Metabolic profiling and pathway analysis in red arillus of *Salacca sumatrana* demonstrate significant pyruvate, sulfur, and fatty acid metabolisms

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Abstract. *Fendiyanto MH, Satrio RD, Darmadi D. 2020. Metabolic profiling and pathway analysis in red arillus of* Salacca sumatrana *demonstrate significant pyruvate, sulfur, and fatty acid metabolisms. Biodiversitas 21: 4361-4368.* Salak or snake fruit (*Salacca* Reinw.; Arecaceae) is a tropical fruit that has high biodiversity in a tropical country such as Indonesia. Several types of salak can be found in Indonesia, one of which is *Salacca sumatrana* (Becc.) Mogea. Research on profiling metabolites in *S. sumatrana* has not been conducted. Therefore, the aim of this study was to determine the metabolite profile and pathway analysis in *S. sumatrana*, especially in red arillus tissue. This research was conducted by the method of gas chromatography-mass spectrometry (GC-MS) and the study of bioinformatics through metabolomics approaches. The results showed that red arillus had metabolites consisting of groups of fatty acids, esters, alcohols, xylene, phenols, etc.. Significant metabolites found were isobutyl acetate, palmitic acid, formic acid, 2-pentanoic acid, ethylic acid, n-hexadecoic acid, hydroxypentanoic acid, etc. There was a tendency that metabolite ethylic acid, identified as C00033 accession, was a key metabolite in either the pyruvate metabolism pathway or sulfur metabolisms with relatively high impact values. Pathway analysis using bioinformatics studies using MetaboAnalyst shows that four of ten pathways detected had a high log-ratio (p)/pathway impact, i.e., pyruvate metabolism, sulfur metabolism, fatty acid biosynthesis, and biosynthesis of unsaturated fatty acids. Thus, pyruvate, sulfur, and fatty acid metabolisms are important pathways in the red arillus of *S. sumatrana*. This study can be used as a reference in early metabolomic studies on *S. sumatrana* using GC-MS and the metabolites identified as metabolite markers can be used for plant breeding and biologists to understand the metabolic mechanism of the red arillus tissues from *S. sumatrana*.

Keywords: Ethylic acid, GC-MS, metabolomics, red arillus, *Salacca sumatrana*

INTRODUCTION

Salak (*Salacca* Reinw.; Arecaceae) is a favorite tropical fruit that has high biodiversity in Indonesia. There are several types of salak that can be found in Indonesia, including Javanese salak (*Salacca zalacca* (Gaertner) Voss), Padang Sidempuan salak (*Salacca sumatrana* (Becc.) Mogea), and Bali salak (*Salacca amboinensis* (Becc.) Mogea) (Yuliamita 2014, Harahap and Ardiarini 2018). *S. zalacca* holds 2-3 seeds and has a dominant characteristic of white arillus meat (Yuliamita 2014). *S. amboinensis* has 1-2 seeds and white to reddish arillus flesh. Specifically for *S. sumatrana*, the arillus fruit is red (Yuliamita 2014; Harahap and Ardiarini 2018). However, some cultivars of *S. sumatrana* were found to vary from white, reddish to red. Harahap and Ardiarini (2018) report that some *S. sumatrana* has various arillus namely white and red. Things that affect the color of the arillus in *S. sumatrana* have not yet known. *S. sumatrana* is not widely studied, even though the fruit is a unique tropical fruit and is asked a lot by many countries such as Indonesia,

Malaysia, Singapore, and Brunei Darussalam. Research on *S. sumatrana* is limited to taxonomic, morphological, anatomical, isozyme markers, and uses relatively simple molecular approaches such as RAPD (Yuliamita 2014; Harahap and Ardiarini 2018; Nikmah et al. 2020). Whereas technological advances in biology such as omic-technology, which includes metabolomics (Zhang et al. 2018), genomics, transcriptomics, EST-omics (Satrio et al. 2019), miRNA-omics, and ion-omics, are very rapid in their development, making it possible to be used as an approach in the study of plant physiology and development. Specifically to metabolomic approaches, therefore, this study focuses on metabolomic analysis to understand the mechanism and pathway of metabolites in the red arillus from the *S. sumatrana* fruit.

