July 21, 2011

Comparative Transcriptome and Metabolite Analysis of Oil Palm and Date Palm Mesocarp that Differ Dramatically in Carbon Partitioning

Fabienne Bourgis
Aruna Kilaru
Xia Cao
Georges Frank Ngando-Ebongue
Noureddine Drira, et al.

Correction

PLANT BIOLOGY

Correction for "Comparative transcriptome and metabolite analysis of oil palm and date palm mesocarp that differ dramatically in carbon partitioning," by Fabienne Bourgis, Aruna Kilaru, Xia Cao, Georges-Frank Ngando-Ebongue, Noureddine Drira, John B. Ohlrogge, and Vincent Arondel, which appeared in issue 30, July 26, 2011, of *Proc Natl Acad Sci USA* (108:12527–12532; first published June 27, 2011; 10.1073/pnas.1106502108).

The authors note the following: "A relevant reference by Tranbarger et al. that describes the regulation of oil palm fruit ripening should be added to the list of references in our article. In addition to providing data that complement our study, Tranbarger et al. reached similar conclusions with regard to the importance of palm orthologs of the Arabidopsis WRINKLED1 transcription factor."

35. Tranbarger TJ, et al. (2011) Regulatory mechanisms underlying oil palm fruit mesocarp maturation, ripening, and functional specialization in lipid and carotenoid metabolism. *Plant Physiol* 156:564–584.

www.pnas.org/cgi/doi/10.1073/pnas.1115243108

Comparative transcriptome and metabolite analysis of oil palm and date palm mesocarp that differ dramatically in carbon partitioning

Fabienne Bourgis[a,b,1], Aruna Kilaru[c,1,2], Xia Cao[d], Georges-Frank Ngando-Ebongue[e], Noureddine Drira[f], John B. Ohlrogge[d], and Vincent Arondel[a,b,3]

[a]Université de Bordeaux Ségalen, Laboratoire de Biogenèse Membranaire, Unité Mixte de Recherche 5200, F-33000 Bordeaux, France; [b]Centre National de la Recherche Scientifique, Laboratoire de Biogenèse Membranaire, Unité Mixte de Recherche 5200, F-33000 Bordeaux, France; [c]Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI 48824; [d]Department of Plant Biology, Michigan State University, East Lansing, MI 48824; [e]Centre de Recherches sur le Palmier à Huile de la Dibamba, Institut de Recherche Agricole pour le Développement, BP243 Douala, Cameroon; and [f]Laboratoire de Biotechnologie Végétale, Faculté des Sciences de Sfax, Sfax 3038, Tunisia

Edited by Maarten J. Chrispeels, University of California San Diego, La Jolla, CA, and approved June 1, 2011 (received for review April 23, 2011)

Oil palm can accumulate up to 90% oil in its mesocarp, the highest level observed in the plant kingdom. In contrast, the closely related date palm accumulates almost exclusively sugars. To gain insight into the mechanisms that lead to such an extreme difference in carbon partitioning, the transcriptome and metabolite content of oil palm and date palm were compared during mesocarp development. Compared with date palm, the high oil content in oil palm was associated with much higher transcript levels for all fatty acid synthesis enzymes, specific plastid transporters, and key enzymes of plastidial carbon metabolism, including phosphofructokinase, pyruvate kinase, and pyruvate dehydrogenase. Transcripts representing an ortholog of the WRI1 transcription factor were 57-fold higher in oil palm relative to date palm and displayed a temporal pattern similar to its target genes. Unexpectedly, despite more than a 100-fold difference in flux to lipids, most enzymes of triacylglycerol assembly were expressed at similar levels in oil palm and date palm. Similarly, transcript levels for all but one cytosolic enzyme of glycolysis were comparable in both species. Together, these data point to synthesis of fatty acids and supply of pyruvate in the plastid, rather than acyl assembly into triacylglycerol, as a major control over the storage of oil in the mesocarp of oil palm. In addition to greatly increasing molecular resources devoted to oil palm and date palm, the combination of temporal and comparative studies illustrates how deep sequencing can provide insights into gene expression patterns of two species that lack genome sequence information.

triacylglycerol biosynthesis | *Elaeis guineensis* | fruit ripening | *Phoenix dactylifera*

Oil palm (*Elaeis guineensis* Jacq) originates from intertropical Africa. It was imported into South Asia where industrial plantations started about 100 years ago. Oil palm is now the most productive world oil crop (3.5 tons/ha/y), with 36% of world production (1). Because present-day genetic material can produce up to 10 tons of oil/ha/y, it is likely that palm oil will keep increasing its share of the market. Most palm oil is derived from the fruit mesocarp where it can comprise up to 90% of the dry weight. This is, by far, the highest oil content reported for any plant tissue. Despite its obvious scientific and economic interest, literature and molecular resources available for oil palm remain scarce (2).

Presently, knowledge of triacylglycerol (TAG) accumulation in plants is based almost entirely on studies of oil seeds, which contain no more than 60% oil (3). The main source of carbon for storage oil synthesis in higher plants is sucrose, which in nongreen tissues is converted to pyruvate via glycolysis and the pentose phosphate pathway (PPP). Pyruvate is the main precursor for the acetyl-CoA molecules destined to fatty acid synthesis. Plastid pyruvate kinase (PK), pyruvate dehydrogenase (PDH), and acetyl-CoA carboxylase are considered as key enzymes for fatty acid synthesis (3) and in oil seeds are regulated by transcription factors, including WRINKLED1 (WRI1) (4, 5).

Fatty acids synthesized from acetyl-CoA in the plastid are exported as acyl-CoA esters to the endoplasmic reticulum where they enter glycerolipid metabolism. Reactions generating fatty acids, phosphatidic acid (PA), and diacylglycerol (DAG) are common to both membrane phospholipid and TAG biosynthetic pathways. Only the last acylation step, conversion of DAG into TAG, is specific to oil synthesis. Oil stored in seeds is used to fuel postgerminative growth of seedlings; however, mesocarp oil provides an attractant for disseminating animals and does not undergo further plant metabolism.

