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TRANSCRIPTOMICS AND METABOLOMICS INVESTIGATION OF MANGOSTEEN FRUIT RIPENING

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Abstract: Mangosteen (*Garcinia mangostana*) is a functional fruit containing several beneficial metabolites such as xanthones which are believed to cure cancer, among others. The molecular mechanisms of xanthone biosynthesis especially during the ripening process of mangosteen are still poorly investigated. Furthermore, mangosteen only ripens when harvested at the middle stage of ripening (appearance of purple colour) but not earlier (mature green stage). Hence, the objective of this study is to investigate the different ripening stages (Stage 0, 2 and 6) using various multi-omics approaches such as transcriptomics and metabolomics. Our result suggests that these approaches are highly useful in elucidating the molecular mechanisms of mangosteen ripening, profiling various transcripts and metabolites related to ripening. Further works in investigating the differentially expressed genes/metabolites from the different ripening stages as well as integrating these different omics are needed to further elucidate the ripening related pathways.

***Keywords*:** fruit ripening, mangosteen, omics, pathway

INTRODUCTION

Ripening is an integral process for fruit to become more palatable and attractive to seed dispersal agents such as animals. Several physiological changes are attributed to ripening such as colour modification, textural softening as well as the build-up of sugars and secondary metabolites. One example is mangosteen (*Garcinia mangostana* L.) fruit which ripens from green to a dark purple colour rind while developing an edible fleshy white pulp. Mangosteen also has been extensively used in medicinal and pharmaceutical products due to having high contents of beneficial secondary metabolites. Xanthones particularly have been widely studied as anti-carcinogenic, pro-apoptotic and anti-inflammatory substances (Gutierrez-Orozco & Failla 2013).

Furthermore, mangosteen has a unique ripening behaviour. Despite being classified as a climacteric fruit (fruit which ripens with a surge of ethylene), mangosteen will only ripen once harvested at the middle of ripening (slight purple colouring on its pericarp) but not earlier during mature green stage (Osman & Milan 2006). Stage-specific molecules are therefore thought to play a significant role in the ripening regulation of mangosteen and hence different ripening stages need to be analysed and compared. Moreover, the molecular mechanisms of mangosteen ripening are still scarcely documented.

Recently, multi-omics platforms have been used to elucidate the ripening of various fruit. This includes tomato (Osorio et al., 2012), strawberry (Bianco et al., 2009) and capsicum (Aizat et al., 2014), which revealed specific molecular and metabolic regulation at various time points/stages of these fruit. One example of these platforms is using transcriptomic approach particularly the RNA-Seq technology. This technology has enabled scientists to accurately identify and quantify the transcripts of any eukaryotic organism, making it as one of the most comprehensive tools for transcriptomic analysis (Wang et al., 2009). The transcriptome of mangosteen during ripening also has not been reported elsewhere and hence, this study employed RNA-Seq technology to analyse the different ripening stages of mangosteen. Furthermore, most studies in mangosteen fruit have been mainly characterizing specific types of xanthones and there are still yet reports on the global metabolite profiling of mangosteen. Therefore, Liquid chromatography- Mass Spectrometry (LC-MS) has been used in this untargeted and global metabolomics analysis.

In this study, both transcriptomics and metabolomics approaches were used to elucidate the ripening process of mangosteen fruit. Three stages were utilized, early (Stage 0), middle (Stage 2) and late (Stage 6) ripening. Our results suggested that these multi-omic approaches are able to elucidate the complexity of mangosteen ripening process. The outcome of this study will not only shed some light into the molecular mechanisms of mangosteen ripening, but also may enhance our understanding of its ripening behaviour which may allow us to develop strategies to prolong its shelf-life for commercialisation purposes.

METHODS

Plant material

Mangosteen fruit were harvested from the UKM mangosteen plot during May to September 2014 period. Early (Stage 0), middle (Stage 2) and late (Stage 6) ripening stages were harvested according to Osman & Milan (2006). At least three biological replicates for each stage were harvested, ground in liquid nitrogen before storage at -80°C.

