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Profiling of Metabolites in Oil Palm Mesocarp at Different Stages of Oil Biosynthesis

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Supporting Information

ABSTRACT: Oil palm is one of the most productive oil producing crops and can store up to 90% oil in its fruit mesocarp. However, the biosynthetic regulation and drivers of palm mesocarp development are still not well understood. Multiplatform metabolomics technology was used to profile palm metabolites during six critical stages of fruit development in order to better understand lipid biosynthesis. Significantly higher amino acid levels were observed in palm mesocarp preceding lipid biosynthesis. Nucleosides were found to be in high concentration during lipid biosynthesis, whereas levels of metabolites involved in the tricarboxylic acid cycle were more concentrated during early fruit development. Apart from insights into the regulation of metabolites during fruit development in oil palm, these results provide potentially useful metabolite yield markers and genes of interest for use in breeding programs.

KEYWORDS: *oil palm mesocarp, metabolomics, mesocarp developmental stages, lipid biosynthesis, oil palm leaves*

INTRODUCTION

Metabolites are small molecules that are the end products of gene expression. Analogous to genomics, which defines all genes in a genome irrespective of their functionality, metabolomics seeks to profile all metabolites in a biological sample irrespective of the chemical and physical properties of these molecules.[1] As small molecules are products of proteins coded by genes, metabolomics is the link between genotypes and phenotypes.[2] In short, plant metabolite profiling is the large-scale, nonbiased, high-throughput phytochemical analysis of a mixture of metabolite compounds or compound classes in typical plant tissue extracts using chromatography platforms. In the field of plant biology, metabolite profiling has become an integral part of plant functional genomics.[3] Although targeted phytochemical analysis has long been a basic component of plant metabolism research, metabolite profiling involving a large-scale, wide range of chemicals allows the establishment of more complete and biologically meaningful metabolic information. Metabolite profiling may actually provide the most direct and closest information of the "-omics" technologies.[4−6] They are the end phenotype associated with valued commodities such as oil, carbohydrates or essential nutrient levels.

Oil palm is a highly efficient oil producing crop and accumulates up to 90% of oil in its fruit. In order to understand lipid production in oil palm, it is important to understand which genes control and regulate the amount of storage oil accumulated by the oil biosynthesis pathway.[7] We applied the metabolomics approach to study the chemical phenotypes associated with oil yield traits in order to understand why a particular palm tree produces more triacylglycerol (TAG) than others, since oil is accumulated as TAGs.

The oil palm fruit is a sessile drupe, consisting of exocarp (skin), mesocarp (pulp) and endocarp (shell) and is produced in bunches containing on average between 1000 and 3000 fruitlets. Oil deposition in the mesocarp starts at about 16 weeks after pollination (WAP) and continues until fruit maturity (20−22 WAP). [8] Using a metabolomics approach, this study compared the mesocarp metabolite concentrations during critical oil production stages of the fruit. Plants produce various metabolites, ranging from simple primary metabolites to highly complex secondary products.[9] Focused analysis of primary metabolites in mesocarp tissue should reveal important changes in key biosynthetic processes that either lead to, or are a result of increased lipid biosynthesis and therefore directs further work on genetic markers for breeding programs and gene expression studies by identifying key productivity-controlling genes. However, one of the key resources for fruit development and lipid biosynthesis are carbohydrates generated from photosynthesis in the leaves. Previous studies on the partitioning between vegetative and reproductive growth in oil palm clearly indicate that bunch yield is source limited by the supply of carbohydrates from leaf photosynthesis.[10] Along with fruit, developing heterotrophic leaves are also sink organs, hence the understanding of leaf (autotrophic: source; heterotrophic: sink) metabolite profiles in comparison to developing mesocarp tissue may provide further insights into

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key drivers of oil yield and guide future studies into carbon and nitrogen partitioning.

Metabolite changes in leaves and fruit throughout the developmental stages needed to be monitored in order to investigate their regulation. To date, no single analytical method can be used to profile all of the plant metabolites accurately. Hence, this study used a multiple platform approach incorporating gas chromatography−mass spectrometry (GC−MS), liquid chromatography−mass spectrometry (LC−MS), and capillary electrophoresis-mass spectrometry (CE−MS) methods, employing both untargeted techniques and specific targeting of relevant primary metabolites. LC−MS LTQ Orbitrap was employed to conduct high-resolution MS profiling of small molecules in an untargeted manner, whereas LC−MS triple quadrupole and CE−MS were used to analyze specific metabolites related to lipid formation and other important biosynthetic pathways, namely, amino acids, organic acids, nucleosides, nucleotides and polyamines. GC−MS complemented these platforms by analyzing metabolites that were unable to be ionized using soft ionization (ESI), such as fatty acids and sugar-related metabolites, while CE−MS enabled the separation of charged and isomeric metabolites, such as phosphorylated sugars. In this study, palm mesocarp during lag, oil biosynthesis and ripening phases together with autotrophic and heterotrophic leaves were sampled and profiled using multiple MS platforms.