To identify features specific to high oil production in nonseed tissues such as oil palm mesocarp, we generated several million ESTs for five developing stages of oil palm mesocarp. To complement and strengthen our study, we carried out similar experiments in date palm (*Phoenix dactylifera*), a closely related species (6) that stores almost exclusively sugars rather than oil (Fig. 1*A*). The 100-fold difference in total fatty acid content in their mesocarp is most striking (Fig. 1*B*). The focus of this temporal and comparative analysis was to gain insight into factors responsible for this dramatic difference in carbon partitioning. In addition, a better understanding of oil accumulation in fruits may present strategies for engineering oil accumulation in other vegetative tissues.

Results and Discussion

As an initial step toward understanding how photosynthate is directed into very different pathways and end products, pyrosequencing ESTs were generated from mesocarp of oil palm and date palm fruits and oil palm leaves. To aid interpretation of the palm transcriptomes, we also conducted metabolite analyses, supplemented with transmission electron microscopy. Aside from lipid and terpenoid compositions, mesocarp metabolites and subcellular organization of oil storage during oil palm ripening are largely unknown.

Author contributions: J.B.O. and V.A. designed research; F.B., X.C., G.-F.N.-E., N.D., and V.A. performed research; A.K., X.C., J.B.O., and V.A. analyzed data; and A.K., J.B.O., and V.A. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: The sequences reported in this paper have been deposited in GenBank Sequence Read Archive (accession nos. SRX059258-62, SRX059116-20, and SRX059798-802) and contig sequences are available at http://www.biomemb.cnrs.fr/contigs.html.

See Commentary on page 12193.

[1]F.B. and A.K. contributed equally to this work.

[2]Present address: Department of Biological Sciences, East Tennessee State University, Johnson City, TN 37614.

[3]To whom correspondence should be addressed. E-mail: vincent.arondel@biomemb.u-bordeaux2.fr.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1106502108/-/DCSupplemental.

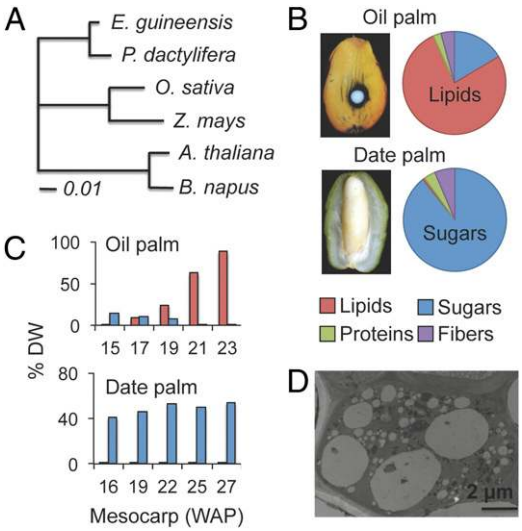

Fig. 1. Oil palm and date palm fruits show a completely different carbon-partitioning pattern. (*A*) Phylogenic tree constructed with *RBCL* gene sequences. Bar indicates the percentage of divergence. (*B*) Open fruits and mesocarp composition (% dry weight) (*SI Materials and Methods*). (*C*) Fatty acid and soluble sugar content of mesocarp during fruit ripening. (*D*) Transmission electron micrograph of cell containing oil droplets from oil palm mesocarp, harvested 20 WAP. See also Fig. S1.

Metabolite Contents Differed Markedly Between Palm Mesocarps. The total fatty acid content in oil palm mesocarp rose from 2% to 88% (dry weight) during ripening whereas it remained below 1% in date palm mesocarp (Fig.1*C*), indicating a 100-fold difference in total fatty acid content. The rate of oil accumulation was 1.6 μmol fatty acid/h/g fresh weight (FW), which is comparable to rapeseed (1.7 μmol fatty acid/h/g FW). Although TAGs accounted for more than 95% of total fatty acids in ripe oil palm, they were undetectable (<0.002% dry weight) in date palm, indicating that storage of fatty acids as TAG differed by more than 1,000-fold. Analysis of fatty acid composition in polar and neutral lipids of oil palm mesocarp indicated less palmitate and fourfold higher linolenate in phosphatidyl choline (PC) compared with TAG (Dataset S1*A*).

Total sugar content increased from 40 to 54% in date palm (Fig. 1*C*). At the last stage, sucrose, fructose, and glucose each represented about one-third of sugar. At the early ripening stage [15 wk after pollination (WAP)], TAG content was extremely low in oil palm whereas sugar content was 14% and decreased to 1.3% at 23 WAP (Dataset S1*B*). This indicated that uptake of sugars, presumably destined to oil synthesis, started before major oil accumulation.

NMR metabolite analysis revealed that organic acids (mostly citrate) of oil palm declined from 3.3% to 0.2%, a trend similar to that of sugars (Dataset S1*B*). In contrast, during date palm mesocarp development, levels of citrate were 20-fold below that of oil palm, and organic acid content remained nearly constant (1.1%). Amino acids were 7- to 10-fold higher in oil palm than in date palm at early stages, and their composition was markedly different in the two species. Starch content increased in the last ripening stages of oil palm to represent more than 1% of dry weight, whereas it was almost undetectable in date palm. The analyses of these major metabolites indicate that sugars represent the major source of carbon available for oil synthesis.

Electron microscopy of 20 WAP oil palm mesocarp showed, most notably, cells with numerous oil droplets, which, in contrast to oil seeds varied greatly in size (0.2–4 μm diameter; Fig. 1*D*). A few cells were almost completely filled by a single oil droplet (Fig. S1*A*). Most plastids showed a chromoplast-like structure (Fig. S1*B*), and a few amyloplasts were visible in ripe fruits (Fig. S1*C*).

