JJBS

Volume 18, Number 3,September 2025
ISSN 1995-6673
Pages 509 – 520

Jordan Journal of Biological Sciences

Comparative Metabolomics Studies Related to Lipid Biosynthesis Indicate Metabolic Pathways Regulation Differences in Mature and Young Seeds (MYS) of *Jatropha curcas*

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Received: October 22, 2024; Revised: March 11, 2025; Accepted: April 6, 2025

Abstract

Jatropha curcas is an alternative bioenergy source that can be developed as a solution to the global energy crisis. This plant represents a promising new alternative energy source. This study aims to conduct a comparative study of the characteristics of metabolites in mature (MS) and young (YS) seeds in *Jatropha* plants through metabolomic studies. Six samples of *Jatropha curcas* seeds (accessions) were used, grown by the cutting method using a completely randomized block design (CRBD) using three replications, and the seed harvesting process was carried out according to the type of development (MS and YS). Seed extraction was tested using the GC-MS method. A total of 25 different metabolites, 19 which of metabolites were highly expressed in mature seeds of *Jatropha curcas*. In contrast, there were only 6 metabolites that were highly expressed in young seeds. In general, the four metabolites that have the highest correlation (strong positive intercorrelation) are the intercorrelation of the metabolites Eicosadienoic acid, Tetracosahexane, Sitosterol, and Aminoethanethiol. Based on the PLS-DA study, the metabolites showed that three MS accessions grouped and were separated from three YS accessions with a total PC value of 91.1%. 24 compounds had the highest impact value on the galactose metabolism pathway. The detected metabolic pathways included the pentose phosphate pathway, pentose and glucuronate interconversions, and amino sugar or nucleotide metabolism. Other relatively significant metabolite pathways that could distinguish between the two types of MS and YS seeds were sesquiterpenoid and triterpenoid biosynthesis and steroid biosynthesis. Thus, mature and young seeds showed differences in the expression of metabolite content and these differences globally occurred in the galactose metabolism, triterpenoid, and steroid biosynthesis pathways. This research is expected to contribute to the study of the selection of the best seeds for lipid synthesis as a raw material for biofuel in *Jatropha curcas.*

Keywords: *Jatropha curcas*, metabolomics, pathway, mature seed (MS), young seed (YS)

1. Introduction

Jatropha curcas is a plant species classified under the class Magnoliopsida or dicotyledons, belonging to the order Malpighiales. This plant is part of the Euphorbiaceae family, a group of plants known for containing various bioactive chemical compounds (Makkar et al., 2008). Taxonomically, Jatropha curcas falls under the genus Jatropha, which includes multiple species with similar characteristics. Jatropha curcas is a perennial shrub or small tree that retains some of its foliage year-round and can grow beyond 6 meters in height (Janick and Paull, 2008; Fendiyanto et al., 2024).

Its remarkable ability to withstand extreme arid conditions allows it to thrive in desert environments. However, the plant contains phorbol esters, which are known to be toxic. Despite this, non-toxic, edible varieties native to Mexico exist and are locally referred to by names such as piñón manso, xuta, chuta, and aishte (Gunjan et al., 2016; Martínez-Herrera et al., 2010). Additionally, J. curcas contains other bioactive compounds, including trypsin inhibitors, phytate, saponins, and a specific type of lectin called curcin (Valdes-Rodríguez et al., 2013). Its seeds have an oil content ranging from 27% to 40% (average 34.4%) (Martínez-Herrera et al., 2012), which can be refined into high-quality biodiesel suitable for use in standard diesel engines (Lin et al., 2010). As a plant capable of withstanding dry conditions, Jatropha curcas is widely utilized for various purposes, including as a source of biodiesel (Fendiyanto et al., 2024).

The utilization of oil-producing plants such as Jatropha curcas to produce alternative energy sources and promote environmental sustainability, as well as to create an energy-independent region, is one of the objectives of developing new energy materials, especially liquid fuels.

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Jatropha is one of the plants that can be utilized by major non-edible oil producers (Openshaw, 2000; Sato et al., 2011). The potential of Jatropha can replace other crops. In addition to having a high level of adaptation to the environment, this plant has many advantages over other edible oil-producing plants such as being resistant to pests, relatively fast growth, and being able to be planted on marginal land that has little soil nutrition (Openshaw 2000; Maes et al., 2009; Mishra, 2009).

Jatropha plants start producing seeds within their first year, but it usually takes about 2 to 3 years for them to reach substantial yields. In the beginning, farmers can expect around 0.4 tons of seeds per hectare, but as the plant matures, this can increase to over 5 tons per hectare by the third year. On average, under moderate soil conditions, jatropha produces about 3.5 tons of seeds per hectare. This translates to roughly 1,590 kilograms of oil per hectare annually, or about 1,892 liters of oil per hectare, making it a promising option for biodiesel production (Valdes-Rodríguez et al., 2013). High adaptability to less fertile environments makes J. curcas an alternative solution to overcome the problem of land shortages due to agricultural land conversion in Indonesia. Based on the category of land suitability of J. curcas, from the total land area of 49.53 million ha, there are 29.72 million ha, or 60% of marginal land that is suitable but has not been utilized efficiently (Fendiyanto et al., 2024). An environmentally friendly fuel called biofuel can be a solution to frequent environmental problems (Fendiyanto et al., 2024). Compared to fossil fuels, biofuels have relatively lower levels of gas emissions in the form of CO and unburned hydrocarbons (CH)n, and can be a solution to global problems as a result of the use of fossil fuels. Unlike non-renewable fossil fuels, J.curcas can be categorized as an alternative renewable bioenergy resource, i.e., edible oil, non-edible oil, and lignocellulosic biomass (Maes et al., 2009). The development of bioenergy resources can be an alternative solution to the problem of the energy crisis in the world, so it is very important to conduct a study of its development.

Although Jatropha curcas has been widely studied as a biodiesel source, there are still many unanswered questions regarding the biochemical and metabolic differences between its young and mature seeds (Fendiyanto et al., 2024). One key area that needs further investigation is how lipid biosynthesis changes throughout seed development. While mature seeds are known to have a high oil content (27–40%), the metabolic processes responsible for this accumulation are not yet fully understood, especially in comparison to young seeds. Exploring these differences could provide valuable insights into the regulatory mechanisms that drive lipid production, potentially leading to improved oil yields through genetic advancements or better farming practices.

