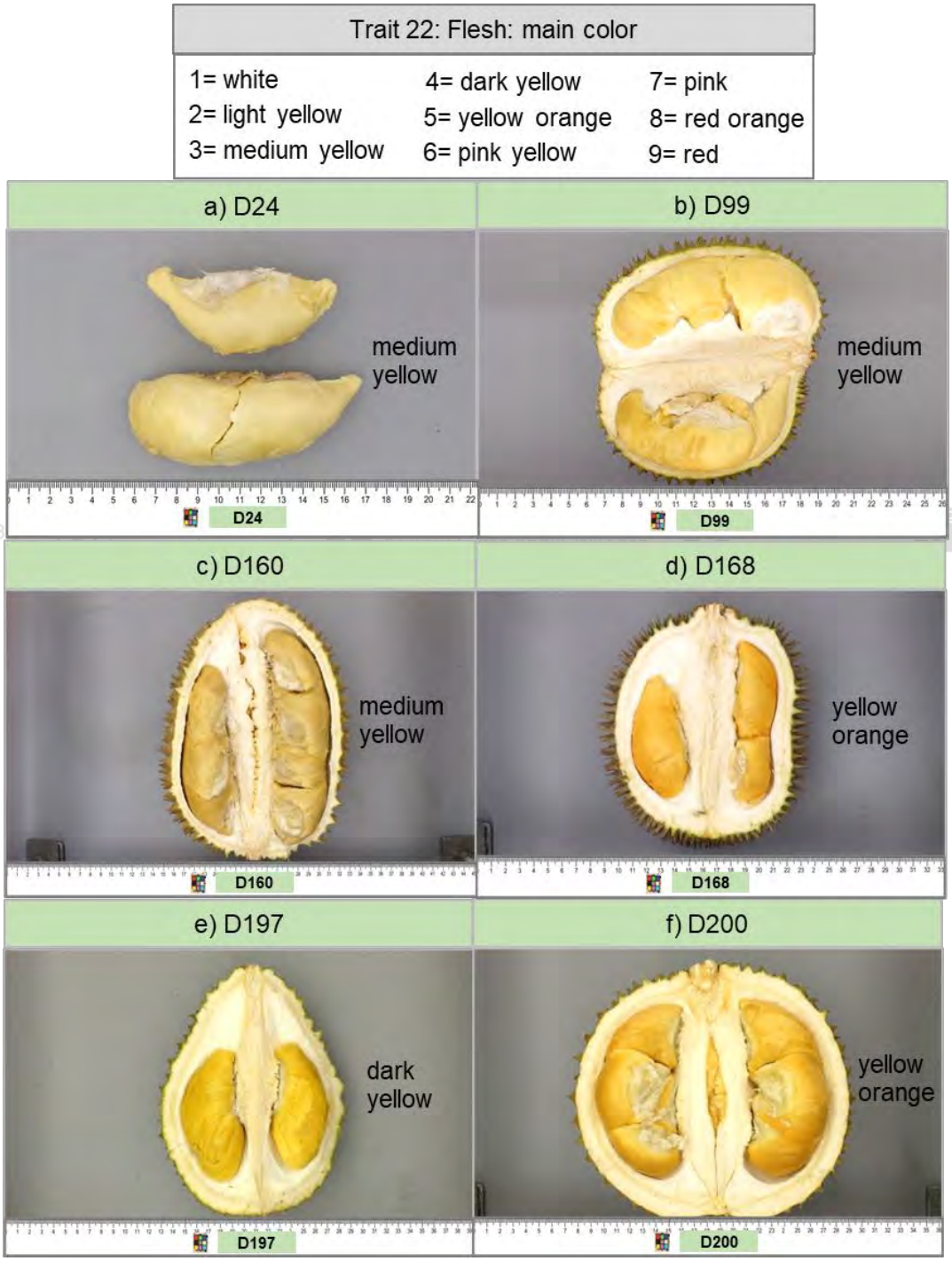
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colour was unique to both D168 and D200 variety, thus enable them to be easily distinguishable from other varieties.

Figure 4.11

Flesh main color. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200



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All five pseudo-qualitative trait (Trait 23-27) values related to aroma, taste and texture were displayed as column charts to ease comparison among varieties (Figure 4.12). Flesh aroma (Trait 23) was displayed in Figure 4.12a. The D168 variety exhibited the highest ranking of aroma, which was ranked strong, while the other varieties were ranked as medium.

Flesh sweetness (Trait 24) was displayed in Figure 4.12b. Among the six varieties, the D99 variety ranked the lowest in sweetness whereas other varieties can reach a strong level. The D168 variety would be in the top ranking in sweetness as all the samples have strong sweetness.

Flesh bitterness (Trait 25) was displayed in Figure 4.12c. The D99 variety has the weakest bitterness among the varieties followed by the D24 and D160 varieties, which can achieve a medium bitterness. In terms of bitterness, D168 and D197 would like to be the top two rankings for strong bitterness, followed by the D200 variety.

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Flesh texture (Trait 26) was displayed in Figure 4.12d. All the varieties have coarse texture to fine texture. However, there are two varieties (D99 and D168) have a finer flesh texture compared to others.

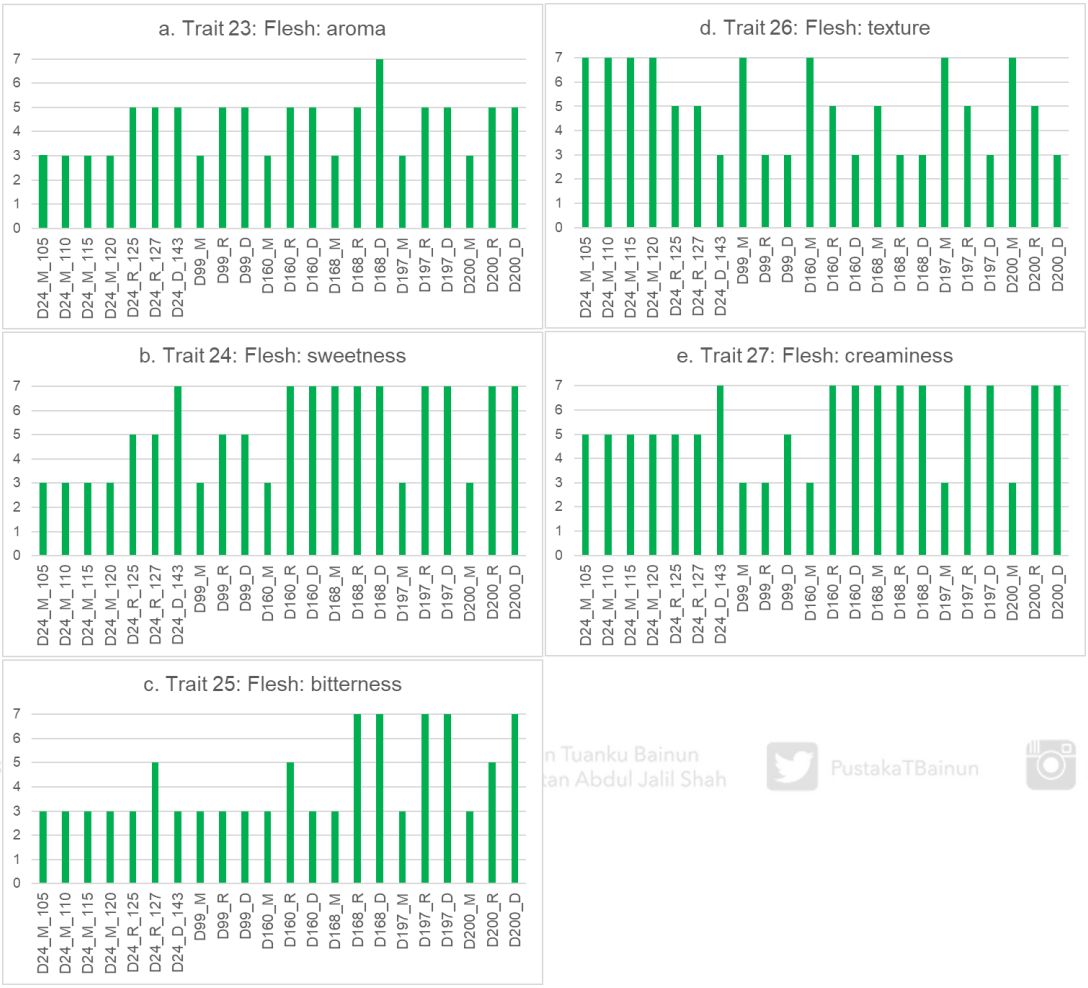
Flesh creaminess (Trait 27) was displayed in Figure 4.12e. The D99 variety would like to be ranked last in creaminess because the samples can only achieve medium creaminess. The D168 variety would rank first in terms of strong creaminess as it achieved strong level for all samples.

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Figure 4.12

Five pseudo-qualitative traits chart of six durian varieties. (a) Flesh: aroma. (b) Flesh: sweetness. (c) Flesh: bitterness. (d) Flesh: texture. (e) Flesh: creaminess



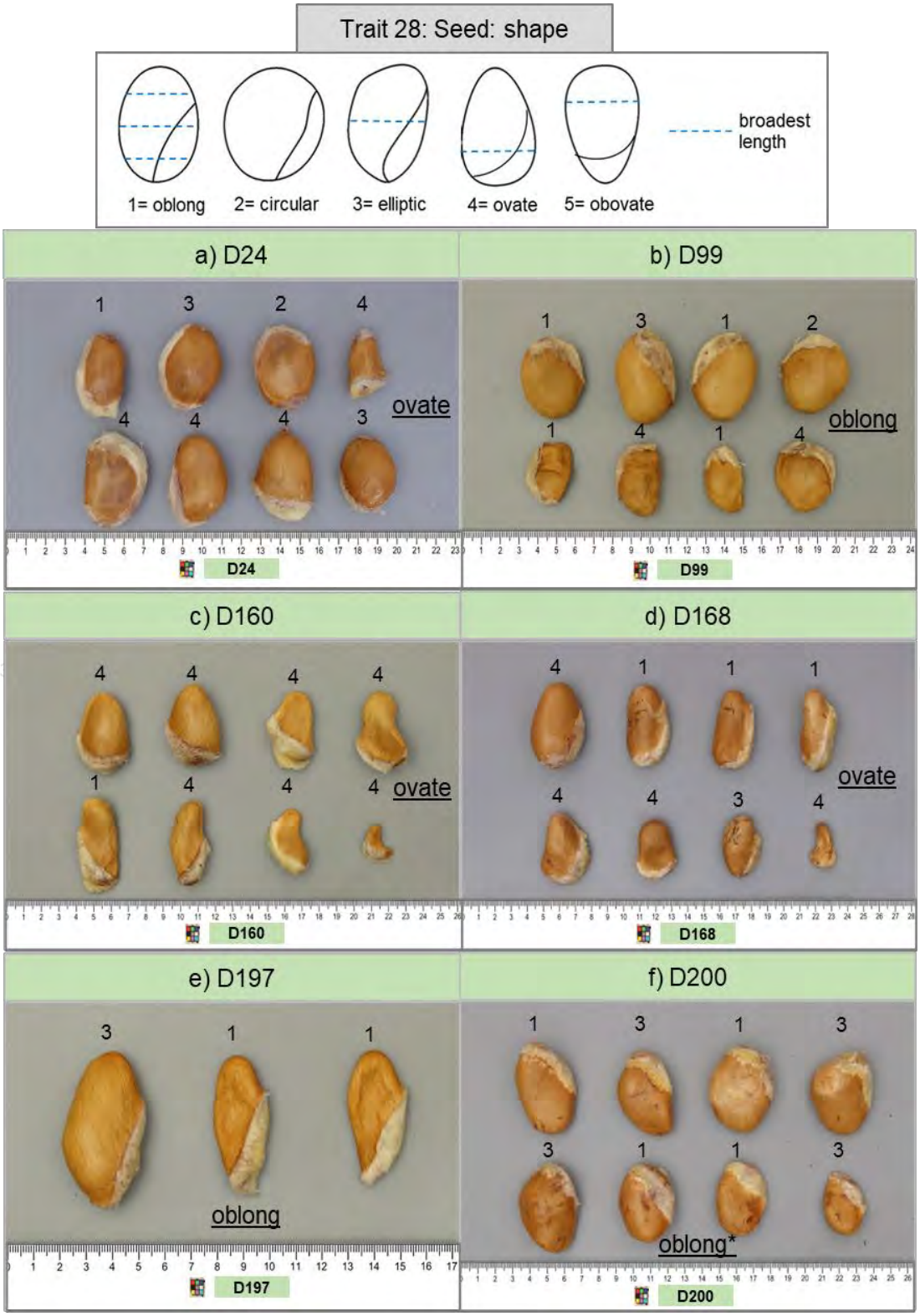
The seed shape (Trait 28) was displayed in Figure 4.13. Three varieties (D24, D160 and D168) had ovate seed shape (Figure 4.13a, 4.13c and 4.13d) whereas D99, D197 and D200 seeds were oblong (Figure 4.13b, 4.13e and 4.13f).

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Figure 4.13

Seed shape. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200



Note, * = Most of the rest of the seeds (not in the picture) have the same shape.

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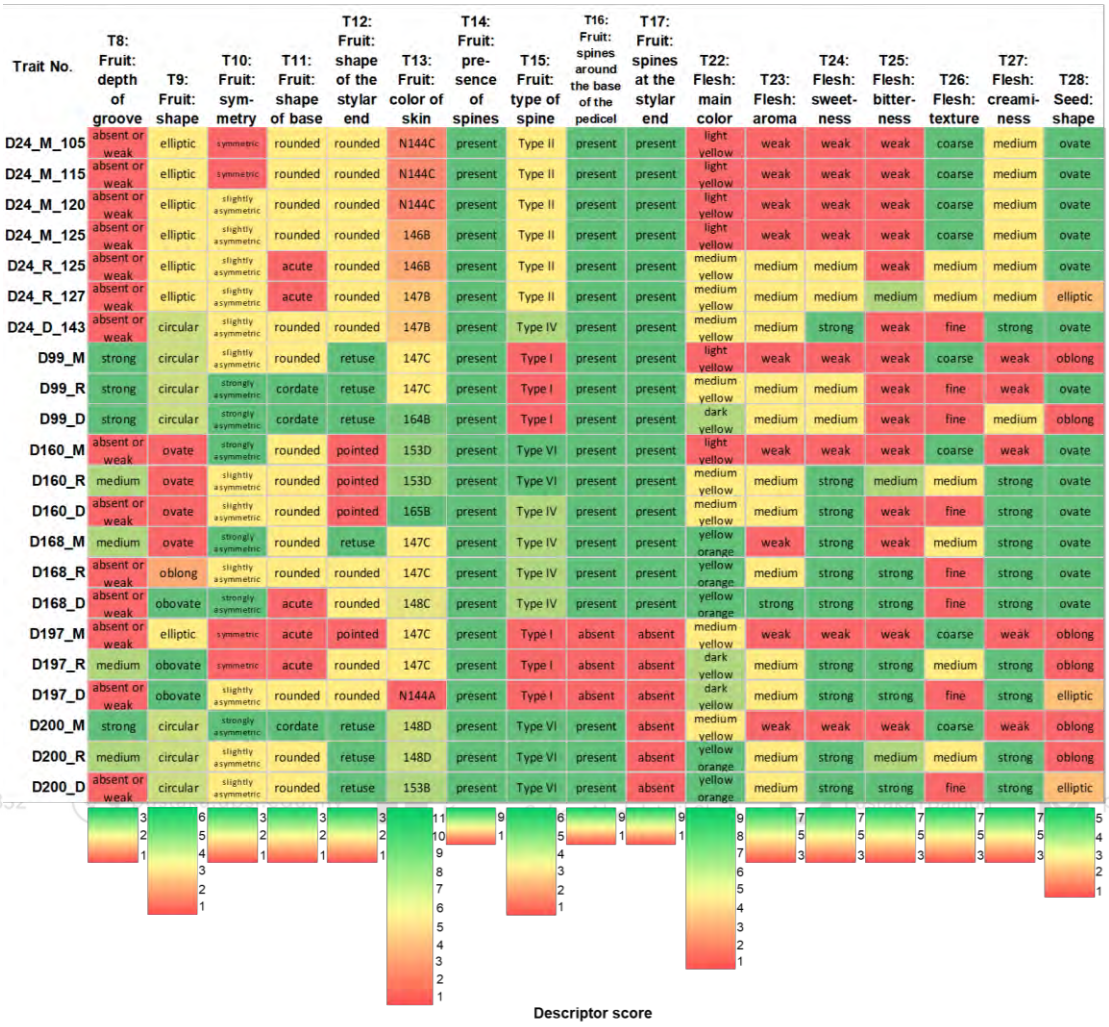
To summarized, all 17 qualitative traits (fruit, flesh, and seed traits) were displayed in the heat map (Figure 4.14). For Trait 8, D99 have strong groove type, the D24, D160, D168 and D197 have unique fruit shapes with an elliptic, ovate, oblong and obovate shape respectively (Trait 9), only the D197 fruits have symmetric trait (Trait 10), D99, D160, D197 and D200 has unique shapes for both base and stylar end traits (Trait 11-12), there are 11 type of colour of skin detected among the six durian varieties with two dominant colours, which are brown green and green brown (Trait 13), D24 and D168 stand out for their Type II and Type IV spine from others (Trait 15), D200 variety has absence of spines at stylar end while D197 variety has absence of spines for both around the base of pedicel and stylar end (Trait 16-17), D168 and D200 have yellow orange flesh colour while D197 has a dark yellow flesh colour (Trait 22), D168 variety has been ranking in top for flesh aroma, flesh sweetness, flesh bitterness, flesh texture and flesh creaminess (Trait 23-27), and there are two seed shapes identified among the varieties; ovate seed shape (D24, D160 and D168) and oblong seed shape (D99, D197 and D200) (Trait 28).

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Figure 4.14

Heat map of 17 QL and PQ traits for six durian varieties



Trait No.	T8: Fruit: depth of groove	T9: Fruit: shape	T10: Fruit: sym-metry	T11: Fruit: shape of base	T12: Fruit: shape of the stylar end	T13: Fruit: color of skin	T14: Fruit: pre-sence of spines	T15: Fruit: type of spine	T16: Fruit: spines around the base of the pedicel	T17: Fruit: spines at the stylar end	T22: Flesh: main color	T23: Flesh: aroma	T24: Flesh: sweet-ness	T25: Flesh: bitter-ness	T26: Flesh: texture	T27: Flesh: creami-ness	T28: Seed: shape
D24_M_105	absent or weak	elliptic	symmetric	rounded	rounded	N144C	present	Type II	present	present	light yellow	weak	weak	weak	coarse	medium	ovate
D24_M_115	absent or weak	elliptic	symmetric	rounded	rounded	N144C	present	Type II	present	present	light yellow	weak	weak	weak	coarse	medium	ovate
D24_M_120	absent or weak	elliptic	slightly asymmetric	rounded	rounded	N144C	present	Type II	present	present	light yellow	weak	weak	weak	coarse	medium	ovate
D24_M_125	absent or weak	elliptic	slightly asymmetric	rounded	rounded	146B	present	Type II	present	present	light yellow	weak	weak	weak	coarse	medium	ovate
D24_R_125	absent or weak	elliptic	slightly asymmetric	acute	rounded	146B	present	Type II	present	present	medium yellow	medium	medium	weak	medium	medium	ovate
D24_R_127	absent or weak	elliptic	slightly asymmetric	acute	rounded	147B	present	Type II	present	present	medium yellow	medium	medium	medium	medium	medium	elliptic
D24_D_143	absent or weak	circular	slightly asymmetric	rounded	rounded	147B	present	Type IV	present	present	medium yellow	medium	strong	weak	fine	strong	ovate
D99_M	strong	circular	slightly asymmetric	rounded	retuse	147C	present	Type I	present	present	light yellow	weak	weak	weak	coarse	weak	oblong
D99_R	strong	circular	strongly asymmetric	cordate	retuse	147C	present	Type I	present	present	medium yellow	medium	medium	weak	fine	weak	ovate
D99_D	strong	circular	strongly asymmetric	cordate	retuse	164B	present	Type I	present	present	dark yellow	medium	medium	weak	fine	medium	oblong
D160_M	absent or weak	ovate	strongly asymmetric	rounded	pointed	153D	present	Type VI	present	present	light yellow	weak	weak	weak	coarse	weak	ovate
D160_R	medium	ovate	slightly asymmetric	rounded	pointed	153D	present	Type VI	present	present	medium yellow	medium	strong	medium	medium	strong	ovate
D160_D	absent or weak	ovate	slightly asymmetric	rounded	pointed	165B	present	Type IV	present	present	medium yellow	medium	strong	weak	fine	strong	ovate
D168_M	medium	ovate	strongly asymmetric	rounded	retuse	147C	present	Type IV	present	present	yellow orange	weak	strong	weak	medium	strong	ovate
D168_R	absent or weak	oblong	slightly asymmetric	rounded	rounded	147C	present	Type IV	present	present	yellow orange	medium	strong	strong	fine	strong	ovate
D168_D	absent or weak	obovate	strongly asymmetric	acute	rounded	148C	present	Type IV	present	present	yellow orange	strong	strong	strong	fine	strong	ovate
D197_M	absent or weak	elliptic	symmetric	acute	pointed	147C	present	Type I	absent	absent	medium yellow	weak	weak	weak	coarse	weak	oblong
D197_R	medium	obovate	symmetric	acute	rounded	147C	present	Type I	absent	absent	dark yellow	medium	strong	strong	medium	strong	oblong
D197_D	absent or weak	obovate	slightly asymmetric	rounded	rounded	N144A	present	Type I	absent	absent	dark yellow	medium	strong	strong	fine	strong	elliptic
D200_M	strong	circular	strongly asymmetric	cordate	retuse	148D	present	Type VI	present	absent	medium yellow	weak	weak	weak	coarse	weak	oblong
D200_R	medium	circular	slightly asymmetric	rounded	retuse	148D	present	Type VI	present	absent	yellow orange	medium	strong	medium	medium	strong	oblong
D200_D	absent or weak	circular	slightly asymmetric	rounded	retuse	153B	present	Type VI	present	absent	yellow orange	medium	strong	strong	fine	strong	elliptic

Note,
Trait 8: Fruit: depth of groove (1=absent or weak; 2= medium; 3= strong) (PQ);
Trait 9: Fruit: shape (1= ovate 2= oblong 3= elliptic 4= circular 5= oblate 6=obovate) (PQ);
Trait 10: Fruit: symmetry (1=symmetric; 2= slightly asymmetric; 3= strongly asymmetric) (PQ);
Trait 11: Fruit: shape of base (1= acute; 2= rounded; 3= cordate) (PQ);
Trait 12: Fruit: shape of the stylar end (1=pointed; 2= rounded; 3= retuse) (PQ);
Trait 13: Fruit: color of skin (RHS colour chart) (PQ);
Trait 14: Fruit: presence of spines (1= absent; 9= present) (PQ);
Trait 15: Fruit: type of spine (1= Type I; 2= Type II; 3= Type III; 4= Type IV; 5= Type V; 6= Type VI) (PQ);
Trait 16: Fruit: spines around the base of the pedicel (1= absent; 9= present) (QL);
Trait 17: Fruit: spines at the stylar end (1= absent; 9= present) (QL);
Trait 22: Flesh: main color (1= white; 2= light yellow; 3= medium yellow; 4= dark yellow; 5= yellow orange; 6= pink yellow; 7= pink; 8= red orange; 9= red) (PQ);
Trait 23: Flesh: aroma (3= weak; 5= medium; 7= strong) (PQ);
Trait 24: Flesh: sweetness (3= weak; 5= medium; 7= strong) (PQ);
Trait 25: Flesh: bitterness (3= weak; 5= medium; 7= strong) (PQ);
Trait 26: Flesh: texture (3= fine; 5= medium; 7= coarse) (PQ);
Trait 27: Flesh: creaminess (3= weak; 5= medium; 7= strong) (PQ);
Trait 28: Seed: shape (1= oblong; 2= circular; 3= elliptic; 4= ovate; 5= obovate) (PQ).

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4.2.3 Qualitative Traits Cluster Analysis

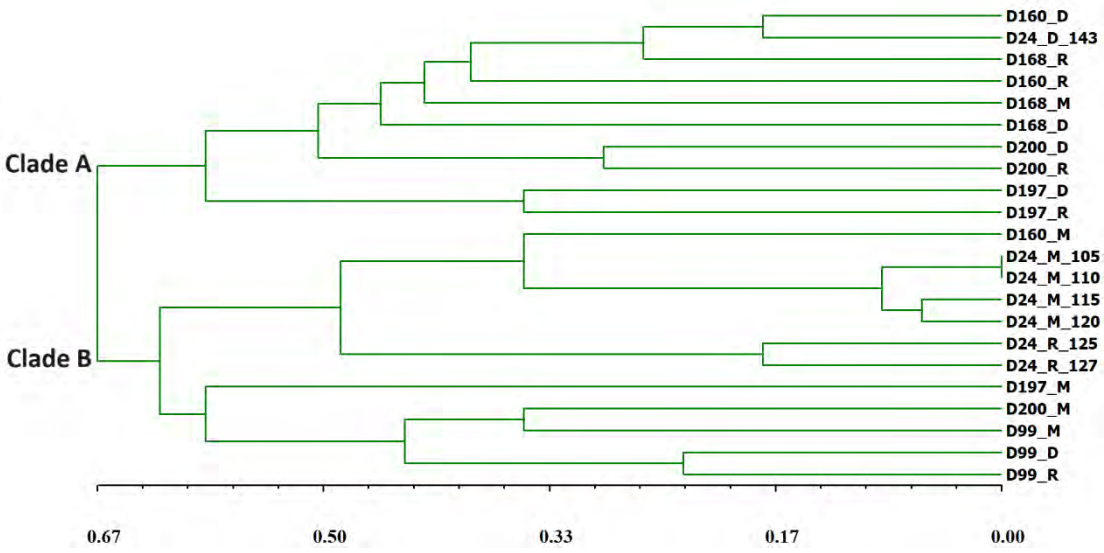
Cluster analysis was performed using data from both the QL and PQ qualitative traits. However, three PQ flower traits (Traits 1-3) were excluded as these traits represent different organs (flower organ) and cannot be compared with the rest of the traits (fruit organ). Thus, only 17 qualitative traits were utilised for cluster analysis, and they are as follows: 10 fruit traits (Trait 8-17), six flesh traits (Trait 22-27) and one seed trait (Trait 28).

A dendrogram generated clustered the six durian varieties into two main groups, labelled as clade A and B (Figure 4.15). Clade A comprises of mostly of D160, D168, D197 and D200 while clade B comprises mainly of D24 and D99 variety samples. However, some of the samples were randomly inter-spread between the two clades.

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Figure 4.15

Unweighted pair group method with an arithmetic mean (UPGMA) dendrogram based on 17 QL and PQ traits



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For genetic distance values, the highest genetic distance was observed between the D160_M sample and the D197_R sample, with a value of 0.9412 (highlighted in yellow) (Table 4.3). The lowest genetic distance was observed between the D24_M_105 sample and the D24_M_110 sample, with a value of 0.0000 (highlighted in yellow), and they were tied together in the dendrogram.

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Table 4.3

Top and bottom of Nei's 1983 pairwise genetic distance of the six durian varieties based on 17 QL and PQ traits

	D160_D	D160_M	D160_R	D168_D	D168_M	D168_R	D197_D	D197_M	D197_R	D200_D	D200_M	D200_R	D24_D_143	D24_M_105	D24_M_110	D24_M_115	D24_M_120	D24_R_125	D24_R_127	D99_D	D99_M	D99_R
D160_D	0.0000																					
D160_M	0.4706	0.0000																				
D160_R	0.2941	0.4706	0.0000																			
D168_D	0.4706	0.6471	0.6471	0.0000																		
D168_M	0.4118	0.4706	0.4118	0.4706	0.0000																	
D168_R	0.2941	0.6471	0.4706	0.2941	0.4118	0.0000																
D197_D	0.5294	0.8235	0.6471	0.5294	0.7647	0.4118	0.0000															
D197_M	0.7059	0.5294	0.8235	0.8235	0.7647	0.8235	0.7059	0.0000														
D197_R	0.7647	0.9412	0.6471	0.5882	0.6471	0.5882	0.3529	0.5294	0.0000													
D200_D	0.4706	0.7059	0.5294	0.5294	0.5882	0.3529	0.3529	0.8235	0.6471	0.0000												
D200_M	0.7647	0.4706	0.7647	0.8235	0.6471	0.8824	0.8824	0.4706	0.8235	0.6471	0.0000											
D200_R	0.5882	0.7647	0.3529	0.7059	0.4706	0.5294	0.5882	0.8235	0.5294	0.2941	0.5294	0.0000										
D24_D_143	0.1765	0.5882	0.4118	0.4118	0.4706	0.2353	0.4706	0.7647	0.7059	0.4118	0.7059	0.5294	0.0000									
D24_M_105	0.5882	0.3529	0.7059	0.6471	0.5882	0.5882	0.7647	0.5294	0.8235	0.7647	0.6471	0.8235	0.5294	0.0000								
D24_M_110	0.5882	0.3529	0.7059	0.6471	0.5882	0.5882	0.7647	0.5294	0.8235	0.7647	0.6471	0.8235	0.5294	0.0000	0.0000							
D24_M_115	0.5294	0.3529	0.6471	0.6471	0.5882	0.5294	0.7059	0.5882	0.8824	0.7059	0.6471	0.7647	0.4706	0.0588	0.0588	0.0000						
D24_M_120	0.5294	0.3529	0.6471	0.6471	0.5882	0.5294	0.7059	0.5882	0.8824	0.7059	0.6471	0.7647	0.4706	0.1176	0.1176	0.0588	0.0000					
D24_R_125	0.4706	0.6471	0.5294	0.5882	0.6471	0.5294	0.7059	0.6471	0.7059	0.7059	0.7647	0.7059	0.4118	0.4118	0.4118	0.3529	0.2941	0.0000				
D24_R_127	0.5882	0.7647	0.5294	0.6471	0.7647	0.5882	0.6471	0.7059	0.7059	0.6471	0.8235	0.6471	0.4706	0.5294	0.5294	0.4706	0.4706	0.1765	0.0000			
D99_D	0.6471	0.7059	0.7647	0.7059	0.6471	0.7059	0.7059	0.7647	0.7059	0.6471	0.4706	0.6471	0.5882	0.7059	0.7059	0.7059	0.7059	0.5882	0.6471	0.0000		
D99_M	0.6471	0.4118	0.7059	0.8235	0.5294	0.6471	0.7647	0.4706	0.7647	0.6471	0.3529	0.5882	0.5882	0.4706	0.4706	0.4118	0.4118	0.7059	0.7647	0.4706	0.0000	
D99_R	0.5294	0.5882	0.6471	0.6471	0.5294	0.5882	0.7647	0.6471	0.7647	0.6471	0.4118	0.7059	0.4706	0.7059	0.7059	0.7059	0.7059	0.5294	0.6471	0.2353	0.4118	0.0000

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4.3 Transcriptomic Analysis on Biological Functions and Molecular Pathway Mechanism Underlying Fruit Developmental Stages

4.3.1 RNA Extraction and Quality Control

A total of 22 durian flesh samples were processed for RNA extraction and library construction. The sample and library quality are summarized in Table 4.4 and Appendix Figure A1-A2. All samples achieved the minimum predetermined RIN value threshold (refer to section 3.4.2). As the flesh sample for the ripe and dehisced stages softens and becomes more deteriorated throughout the fruit's development, the RIN values declined. However, the data obtained still fulfilled the QC for downstream applications.

Three samples (D24_D_143, D160_D and D197_D) were upgraded with an additional 10Gb of data to ensure that extra effective reads were obtained as a matter of perfection before analysis. However, one sample, the D200_D, cannot be sequenced due to failed library QC. Thus, only 21 durian flesh samples were processed for RNA sequencing (Table 4.4).

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Table 4.4

RNA quantification, purity, and RIN for 22 durian flesh samples collected

No.	Sample	Amount (ng)	Volume (µl)	Concentration (ng/ µl)	Purity		RIN
	Require-ment	≥ 400 ng	≥ 20 µl	≥ 20ng/µl	OD 260/280 ≥ 2.0	OD 260/230 ≥ 2.0	≥ 6.3
1	D24 _M_105	7,060 ng	50 µl	141.28 ng/µl	2.206	2.321	8.1
2	D24_M_110	8,940 ng	50 µl	178.72 ng/µl	2.187	2.235	7.1
3	D24_M_115	7,060 ng	50 µl	141.28 ng/µl	2.217	1.929	7.2
4	D24_M_120	5,680 ng	50 µl	113.60 ng/µl	2.196	1.882	7.0
5	D24_R_125	3,820 ng	50 µl	76.48 ng/µl	2.188	2.369	6.6

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No.	Sample	Amount (ng)	Volume (µl)	Concentration (ng/ µl)	Purity	RIN	No.
	Require-ment	≥ 400 ng	≥ 20 µl	≥ 20ng/µl	OD 260/280 ≥ 2.0	OD 260/230 ≥ 2.0	≥ 6.3
6	D24_R_127	11,590 ng	50 µl	231.84 ng/µl	2.207	2.166	5.0
7	D24_D_143	19,520 ng	50 µl	390.36 ng/µl	2.212	2.373	3.1
8	D99_M	16,840 ng	50 µl	336.88 ng/µl	2.191	1.788	7.8
9	D99_R	18,500 ng	50 µl	246.96 ng/µl	2.214	2.175	8.0
10	D99_D	7,950 ng	50 µl	158.96 ng/µl	2.207	1.466	4.0
11	D160_M	8,350 ng	50 µl	167.12 ng/µl	2.207	2.052	8.2
12	D160_R	33,440 ng	50 µl	376.32 ng/µl	2.231	2.190	5.5
13	D160_D	24,400 ng	50 µl	487.96 ng/µl	2.221	2.214	4.3
14	D168_M	26,220 ng	50 µl	524.36 ng/µl	2.234	2.014	7.1
15	D168_R	27,960 ng	50 µl	559.28 ng/µl	2.233	2.271	6.8
16	D168_D	18,230 ng	50 µl	364.68 ng/µl	2.203	2.287	5.9
17	D197_M	12,140 ng	50 µl	242.76 ng/µl	2.220	2.033	7.0
18	D197_R	26,350 ng	50 µl	335.24 ng/µl	2.218	2.239	7.4
19	D197_D	39,350 ng	50 µl	786.96 ng/µl	2.240	2.325	3.4
20	D200_M	18,510 ng	50 µl	370.28 ng/µl	2.194	2.204	8.1
21	D200_R	16,530 ng	50 µl	330.64 ng/µl	2.099	2.256	6.4
22	D200_D	21,570 ng	50 µl	431.40 ng/µl	2.208	2.351	2.0

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4.3.2 Bioinformatic Analysis Pipeline Profiling

The profiling of bioinformatics analysis includes data quality control, alignment and mapping, novel gene prediction, gene expression quantification, differential gene expression analysis and functional annotation.

4.3.2.1 Data Quality Control

The data quality assessment for RNA sequencing is summarized in Table 4.5. All the sequencing libraries produced at least 81 million raw data except for library constructed for D99 variety at riped stage (D99_R) which obtained raw reads of approximately 67 million (Table 4.5). Sequencing reads for all libraries achieved a

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Q20 percentage (percentage of sequences with sequencing error rate lower than 1%) over 97%. On the other hand, sequencing reads for all libraries at Q30 percentage (percentage of sequencing error rate lower than 0.1%) were over 92%. These raw data supported that the sequencing quality was high enough to proceed with downstream analysis.

Table 4.5

Sample Data Quality Summary

Sample Name	Raw Reads	Raw Bases	Clean Reads	Clean Bases	Error Rate	Q20	Q30	GC (%)
D24_M_105	84,949,286	12.74G	84,452,040	12.67G	0.03	97.68	93.27	43.35
D24_M_110	87,077,690	13.06G	86,424,936	12.96G	0.03	97.84	93.65	43.68
D24_M_115	88,758,916	13.31G	87,535,868	13.13G	0.03	97.84	93.72	43.41
D24_M_120	98,926,972	14.84G	98,322,964	14.75G	0.03	97.87	93.61	43.58
D24_R_125	88,895,450	13.33G	87,769,646	13.17G	0.03	97.82	93.51	43.72
D24_R_127	89,892,528	13.48G	88,946,308	13.34G	0.03	97.57	93.21	44.48
D24_D_143	234,966,562	35.24G	130,816,254	19.62G	0.03	97.32	92.27	45.86
D99_M	84,016,300	12.6G	83,582,182	12.54G	0.03	97.92	93.84	43.77
D99_R	67,481,114	10.12G	66,727,950	10.01G	0.03	97.84	93.6	43.65
D99_D	81,598,812	12.24G	77,548,074	11.63G	0.02	97.98	94.35	44.9
D160_M	100,189,054	15.03G	99,730,740	14.96G	0.03	97.9	93.68	43.98
D160_R	86,572,106	12.99G	81,732,158	12.26G	0.03	97.73	93.7	45.76
D160_D	116,958,946	17.54G	73,393,138	11.01G	0.03	97.15	92.06	47.01
D168_M	85,761,402	12.86G	85,040,444	12.76G	0.03	97.69	93.4	44.26
D168_R	83,657,006	12.55G	80,443,008	12.07G	0.03	97.81	93.83	43.83
D168_D	87,247,146	13.09G	82,689,642	12.4G	0.03	97.58	93.35	44.36
D197_M	86,145,144	12.92G	85,614,852	12.84G	0.03	97.72	93.38	43.5
D197_R	85,111,440	12.77G	80,959,798	12.14G	0.03	97.82	93.84	44.19
D197_D	96,807,806	14.52G	78,974,080	11.85G	0.03	97.37	92.76	44.65
D200_M	90,478,396	13.57G	89,715,084	13.46G	0.03	97.6	93.16	43.93
D200_R	84,179,334	12.63G	79,633,434	11.95G	0.03	97.89	94.01	44.07

Note,
Q20 = The percentage of the bases whose QPhred values is greater than 20. (Number of bases with QPhred value > 20) / (Number of total bases) *100;
Q30 = The percentage of the bases whose QPhred values is greater than 30. (Number of bases with QPhred value > 30) / (Number of total bases) *100.

4.3.2.1.1 Error Rate Distribution

The sample sequencing error rate distribution for 21 samples are shown in the graph as displayed in Figure 4.16. Pair-end sequencing data are plotted together, with the

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first read of paired ends 150bp representing read 1 and the following paired ends 150bp for read 2. The x-axis shows the base position along with each sequencing read and the y-axis shows the base error rate. In general, all the samples sequenced have a less than 1% single-base error rate (Table 4.5).