Oil Palm and Date Palm Transcript Sequences Were Highly Similar and Closely Matched with *Arabidopsis* Orthologs. For this study, about 4 and 2 million ESTs, with an average read length of 389 and 362 nt, were obtained for oil palm and date palm mesocarp, respectively (Dataset S1*C*). After oil palm EST assembly, 95.9% of contigs with ≥50 ESTs were highly similar (BLASTX *E*-value < 10^{-10}) to *Arabidopsis* proteins. Comparable results were obtained for date palm (Dataset S1*C*). Contigs were annotated on the basis of *Arabidopsis* proteins because it is by far the best-annotated proteome among the plant kingdom. ESTs were assigned to the most closely related *Arabidopsis* protein with the understanding that palms may have gene families with different complexity that may not be reflected in these assignments. Genes most relevant to this study are listed in Dataset S2*A* and *B*, with expression data for each stage of development. Oil palm and date palm nucleotide sequences were highly conserved (92% identity). This high identity is further discussed in relation to palm phylogeny in *SI Materials and Methods*.

Analysis of Lipid-Related Genes: Transcripts for All Fatty Acid Synthesis Enzymes Showed Up-Regulation Patterns. Annotations of over 600 genes of acyl-lipid metabolism in *Arabidopsis* were recently updated (7). Orthologs of about 450 of these genes were expressed in oil palm mesocarp during fruit ripening, and these were categorized on the basis of their biochemical pathway and subcellular localization (Dataset S2*A*). Unexpectedly, for almost every pathway of lipid metabolism, EST levels were similar in oil palm and date palm (Fig. 2*A*) and did not show temporal changes during palm ripening (Fig. 2*B*). Only the fatty acid synthesis subcategory displayed major differences between oil palm and date palm together with distinct temporal increases during oil palm ripening.

EST levels for 18 plastidial proteins that are involved in conversion of pyruvate to fatty acids were, on average, 13-fold higher in oil palm than in date palm (Fig. 2*A* and Fig. 3). The largest individual differences were noted for ketoacyl-acyl carrier protein (ACP) reductase, ketoacyl-ACP dehydratase, and the E3 and E1α subunits of PDH, for which the ESTs were more than 50-fold higher in oil palm relative to date palm (Dataset S2*A*).

In addition to the high oil-palm-to-date-palm ratio, ESTs for fatty acid synthesis from pyruvate increased on average 5.7 ± 1.3-fold between 15 and 21 WAP (Fig. 2*B*). These increases were within a narrow range and displayed a highly correlated temporal expression pattern (R^2 = 0.99; Fig. S2). These data indicate closely coordinated expression during oil synthesis in a nonseed tissue, a pattern also noted in *Arabidopsis* and other seeds (3).

It is clear from both the species comparisons and the temporal data that the very high oil content of oil palm mesocarp correlates with high and increasing levels of transcripts coding for all fatty acid synthesis proteins. Contrary to oil seeds (3), the levels

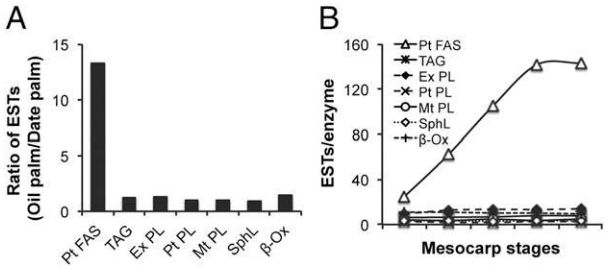

Fig. 2. Lipid subcategories except for fatty acid synthesis show similar expression pattern in oil palm and date palm. (*A*) The ratio of ESTs in oil palm versus date palm for each lipid category (calculated per enzyme to account for different number of enzymes per pathway). (*B*) Temporal profile of ESTs for lipid categories in developing mesocarp of oil palm. See Dataset S2*A* for the list of genes included in each category. Pt, plastid; TAG, triacylglycerol; ExPL, extraplastidial polar lipids; PL, polar lipids; Mt, mitochondria; SphL, sphingolipids; β-ox, β-oxidation.

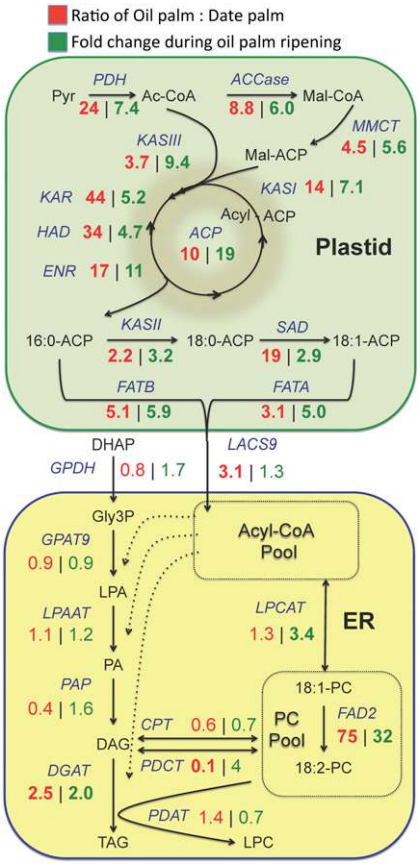

Fig. 3. Transcript patterns for enzymes involved in plastidial and extraplastidial reactions of oil synthesis differ greatly. Values in red indicate the ratio of oil palm to date palm ESTs, calculated as the ratio of sum of ESTs for five stages. Values in green indicate fold increase in ESTs during oil palm ripening, which are calculated as ratio of ESTs at 23 versus 15 WAP. Ratios ≥ twofold and significant at P value < 0.05 are indicated in boldface type. ESTs for enzymes with multiple subunits or isoforms were summed. For details on abbreviations, annotations, and EST levels at each stage, see Dataset S2*A*. 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid.

of fatty acid synthesis transcripts remained high up to the end of oil accumulation in oil palm mesocarp.