MATERIALS AND METHODS

Material. A total of 16 *Elaeis guineensis* palms derived from Serdang Avenue dura and AVROS pisifera crosses were collected from Carey Island, Malaysia. Palms were of the same age and planted in the same commercial plot.

Palm Mesocarp. One bunch per palm was sampled for each time point (12, 14, 16, 18, 20, and 22 WAP) according to different developmental stages preceding, during and after the major oil biosynthesis period.[8,11,12] All palm fruitlets were detached from the bunch and 20 were randomly selected for analysis.

Palm Leaves. Six randomized leaflets from the mid-section of the rachis of heterotrophic leaf number zero (unopened spear, leaf #0) and autotrophic leaf 12 (leaf #12) were sampled on the same sunny day between 10 a.m. and 2 p.m.

Oil palm mesocarp and leaf tissues were harvested and snap frozen in liquid N_2 in order to quench metabolism of the plant tissues and enzyme activity, then stored in the dark at −80 °C until used for metabolite analysis. Prior to extraction, plant material was ground to a fine powder under liquid nitrogen using a pestle and mortar

Extraction. *Palm Mesocarp.* The extraction of polar and lipid metabolites from oil palm tissues was carried out in a single integrated procedure.[13,14] Ground samples (100 mg) were extracted with isopropyl alcohol(2 mL) containing 0.05% butylatedhydrotoluene (BHT) and heated to 75 °C for 15 min. After allowing the sample to cool, a mixture of chloroform/methanol/water (1:1:1, 3 mL) was added along with ribitol (60 μL, 0.2 mg/mL in H_2O) and phenanthrene (60 μL, 0.2 mg/mL in $CHCl_3$) as internal standards. The samples were mixed by vortex for 1 min and shaken at 60 °C in a thermomixer at 750 rpm for 1 h, followed by centrifuging at 4000 rpm for 2 min. The supernatant was then removed while the residue was washed again by the addition of chloroform and water (1 mL each), followed by shaking at 60 °C in a thermomixer at 750 rpm for 30 min and centrifuging. The combined supernatants were then centrifuged at 4000 rpm, 4 °C for 5 min to yield two layers as the polar and nonpolar fractions. The polar fraction was dried under vacuum while the nonpolar fraction was further washed with equal volume of 1 M KCl before being dried under nitrogen gas. Weights of both polar and nonpolar fractions were recorded.

Palm Leaves. Powdered leaf tissues (500 mg) were extracted using 5 mL of 80% (v/v) methanol in water with 0.1% BHT. Ribitol (0.2 mg/mL) was added to the tissue as an internal standard. The mixture was shaken for 30 s using a vortex mixer and incubated at 4 °C for 5 min at 500 rpm, followed by sonication for 5 min at 10 °C. The incubation and sonication procedures were repeated three times then followed by centrifuging at 4 °C, 4000 rpm for 20 min. The methanol extract was transferred into four preweighed new tubes (1 mL each). The extracts were dried using nitrogen gas and the weights were recorded.

All extracts were kept dry at −80 °C until further use. The analysis comprised of targeted and untargeted LC−MS, GC−MS and CE−MS analysis.

Profiling and Spectrometry Methods. *Untargeted LC−MS Analysis.* LC−MS data were acquired using Accela LTQ Orbitrap instrument (Thermo Fisher, Germany). Sample analysis was carried out in positive and negative ion modes of detection. The mass scanning range was 100−2000 m/z, while capillary temperature was 300 °C and sheath gas auxiliary gas flow rates were 35 and 15 arb unit (arbitrary unit), respectively. The sweep gas flow rate was set at 1 arb, I-spray voltage at 4.5 kV. The resolution was 30 000 at 1 microscan. The capillary voltage and tube lens were set at 40 and 80 V, respectively, for positive ion modes. Both respective parameters were set at −2.00 and −47.44 V for negative ion mode. The MS/MS spectra of metabolites were obtained by collision energy ramp at 35 V. Autosampler temperature was set at 10 °C with 3.0 μL injection volume. The LC-MS system (controlled by Xcalibur version 2.0, Thermo Fisher Corporation) was run in binary gradient mode. Solvent A was 0.1% (v/v) formic acid/water and solvent B was acetonitrile (ACN) containing 0.1% (v/v) formic acid; the flow rate was 0.2 mL/min. An Acquity UPLC HSS T3 column (1.8 μm, 2.1 × 100 mm; Waters, Malaysia) set at 45 °C was used for analyses. The gradient was as follows: 0−1.8 min isocratic at 99% solvent A, 1.8−3 min linear gradient to 90% solvent A, 3−23 min linear gradient to 60% solvent A, 23−26 min hold at 60% solvent A, 26−29 min linear gradient to 10% solvent A and 29−35 min to 99% solvent A. The raw data was processed and compared using Sieve version 1.2 (Thermo Fisher, Alpha Analytical, Malaysia) with the frame time and m/z width set at 1.5 min and 0.002, respectively.