Metabolomics provides a powerful tool for unraveling the biochemical pathways involved in lipid biosynthesis in Jatropha curcas. By analyzing the metabolites present in both young and mature seeds, scientists can identify key compounds and regulatory factors that influence fatty acid and triacylglycerol (TAG) production (Fendiyanto et al., 2024). For example, shifts in carbohydrate metabolism, amino acid composition, and hormone activity could indicate how metabolic priorities change as the seed matures, transitioning from early growth to oil storage (Fendiyanto et al., 2024). Moreover, combining metabolomics with transcriptomics and proteomics could create a more detailed picture of how genes, enzymes, and metabolites interact to regulate lipid production.

A deeper understanding of how lipid metabolism differs between young and mature Jatropha curcas seeds could pave the way for strategies to boost oil production, enhance biodiesel quality, and refine cultivation techniques. Addressing these knowledge gaps would help researchers identify key metabolic limitations and develop Jatropha varieties with higher oil content and better adaptability to diverse environmental conditions. Thus, this study aimed to conduct a comprehensive study to characterize the potential of the oil plant (Jatropha curcas) as a renewable bioenergy source through a metabolomic approach.

2. Materials and Methods

2.1. Plant materials

Plant materials used in this research were the young and mature seeds of Jatropha curcas. Comparative study of J. curcas that was at least 2 years old, cuttings in polybags with 12 experimental units and grouped into two types of treatments, namely mature seed type (MS) and young seed type (YS) using a completely randomized block design (RCBD). We conducted the study three times a year, across three different periods. In addition, J. curcas from existing land or gardens were also used in this study, especially for metabolomic testing. The growth and development of J. curcas potential were performed by the following Fendiyanto et al., (2024). In addition, J. curcas harvesting in this study was carried out when the fruit was ripe and the fruit turned yellow for mature seed (MS) samples, which were 5-6 months old, while the fruit was green for young seeds (YS), which were 1-2 months old after flowering. Seed collection was carried out by cutting and then the sample was soaked in liquid nitrogen.

2.2. Metabolites extraction and identification

GC-MS study of J. curcas seeds was carried out to identify chemical components using a modified method from Fendiyanto et al., (2024). In the initial stage, 10 grams of seeds were extracted using ethyl acetate solvent at a temperature of 25°C for 1 hour, where the solution was continuously stirred with a shaker at a speed of 50 rpm. The mixture obtained was then evaporated for 60 mins with an evaporator (Caliper-Life-Science, USA), and then injected into the GC-MS instrument. The GC-MS system used includes a main unit (Agilent Tech-Palo Alto-USA), an auto-sampler (Agilent Tech-Palo Alto-USA), and a mass selective detector (inert MSD Detector, Agilent Tech-Palo Alto-USA) (Fendiyanto et al., 2021).

We performed GC-MS Column Specifications, particularly column Type Capillary column (Agilent HP-5MS), Column Dimensions in 30 m × 0.25 mm ID × 0.25 µm film thickness, Stationary Phase in 5% Phenyl / 95% Dimethylpolysiloxane. In addition, we used temperature gradient (oven program), i.e. initial temperature in 50°C, hold for 1–2 min, Ramp in 10–15°C/min to 250–300°C, and Final temperature in hold for 5–10 min. We conducted Ionization Parameters (MS Conditions) particularly Ionization Mode in Electron Ionization (EI), Ionization Energy in 70 eV, Mass Scan Range in 50–600 m/z,

Detector Voltage in Optimized based on instrument specifications, Source Temperature in 230–250°C, and Quadrupole Temperature in ~150°C. The final stage of this analysis includes mass spectrum detection and metabolite identification, which is performed by referring to the operational procedures of Fendiyanto et al., (2020). Thus, this method is expected to optimize the detection of chemical compounds in J. curcas seed samples.

2.3. Comparative Fold Change Study and Data Analysis

Metabolomics data were analyzed using MetaboAnalyst.R (Xia and Wishart, 2016) with a fold change approach. The settings used in the analysis included the analysis type 'unpaired', the fold change threshold '2', the comparison type 'Mature/Young', and the significance threshold of '85%'. Furthermore, the correlation between metabolites was analyzed using the 'feature' dimension setting with the 'Spearman rank correlation' distance measure. The display mode was set as 'overview', with red/green color contrast, without fixed color distribution, and without clustering. For heatmap analysis, the distance was measured using the 'Euclidean' method, the 'single' clustering algorithm, and standardization using the autoscale feature, with normalized data sources. The selected display mode was 'overview with T-test/ANOVA' without additional display options. Then, all metabolites were compared with the KEGG, HMDB (Wishart et al., 2018), and PubChem databases based on the adapted guidelines from Fendiyanto et al., (2020). Further analysis was carried out using univariate and multivariate data (Fendiyanto et al., 2019a; Fendiyanto et al., 2019b; Satrio et al., 2019) with R (Lander, 2014) and MetaboAnalystR (Chong et al., 2018; Chong and Xia, 2018; Chong et al., 2019; Pang et al., 2020). In addition, morphological identification (Sunil et al., 2013) of potential J. curcas was done by direct observation following Fendiyanto et al., (2024).

3. Results

3.1. Comparative study of metabolites in mature and young seeds of Jatropha curcas

Metabolite content in mature and young seeds showed differences in metabolite content expression. Twenty-five metabolites were detected, with 19 showing high expression in mature J. curcas seeds. In contrast, there were only 6 metabolites that were highly expressed in young seeds (Figure 1, Table 1). The compounds that were highly expressed in mature seeds included butanoic acid, benzene acetaldehyde, hexahydro, germa-cyclo pentane, benzodioxane, 3 oxobutyric acid, pyrazol, galactose, and others. The compounds that were highly expressed in young seeds included hexadecenoic acid, eicosadienoic acid, tetracohexane, sitosterol, amino ethathiol, and octadecadiene (Figure 1). The tissue in mature seeds that had the highest significant metabolite expression was MS01 followed by MS02 and MS03. The highest metabolite expression in young seeds was YS01 followed by YS02, while accession YS03 had relatively low global lipid metabolite expression (Figure 1).