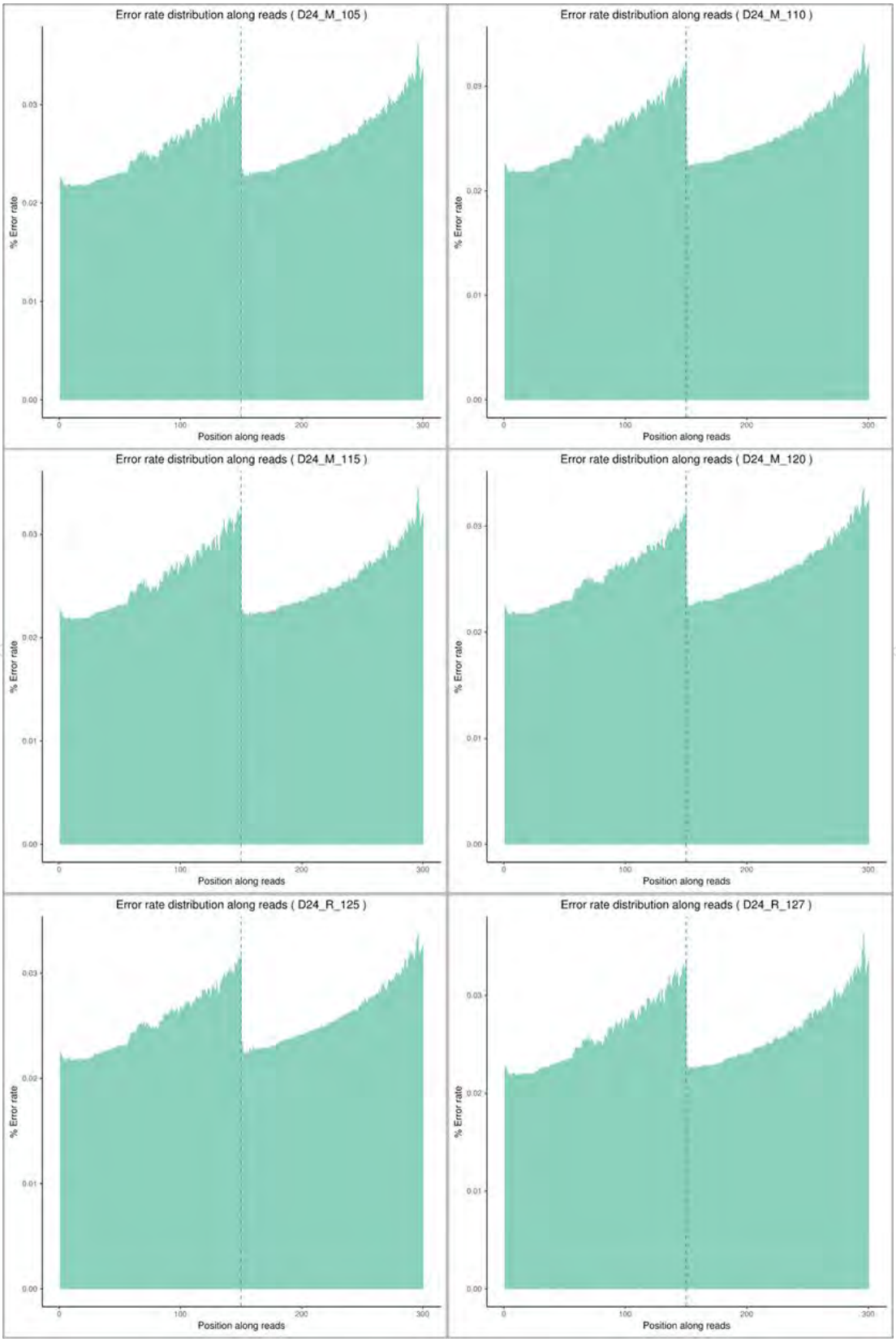
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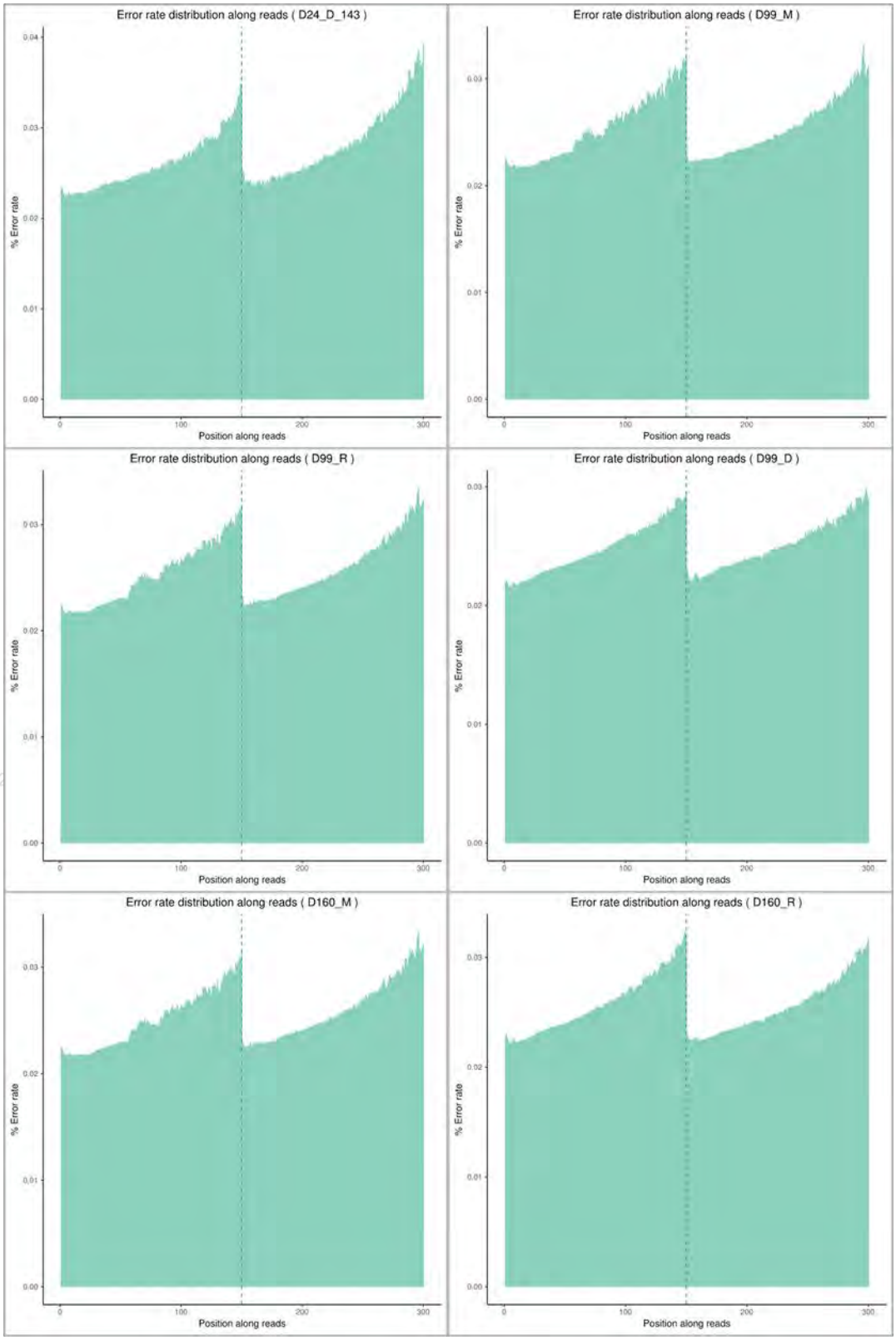
Figure 4.16

Sequencing data error rate distribution for 21 durian samples



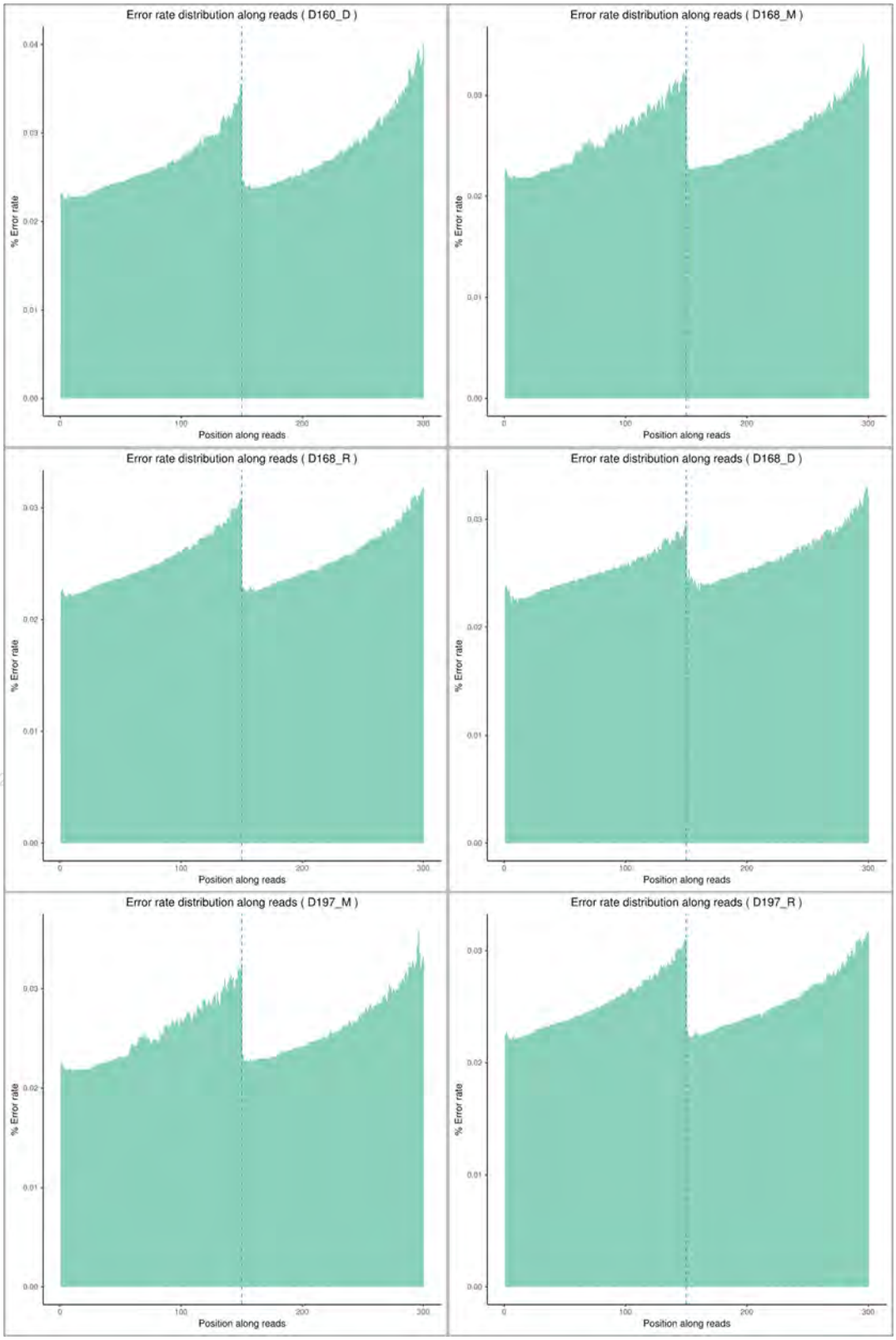
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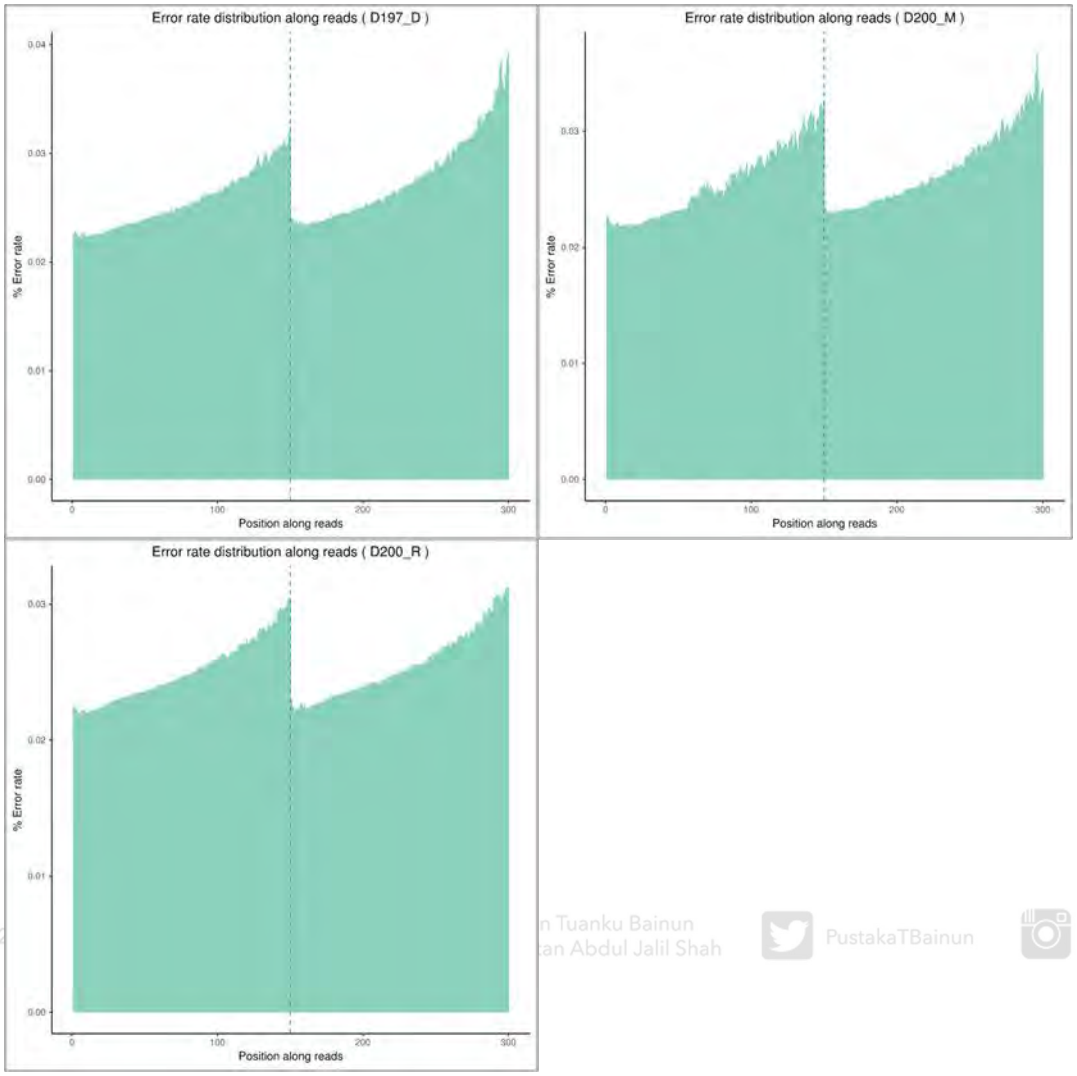
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4.3.2.1.2 GC Content Distribution

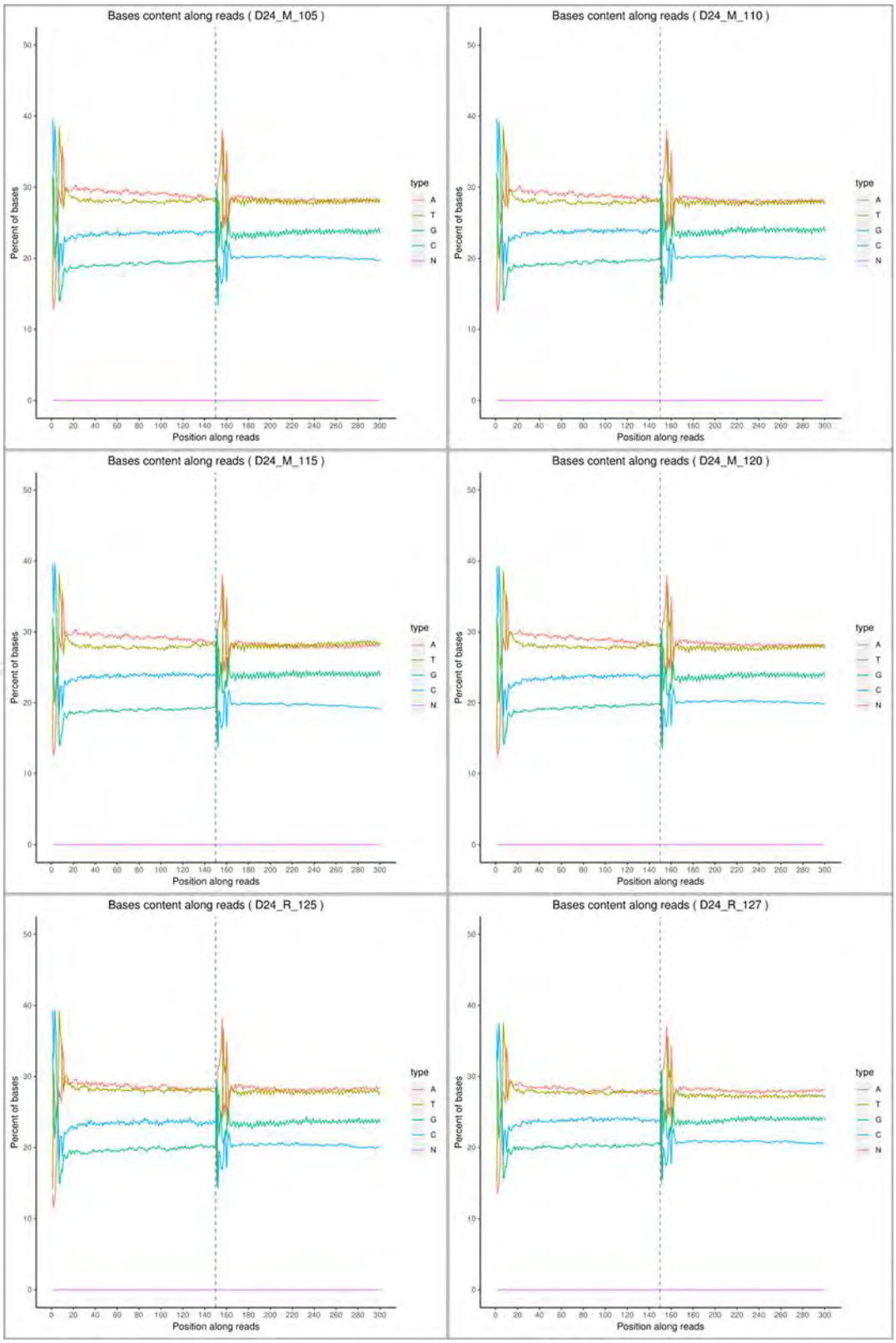
The GC content distribution for 21 samples is displayed in Figure 4.17. The x-axis shows each base position within a read, and the y-axis shows the percentage of each base, with each base represented by a different colour. The left side of the vertical dashed line is the GC-content of read 1, the right side is the GC-content of read 2. Based on the distribution pattern depicted in Table 4.5, all the sequenced samples have approximately equal G, C, A, and T content.

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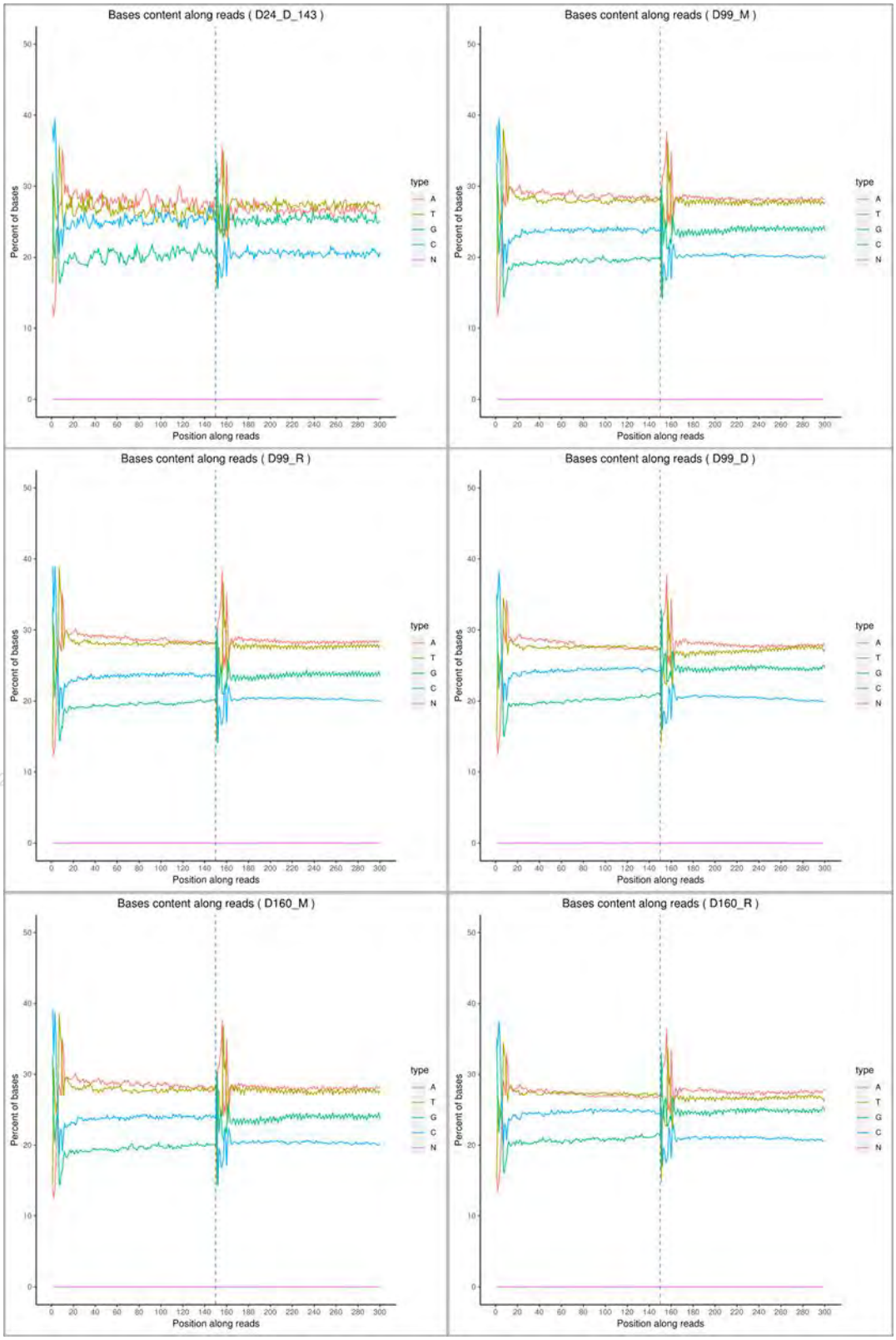
Figure 4.17

GC content distribution for 21 durian samples



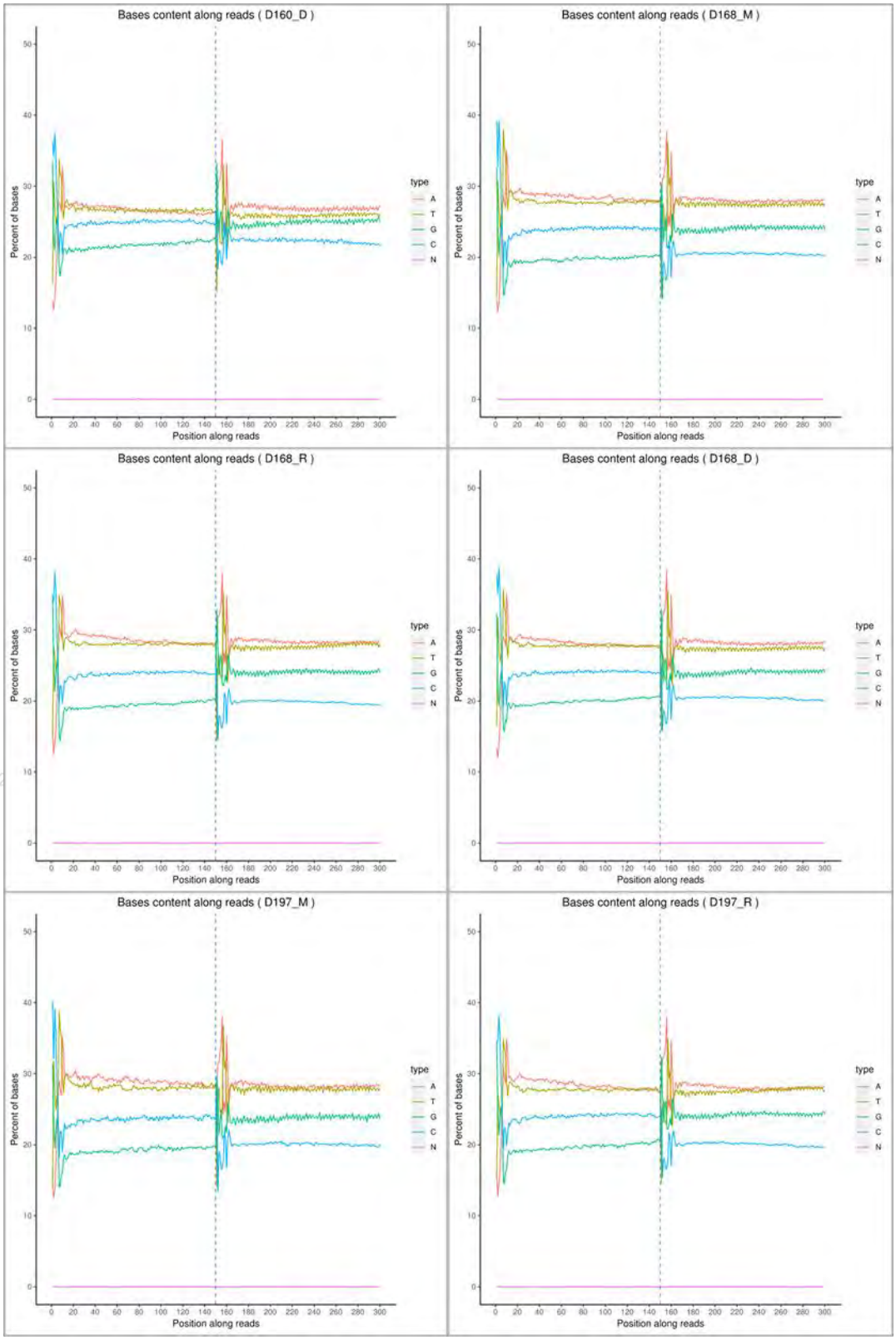
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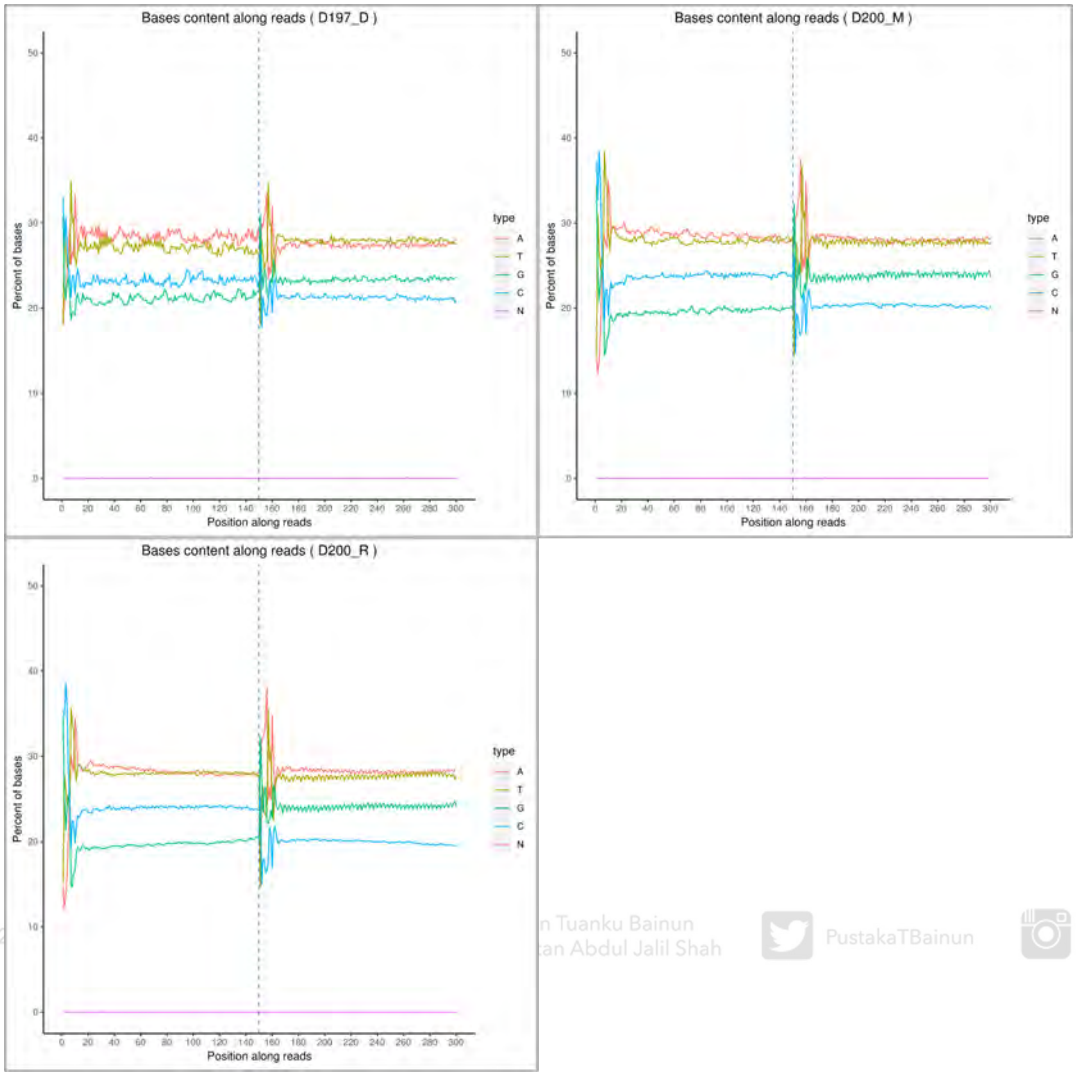
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4.3.2.1.3 Data Filtering

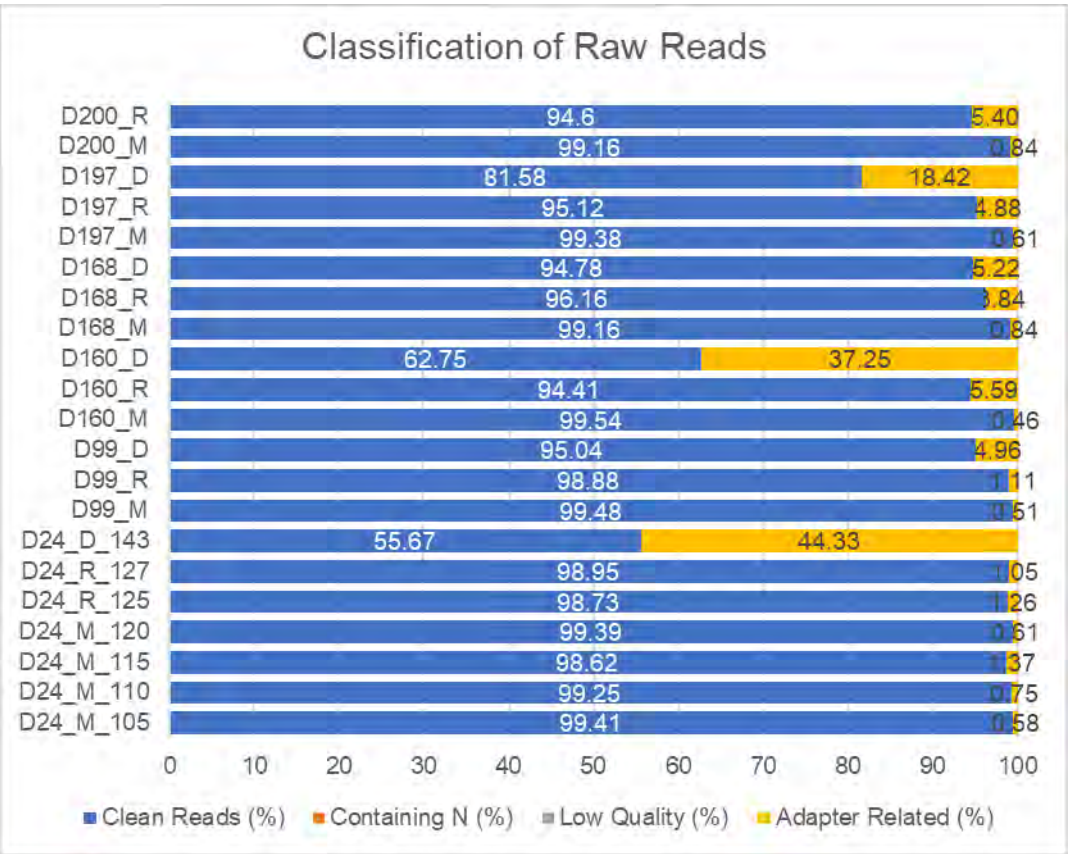
The raw reads filtering for each sample's sequencing data is displayed in Figure 4.18. The proportions of the different colours in the stack bar chart represent the proportion of different components. All filtered data recorded a 0.00% for both containing N and low-quality components. Only three samples from the dehiscence stage (D24_D_143, D160_D and D197_D), recorded clean reads of less than 90%, due to the presence of adapter-related reads (> 10%). Therefore, all filtered data are considered achieved an acceptable clean read.

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Figure 4.18

Sample Sequencing Data Filtering



Note,
Clean reads = clean reads / total raw reads;
Containing N (uncertain nucleotides) = reads with more than 10% N / total raw reads;
Low quality = reads of low quality / total raw reads;
Adapter related = reads containing adapter / total raw reads.

4.3.2.2 Mapping Statistics

The mapping percentages of samples reflect the similarity between each sample and the reference genome. The column chart displayed in Figure 4.19 provides an overview of the percentage of the samples that have been mapped to the reference genome. Majority of the samples have more than 90% reads that were successfully mapped to the *D. zibethinus* reference genome, which achieved the suggested minimum threshold of 70%, except for two libraries, namely D24_D_143 (44%) and

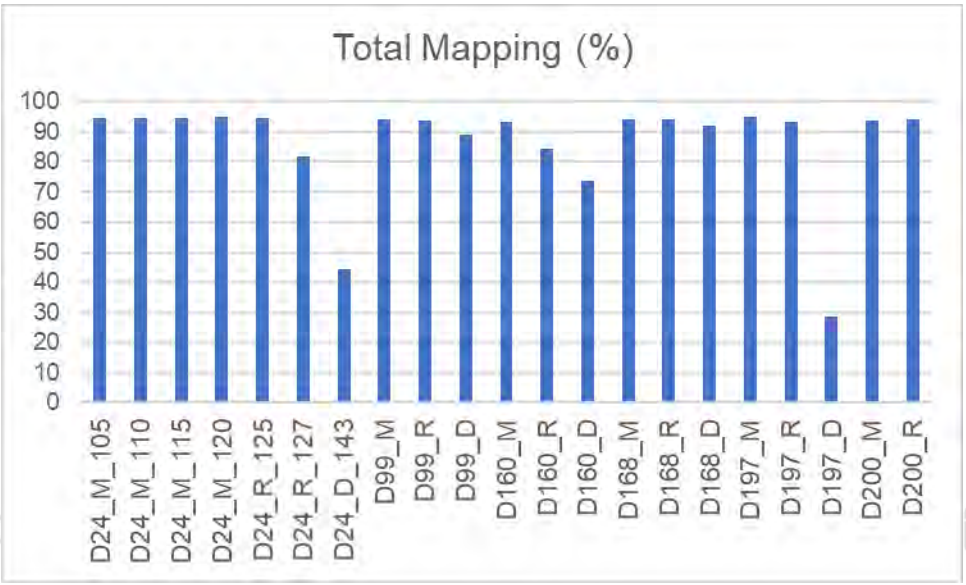
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D197_D (28%) (Figure 4.19). Appendix Table A2 provides a summary of the mapping statistics for sample and reference genome comparisons.

Figure 4.19

The total mapping statistics of 21 durian flesh samples to the D. zibethinus reference genome



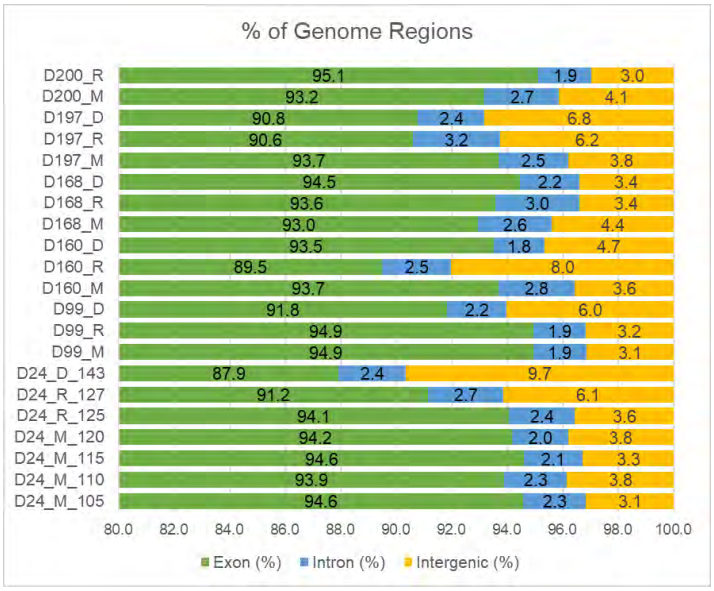
Mapped regions were classified as exons, introns, or intergenic regions. In general, the mapping classification principle applied is that if the reference genome is properly annotated, exon-mapped reads should be the highest abundance reads, while reads mapped to intergenic regions are mostly due to the reference genome's weak annotation (Manasseh, Sathuvalli, & Pappu, 2024). Figure 4.20 below displays the distribution of sequencing reads across all 21 samples within the genomic region. The most abundant type of reads exhibited in all libraries were exon-mapped reads (at least 88% and above), followed by intergenic-mapped reads (no more than 9.7%) and the least class of reads was intron-mapped reads (no more than 3.2%).

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Figure 4.20

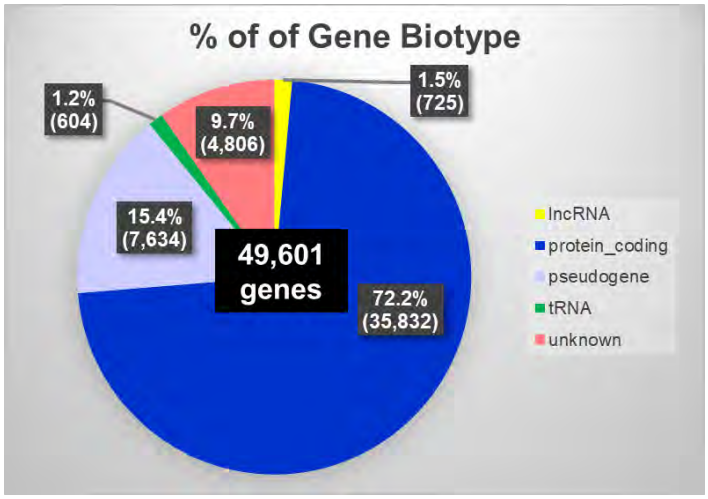
Distribution of sequencing reads in the genomic region



The mapped reads of each sample were then assembled by StringTie, which produced a total of 49,601 genes, which also included novel genes (Figure 4.21). The biggest gene biotype is protein coding genes, with a total of 35,832 genes (72.2%), followed by pseudogene (7,634 genes, 15.4%), unknown (4,806 gene, 9.7%), lncRNA (725 gene, 1.5%) and tRNA (604 gene, 1.2%).