Targeted LC−MS Analysis. An Acquity UPLC system coupled to an MS detector, Xevo TQs (triple quadrupole system) was used for analytical determination of the targeted metabolites in the leaf samples. Chromatography was conducted with an Acquity UPLC HSS T3 column (1.8 μm, 2.1 × 100 mm; Waters, Malaysia). Column and sample were maintained at 45 and 4 °C, respectively. The LC mobile phases used were 0.1% formic acid in water (solvent A) and 0.1% formic acid in ACN (solvent B). The flow rate was 0.3 mL/min. The elution gradient was as follows: initial at 95% solvent A; 0−3 min linear gradient to 60% solvent A; 3−5 min 5% solvent A; 5−5.1 min linear gradient to 95% solvent A and hold to 7 min. Injection volume was 3 μL. The mass spectrometer was operated in both positive and negative modes using ESI with multiple reactions monitoring (MRM). The capillary voltage was set to 2.9 kV, desolvation gas set at 800 L/h at temperature of 350 °C. The collision gas flow was set at 0.15 mL/min. The MRM settings in the MS/MS function with corresponding cone voltage and collision energy were optimized for each standard compound. Auto dwell times were set for positive and negative modes, respectively. Total acquisition durations for both UPLC and MS were set to 15 min. Data was acquired and processed using MassLynx V4.1 and TargetLynx, respectively. Each standard compound was weighed and dissolved with 5% (v/v) ACN in Milli-Q water to make a stock solution with final concentration of 1 mg/mL (1000 ppm). The stock solutions were diluted to 1 μg/mL as working stock solutions. A mix standard at concentration of 1 μg/mL was prepared and injected into LC−MS daily to check system sensitivity and reproducibility. The samples were resuspended in 5% (v/v) ACN to final concentration of 10 mg/mL for analysis.

Targeted GC−MS Analysis. Samples were analyzed using an Agilent 6890N Gas Chromatograph (GC) coupled with an Agilent 5973i Mass Detector and 7683 series autosampler. Polar metabolites were separated on a HP-5 ms column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness. Seven types of derivatized sugars were detected,

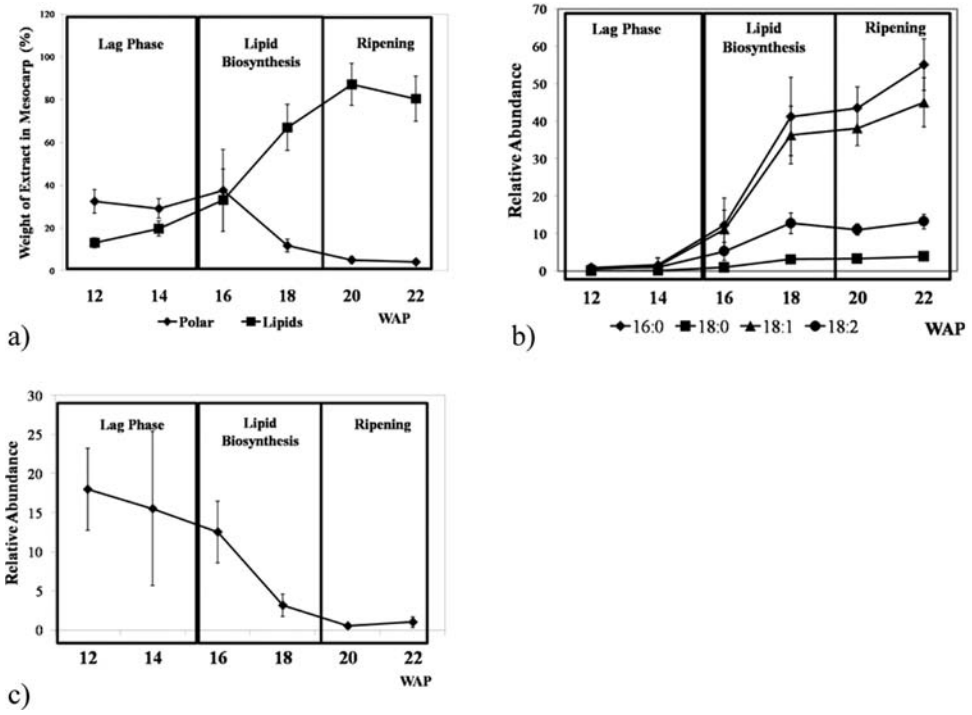

Figure 1. (a) Total lipid and polar extracts (n = 16) of palm mesocarp at 6 critical time points; (b) fatty acid composition of palm lipids (relative abundance to phenanthrene, 2 μg/mL); (c) total sugar content in mesocarp tissue (relative abundance to ribitol, 2 μg/mL) during the critical period of oil palm fruit development.