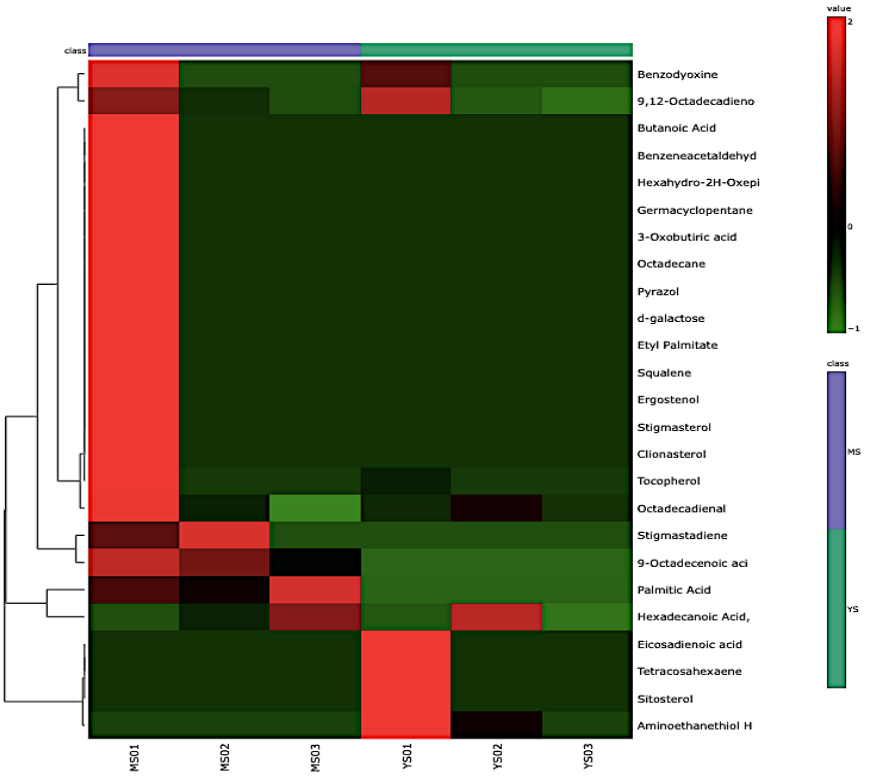


Figure 1. Differences in metabolite content in young and mature seeds of *Jatropha curcas*. There were 25 compounds detected significantly different by the gas chromatography-mass spectrophotometry (GC-MS) test. The heatmap comparison difference value ranged from -1 to 2. The class groups used were mature seed (MS, purple) and young seed (YS, green). MS01: mature seed repeat 1, MS02: mature seed repeat 2, MS03: mature seed repeat 3, YS01: young seed repeat 1, YS02: young seed repeat 2, YS03: young seed repeat 3.

Table 1. List of significant metabolite differences between mature seeds (MS) and young seeds (MS) of *Jatropha curcas*

Compound	HMDB	PubChem	KEGG
Benzeneacetaldehyde	HMDB0006236	998	C00601
Butanoic Acid	HMDB0000039	264	C00246
Hexahydro-2H-Oxepino	NA	NA	NA
Germacyclopentane	HMDB0031407	8452	C00557
Hexadecanoic Acid, Methyl hexanoate	HMDB0035238	7824	NA
3-Oxobutiric acid	HMDB0000005	58	C00109
Octadecane	HMDB0033721	11635	NA
Pyrazol	NA	NA	NA
d-galactose	HMDB0000143	439357	C00124
Ethyl Palmitate	HMDB0061709	164860	NA
Palmitic Acid	HMDB0000220	985	C00249
9,12-Octadecadienoic Acid (9z)	NA	NA	NA
Octadecadienal	HMDB0005047	5282796	C04056
Squalene	HMDB0000256	638072	C00751
9-Octadecenoic acid	HMDB0000207	445639	C00712
Tocopherol	HMDB0001492	92729	C02483
Benzodyoxine	HMDB0040528	4685450	NA
Ergosterol/Phytosterol	HMDB0000878	444679	C01694
Stigmasterol	HMDB0000937	5280794	C05442
Clionasterol	HMDB0000649	457801	C19654
Stigmastadiene	HMDB0000937	5280794	C05442
Aminoethanethiol Hydrogene Sulphate	NA	NA	NA
Tetracosahexaene	HMDB0000256	638072	C00751
Eicosadienoic acid	HMDB0005060	6439848	C16525
Sitosterol	HMDB0000649	457801	C19654

3.2. Correlation study between metabolites in two types of Jatropha curcas seeds

The content of metabolite compounds obtained from two types of J. curcas seeds showed three types of groups, namely: groups of compounds that have strong positive correlation values, compounds that have strong negative correlations, and compounds that have low correlations (Figure 2). In general, the four metabolites that have the highest correlation (strong positive intercorrelation) are the intercorrelation of the metabolites Eicosadienoic acid, Tetracosahexane, Sitosterol, and Aminoethathiol. The four compounds included in the strong negative correlation type group and have the most negative values are Aminoethanethiol-Octadecanoic acid, Benzodioxane-Hexadeconoic Acid, Aminoethanethiol-Palmitic acid, and Octadecenoic acid-Hexadeconoic Acid (Figure 2).

