MORPHOLOGICAL OBSERVATION AND MOLECULAR CHARACTERIZATION ON FRUIT DEVELOPMENTAL STAGES OF SELECTED MALAYSIAN DURIAN VARIETIES

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

2024

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DISSERTATION PRESENTED TO QUALIFY FOR A MASTER'S IN SCIENCE (RESEARCH MODE)

FACULTY OF SCIENCE AND MATHEMATICS
SULTAN IDRIS EDUCATION UNIVERSITY

2024

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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.

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.

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

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

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

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).

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).

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).

Figure 1.1

Percentage of hectare area occupied for durian plantation by variety

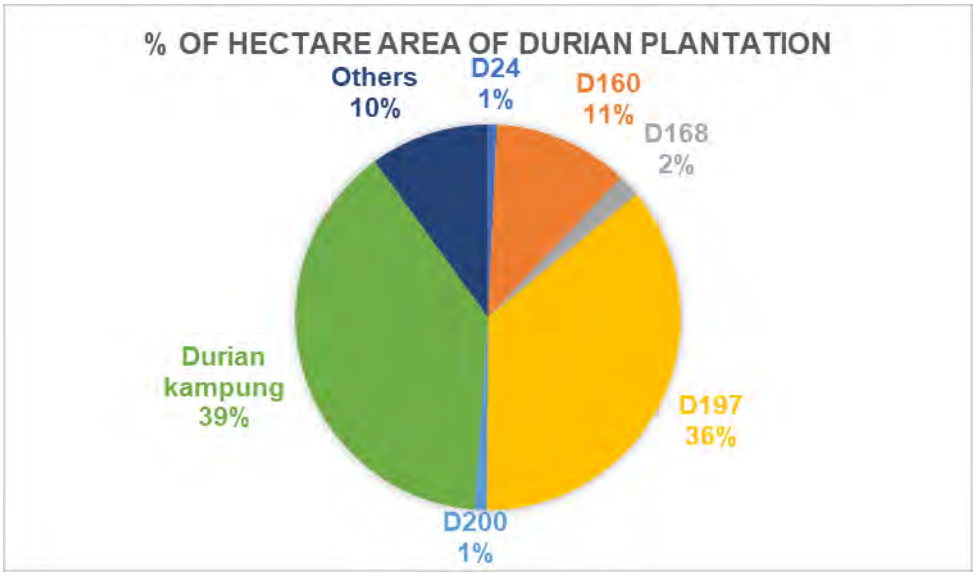
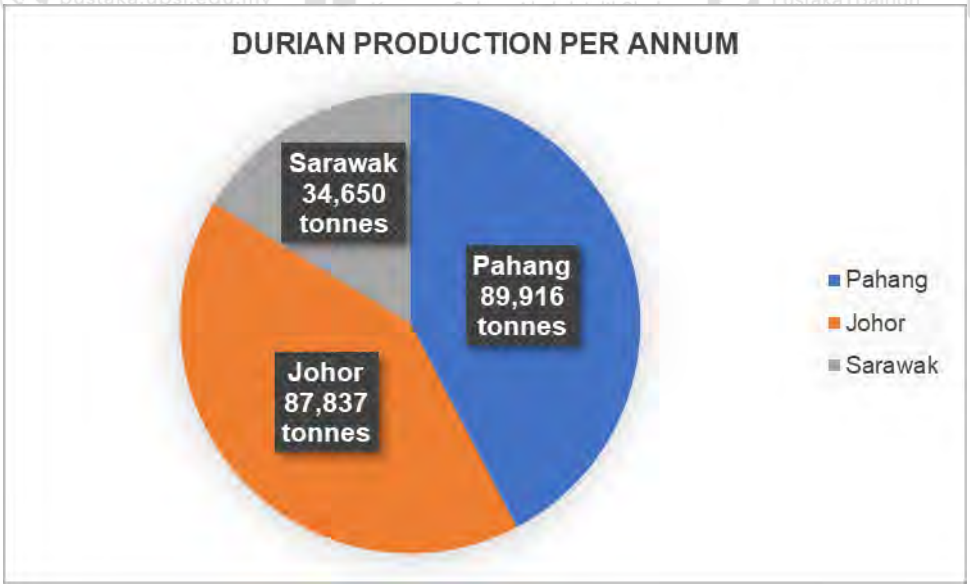


Figure 1.2

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

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

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

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

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

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.

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

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

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).

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.

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.

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

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.