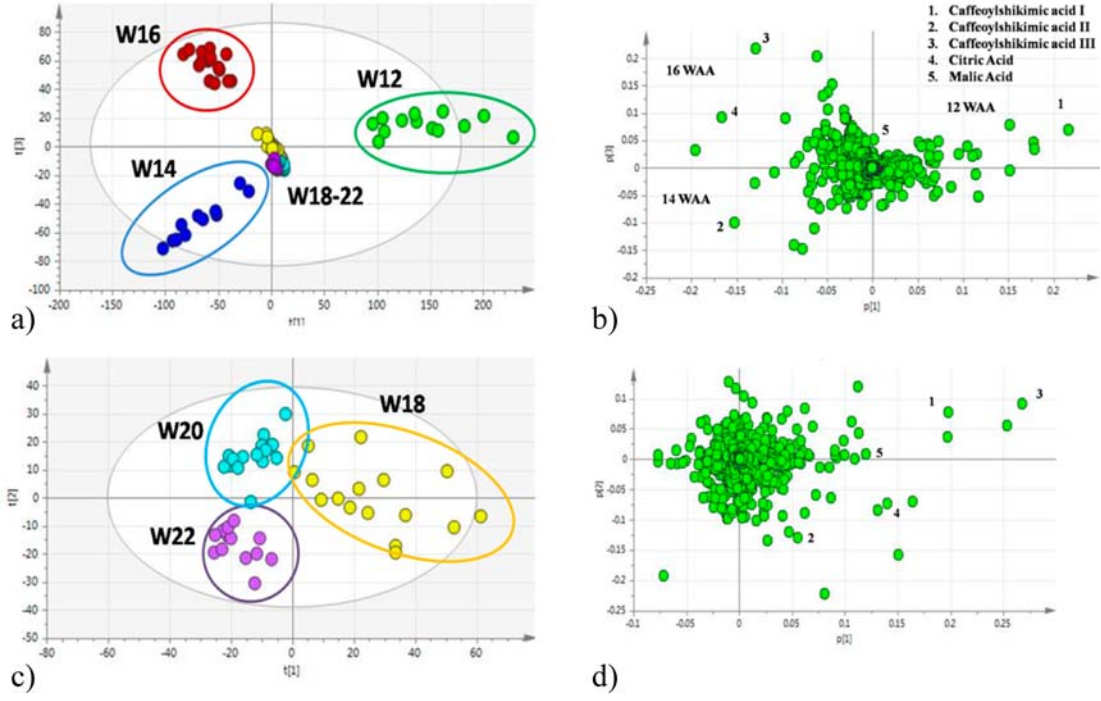

Figure 2. (a) Principle component analysis (PCA) score plot, and (b) loading plot of 12−22 WAP; (c) expansion score plot; and (d) loading plot of 18−22 WAP mesocarp polar extract metabolites, obtained from untargeted analysis using LC−MS Orbitrap. High-loading metabolites with MS/MS identification are indicated.

namely, D-fructose, D-glucose, sucrose, D-mannose, D-sorbitol, arabinose and D-galactose. The derivatization method and analysis were done according to methodologies described by Tarpley et al.[15] with minor modifications. Esterification of lipid extracts was performed using a modified method based on Malaysian Palm Oil Board (MPOB) test methodology.[16] The fatty acid methyl esters were separated on a DB-Wax column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness. The temperature program was set at 195 °C, followed by a 5 °C per minute ramp to 240 °C, and holding at this temperature for 6 min. The injection port was split (1:100) at 250 °C while column flow was maintained constant at 1.2 mL/min of Helium gas. The MS source was set at 230 °C with a scanning range of m/z 50−800.