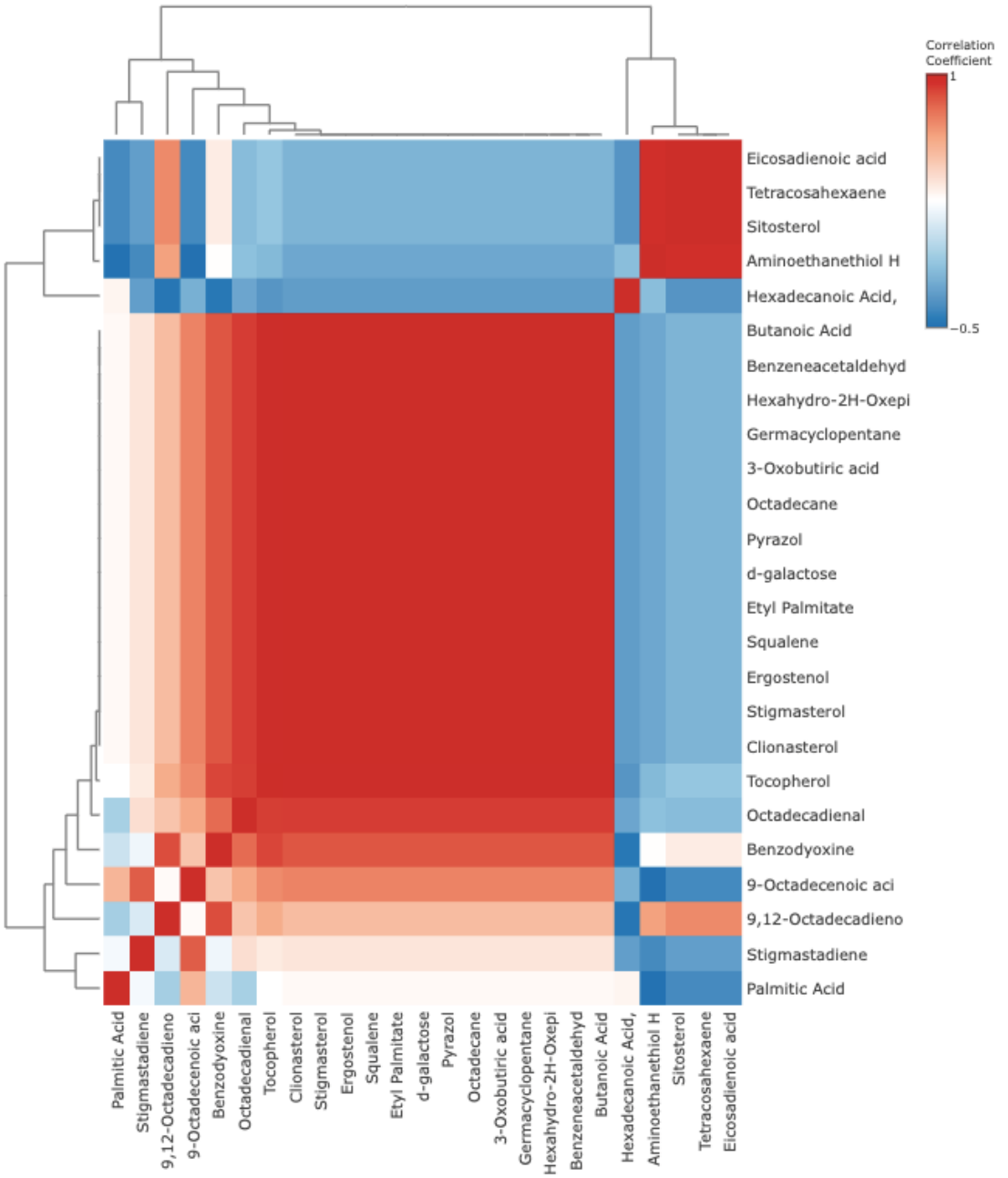


Figure 2. Correlation between metabolites in young and mature seeds of *Jatropha curcas*. Testing was done using correlation heatmap and statistical testing. Correlation values range from -1 to +1 with threshold values of (-0.5 and 1). The red color indicates a strong positive correlation value. The blue color indicates a strong negative correlation value. The white color indicates no correlation.

3.3. Metabolite profiles in two types of Jatropha curcas seeds

The metabolite profiles showed normal distribution based on a comparative study using multi boxplot (Figure 2A). The metabolite that showed a wide distribution was the compound 9,12-Octadecanoic acid, while the narrowest distribution was Eicosadinoic acid and Benzeneacetaldehyde (Figure 3A). Globally, the metabolite profiles in *Jatropha curcas* seeds showed a division into three clusters, namely types 1, 2, and 3. Based on a comparative study of the concentration between mature (MS) and young (YS) seeds, Cluster type 1 had a relatively moderate fluctuation value, cluster type 2 showed a relatively low value, while cluster type 3 showed a high value (Figure 3B). Based on the sensitivity study of the metabolite profile, the metabolites in both types of *J. curcas* seeds showed differences in Var.AUC and CI value groups were different and grouped into 6 types with specificity values from 0 to 1 (Figure 3C).

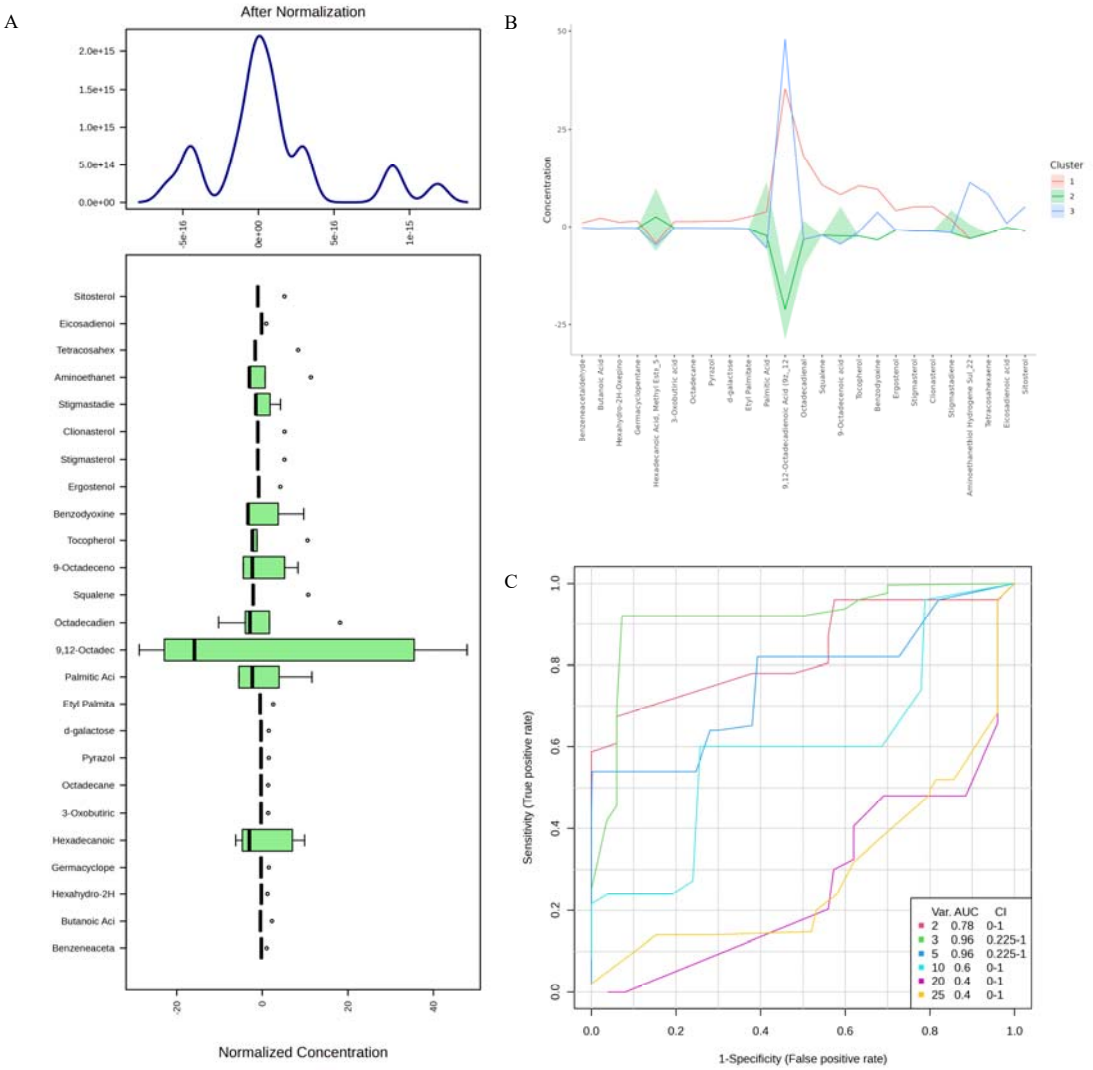


Figure 3. Comparison of the concentration of compounds in the lipid metabolism pathway in mature and young seeds of *Jatropha curcas*. The normality value of each metabolite compound detected based on the boxplot in each compound (A). The difference in concentration of 25 metabolites is divided into 3 clusters, namely clusters 1, 2, and 3 (B). The sensitivity rate value of each metabolite in *J. curcas*, especially in the difference in seed development stages (C).