TAG Assembly Transcript Levels Were Similar in Oil and Date Palm and Remained Mostly Constant. In sharp contrast to the EST patterns for plastidial fatty acid enzymes, ESTs for most enzymes involved in TAG assembly showed low or no up-regulation during ripening and no substantial difference between oil palm and date palm (Fig. 3 and Fig. S3). For example, the acylating enzymes G3P acyltransferase (GPAT9); LysoPA acyltransferases (LPAATs); and phospholipid:DAG acyltransferase (PDAT), an enzyme that catalyzes the transfer of an acyl group from PC to DAG, showed no up-regulation during oil palm ripening and a less than twofold difference between palm mesocarps (Fig. 3 and Dataset S2*A*). Although patterns differed for the two isoforms of DAG:acyl-CoA acyltransferase (DGAT1 and -2) that catalyze the last (acyl-CoA–dependent) acylation step to TAG, the sum of their ESTs increased only 2-fold during ripening and were 2.5-fold higher in oil palm than in date (Fig. 3 and Fig. S3).

Taken together, our data implicate transcriptional regulation of fatty acid synthesis rather than of TAG assembly enzymes as a major factor associated with very high oil synthesis in oil palm mesocarp.

PA Phosphatase Isoforms Were Distinct. Although the overall EST levels of TAG assembly enzymes did not show substantial differences temporally or between oil and date palm, in some cases a specific isoform varied more (Fig. S3). Presently, it is unclear which isoforms of PA phosphatase (PAP) are involved in TAG biosynthesis (8). Orthologs of *Arabidopsis* PAH1 and PAH2, which are involved in phospholipid synthesis but apparently not in TAG assembly, showed much lower EST levels in oil palm than in date palm mesocarp. Therefore, more probable candidates for generating DAG destined to TAG are two other putative PAPs, LPPβ and LPPδ (9), which remain largely uncharacterized. LPPδ was expressed at similar levels in both palms and was not up-regulated during oil palm ripening (Dataset S2*A*). However, oil palm ESTs for LPPβ were 13 times higher than in date palm, were not detected in leaves, and increased during oil palm ripening (Fig. S3). The EST levels of LPPβ were comparable to that of GPAT and LPAAT and showed a pattern that correlated with oil synthesis (Fig. S3). Thus, it is tempting to speculate that LPPβ might generate a DAG pool destined to oil synthesis in oil palm mesocarp.

PC-Related Enzymes Showed Distinct Changes. Although detailed understanding of the molecular mechanisms involved is still lacking, flux analyses and other studies have demonstrated the importance of PC metabolism and acyl editing in the process of TAG assembly (10–12). Indeed, ESTs coding for some enzymes possibly involved in these processes showed important variations (Fig. S4). For example, PC synthesis can proceed via either the methylation or nucleotide pathway (13). The methylation pathway was down-regulated in oil palm compared with date palm, whereas the nucleotide pathway was up-regulated. Interestingly, down-regulation of the methylation pathway was linked to higher oil content in yeast (14), liver (15), and *Brassica napus* transgenic lines overexpressing DGAT (16). Surprisingly, PC:DAG phosphocholine transferase (PDCT), an enzyme that plays an important role in TAG composition of *Arabidopsis* (12), showed 17-fold less ESTs in oil palm than in date palm. These data provide leads for future research on the interconnection of pathways involved in phospholipid and TAG synthesis and their possible importance for high oil synthesis.

A Transcription Factor with High Similarity to WRI1 Was Strongly Up-Regulated. Of 784 transcription factors (with >40 ESTs), only 6 had EST levels at least 15 times higher in oil palm than in date palm and were up-regulated at least fivefold during oil palm ripening. One of these six transcription factors is an AP2-domain–containing transcription factor with a very high similarity to maize (E-value < 10^{-57}) (17, 18) and *Arabidopsis* WRI1 (E-value < 10^{-59}; Fig. S5). The EST levels for WRI1-like were 57-fold higher in oil palm mesocarp, relative to date palm, and also increased by 7.5-fold during ripening (Fig. 4). This suggests that a WRI1 ortholog in oil palm might play a role similar to that in *Arabidopsis*. This hypothesis is also strongly supported by the higher oil palm EST levels for each of the 10 genes that are reported to be WRI1-regulated (4, 5, 17). Furthermore, as with *Arabidopsis* seeds, the temporal profile of ESTs coding for WRI1-like in oil palm was similar to that of its putative targets (Fig. 4). Our data provide a strong indication that a WRI1 ortholog plays a major role in oil accumulation not only in seeds but also in nonseed vegetative tissues such as mesocarp. In *Arabidopsis*, WRI1 is under the control of seed maturation master regulators such as LEAFY COTYLEDON1 and -2, FUSCA3 and ABSCISIC ACID INSENSITIVE3 (19, 20). However, no obvious homologs to these genes were identified in oil palm mesocarp, suggesting that *Eg*WRI1-like is likely to control oil synthesis independently of the upstream factors that participate in seed development and may involve a different regulatory network, possibly fruit-specific.

Increased Transcripts for Specific Enzymes and Transporters That Provide Pyruvate for Fatty Acid Synthesis. A major flux through glycolysis is expected to provide the large amounts of pyruvate required for high oil synthesis in oil palm. Each of the eight steps