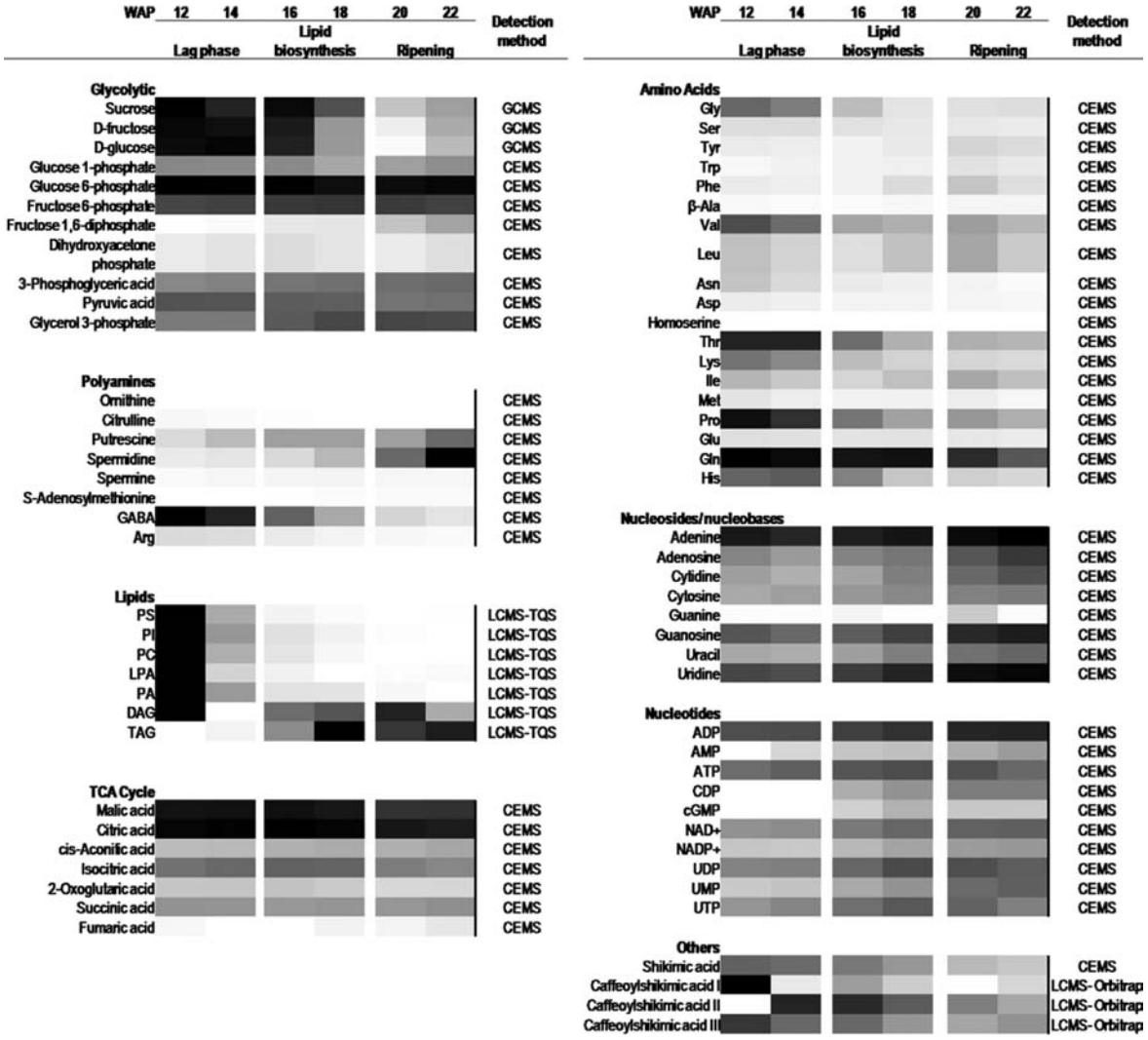

Figure 3. Heatmap of selected lipid and polar metabolite concentrations in key biosynthetic and cellular pathways of palm mesocarp at 12−22 WAP using multiple MS platform analyses (CE−MS, LC−MS and GS−MS). Scale from white (lowest) to black (highest concentration) within each metabolite class.

Sample (1 μL) was injected into a programmable injector. The total run time was 15 min. Fatty acid composition was calculated based on relative abundance to internal standard, phenanthrene.