3.4. etabolite profile clustering and network analysis test

Mature seeds (MS) tend to have high loading and frequency values, while young seeds (YS) have low values. The differences that occur are due to the frequency of expression of compounds 9 Octadecanoic acid, palmitic acid, Hexadecanoic acid, Stigmastadiene, 9.12 Octadecadienoic acid, and Octadecadienal. The expression value of 9 Octadecanoic acid shows a relatively high-frequency value, which is close to 1 (Figure 4A). The predictive accuracy value of *J. curcas* seed metabolites shows as much as 93% at the number of features 3, while the lowest value is 32% at the number of features 20 (Figure 4B). Based on the PLS-DA study, metabolites show three MS accessions grouped into one and separated from three YS accessions with a total PC value of 91.1% (Figure 4C). The network analysis test showed that 25 metabolites were closely related to each other and centered on Oxobutyric acid (Figure 4D).

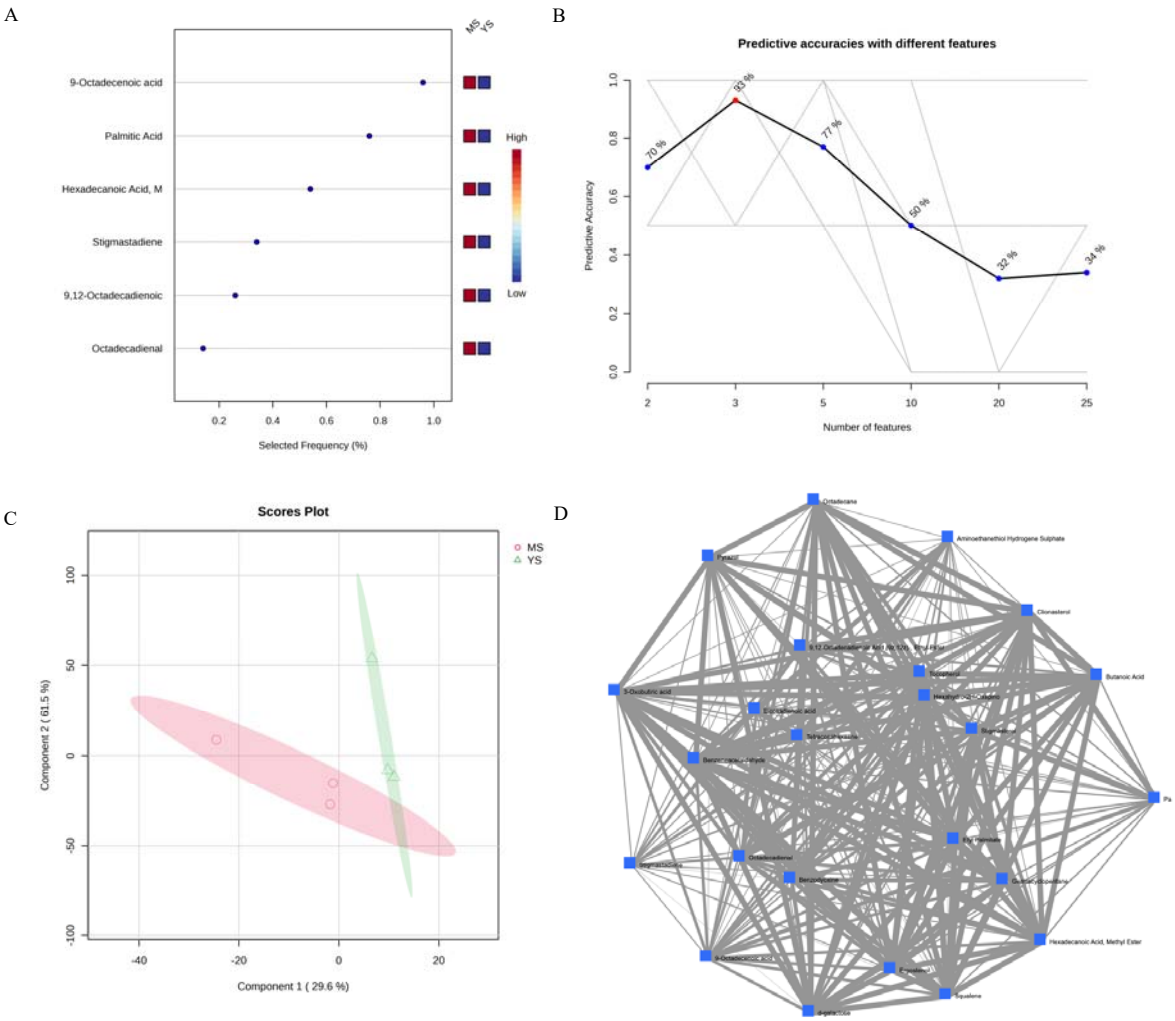
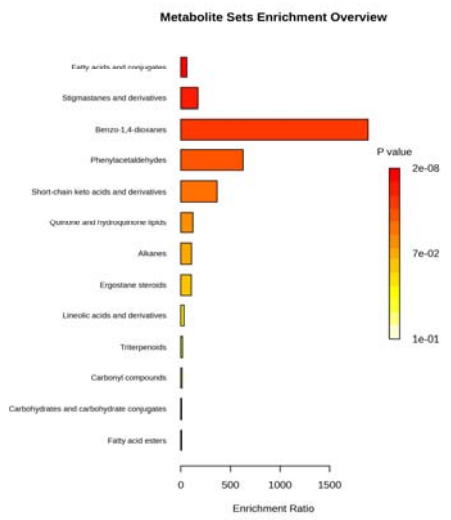


Figure 4. Frequency loading plot (A), Predictive accuracy (B), PLS-DA clustering (C), and network analysis (D) of 25 metabolites in young and mature seeds of *Jatropha curcas.*