Morphological characteristics can be used as a distinguishing character between several species or cultivars in salak fruit, one of which is arillus (Harahap and Ardiarini 2018). Arilus in *S. zalacca* can be used as a differentiator of types or cultivars in *S. sumatrana*. Arillus also influences the preferences of the community, so this

character is important to be studied (Yuliamita 2014). Several types of arillus can distinguish several accessions in *S. sumatrana*. However, the mechanism of the differences in compounds and expression of metabolites that affect the color of arillus have been few studied so far. Some of these accessions are only morphology and biochemically distinguished at the isozyme level, but most have not been studied at the level of metabolites and/or genes. Meanwhile, to determine the role of a metabolic pathway in the development of fruit arillus, specifically *S. sumatrana*, it is necessary to do a metabolomic analysis. In addition to looking for metabolite markers related to arillus color, the metabolomic approach can enable us to know and understand the metabolic pathways that occur in the arillus of the *S. sumatrana*.

The metabolomic approach (MA) is omic-technology which can be used to study the mechanism of metabolites that play a role in metabolism or growth and development in some plants. Omic technology can be divided into three approaches namely genomic, transcriptomic, and metabolomics (Do Amaral et al. 2016). Metabolomics are widely used for studying tropical fruit especially the genus *Salacca*. This approach is used to study stress physiological processes in plants (Jorge et al. 2016; Quinet et al. 2012), fruit development (Yuliamita 2014), photosynthesis regulation (Quinet et al. 2012; Hiremath et al. 2017), and plant mineral nutrition (Zhang et al. 2018), and photoperiodism (Hiremath et al. 2017). The use of metabolomics in dissecting the differences in metabolites in *S. sumatrana*, particularly arillus tissues has not been done before. Therefore, this study aims to determine differences in metabolites profiling in the Arillus of *S. sumatrana*, understand the metabolites pathway and its expression in the red arillus.

MATERIALS AND METHODS

Materials

Salacca sumatrana (Becc.) Mogea was explored and collected from Medan, North Sumatra, Indonesia, and Bogor, West Java, Indonesia. We used only red arillus of snake fruit to understand their extract metabolites. We analyzed six red arillus replicates in different populations, consisting of three populations from Medan and three populations from Bogor, respectively. Two types of ecotypes were represented by Medan to represent lowland area ecotypes and the original population of *S. sumatrana*, while Bogor ecotypes represented highland areas and ex-situ conservation populations. Arillus from *S. sumatrana* was collected and stored in an icebox, then freezing was done. Each collected fruit snake was frozen using liquid-N_2 and stored at-40 °C. The frozen cell and tissue had a relatively similar physiological process, paused temporarily, and avoids fruit senescence so that it would reactivate during the extraction analysis process and gas chromatography-mass spectrometry (GC-MS).

Arillus-metabolites extraction of *Salacca sumatrana*

We used frozen arillus tissues to extract the metabolites. The extraction methods were performed using Pratami (2020) method with slight modification. We extracted 2 g of arillus using absolute ethanol at 25°C for 5 d. We used 2 g sample and diluted up to 10 ml of solvent (ethanol). Separated extract of arillus was evaporated using a turbo-evaporator (Caliper Life Sci, USA) at 44 °C for 60 min. The extract was then used to perform a gas chromatography-mass spectrometry (GC-MS).

GC-MS of *Salacca sumatrana*

GC-MS analysis was performed following Pratami (2020) methods with slight modification. Arillus extracts (5 µl) were injected in a GC-MS (Model 7890, Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler (Model 7693, Agilent Technologies, Palo Alto, CA, USA), which were connected to a Mass Selective Detector and a Chemstation Data System (Model 5975C inert MSD with Triple-Axis Detector, Agilent Technologies, Palo Alto, CA, USA). The HP ultra 2 column capillary (0.11 µm) was used in this method. We used instruments with settings, i.e., ion source temperature (230°C), injection temperature (250°C), quadrupole temperature (140°C), and interface temperature (280°C). For carrier gas, we performed Helium with a flow rate of 1.2 ml/min. The detected mass spectrum was used in a mass-to-charge range of 20-500 m/z. Obtained metabolites were identified according to the Wiley W8N08.L database.

Metabolites confirmation and data analysis

Salacca sumatrana metabolites and pathways data were analyzed using MetaboAnalystR 4.0 (Chong et al. 2018, Chong et al. 2019) through univariate and multivariate analysis and R program version 3.5.1 (https: //cran.r-project.org/, Lander 2014). For multivariate analysis, we used R program according to syntax from Fendiyanto et al. (2019a,b). For univariate analysis, we performed R program based on Satrio et al. (2019) syntax. We performed the data to identify compounds for red arillus in *S. sumatrana*, in particular, compounds identification, pathway analysis, VIP score analysis, and analysis of variance (ANOVA) to determine the most important metabolite markers correlated to red arillus. The obtained metabolite was characterized using The Human Metabolome Database (HMDB) (http: //www.hmdb.ca/; Wishart et al. 2017), Pubchem (https: //pubchem.ncbi. nlm.nih.gov/), Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database (http: //www.genome.jp/kegg/pathway.html), LIPID MAPS (http: //www.lipidmaps.org/), ChemSpider (http: //www. chemspider.com/), and CAMEO (https: //cameochemicals. noaa.gov/). Each metabolite was verified with a literature study approach based on Saleh et al. (2018) and the MetaboAnalystR version 3.0 database (https: //www. metaboanalyst.ca/, Xia and Wishart 2016, Chong and Xia 2018, Chong et al. 2019, Pang et al. 2020). Every metabolite data was processed using MS Spectra Processing according to Pratami (2020).