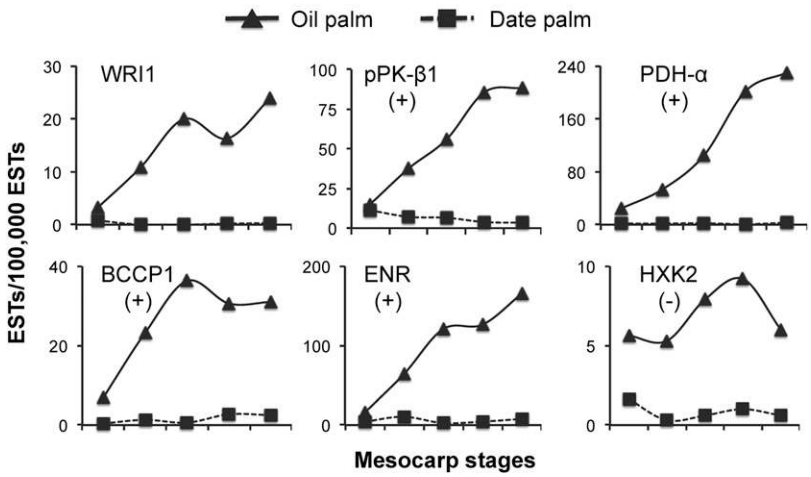

Fig. 4. Temporal pattern of EST levels for palm orthologs of Wrinkled1 (WRI1) (At3g54320) and select enzymes that are known to be WRI1-regulated (+) or not (–). pPK, plastid pyruvate kinase (At5g52920); PDH-α, pyruvate dehydrogenase (At1g01090); BCCP1, biotin carboxyl carrier protein1 (At5g16390); ENR, enoyl-ACP reductase (At2g05990); HXK2, hexokinase (At2g19860).

of glycolysis occurs in both cytosol and plastid. Notably, only 6 glycolytic enzymes of 16 increased, either temporally during oil palm ripening or when comparing oil palm and date palm (Fig. 5). Only two of these six differed by both criteria. These were plastid isoforms of ATP-dependent phosphofructokinase (PFK) and PK, which were 6.3- and 4.1-fold higher than in date palm, respectively (Fig. 5). These two enzymes also increased 5- and 3.3-fold during ripening of oil palm (Fig. 5). Both PK and PFK are considered to catalyze key regulatory steps of glycolysis (21), whereas most other enzymes of glycolysis are estimated to be in large excess with regards to flux in *B. napus* embryos (22). Therefore, our results imply that an increase in expression of plastid glycolysis plays a major role in providing pyruvate for high rates of fatty acid synthesis.

Unlike the plastid isoforms, none of the ESTs for cytosolic glycolysis showed increases during ripening of oil palm mesocarp (Fig. 5). In addition, EST levels for cytosolic glycolysis were similar in both palms, except for PFK (Fig. 5). The ESTs coding for cytoplasmic PFK, which is an ATP-dependent enzyme, were 3.4 times higher in oil palm than in date palm mesocarp whereas pyrophosphate-dependent phosphofructokinase (PFP) EST levels remained unchanged during ripening and were at similar levels compared with date mesocarp (Fig. S6*A*). This strongly contrasts with *Arabidopsis* and *B. napus* seeds, where PFP is

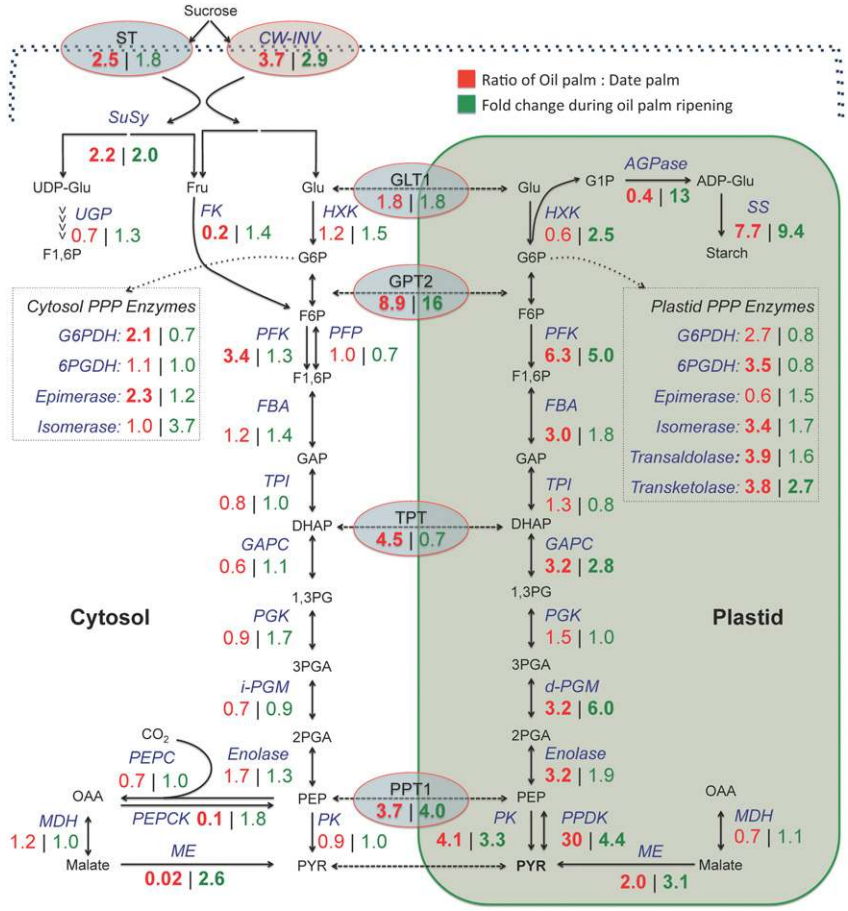

Fig. 5. Transcript patterns for enzymes involved in generating pyruvate from sucrose. Values in red indicate the ratio of oil palm to date palm ESTs, calculated as the ratio of sum of ESTs for five stages. Values in green indicate temporal changes during oil palm ripening, which is calculated as the ratio of ESTs at 23 versus 15 WAP. Ratios ≥ twofold significance at P value < 0.05 are indicated in boldface type. ESTs for enzymes with multiple subunits or isoforms were summed. For details on abbreviations, annotations, and EST levels at each stage, see Dataset S2*B*.

much more highly expressed than PFK and has been hypothesized to carry most of the flux through glycolysis (23).