Figure 4.21

Total number of genes discovered and the percentages of their gene biotype



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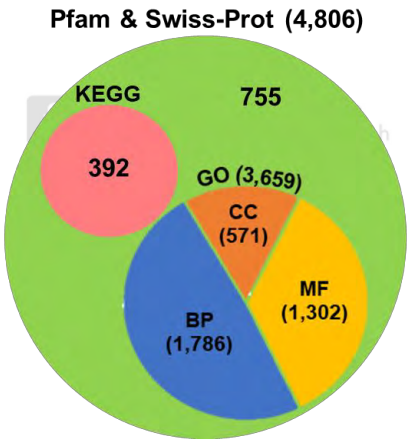
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4.3.2.3 Novel Gene Annotation

The novel gene annotation statistics are displayed in Figure 4.22. Overall, there were 4,806 novel genes identified by the annotations in the Pfam and Swiss-Prot databases. Out of 4,806 novel genes, 3,659 novel genes were annotated in the GO database, and 392 novel genes were annotated in the KEGG database. The GO classification divided the novel genes into three components: biological process (1,786 novel genes), cellular component (571 novel genes), and molecular function (1,302 novel genes).

Figure 4.22

Novel Gene Annotation



4.3.2.4 Gene Expression Quantification

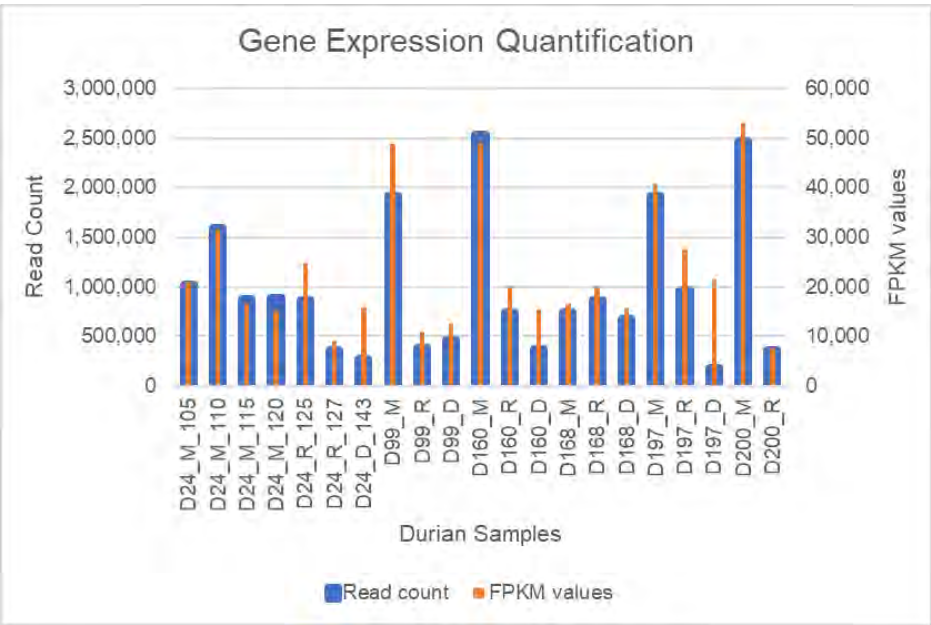
The gene expression quantification for all samples are displayed in Figure 4.23. The left y-axis represents the read count value ranges, while the right y-axis represents the FPKM value ranges (gene expression level) for the 49,601 genes discovered for each sample. The D160_M and D200_M samples stood out as the top two, showcasing the highest levels of gene expression.

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Figure 4.23

Gene expression quantification for 21 durian flesh samples



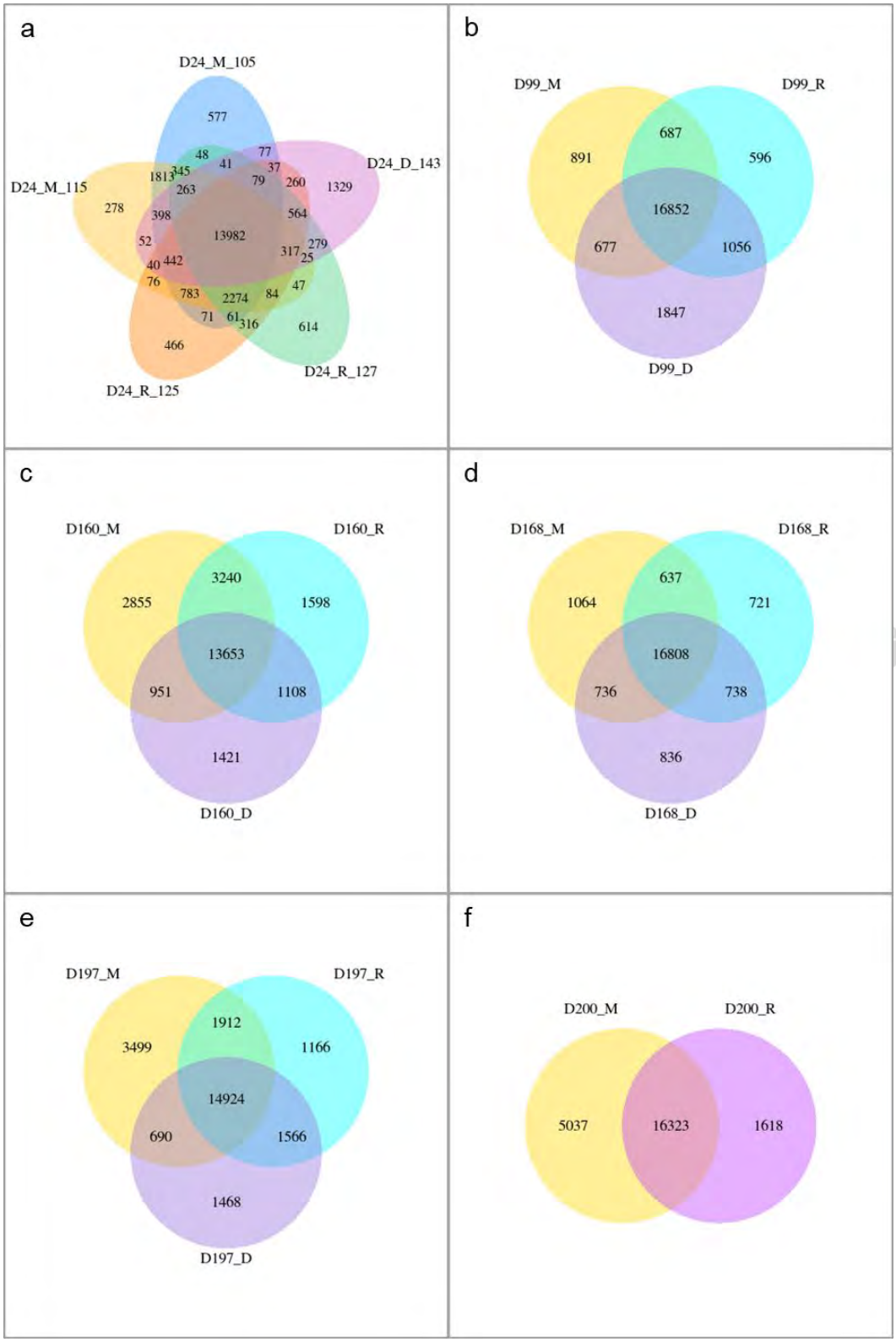
The co-expression Venn diagrams are displayed in Figure 4.24. The highest gene number that is co-expressed between the fruit developmental stages is in the D99 variety with a total of 16,852 genes (Figure 4.24b), and the lowest gene number that is co-expressed is in the D160 variety with 13,653 genes (Figure 4.24c).

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Figure 4.24

Co-expression Venn diagram. (a) D24. (b) D99. (c) D160. (d) D168. (e) D197. (f) D200



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4.3.2.4.1 Correlation Analysis

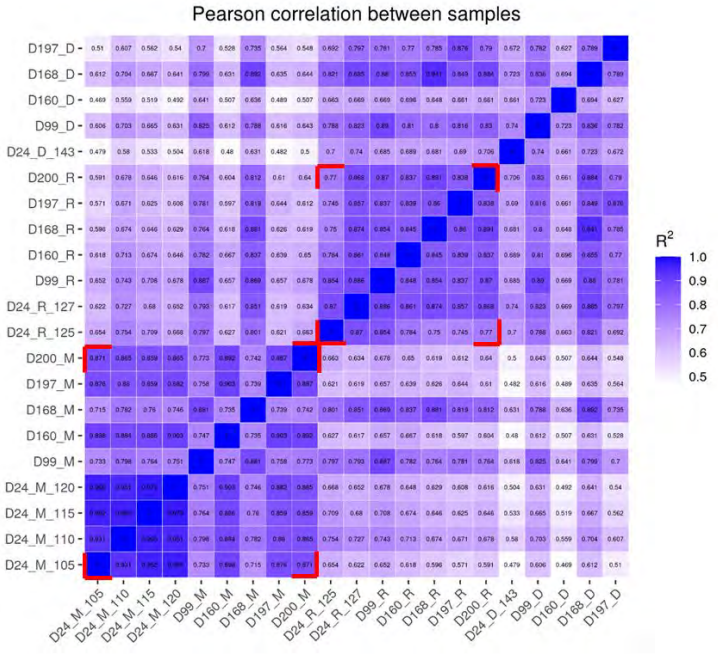
To view gene expression patterns throughout fruit developmental stages, all gene expression level (FPKM) were visualized in heat maps to give overall understanding about the gene expression level between samples (Figure 4.25 and Appendix Table A3). It can be intuitive to show sample expression level differences and repeat cases between groups. The degree of gene expression similarity between the samples increases as the correlation coefficient approaches to 1. The square of Pearson correlation coefficient (R^2) should be greater than 0.8 and 0.92 (under ideal experiment conditions). The results showed that the range of R^2 value obtained are from 0.469 to 0.973 (Appendix Table A3). Expression profiles for all transcripts are between two samples exhibited correlation value of R2 > 0.5 except for six groups (highlighted in yellow), belonging to different fruit developmental stage (Appendix Table A3). Samples belonging to the matured and riped stages (highlighted in red boxes in Figure 4.25) both exhibited a correlation value of R2 > 0.7. As for dehisced stage, sample groups display correlation value of R2 > 0.6 (Figure 4.25 and Appendix Table A3).

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Figure 4.25

Pearson correlation matrix



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4.3.2.5 Differential Gene Expression Analysis

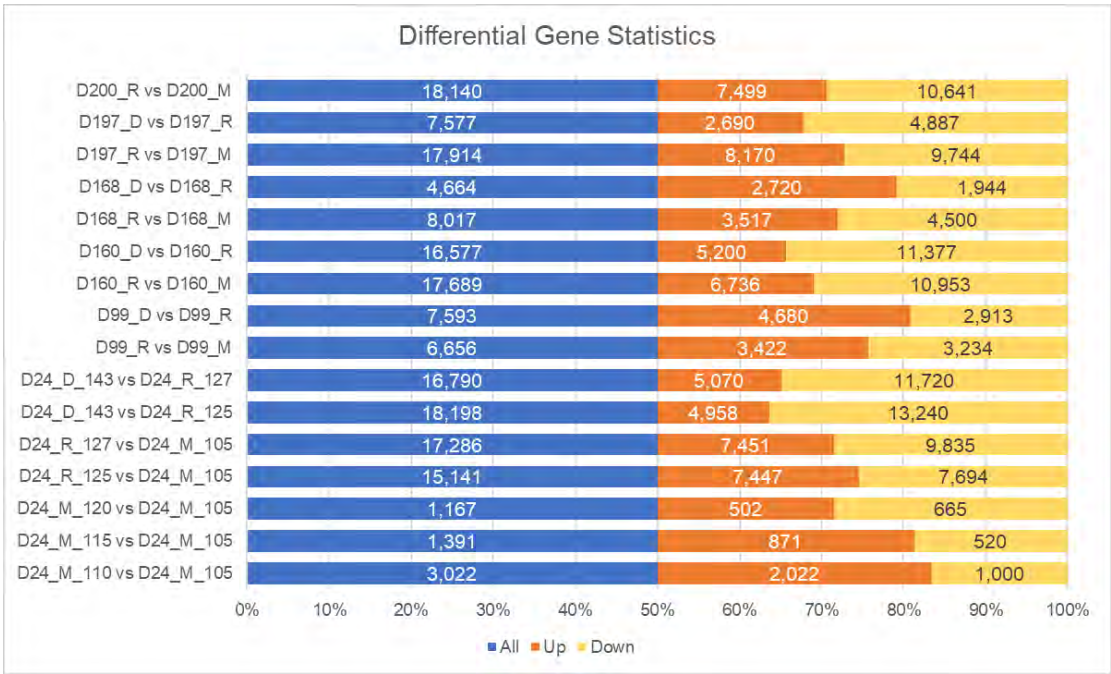
Differential and expression analysis identify number of genes that had been up-regulated and down-regulated in relative to control group (Figure 4.26). There were 1,167 to 18,198 genes that were differentially expressed, of which 502 to 8,170 genes were upregulated and 520 to 13,240 genes were downregulated. Out of 16 differential group samples, only five groups (D24_M_110 vs D24_M_105, D24_M_115 vs D24_M_105, D99_R vs D99_M, D99_D vs D99_R and D168_D vs D168_R) that have more up-regulated genes than down-regulated genes.

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Figure 4.26

Differential gene expression that showed up and down-regulated genes



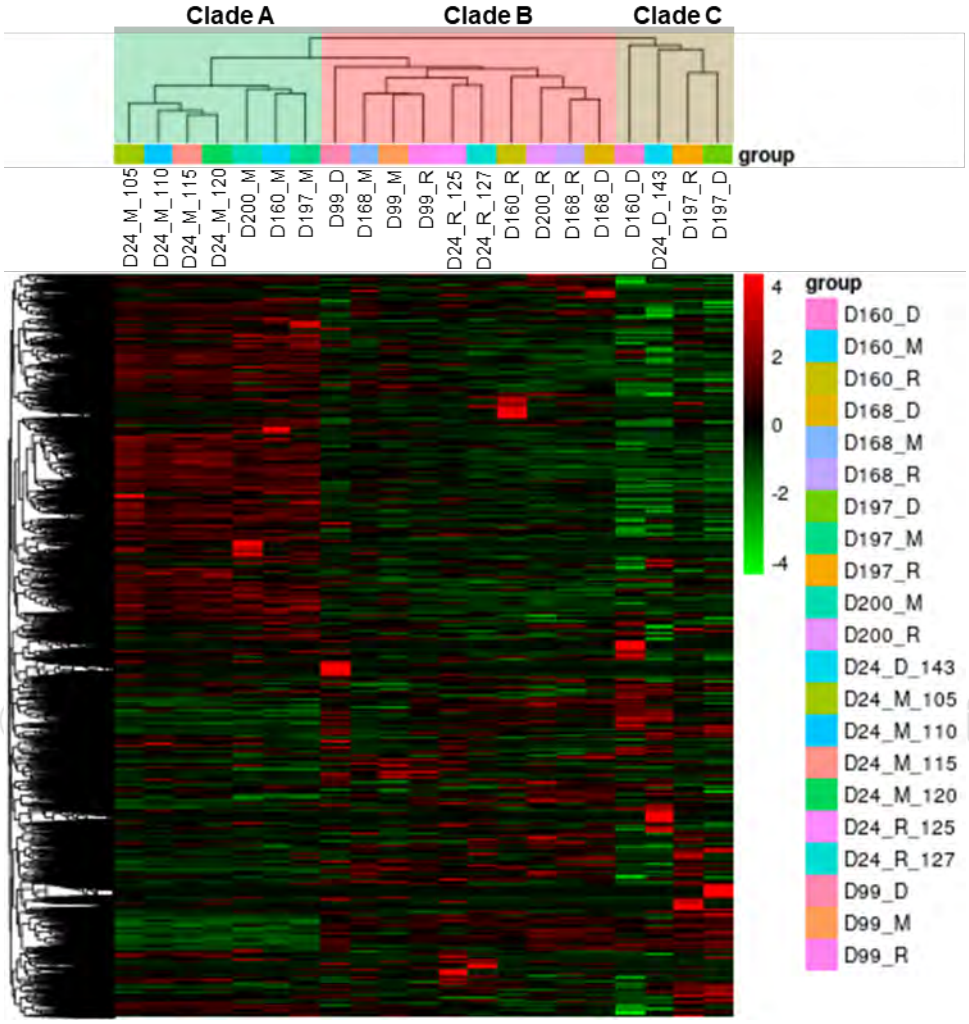
The heat map of differentially expressed genes are displayed in Figure 4.27. The heat map clustered the samples into three main groups, labelled as clade A, clade B and clade C. Clade A consists mainly of matured samples, while clade B has a mixture of matured, riped and dehisced samples, and clade C has a mixture of riped and dehisced samples. All the D99 and D168 samples are grouped together in clade B, while the D24, D160, and D200 samples have been scattered among the three clades according to stages (matured samples in clade A, riped samples in clade B, and dehisced samples in clade C), and the D197 samples have been separated at particular stages of fruit development (matured sample in clade A and riped and dehisced samples in clade C).

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Figure 4.27

Differential expression gene clustering heatmap among the differential group samples. The red colour represents genes with high expression levels, whereas the green colour represents genes with low expression levels



4.3.2.6 Functional Annotation

A total of 49,601 genes were annotated to two public databases: Gene Ontology (GO) enrichment analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Figure 4.28). Among them, 26,212 genes (including 3,659 novel genes) were annotated by GO database while 7,205 genes (including 392 novel genes) were annotated by KEGG database. GO enrichment analysis classified 26,212 genes into

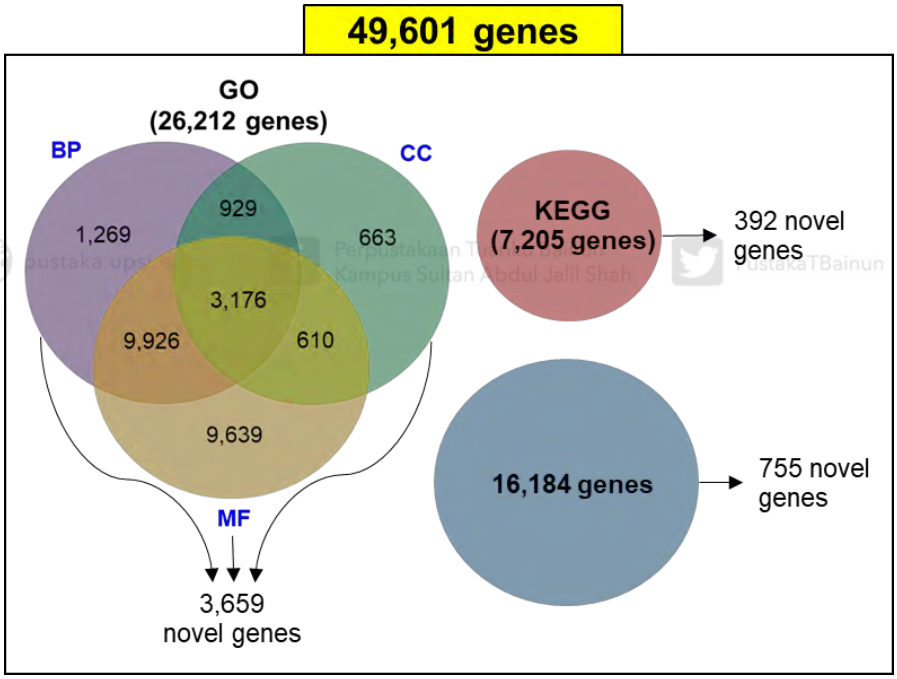
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three main gene ontology categories: cellular component (CC), biological process (BP), and molecular function (MF). Among the three categories, a total of 15,300 genes were annotated under biological process, 5,378 genes were annotated under cellular component and 23,351 genes were annotated under molecular function. However, a total of 16,184 (including 755 novel genes) cannot be annotated in either the GO or KEGG databases. This may be due to insufficient information and poorly annotated genomes (Primmer, Papakostas, Leder, Davis, & Ragan, 2013).

Figure 4.28

Functional annotation by GO and KEGG databases



4.3.2.6.1 GO Enrichment Analysis

In the results of GO enrichment analysis, the top 15 most common GO terms which were significant (padj values < 0.05) among the 21 samples are displayed in Figure 4.29. Cellular carbohydrate metabolic process, cell wall and hydrolase activity, acting

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on glycosyl bonds, are the top-hit GO terms. The highest number of differential sample groups representation that share the top GO terms were associated with cell wall, external encapsulating structure, extracellular region, cell periphery, hydrolase activity, acting on glycosyl bonds, hydrolase activity, hydrolyzing O-glycosyl compounds, apoplast and oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen (Appendix Table A4). Descriptions for each GO term is listed in Table 4.6.

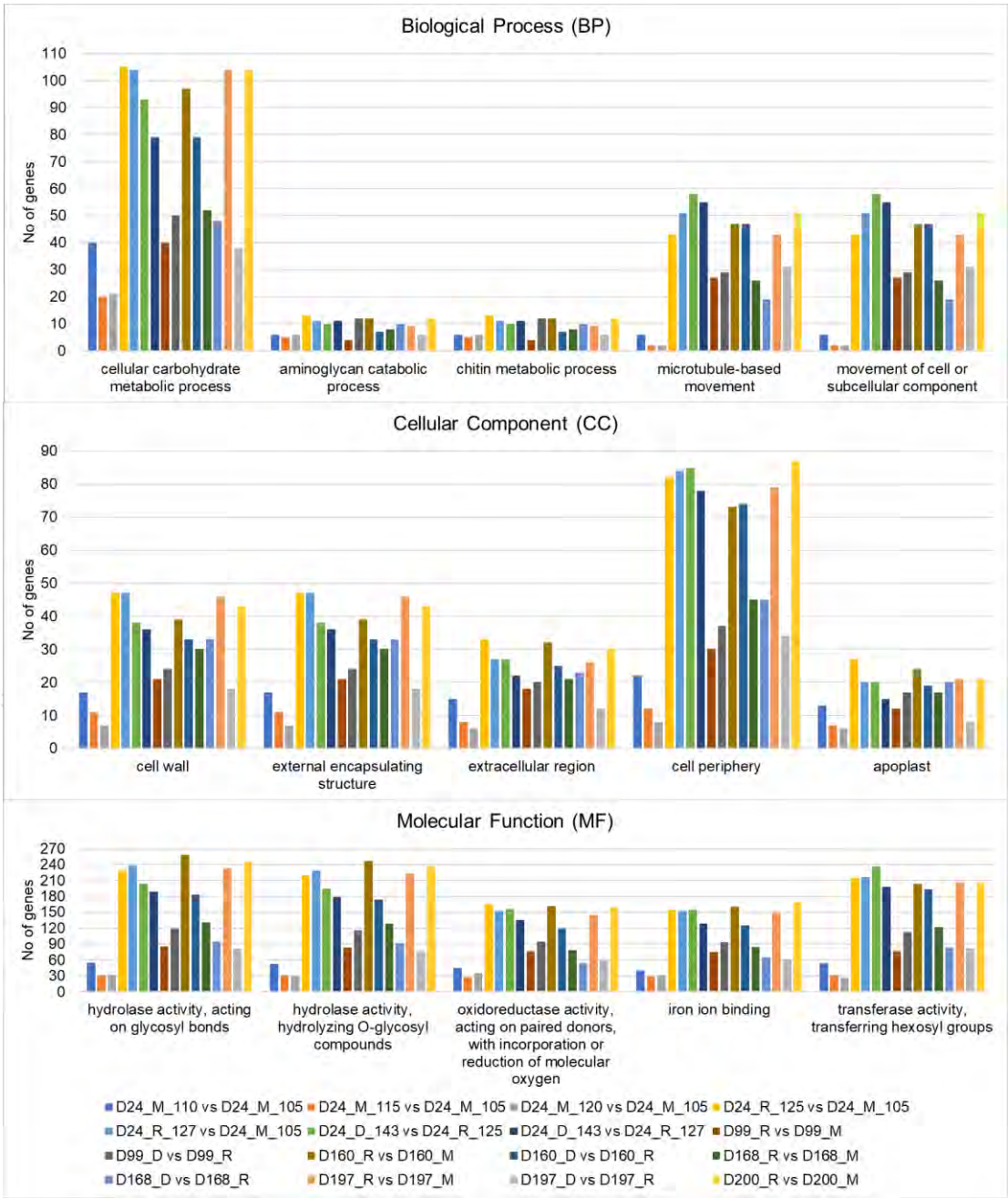
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Figure 4.29

Fifteen most common GO terms share biological process, cellular components, and molecular functions across all stages of fruit development



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Table 4.6

Description of GO terms

GO term	GO ID	Description
	Biological Process	
cellular carbohydrate metabolic process	GO:0044262	The chemical reactions and pathways involving carbohydrates, any of a group of organic compounds based of the general formula Cx(H2O)y, as carried out by individual cells.
aminoglycan catabolic process	GO:0006026	The chemical reactions and pathways resulting in the breakdown of aminoglycans, any polymer containing amino groups that consists of more than about 10 monosaccharide residues joined to each other by glycosidic linkages.
chitin metabolic process	GO:0006030	The chemical reactions and pathways involving chitin, a linear polysaccharide consisting of beta-(1->4)-linked N-acetyl-D-glucosamine residues.
microtubule-based movement	GO:0007018	A microtubule-based process that results in the movement of organelles, other microtubules, or other cellular components. Examples include motor-driven movement along microtubules and movement driven by polymerization or depolymerization of microtubules.
movement of cell or subcellular component	GO:0006928	The directed, self-propelled movement of a cell or subcellular component without the involvement of an external agent such as a transporter or a pore.
	Cellular Component	
cell wall	GO:0005618	The rigid or semi-rigid envelope lying outside the cell membrane of plant, fungal, most prokaryotic cells and some protozoan parasites, maintaining their shape and protecting them from osmotic lysis. In plants it is made of cellulose and, often, lignin; in fungi it is composed largely of polysaccharides; in bacteria it is composed of peptidoglycan; in protozoan parasites such as Giardia species, it's made of carbohydrates and proteins.
external encapsulating structure	GO:0030312	A structure that lies outside the plasma membrane and surrounds the entire cell or cells. This does not include the periplasmic space.

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GO term	GO ID	Description
	Cellular Component	
extracellular region	GO:0005576	The space external to the outermost structure of a cell. For cells without external protective or external encapsulating structures this refers to space outside of the plasma membrane. This term covers the host cell environment outside an intracellular parasite.
cell periphery	GO:0071944	The broad region around and including the plasma membrane of a cell, encompassing the cell cortex (inside the cell), the plasma membrane, and any external encapsulating structures.
apoplast	GO:0048046	The cell membranes and intracellular regions in a plant are connected through plasmodesmata, and plants may be described as having two major compartments: the living symplast and the non-living apoplast. The apoplast is external to the plasma membrane and includes cell walls, intercellular spaces, and the lumen of dead structures such as xylem vessels. Water and solutes pass freely through it.
	Molecular Function	
hydrolase activity, acting on glycosyl bonds	GO:0016798	Catalysis of the hydrolysis of any glycosyl bond.
hydrolase activity, hydrolyzing O-glycosyl compounds	GO:0004553	Catalysis of the hydrolysis of any O-glycosyl bond.
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	GO:0016705	Catalysis of an oxidation-reduction (redox) reaction in which hydrogen or electrons are transferred from each of two donors, and molecular oxygen is reduced or incorporated into a donor.
iron ion binding	GO:0005506	Binding to an iron (Fe) ion.
transferase activity, transferring hexosyl groups	GO:0016758	Catalysis of the transfer of a hexosyl group from one compound (donor) to another (acceptor).

Up and down regulated genes for each differential sample groups are displayed in Table 4.7a and 4.7b. The top 15 GO terms were listed into major categories of biological process (BP), cellular component (CC) and molecular

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function (MF) for up and down regulated expressed genes. In general, the number of up and down regulated expressed genes were higher in molecular function compared to biological process and cellular component. This indicated that the genes annotated in durian are more active in molecular function, in which the genes encode a gene product and carry out a molecular-level process or perform specific tasks, such as enzymes, receptors, or transporters (Thomas, 2017)

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Table 4.7a

Up and down regulated genes for 16 differential sample groups

No	Samples	D24_M_110 vs D24_M_105		D24_M_115 vs D24_M_105		D24_M_120 vs D24_M_105		D24_R_125 vs D24_M_105		D24_R_127 vs D24_M_105		D24_D_143 vs D24_R_125		D24_D_143 vs D24_R_127	
	GO terms	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
							Biological Process								
1	cellular carbohydrate metabolic process	25	15	8	12	7	14	65	40	42	62	19	74	32	47
2	aminoglycan catabolic process	2	4	4	1	2	4	11	2	6	5	2	8	9	2
3	chitin metabolic process	2	4	4	1	2	4	11	2	6	5	2	8	9	2
4	microtubule-based movement	2	4	1	1	0	2	18	25	20	31	5	53	6	49
5	movement of cell or subcellular component	2	4	1	1	0	2	18	25	20	31	5	53	6	49
							Cellular Component								
6	cell wall	13	4	7	4	3	4	33	14	21	26	5	33	12	24
7	external encapsulating structure	13	4	7	4	3	4	33	14	21	26	5	33	12	24
8	extracellular region	12	3	5	3	2	4	25	8	14	13	3	24	8	14
9	cell periphery	18	4	8	4	4	4	55	27	35	49	11	74	21	57
10	apoplast	10	3	4	3	2	4	21	6	9	11	2	18	7	8

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No	Samples	D24_M_110 vs D24_M_105		D24_M_115 vs D24_M_105		D24_M_120 vs D24_M_105		D24_R_125 vs D24_M_105		D24_R_127 vs D24_M_105		D24_D_143 vs D24_R_125		D24_D_143 vs D24_R_127	
	GO terms	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
							Molecular Function								
11	hydrolase activity, acting on glycosyl bonds	26	29	18	13	12	20	116	113	82	156	28	175	56	133
12	hydrolase activity, hydrolyzing O-glycosyl compounds	26	27	18	13	12	18	116	103	80	148	26	169	54	124
13	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	22	22	16	12	11	24	88	77	60	92	38	118	43	92
14	iron ion binding	18	22	15	14	12	19	81	74	62	91	36	119	41	88
15	transferase activity, transferring hexosyl groups	43	11	22	9	12	14	119	96	79	137	46	191	58	140

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Table 4.7b

Up and down regulated genes for 16 differential sample groups

No	Samples	D99_R vs D99_M		D99_D vs D99_R		D160_R vs D160_M		D160_D vs D160_R		D168_R vs D168_M		D168_D vs D168_R		D197_R vs D197_M		D197_D vs D197_R		D200_R vs D200_M	
	GO terms	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
	Biological Process																		
1	cellular carbohydrate metabolic process	22	18	19	31	40	57	27	52	16	36	36	12	44	60	16	22	33	71
2	aminoglycan catabolic process	0	4	10	2	7	5	6	1	0	8	10	0	4	5	5	1	5	7
3	chitin metabolic process	0	4	10	2	7	5	6	1	0	8	10	0	4	5	5	1	5	7
4	microtubule-based movement	22	5	4	25	14	33	11	36	15	11	3	16	14	29	7	24	24	27
5	movement of cell or subcellular component	22	5	4	25	14	33	11	36	15	11	3	16	14	29	7	24	24	27
	Cellular Component																		
6	cell wall	16	5	11	13	21	18	15	18	7	23	28	5	25	21	13	5	15	28
7	external encapsulating structure	16	5	11	13	21	18	15	18	7	23	28	5	25	21	13	5	15	28
8	extracellular region	14	4	8	12	22	10	11	44	3	18	19	4	20	6	7	5	11	19
9	cell periphery	22	8	17	20	33	40	25	49	11	34	39	6	34	45	14	20	33	54

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No	Samples	D99_R vs D99_M		D99_D vs D99_R		D160_R vs D160_M		D160_D vs D160_R		D168_R vs D168_M		D168_D vs D168_R		D197_R vs D197_M		D197_D vs D197_R		D200_R vs D200_M	
	GO terms	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
	Cellular Component																		
10	apoplast	12	0	6	11	17	7	9	10	3	14	17	3	16	5	6	2	9	12
	Molecular Function																		
11	hydrolase activity, acting on glycosyl bonds	33	52	57	62	93	165	71	112	24	107	81	13	90	143	29	52	73	173
12	hydrolase activity, hydrolyzing O-glycosyl compounds	32	51	57	59	91	156	70	104	23	106	80	12	87	136	25	51	70	166
13	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	22	54	58	37	57	105	52	68	21	58	42	12	57	88	23	36	47	112
14	iron ion binding	22	53	56	37	58	102	50	75	24	60	51	14	59	90	26	36	48	120
15	transferase activity, transferring hexosyl groups	37	39	58	55	75	128	59	134	29	93	62	21	79	127	34	48	58	148

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Table 4.8 shows the number of differential groups for top-hit genes for the 15 most common GO terms. There are at least thirteen differential sample groups that share the top-hit genes. The number of differential groups for up-regulated top-hit genes was greater than down-regulated top-hit genes for majority of the GO terms. This indicated that gene expression in durian has increased in response to fruit development, which intensifies the synthesis of the corresponding proteins.

Table 4.8

Number of differential groups for top-hit up and down-regulated genes for each of the 15 most common GO terms

No.	GO terms	NCBI-GeneID	Enzyme	Total Up	Total Down	No. All
1	cellular carbohydrate metabolic process	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14
2	aminoglycan catabolic process	LOC111308707	endochitinase 1-like	3	11	14
3	chitin metabolic process	LOC111292022	basic endochitinase-like	12	2	14
4		LOC111308707	endochitinase 1-like	3	11	14
5	microtubule-based movement	LOC111275625	kinesin-like protein KIN-12F	2	11	13
6	movement of cell or subcellular component	LOC111275625	kinesin-like protein KIN-12F	2	11	13
7	cell wall	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14
8	external encapsulating structure	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14
9	extracellular region	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14
10	cell periphery	LOC111285714	xyloglucan endotransglucosylase/hydrolase	12	2	14

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No.	GO terms	NCBI-GeneID	Enzyme	Total Up	Total Down	No. All
			protein 22-like			
11	apoplast	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14
12	hydrolase activity, acting on glycosyl bonds	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14
13		LOC111292022	basic endochitinase-like	12	2	14
14		LOC111308707	endochitinase 1-like	3	11	14
15	hydrolase activity, hydrolyzing O-glycosyl	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14
16	compounds	LOC111292022	basic endochitinase-like	12	2	14
17		LOC111308707	endochitinase 1-like	3	11	14
18	oxidoreductase activity,	LOC111275604	premnaspirodiene oxygenase-like	9	4	13
19	acting on paired donors,	LOC111276069	premnaspirodiene oxygenase-like	3	10	13
20	with incorporation or reduction of	LOC111279674	squalene monooxygenase-like	9	4	13
21	molecular oxygen	LOC111308820	cytochrome P450 84A1-like	9	4	13
22		LOC111314425	cytochrome P450 82G1-like	3	10	13
23		LOC111318668	cytochrome P450 710A1-like	12	1	13
24	iron ion binding	LOC111312087	ferritin-3, chloroplastic	11	4	15
25	transferase activity, transferring hexosyl groups	LOC111285714	xyloglucan endotransglucosylase/hydrolase protein 22-like	12	2	14

Note,
No. All = Total number of sample groups that being differentially expressed;
Up = Total number of samples group that being up-regulated;
Down = Total number of samples group that being down-regulated.

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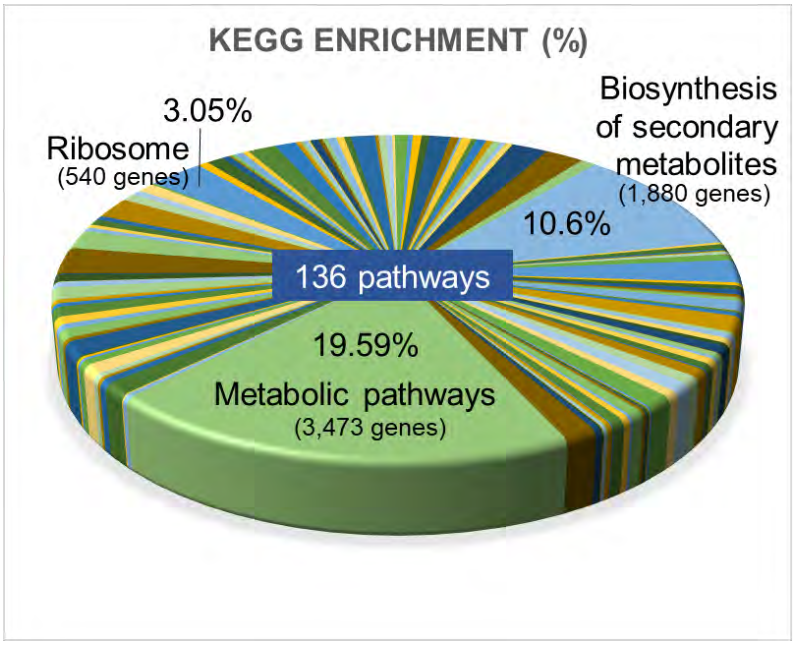
4.3.2.6.2 KEGG Enrichment Analysis

KEGG enrichment analysis annotated 7,205 genes into 136 KEGG pathways (Figure 4.30). Top three major pathways annotation with highest genes representation were metabolic pathways (19.59%) followed by biosynthesis of secondary metabolites (10.6%) and ribosome (3.05%). In the results of the KEGG enrichment analysis, the most significant and common five terms are summarized in interactive pathways (Figure 4.31). The top five most common KEGG pathways which were significant (padj values < 0.05) among the differential sample groups are associated with carotenoid biosynthesis, fatty acid biosynthesis, starch and sucrose metabolism, phenylpropanoid biosynthesis and galactose metabolism (Figure 4.32). The interactions of multiple genes may be involved in certain biological functions. Detailed pathway maps depicted in Figure 4.31 are displayed in Appendix Figure B1 and Appendix C-G.