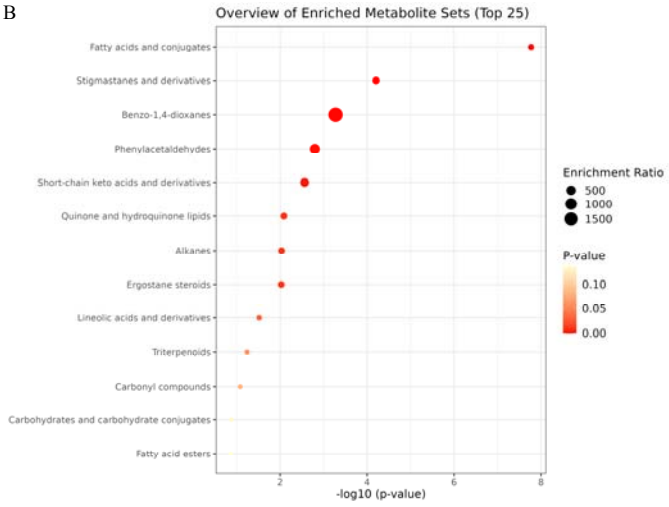
3.5. Metabolite set enrichment studies

Metabolite set enrichment showed that the metabolites detected in the two types of seeds were found in the Benzo 1.4 dioxanes, Phenylacetaldehyde, and keto acid and its derivatives groups. The highest enrichment ratio value was shown in Benzo 1.4 dioxanes, while the lowest was found in fatty acid esters (Figure 5A). If the -log10 ratio value is tested, the highest value is fatty acids and conjugates, followed by stigmastanes and Benzo 1.4 dioxanes (Figure 5B). Globally, these metabolites are also included in the fatty acids and conjugates compound set group along with stigmastanes and derivatives. Other groups include alkanes, benzo 1.4-dyoxans, carbohydrates, carbonyl compounds, ergostane steroids, fatty acid esters, linolenic acid, phenylacetaldehyde, quinone & hydroxyquinone lipids, keto acids and derivatives, and triterpenoids (Figure 5C). The highest pathway impact pathway value reached a value of 0.30 while the highest hold value reached a value of 2.6 (Figure 5D). The highest pathway value that has the highest hits value is fatty acids & conjugates, and the lowest is fatty acid ester (Figure 5E).

A



B



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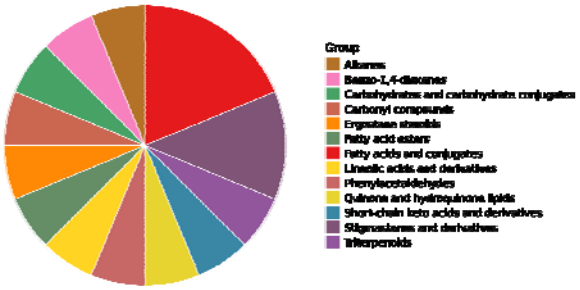

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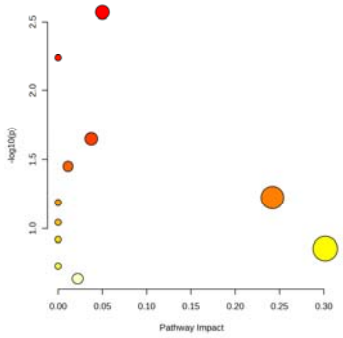

E

	Metabolite Set	Total	Hits	Expect	P value	Holm P	FDR
	Fatty acids and conjugates	941	5	0.0831	1.69E-8	1.51E-5	1.51E-5
	Stigmastanes and derivatives	131	2	0.0116	6.23E-5	0.0554	0.0277
	Benzo-1,4-dioxanes	6	1	5.3E-4	5.3E-4	0.471	0.157
	Phenylacetaldehydes	18	1	0.00159	0.00159	1.0	0.354
	Short-chain keto acids and derivatives	31	1	0.00274	0.00274	1.0	0.487
	Quinone and hydroquinone lipids	93	1	0.00822	0.00818	1.0	1.0
	Alkanes	105	1	0.00928	0.00924	1.0	1.0
	Ergostane steroids	107	1	0.00945	0.00941	1.0	1.0
	Lineolic acids and derivatives	348	1	0.0307	0.0303	1.0	1.0
	Triterpenoids	672	1	0.0594	0.0577	1.0	1.0
	Carbonyl compounds	975	1	0.0861	0.0827	1.0	1.0
	Carbohydrates and carbohydrate conjugates	1600	1	0.141	0.132	1.0	1.0
	Fatty acid esters	1650	1	0.146	0.136	1.0	1.0

Figure 5. Set of metabolite enrichment (A), enrichment ratio (B), grouping of compound types (C), pathway impact (D), and pathway analysis group (E) in young and mature seeds of *Jatropha curcas.*

3.6. KEGG Pathway

The metabolite pathway in galactose metabolism showed significant compounds detected in two types of seeds relatively high. 24 compounds had the highest impact value on the galactose metabolism pathway. The detected metabolic pathways included the pentose phosphate pathway, pentose and glucuronate interconversions, and amino sugar or nucleotide metabolism (Figure 6). Significant metabolites included the farnesol, germacrene, lupeol, squalene, and farnesyl-PP groups (Figure 7). A total of 41 metabolites played a role in the steroid biosynthesis pathway. This pathway is also related to the biosynthesis pathways of brassinosteroids, phytosterols, bile acid biosynthesis, steroid hormone biosynthesis, and phytosterols biosynthesis (Figure 8).

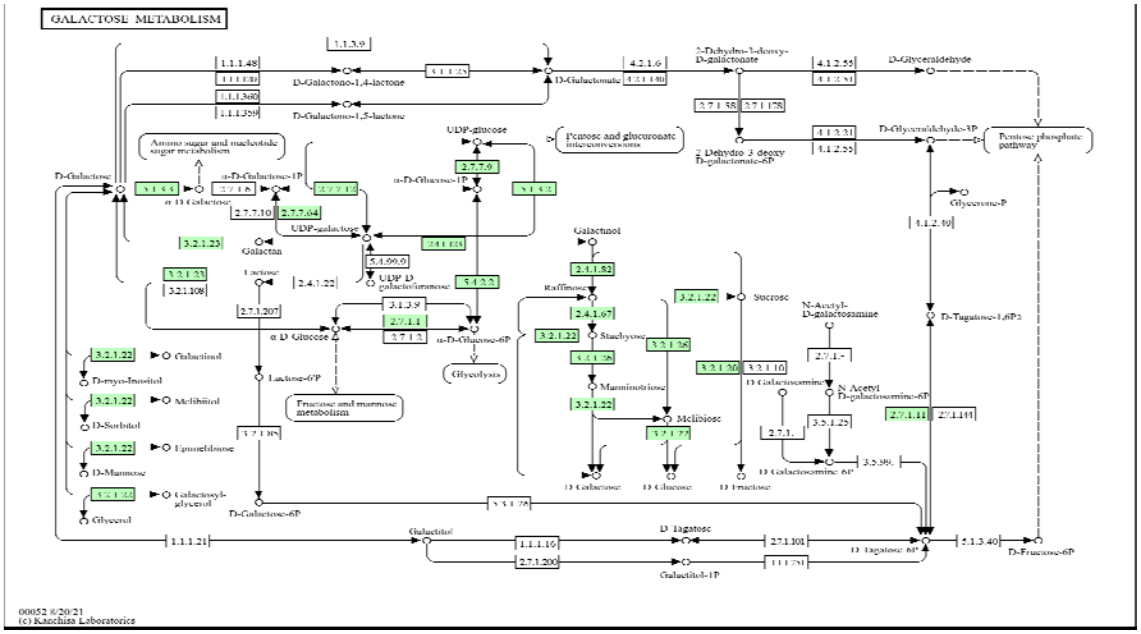

Figure 6. Metabolite pathway of galactose metabolism in *Jatropha curcas* seeds. Green marks indicate the significance of metabolites that affect galactose metabolism.