Lipid Species LC−MS Analysis. Targeted profiling of lipid compounds was carried out at Kansas Lipidomics Research Centre, Kansas State University, KS, as per previously published method.[13]

Targeted Profiling of Polar Metabolites Using CE−MS. Targeted profiling of 191 metabolites using CE−MS was carried out by Human Metabolome Technologies, Inc. (HMT), Japan, as per previously published method. [17]

Multivariate and Statistical Analysis. Principle component analysis (PCA) using Simca-P version 1.3 (Umetrics) was used to identify metabolites that reflected the differences between the stages of mesocarp maturation and two types of leaves. The *t* test algorithm of Excel 2000 (Microsoft) was used for determining if observations were significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Fruitlets from bunches sampled during the critical stages of oil production were initially analyzed to determine lipid and polar metabolite total concentrations, during and after the major lipid production stages.[12] Figure 1a shows the significant increase in lipid content between 16 and 18 WAP, reaching optimal fruit harvest time of 20−22 WAP. Conversely to lipid production, the sugar content (Figure 1c) along with overall polar extractable components of the fruit decreased to very low levels by 18 WAP. These results were consistent with several previous studies that investigated the developmental stages of oil palm fruit and highlight the three major developmental events of oil production: lag phase preceding lipid biosynthesis, 12−16 WAP; fruit maturation/oil biosynthesis, 16−20 WAP; and fruit ripening, 20−22 WAP.[11,17−20] For each mesocarp development time point, the polar extracts of individual palms were profiled using an untargeted LC−MS approach in order to gather information about as many metabolites as possible.[21,22] A total of 1217 and 4761 components (m/z) were detected from positive and negative ionization modes, respectively. Multivariate analysis showed that polar mesocarp extracts were well clustered according to the six time points (Figure 2a,c), indicating the altered metabolite profiles associated with the development stages of palm mesocarp. However, as the overall concentration of polar components in the mesocarp decreased when lipid production commences (>16 WAP), the distinction was less evident (Figure 2a). Several metabolites that showed relatively higher concentration during early development were elucidated based on their MS/MS fragmentation profiles and

included primary metabolites such as arginine and homoarginine, as well as several organic acids. Major components of the mesocarp extracts were identified to be three isomeric caffeoylshikimic acids (CSA) as elucidated using both positive and negative MS ionization at retention times 9.31, 9.72, and 10.75 min (labeled as CSA I, II and III, respectively, in Figure 2b,d). This group of CSA isomers consisted of 3-*O*-caffeoylshikimic acid, 4-*O*-caffeoylshikimic acid and 5-*O*-caffeoylshikimic acid with same *m*/*z* 337.0901 $[M + H]^+$ and 335.0740 $[M - H]^-$.[23] MS/MS of these parent ions yielded distinctive fragments of *m*/*z* (%) 319 (10), 181 (10), 163 (100) in positive mode,[24] and 161 (100), 179 (70), 135 (10) in negative mode.[25] Regiochemical assignment of the isomers was not conducted. PCA loading plots (Figure 2b,d) show that CSA I, II and III concentrations change significantly during fruit development. The concentration of the CSA isomers generally decreased after 12−14 WAP (Figure 3).

Subsequently, extensive analysis of metabolites involved in key biosynthetic and cellular processes was conducted (Supporting Information). This investigation revealed significant differences across many of the key metabolite classes at different stages of fruit development, including lipids, glycolysis, TCA cycle organic acids, amino acids, polyamines and nucleosides (Figure 3).

Simple sugars such as sucrose, fructose and glucose, along with glucose-6-phosphate (G6P), were found to be at their highest concentrations during lag phase and early lipid biosynthesis, whereas the intermediate metabolites downstream in glycolysis exhibited higher concentration at the later stages of fruit development. In particular, fructose-6-phosphate, fructose-1,6-biphosphate and glycerol-3-phosphate all appeared at higher levels toward the end of lipid biosynthesis. However, upon completion of lipid biosynthesis, G6P levels were found to increase at 22 WAP. These results may be associated with the findings of Bourgis et al. who reported that the G6P transporter (GPT2) that transports G6P from the cytosol to plastid increases 16-fold during ripening. GPT2 was identified as one of the key drivers for increasing carbon flux through glycolysis to pyruvate for eventual accumulation of 90% oil in palm mesocarp.[18] In addition to pyruvate for fatty acid assembly, glycerol-3-phosphate is the backbone of lipid (TAG) molecules, and was found to accumulate toward the lipid biosynthesis phase. It has been demonstrated in previous studies that diversion of carbon flux through glycolysis to produce more glycerol-3-phosphate can lead to higher levels of lipid production in seed oils.[26−28]

In biosynthesis of castor oil, polyamines are essential for the synthesis of phosphatidic acid (PA) from ricinoleoyl-CoA and lysophosphatidic acid (LPA) by castor LPA acyltransferase reaction, suggesting polyamines modulate enzyme affinity for the acyl-CoA substrate *in vivo*.[29] In oil palm, it appears that putrescine, spermidine and PA/LPA concentration coincide with Tomosugi et al. findings that showed the concentrations of PA were inversely proportional to polyamine concentrations (Figure 3).