The most striking changes with regards to carbohydrate pathways concerned plastid transporters (Fig. 6*B*). Oil palm ESTs coding for a GPT2 ortholog, which in *Arabidopsis* transports hexose, pentose, and triose phosphate (24), increased 16-fold during ripening, were on average 9 times higher than in date (Fig. 6 *B* and *C*), and represented one of the highest EST counts of all carbohydrate-related genes of oil palm mesocarp. Interestingly, antisense inhibition of a GPT transporter in *Vicia* seeds altered carbon partitioning and promoted protein storage at the expense of carbohydrates (25). ESTs for the phosphoenolpyruvate (PEP) transporter PPT1 were 3.7 times higher than in date palm and increased fourfold during oil palm ripening (Figs. 5 and 6*B*).

PPP and malate have been shown to provide some contribution to oil synthesis, both as carbon units and as a reductant source (3). Although plastid-localized enzymes of these pathways showed EST increases in oil palm, these were moderate compared with plastid PK, PFK, and GPT2 and fatty acid synthesis enzymes (Fig. 5 and Dataset S2*B*). Only a strong decrease in cytosolic malic enzyme was noted in oil palm compared with date palm.

Taken together, our results suggest that (*i*) plastid glycolysis is up-regulated in oil palm compared with date palm and temporally during ripening, and (*ii*) GPT2 and PPT provide glycolytic substrates (hexose P) and intermediates (triose phosphate, PEP) to the plastid by transport from the cytosol, a trend that is accentuated during ripening. This implies a strong funneling of carbon toward pyruvate in the plastids of oil palm but not of date palm. Thus, strongly increased fatty acid synthesis (Figs. 2 and 3), together with plastid carbon supply, is likely crucial for the eventual accumulation of 90% oil in the mesocarp.

Ratio of Cell-Wall Invertase to Sucrose Synthase ESTs Is Much Higher in Oil Palm than in Oil Seeds. Carbon is supplied to the fruit mostly as apoplastic sucrose. Higher cell-wall invertase activity is known to lead to increased fruit sugar levels and increased seed weight in tomato fruit (26). Because the sum of ESTs encoding cell-wall invertases increased about 3-fold during oil palm ripening and were 3.7-fold higher than in date palm (Fig. 5 and Fig. S6*B*) and 50-fold higher than in oil palm leaf (Dataset S2*B*), we speculate that a significant part of sucrose is hydrolyzed at the cell wall. The level of ESTs coding for sucrose synthase, which initiates a pyrophosphate-dependent pathway to hexose-P via UDPG pyrophosphorylase, was similar in oil palm mesocarp and in leaf, was 2.2-fold higher than in date, and increased by 2-fold during oil palm ripening. The ratio of cell-wall invertase to sucrose synthase ESTs was 35-fold higher than this ratio in *Arabidopsis* seeds (4). This suggests, as noted above for the PFP versus PFK ratios, that oil palm mesocarp and oil seeds differently use ATP- and pyrophosphate-dependent enzymes.

Aside from glycolysis, hexoses provide carbon for starch biosynthesis. ESTs coding for ADPG pyrophosphorylase (AGPase) and starch synthase showed 13- and 9.4-fold increases during oil palm ripening, respectively (Fig. 5, Fig. S6*C*, and Dataset S2*B*). Interestingly, plastid phosphoenolpyruvate orthophosphate dikinase (PPDK) showed a similar expression pattern (Fig. S6*C*). In maize, PPDK belongs, together with AGPase, to a starch synthesizing multienzyme complex (27) that likely facilitates pyrophosphate exchange between AGPase and PPDK. Taken together, these data explain the late increase of starch that was revealed by our metabolite analysis of oil palm mesocarp (Dataset S1*B*).

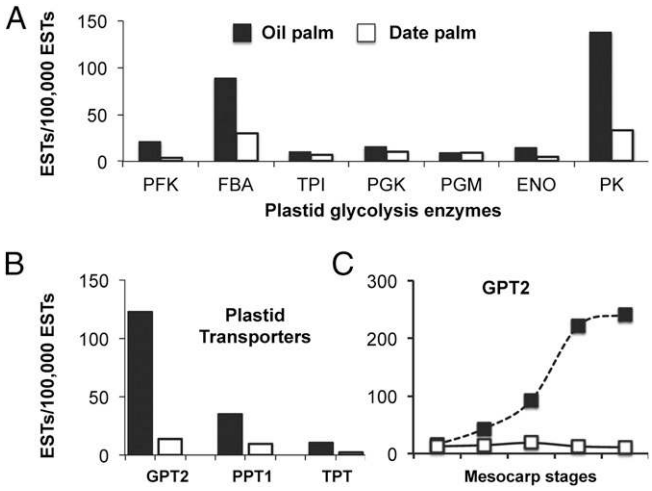

Fig. 6. Plastid glycolytic enzymes and transporters. (*A*) Average EST levels for select plastidial glycolytic enzymes and (*B*) plastid transporters and (*C*) temporal profile of glucose-6-phosphate transporter (GPT2) expression in five stages of oil palm and date palm mesocarp. For details on EST levels for cytosolic enzymes, abbreviations, annotations, and EST levels for each stage, see Dataset S2*B*.

Conclusions

The temporal and cross-species comparisons made in this study provide a concurrent exploration of transcripts and metabolites associated with major species-specific differences in central carbon partitioning. In almost all cases, if transcripts increased more than threefold during oil palm ripening, there was also a greater or similar difference between oil and date palm. Thus, the comparison between oil and date palm reinforced and strengthened the interpretations derived from transcript temporal patterns.

The high oil content of oil palm was not associated with increased gene expression for many of the enzymes required for hexose-to-TAG metabolism. Instead, high oil production correlated most strongly with temporal increases and high oil-palm-to-date palm ratios for transcripts coding for all fatty acid synthesis enzymes (including PDH), specific plastid transporters, select plastid glycolytic enzymes (namely PFK and PK), and a WRI1-like transcription factor. The evidence for WRI1-like involvement in oil palm mesocarp, and that all its known targets were up-regulated, implies a remarkable similarity between regulation of fatty acid synthesis destined to oil in seeds and in nonseed tissues. Intriguingly, although oil palm and date palm appeared to express similar levels of transcripts for the complete pathway of TAG synthesis from plastid fatty acids, date palm accumulates no detectable TAG.