RESULTS AND DISCUSSION

Metabolites profiling

A total of twenty-six metabolites were found in this study (Table 1), indicating the GC-MS method used was very suitable for extracting metabolites in red arillus from *S. sumatrana*. In general, some metabolites consisted of groups of fatty acids, esters, alcohols, xylene, phenols, and other compounds (Table 1). Metabolites that can be categorized as fatty acids in this study were isobutyl acetate, palmitic acid, formic acid, 2-pentanoic acid, ethylic acid, n-hexadecoic acid, and hydroxypentanoic acid. The metabolites classified as esters were methyl esters, and formic acid, ethyl esters. Metabolites classified as alcohol were n-cetyl alcohol and phenethyl alcohol. Metabolites classified as xylene were 2,4-xylene and 3,4-xylene. Metabolites classified as phenols were phenol, 2-methoxy-4-(2-propenyl), 2-methoxy-4-vinyl phenol, benzene, 1,2-dimethyl, and 4-vinyl phenol. Metabolites that were classified as other compounds were phenylethane and hexyl chloroformate (Table 1).

The database in the MetaboAnalyst application was a bioinformatics feature that integrated three databases at once, i.e., HMDB, PubChem, KEGG. In this study, a total of twenty compounds were identified and six compounds were not identified by the three databases. The compounds identified in the HMDB database were twenty out of a total of twenty-six compounds. The compounds identified in the PubChem database were twenty out of a total of twenty-six compounds. The compounds identified in the KEGG database were fourteen out of twenty-six total compounds (Table 1). In general, the compounds identified as metabolites in this study were 2-methyl propyl acetate, 1-phenylethylamine, 2,6-dimethylbenzenethiol, 3,4-dimethylphenol, methyl methacrylate, acetic acid, octanol, butyric acid, methylacetoacetic acid, 1-phenyl ethanol, aceteugenol, stearic acid, 2-methoxy-4-vinyl phenol, stearoylethanolamide, ethyl formate, 4-hydroxy styrene, and palmitic acid (Table 1).

Pathway analysis of *Salacca sumatrana* arillus

Pathway analysis was a way to find out whether metabolites detected by GC-MS had a significant effect on metabolism in plants. The pathway results showed that 10 pathways were detected in this study, where the metabolic pathway of biosynthesis of unsaturated fatty acids, fatty acid biosynthesis, and sulfur metabolism had the highest-LOG (p) values with 4.5413, 2.7820, and 2.2430, respectively (Table 2). Based on expected metabolic values, the pathways that have the highest values were fatty acid biosynthesis, fatty acid degradation, and glyoxylate-dicarboxylate metabolism with values of 0.4154, 0.2744, and 0.2151, respectively (Table 2). Holm adjusts and FDR values in this study indicate a value of 1 (Table 2), indicating that the metabolic that was in accordance with the MetaboAnalyst database had a very high impact value. The highest Hits value was the pathway of biosynthesis of unsaturated fatty acids and fatty acid biosynthesis with a metabolite hit value of two. The highest impact metabolite value on the pathway was found in pyruvate metabolism,

while the lowest was found in the pathway biosynthesis of unsaturated fatty acids, butanoate metabolism, cutin, suberin and wax biosynthesis, fatty acid elongation, glyoxylate and dicarboxylate metabolism, and fatty acid degradation (Table 2). P-value for pathway analysis in this study ranged from 0.0107 to 0.2434 (Table 2).