All of the organic acids in the TCA cycle were at their lowest concentration during the final stages of fruit ripening. Citric acid, isocitric acid and, more markedly, malic acid all appeared higher in concentration preceding and during the early stages of lipid biosynthesis (14−18 WAP). Malic acid and citric acid were also found as the major components in mesocarp polar extract detected using an untargeted approach. Using PCA analysis, citric acid was distinct at both 16 and 18 WAP (Figure 2b,d). In addition, the differing ratio of malic acid and citric acid during fruit maturation can be seen in Figure 4, whereby the

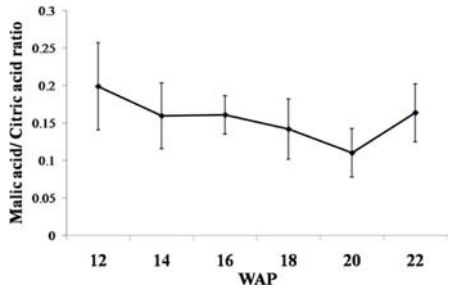

Figure 4. Malic acid/citric acid ratio of palm mesocarp samples during 12−22 WAP. Data obtained from LC−MS-Orbitrap analysis with validation using CE−MS.

ratio of malic acid to citric acid clearly decreases during lipid biosynthesis until 20 WAP. High malate to citrate ratio during lipid biosynthesis in the mesocarp of olive has been reported to be an important feature in higher oil producing ability.[30] Malic acid has also been shown to be crucial for fatty acid synthesis in developing castor endosperm,[31] while the relatively low concentrations of 2-oxoglutaric acid observed in palm during lipid biosynthesis is likely to be a result of higher utilization for fatty acid production from acetyl-CoA, and possibly other major biosynthetic precursors leading to amino acid and protein production.

Overall it was found that the concentrations of most amino acids declined from 12 to 22 WAP with the highest concentrations observed before commencement of lipid biosynthesis (Figure 3). The accumulation of amino acids at this stage could possibly support production of the proteins necessary for lipid production and cell division required later during maturation and mesocarp development. Interestingly, the comparison of oil palm with date palm also revealed significantly higher concentrations of amino acids in oil palm compared to the nonoil producing relative.[18]

Nucleosides such as adenine, cytidine, uracil and uridine were all found to be significantly higher during the later stages of fruit development (18−22 WAP), as also seen in Figure 3. Overall, it can be seen that the concentration of these nucleosides increase during fruit development to a maximum at 22 WAP. Purines and pyrimidines are important building blocks for nucleic acids, as well as being indirectly involved in many other biochemical processes, including sucrose and cell wall polysaccharide metabolism[32] and lipid production.[33,34]

As palm is a source limited plant, information on the partitioning of nitrogen and carbon assimilated from autotrophic leaves to various sink organs, particularly developing mesocarp and heterotrophic leaves for reproductive and vegetative growth will be valuable to palm breeding and genomic studies. The correlation of fat, sugars and protein nitrogen between olive leaves and fruit has been reported[30] and supports the transportation of carbon and nitrogen from leaves to fruit for lipid synthesis in the mesocarp. A study on oil palm leaf biomass, nitrogen, sugars and photosynthesis using leaf ranks −6 to 57 suggested that carbon isotope composition of heterotrophic spear leaves (<rank 0) were ^{13}C sugar-enriched due to remobilization of reserve carbon from source organs.[35] While another study of oil palm has shown that the demands for vegetative growth are met first and then excess assimilates are used for generative growth.[36] To date, there has not been a