RNA extraction and transcriptomics analysis

Total RNA was isolated using modified CTAB method as detailed previously (Abdul Rahman et al., 2017a). High quality RNA (based on QC using Bioanalyzer instrument) was chosen for the subsequent RNA-seq run. The instrument used was Illumina Hiseq 4000 platform and all analyses were detailed in Abdul Rahman et al. (2017b).

Metabolite extraction and LC-MS analysis

Metabolites from the three stages of ripening were extracted using either Cadahia et al. (2015) (methanol:chloroform:water 3:1:1 v/v) or De Vos et al. (2007) (methanol acidified with formic acid 599:1 v/v) techniques. LC-MS analysis was performed as detailed in Rosli et al. (2017).

RESULTS AND DISCUSSION

Three stages of mangosteen (Stage 0, 2 and 6) were harvested according to the Malaysian Maturity Indices (Osman & Milan 2006). Fruit at stage 0 is light green, stage 2 is light pink and stage 6 is dark purple (Figure 1). Stage 6 did not possess latex deposition compared to the other two stages.

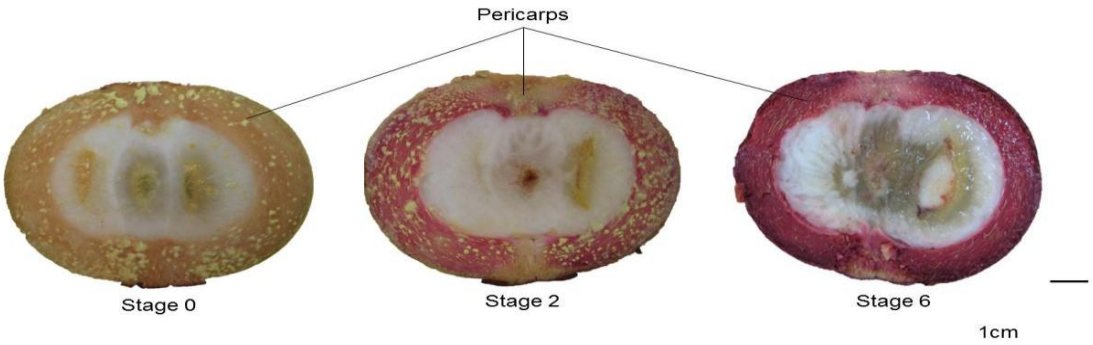

Figure 1. The early (Stage 0), middle (Stage 2) and late (Stage 6) ripening stages of mangosteen ripening (Reference: Adapted from Abdul-Rahman et al., (2017a)).

Total RNA was extracted from these ripening stages and sent for RNA-seq analysis. More than 650 million processed reads were obtained which comprised of more than 250 thousand unique transcripts (Table 1). Further work in confirming various differentially expressed transcripts are currently in progress.

Table 1: Transcriptome sequencing statistics of mangosteen ripening.

Data	Value
Total processed reads	650,887,650
Number of unique transcripts	250,682

(Reference: Adapted from Abdul-Rahman et al., (2017b)

For an optimization experiment, total metabolites were extracted from only the Stage 6 pericarp tissue using either Cadahia et al. (2015) or De Vos et al. (2007) methods (Table 2). These two methods employed different solvent extractions ie. methanol:chloroform:water 3:1:1 v/v or methanol acidified with formic acid 599:1 v/v, respectively. De Vos et al. (2007) method produced 138 compound peaks, much higher compared to only 30 peaks from Cadahia et al. (2015) method. Such differences might be attributed to the solvent differences between these methods. We are currently investigating the metabolic differences between the three stages of mangosteen ripening.

Table 2: Optimization of LC-MS run using two different metabolite extraction techniques, Cadahia et al., (2015) and De Vos et al., (2007).

Extraction method	Solvent	Number of compound peaks
Cadahia et al., (2015)	methanol:chloroform:water 3:1:1 v/v	30
De Vos et al., (2007)	methanol acidified with formic acid 599:1 v/v	138

CONCLUSIONS

Both transcriptomics and metabolomics approaches were able to profile considerable number of transcripts and metabolites respectively. More works need to be done to elucidate the molecular pathways related to ripening in mangosteen.

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