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Figure 4.30

Distribution of genes percentage annotated in the KEGG enrichment pathways

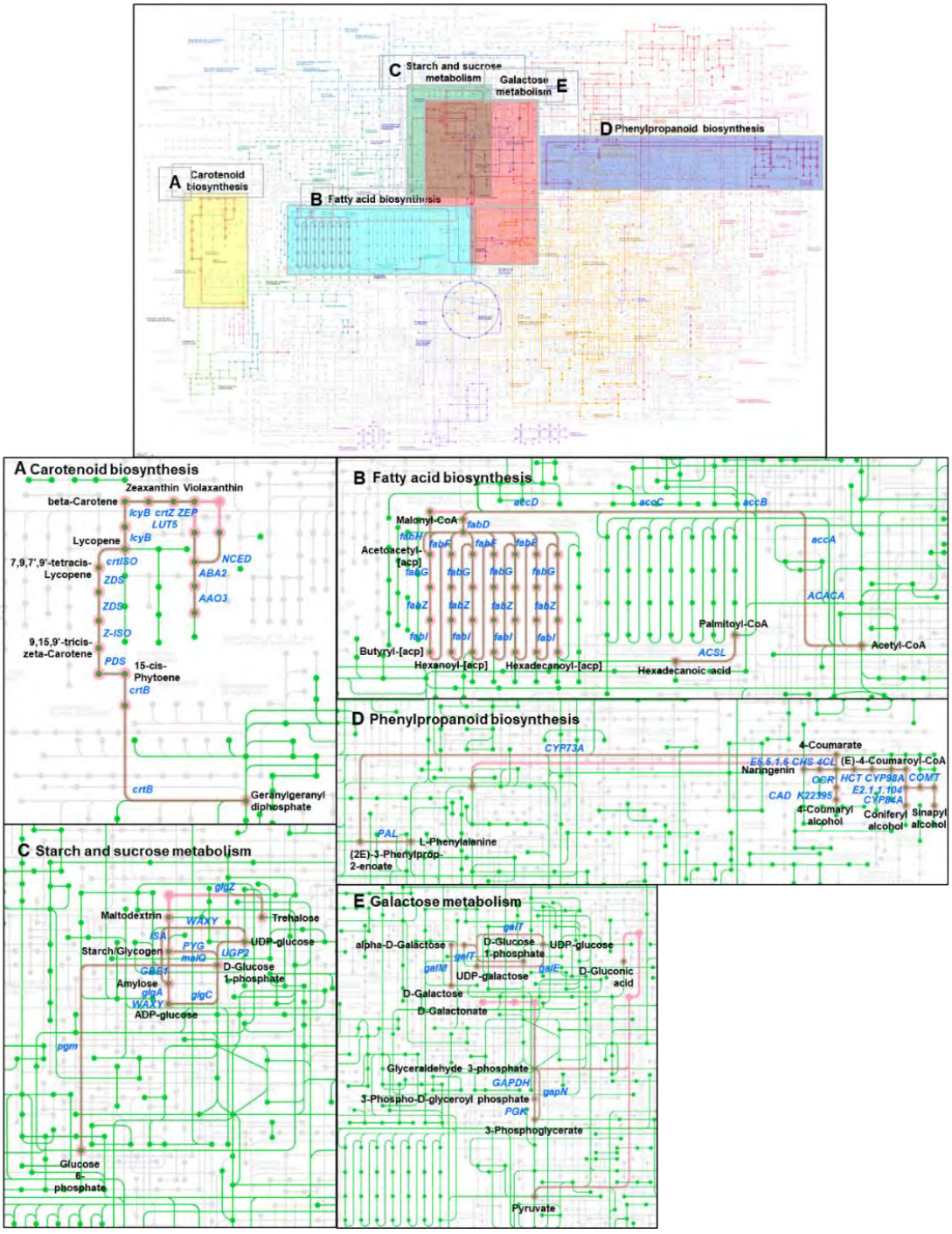


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Figure 4.31

Interactive pathways analysis during durian fruit developmental stages. (A) Carotenoid biosynthesis. (B) Fatty acid biosynthesis. (C) Starch and sucrose metabolism. (D) Phenylpropanoid biosynthesis. (E) Galactose metabolism

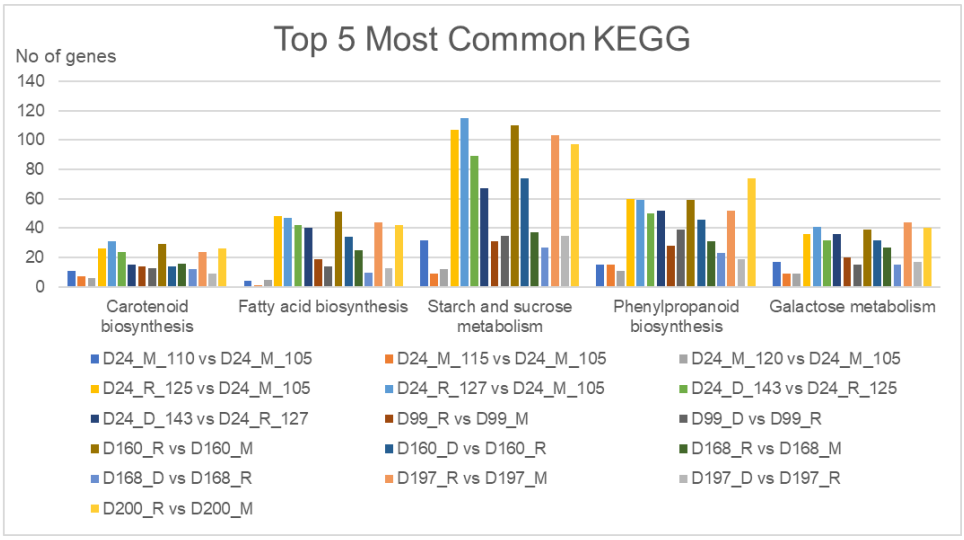


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Figure 4.32

The top five most common KEGG terms share biological pathways across all stages of fruit development



The highest number of differential sample groups representation that share the top KEGG pathways is carotenoid biosynthesis (12), fatty acid biosynthesis (11), starch and sucrose metabolism (11), phenylpropanoid biosynthesis (9) and galactose metabolism (9) (Table 4.9). These KEGG pathways were on the list of the top 20 KEGG terms for each differential sample groups.

Up- and down regulated genes for each differential sample group of the top five most common KEGG pathways are displayed in Table 4.10. To narrow down the KEGG enrichment analysis, the top-hit genes for each of the five most common KEGG pathways are listed in Table 4.11. There are at least 11 differential sample groups that share the top-hit genes. The number of top-hit genes listed for the respective KEGG pathways listed is 4-7 genes.

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Table 4.9

The top five most common KEGG terms share biological pathways across all stages of fruit development. Gene ratio of differentially expressed genes were displayed in the table

No.	Differential Sample Group	Carotenoid biosynthesis	Fatty acid biosynthesis	Starch and sucrose metabolism	Phenyl-propanoid biosynthesis	Galactose metabolism
1	D24_M_110 vs D24_M_105	11/467	4/467	32/467	15/467	17/467
2	D24_M_115 vs D24_M_105	7/246	1/246	9/246	15/246	9/246
3	D24_M_120 vs D24_M_105	6/206	5/206	12/206	11/206	9/206
4	D24_R_125 vs D24_M_105	26/2568	48/2568	107/2568	60/2568	36/2568
5	D24_R_127 vs D24_M_105	31/3017	47/3017	115/3017	59/3017	41/3017
6	D24_D_143 vs D24_R_125	24/2999	42/2999	89/2999	50/2999	32/2999
7	D24_D_143 vs D24_R_127	15/2831	40/2831	67/2831	52/2831	36/2831
8	D99_R vs D99_M	14/1191	19/1191	31/1191	28/1191	20/1191
9	D99_D vs D99_R	13/1315	14/1315	35/1315	39/1315	15/1315
10	D160_R vs D160_M	29/2972	51/2972	110/2972	59/2972	39/2972

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No.	Differential Sample Group	Carotenoid biosynthesis	Fatty acid biosynthesis	Starch and sucrose metabolism	Phenyl-propanoid biosynthesis	Galactose metabolism
11	D160_D vs D160_R	14/2713	34/2713	74/2713	46/2713	32/2713
12	D168_R vs D168_M	16/1361	25/1361	37/1361	31/1361	27/1361
13	D168_D vs D168_R	12/667	10/667	27/667	23/667	15/667
14	D197_R vs D197_M	24/2994	44/2994	103/2994	52/2994	44/2994
15	D197_D vs D197_R	9/1143	13/1143	35/1143	19/1143	17/1143
16	D200_R vs D200_M	26/3120	42/3120	97/3120	74/3120	40/3120
	Count	12	11	11	9	9

Note,
Gene ratio = Ratio between the number of differentially expressed genes in each pathway and all differentially expressed genes that can be found in KEGG database;
Colour highlights = Green (significant and in the list of top 20 KEGG terms), Orange (not significant but in the list of top 20 KEGG terms), no highlight (not significant and/or not in the list of top 20 KEGG terms);
Count = Number of KEGG terms that highlighted by green and orange colour cells.

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Table 4.10

Up and down regulated genes for 16 differential sample groups

No.	Differential Sample Group	Gene Regulation	Carotenoid biosynthesis	Fatty acid biosynthesis	Starch and sucrose metabolism	Phenyl-propanoid biosynthesis	Galactose metabolism
1	D24_M_110 vs D24_M_105	Up	7	3	20	13	15
		Down	4	1	12	2	2
2	D24_M_115 vs D24_M_105	Up	3	0	5	12	8
		Down	4	1	4	3	1
3	D24_M_120 vs D24_M_105	Up	2	1	5	10	8
		Down	4	4	7	1	1
4	D24_R_125 vs D24_M_105	Up	10	7	40	34	24
		Down	16	41	67	26	12
5	D24_R_127 vs D24_M_105	Up	10	4	43	25	23
		Down	21	43	72	34	18
6	D24_D_143 vs D24_R_125	Up	3	5	21	10	10
		Down	21	37	68	40	22
7	D24_D_143 vs D24_R_127	Up	3	8	14	17	13
		Down	12	32	53	35	23
8	D99_R vs D99_M	Up	2	2	8	4	10
		Down	12	17	23	24	10
9	D99_D vs D99_R	Up	5	2	17	31	8
		Down	8	12	18	8	7
10	D160_R vs D160_M	Up	9	6	32	22	19
		Down	20	45	78	37	20
11	D160_D vs D160_R	Up	2	8	22	22	10
		Down	12	26	52	24	22
12	D168_R vs D168_M	Up	2	1	13	8	10
		Down	14	24	24	23	17
13	D168_D vs D168_R	Up	10	7	12	15	9
		Down	2	3	15	8	6
14	D197_R vs D197_M	Up	8	3	39	21	21
		Down	16	41	64	31	23
15	D197_D vs D197_R	Up	1	1	1	10	4
		Down	8	12	34	9	13
16	D200_R vs D200_M	Up	6	1	29	22	20
		Down	20	41	68	52	20

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Table 4.11

Top-hit up and down-regulated genes for each of the five most common KEGG pathways

No.	KEGG Pathways	NCBI-GeneID	Gene Name	Enzyme	Total Up	Total Down	No .Al l
1	Carotenoid biosynthesis	LOC111280882	NCED5	probable 9-cis-epoxycarotenoid dioxygenase	9	3	12
2		LOC111296235	CYP707A 1	abscisic acid 8'-hydroxylase 4-like isoform X1	6	6	12
3		LOC111276007	NCED3	9-cis-epoxycarotenoid dioxygenase	8	3	11
4		LOC111280188	NCED6	9-cis-epoxycarotenoid dioxygenase	4	7	11
5		LOC111290894	CYP707A 1	abscisic acid 8'-hydroxylase 1-like	1	10	11
6	Fatty acid biosynthesis	LOC111283778	FATA	oleoyl-acyl carrier protein thioesterase 1	3	8	11
7		LOC111288503	fabG	3-oxoacyl-[acyl-carrier-protein] reductase	0	11	11
8		LOC111293698	fabG	NADPH-dependent aldehyde reductase-like protein	8	3	11
9		LOC111315633	ACSL	long chain acyl-CoA synthetase 9	0	11	11
10		LOC111317969	fabG	3-oxoacyl-[acyl-carrier-protein] reductase 4-like isoform X1	1	10	11
11	Starch and sucrose metabolism	LOC111287553	GN1_2_3	glucan endo-1	3	10	13
12		LOC111277804	bglX	beta-glucosidase BoGH3B-like	0	12	12
13		LOC111279832	E2.4.1.14	probable sucrose-phosphate synthase 3	6	6	12
14		LOC111281760	TPS	alpha,alpha-trehalose-phosphate synthase [UDP-forming] 6-like	0	12	12
15	Phenylpropanoid biosynthesis	LOC111292073	E1.11.1.7	peroxidase 51-like	9	5	14
16		LOC111308820	CYP84A	cytochrome P450 84A1-like	9	4	13
17		LOC111317224	CYP84A	cytochrome P450 84A1-like	2	10	12

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No.	KEGG Pathways	NCBI-GeneID	Gene Name	Enzyme	Total Up	Total Down	No. All
18		LOC111285045	CYP73A	trans-cinnamate 4-monooxygenase	3	8	11
19		LOC111296231	PAL	phenylalanine ammonia-lyase	7	4	11
20	Phenylpropanoid biosynthesis	LOC111299575	E1.11.1.7	peroxidase 31-like	5	6	11
21		LOC111317211	E1.11.1.7	peroxidase 72-like isoform X1	4	7	11
22	Galactose metabolism	LOC111292111	galM	aldose 1-epimerase-like	4	10	14
23		LOC111292856	E2.4.1.82	probable galactinol--sucrose galactosyltransferase 5	7	6	13
24		LOC111293659	E2.4.1.82	probable galactinol--sucrose galactosyltransferase 2	12	1	13
25		LOC111313380	GOLS	galactinol synthase 1-like	12	0	12
26		LOC111316628	E2.4.1.82	probable galactinol--sucrose galactosyltransferase 5	8	4	12

Note,
No. All = Total number of sample groups that being differentially expressed;
Up = Total number of samples group that being up-regulated;
Down = Total number of samples group that being down-regulated.

4.4 Summary

In conclusion, both quantitative (QN) and qualitative trait (QL) analysis of flowers and fruits are able to show differences between the six durian varieties under study. Analyses of variance (ANOVA) for QN traits results showed that only pedicel length (Trait 4) showed significant difference ($P<0.05$) between D168 variety with the other five varieties. For QL traits, in general were quite varied, each variety has its' own unique combinations and descriptions.

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Transcriptomic analysis results disclosed by KEGG in this study showed that the top three major pathways annotation with highest genes representation were metabolic pathways followed by biosynthesis of secondary metabolites and ribosome, suggesting these pathways activities were prominent during fruit development which begins with matured, riped and dehisced. Moreover, carotenoid biosynthesis, fatty acid biosynthesis, starch and sucrose metabolism, phenylpropanoid biosynthesis and galactose metabolism play important roles during fruit development, which explains the physiological changes in durian fruit and their associations with taste, texture and aroma of fruit arils. The combination of molecular data with morphology information can improve understanding of durian fruit developmental stages.

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CHAPTER 5

DISCUSSION

5.1 Introduction

The discussion presented here was divided into two sections based on objectives. Section 5.2 represent the discussion related to findings of characterization of six commercial durian varieties using morphological observation, while section 5.3 discussed findings regarding transcriptomic analysis of six commercial durian varieties based on three fruit developmental stages (matured, riped, and dehisced).

5.2 Characterization of Six Commercial Durian Varieties in Malaysia Using Morphological Observation

Morphological observations reported in this study could serve as an important resource to differentiate between six Malaysian durian varieties. The flowers and fruits of six durian varieties were evaluated for eight quantitative (QN), two qualitative (QL) and 18 pseudo-qualitative (PQ) traits. Obviously, some morphological durian traits were easy to distinguish and very outstanding in providing valuable reference to durian growers and consumers for variety differentiation. However, some of the traits

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are difficult to describe, a bit confusing and need specialized equipment for evaluation.

It was noted that there are five quantitative traits that were most outstanding in the D200 variety (Figure 4.1). They are pedicel length, fruit weight, weight of peel, weight of seeds and number of seeds per fruit. In addition, D200 fruit tends to have average length and wider fruit, making the fruit size huge. Huge fruit size, which contributes to heavier weight, is a highly desirable trait among durian growers because the price is determined by weight. The D200 fruit sampled in this study, which exceeds the average marketable fruit weight (1.5kg to 3.5kg) (Paull & Ketsa, 2014), is attractive in terms of profit to durian growers. Contrarily, D99 riped fruit has the lowest fruit weight and the shortest fruit length and width. Moreover, this variety has the smallest fruit size comparatively, making it easily distinguishable among the varieties studied. Thus, the fruit size could be an indicator to differentiate between the D200 and D99 varieties, however, future research should include more samples to enhanced validity.

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Another durian variety, D160, or better known as Musang Queen has two outstanding quantitative traits (Figure 4.1). They have the greatest number of seeds per fruit ($M = 13.00, SD = 7.55$) and number of flattened seeds per fruit ($M = 6.67, SD = 6.35$). Many consumers were devoted to this trait, as flattened seeds could offer more fruit meat. However, the standard deviations are quite high, which indicates greater variability between the D160 fruit samples. Therefore, a larger sample size should be included to fully understand if these traits are applicable to differentiate the varieties.

In Thailand, pedicel length is customarily used by orchard owners to identify the Kan Yao durian variety from others (Songnuan et al., 2019). Kan Yao, which

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means long stalk in the Thai language, as the fruit stalk for this variety could reach up to 10-14 cm in length (Ketsa et al., 2020). In this study, statistical analysis showed that pedicel length was significant different ($P<0.05$), between D168 variety with the other five varieties. This trait was found to be the longest in D168 variety (M = 6.83 cm, SD = 1.22 cm) comparatively to the other five varieties under study. According to field observation by local farmers, pedicel length could also be influenced by the age of the trees within the same variety, but no scientific record was made and evaluate. Hence, our study suggests that pedicle length could be one of the traits that is useful to differentiate the D168 variety from the other five durian varieties studied.

In general, qualitative traits observations among the six varieties were diverse and varied according to variety. Each variety has its own unique trait characters. However, qualitative traits within the same variety could also be different, resulting difficulties in identification via fruit morphology. Due to this, a few trait characters were meticulously studied, described, and recorded based on common features. For instance, D197 does not have spines at both around the base of the pedicel and at stylar end. These common features may be useful as a key to identifying and distinguishing the six durian varieties apart from each other.

Interestingly, our observation showed that flowers in the D99 and D200 varieties displayed common features that were quite different from the D24, D160, D168 and D197 varieties. Both D99 and D200 have flower bud that is circular (Trait 1) with a rounded bud apex (Trait 2). Moreover, only D99 flower's stigma is located below the anther (Trait 3) (Figure 4.4b). The uniqueness of D99 variety flower traits could be attributed to differences in country of origin, as D99 is native to Thailand while the other varieties are of Malaysian origin. Durian flower could be a reliable trait for variety differentiation because it is genetically inherited and less influenced by the environment (Idris, 1996). However, they are rarely used in folk groupings for

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identification purpose due to their limited seasonal availability (Songnuan et al., 2019).

Based on the heat map of 17 QL and PQ traits (fruit, flesh, and seed traits), all the traits are unique and distinctive among the six durian varieties except for the presence of spines (Trait 14) (Figure 4.14). The evaluation of sensory traits can also be bias as taste is highly depending on the sentiment of the observer (Songnuan et al., 2019). The subjectivity in the analysis could become an argument among the observers, especially on the qualitative traits and different management practises could contribute to morphological variation (Weising, Nybom, Wolff, & Kahl, 2005). Hence, more precise analytic methods such as Fuzzy Relevance Vector Machine (FRVM) (VijayaLakshmi & Mohan, 2016), gas chromatography-mass spectrometry (GC-MS) (Li et al., 2012), high-performance liquid chromatography (HPLC) (Huang et al., 2021), electronic nose (e-nose) and electronic tongue (e-tongue) (Xiao, Niu, & Niu, 2022) should be utilized in future studies for precision and reliability.

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The dendrogram depicted in Figure 4.15 showed that varieties were separated into two main clades: clade A and B. Since all the D99 and D168 variety samples were clustered together in Clade B and Clade A, respectively, this indicates that there is not much variation in the D99 and D168 varieties as compared to others. This may indicate that the traits do not vary much over the period of the fruit's development. The highest variation detected is in the D160 variety, as all D160 samples have been scattered between both clades. The high variation in these varieties could be attributed to the fact that observations are difficult to determine and hardly correspond well with the descriptors. For instance, given a large spectrum of colours, determining the colour traits may be difficult. Songnuan et al. (2019) also recommended narrowing down the colour options because clustering would be challenging with so many options.

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Then, more mature fruit samples were clustered in Clade B, whereas more riped and dehisced fruit were clustered in Clade A. Clade A and Clade B were separated by genetic distance with a value of 0.67 (Figure 4.15). As roughly, this indicates that the variation from Clade A to Clade B is about 67%, with 33% similarity between them. Among the samples, the highest genetic distance that was discovered is between the D160_M sample and the D197_R sample, with a value of 0.9412, which is nearly equal to one, indicating that the two samples are completely distinct from one another (different varieties) and are at different stages of fruit development (Table 4.3). Then, the lowest genetic distance discovered is between the D24_M_105 sample and the D24_M_110 sample, with a value of 0.0000 (Table 4.3), indicating that the samples are very similar (same variety) and are at the same stages of fruit development.

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5.3 Transcriptomic Analysis on Biological Functions and Molecular Pathway Mechanism Underlying Fruit Developmental Stages

5.3.1 Samples Bioinformatic Analysis Pipeline Profiling

In general, the profiling of bioinformatics analysis of 21 samples had achieved all requirements which translated into data reliability. These include data quality control (low sequencing error rate, <1%), alignment and mapping (90% reads in majority of the samples were successfully mapped to the *D. zibethinus* reference genome), novel gene prediction (out of 4,806 novel genes, 3,659 novel genes were annotated in the GO database, and 392 novel genes were annotated in the KEGG database), high gene expression quantification (quantified via both read counts and FPKM), differential gene expression analysis (a total of 1,167 to 18,198 genes that were differentially expressed, of which 502 to 8,170 genes were upregulated and 520 to

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13,240 genes were downregulated) and functional annotation by GO and KEGG. Focus was made to discuss on all the genes that map to the top KEGG pathways that are involved in gene expression in the metabolism of terpenoids and polyketides (carotenoid biosynthesis), lipid metabolism (fatty acid biosynthesis), carbohydrate metabolism (starch and sucrose metabolism and galactose metabolism) and biosynthesis of secondary metabolites (phenylpropanoid biosynthesis).

5.3.2 GO Enrichment

Go enrichment analysis showed that most of the top-hit genes have a greater number of up-regulated genes than down-regulated genes, indicating that the GO terms have been significantly expressed throughout fruit developmental stages (Table 4.8). Interestingly, among the top-hit genes, the LOC111285714 gene is associated with nine GO terms (GO no. 1, 7, 8, 9, 10, 11, 12, 15 and 25) that cover each of the GO term categories (biological process, cellular component, and molecular function). This gene is regulated by xyloglucan endotransglucosylase/hydrolase protein 22-like enzyme (XTH). The XTH gene has been reported to play roles in rice growth (Hara, Yokoyama, Osakabe, Toki, & Nishitani, 2014) and also during tomato and apple fruit ripening by loosening the cell wall (Muñoz-Bertomeu, Miedes, & Lorences, 2013). Therefore, this indicates the gene is very significant throughout the fruit developmental stages, and further study may be necessary to look up the role of the XTH gene in durian.

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5.3.3 KEGG Enrichment

In this empirical study, it is suggested that during fruit development process, there are four main dynamic changes were observed in durian fruit arils. First, the durian fruit aril's colour changes to a yellowish or golden colour. Second, enhancement of the buttery taste and texture of durian fruit arils. Third, the increment of sweetness in durian fruit arils. Fourth, defence mechanisms against pathogens by secondary metabolites.

These changes are in line with the transcriptomic analysis performed in this study, which showed that the most common KEGG pathways throughout fruit developmental stages were carotenoid biosynthesis (6-31 genes), followed by fatty acid biosynthesis (1-51 genes), starch and sucrose metabolism (9-115 genes), phenylpropanoid biosynthesis (11-74 genes) and galactose metabolism (9-44 genes) (Table 4.9).

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There is some correlation between GO enrichment and KEGG enrichment findings. For instance, the GO term (aminoglycan catabolic process) involves chemical reactions and pathways resulting in the breakdown of aminoglycans to produce glucose, which is then utilised in the KEGG pathways for the starch and sucrose metabolism and galactose metabolism (further explained in section 5.2.2.3 and 5.2.2.5). There is also one top-hit gene in KEGG pathways (Table 4.11) that parallels top-hit genes in GO terms (Table 4.8). The gene is LOC111308820 (CYP84A) (Table 4.11) that is expressed in phenylpropanoid biosynthesis, which is parallel with GO terms under the molecular function category (oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen). This comprehensive investigation of the discovered GO terms and KEGG pathways reveals that they all participate in the durian fruit's development.

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5.3.3.1 Carotenoid Biosynthesis

Carotenoid biosynthesis in durian involves two KEGG modules; the first is in beta-Carotene biosynthesis and the second module is abscisic acid biosynthesis (Appendix Figure C1A1-A2). The top five genes that listed in Table 4.11 were differentially expressed in 11 to 12 sample groups. There are 43 genes expressed in both modules and downstream of the carotenoid biosynthesis pathway (Appendix Figure C1A). *crtB, PDS, Z-ISO, ZDS, crtISO* and *lcyB* genes were involved in the beta-Carotene biosynthesis, *crtZ, LUT5, ZEP, NCED, ABA2* and *AAO3* genes were involved in the abscisic acid biosynthesis and *CYP707A, CCD7, LUT1* and *VDE* genes were being expressed in the downstream pathway of carotenoid biosynthesis. These four genes (*CYP707A, CCD7, LUT1* and *VDE*) are involved in the pathway, however they only appear downstream, and changes in their expression levels may have little effect on the given pathway (Draghici, 2023).

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Briefly, the first step in beta-Carotene biosynthesis is the condensation of geranylgeranyl-diphosphate to form phytoene in two conversion steps. Phytoene undergoes five sequential steps to form lycopene. The *lcyB* gene completes the two conversion steps from lycopene to beta-carotene (Appendix Figure C1A1). There are only four genes (*crtB, Z-ISO, ZDS* and *crtISO*) participating in beta-carotene biosynthesis (Appendix Figure C1A). The genes showed non-significant expression change during the maturation stage and were further down-regulated during the ripening and dehiscence stages and thus reduce beta-carotene synthesis.

At this point, there are three probable hypothesis regarding the association between beta-carotene biosynthesis and durian fruit colouration. First, accumulation of beta-carotene occurs prior to the onset of fruit maturation and is required only as a precursor for synthesis of abscisic acid (Wisutiamonkul et al., 2017). Second, there is

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a more prominent role in post-harvest stress response by production of abscisic acid (Chen, Mao, Lu, Ying, & Luo, 2016). Third, abscisic acid biosynthesis might unintentionally enhanced yellow colour in fruit arils. In fact, studies on ripening process in grapes had discovered that abscisic acid concentration increases during the onset of maturation, triggers the anthocyanin pigment accumulation which causes colour change in black grapes (Palma, Corpas, & del Río , 2011). Despite these, the importance of beta-carotene biosynthesis in durian fruit colour must not be underestimated, and further research should be studied.

Next, the genes involved in abscisic acid biosynthesis were found to be both upregulated (*crtZ, ZEP, NCED, ABA2* and *AAO3*) and downregulated (*LUT5*) across the fruit development stages (Appendix Figure C1A). *NCED* gene family (*NCED 3, NCED 5* and *NCED 6*) were among the top-hit genes in the carotenoid biosynthesis which were expressed in 11 to 12 differential sample groups (Table 4.11). For instance, the *NCED 5* gene were up-regulated during maturation and ripening stages but was down-regulated when reaching dehiscence stage (Table 4.11 and Appendix Figure C1A). The *NCED* gene is required to mediate the conversion of 9-cis-Violaxanthin and 9'-cis-Neoxanthin by converting them to xanthoxin (Appendix Figure C1A2). Xanthoxin is the substrate for *ABA2* gene which converts the xanthoxin to abscisic aldehyde. Then, abscisic aldehyde was further regulated by *AA03* gene which is subsequently converted to abscisic acid.

In contrast to *NCED* genes, the gene expression of *ABA2* were down-regulated during ripening and more likely to be up-regulated during dehiscence (Appendix Figure C1A). *AA03* gene (LOC111286289) encoding for abscisic-aldehyde oxidase enzyme were also correlated with *ABA2* genes. An increase in abscisic acid was also observed in postharvest strawberries, which experienced accelerated ripening and senescence processes (Chen et al., 2016). It has been demonstrated

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that *NCED* genes regulates *ABA2* synthesis in response to a water deficit in rosettes (Frey et al., 2012) and in cacao (Santos et al., 2014). According to Razali et al. (2020), when the durian ripens, water losses in the fruit cause the husk to shrink, making the fruit prone to cracking. Prolonged ripening may result in increased water loss in the fruit about 10-14% (Palapol et al., 2015). Dehisced durian fruit seems to shrink more and dry, which may be explained by the balanced expression of the *NCED, ABA2*, and *AA03* genes. Thus, the abscisic acid accumulation across the three stages of fruit development is regulated by the balanced expression of the *NCED, ABA2* and *AA03* genes and also supported by the expression of *crtZ* and *ZEP* genes.

5.3.3.2 Fatty Acid Biosynthesis

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Fatty acid biosynthesis pathway is the second KEGG term that is significant during durian fruit developmental stages (Figure 4.32). There are three KEGG modules involve in the fatty acid biosynthesis; fatty acid biosynthesis (initiation), fatty acid biosynthesis (elongation) and beta-oxidation (acyl-CoA synthesis) (Appendix Figure D1B1-B3). A series of gene expressions for *ACACA, accA, accB, accC*, and *accD* initiates the fatty acid biosynthesis for the conversion of acetyl-CoA to malonyl-CoA (Appendix Figure D1B1). Following that, the *fabD* gene regulates the conversion of malonyl-CoA to malonyl-[acp]. The *fabH* gene encoding the 3-oxoacyl-[acyl-carrier-protein] synthase III enzyme then completes the module's step by directing the conversion of malonyl-CoA to acetoacetyl-[acp].

Then, malonyl-[acp] is catalysed again in the elongation of fatty acid biosynthesis by a different enzyme, 3-oxoacyl-[acyl-carrier-protein] synthase II encoded by the *fabF* gene, which converts to the same product, acetoacetyl-[acp]

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(Appendix Figure D1B2). The expression of *fabG, fabZ*, and *fabI* genes then further regulates another three-step conversion until butyryl-[ACP] is produced in the first cycle. The first cycle's end products, butyryl-[ACP] were then introduced into the second cycle with malonyl-[acp] and catalysed further by the same series expression of the *fabF, fabG, fabZ*, and *fabI* genes. The end's product of the second cycle is hexanoyl-[acp] compound. The cycle was repeated by the same series of expression of the *fabF, fabG, fabZ,* and *fabI* genes with malonyl-[acp], making a total of around for seven cycles in the fatty acid elongation. Octanoyl-[acp], decanoyl-[acp], dodecanoyl-[acp], tetradecanoyl-[acp] and hexadecanoyl-[acp] are the end products for the third, fourth, fifth, sixth and seventh cycles, respectively. Then, in the last module of fatty acid biosynthesis, a simple step of beta-oxidation occur, in which *ACSL* genes regulates the conversion of hexadecanoic acid to palmitoyl-CoA (Appendix Figure D1B3).

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The top five genes that listed in Table 4.11 were differentially expressed in 11 sample groups. Among the top genes, three *fabG* genes were associated with elongation of fatty acid biosynthesis, the *ACSL* gene was associated with acyl-CoA synthesis by beta-oxidation, and the *FATA* gene was involved in the downstream of the pathway (Table 4.11 and Appendix Figure D1B). A total of 67 genes were found to be expressed across the whole fatty acid biosynthesis pathway (Appendix Figure D1B).

Malonyl-CoA substrate that is produced in the initiation of fatty acid biosynthesis (Appendix Figure D1B1) was reported to be an inhibitor of fatty acid oxidation (Foster, 2012). It plays a key role in the partitioning of fatty acids, which determines the switch between fatty acid synthesis and oxidation. Most genes in the initiation of fatty acid biosynthesis module were found to be down-regulated as the fruit progresses from the maturation stage to the dehiscence stage, implying that

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malonyl-CoA production has also been reduced, thus activating fatty acid oxidation (Appendix Figure D1B). Some of the *ACSL* genes found to be upregulated during the ripening and dehiscence stages, to complete conversion to form palmitoyl-CoA (Appendix Figure D1B). This observation suggests that accumulation of malonyl-CoA has been reduced before or upon reaching maturation stage, whereas oxidation of fatty acids to palmitoyl-CoA is activated as the fruit developmental stages progress from ripening to the dehiscence stage.

In the elongation of fatty acids, the *fabG* gene (LOC111293698) seems to balance the expression of other genes involved in the modules where it is elevated at every stage (Appendix Figure D1B). The *fabG* gene was also found to be two- to three-fold overexpressed in *Escherichia coli*, demonstrating its importance in the synthesis of fatty acids (Eunyoung et al., 2012; Zhang & JR, 1998). Then, the *fabF, fabZ* and *fabI* genes tend to be down-regulated at each stage, except for some of the *fabF* and *fabZ* genes that were only up-regulated during the dehiscence stage (Appendix Figure D1B). The butyryl-[ACP] or butyric acid that is produced in the first cycle of elongation of fatty acids (Appendix Figure D1B2) was initially claimed as being responsible for the buttery smell (Chevreul, 1815). It can be present in animal fats and plant oils, bovine milk, breast milk, butter, parmesan cheese, body odour, vomit, and as a by-product of anaerobic fermentation (including in the colon) (McNabney & Henagan, 2017; Morrison & Preston, 2016). Low-molecular-weight esters of butyric acid, such as methyl butyrate, have usually pleasant aroma and tastes and are thus employed as food and perfume additives (Riemenschneider, 2012). Therefore, this compound may be responsible for contributing to the creamy taste and aroma of durian. Since the *fabG* gene (LOC111293698) was up-regulated (four-fold overexpressed) in each stage, it has a big potential to be further exploited to enhance the durian taste and aroma in the future.

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5.3.3.3 Starch and Sucrose Metabolism

Starch and sucrose metabolism pathway involve three KEGG modules; glycogen biosynthesis (convert glucose-1P to glycogen/starch), glycogen degradation (degrade glycogen into glucose-6P) and trehalose biosynthesis (convert D-glucose 1P to trehalose) (Appendix Figure E1C1-C3). The top four genes (*GN1_2_3, bglX, E2.4.1.14*, and *TPS*) that listed in the Table 4.11 were found to be expressed downstream (Appendix Figure E1C) and were differentially expressed in 12 to 13 sample groups. There were 130 genes that were differentially expressed in the starch and sucrose metabolism pathway across fruit developmental stages (Appendix Figure E1C).

During maturation, *GBE1* gene (LOC111311749) was found to be up-regulated to synthesize starch or glycogen from amylose (Appendix Figure E1C). During ripening, *UGP2* gene (LOC111305812) and *glgA* genes (LOC111274881 and LOC111291363) take place to further synthesize glycogen. The *UGP2* gene (LOC111296541), *glgA* genes (LOC111274881 and LOC111291363), and *GBE1* genes (LOC111275656 and LOC111311749) then prolong glycogen synthesis during the dehiscence stage.

For glycogen degradation, *PYG* gene (LOC111280900) was found to be up-regulated during maturation stage to degrade the glycogen into D-Glucose 1-phosphate (Appendix Figure E1C and Appendix Figure E1C2). Then, *malQ* gene (LOC111293942) take over to regulate the same conversion during ripening stage (Appendix Figure E1C). In dehiscence stage, *PYG* gene (LOC111292628) was elevated again to further accumulate D-Glucose 1-phosphate.

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Then, the accumulation of D-Glucose 1-phosphate were further used in the trehalose biosynthesis module (Appendix Figure E1C3). Same as glycogen synthesis, the same genes; *GBE1* genes were elevated during maturation and dehiscence and glgA genes elevated during ripening and dehiscence stages in order to synthesis glycogen. At this point, it is notable that D-Glucose 1-phosphate was converted back again into glycogen. Glycogen produced was subsequently converted by *ISA* gene (LOC111305172) that is being up-regulated and further converts the glycogen into maltodextrin during ripening and dehiscence stages (Appendix Figure E1C and Appendix Figure E1C3). The pathway in this study, however, ends with the synthesis of maltodextrin because neither the *glgY* nor the *glgZ* genes were expressed to carry out the further steps in the production of trehalose.

Maltodextrin is a low-sweet saccharide polymer that is commonly utilized as additives in ice cream, dried instant food formulations, confectionary, cereals, snacks, and drinks (Hofman, Buul, & Brouns, 2016). Even though maltodextrin has a low level of sweetness, the enhanced sweetness in durian fruit, especially during ripening and dehiscence, could be attributable to the formation of other types of sugar such as glucose, sucrose, maltose, glucan, and fructose (Appendix Figure E1C). In this study, *AMY, bglX, E2.4.1.14, E3.2.1.2, E3.2.1.4, E3.2.1.21, INV*, and *SUS* genes were up-regulated in the downstream (Appendix Figure E1C) and produce the sugars aforementioned. These genes were up-regulated in durian, with *AMY* produced maltose, *bglX* produced glucose, *E2.4.1.14* produced sucrose, *E3.2.1.2* produced glucose, *E3.2.1.4* produced glucan, *E3.2.1.21* produced glucose, *INV* produced glucose and fructose, and *SUS* produced glucose, sucrose, and fructose. Among the sugar produced, fructose is known as the sweetest of all natural carbohydrates (Hanover & White, 1993). Other than that, it was also discovered that

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durian fruit contains a significantly higher concentration of sucrose than other types of sugar (Xiao et al., 2022).

5.3.3.4 Phenylpropanoid Biosynthesis

Phenylpropanoid biosynthesis pathway has two KEGG modules; monolignol biosynthesis (conversion of phenylalanine/tyrosine to monolignol) and flavanone biosynthesis (conversion of phenylalanine to naringenin) (Appendix Figure F1D1-D2). A total of 148 genes have been expressed in the pathway across fruit developmental stages (Appendix Figure F1D). The top seven genes listed in the Table 4.11 were differentially expressed in 11 to 14 sample groups. Three genes (*CYP84A, CYP73A* and *PAL*) have been expressed in the KEGG modules whereas *E1.11.1.7* genes have been expressed in the downstream (Appendix Figure F1D).

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There are 11 genes involve in the monolignol biosynthesis, five genes in the flavanone biosynthesis and six genes in the downstream (Appendix Figure F1D). In brief, phenylpropanoids biosynthesis begins with the two-step conversion of phenylalanine to coumarate by the *PAL* and *CYP73A* genes. These two genes were found to be up-regulated during the three stages of fruit development. Next, *4CL* genes that were up-regulated aided the ATP-dependent synthesis of coumaroyl-CoA. Several other genes, such as *HCT, CYP98A, E2.1.1.104, CCR, CYP84A, COMT* and *CAD,* were up-regulated to complete the conversion steps, and end with the formation of sinapyl alcohol in this module.

In flavanone biosynthesis, there are five genes that have been expressed in the module, which are *PAL, CYP73A, 4CL, CHS,* and *E5.5.1.6* (Appendix Figure F1D2). All genes have been differentially expressed to be up- or down-regulated

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except for *CHS* and *E5.5.1.6*. Thus, naringenin (a type of flavonoid) is not significantly produced in durian and is therefore not further explored.

Sinapyl alcohol is the end product in monolignol biosynthesis (Appendix Figure F1D1) which is responsible as precursor to lignin (Boerjan, Ralph, & Baucher, 2003). Lignin is crucial in the formation of cell wall that build up the structural integrity, stiffness and strength of the stem (Boerjan et al., 2003; Chabannes et al., 2001), which could act as a barrier against microbial attacks. There are two potential association between monolignol biosynthesis with its function. First, lignin synthesis could be regarded of as pathogen-induced lignification that protects the fruit after it has fallen from the tree. Second, the presence of sinapyl alcohol and lignin may be responsible for the bitterness taste (Lu, Ma, Liu, An, & Ludlow, 2020) in durian fruit. Moreover, plants have been reported produce little sinapyl alcohol due to being severely depleted in *COMT* (Boerjan et al., 2003). The expression of *COMT* gene in chrysanthemum pedicel is correlated with the rigidity of the pedicel (Lv et al., 2011).