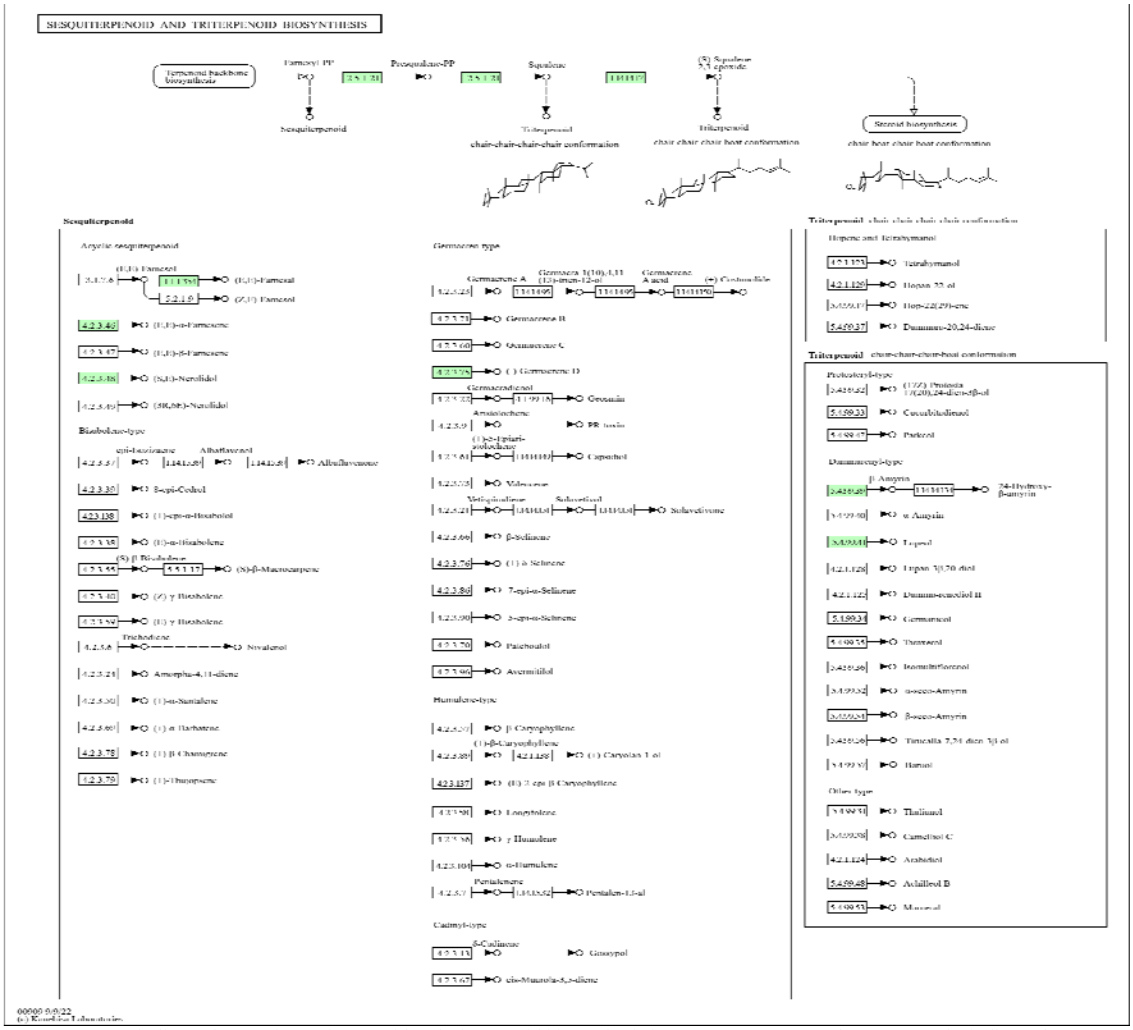

Figure 7. Metabolite pathway of sesquiterpenoid and triterpenoid biosynthesis in *Jatropha curcas* seeds. Green marks indicate the significance of metabolites that affect sesquiterpenoid and triterpenoid biosynthesis.