Narrow deep impact using-log(p)/pathway impact ratio

The significance of the influence of the pathway could be measured by determining the value of the ratio-log (p)/pathway impact of each metabolic pathway. This study showed that four of the ten pathways detected have a high log-ratio (p)/pathway impact, i.e., pyruvate metabolism, sulfur metabolism, fatty acid biosynthesis, and biosynthesis of unsaturated fatty acids (Figure 1). Pyruvate metabolism had a pathway impact value above 0.06 and-log (p) above 0.6. Sulfur metabolism had a pathway impact value above 0.06 and-log (p) above 2.0. Fatty acid biosynthesis had pathway impact values above 0.01 and-log (p) above 2.5. Biosynthesis of unsaturated fatty acids had a pathway impact value above 0.002 and-log (p) above 4.5 (Figure 1). Based on the cloud color intensity, the highest cloud color intensity was shown by fatty acid biosynthesis, and biosynthesis of unsaturated fatty acids, representing these two pathways having the highest ratio and having a vital role in the red arillus fruit of *S. sumatrana*. Based on the log-ratio (p)/pathway impact, the highest value was indicated by the pyruvate metabolism and sulfur metabolism pathway (Figure 1), indicating that both pathways have a metabolite significance to the pathway in the red arillus of *S. sumatrana*. Therefore, pyruvate metabolism and sulfur metabolism were further analyzed its pathway to find out the metabolites playing a role in the red arillus of *S. sumatrana*.

Significance metabolites correlated to pyruvate and sulfur metabolisms

Pyruvate metabolism and sulfur katabolism were two pathways that had a high log-ratio (p)/pathway impact, so the metabolite mechanism in this pathway was important to understand. The metabolite that had a significant value in the pyruvate metabolism pathway was C00033. The accession of C00033 showed metabolite ethylic acid or acetic acid. C00033 accession was a key compound in the pyruvate metabolism pathway of the red arillus of *S. sumatrana*. Other accessions that play a role in pyruvate metabolism were C05993, C00084, and C00227 (Figure 2). C05993 compounds could provide negative feedback effects, while C00084 and C00227 could be used as precursors for the formation of ethylic acid compounds (Figure 2).

The metabolite that has significant value on the sulfur metabolism pathway in *S. sumatrana* was C00033 (Figure 2). C00033 accession showed the metabolites of ethylic acid or acetic acid in red arillus of *S. sumatrana*. C00033 accession was the key compound in the sulfur metabolism pathway of the red arillus of *S. sumatrana*. Besides C00033, other accessions that play a role in sulfur metabolism were C00979 and C00283 (Figure 2). The accession of metabolites C00979 and C00283 can be used

as a key precursor of the acetic acid compound in red arillus of *S. sumatrana*. The C00979 accession was regulated by C00065 compound expression, while the C00283 accession was regulated directly by the expression of the C00409 metabolite or indirectly by C00094 (Figure 2).

Metabolite ethylic acid had the synonym name of acetic acid which was identified as C00033 accession based on MetaboAnalyst Databases. This accession was a key metabolite in either the pyruvate metabolism pathway or sulfur metabolisms with relatively high impact values (Figure 2).

Table 1. Metabolite profiling of red arillus of *Salacca sumatrana* using gas chromatography-mass spectrophotometry (GC-MS)

Compounds	Database	HMDB	PubChem	KEGG
Isobutyl acetate	2-Methylpropyl Acetate	HMDB0031246	8038	NA
Phenylethane	1-Phenylethylamine	HMDB0002017	7408	C02455
Benzene, 1,2-dimethyl	Na	NA	NA	NA
2,4-xylene	2,6-dimethylbenzenethiol	HMDB0032019	61045	NA
3,4-xylene	3,4-dimethylphenol	HMDB0032151	7249	NA
2-pentanoic acid, 3-methyl-, methyl ester	Methyl methacrylate	HMDB0032385	6658	C19504
Hexyl chloroformate	Na	NA	NA	NA
Ethylic acid	Acetic acid	HMDB0000042	176	C00033
N-octyl alcohol	Octanol	HMDB0001183	957	C00756
Butyric acid	Butyric acid	HMDB0000039	264	C00246
Butanoic acid, 2-methyl	Methylacetoacetic acid	HMDB0000310	7757	NA
2-methyl-1-octene	Na	NA	NA	NA
Phenethyl alcohol	1-phenylethanol	HMDB0032619	7409	C07112
Phenol, 2-methoxy-4-(2-propenyl)	Aceteugenol	HMDB0034122	7136	C14567
Octadecanoic acid	Stearic acid	HMDB0000827	5281	C01530
Stearic acid	Stearic acid	HMDB0000827	5281	C01530
2-methoxy-4-vinylphenol	2-methoxy-4-vinylphenol	HMDB0013744	332	C17883
N-octadecanoic acid	Stearic acid	HMDB0000827	5281	C01530
N-octadecanoic	Stearoylethanolamide	HMDB0013078	27902	NA
(3s)-3-methyl-3-hydroxypentanoic acid	Na	NA	NA	NA
Formic acid, ethyl ester	Ethyl formate	HMDB0031229	8025	NA
4-vinylphenol	4-hydroxystyrene	HMDB0004072	62453	C05627
2-furancarboxaldehyde, 5-(hydroxymethyl)	Na	NA	NA	NA
5,5'-oxy-dimethylene-bis(2-furaldehyde)	Na	NA	NA	NA
N-hexadecoic acid	Palmitic acid	HMDB0000220	985	C00249
Palmitic acid	Palmitic acid	HMDB0000220	985	C00249

Note: NA was compound which was not identified by Metaboanalyst Database.