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5.3.3.5 Galactose Metabolism

Galactose metabolism pathway has four KEGG modules; Semi-phosphorylative Entner-Doudoroff pathway (conversion of gluconate/galactonate to glycerate-3P), Galactose degradation [Leloir pathway (conversion of galactose to alpha-D-glucose-1P)], D-galactonate degradation [De Ley-Doudoroff pathway (conversion of D-galactonate to glycerate-3P)] and Nucleotide sugar biosynthesis (conversion of galactose to UDP-galactose) (Appendix Figure G1E1-E4). A total of 78 genes has been expressed in the pathway across fruit developmental stages (Appendix Figure G1E). The top five genes listed in the Table 4.11 were differentially expressed in 12 to 14 sample groups. There is only one gene (*galM*) has been expressed in the

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KEGG module whereas others are expressed in the downstream (Appendix Figure G1E).

All the KEGG modules in the galactose metabolism pathways are either incomplete or have one block missing (Appendix Figure G1E1-E4). Only one gene (*gapN*) has been expressed in the semi-phosphorylative Entner-Doudoroff pathway module; however, the gene is not expressed differentially enough to be either up- or down-regulated (Appendix Figure G1E1). In the Leloir pathway module, there is one block missing (*galK*), whereas *galM* and *galE* are either up- or down-regulated, and *galT* is not differentially expressed (Appendix Figure G1E2). The De Ley-Doudoroff pathway module is also incomplete where only two genes have been expressed (*GAPDH* and *PGK*), and they have not been expressed differentially enough to be either up- or down-regulated (Appendix Figure G1E3). Nucleotide sugar biosynthesis also has one block missing (*galK*), and *galT* has been expressed but not differentially enough to be either up- or down-regulated (Appendix Figure G1E4). However, the gene activity in the galactose metabolism pathway is actually more active in the downstream part, where *E2.4.1.82, GOLS, HK, INV, pfkA,* and *UGP2* were found to be up-regulated (Appendix Figure G1E).

Based on Appendix Figure G1E, the only active gene expression among the four modules is regulated by *galE* gene expression, which is up-regulated at each stage of fruit development. The *galE* genes are responsible for the conversion of UDP-galactose into UDP-glucose (Appendix Figure G1E2). Galactose is a monosaccharide sugar that is roughly as sweet as glucose and about 65% as sweet as sucrose (Spillane, 2006). Since glucose is more stable than galactose, galactose metabolism transforms galactose into glucose. Many people believe that this is why a pathway for rapid conversion of galactose to glucose has been highly conserved in a

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lot of species (Walter & Fridovich-Keil, 2019). However, future studies need to be carried out for validation.

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CHAPTER 6

CONCLUSION

Overall, these six Malaysian durian varieties demonstrated a considerable level of variation at the morphological and molecular level. Based on quantitative trait variation, the D200 variety is the most outstanding, having superiority traits in terms of pedicel length, fruit weight, weight of peel, weight of seeds and number of seeds per fruit. The D160 then had the most seeds and the most flattened seeds. Based on the statistical ANOVA and canonical discriminant analysis, the pedicel length trait is the most significant trait to differentiate the varieties and separate the D168 variety from the rest.

The evaluation of qualitative trait variation has revealed three unique flower traits and 16 distinctive fruit traits for classifying and differentiating the six durian varieties. The distinctive flower traits of D99 variety could be used for identification during the flowering stage. D200's flowers can still be distinguished from D24, D160, D168, and D197 varieties by their circular and rounded flower bud shapes. The absence of spines at both around the base of the pedicel and at stylar end is unique to D197 variety. The dendrogram generated by cluster analysis classified the

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varieties into two clades: Clade A (mostly of D160, D168, D197, and D200) and Clade B (mostly of D24 and D99).

Therefore, the morphological information elucidated in this study could serve as a preliminary reference or simple guidelines to differentiate the six durian varieties in Malaysia. Even though many durian varieties are still partially characterised, have overlapping traits among them, and the classification is continuously debatable among the experts, comprehensive studies on durian morphology need to be conducted continuously to support durian germplasm conservation in Malaysia.

Next, molecular findings elucidated in this study allow an understanding of gene expression in durian throughout different fruit developmental stages (matured, riped and dehisced). The 49,601 genes that were discovered and annotated in this study serve as an ideal starting point for future genetic and functional genomic research. A total of 1,167 to 18,198 genes showed differential expression, with 502 to 8,170 being upregulated and 520 to 13,240 being downregulated. Five groups (D24_M_110 vs D24_M_105, D24_M_115 vs D24_M_105, D99_R vs D99_M, D99_D vs D99_R and D168_D vs D168_R) have more up-regulated genes than down-regulated genes, while the rest have the opposite.

There are 26,212 genes (including 3,659 novel genes) that could be annotated to the GO database, and 7,205 genes (including 392 novel genes) could be annotated to the KEGG database. The most significant GO terms were associated with cell wall, external encapsulating structure, extracellular region, cell periphery, hydrolase activity, acting on glycosyl bonds, hydrolase activity, hydrolyzing O-glycosyl compounds, apoplast, and oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen. The role of XTH gene that

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associated with nine GO terms suggested to be further study in durian as the gene have been reported involve in development of rice, tomato, and apple.

The KEGG pathway analysis found that carotenoid biosynthesis is the top biological pathway expressed in durian followed by fatty acid biosynthesis, starch and sucrose metabolism, phenylpropanoid biosynthesis and galactose metabolism. The carotenoid biosynthesis pathway might have a more prominent role in the accumulation of abscisic acid than the synthesis of beta-carotene and thus stimulate the post-harvest stress response in durian and enhanced fruit colouration as a by-product effect. In fatty acid biosynthesis pathway, the butyryl-[ACP] or butyric acid that is produced in the first cycle of elongation of fatty acids was claimed to be responsible for buttery smell, and since the *fabG* gene (LOC111293698) was up-regulated in each stage, it has the potential to be further utilized in the future to improve the creamy taste and aroma of durian.

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The elevated sweetness level in durian could be due to the accumulation of maltodextrin, and other types of sugars such as glucose, sucrose, maltose, glucan, and fructose by starch and sucrose metabolism pathway. As for the phenylpropanoid biosynthesis pathway, sinapyl alcohol is responsible as a precursor to lignin synthesis, which then acts as pathogen-induced lignification, and the lignin content most likely contributes to the bitter taste in durian. Next, the final top-five significant and common KEGG pathway in durian is galactose metabolism; however, all the modules in this pathway are either incomplete or have one block missing.

Morphological and molecular work should be carried out in parallel with each other. Durian morphology could be served as the fundamental information that complement with the molecular works. The NGS data could be further explored exclusively for each fruit variety and the key genes especially those related to top five

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most common KEGG pathways should be validated using quantitative PCR (qPCR) analysis in future studies. This study will advance knowledge of fruit development stages regarding physiological processes, genetics, and molecular mechanisms in response to consumer and agro-biotechnology industry demand. Moreover, this knowledge could be used to set up strategies for breeding and crop improvement, as well as post-harvest strategies for improving the quality of durian fruit.

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APPENDICES

Appendix A

Table A1

Shapiro-Wilk and Kolmogorov-Smirnov normality test for quantitative traits

Trait	Shapiro-Wilk			Kolmogorov-Smirnov		
	Statistic	df	Sig.	Statistic	df	Sig.
Trait 4: Pedicel: length (cm)	0.912	22	0.052	0.217	22	0.008
Trait 5: Fruit: weight (kg)	0.941	22	0.203	0.193	22	0.032
Trait 6: Fruit: length (cm)	0.982	22	0.947	0.114	22	0.200
Trait 7: Fruit: width (cm)	0.971	22	0.723	0.099	22	0.200
Trait 18: Fruit: weight of peel (kg)	0.962	22	0.533	0.130	22	0.200
Trait 19: Fruit: weight of seeds (kg)	0.974	22	0.808	0.108	22	0.200
Trait 20: Fruit: number of seeds per fruit	0.956	22	0.421	0.154	22	0.189
Trait 21: Fruit: number of flattened seeds per fruit	0.831	22	0.002	0.222	22	0.006

Note,

If $p \leq 0.05$: then the null hypothesis can be rejected (not normally distributed);

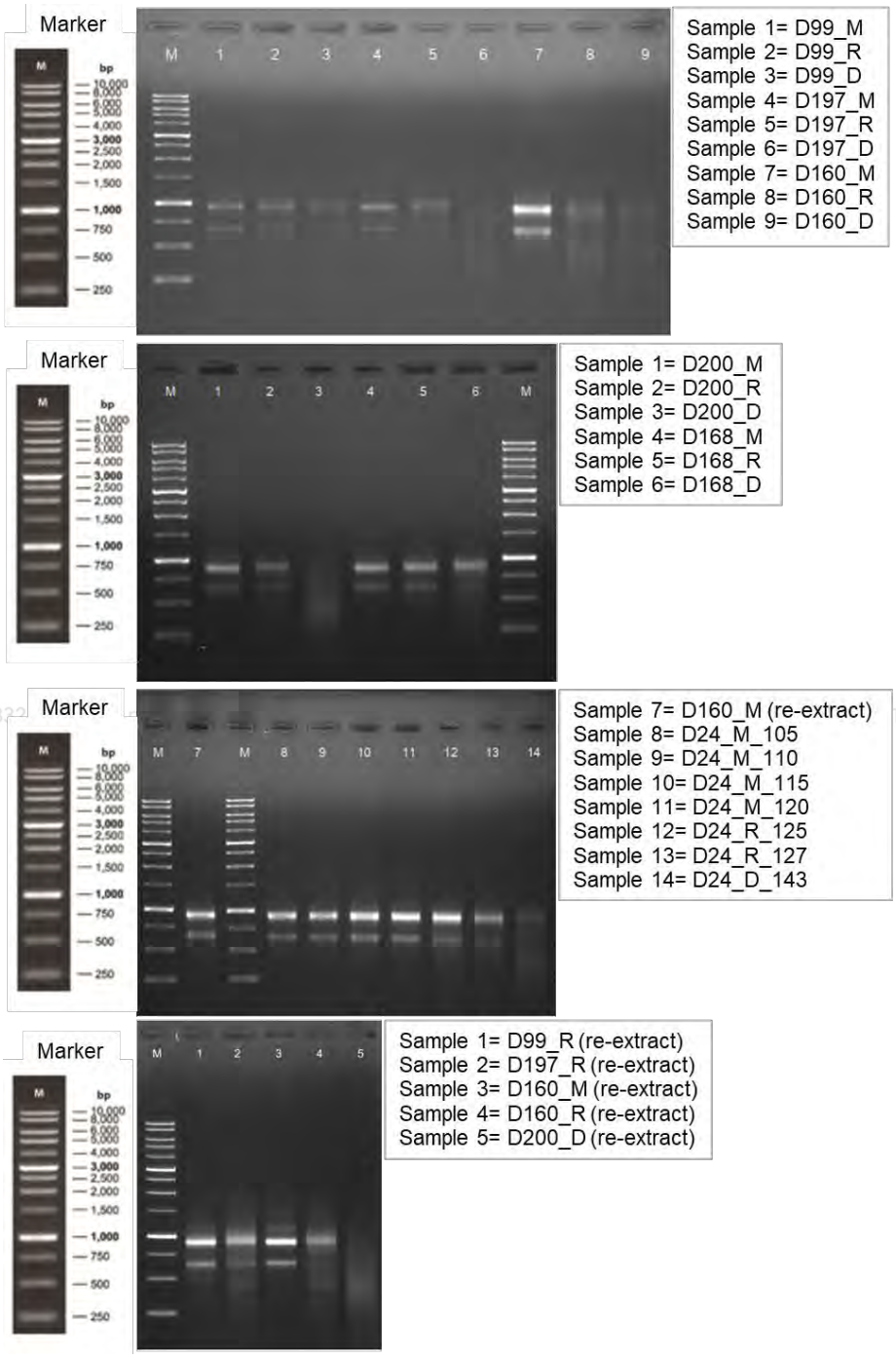
If $p > 0.05$: then the null hypothesis cannot be rejected (normally distributed).

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Figure A1

RNA qualification for 22 durian flesh samples collected (including RNA re-extraction samples

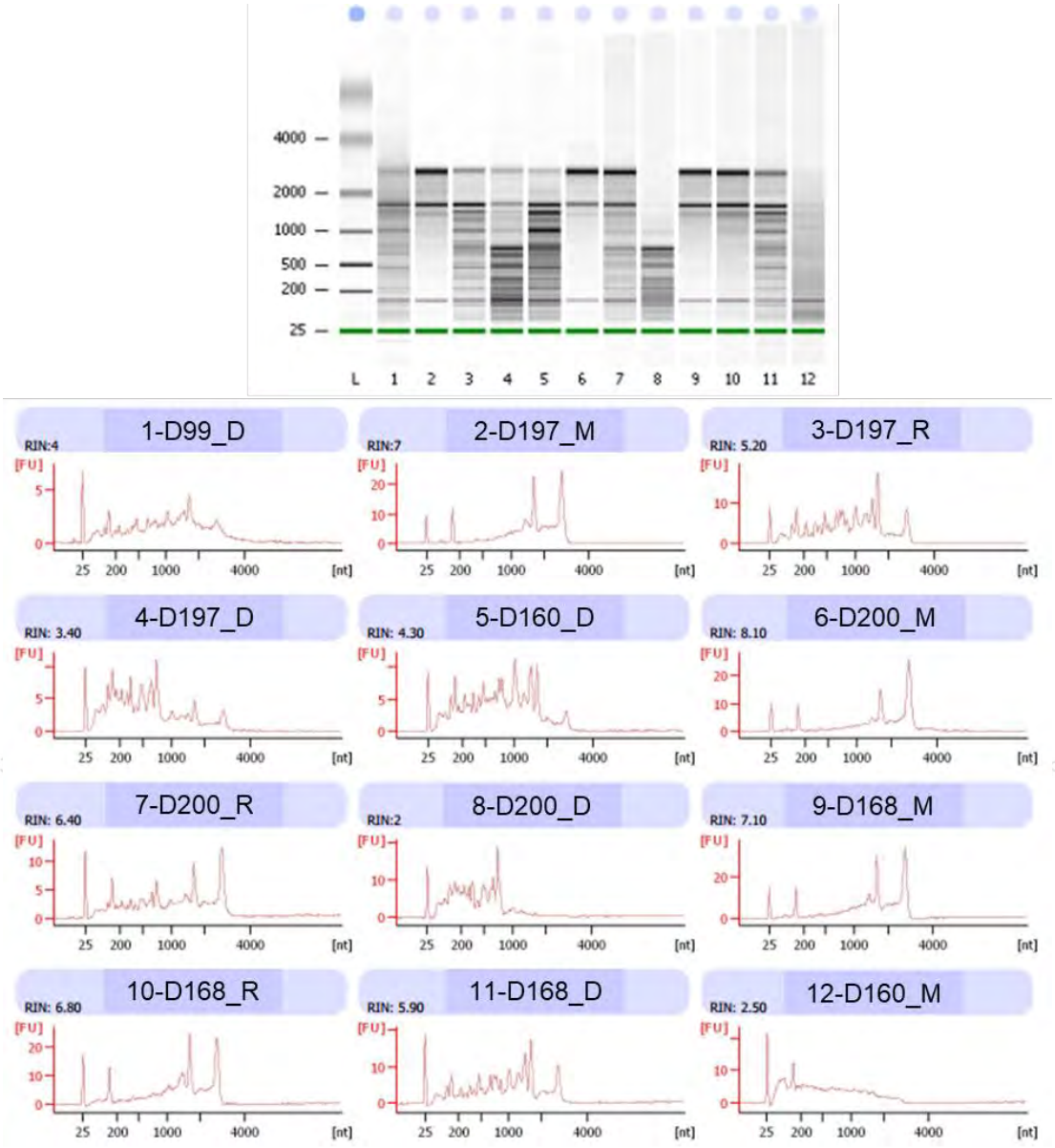


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Figure A2 (a)

RNA Integrity Number (RIN) for 22 durian flesh samples collected

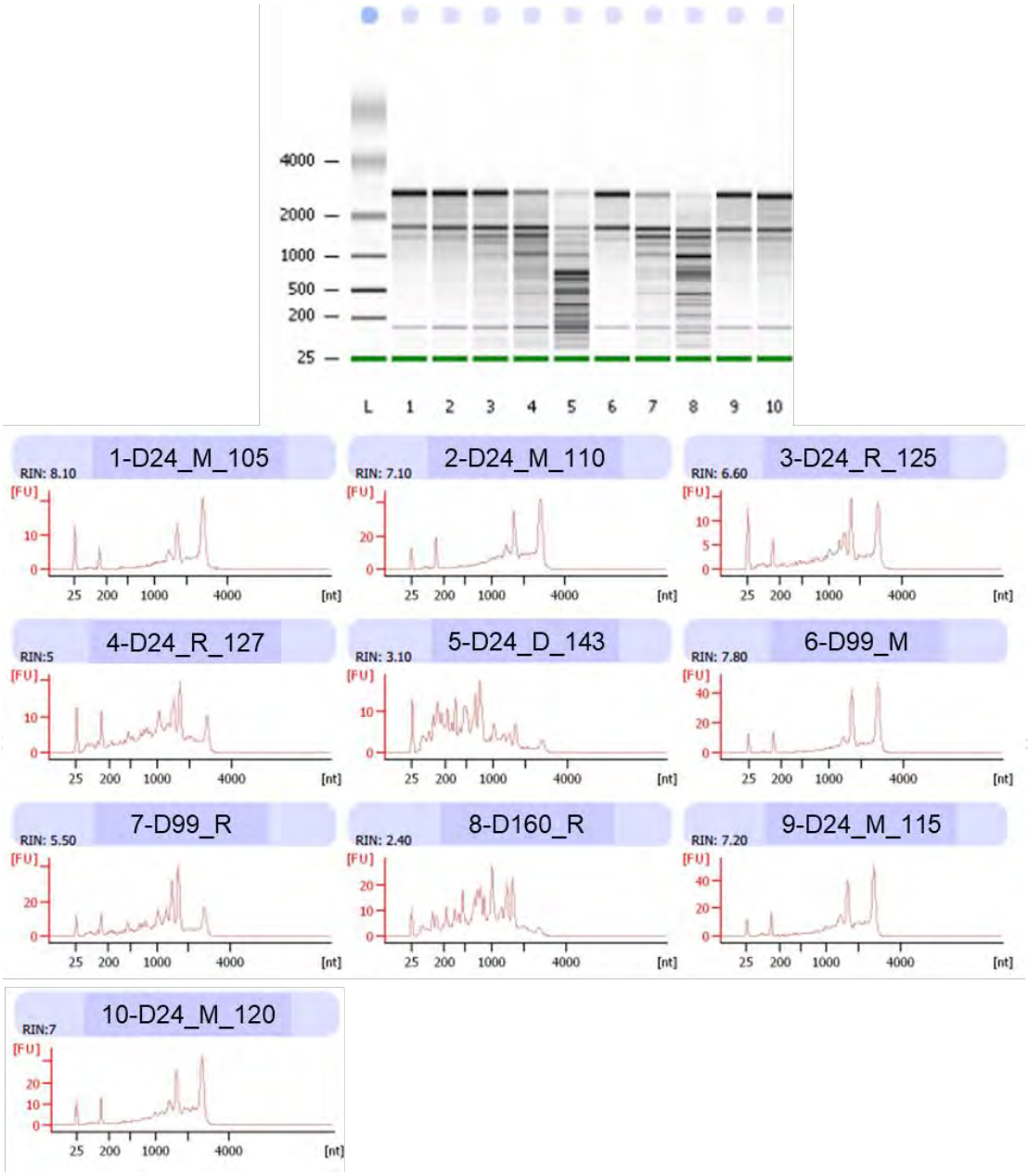


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Figure A2 (b)

RNA Integrity Number (RIN) for 22 durian flesh samples collected



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Table A2

Summary of mapping statistics of 21 durian flesh samples to the D. zibethinus reference genome

Sample	Total Map (%)	Unique Map (%)	Multi Map (%)	Read1 Map (%)	Read2 Map (%)	Positive Map (%)	Negative Map (%)	Splice Map (%)	Unsplice Map (%)	Proper Map (%)	Exon (%)	Intron (%)	Intergenic (%)
D24_M_105	94.58	90.84	3.74	45.37	45.47	45.38	45.46	39.14	51.7	87.91	94.59	2.26	3.15
D24_M_110	94.55	89.55	4.99	44.66	44.89	44.74	44.82	38.11	51.45	86.82	93.89	2.27	3.83
D24_M_115	94.58	90.47	4.11	45.05	45.42	45.19	45.28	38.36	52.11	87.62	94.61	2.12	3.26
D24_M_120	95.07	89.83	5.24	44.81	45.02	44.88	44.95	38.48	51.35	87.24	94.19	2.01	3.80
D24_R_125	94.49	89.1	5.39	44.47	44.63	44.51	44.59	33.78	55.31	86.44	94.06	2.38	3.56
D24_R_127	81.66	73.89	7.77	36.83	37.06	36.86	37.03	28.4	45.49	71.27	91.17	2.69	6.14
D24_D_143	44.49	37.27	7.22	18.72	18.55	18.54	18.73	12.96	24.31	35.61	87.94	2.40	9.66
D99_M	94.21	89.11	5.1	44.41	44.7	44.49	44.61	38.71	50.4	86.45	94.95	1.91	3.14
D99_R	93.54	89.87	3.67	44.83	45.03	44.88	44.99	36.53	53.33	87.21	94.95	1.89	3.16
D99_D	89.17	82.79	6.37	41.36	41.43	41.34	41.45	31.32	51.47	79.85	91.83	2.16	6.01
D160_M	93.4	86.67	6.73	43.32	43.35	43.2	43.46	38.78	47.89	83.94	93.69	2.76	3.56
D160_R	84.45	72.08	12.37	36.01	36.07	35.95	36.13	28.56	43.52	69.48	89.48	2.51	8.01
D160_D	73.48	70.4	3.08	35.26	35.14	35.11	35.28	27.09	43.3	68.28	93.52	1.83	4.65
D168_M	94.23	85.54	8.69	42.72	42.82	42.68	42.86	35.01	50.53	82.83	92.96	2.65	4.40
D168_R	94.28	89.83	4.46	44.91	44.92	44.82	45.01	34.77	55.06	86.94	93.57	3.03	3.40
D168_D	92.07	87.35	4.71	43.81	43.54	43.6	43.76	34.05	53.31	84.65	94.46	2.15	3.39
D197_M	94.78	89.56	5.22	44.71	44.85	44.72	44.84	38.78	50.77	86.98	93.70	2.52	3.78
D197_R	93.09	86.92	6.16	43.47	43.46	43.39	43.54	33.36	53.56	84.2	90.60	3.16	6.24
D197_D	28.49	26.57	1.92	13.3	13.27	13.26	13.31	9.82	16.75	25.67	90.79	2.40	6.81
D200_M	93.78	86.74	7.04	43.35	43.39	43.31	43.44	37.6	49.15	83.96	93.19	2.69	4.12
D200_R	94.22	90.2	4.01	45.07	45.13	45.04	45.17	33.6	56.61	87.2	95.13	1.90	2.97

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Table A3

Pearson correlation matrix

Sample	D24_M_105	D24_M_110	D24_M_115	D24_M_120	D99_M	D160_M	D168_M	D197_M	D200_M	D24_R_125	D24_R_127	D99_R	D160_R	D168_R	D197_R	D200_R	D24_D_143	D99_D	D160_D	D168_D	D197_D
D24_M_105	1	0.931	0.962	0.966	0.733	0.898	0.715	0.876	0.871	0.654	0.622	0.652	0.618	0.596	0.571	0.591	0.479	0.606	0.469	0.612	0.51
D24_M_110	0.931	1	0.965	0.951	0.798	0.884	0.782	0.86	0.865	0.754	0.727	0.743	0.713	0.674	0.671	0.678	0.58	0.703	0.559	0.704	0.607
D24_M_115	0.962	0.965	1	0.973	0.764	0.886	0.76	0.859	0.859	0.709	0.68	0.708	0.674	0.646	0.625	0.646	0.533	0.665	0.519	0.667	0.562
D24_M_120	0.966	0.951	0.973	1	0.751	0.903	0.746	0.882	0.865	0.668	0.652	0.678	0.648	0.629	0.608	0.616	0.504	0.631	0.492	0.641	0.54
D99_M	0.733	0.798	0.764	0.751	1	0.747	0.881	0.758	0.773	0.797	0.793	0.887	0.782	0.764	0.781	0.764	0.618	0.825	0.641	0.799	0.7
D160_M	0.898	0.884	0.886	0.903	0.747	1	0.735	0.903	0.892	0.627	0.617	0.657	0.667	0.618	0.597	0.604	0.48	0.612	0.507	0.631	0.528
D168_M	0.715	0.782	0.76	0.746	0.881	0.735	1	0.739	0.742	0.801	0.851	0.869	0.837	0.881	0.819	0.812	0.631	0.788	0.636	0.892	0.735
D197_M	0.876	0.86	0.859	0.882	0.758	0.903	0.739	1	0.887	0.621	0.619	0.657	0.639	0.626	0.644	0.61	0.482	0.616	0.489	0.635	0.564
D200_M	0.871	0.865	0.859	0.865	0.773	0.892	0.742	0.887	1	0.663	0.634	0.678	0.65	0.619	0.612	0.64	0.5	0.643	0.507	0.644	0.548
D24_R_125	0.654	0.754	0.709	0.668	0.797	0.627	0.801	0.621	0.663	1	0.87	0.854	0.784	0.75	0.745	0.77	0.7	0.788	0.663	0.821	0.692
D24_R_127	0.622	0.727	0.68	0.652	0.793	0.617	0.851	0.619	0.634	0.87	1	0.886	0.861	0.874	0.857	0.868	0.74	0.823	0.669	0.885	0.797
D99_R	0.652	0.743	0.708	0.678	0.887	0.657	0.869	0.657	0.678	0.854	0.886	1	0.848	0.854	0.837	0.87	0.685	0.89	0.669	0.88	0.781
D160_R	0.618	0.713	0.674	0.648	0.782	0.667	0.837	0.639	0.65	0.784	0.861	0.848	1	0.845	0.839	0.837	0.689	0.81	0.696	0.855	0.77
D168_R	0.596	0.674	0.646	0.629	0.764	0.618	0.881	0.626	0.619	0.75	0.874	0.854	0.845	1	0.86	0.891	0.681	0.8	0.648	0.941	0.785
D197_R	0.571	0.671	0.625	0.608	0.781	0.597	0.819	0.644	0.612	0.745	0.857	0.837	0.839	0.86	1	0.838	0.69	0.816	0.661	0.849	0.876
D200_R	0.591	0.678	0.646	0.616	0.764	0.604	0.812	0.61	0.64	0.77	0.868	0.87	0.837	0.891	0.838	1	0.706	0.83	0.661	0.884	0.79
D24_D_143	0.479	0.58	0.533	0.504	0.618	0.48	0.631	0.482	0.5	0.7	0.74	0.685	0.689	0.681	0.69	0.706	1	0.74	0.661	0.723	0.672
D99_D	0.606	0.703	0.665	0.631	0.825	0.612	0.788	0.616	0.643	0.788	0.823	0.89	0.81	0.8	0.816	0.83	0.74	1	0.723	0.836	0.782
D160_D	0.469	0.559	0.519	0.492	0.641	0.507	0.636	0.489	0.507	0.663	0.669	0.669	0.696	0.648	0.661	0.661	0.661	0.723	1	0.694	0.627
D168_D	0.612	0.704	0.667	0.641	0.799	0.631	0.892	0.635	0.644	0.821	0.885	0.88	0.855	0.941	0.849	0.884	0.723	0.836	0.694	1	0.789
D197_D	0.51	0.607	0.562	0.54	0.7	0.528	0.735	0.564	0.548	0.692	0.797	0.781	0.77	0.785	0.876	0.79	0.672	0.782	0.627	0.789	1

Note,

$R^2 > 0.4$; $R^2 > 0.6$; $R^2 > 0.7$; $R^2 > 0.8$; $R^2 > 0.9$; $R^2 = 1.0$.

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Table A4

The top 15 most common GO terms share biological functions across all stages of fruit development. Gene ratio of differentially expressed genes is displayed in the table

No.	Go Terms	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_R vs D99_M	D99_D vs D99_R	D160_R vs D160_M	D160_D vs D160_R	D168_R vs D168_M	D168_D vs D168_R	D197_R vs D197_M	D197_D vs D197_R	D200_R vs D200_M	Count
Biological Process																		
1	cellular carbohydrate metabolic process	40/1067	20/508	21/417	105/5036	104/5580	93/5730	79/5177	40/2152	50/2577	97/5580	79/4984	52/2623	48/1540	104/5535	38/2135	104/5761	6
2	aminoglycan catabolic process	6/1067	5/508	6/417	13/5036	11/5580	10/5730	11/5177	4/2152	12/2577	12/5580	7/4984	8/2623	10/1540	9/5535	6/2135	12/5761	5
3	chitin metabolic process	6/1067	5/508	6/417	13/5036	11/5580	10/5730	11/5177	4/2152	12/2577	12/5580	7/4984	8/2623	10/1540	9/5535	6/2135	12/5761	5
4	microtubule-based movement	6/1067	2/508	2/417	43/5036	51/5580	58/5730	55/5177	27/2152	29/2577	47/5580	47/4984	26/2623	19/1540	43/5535	31/2135	51/5761	5
5	movement of cell or subcellular component	6/1067	2/508	2/417	43/5036	51/5580	58/5730	55/5177	27/2152	29/2577	47/5580	47/4984	26/2623	19/1540	43/5535	31/2135	51/5761	5
Cellular Component																		
1	cell wall	17/308	11/160	7/138	47/1710	47/1984	38/2095	36/1943	21/720	24/1008	39/1935	33/1893	30/992	33/488	46/1938	18/807	43/2084	15
2	external encapsulating structure	17/308	11/160	7/138	47/1710	47/1984	38/2095	36/1943	21/720	24/1008	39/1935	33/1893	30/992	33/488	46/1938	18/807	43/2084	14
3	extracellular region	15/308	8/160	6/138	33/1710	27/1984	27/2095	22/1943	18/720	20/1008	32/1935	25/1893	21/992	23/488	26/1938	12/807	30/2084	13
4	cell periphery	22/308	12/160	8/138	82/1710	84/1984	85/2095	78/1943	30/720	37/1008	73/1935	74/1893	45/992	45/488	79/1938	34/807	87/2084	13
5	apoplast	13/308	7/160	6/138	27/1710	20/1984	20/2095	15/1943	12/720	17/1008	24/1935	19/1893	17/992	20/488	21/1938	8/807	21/2084	10
Molecular Function																		
1	hydrolase activity, acting on glycosyl bonds	55/1503	31/689	32/567	229/7502	238/8416	203/8745	189/7991	85/3263	119/3653	258/8476	183/7885	131/3924	94/2194	233/8546	81/3348	246/8938	12
2	hydrolase activity, hydrolyzing O-glycosyl compounds	53/1503	31/689	30/567	219/7502	228/8416	195/8745	178/7991	83/3263	116/3653	247/8476	174/7885	129/3924	92/2194	223/8546	76/3348	236/8938	11

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No.	Go Terms	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_R vs D99_M	D99_D vs D99_R	D160_R vs D160_M	D160_D vs D160_R	D168_R vs D168_M	D168_D vs D168_R	D197_R vs D197_M	D197_D vs D197_R	D200_R vs D200_M	Count
3	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	44/1503	28/689	35/567	165/7502	152/8416	156/8745	135/7991	76/3263	95/3653	162/8476	120/7885	79/3924	54/2194	145/8546	59/3348	159/8938	10
4	iron ion binding	40/1503	29/689	31/567	155/7502	153/8416	155/8745	129/7991	75/3263	93/3653	160/8476	125/7885	84/3924	65/2194	149/8546	62/3348	168/8938	9
5	transferase activity, transferring hexosyl groups	54/1503	31/689	26/567	215/7502	216/8416	237/8745	198/7991	76/3263	113/3653	203/8476	193/7885	122/3924	83/2194	206/8546	82/3348	206/8938	7

Note,
GeneRatio = Ratio between the number of differentially expressed genes in each GO term and all differentially expressed genes that can be found in GO database;
Colour highlights = Green (significant and in the list of top 30 GO terms), Orange (not significant but in the list of top 30 GO terms), no highlight (not significant and/or not in the list of top 30 GO terms);
Count = Number of GO terms that highlighted by green and orange colour cell.

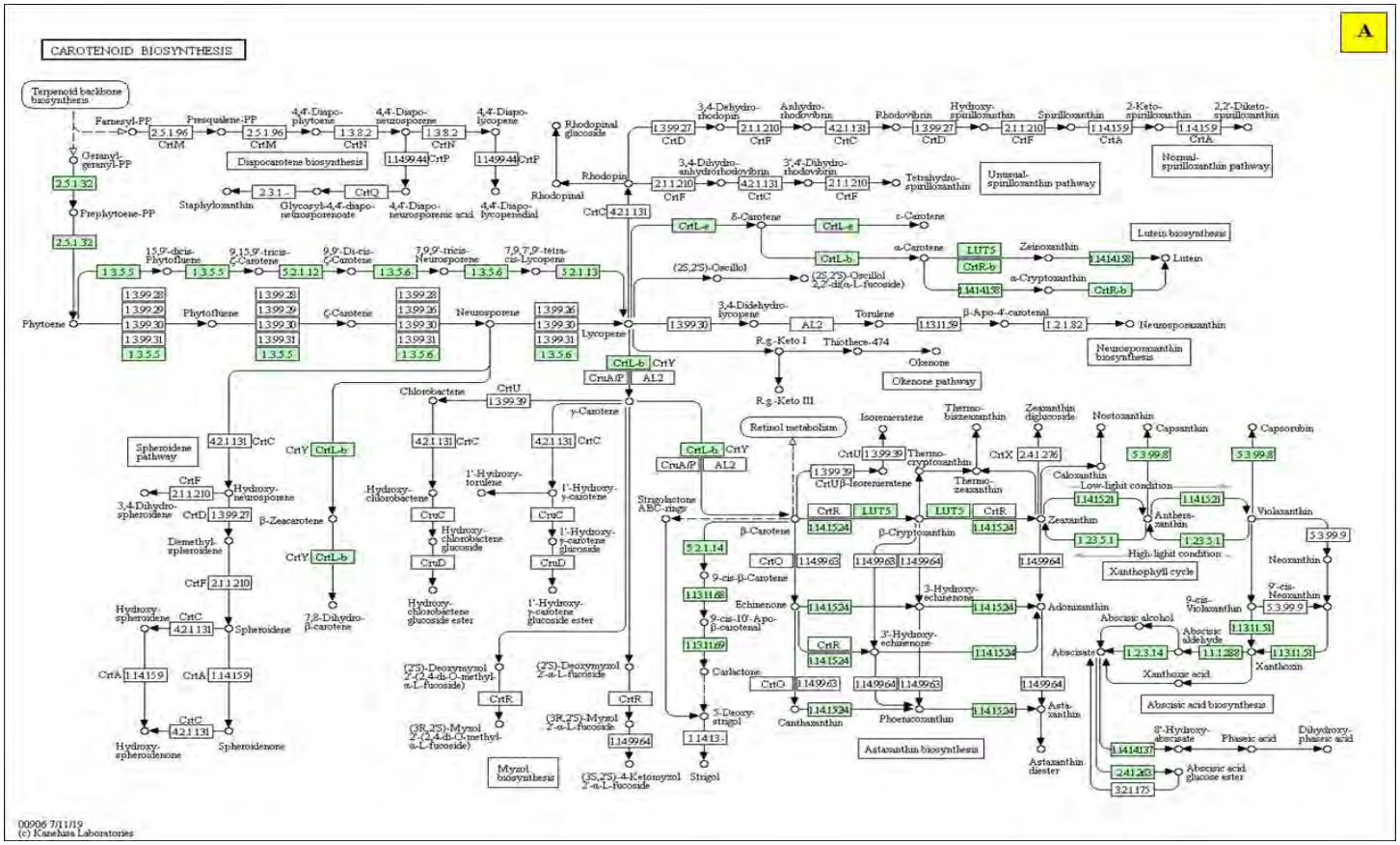
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Appendix B

Figure B1

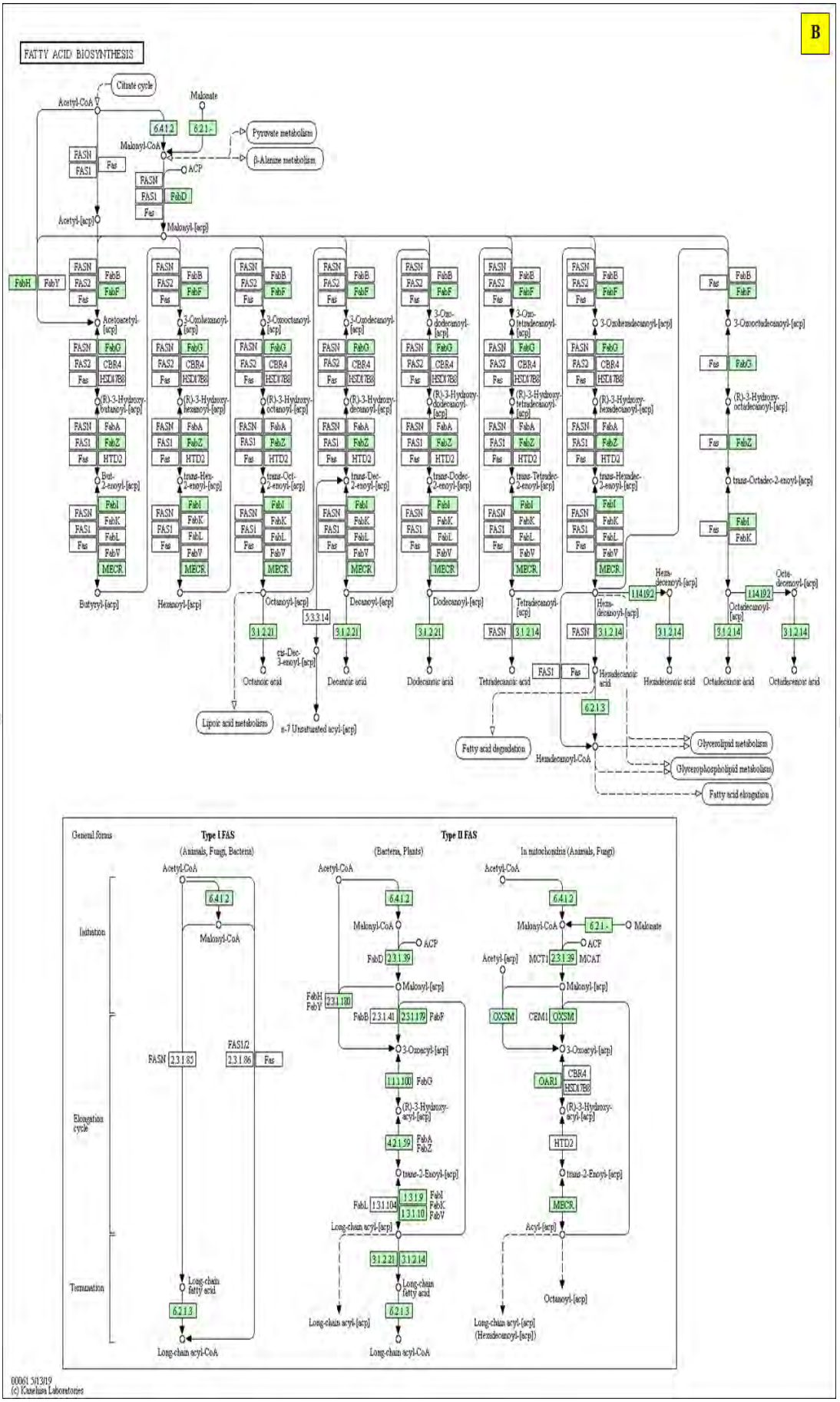
The top five most common KEGG enrichment analysis that share biological pathways across all stages of fruit development. A Carotenoid biosynthesis; B Fatty acid biosynthesis; C Starch and sucrose metabolism; D Phenylpropanoid biosynthesis; E Galactose metabolism. The green shades indicate all the genes that were expressed in D24, D99, D160, D168, D197 and D200 varieties