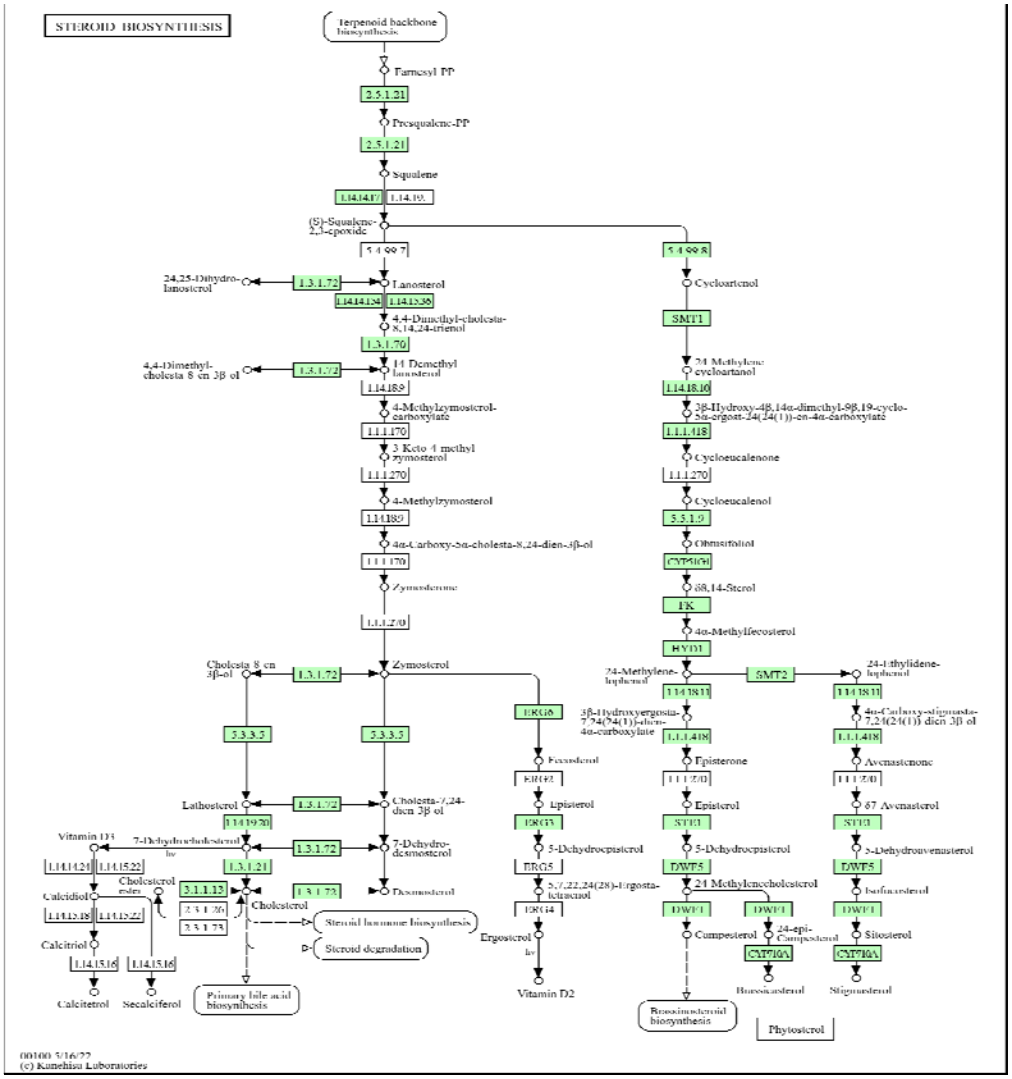

Figure 8. Metabolite pathway of steroid biosynthesis in *Jatropha curcas* seeds. Green marks indicate the significance of metabolites that affect steroid biosynthesis.

4. Discussion

Metabolomics in plants like *Jatropha* aims to characterize organ-specific metabolite profiles under defined conditions (Debnath *et al.,* 2011). The target of metabolomics research is low molecular weight compounds that are synthesized by an organism at a certain time and condition (Mastrangelo *et al.,* 2015). Plants are estimated to have more than 200 thousand types of metabolites (Valdes-Rodríguez *et al.,* 2013). Metabolomics, especially in plants, investigates compound identification strategies with statistical approaches to measure metabolite profiles found in cells and tissues, i.e. in *Pistia stratiotes* (Tyagi and Agarwal, 2017). Compared to transcriptomics analysis, metabolomics is an easier method to study comparative analysis in plant abiotic stress (Amaral *et al.,* 2016). Metabolomics studies consist of metabolites profiling, which is a quantitative estimate of a certain group of metabolites, metabolic fingerprinting, and isotope-based analysis which is intended to analyze specific compounds from intermediate metabolites from a metabolic pathway (Kibazohi and Sangwan, 2011).

Compared to Nuclear Magnetic Resonance (NMR), metabolomics studies using GC-MS or LC-MS are considered relatively easy because the methods do not require isotopes in their use. The application of metabolomics research has been used in studying plant responses to abiotic stress particularly stress to waterlogging conditions, drought, and other stresses. Gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE) are types of Mass Spectrometry (MS) analytical tools that can be used to estimate quantitatively the mass-to-charge ratio of one or more molecules in a sample used for the study. GC-MS is a sophisticated omics study in metabolite investigation with high chromatographic resolution. However, GC-MS cannot be used to detect polar compounds. Therefore, to cover this weakness, HPLC-MS is used which has a wide range in analyzing compounds including with lower chromatographic resolution. Integrated omics studies like genomic (Satrio *et al.,* 2021; Halim *et al.,* 2021; Pratami *et al.,* 2022), transcriptomic and gene expression (Wang *et al.,* 2013; Satrio *et al.,* 2019; Ratnadewi *et al.,* 2021), and metabolomic (Bates *et al.,* 2007) can combine each other

to understand comprehensive studies of oil producing pathway, particularly in *J. curcas*.

Jatropha curcas plants do not require special growing conditions (Abdelgadir *et al.,* 2012). This plant is widely planted in tropical areas as a hedge around fields and villages. *Jatropha curcas* easily adapts to the environment including critical and marginal environmental conditions, this plant can also be planted for reforestation of eroded areas. *Jatropha curcas* can live at an altitude of 0-2000 m above sea level, rainfall of 300-1200 mm per year, and temperatures ranging from 18-30ºC. In areas with low temperatures (< 18ºC) it can inhibit growth, while at high temperatures (>35ºC) it can cause leaves and flowers to fall, and the fruit becomes dry so that the production of this plant decreases. *Jatropha curcas* can grow in less fertile areas but must have good drainage, not be flooded, and a soil pH of 5.0-6.5 (Prihandana and Hendroko, 2006). Such growing habits can be used as a preference for obtaining oil metabolites in *J. curcas*. *Jatropha curcas* can produce 15-20 times more lipids than lipid-producing plants (oil palm) in its best condition, 1000 per year (Valdes-Rodríguez *et al.,* 2013). *Jatropha curcas* has the greatest potential as a producer of biodiesel raw materials compared to other plants. Unlike other plants, *J. curcas* can produce very high lipids for biodiesel raw materials with a fast harvest time. This research is expected to contribute to the study of the selection of the best seeds for lipid synthesis as a raw material for biofuel in *J. curcas.*

5. Conclusion

Mature (MS) and young (YS) seeds showed differences in metabolite content expression and these differences globally occurred in the galactose metabolism, triterpenoid, and steroid biosynthesis pathways. A total of 25 different metabolites, 19 which of metabolites were highly expressed in mature seeds of *Jatropha curcas*. In contrast, there were only 6 metabolites that were highly expressed in young seeds. In general, the four metabolites that have the highest correlation (strong positive intercorrelation) are the intercorrelation of the metabolites Eicosadienoic acid, Tetracosahexane, Sitosterol, and Aminoethanethiol. This funding showed that mature seeds (MS) have significantly higher metabolite expression than young seeds (YS) in *Jatropha curcas*. Therefore, the best seed selection synthesis as a raw material for biofuel in *J. curcas* is mature seed (MS). This funding has practical implications for harvesting seeds from *J. curcas* at a mature age seeds (MS) to produce high lipid production as biofuel which is characterized by high expression of metabolites related to lipid biosynthesis.

Acknowledgments

This research was fully supported by Trunojoyo Madura University (UTM) through the Mandiri Research (Risman) scheme (PI: E.S). We thank the Biology Laboratory, Indonesia Defense University for metabolites extraction. We thank the Health Laboratory of DKI Jakarta for supporting the metabolite identification of *J. curcas.*

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