Although many features of the transcription patterns described here showed similarities to that in oil seeds, important differences could be noted. WRI1-like does not appear to be under control of the same upstream factors (e.g., LEC2) as in oil seeds. This may explain in part the very different temporal profiles of expression between oil palm and oil seeds. For most oil seeds, transcripts related to lipid synthesis peak and decline well before the later stages of TAG accumulation (3). In contrast, in oil palm, almost all of the up-regulated transcripts did not decline but continued to increase until the end of oil accumulation. Also, in contrast to oil seeds, oil palm showed a preferential up-regulation of ATP-dependent enzymes rather than one for pyrophosphate-dependent steps of sucrose breakdown and early glycolysis, suggesting that ATP availability might be an important factor related to higher levels of oil. Finally, such a large change in expression of the GPT plastid transporter gene was not reported in oil seeds. All these differences may provide insights into how oil palm mesocarp accumulates more oil than oil seeds do.

The oil palm and date palm EST datasets also provide information on several lipid-related enzymes and their specific isoforms, such as PAPs, which may give possible leads for future research. More generally, the data illustrate that such a dual developmental and comparative approach, applied to diverse biological systems of interest, can be as powerful as developmental transcriptomic studies carried out in model organisms. Finally, our results increase tremendously the molecular resources available for palms and provide a large EST collection for fruits. In addition to knowledge generated on the regulation of carbon partitioning in oil palm mesocarp, these resources should also

allow breeders to identify candidate genes involved in high oil synthesis. For example, polymorphism of WRI1 in rapeseed relates highly to oil content (28). This will pave the way to marker-assisted selection of oil palm, a key technique for more rapid improvement of crop trees.

Materials and Methods

For complete details, see *SI Materials and Methods*.

Biological Material. Oil and date palm fruits were harvested at five comparable developing stages and were flash-frozen. After removal of pericarp, mesocarp was dissected and powdered in liquid nitrogen for metabolite analyses and RNA extraction.

Metabolite Analyses. Mesocarp tissue was analyzed for polar metabolites by 1H-NMR (29) and nonsoluble residue for starch content (30). Total lipids were extracted from the mesocarp and neutral and polar lipids were separated by 1D or 2D TLC and quantified using gas chromatography.

Electron Microscopy. Oil palm mesocarp (20 WAP) was subjected to cryofixation and cryosubstitution before sectioning. Slices of 50–80 nm thickness were observed by transmission electron microscope mostly as described (31).

cDNA Library Construction and Sequencing. Total RNA was extracted from five stages of oil palm (32) and date palm mesocarp (33) and oil palm leaves. Oil palm and date palm cDNA libraries were synthesized according to GS20 DNA Library Preparation (Roche) and Roche cDNA Rapid Library Preparation Method, respectively. cDNA libraries were subjected to emulsion PCR, and beads with amplified library were loaded onto a picotiter plate and sequenced. Pyrosequencing was performed at the Department of Energy Joint Genome Institute following protocols for the Genome Sequencer GS FLX Titanium System (Roche Diagnostic).

Bioinformatics and Data Analyses. EST reads obtained from 454 Sequencing were trimmed and filtered to remove low-quality sequences. De novo assembly was by CAP3 software. Blast similarity search using BLASTX (E-value < 10^{-10}) against the The Arabidopsis Information Resource 8 and RefSeq (National Center for Biotechnology Information) databases was performed to assign an annotation to the transcripts. EST sequences generated from this study are available from National Center for Biotechnology Information Sequence Read Archive (accession nos. SRX059258-62, SRX059116-20, and SRX059798-802). Information on data files containing annotation and temporal expression along with unprocessed sequences is provided in *SI Materials and Methods*. Contigs with more than 10 ESTs are listed in Dataset S2C and sequences can be downloaded at http://www.biomemb.cnrs.fr/contigs.html.

The number of ESTs/100,000 ESTs was used as an estimate of gene expression to enable fold change comparison between stages and species. Values greater or equal to twofold that are presented in figures or text are significant at $P < 0.05$ on the basis of the DEGseq method (34).

ACKNOWLEDGMENTS. We thank J. Malek and R. Sambanthamurthi for sharing unpublished results, A. Troncoso-Ponce for help with data analysis and P. Koona for support. Metabolite analyses were carried out at the Metabolome Facility of Bordeaux Functional Genomics Center (University of Bordeaux-Ségalen/Institut National de la Recherche Agronomique/Centre National de la Recherche Scientifique) by M. Maucourt and C. Deborde (NMR) and by Y. Gibon and V.A. (starch). Electron microscopy was carried out at the Bordeaux Imaging Center (Plant Imaging Facility) by M. Peypelut, with help from B. Batailler, C. Cheniclet, and V. Rouyère. DNA sequencing was supervised by E. Lindquist and C. Pennacchio. EST assembly and databases were provided by Curt Wilkerson and Nick Thrower (Michigan State University). F.B. was the recipient of a fellowship from the Conseil Régional d'Aquitaine. G-F.N-E. was the recipient of a short-term European Molecular Biology Organization fellowship. This work was supported in part by the Office of Science of the Department of Energy under Contract DE-AC02-05CH11231.