Table 2. Pathway analysis of *Salacca sumatrana*

| Pathway	Total	Expected	Hits	P-Value	-LOG(p)	Holm adjust	FDR	Impact
Biosynthesis of unsaturated fatty acids	22	0.1632	2	0.0107	4.5413	1	1	0.0000
Fatty acid biosynthesis	56	0.4154	2	0.0619	2.7820	1	1	0.0112
Sulfur metabolism	15	0.1113	1	0.1061	2.2430	1	1	0.0608
Butanoate metabolism	17	0.1261	1	0.1195	2.1245	1	1	0.0000
Cutin, suberin and wax biosynthesis	18	0.1335	1	0.1261	2.0707	1	1	0.0000
Pyruvate metabolism	22	0.1632	1	0.1521	1.8834	1	1	0.0750
Fatty acid elongation	23	0.1706	1	0.1585	1.8422	1	1	0.0000
Glycolysis/Gluconeogenesis	26	0.1929	1	0.1774	1.7296	1	1	0.0015
Glyoxylate and dicarboxylate metabolism	29	0.2151	1	0.1959	1.6304	1	1	0.0000
Fatty acid degradation	37	0.2744	1	0.2434	1.4132	1	1	0.0000
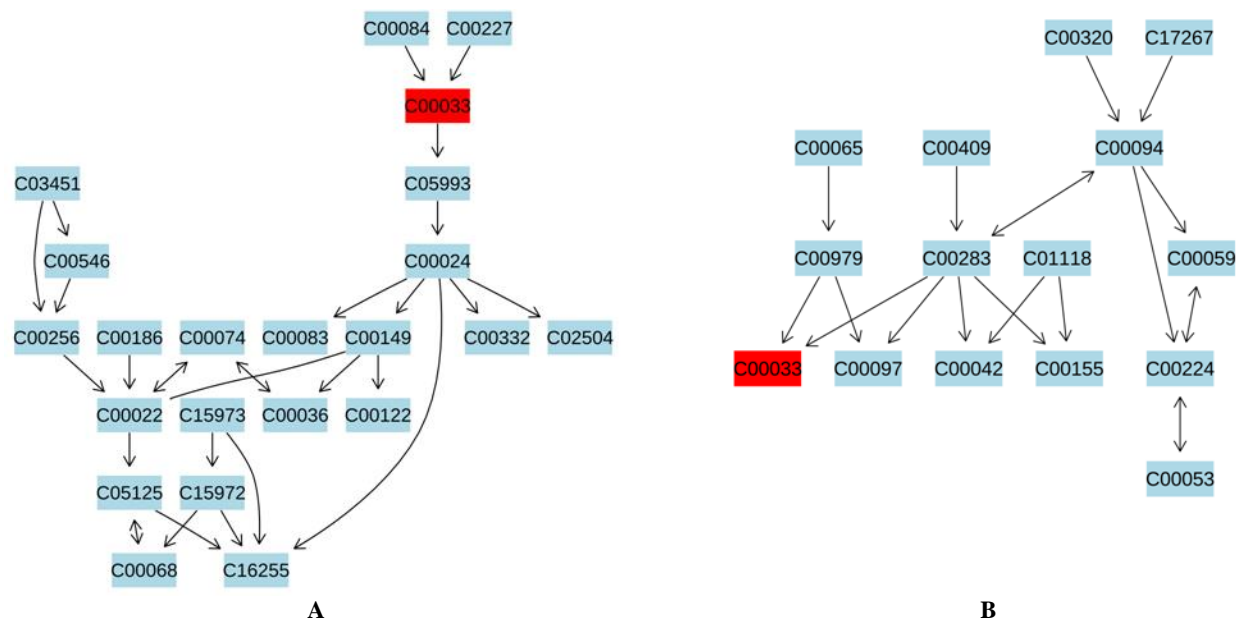
Figure 2. Significance metabolism pathway of *Salacca sumatrana* particularly pyruvate (A) and sulfur metabolisms (B). The red square represents a significant metabolite with the highest impact value. The blue square represents a significance metabolite with a low or moderate impact value
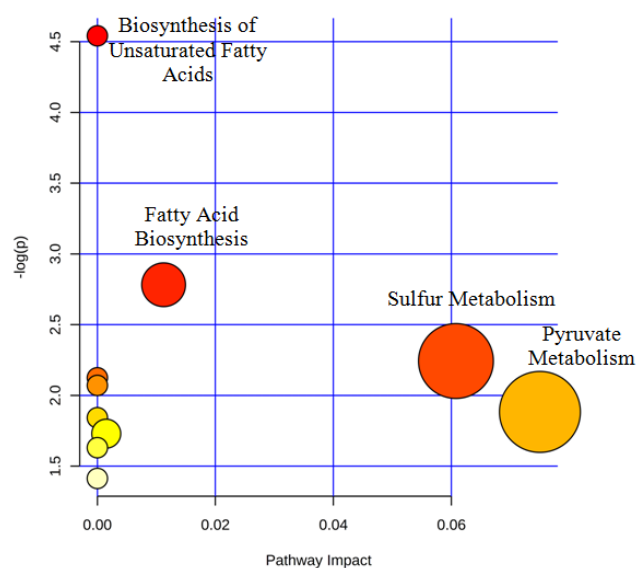