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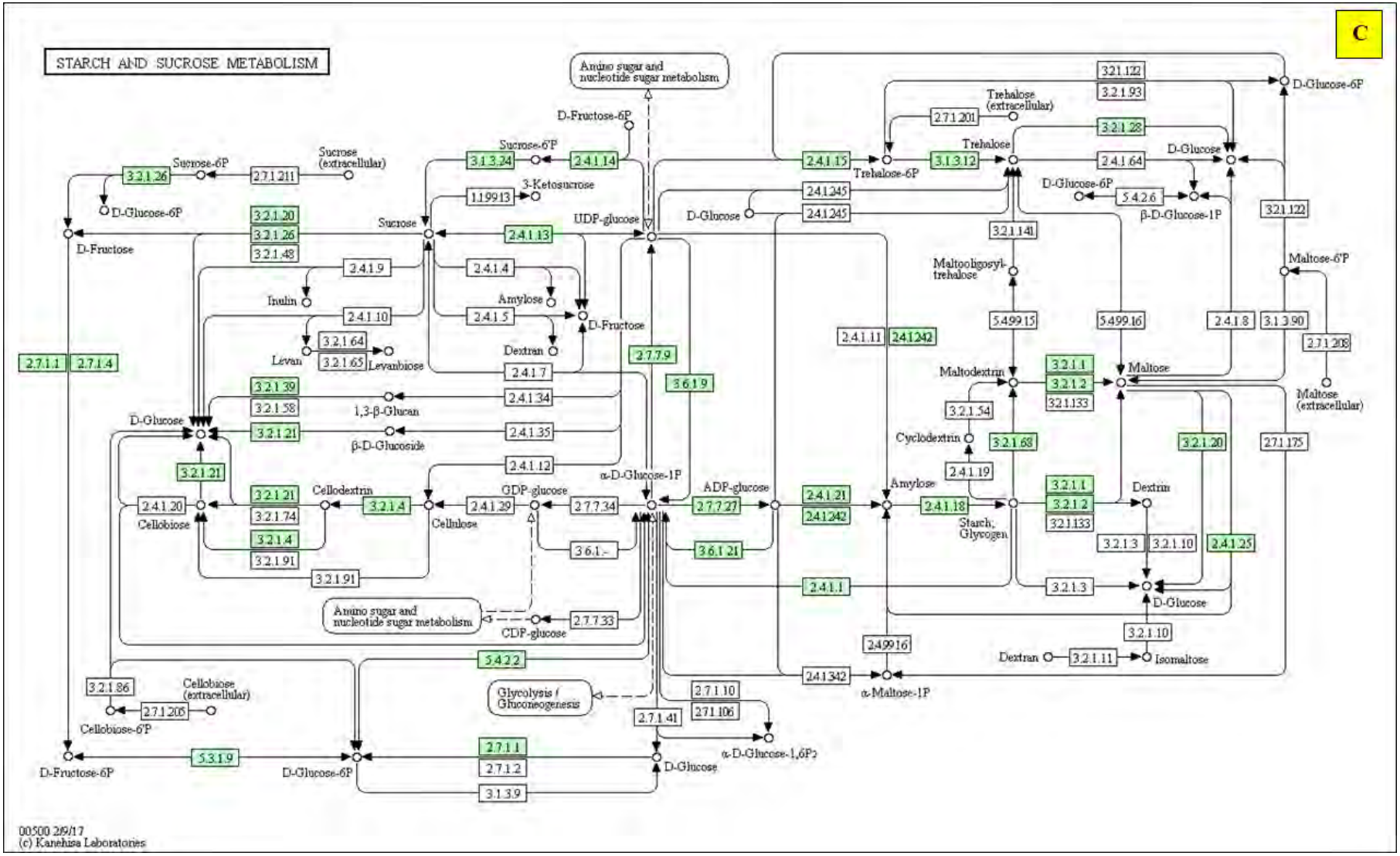
B



00061 5/13/19
(c) Kanehisa Laboratories

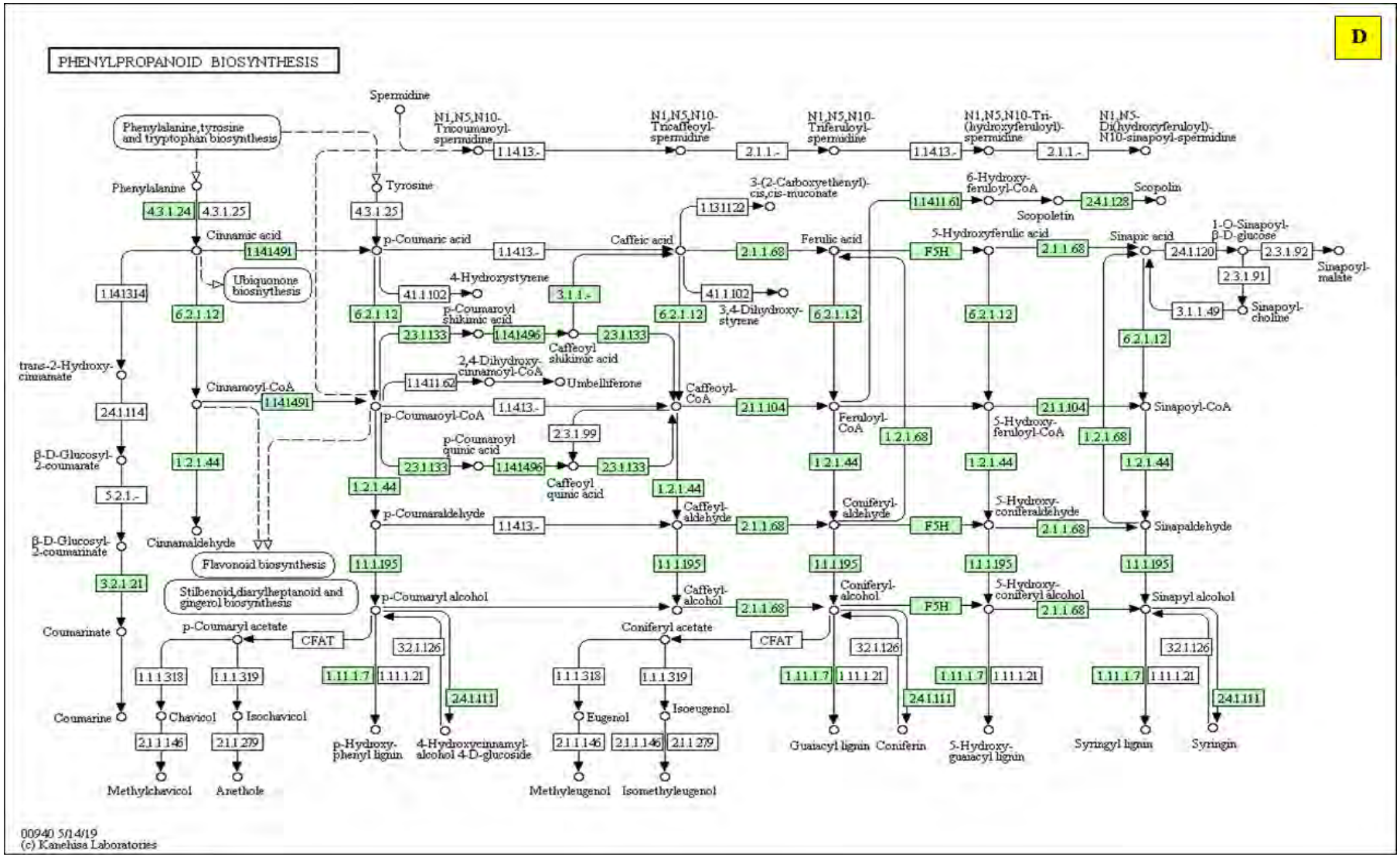
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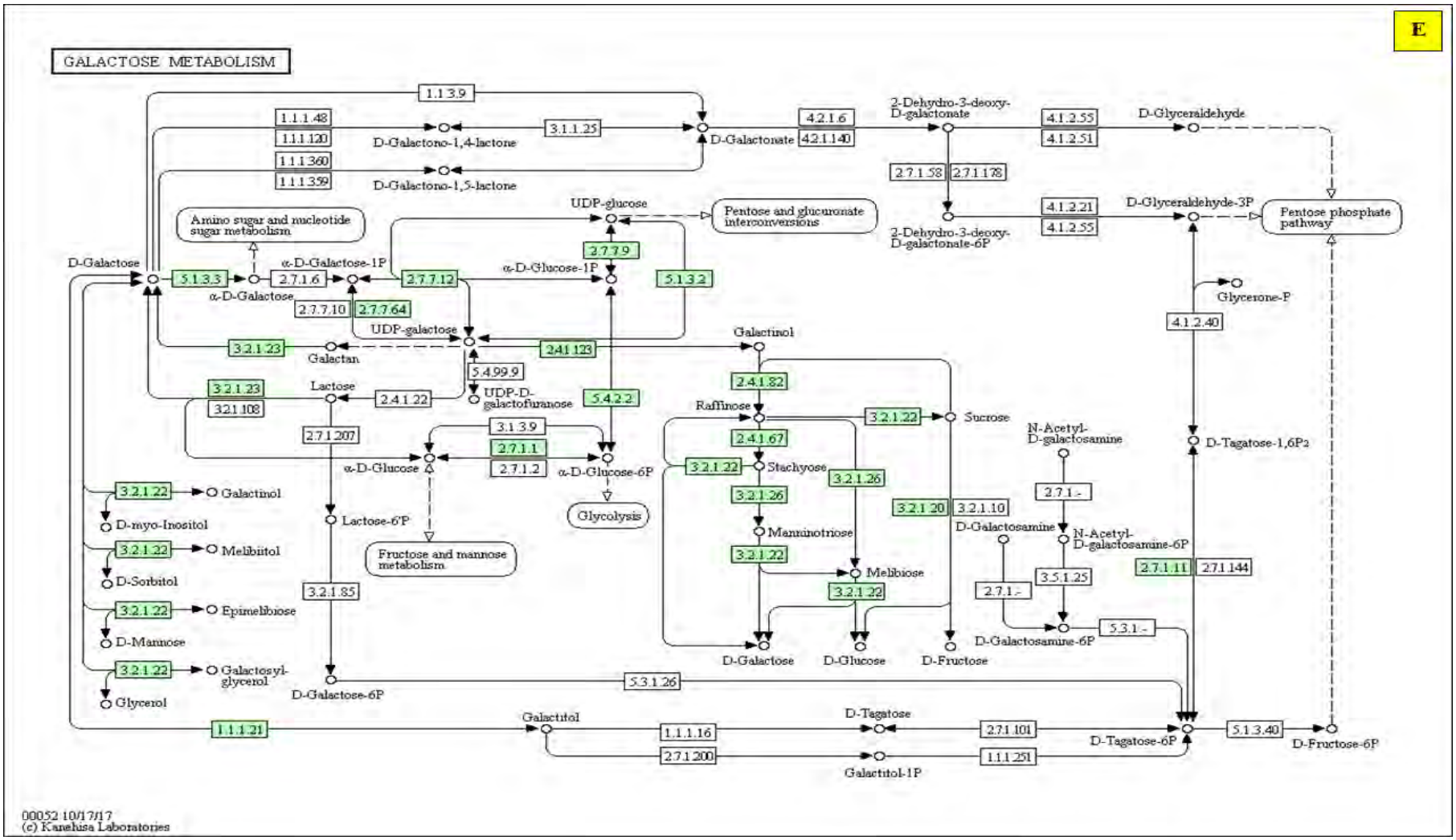
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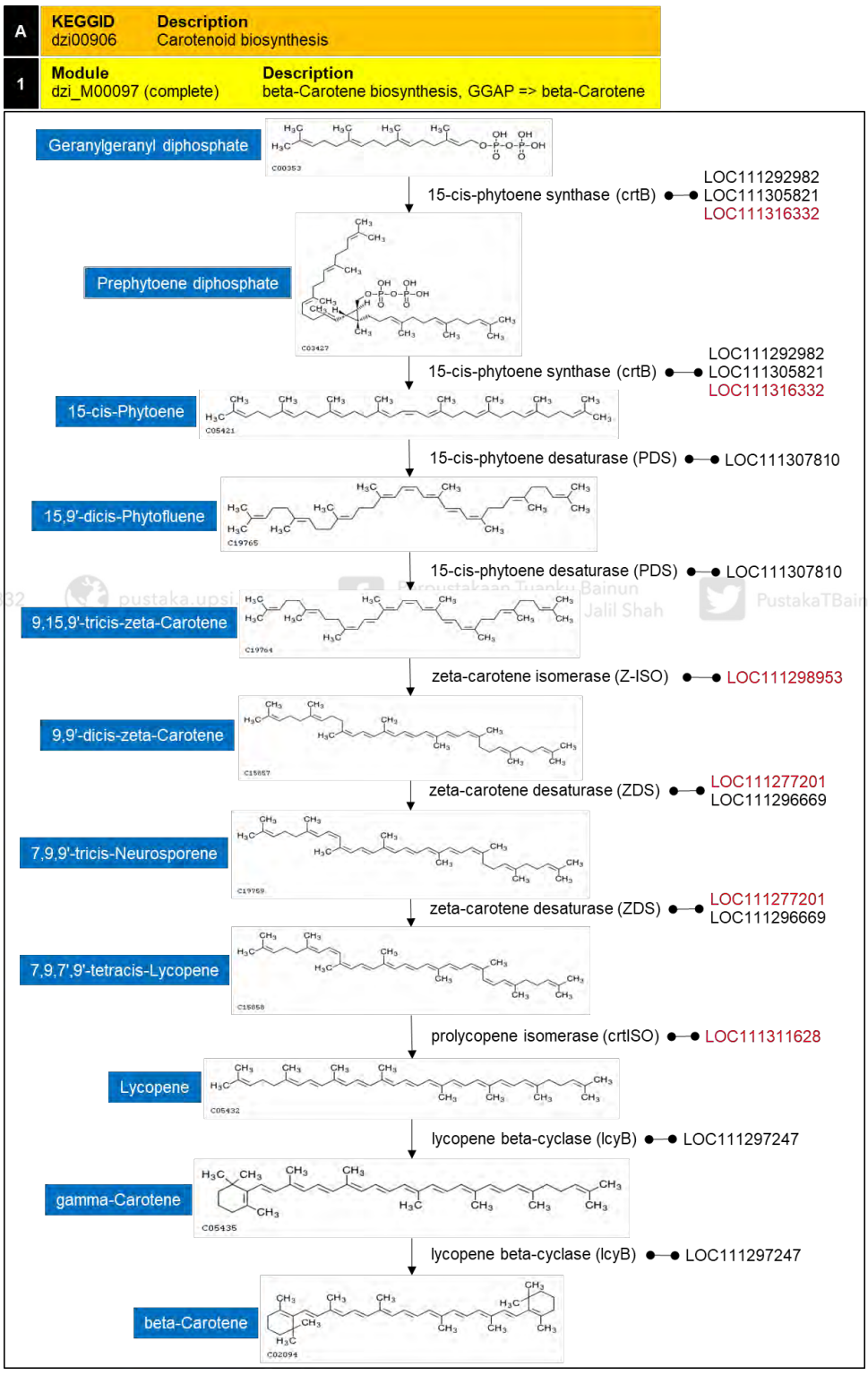
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Appendix C

Figure C1

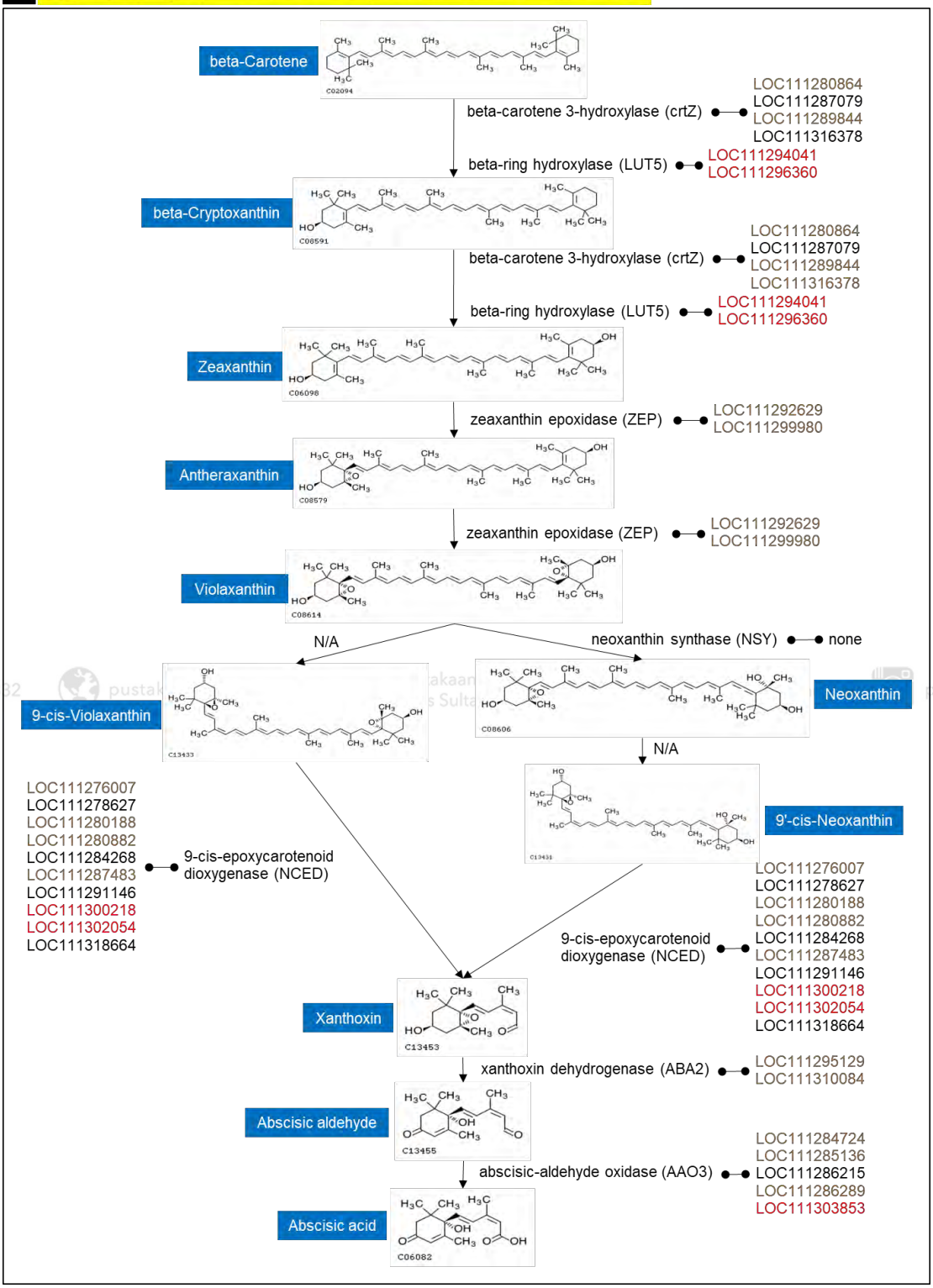
Carotenoid biosynthesis. (A1-A2) Graphical diagrams for KEGG modules for Carotenoid biosynthesis. (A) Genes up- and down-regulated in relation to the differential sample groups for Carotenoid biosynthesis



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A	KEGGID	Description
	dzi00906	Carotenoid biosynthesis
2	**Module**	**Description**
	dzi_M00372 (complete)	Abscisic acid biosynthesis, beta-carotene => abscisic acid



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A	KEGGID	Description
	dzi00906	Carotenoid biosynthesis

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
1	dzi_M00097 (complete): beta-Carotene biosynthesis, GGAP => beta-Carotene	15-cis-phytoene synthase (crtB)	LOC111292982																
2			LOC111305821																
3			LOC111316332						crtB	crtB	crtB	crtB		crtB					
4		15-cis-phytoene desaturase (PDS)	LOC111307810																
5		zeta-carotene isomerase (Z-ISO)	LOC111298953				Z-ISO	Z-ISO		Z-ISO	Z-ISO	Z-ISO	Z-ISO	Z-ISO	Z-ISO	Z-ISO			
6		zeta-carotene desaturase (ZDS)	LOC111277201							ZDS				ZDS		ZDS			
7			LOC111296669																
8		prolycopene isomerase (crtISO)	LOC111311628				crtISO	crtISO	crtISO	crtISO	crtISO	crtISO	crtISO						
9		lycopene beta-cyclase (lcyB)	LOC111297247																
10	dzi_M00372 (complete): Abscisic acid biosynthesis, beta-carotene => abscisic acid	beta-carotene 3-hydroxylase (crtZ)	LOC111280864		crtZ		crtZ		crtZ	crtZ	crtZ			crtZ			crtZ		
11			LOC111287079																
12			LOC111289844	crtZ			crtZ	crtZ		crtZ		crtZ	crtZ	crtZ	crtZ			crtZ	crtZ
13			LOC111316378				crtZ	crtZ		crtZ	crtZ	crtZ	crtZ	crtZ			crtZ	crtZ	
14		beta-ring hydroxylase (LUT5)	LOC111294041				LUT5	LUT5	LUT5	LUT5	LUT5	LUT5	LUT5	LUT5	LUT5				
15			LOC111296360				LUT5	LUT5	LUT5	LUT5	LUT5	LUT5	LUT5				LUT5		
16		zeaxanthin epoxidase (ZEP)	LOC111292629				ZEP	ZEP		ZEP	ZEP		ZEP		ZEP		ZEP		ZEP
17			LOC111299980	ZEP				ZEP	ZEP	ZEP			ZEP	ZEP	ZEP		ZEP		ZEP
18		9-cis-epoxycarotenoid dioxygenase (NCED)	LOC111276007	NCED	NCED		NCED	NCED		NCED	NCED	NCED	NCED	NCED	NCED			NCED	
19			LOC111278627																
20			LOC111280188	NCED	NCED	NCED		NCED		NCED	NCED	NCED		NCED	NCED		NCED	NCED	
21			LOC111280882	NCED	NCED	NCED	NCED	NCED	NCED	NCED		NCED	NCED	NCED	NCED	NCED			
22			LOC111284268																
23			LOC111287483		NCED		NCED	NCED		NCED			NCED				NCED	NCED	NCED
24			LOC111291146																
25			LOC111300218							NCED									
26			LOC111302054	NCED		NCED	NCED	NCED											
27			LOC111318664																
28		xanthoxin dehydrogenase (ABA2)	LOC111295129				ABA2	ABA2	ABA2	ABA2	ABA2	ABA2	ABA2	ABA2				ABA2	
29			LOC111310084				ABA2	ABA2		ABA2		ABA2	ABA2			ABA2		ABA2	
30		abscisic-aldehyde oxidase (AAO3)	LOC111284724	AA03			AA03						AA03	AA03				AA03	
31			LOC111285136				AA03	AA03		AA03	AA03	AA03	AA03	AA03	AA03	AA03	AA03		
32			LOC111286215																
33			LOC111286289				AA03	AA03		AA03		AA03	AA03	AA03	AA03	AA03	AA03	AA03	
34			LOC111303853	AA03	AA03	AA03	AA03	AA03				AA03	AA03				AA03		
35	Downstream pathway	(+)-abscisic acid 8'-hydroxylase (CYP707A)	LOC111275534	CYP707A															
36			LOC111290894					CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A		CYP707A	CYP707A
37			LOC111296235				CYP707A	CYP707A		CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A	CYP707A
38			LOC111303652			CYP707A	CYP707A	CYP707A				CYP707A		CYP707A	CYP707A	CYP707A		CYP707A	
39			LOC111308934				CYP707A	CYP707A		CYP707A		CYP707A	CYP707A	CYP707A	CYP707A	CYP707A			CYP707A
40			LOC111314846					CYP707A		CYP707A							CYP707A		
41		9-cis-beta-carotene 9',10'-cleaving dioxygenase (CCD7)	LOC111304574											CCD7	CCD7				CCD7
42		carotenoid epsilon hydroxylase (LUT1)	LOC111296450				LUT1	LUT1	LUT1	LUT1		LUT1	LUT1	LUT1		LUT1			
43		violaxanthin de-epoxidase (VDE)	LOC111277831					VDE	VDE	VDE				VDE					

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Note,

Highlight green = Up-regulated genes;

Highlight red = Down-regulated genes;

Highlight brown = Both up and down-regulated genes;

Highlight black = Genes with no differential sample groups associated (indicate the genes with non-significant expression change).

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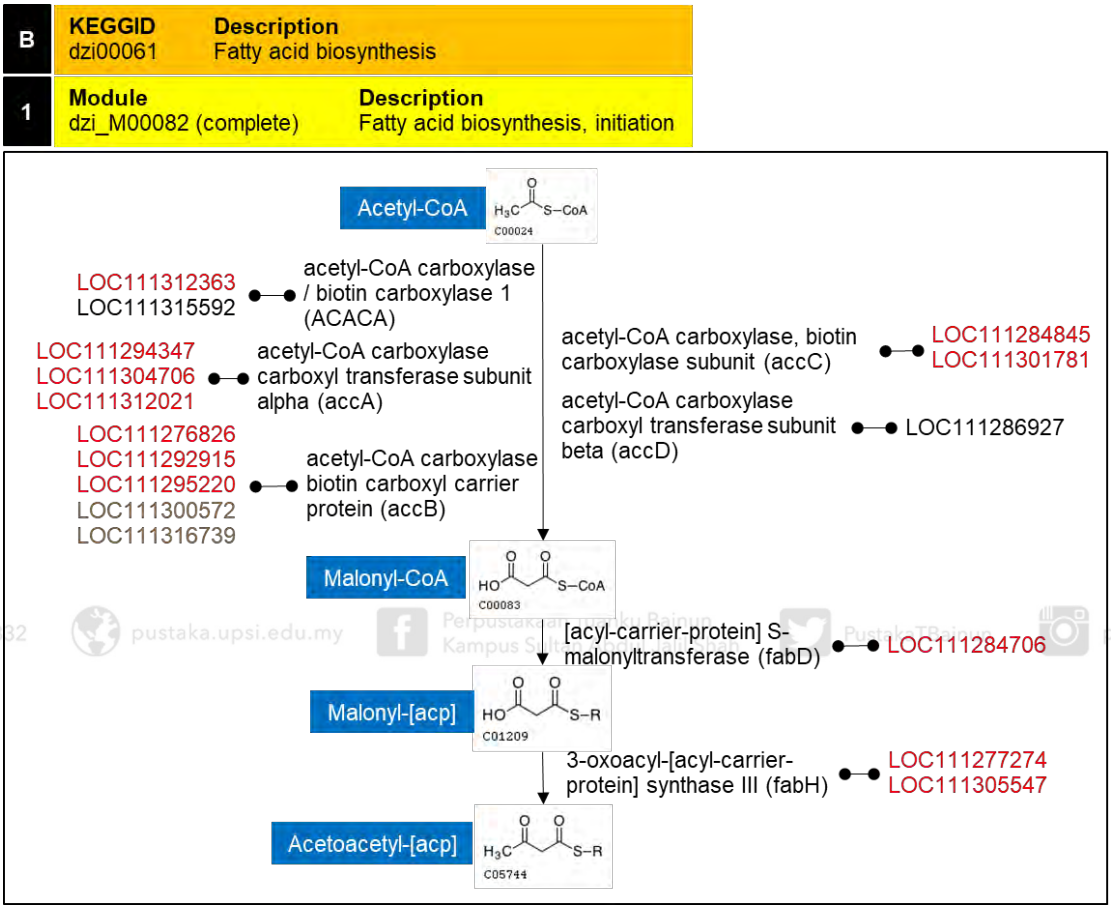
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Appendix D

Figure D1

Fatty acid biosynthesis. (B1-B3) Graphical diagrams for KEGG modules for Fatty acid biosynthesis. (B) Genes up- and down-regulated in relation to the differential sample groups for Fatty acid biosynthesis

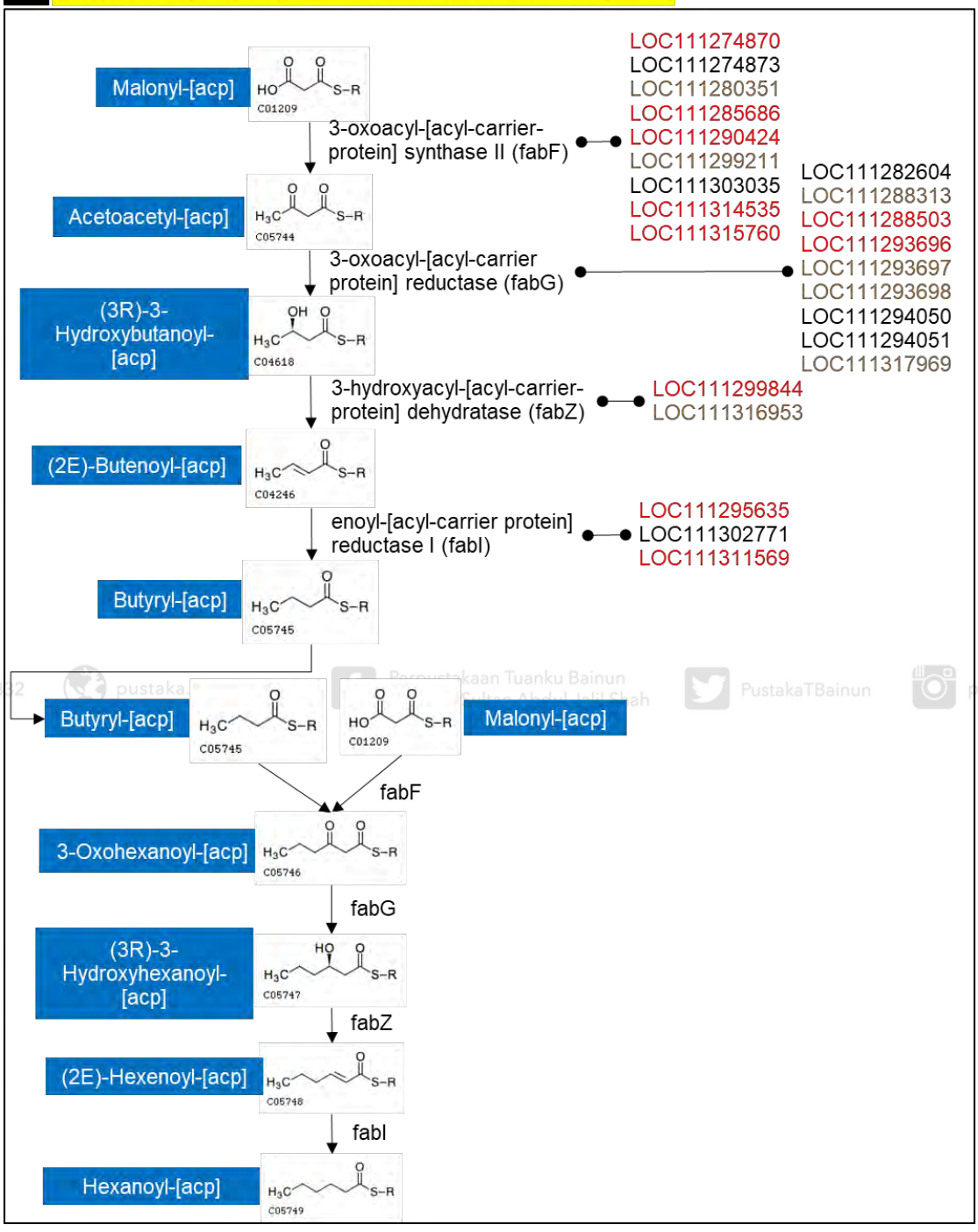


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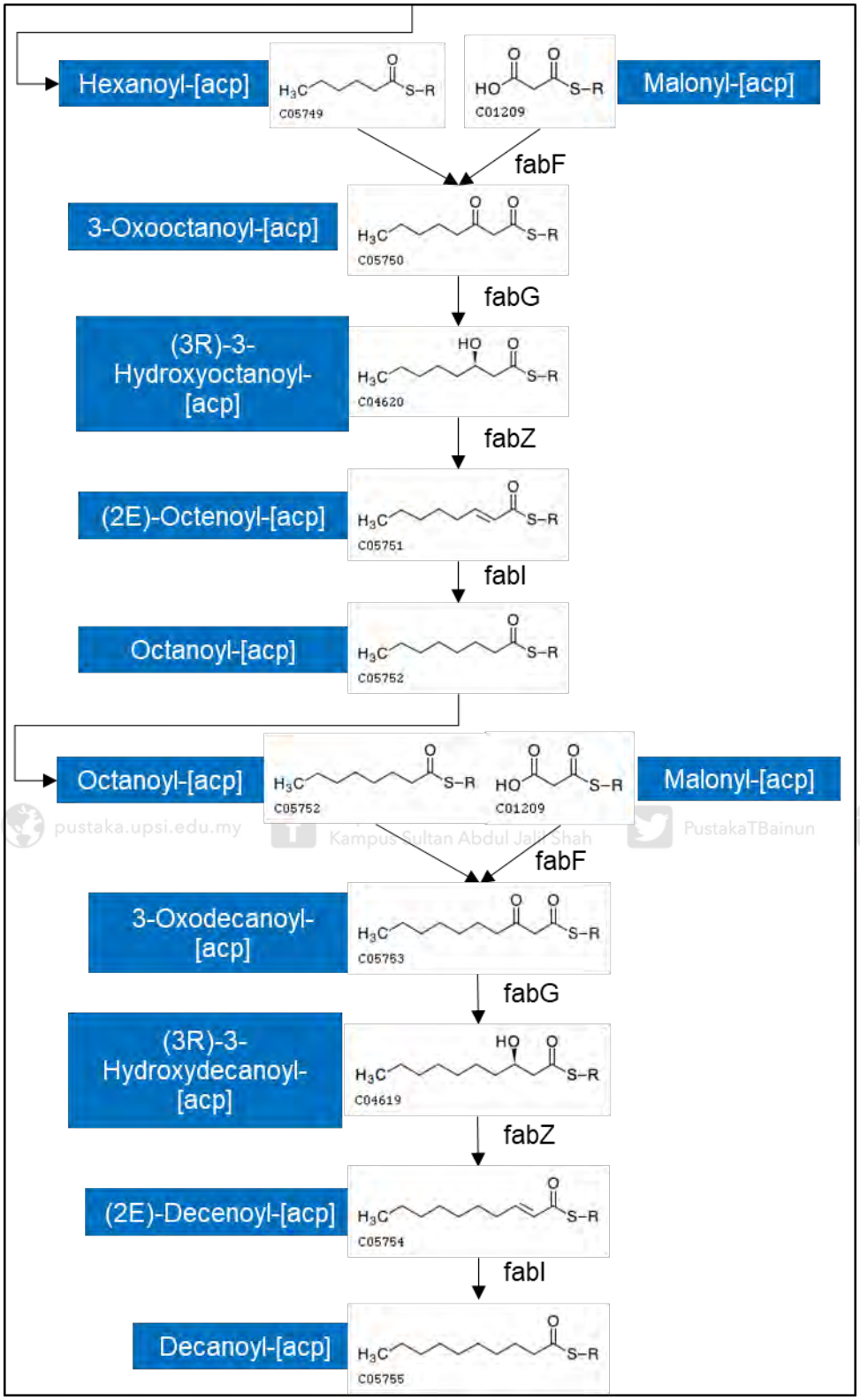
B	KEGGID	Description
	dzi00061	Fatty acid biosynthesis