1. Ngando Ebongue GF, Ajambang WN, Koona P, Lalu Firman B, Arondel V (2011) Breeding of the oil palm: Past, present and prospects. *Technological Innovations in Major World Oil Crops*, ed Gupta SK (Springer Verlag, Berlin), Vol 1, in press.
2. Sambanthamurthi R, Sundram K, Tan Y (2000) Chemistry and biochemistry of palm oil. *Prog Lipid Res* 39:507–558.
3. Baud S, Lepiniec L (2010) Physiological and developmental regulation of seed oil production. *Prog Lipid Res* 49:235–249.
4. Ruuska SA, Girke T, Benning C, Ohlrogge JB (2002) Contrapuntal networks of gene expression during Arabidopsis seed filling. *Plant Cell* 14:1191–1206.
5. Baud S, Wuillème S, To A, Rochat C, Lepiniec L (2009) Role of WRINKLED1 in the transcriptional regulation of glycolytic and fatty acid biosynthetic genes in Arabidopsis. *Plant J* 60:933–947.
6. Janssen TBK (2004) The age of major monocot groups inferred from 800+ *rbcl* sequences. *Bot J Linn Soc* 146:385–398.
7. Li-Beisson Y, et al. (2010) Acyl-lipid metabolism. *The Arabidopsis Book*, ed Last R (American Society of Plant Biologists, Rockville, MD), pp 1–65.
8. Eastmond PJ, et al. (2010) Phosphatidic acid phosphohydrolase 1 and 2 regulate phospholipid synthesis at the endoplasmic reticulum in Arabidopsis. *Plant Cell* 22: 2796–2811.
9. Nakamura Y, Tsuchiya M, Ohta H (2007) Plastidic phosphatidic acid phosphatases identified in a distinct subfamily of lipid phosphate phosphatases with prokaryotic origin. *J Biol Chem* 282:29013–29021.
10. Bates PD, Durrett TP, Ohlrogge JB, Pollard M (2009) Analysis of acyl fluxes through multiple pathways of triacylglycerol synthesis in developing soybean embryos. *Plant Physiol* 150:55–72.
11. Bafor M, Smith MA, Jonsson L, Stobart K, Stymne S (1991) Ricinoleic acid biosynthesis and triacylglycerol assembly in microsomal preparations from developing castor-bean (Ricinus communis) endosperm. *Biochem J* 280:507–514.
12. Lu C, Xin Z, Ren Z, Miquel M, Browse J (2009) An enzyme regulating triacylglycerol composition is encoded by the ROD1 gene of Arabidopsis. *Proc Natl Acad Sci USA* 106: 18837–18842.
13. Keogh MR, Courtney PD, Kinney AJ, Dewey RE (2009) Functional characterization of phospholipid N-methyltransferases from Arabidopsis and soybean. *J Biol Chem* 284: 15439–15447.
14. Malanovic N, et al. (2008) S-adenosyl-L-homocysteine hydrolase, key enzyme of methylation metabolism, regulates phosphatidylcholine synthesis and triacylglycerol homeostasis in yeast: Implications for homocysteine as a risk factor of atherosclerosis. *J Biol Chem* 283:23989–23999.
15. Li Z, Vance DE (2008) Phosphatidylcholine and choline homeostasis. *J Lipid Res* 49: 1187–1194.
16. Sharma N, et al. (2008) Transgenic increases in seed oil content are associated with the differential expression of novel Brassica-specific transcripts. *BMC Genomics* 9:619.
17. Cernac A, Benning C (2004) WRINKLED1 encodes an AP2/EREB domain protein involved in the control of storage compound biosynthesis in Arabidopsis. *Plant J* 40:575–585.
18. Shen B, et al. (2010) Expression of ZmLEC1 and ZmWRI1 increases seed oil production in maize. *Plant Physiol* 153:980–987.
19. Baud S, et al. (2007) WRINKLED1 specifies the regulatory action of LEAFY COTYLEDON2 towards fatty acid metabolism during seed maturation in Arabidopsis. *Plant J* 50: 825–838.
20. Wang H, Guo J, Lambert KN, Lin Y (2007) Developmental control of Arabidopsis seed oil biosynthesis. *Planta* 226:773–783.
21. Plaxton WC (1996) The organization and regulation of plant glycolysis. *Annu Rev Plant Physiol Plant Mol Biol* 47:185–214.
22. Junker BH, Lonien J, Heady LE, Rogers A, Schwender J (2007) Parallel determination of enzyme activities and in vivo fluxes in Brassica napus embryos grown on organic or inorganic nitrogen source. *Phytochemistry* 68:2232–2242.
23. Baud S, Graham IA (2006) A spatiotemporal analysis of enzymatic activities associated with carbon metabolism in wild-type and mutant embryos of Arabidopsis using in situ histochemistry. *Plant J* 46:155–169.
24. Linka N, Weber AP (2010) Intracellular metabolite transporters in plants. *Mol Plant* 3: 21–53.
25. Rolletschek H, et al. (2007) Antisense inhibition of the plastidial glucose-6-phosphate/phosphate translocator in Vicia seeds shifts cellular differentiation and promotes protein storage. *Plant J* 51:468–484.
26. Jin Y, Ni DA, Ruan YL (2009) Posttranslational elevation of cell wall invertase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. *Plant Cell* 21:2072–2089.
27. Hennen-Bierwagen TA, et al. (2009) Proteins from multiple metabolic pathways associate with starch biosynthetic enzymes in high molecular weight complexes: A model for regulation of carbon allocation in maize amyloplasts. *Plant Physiol* 149:1541–1559.
28. Liu J, et al. (2010) Increasing seed mass and oil content in transgenic Arabidopsis by the overexpression of wri1-like gene from Brassica napus. *Plant Physiol Biochem* 48:9–15.
29. Moing A, et al. (2004) Quantitative metabolic profiling by 1-dimensional H-1-NMR analyses: Application to plant genetics and functional genomics. *Funct Plant Biol* 31: 889–902.
30. Gibon Y, et al. (2004) A Robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: Comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. *Plant Cell* 16:3304–3325.
31. Melser S, et al. (2010) Glucosylceramide biosynthesis is involved in Golgi morphology and protein secretion in plant cells. *Traffic* 11:479–490.
32. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18: 5294–5299.
33. Meisel L, et al. (2005) A rapid and efficient method for purifying high quality total RNA from peaches (Prunus persica) for functional genomics analyses. *Biol Res* 38:83–88.
34. Wang L, Feng Z, Wang X, Wang X, Zhang X (2010) DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* 26: 136–138.