Figure 1. Cloud plot of top hits-log(p)/pathway impact ratio of *S. sumatrana*. A Cloud plot was performed using Metaboanalyst. Cloud color in white to orange represents low to high of-log(p)/pathway impact ratio. The big circle represents the impact value of metabolites to metabolism pathways in red arillus of *S. sumatrana*

Discussion

Metabolomics is one of the scientific approaches that study the regulation of metabolites in organisms whether animal, plant, or microbes (Saleh et al. 2018; Pratami 2020). Metabolomics is one omic technology which generally studies the central dogma from DNA to protein. Metabolomic studies are closely related to enzyme regulation where enzymes are studied at the protein level.

All enzymes are proteins, and the products of enzymes can be either primary or secondary metabolites. Metabolomic analysis can be used as a reference in determining the level of metabolite expression in some plants such as rice, corn, wheat, and arabidopsis. The study of metabolomics analysis on *S. sumatrana* has never been carried out, so research on metabolite profiling using GC-MS is the first to be performed, especially for Indonesian unique salak fruit (*S. sumatrana*).

The metabolomic analysis is one of the bioinformatics analyzes which is important in determining the global pathway of metabolism and biological processes of organisms. Other bioinformatics analyzes are transcriptomic analysis, genetic regulation of a gene, subcellular localization, protein characterization, gene characterization, transcription factor (TF) function, promoter analysis, and untranslated region (UTR) analysis. Such studies are limited to the genome or gene level. Meanwhile, metabolomic studies with added bioinformatics metabolite analysis can be used to look for putative genes that are responsive through comparative studies of metabolites. In this study, a metabolic profiling study was carried out to ascertain which metabolites were involved in the formation of red arillus in *S. sumatrana*.

Red arillus is part of the salak fruit that is consumable, and only red arillus is used in this research. Morphological, anatomical, or taxonomic studies of *S. sumatrana* has been done, but studies on the profile of metabolites in *S. sumatrana* are very few. This research confirms that there are some significant metabolites found in the red arillus tissues of *S. sumatrana*, i.e., isobutyl acetate, palmitic acid, formic acid, 2-pentanoic acid, ethylic acid, n-hexadecoic acid, hydroxypentanoic acid, methyl acid esther, ethyl esther n-octyl alcohol, phenethyl alcohol, 2,4-xylene, 3,4-

xylene, phenol, 2-methoxy-4-(2-propenyl), 2-methoxy-4-vinyl phenol, benzene, 1,2-dimethyl, 4-vinyl phenol, phenylethane, and hexyl chloroformate (Table 1). Isobutyl acetate plays a role in fruit ripening in tomato, particularly *S. lycopersicum. S. cheesmaniae*, and *S. galapagense* (Goulet et al. 2012). Palmitic acid and formic acid were biological properties of palm oil and had important rules in physiological processes (Mancini et al. 2015). 2-pentanoic acid is a common compound found in berries, apple, and other fruits (Goulet et al. 2012). Ethylic acid plays a role in physiological processes. n-hexadecanoic acid had the activity related to antioxidant, hypocholesterolemic, nematicide, anti-androgenic, flavor, and hemolytic in *Eichhornia crassipes* (Mart.) Solms. Methyl ester can be used as antimicrobial, anticancer, hepatoprotective, anti-arthritic, anti-asthma, and diuretic activity. Ethyl ester n-octyl alcohol plays a role as antimicrobial. Phenethyl alcohol had antioxidant activity. 2,4-xylene and 3,4-xylene were common and found in odor activity in fruit. Phenol, 2-methoxy-4-(2-propenyl) plays a role in the physiological process in fruit formation. 2-methoxy-4-vinyl phenol is a common compound in fruit. 1,2-dimethyl-4-vinyl phenol and phenylethane play a role in fatty acid metabolism in plants. Hexyl chloroformate plays as an antioxidant (Tyagi and Agarwal 2017). Thus, in this study, these metabolites play a vital role in the red arillus section of *S. sumatrana*. In addition to the study, some of the metabolites found were thought to be similar to some candidate genes found by Fendiyanto et al. (2019a) and Satrio et al. (2019), indicating there is a relationship between metabolite compounds associated with abiotic stress.