2	Module	Description
	dzi_M00083 (complete)	Fatty acid biosynthesis, elongation



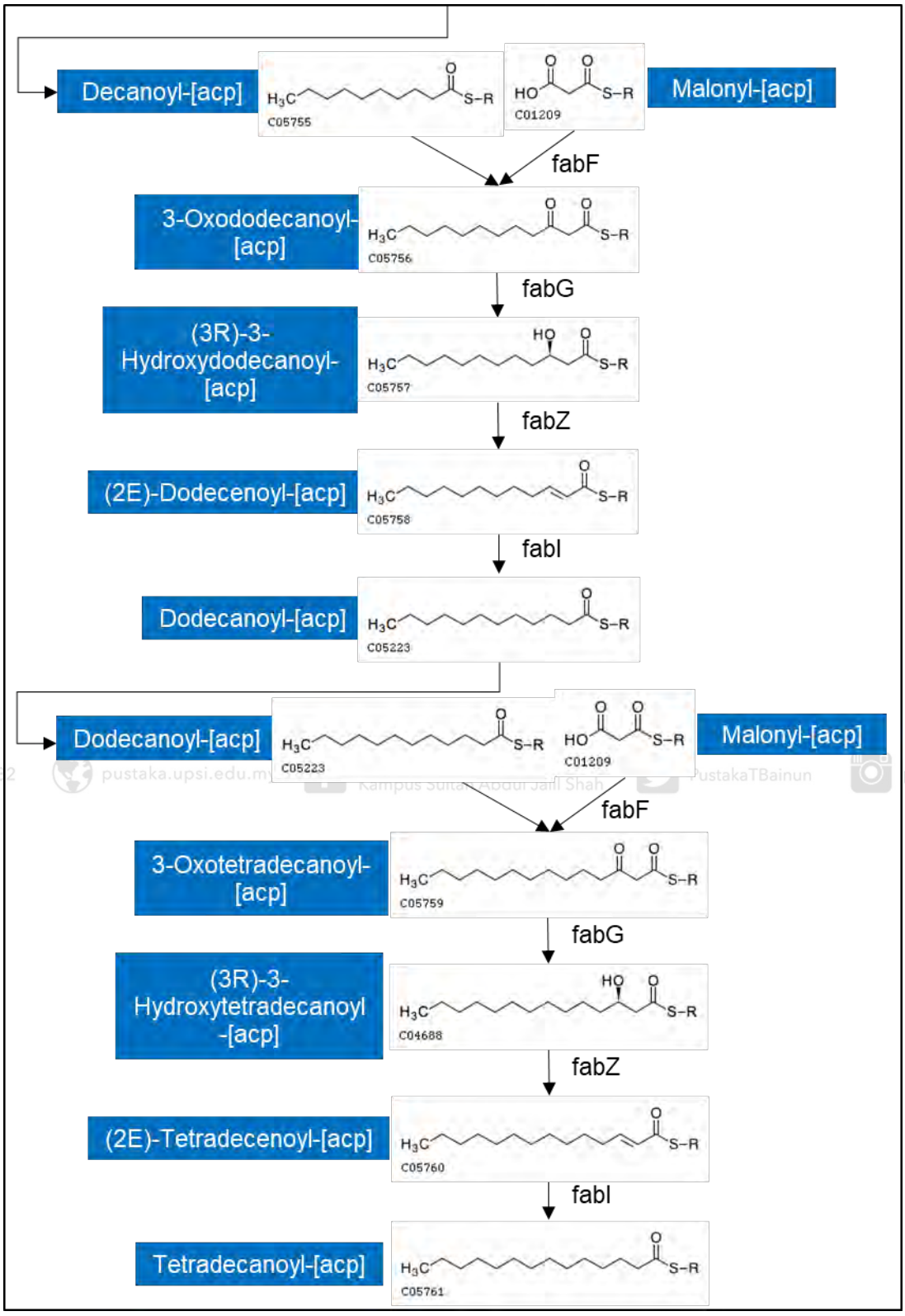
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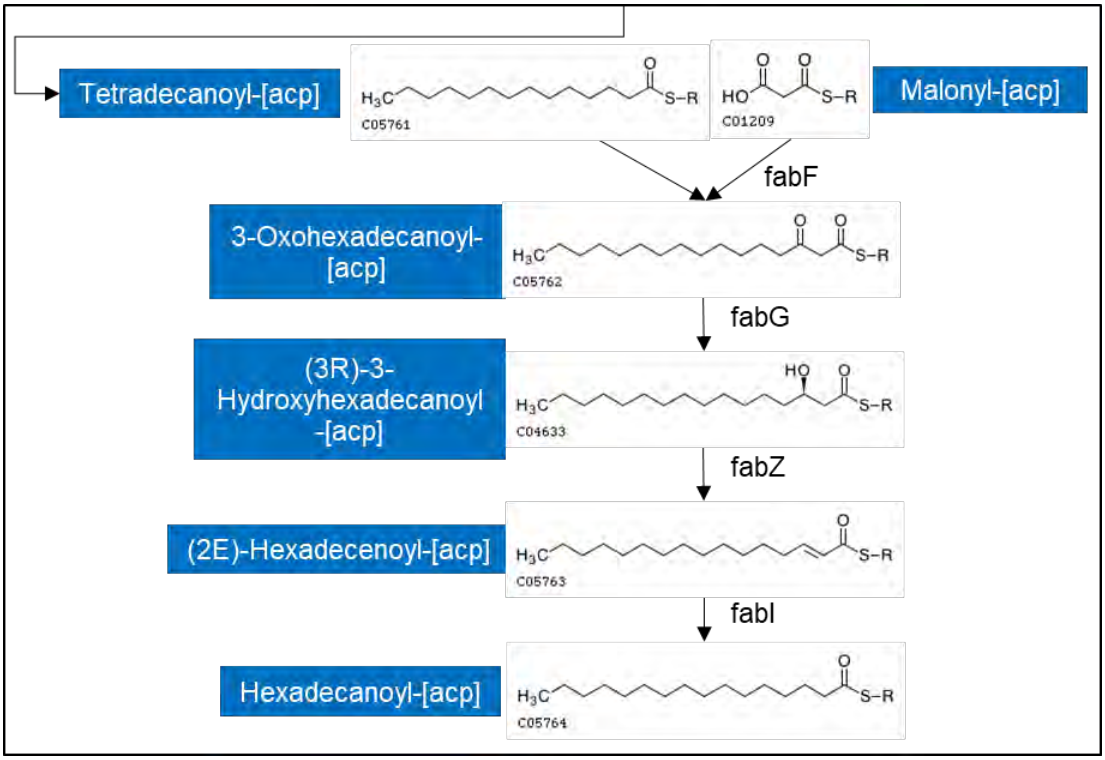
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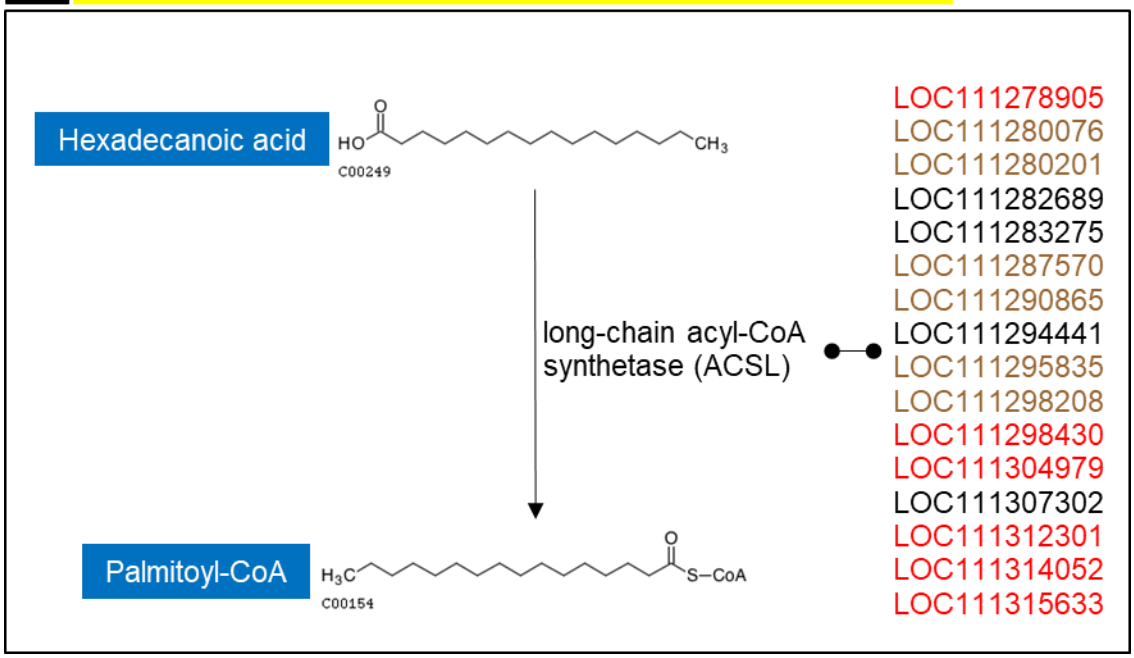
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B	KEGGID	Description
	dzi00061	Fatty acid biosynthesis

3	Module	Description
	dzi_M00086 (complete)	beta-Oxidation, acyl-CoA synthesis



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B **KEGGID** dzi00061 **Description** Fatty acid biosynthesis

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
1	Fatty acid biosynthesis, initiation	acetyl-CoA carboxylase / biotin carboxylase 1 (ACACA)	LOC111312363							ACACA		ACACA							ACACA
2			LOC111315592																
3		acetyl-CoA carboxylase carboxyl transferase subunit alpha (accA)	LOC111294347				accA	accA		accA		accA	accA	accA	accA	accA	accA		accA
4			LOC111304706						accA										
5			LOC111312021				accA	accA		accA	accA	accA	accA	accA	accA	accA			
6		acetyl-CoA carboxylase biotin carboxyl carrier protein (accB)	LOC111276826								accB			accB	accB		accB		
7			LOC111292915				accB	accB		accB			accB				accB		
8			LOC111295220				accB	accB		accB	accB	accB	accB				accB		
9			LOC111300572				accB	accB		accB		accB	accB	accB	accB		accB		
10			LOC111316739				accB	accB		accB	accB	accB	accB		accB		accB		
11		acetyl-CoA carboxylase, biotin carboxylase subunit (accC)	LOC111284845				accC	accC	accC	accC	accC	accC	accC	accC	accC	accC			
12			LOC111301781				accC	accC		accC	accC	accC	accC	accC	accC				accC
13		acetyl-CoA carboxylase carboxyl transferase subunit beta (accD)	LOC111286927																
14		[acyl-carrier-protein] S-malonyltransferase (fabD)	LOC111284706				fabD	fabD		fabD		fabD					fabD		
15		3-oxoacyl-[acyl-carrier-protein] synthase III (fabH)	LOC111277274							fabH			fabH		fabH				
16			LOC111305547				fabH	fabH		fabH	fabH	fabH	fabH	fabH	fabH				fabH
17	Fatty acid biosynthesis, elongation	3-oxoacyl-[acyl-carrier-protein] synthase II (fabF)	LOC111274870				fabF	fabF		fabF		fabF		fabF	fabF				
18			LOC111274873																
19			LOC111280351				fabF	fabF		fabF	fabF	fabF	fabF	fabF			fabF	fabF	fabF
20			LOC111285686				fabF	fabF		fabF	fabF	fabF	fabF	fabF	fabF		fabF		fabF
21			LOC111290424				fabF	fabF		fabF	fabF	fabF	fabF	fabF	fabF	fabF			
22			LOC111299211				fabF	fabF		fabF	fabF	fabF	fabF		fabF		fabF	fabF	
23			LOC111303035																
24			LOC111314535				fabF	fabF				fabF		fabF	fabF		fabF		fabF
25			LOC111315760				fabF	fabF	fabF	fabF		fabF	fabF	fabF					
26		3-oxoacyl-[acyl-carrier protein] reductase (fabG)	LOC111282604																
27			LOC111288313				fabG	fabG	fabG	fabG	fabG	fabG	fabG				fabG		fabG
28			LOC111288503				fabG	fabG	fabG	fabG		fabG	fabG	fabG	fabG		fabG	fabG	fabG
29			LOC111293696										fabG						
30			LOC111293697							fabG							fabG		
31			LOC111293698	fabG		fabG	fabG	fabG	fabG	fabG		fabG		fabG	fabG	fabG	fabG		
32			LOC111294050																
33			LOC111294051																
34			LOC111317969				fabG	fabG		fabG	fabG	fabG	fabG	fabG	fabG		fabG	fabG	fabG
35		3-hydroxyacyl-[acyl-carrier-protein] dehydratase (fabZ)	LOC111299844				fabZ	fabZ			fabZ	fabZ	fabZ	fabZ	fabZ				
36			LOC111316953				fabZ	fabZ	fabZ	fabZ	fabZ	fabZ	fabZ	fabZ				fabZ	
37		enoyl-[acyl-carrier protein] reductase I (fabI)	LOC111295635				fabI	fabI	fabI	fabI	fabI	fabI	fabI	fabI		fabI	fabI		
38			LOC111302771																
39			LOC111311569				fabI	fabI		fabI	fabI	fabI	fabI	fabI	fabI	fabI	fabI		

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B	KEGGID	Description
	dzi00061	Fatty acid biosynthesis

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
40	beta-Oxidation, acyl-CoA synthesis	long-chain acyl-CoA synthetase (ACSL)	LOC111278905				ACSL	ACSL	ACSL	ACSL		ACSL	ACSL	ACSL			ACSL		
41			LOC111280076			ACSL				ACSL	ACSL	ACSL		ACSL	ACSL			ACSL	
42			LOC111280201				ACSL			ACSL				ACSL			ACSL		
43			LOC111282689																
44			LOC111283275																
45			LOC111287570				ACSL	ACSL	ACSL	ACSL		ACSL	ACSL	ACSL	ACSL				
46			LOC111290865							ACSL				ACSL	ACSL		ACSL	ACSL	ACSL
47			LOC111294441																
48			LOC111295835			ACSL	ACSL	ACSL		ACSL							ACSL	ACSL	
49			LOC111298208					ACSL		ACSL	ACSL	ACSL	ACSL	ACSL	ACSL		ACSL		
50			LOC111298430		ACSL		ACSL	ACSL					ACSL						
51			LOC111304979						ACSL					ACSL	ACSL				
52			LOC111307302																
53			LOC111312301							ACSL				ACSL	ACSL				
54			LOC111314052				ACSL	ACSL		ACSL		ACSL	ACSL	ACSL	ACSL		ACSL		ACSL
55			LOC111315633				ACSL	ACSL	ACSL	ACSL	ACSL	ACSL	ACSL	ACSL	ACSL	ACSL	ACSL		
56	Downstream pathway	malonyl-CoA/methylmalonyl-CoA synthetase (ACSF3)	LOC111278937				ACSF3	ACSF3		ACSF3		ACSF3	ACSF3	ACSF3	ACSF3		ACSF3		ACSF3
57		acyl-[acyl-carrier-protein] desaturase (FAB2)	LOC111279287				FAB2	FAB2	FAB2	FAB2			FAB2		FAB2		FAB2	FAB2	
58			LOC111284142					FAB2	FAB2	FAB2		FAB2		FAB2	FAB2		FAB2		
59			LOC111287575				FAB2	FAB2		FAB2	FAB2	FAB2	FAB2	FAB2	FAB2	FAB2	FAB2		
60			LOC111294425				FAB2	FAB2		FAB2	FAB2	FAB2		FAB2	FAB2		FAB2		
61		fatty acyl-ACP thioesterase A (FATA)	LOC111283384				FATA	FATA	FATA	FATA		FATA	FATA		FATA				
62			LOC111283778	FATA			FATA	FATA	FATA		FATA	FATA	FATA	FATA	FATA	FATA	FATA		
63		fatty acyl-ACP thioesterase B (FATB)	LOC111284999	FATB			FATB	FATB		FATB		FATB		FATB	FATB			FATB	
64			LOC111299072				FATB	FATB		FATB	FATB	FATB	FATB	FATB	FATB	FATB			
65			LOC111303232	FATB			FATB	FATB	FATB	FATB		FATB	FATB	FATB		FATB	FATB		
66		mitochondrial enoyl-[acyl-carrier protein] reductase / trans-2-enoyl-CoA reductase (MECR)	LOC111285194				MECR	MECR	MECR	MECR	MECR	MECR	MECR	MECR	MECR		MECR		
67		Related to carboxyl transferase	novel.3671						NOVEL	NOVEL						NOVEL			

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Note,

Highlight green = Up-regulated genes;

Highlight red = Down-regulated genes;

Highlight brown = Both up and down-regulated genes;

Highlight black = Genes with no differential sample groups associated (indicate the genes with non-significant expression change).

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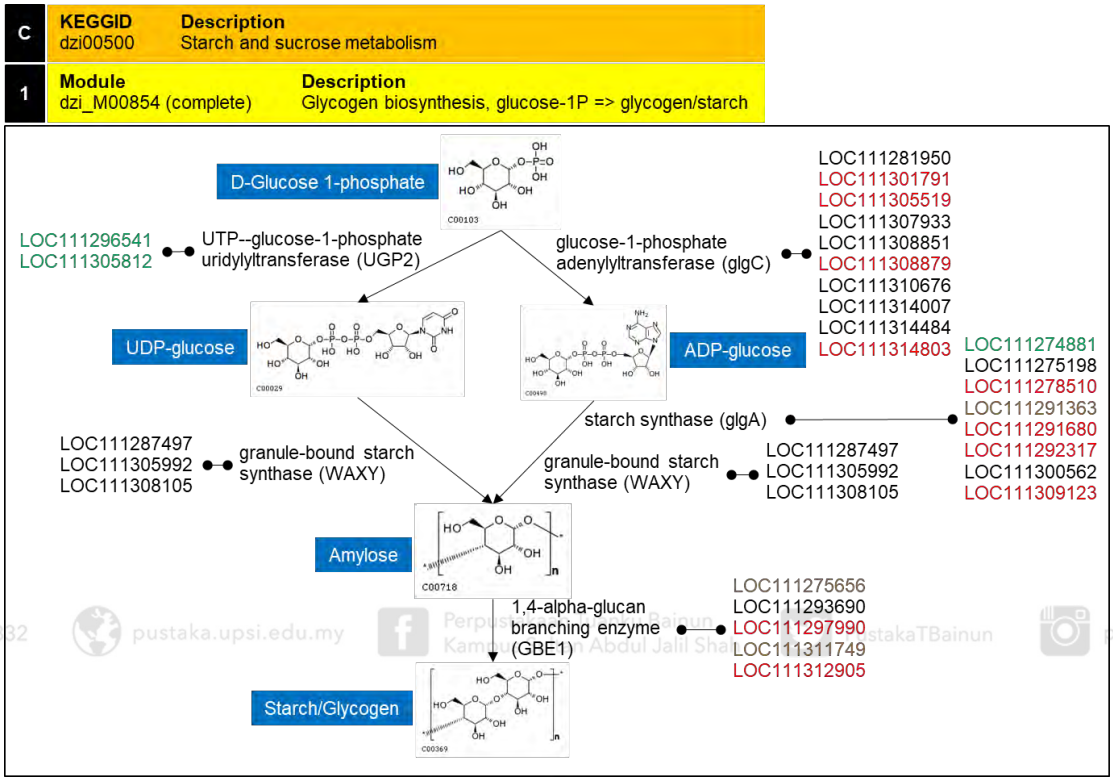
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Appendix E

Figure E1

Starch and sucrose metabolism. (C1-C3) Graphical diagrams for KEGG modules for Starch and sucrose metabolism. (C) Genes up- and down-regulated in relation to the differential sample groups for Starch and sucrose metabolism

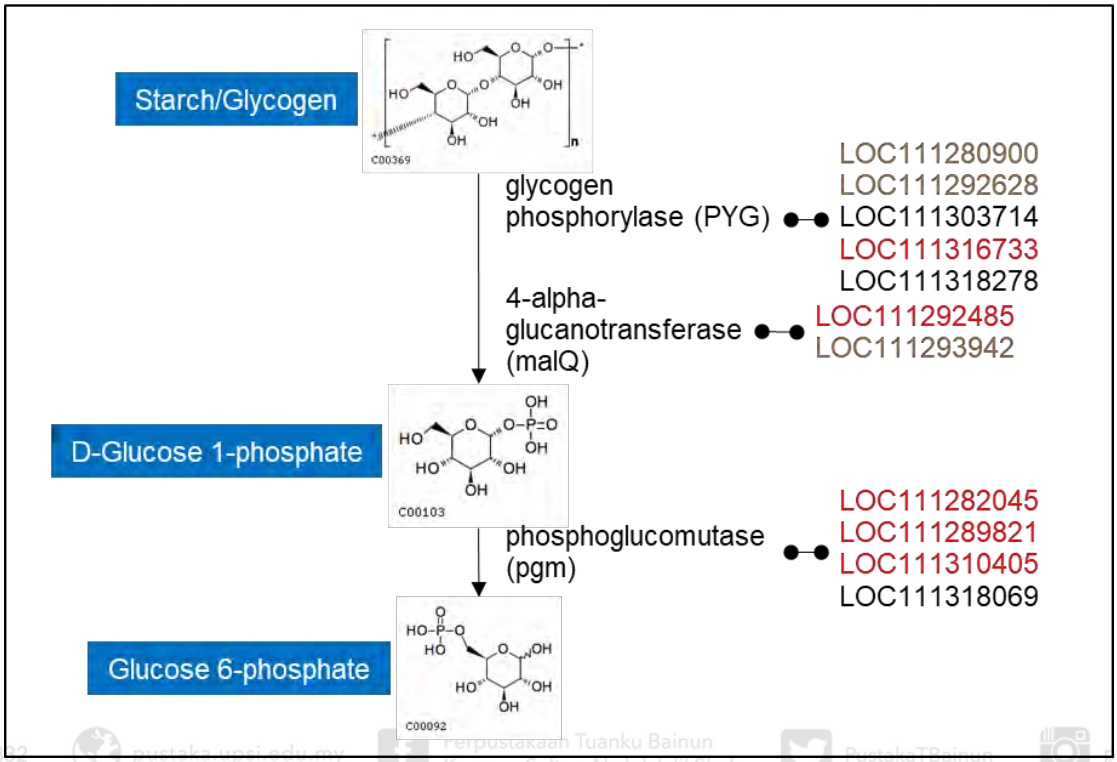


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C	KEGGID	Description
	dzi00500	Starch and sucrose metabolism

2	Module	Description
	dzi_M00855 (1 block missing)	Glycogen degradation, glycogen => glucose-6P

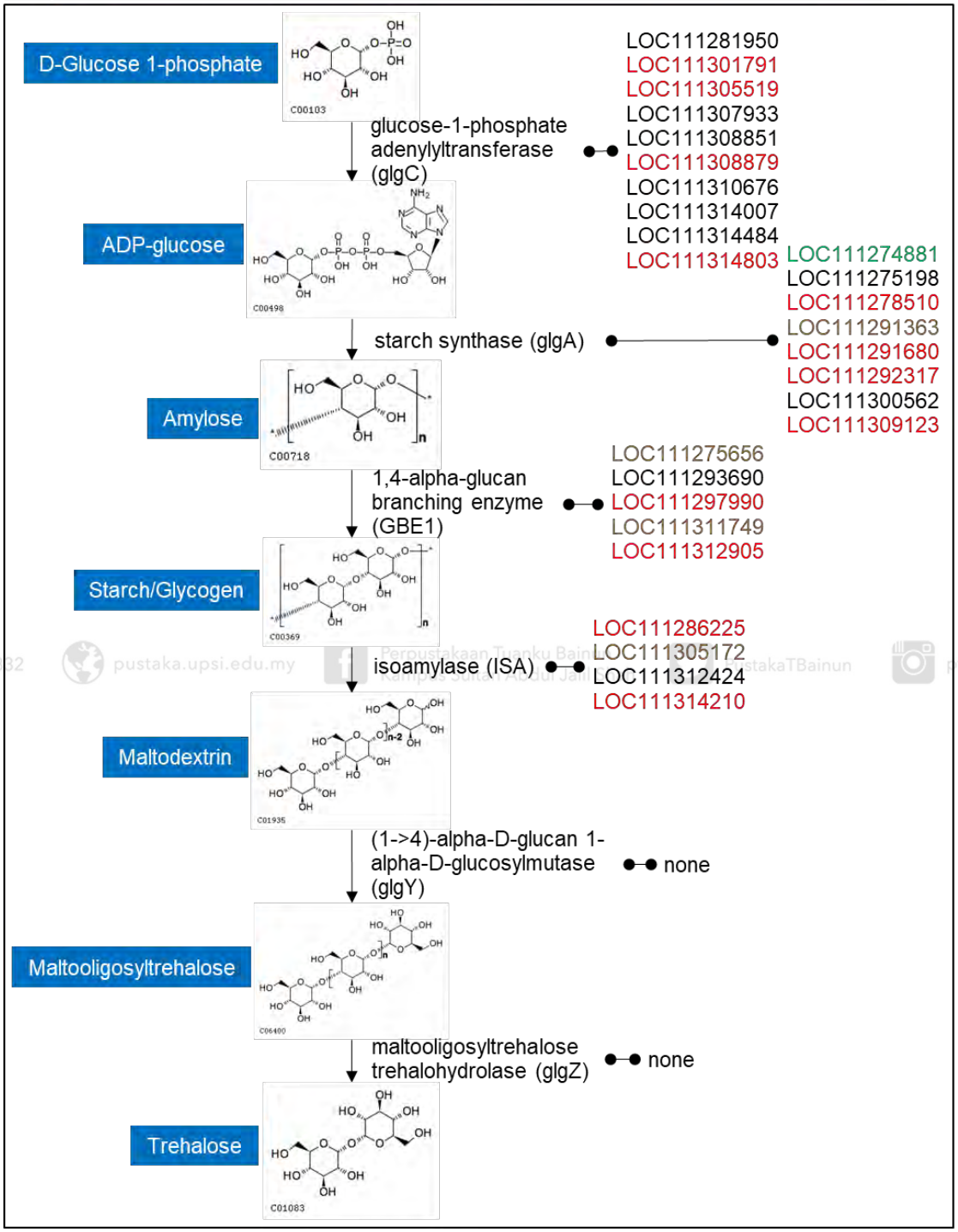


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C	KEGGID	Description
	dzi00500	Starch and sucrose metabolism

3	Module	Description
	dzi_M00565 (2 block missing)	Trehalose biosynthesis, D-glucose 1P => trehalose



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C	KEGGID	Description
	dzi00500	Starch and sucrose metabolism

				MATURATION					RIPENING					DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
1	Glycogen biosynthesis, glucose-1P => glycogen/starch	UTP--glucose-1-phosphate uridylyltransferase (UGP2)	LOC111296541											UGP2					
2			LOC111305812									UGP2	UGP2						
3	Glycogen biosynthesis, glucose-1P => glycogen/starch & Trehalose biosynthesis, D-glucose 1P => trehalose	glucose-1-phosphate adenylyltransferase (glgC)	LOC111281950																
4			LOC111301791							glgC									glgC
5			LOC111305519									glgC			glgC		glgC		glgC
6			LOC111307933																
7			LOC111308851																
8			LOC111308879				glgC	glgC		glgC		glgC	glgC						
9			LOC111310676																
10			LOC111314007																
11			LOC111314484																
12			LOC111314803							glgC									
13	Glycogen biosynthesis, glucose-1P => glycogen/starch	granule-bound starch synthase (WAXY)	LOC111287497																
14			LOC111305992																
15			LOC111308105																
16	Glycogen biosynthesis, glucose-1P => glycogen/starch & Trehalose biosynthesis, D-glucose 1P => trehalose	starch synthase (glgA)	LOC111274881					glgA			glgA			glgA					
17			LOC111275198																
18			LOC111278510				glgA			glgA			glgA		glgA				
19			LOC111291363					glgA		glgA		glgA			glgA		glgA		
20			LOC111291680				glgA			glgA		glgA					glgA		
21			LOC111292317				glgA	glgA	glgA	glgA		glgA	glgA	glgA	glgA				glgA
22			LOC111300562																
23			LOC111309123				glgA	glgA	glgA	glgA	glgA	glgA	glgA	glgA	glgA		glgA		
24	Glycogen biosynthesis, glucose-1P => glycogen/starch & Trehalose biosynthesis, D-glucose 1P =>	1,4-alpha-glucan branching enzyme (GBE1)	LOC111275656				GBE1	GBE1		GBE1			GBE1				GBE1		
25			LOC111293690																
26			LOC111297990				GBE1	GBE1	GBE1	GBE1	GBE1	GBE1	GBE1	GBE1		GBE1			GBE1
27			LOC111311749	GBE1	GBE1	GBE1	GBE1	GBE1		GBE1				GBE1	GBE1		GBE1		GBE1
28			LOC111312905				GBE1	GBE1	GBE1	GBE1		GBE1	GBE1	GBE1		GBE1			
29	Glycogen degradation, glycogen => glucose-6P	glycogen phosphorylase (PYG)	LOC111280900	PYG						PYG		PYG							
30			LOC111292628					PYG	PYG		PYG	PYG	PYG	PYG		PYG	PYG		
31			LOC111303714																
32			LOC111316733				PYG	PYG				PYG		PYG	PYG		PYG	PYG	
33			LOC111318278																
34	Glycogen degradation, glycogen => glucose-6P	4-alpha-glucanotransferase (malQ)	LOC111292485				malQ	malQ	malQ	malQ			malQ		malQ		malQ		
35			LOC111293942				malQ	malQ		malQ		malQ	malQ	malQ	malQ	malQ	malQ		
36	Glycogen degradation, glycogen => glucose-6P	phosphoglucomutase (pgm)	LOC111282045				pgm	pgm		pgm		pgm	pgm						pgm
37			LOC111289821												pgm	pgm	pgm	pgm	
38			LOC111310405				pgm	pgm		pgm		pgm	pgm	pgm	pgm	pgm	pgm		pgm
39			LOC111318069																
40	Trehalose biosynthesis, D-glucose 1P => trehalose	isoamylase (ISA)	LOC111286225				ISA	ISA		ISA	ISA	ISA	ISA	ISA	ISA		ISA		
41			LOC111305172				ISA	ISA		ISA						ISA	ISA		
42			LOC111312424																
43			LOC111314210				ISA	ISA		ISA		ISA	ISA	ISA	ISA		ISA		ISA

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C	KEGGID	Description
	dzi00500	Starch and sucrose metabolism

				MATURATION						RIPENING						DEHISCENCE				
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
44	Downstream pathway	alpha-amylase (AMY)	LOC111282304					AMY				AMY			AMY				AMY
45			LOC111287216					AMY	AMY		AMY	AMY	AMY	AMY		AMY		AMY	
46			LOC111292875						AMY	AMY							AMY		AMY
47			LOC111312610				AMY	AMY		AMY		AMY		AMY	AMY		AMY		
48			LOC111312611				AMY	AMY		AMY	AMY	AMY	AMY						
49			LOC111312868				AMY	AMY		AMY		AMY	AMY	AMY	AMY	AMY	AMY		
50			LOC111318372				AMY	AMY		AMY		AMY	AMY				AMY		AMY
51		beta-glucosidase (bglB)	LOC111290180										bglB						
52			LOC111302307														bglB		
53		beta-glucosidase (bglX)	LOC111277028							bglX						bglX			
54			LOC111277804	bglX			bglX	bglX		bglX	bglX	bglX	bglX	bglX	bglX	bglX	bglX		bglX
55			LOC111295127				bglX	bglX	bglX	bglX	bglX	bglX		bglX	bglX	bglX	bglX	bglX	
56			LOC111296009				bglX							bglX					
57			LOC111303724				bglX	bglX		bglX			bglX	bglX	bglX			bglX	bglX
58			LOC111303852				bglX			bglX		bglX	bglX		bglX		bglX		bglX
59			LOC111309915							bglX			bglX			bglX			
60		sucrose-phosphate synthase (E2.4.1.14)	LOC111276696				E2.4.1.14	E2.4.1.14		E2.4.1.14		E2.4.1.14	E2.4.1.14			E2.4.1.14			
61			LOC111279832	E2.4.1.14		E2.4.1.14	E2.4.1.14	E2.4.1.14	E2.4.1.14	E2.4.1.14		E2.4.1.14		E2.4.1.14	E2.4.1.14		E2.4.1.14	E2.4.1.14	E2.4.1.14
62			LOC111284186	E2.4.1.14			E2.4.1.14	E2.4.1.14		E2.4.1.14	E2.4.1.14	E2.4.1.14	E2.4.1.14		E2.4.1.14	E2.4.1.14	E2.4.1.14		E2.4.1.14
63			LOC111299386					E2.4.1.14		E2.4.1.14	E2.4.1.14		E2.4.1.14	E2.4.1.14			E2.4.1.14	E2.4.1.14	
64			LOC111303296				E2.4.1.14	E2.4.1.14				E2.4.1.14		E2.4.1.14			E2.4.1.14		
65		fructokinase (E2.7.1.4)	LOC111287537	E2.7.1.4			E2.7.1.4	E2.7.1.4		E2.7.1.4		E2.7.1.4							
66			LOC111307244							E2.7.1.4									
67			LOC111313184				E2.7.1.4	E2.7.1.4		E2.7.1.4	E2.7.1.4		E2.7.1.4	E2.7.1.4	E2.7.1.4		E2.7.1.4		E2.7.1.4
68		beta-amylase (E3.2.1.2)	LOC111289613					E3.2.1.2			E3.2.1.2	E3.2.1.2					E3.2.1.2	E3.2.1.2	
69			LOC111289823				E3.2.1.2	E3.2.1.2		E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2		E3.2.1.2		
70			LOC111296125							E3.2.1.2			E3.2.1.2	E3.2.1.2	E3.2.1.2				E3.2.1.2
71			LOC111296153					E3.2.1.2					E3.2.1.2	E3.2.1.2			E3.2.1.2		
72			LOC111304731									E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2				
73			LOC111305012				E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2		E3.2.1.2	E3.2.1.2		E3.2.1.2	E3.2.1.2	E3.2.1.2		E3.2.1.2
74			LOC111306779	E3.2.1.2			E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2	E3.2.1.2					
75		endoglucanase (E3.2.1.4)	LOC111275321					E3.2.1.4		E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4			E3.2.1.4		
76			LOC111281000				E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4		E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4			
77			LOC111286386				E3.2.1.4	E3.2.1.4		E3.2.1.4		E3.2.1.4	E3.2.1.4	E3.2.1.4		E3.2.1.4			
78			LOC111287717											E3.2.1.4	E3.2.1.4	E3.2.1.4			
79			LOC111290701				E3.2.1.4	E3.2.1.4		E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4		E3.2.1.4		E3.2.1.4		
80			LOC111294191				E3.2.1.4							E3.2.1.4		E3.2.1.4			
81			LOC111294284	E3.2.1.4		E3.2.1.4	E3.2.1.4	E3.2.1.4		E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4	E3.2.1.4				E3.2.1.4	
82			LOC111297235							E3.2.1.4			E3.2.1.4	E3.2.1.4	E3.2.1.4				
83			LOC111306051				E3.2.1.4	E3.2.1.4		E3.2.1.4		E3.2.1.4	E3.2.1.4						
84		beta-glucosidase (E3.2.1.21)	LOC111275745			E3.2.1.21	E3.2.1.21	E3.2.1.21		E3.2.1.21		E3.2.1.21	E3.2.1.21	E3.2.1.21					
85			LOC111276820				E3.2.1.21	E3.2.1.21		E3.2.1.21		E3.2.1.21		E3.2.1.21	E3.2.1.21				
86			LOC111318270							E3.2.1.21	E3.2.1.21	E3.2.1.21	E3.2.1.21	E3.2.1.21	E3.2.1.21	E3.2.1.21			
87		ectonucleotide pyrophosphatase/phosphodiesterase family member 1/3 (ENPP1_3)	LOC111284529				ENPP1_3	ENPP1_3	ENPP1_3	ENPP1_3	ENPP1_3	ENPP1_3	ENPP1_3	ENPP1_3	ENPP1_3		ENPP1_3		
88			LOC111300869				ENPP1_3	ENPP1_3		ENPP1_3		ENPP1_3	ENPP1_3						

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C	KEGGID	Description
	dzi00500	Starch and sucrose metabolism

				MATURATION					RIPENING					DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
89	Downstream pathway	glucan endo-1,3-beta-glucosidase 1/2/3 (GN1_2_3)	LOC111275206					GN1_2_3		GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	
90			LOC111280923				GN1_2_3	GN1_2_3					GN1_2_3	GN1_2_3			GN1_2_3		
91			LOC111283670					GN1_2_3		GN1_2_3			GN1_2_3						
92			LOC111305721				GN1_2_3	GN1_2_3		GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3	GN1_2_3		GN1_2_3	GN1_2_3	
93			LOC111318079	GN1_2_3			GN1_2_3	GN1_2_3											
94		glucan endo-1,3-beta-glucosidase 4 (GN4)	LOC111284621				GN4	GN4		GN4		GN4	GN4	GN4	GN4				
95			LOC111301805				GN4	GN4		GN4		GN4	GN4	GN4				GN4	
96		glucan endo-1,3-beta-glucosidase 5/6 (GN5_6)	LOC111285347				GN5_6						GN5_6				GN5_6		
97			LOC111302810				GN5_6	GN5_6		GN5_6		GN5_6						GN5_6	
98		glucose-6-phosphate isomerase (GPI)	LOC111312699											GPI	GPI				
99			LOC111315361				GPI		GPI			GPI		GPI					
100			LOC111315541				GPI			GPI		GPI		GPI			GPI		
101		beta-fructofuranosidase (INV)	LOC111274633	INV	INV		INV	INV		INV		INV		INV		INV	INV	INV	
102			LOC111277296	INV				INV		INV					INV		INV		
103			LOC111289759					INV				INV			INV	INV			
104			LOC111290775		INV		INV	INV	INV	INV		INV	INV	INV		INV		INV	INV
105			LOC111311698					INV									INV	INV	
106		alpha-glucosidase (malZ)	LOC111281283						malZ	malZ	malZ	malZ	malZ			malZ	malZ		
107		ADP-sugar diphosphatase (NUDX14)	LOC111305872				NUDX14	NUDX14					NUDX14	NUDX14	NUDX14				
108		trehalose 6-phosphate phosphatase (otsB)	LOC111277311				otsB	otsB		otsB		otsB	otsB						
109			LOC111285894				otsB			otsB		otsB	otsB	otsB	otsB	otsB	otsB		
110			LOC111289615				otsB	otsB	otsB				otsB	otsB	otsB		otsB		otsB
111			LOC111289683					otsB		otsB				otsB	otsB		otsB		otsB
112			LOC111292894	otsB		otsB	otsB	otsB					otsB						
113			LOC111301018	otsB	otsB	otsB	otsB	otsB	otsB	otsB		otsB	otsB						
114			LOC111305563	otsB		otsB	otsB	otsB		otsB					otsB	otsB	otsB		
115			LOC111309311	otsB															
116			LOC111310776						otsB	otsB									
117			LOC111314952					otsB		otsB	otsB				otsB				
118			LOC111316956	otsB	otsB	otsB	otsB	otsB		otsB		otsB	otsB	otsB					
119		sucrose-6-phosphatase (SPP)	LOC111293650				SPP	SPP					SPP	SPP					
120			LOC111310424					SPP		SPP		SPP	SPP	SPP			SPP		
121			LOC111311629				SPP	SPP	SPP	SPP		SPP	SPP				SPP		
122		sucrose synthase (SUS)	LOC111285632				SUS	SUS		SUS			SUS						
123			LOC111286797				SUS	SUS		SUS	SUS	SUS		SUS	SUS			SUS	
124			LOC111289721							SUS									
125			LOC111299212				SUS	SUS	SUS	SUS	SUS	SUS	SUS	SUS	SUS				
126			LOC111303031				SUS	SUS		SUS		SUS							
127			LOC111305045					SUS				SUS		SUS					SUS
128		trehalose 6-phosphate synthase/phosphatase (TPS)	LOC111282926				TPS			TPS	TPS		TPS	TPS	TPS				TPS
129		Related to photosystem II reaction centre I protein	novel.1808		NOVEL			NOVEL		NOVEL	NOVEL	NOVEL	NOVEL				NOVEL		
130		Related to UGPA_SOLTU UTP--glucose-1-phosphate uridylyltransferase	novel.2810						NOVEL	NOVEL		NOVEL	NOVEL				NOVEL		

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Note,

Highlight green = Up-regulated genes;

Highlight red = Down-regulated genes;

Highlight brown = Both up and down-regulated genes;

Highlight black = Genes with no differential sample groups associated (indicate the genes with non-significant expression change).