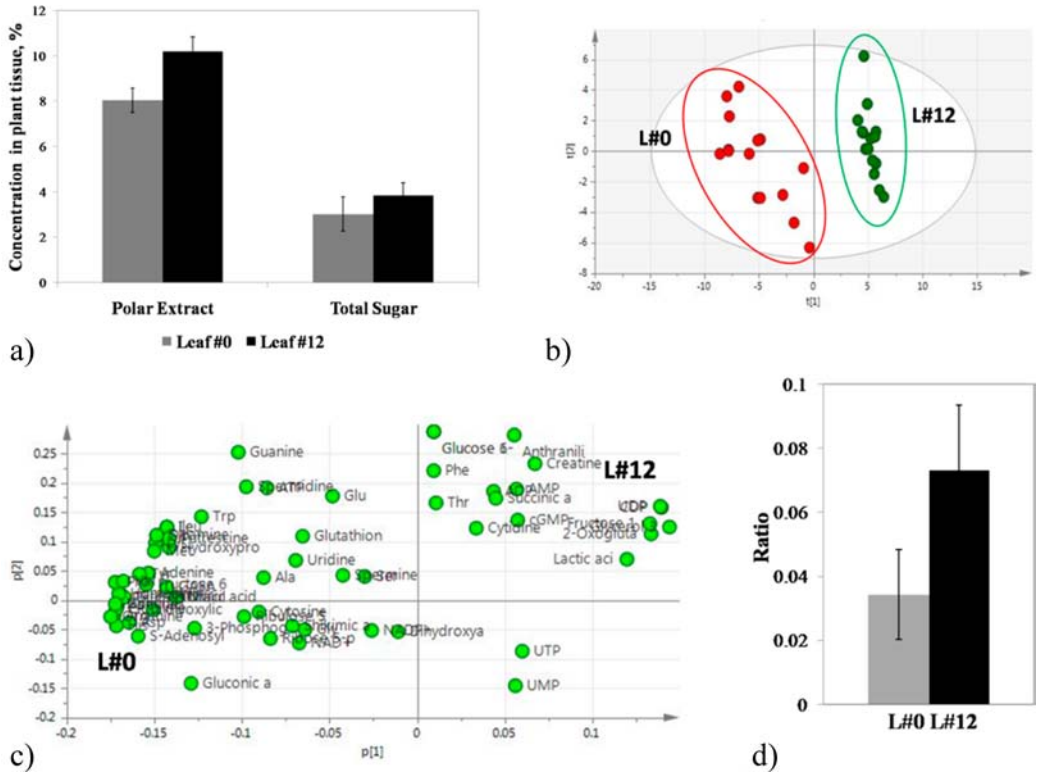

Figure 5. (a) Polar extract using 80% aqueous methanol and total sugar content in oil palm leaves according to leaf age, (b) PCA score, and (c) loading plot of palm leaves displaying clustering of leaves samples due to differentiation of 68 metabolites concentration using LC−MS-TQS, (d) Malic acid/citric acid ratio in source (leaf#12) and sink (leaf#0).

detailed study published on the metabolite changes in palm mesocarp and how leaf photosynthesis contributes to mesocarp development. The current study conducted an initial investigation of leaf metabolite profiles as a platform for future comprehensive studies on palm carbon and nitrogen assimilation. In the unopened spear (Leaf#0), which is heterotrophic (sink organ), the leaf sugar contents (Figure 5a) were lower, presumably to support leaf growth.[37] Concordant with the lower amount of extract obtained from unopened spear, higher biomass was assumed compared to leaf #12. The rapid increase in spear leaf biomass required a carbon allocation presumably from both heterotrophic (trunk apex) and autotrophic organs (older functional leaves, such as leaf #12).[35] Total sugar content produced from photosynthesis may vary and is believed to be related to leaf rank (heterotrophy to autotrophy).[37,38] The lower sugar concentration in the unopened spear suggests that sugar participates in leaf growth during the fast elongation stage. [35]

Extracts of different aged leaves (Leaf#0 and #12) also demonstrated significant clustering (Figure 5b). Most of the amino acids such as arginine, asparagine, proline, valine and tyrosine together with adenine were more concentrated in leaf#0 compared to leaf #12 (Figure 5c). In leaves, the biosynthesis of amino acids is developmentally regulated, usually being most active in young leaves.[39−41] This trend is similar to that observed in developing mesocarp tissue before lipid biosynthesis commences. It has been reported that nucleosides in young leaves are converted to salvage products (nucleotides and nucleic acids), to degradation products and to purine alkaloids, such as caffeine. Almost 75% reduction of purine alkaloids was observed throughout leaf development.[42,43] The concentration of nucleosides was also found to be markedly lower in matured leaves in this study. As discussed earlier, the regulation of nucleosides also appears to play an important role in fruit development and lipid production in mesocarp. A higher malate to citrate ratio in leaf #12 compared to leaf #0 was also evident from this analysis (Figure 5d), but both were significantly lower than that observed in developing mesocarp (Figure 4).

In conclusion, the study of metabolite profiles during oil palm fruit development revealed global changes of amino acids, organic acids, sugars, polyamines, lipid content and nucleosides levels. It is possible that the higher levels of amino acids (12−16 WAP) and nucleosides (18−22 WAP) are associated with protein biosynthesis preceding oil biosynthesis and later fruit expansion to support lipid production and fits well with current knowledge of the fruit development stages.[12] Differences in the metabolites that are more directly linked to lipid production in the glycolysis pathway and TCA cycle exhibited more complex trends. Amino acids, nucleosides and organic acids concentrations also appear to play an important role in developing leaves compared to mature leaves. Taken together, these results provide useful information about the regulatory control of processes that lead to increased oil yield in commercial oil palm populations, both directly and indirectly. Future studies that investigate the role that these key metabolites play in high yielding palms as well as nitrogen and carbon partitioning between different plant organs are warranted.

■ ASSOCIATED CONTENT

Supporting Information

Analysis of metabolites involved in key biosynthetic and cellular processes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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