Biosynthesis of unsaturated fatty acids and fatty acid biosynthesis is a pathway that is commonly found in the process of physiology and plant growth both directly or indirectly, i.e, photosynthesis (Ohki 1986), chlorophyll formation (Ohki 1986; Saleh et al. 2018; Fendiyanto et al. 2019a), cell growth rate (Zaini et al. 2013; Saleh et al. 2018), biotic and abiotic stress (Aralas et al. 2019), aluminum stress (Kochian et al. 2015; Fendiyanto et al. 2019a), drought stress (Satrio et al. 2019), salinity stress (Aralas et al. 2019), and fruit and flower formation (Wong and Tie 1993; Priyatno et al. 2012; Li et al. 2013). However, there was very little information about the relationship between lipid biosynthesis in red arillus from *S. sumatrana*, therefore this study confirms that lipid biosynthesis is related to arillus color formation. However, this study also found other important pathways related to the formation of arillus from *S. sumatrana*, such as butanoate metabolism, cutin, suberin and wax biosynthesis, fatty acid elongation, glyoxylate and dicarboxylate metabolism, and fatty acid degradation.

Chloroplasts contain a complex photosynthetic membrane system that dominates the total membrane content of leaves (Harwood 1989) and are the main location for lipid synthesis and assembly (Browse et al. 1991). In thylakoids, the chloroplast-specific glycoglycerolipids monogalactosyl diacylglycerol, digalactosyl diacylglycerol, sulfo quinovosyl diacylglycerol, and phosphatidylglycerol construct a special hydrophobic matrix for pigment-protein complexes which are essential for photosynthesis. Some of the fatty acids synthesized in chloroplasts are directly assembled into thylakoid lipids, while others are exported to the endoplasmic reticulum (ER). Chloroplast lipid precursors originate from different locations and are primarily assembled at the chloroplast envelope membranes (Harwood 1996; Wang and Benning 2012). All of the membrane lipids and storage lipids in the plant, including fatty acids that derive from plastid-synthesized palmitic acid (16: 0) and oleic acid (18: 1) are biosynthesized in the stroma of the chloroplasts. Once formed, a part of the fatty acids is directly synthesized into glycerolipids (galactolipids, sulfolipids, and phosphatidylglycerol) within the envelopes, while the other parts are exported across the envelopes to the ER in which they are constructed into phospholipids, especially phosphatidylcholine (Joyard and Douce 1987; Bates et al. 2007).

An important precursor in lipid biosynthesis, pyruvate metabolism, and sulfur is pyruvate. Pyruvate can be found in all three pathways and has an important role. Pyruvate is a product of glycolysis and plays a role in many metabolic processes, such as respiration, photosynthesis, the pentose phosphate pathway, and photorespiration (Saleh et al. 2018).

Other important pathways in the arillus of *S. sumatrana* are pyruvate metabolism and sulfur metabolism (Figure 1). Pyruvate metabolism has a pathway impact value above 0.06 and-log (p) above 0.6. Sulfur metabolism has a pathway impact value above 0.06 and-log (p) above 2.0. Based on the log-ratio (p) / pathway impact, the highest value is shown by the pyruvate metabolism and sulfur metabolism pathway (Figure 1), indicating that both pathways have a metabolite significance to the pathway in the red arillus *S. sumatrana*. Therefore, pyruvate metabolism and sulfur metabolism are further analyzed its pathway to find out the metabolites playing a role in the red arillus *S. sumatrana*. This is consistent with the study of Ragasa et al. (2016) which shows that pyruvate metabolism and sulfur metabolism play an important role in fruit formation.