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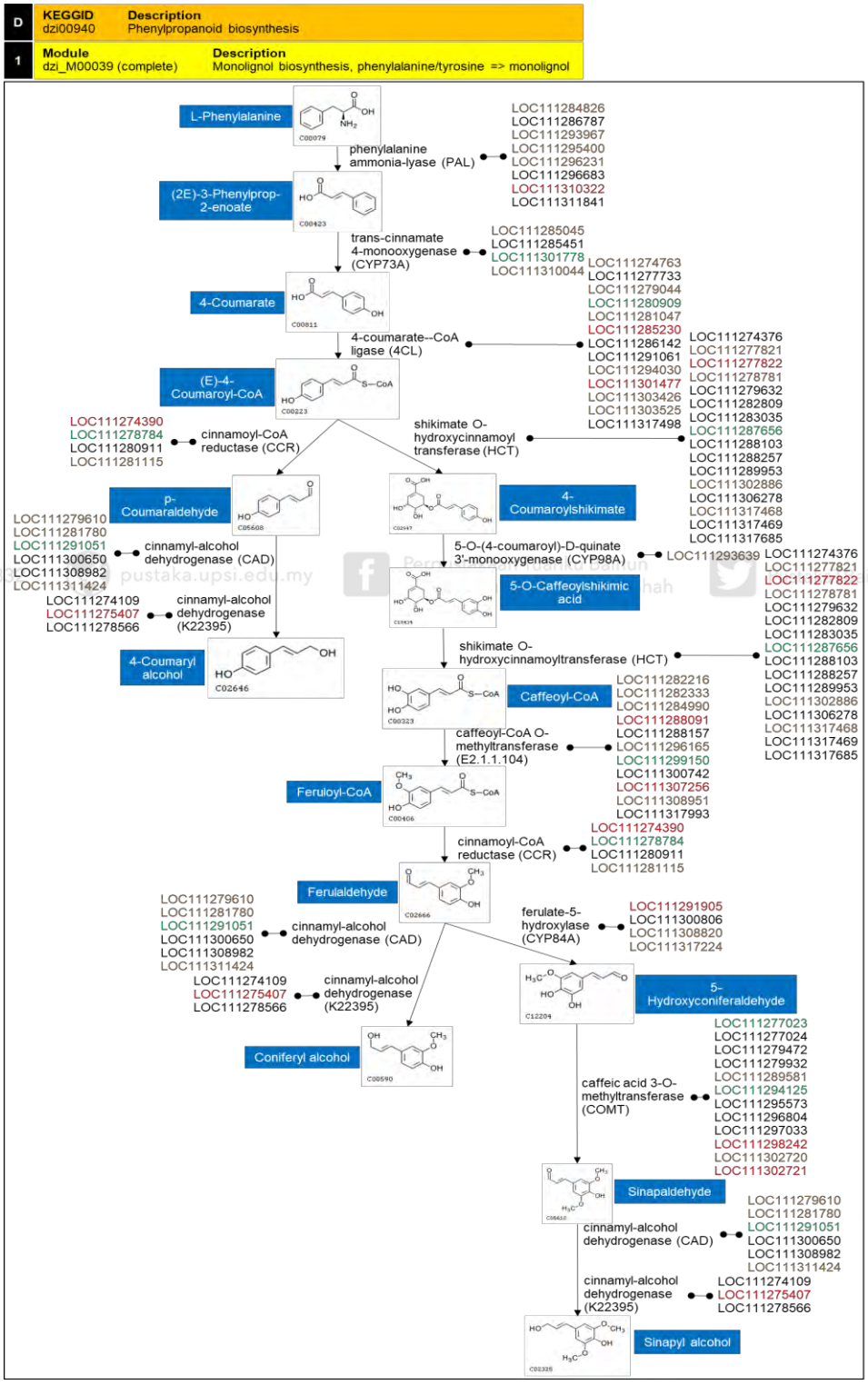
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Appendix F

Figure F1

Phenylpropanoid biosynthesis. (D1-D2) Graphical diagrams for KEGG modules for Phenylpropanoid biosynthesis. (D) Genes up- and down-regulated in relation to the differential sample groups for Phenylpropanoid biosynthesis

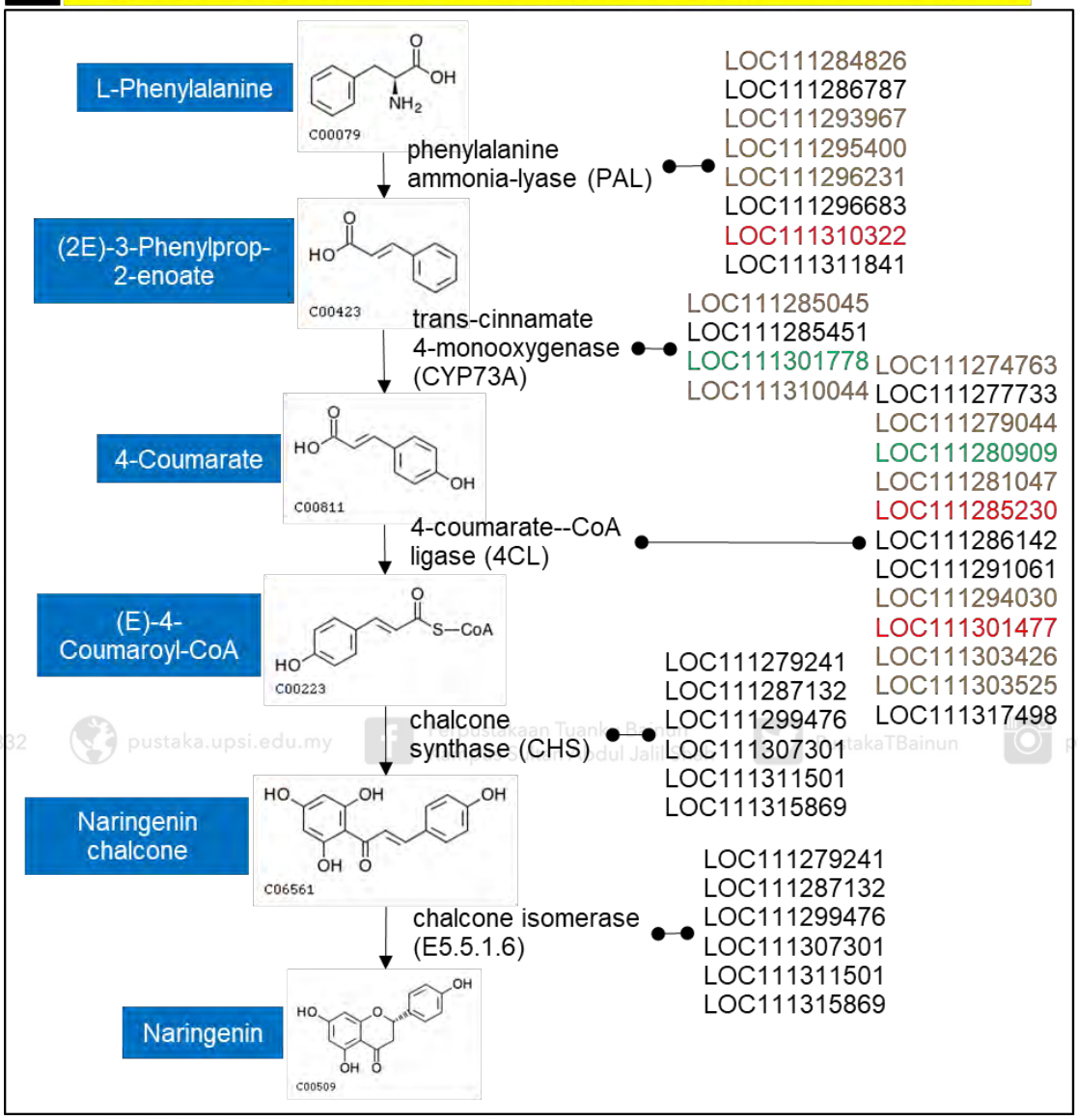


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D	KEGGID	Description
	dzi00940	Phenylpropanoid biosynthesis

2	Module	Description
	dzi_M00137 (complete)	Flavanone biosynthesis, phenylalanine => naringenin



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D	KEGGID	Description
	dzi00940	Phenylpropanoid biosynthesis

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
1	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol & Flavanone biosynthesis, phenylalanine => naringenin	phenylalanine ammonia-lyase (PAL)	LOC111284826				PAL	PAL		PAL	PAL	PAL	PAL					PAL	
2			LOC111286787																
3			LOC111293967				PAL							PAL		PAL			
4			LOC111295400							PAL		PAL							
5			LOC111296231	PAL	PAL		PAL	PAL		PAL	PAL	PAL	PAL	PAL	PAL	PAL			
6			LOC111296683																
7			LOC111310322							PAL									
8			LOC111311841																
9	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol & Flavanone biosynthesis, phenylalanine => naringenin	trans-cinnamate 4-monooxygenase (CYP73A)	LOC111285045				CYP73A	CYP73A	CYP73A	CYP73A	CYP73A	CYP73A	CYP73A	CYP73A	CYP73A	CYP73A	CYP73A		
10			LOC111285451																
11			LOC111301778		CYP73A		CYP73A								CYP73A	CYP73A			
12			LOC111310044				CYP73A	CYP73A	CYP73A				CYP73A	CYP73A	CYP73A	CYP73A	CYP73A		CYP73A
13	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol & Flavanone biosynthesis, phenylalanine => naringenin	4-coumarate--CoA ligase (4CL)	LOC111274763		4CL		4CL	4CL			4CL			4CL	4CL		4CL	4CL	
14			LOC111277733																
15			LOC111279044	4CL			4CL	4CL		4CL		4CL	4CL			4CL			
16			LOC111280909													4CL			
17			LOC111281047					4CL	4CL	4CL				4CL	4CL	4CL	4CL		
18			LOC111285230					4CL					4CL	4CL	4CL	4CL	4CL		
19			LOC111286142																
20			LOC111291061																
21			LOC111294030				4CL			4CL		4CL	4CL		4CL			4CL	4CL
22			LOC111301477							4CL		4CL	4CL						
23			LOC111303426				4CL	4CL	4CL	4CL		4CL		4CL	4CL	4CL	4CL		
24			LOC111303525	4CL		4CL	4CL			4CL			4CL		4CL				4CL
25			LOC111317498																
26	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	cinnamoyl-CoA reductase (CCR)	LOC111274390				CCR	CCR				CCR	CCR	CCR			CCR		
27			LOC111278784				CCR	CCR		CCR		CCR	CCR				CCR		
28			LOC111280911																
29			LOC111281115				CCR	CCR	CCR	CCR		CCR	CCR		CCR	CCR			
30	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	shikimate O-hydroxycinnamoyltransferase (HCT)	LOC111274376																
31			LOC111277821				HCT	HCT		HCT		HCT	HCT	HCT	HCT				
32			LOC111277822		HCT		HCT	HCT		HCT			HCT						
33			LOC111278781	HCT	HCT		HCT	HCT		HCT			HCT	HCT	HCT		HCT		
34			LOC111279632																
35			LOC111282809																
36			LOC111283035																
37			LOC111287656	HCT															
38			LOC111288103																
39			LOC111288257																
40			LOC111289953																
41			LOC111302886				HCT		HCT		HCT	HCT	HCT	HCT				HCT	
42			LOC111306278																
43			LOC111317468					HCT		HCT	HCT						HCT		
44			LOC111317469																
45			LOC111317685																

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D	KEGGID	Description
	dzi00940	Phenylpropanoid biosynthesis

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
46	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	cinnamyl-alcohol dehydrogenase (CAD)	LOC111279610				CAD	CAD		CAD		CAD	CAD	CAD	CAD				
47			LOC111281780				CAD	CAD	CAD		CAD		CAD	CAD	CAD			CAD	CAD
48			LOC111291051										CAD	CAD	CAD		CAD		
49			LOC111300650																
50			LOC111308982																
51			LOC111311424			CAD	CAD			CAD		CAD	CAD	CAD			CAD		
52	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	cinnamyl-alcohol dehydrogenase (K22395)	LOC111274109																
53			LOC111275407										K22395						
54			LOC111278566																
55	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase (CYP98A)	LOC111293639				CYP98A	CYP98A	CYP98A	CYP98A		CYP98A	CYP98A		CYP98A	CYP98A	CYP98A		
56	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	caffeoyl-CoA O-methyltransferase (E2.1.1.104)	LOC111282216				E2.1.1.104	E2.1.1.104	E2.1.1.104		E2.1.1.104		E2.1.1.104	E2.1.1.104	E2.1.1.104		E2.1.1.104		E2.1.1.104
57			LOC111282333					E2.1.1.104		E2.1.1.104			E2.1.1.104		E2.1.1.104		E2.1.1.104		
58			LOC111284990	E2.1.1.104		E2.1.1.104	E2.1.1.104	E2.1.1.104				E2.1.1.104	E2.1.1.104		E2.1.1.104				E2.1.1.104
59			LOC111288091									E2.1.1.104	E2.1.1.104						
60			LOC111288157																
61			LOC111296165									E2.1.1.104				E2.1.1.104			E2.1.1.104
62			LOC111299150				E2.1.1.104				E2.1.1.104								
63			LOC111300742																
64			LOC111307256							E2.1.1.104			E2.1.1.104						
65			LOC111308951	E2.1.1.104		E2.1.1.104	E2.1.1.104	E2.1.1.104		E2.1.1.104		E2.1.1.104	E2.1.1.104		E2.1.1.104		E2.1.1.104		
66			LOC111317993																
67	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	ferulate-5-hydroxylase (CYP84A)	LOC111291905	CYP84A					CYP84A		CYP84A		CYP84A	CYP84A	CYP84A	CYP84A	CYP84A		
68			LOC111300806																
69			LOC111308820		CYP84A		CYP84A	CYP84A	CYP84A	CYP84A	CYP84A	CYP84A	CYP84A		CYP84A	CYP84A	CYP84A	CYP84A	CYP84A
70			LOC111317224				CYP84A	CYP84A	CYP84A	CYP84A	CYP84A	CYP84A	CYP84A	CYP84A	CYP84A		CYP84A	CYP84A	CYP84A
71	Monolignol biosynthesis, phenylalanine/tyrosine => monolignol	caffeic acid 3-O-methyltransferase (COMT)	LOC111277023											COMT	COMT	COMT			
72			LOC111277024																
73			LOC111279472																
74			LOC111279932																
75			LOC111289581					COMT	COMT		COMT		COMT		COMT	COMT		COMT	
76			LOC111294125													COMT			
77			LOC111295573																
78			LOC111296804																
79			LOC111297033																
80			LOC111298242					COMT		COMT		COMT		COMT	COMT				
81			LOC111302720					COMT	COMT	COMT	COMT		COMT	COMT	COMT		COMT		COMT
82			LOC111302721				COMT	COMT	COMT	COMT	COMT		COMT				COMT	COMT	

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D	KEGGID	Description
	dzi00940	Phenylpropanoid biosynthesis

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
83	Flavanone biosynthesis, phenylalanine => naringenin	chalcone synthase (CHS)	LOC111279241																
84			LOC111287132																
85			LOC111299476																
86			LOC111307301																
87			LOC111311501																
88			LOC111315869																
89	Flavanone biosynthesis, phenylalanine => naringenin	chalcone isomerase (E5.5.1.6)	LOC111279241																
90			LOC111287132																
91			LOC111299476																
92			LOC111307301																
93			LOC111311501																
94			LOC111315869																
95	Downstream pathway	caffeoylshikimate esterase (CSE)	LOC111274117				CSE	CSE	CSE	CSE		CSE	CSE	CSE			CSE	CSE	
96			LOC111296902	CSE			CSE	CSE		CSE		CSE	CSE		CSE		CSE		
97			LOC111305745										CSE		CSE				
98			LOC111310247			CSE	CSE		CSE				CSE	CSE	CSE			CSE	
99			LOC111313402					CSE				CSE		CSE	CSE			CSE	CSE
100			LOC111314704					CSE	CSE	CSE			CSE				CSE		
101			LOC111317385				CSE	CSE	CSE	CSE	CSE	CSE	CSE	CSE			CSE		
102		peroxidase (E1.11.1.7)	LOC111274525					E1.11.1.7		E1.11.1.7					E1.11.1.7		E1.11.1.7		
103			LOC111275194				E1.11.1.7	E1.11.1.7		E1.11.1.7		E1.11.1.7	E1.11.1.7						
104			LOC111275195				E1.11.1.7	E1.11.1.7		E1.11.1.7		E1.11.1.7	E1.11.1.7						
105			LOC111276611							E1.11.1.7								E1.11.1.7	
106			LOC111281470				E1.11.1.7						E1.11.1.7	E1.11.1.7	E1.11.1.7			E1.11.1.7	
107			LOC111282364				E1.11.1.7	E1.11.1.7				E1.11.1.7	E1.11.1.7				E1.11.1.7		
108			LOC111284369										E1.11.1.7						
109			LOC111284872				E1.11.1.7	E1.11.1.7		E1.11.1.7				E1.11.1.7	E1.11.1.7		E1.11.1.7		
110			LOC111285639	E1.11.1.7		E1.11.1.7	E1.11.1.7			E1.11.1.7		E1.11.1.7		E1.11.1.7			E1.11.1.7		
111			LOC111285680		E1.11.1.7	E1.11.1.7			E1.11.1.7				E1.11.1.7						
112			LOC111288037																E1.11.1.7
113			LOC111288270										E1.11.1.7						
114			LOC111290243			E1.11.1.7	E1.11.1.7	E1.11.1.7			E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7		E1.11.1.7	E1.11.1.7	
115			LOC111291695				E1.11.1.7	E1.11.1.7	E1.11.1.7		E1.11.1.7			E1.11.1.7	E1.11.1.7				
116			LOC111291984										E1.11.1.7						
117			LOC111292073	E1.11.1.7	E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7
118			LOC111292660	E1.11.1.7	E1.11.1.7	E1.11.1.7			E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7						
119			LOC111296160	E1.11.1.7		E1.11.1.7				E1.11.1.7	E1.11.1.7		E1.11.1.7			E1.11.1.7	E1.11.1.7		

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D	KEGGID	Description
	dzi00940	Phenylpropanoid biosynthesis

				MATURATION						RIPENING							DEHISCENCE				
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
120	Downstream pathway	peroxidase (E1.11.1.7)	LOC111297732					E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7
121			LOC111299575		E1.11.1.7		E1.11.1.7	E1.11.1.7			E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7
122			LOC111299997				E1.11.1.7	E1.11.1.7		E1.11.1.7		E1.11.1.7	E1.11.1.7						
123			LOC111300167				E1.11.1.7	E1.11.1.7				E1.11.1.7	E1.11.1.7						
124			LOC111300720										E1.11.1.7						
125			LOC111302385				E1.11.1.7	E1.11.1.7						E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7		
126			LOC111302386									E1.11.1.7							
127			LOC111304021				E1.11.1.7	E1.11.1.7		E1.11.1.7		E1.11.1.7	E1.11.1.7						
128			LOC111307212													E1.11.1.7			
129			LOC111307214													E1.11.1.7			
130			LOC111307215													E1.11.1.7			
131			LOC111307216													E1.11.1.7			
132			LOC111307220													E1.11.1.7			
133			LOC111307464					E1.11.1.7							E1.11.1.7				
134			LOC111307639				E1.11.1.7							E1.11.1.7		E1.11.1.7			
135			LOC111307640	E1.11.1.7	E1.11.1.7		E1.11.1.7		E1.11.1.7		E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	
136			LOC111308192				E1.11.1.7	E1.11.1.7		E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7		E1.11.1.7	E1.11.1.7	
137			LOC111308367											E1.11.1.7	E1.11.1.7				
138			LOC111309670				E1.11.1.7	E1.11.1.7	E1.11.1.7			E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7				
139			LOC111311043							E1.11.1.7									
140			LOC111315890								E1.11.1.7							E1.11.1.7	
141			LOC111317211		E1.11.1.7	E1.11.1.7	E1.11.1.7		E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7	E1.11.1.7			E1.11.1.7	
142		peroxiredoxin 6 (PRDX6)	LOC111279845									PRDX6	PRDX6						
143			LOC111279846											PRDX6	PRDX6	PRDX6	PRDX6		PRDX6
144		coniferyl-aldehyde dehydrogenase (REF1)	LOC111284558	REF1	REF1		REF1	REF1	REF1	REF1	REF1	REF1	REF1						
145		coniferyl-alcohol glucosyltransferase (UGT72E)	LOC111291807				UGT72E	UGT72E			UGT72E			UGT72E	UGT72E	UGT72E	UGT72E		
146			LOC111292185		UGT72E			UGT72E		UGT72E			UGT72E	UGT72E					
147			LOC111316230				UGT72E							UGT72E					
148		Related to 4-coumarate--CoA ligase-like	novel.3355							NOVEL				NOVEL	NOVEL				

Note,

Highlight green = Up-regulated genes;

Highlight red = Down-regulated genes;

Highlight brown = Both up and down-regulated genes;

Highlight black = Genes with no differential sample groups associated (indicate the genes with non-significant expression change).

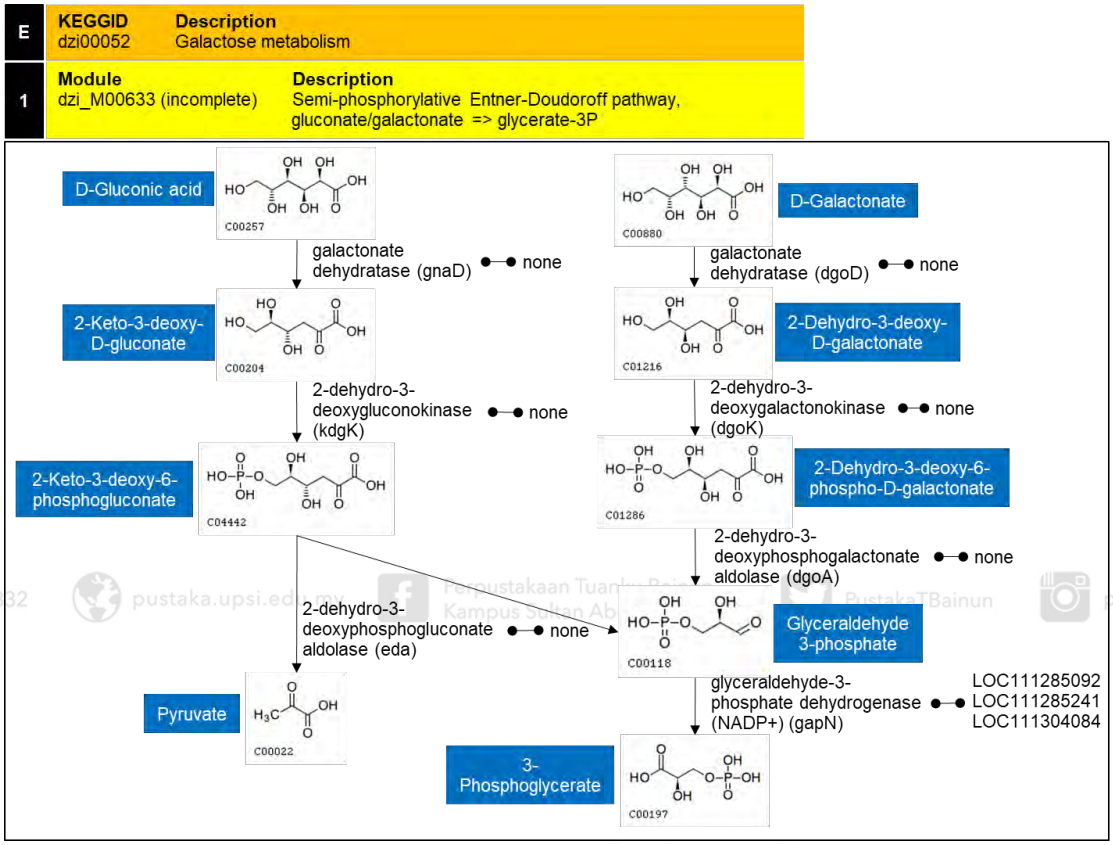
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Appendix G

Figure G1

Galactose metabolism. (E1-E4) Graphical diagrams for KEGG modules for Galactose metabolism. (E) Genes up- and down-regulated in relation to the differential sample groups for Galactose metabolism

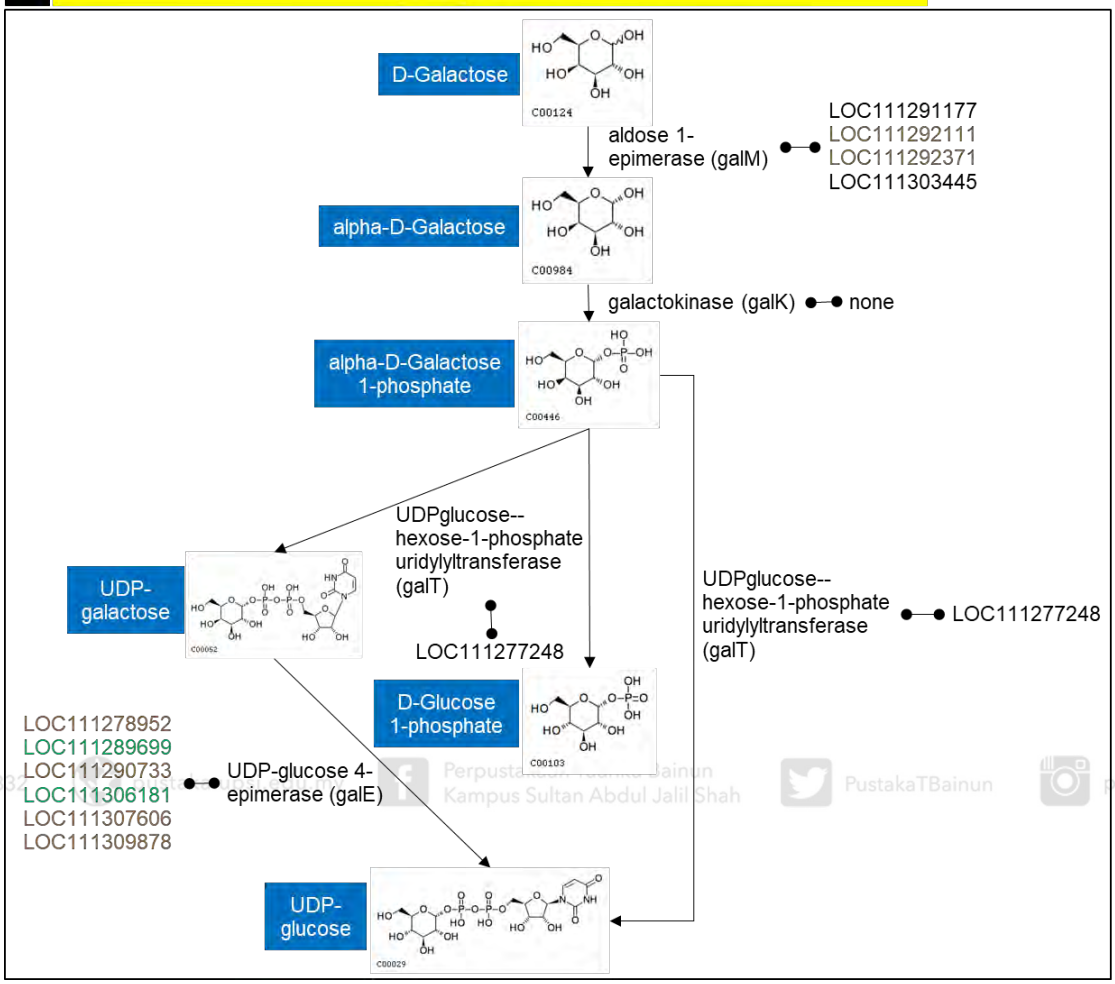


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E	KEGGID	Description
	dzi00052	Galactose metabolism

2	Module	Description
	dzi_M00632 (1 block missing)	Galactose degradation, Leloir pathway, galactose => alpha-D-glucose-1P

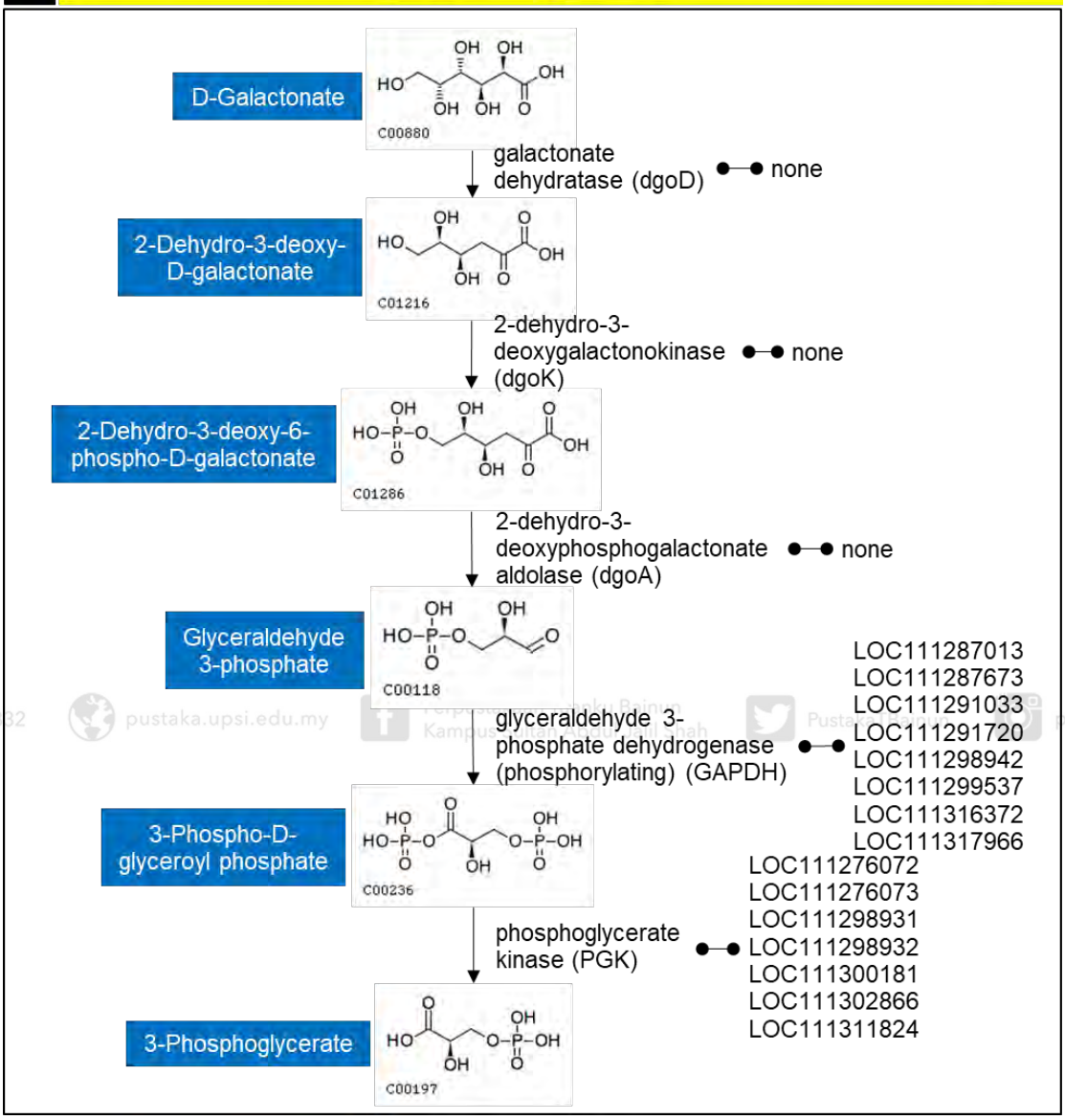


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E	KEGGID	Description
	dzi00052	Galactose metabolism

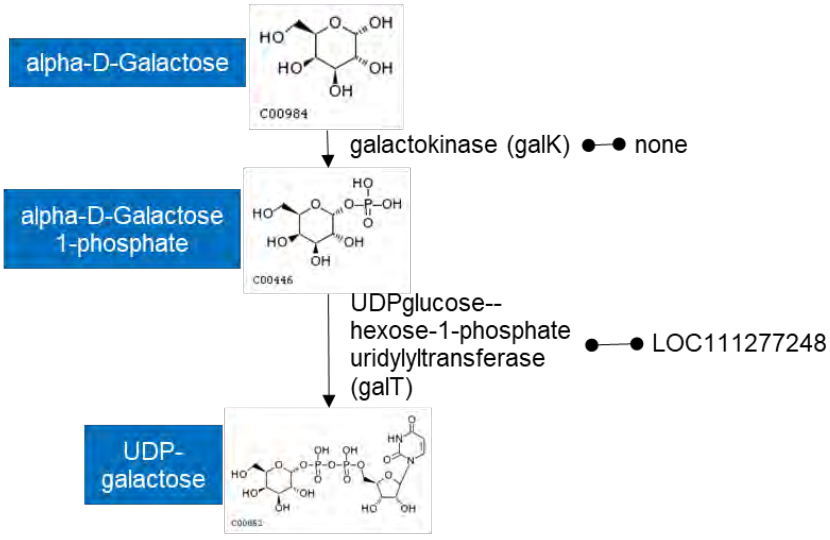
3	Module	Description
	dzi_M00552 (incomplete)	D-galactonate degradation, De Ley-Doudoroff pathway, D-galactonate => glycerate-3P



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E	**KEGGID** dzi00052	**Description** Galactose metabolism
4	**Module** dzi_M00554 (1 block missing)	**Description** Nucleotide sugar biosynthesis, galactose => UDP-galactose



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E	KEGGID	Description
	dzi00052	Galactose metabolism

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
1	Semi-phosphorylative Entner-Doudoroff pathway, gluconate/galactonate => glycerate-3P	glyceraldehyde-3-phosphate dehydrogenase (NADP+) (gapN)	LOC111285092																
2			LOC111285241																
3			LOC111304084																
4	Galactose degradation, Leloir pathway, galactose => alpha-D-glucose-1P	aldose 1-epimerase (galM)	LOC111291177																
5			LOC111292111	galM	galM	galM	galM	galM	galM	galM	galM	galM		galM	galM		galM	galM	galM
6			LOC111292371					galM			galM	galM	galM					galM	
7			LOC111303445																
8	Galactose degradation, Leloir pathway, galactose => alpha-D-glucose-1P & Nucleotide sugar biosynthesis, galactose => UDP-galactose	UDPglucose--hexose-1-phosphate uridylyltransferase (galT)	LOC111277248																
9	Galactose degradation, Leloir pathway, galactose => alpha-D-glucose-1P	UDP-glucose 4-epimerase (galE)	LOC111278952				galE	galE	galE	galE	galE	galE	galE	galE	galE	galE	galE		
10			LOC111289699	galE	galE	galE	galE	galE	galE	galE	galE	galE	galE						
11			LOC111290733				galE		galE				galE		galE		galE		
12			LOC111306181				galE	galE			galE	galE	galE		galE	galE	galE		
13			LOC111307606	galE	galE	galE	galE	galE		galE		galE	galE				galE		
14			LOC111309878				galE		galE	galE	galE	galE		galE	galE		galE		
15	D-galactonate degradation, De Ley-Doudoroff pathway, D-galactonate => glycerate-3P	glyceraldehyde 3-phosphate dehydrogenase (phosphorylating) (GAPDH)	LOC111287013																
16			LOC111287673																
17			LOC111291033																
18			LOC111291720																
19			LOC111298942																
20			LOC111299537																
21			LOC111316372																
22			LOC111317966																
23		phosphoglycerate kinase (PGK)	LOC111276072																
24			LOC111276073																
25			LOC111298931																
26			LOC111298932																
27			LOC111300181																
28			LOC111302866																
29			LOC111311824																
30	Downstream pathway	stachyose synthetase (E2.4.1.67)	LOC111306029							E2.4.1.67		E2.4.1.67	E2.4.1.67	E2.4.1.67	E2.4.1.67				
31			LOC111314057							E2.4.1.67		E2.4.1.67							
32		raffinose synthase (E2.4.1.82)	LOC111276406				E2.4.1.82	E2.4.1.82		E2.4.1.82		E2.4.1.82	E2.4.1.82						
33			LOC111286096	E2.4.1.82	E2.4.1.82		E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82	E2.4.1.82		E2.4.1.82	E2.4.1.82	E2.4.1.82		
34			LOC111287566				E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82				
35			LOC111292856	E2.4.1.82		E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82
36			LOC111293659	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82	E2.4.1.82		E2.4.1.82
37			LOC111303287				E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82	E2.4.1.82							
38			LOC111311522						E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82				E2.4.1.82		E2.4.1.82
39			LOC111316628	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82			E2.4.1.82	E2.4.1.82	E2.4.1.82		E2.4.1.82

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E	KEGGID	Description
	dzi00052	Galactose metabolism

				MATURATION			RIPENING							DEHISCENCE					
No.	KEGG Module	Enzyme/Gene Name	NCBI-GeneID	D24_M_110 vs D24_M_105	D24_M_115 vs D24_M_105	D24_M_120 vs D24_M_105	D24_R_125 vs D24_M_105	D24_R_127 vs D24_M_105	D99_R vs D99_M	D160_R vs D160_M	D168_R vs D168_M	D197_R vs D197_M	D200_R vs D200_M	D24_D_143 vs D24_R_125	D24_D_143 vs D24_R_127	D99_D vs D99_R	D160_D vs D160_R	D168_D vs D168_R	D197_D vs D197_R
40	Downstream pathway	alpha-galactosidase (E3.2.1.22B)	LOC111281622										E3.2.1.22B						
41			LOC111281926				E3.2.1.22B	E3.2.1.22B	E3.2.1.22B		E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B		E3.2.1.22B		
42			LOC111282053				E3.2.1.22B	E3.2.1.22B		E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B		E3.2.1.22B			E3.2.1.22B	
43			LOC111293625				E3.2.1.22B	E3.2.1.22B		E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B				
44			LOC111308381						E3.2.1.22B	E3.2.1.22B									
45			LOC111309183					E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B		E3.2.1.22B		E3.2.1.22B	
46			LOC111310037				E3.2.1.22B	E3.2.1.22B		E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B		E3.2.1.22B		E3.2.1.22B		
47			LOC111315863										E3.2.1.22B	E3.2.1.22B	E3.2.1.22B				
48			LOC111315867				E3.2.1.22B				E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B	E3.2.1.22B				
49		beta-galactosidase (GLB1)	LOC111274640									GLB1		GLB1	GLB1			GLB1	
50			LOC111280838				GLB1	GLB1		GLB1	GLB1	GLB1	GLB1	GLB1	GLB1		GLB1		GLB1
51		inositol 3-alpha-galactosyltransferase (GOLS)	LOC111296264	GOLS	GOLS	GOLS		GOLS		GOLS	GOLS		GOLS		GOLS		GOLS	GOLS	GOLS
52			LOC111313380			GOLS	GOLS	GOLS	GOLS		GOLS	GOLS	GOLS	GOLS	GOLS	GOLS	GOLS	GOLS	
53			LOC111317875	GOLS			GOLS	GOLS		GOLS		GOLS		GOLS	GOLS				GOLS
54		hexokinase (HK)	LOC111274573				HK	HK											
55			LOC111278957											HK	HK				
56			LOC111281155				HK	HK				HK	HK	HK			HK	HK	HK
57			LOC111290828														HK		HK
58			LOC111303165	HK			HK	HK					HK	HK					
59			LOC111303941	HK			HK	HK		HK		HK	HK	HK	HK				HK
60		beta-fructofuranosidase (INV)	LOC111274633	INV	INV		INV	INV		INV		INV		INV		INV	INV	INV	
61			LOC111277296	INV				INV		INV					INV		INV		
62			LOC111289759					INV				INV			INV	INV			
63			LOC111290775		INV		INV	INV	INV	INV		INV	INV	INV		INV		INV	INV
64			LOC111311698					INV									INV	INV	
65		beta-galactosidase (lacZ)	LOC111309977				lacZ	lacZ		lacZ		lacZ	lacZ	lacZ	lacZ				
66		alpha-glucosidase (malZ)	LOC111281283						malZ	malZ	malZ	malZ	malZ			malZ	malZ		
67		6-phosphofructokinase 1 (pfkA)	LOC111274579					pfkA		pfkA	pfkA		pfkA	pfkA	pfkA		pfkA	pfkA	pfkA
68			LOC111285266	pfkA			pfkA	pfkA	pfkA	pfkA		pfkA	pfkA				pfkA		
69			LOC111286103	pfkA			pfkA		pfkA					pfkA	pfkA		pfkA	pfkA	pfkA
70			LOC111289246							pfkA		pfkA		pfkA	pfkA				
71			LOC111292372								pfkA	pfkA			pfkA	pfkA	pfkA		
72			LOC111316139				pfkA	pfkA						pfkA	pfkA				
73		phosphoglucomutase (pgm)	LOC111282045				pgm	pgm		pgm		pgm	pgm						pgm
74			LOC111289821												pgm	pgm	pgm	pgm	
75			LOC111310405				pgm	pgm		pgm		pgm	pgm	pgm	pgm	pgm	pgm		pgm
76		UTP--glucose-1-phosphate uridylyltransferase (UGP2)	LOC111296541											UGP2					
77			LOC111305812									UGP2	UGP2						
78		Related to glucose-1-phosphate uridylyltransferase	novel.2810						NOVEL	NOVEL		NOVEL	NOVEL				NOVEL		

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