Salacca cultivars have been studied for their phytochemical constituents using various techniques. Wong and Tie (1993) reported on the volatile compounds present in the pulp of *Salacca* Reinw. The identification of about 46 compounds were detected consisting of mostly carboxylic acids (15.9%), alcohols (1.3%), aldehydes (0.8%), ketones (0.7%), sulfur-containing compounds (0.2%), and aromatic hydrocarbons (0.3%). Specifically, the most prominent compounds viz., methyl 3-hydroxy-3-methyl pentanone, and methyl (E)-3-methyl penta-2-enoate were found to be about 25.0% and 23.4%, respectively (Kanlayavattanakul et al. 2013). This study also found compounds that are similar to these compounds, namely 2-pentanoic acid, ethylic acid, n-hexadecoic acid, hydroxypentanoic acid.

Comparing to *S. sumatrana*, the investigation on *S. zalacca* fruit juice using its ripe arillus showed a remarkable antioxidant activity with the initial screening of total phenolic content of 175.99 μg GAE and 98.28 μg AAE of vitamin C per g sample, respectively. Meanwhile,

the DPPH and FRAP activities were observed as 421.56 μg vitamin C equivalent antioxidant capacity per gram and 1 556.79 μg TE antioxidant capacity per g sample, respectively (Saleh et al. 2018). This is followed by a study where the salak core (fruit flesh) and shell were analyzed for their antioxidant activity using the DPPH scavenging activity and total flavonoid content. The core and shell hydro-alcoholic extracts were found to be active in the DPPH assay at 82.67 and 73.13%, respectively (Saleh et al. 2018). Antioxidant compounds found in salak fruit (*S. zalacca*) and found in this study, especially in *S. sumatrana* are methyl 3-hydroxy-3-methyl pentanone, methyl 3-hydroxy-3-methyl pentanone, and pentanoic acid (Table 1).

Pyruvate and sulfur metabolism are two pathways that have a high log-ratio (p)/pathway impact, so the metabolite mechanism in this pathway is important to understand. Metabolites that have significant values in the pathway of pyruvate metabolism and sulfur metabolism were C00033. The accession of C00033 shows metabolite ethylic acid or acetic acid. C00033 accession is a key compound in the pyruvate metabolism pathway of the red arillus *S. sumatrana*. Other accessions that play a role in pyruvate metabolism are C05993, C00084, and C00227 (Figure 2). The C05993 compounds can provide negative feedback effects, while C00084 and C00227 can be used as precursors for the formation of ethylic acid compounds (Figure 2). The C00033 accession shows the metabolites of ethylic acid or acetic acid in red arillus *S. sumatrana*. The C00033 is the key compound in the sulfur metabolism pathway of the red arillus *S. sumatrana*. Besides C00033, other accessions that play a role in sulfur metabolism are C00979 and C00283 (Figure 2). The accession of metabolites C00979 and C00283 can be used as a key precursor of the acetic acid compound in red arillus *S. sumatrana*. The C00979 accession is regulated by C00065 compound expression, while the C00283 accession is regulated directly by the expression of the C00409 metabolite or indirectly by C00094 (Figure 2). The same thing was also found in the research of Saleh et al. (2018) of *S. zalacca*. Determination of markers for the selection process in plant breeding programs can be done at the level of DNA markers such as ISSR (Pratami et al. 2020) and SNP (Fendiyanto et al. 2019a), morphological markers (Yuliamita 2014, Pratami et al. 2019), isozymes (Yuliamita 2014), and metabolite markers (Pratami 2020). Therefore both molecular approaches (Nguyen et al. 2001; Nguyen et al. 2003; Yamaji et al. 2009; Tsutsui et al. 2011; Ma et al. 2014), morphology (Yuliamita 2014; Pratami et al. 2019), metabolomics (Pratami 2020), and comprehensive anatomy (Nikmah et al. 2020; Pratami 2020) can be done in the future to characterize the genes and potential compounds in the red arillus *S. sumatrana*.

Lipid, sulfur, and pyruvate metabolism are important pathways in the red arillus of *S. sumatrana*. These three pathways can be used as a reference to determine the physiological processes of red arillus, specifically *S. sumatrana*. Important compounds that can be found in this study are isobutyl acetate, palmitic acid, formic acid, 2-pentanoic acid, ethylic acid, n-hexadecoic acid, hydroxypentanoic acid, methyl ester, ethyl ester, n-octyl

alcohol, phenethyl alcohol, 2,4-xylene, 3,4-xylene, phenol, 2-methoxy-4-(2-propenyl), 2-methoxy-4-vinyl phenol, benzene, 1,2-dimethyl-4-vinyl phenol, phenylethane, and hexyl chloroformate. In addition, compounds that play an important role in the metabolism of pyruvate and sulfur are ethylic acid. The biosynthetic compounds and pathways in this study can be used as markers for future breeding programs, especially in *S. sumatrana.*

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