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# Biochemical and gene expression analysis for the selection of elite oil palm ortets

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**Abstract**

The rising global demand for palm oil necessitates innovative strategies to accelerate yield improvement and ensure sustainable production. Conventional propagation methods are limited by long regeneration cycles, making tissue culture a more practical alternative that relies on elite ortet identification. This preliminary study aimed to evaluate crude palm oil quality, leaf metabolite profiles, and gene expression as early indicators for selecting elite ortets. Mature fruits were used to assess oil yield, free fatty acid, and β-carotene contents, while leaves were analyzed for chlorophyll and total phenolic content. Based on biochemical screening, three candidate ortets were further evaluated for the expression of acetyl-CoA carboxylase (*ACCase*) and phytoene synthase (*PSY*) genes. Oil yield ranged from 77-83%, FFA 5-9%, and β-carotene 200-450 mg $kg^{-1}$ CPO across candidates. Candidate 3.7 exhibited the highest oil yield and elevated *ACCase* expression, while candidate 6.12 showed the highest β-carotene content and *PSY* expression. Candidate 5.7 demonstrated relatively low FFA and favorable chlorophyll levels. These results suggest an association between *ACCase* expression and oil yield, as well as between *PSY* expression and β-carotene accumulation. Collectively, these findings support a proof-of-concept for an integrated profiling strategy to select elite ortets for advanced tissue culture propagation.

[Keywords: carotene content, clonal propagation, crude palm oil, *Elaeis guineensis*]

## Introduction

Crude palm oil (CPO) is one of the world's largest vegetable oil commodities, serving as a key raw material for the food, textile, cosmetics, pharmaceutical, and biodiesel industries (Hambali & Muzdalifah, 2020). Global demand continues to rise, with palm oil accounting for 51.14% of global vegetable oil consumption (Mustafa, 2022). In 2022, production exceeded 400 million tons, representing an increase of ~160% compared to 2000 (FAOSTAT, 2023). This sharp growth highlights the need for more efficient propagation systems to generate high-yielding and superior planting materials. Conventional propagation is limited by long growth cycles, whereas tissue culture provides a scalable alternative for producing uniform seedlings (Azahra & Ernayunita, 2023).

Tissue culture involves isolating plant tissues and cultivating them in an artificial medium enriched with nutrients and growth regulators, enabling the mass production of uniform seedlings (Kushairi et al., 2010; Baharuddin et al., 2025). This technique relies on explants derived from carefully selected parent plants, or ortets (Azahra & Ernayunita, 2023). Ortets are typically chosen based on agronomic traits such as oil yield and quality, growth vigor, crown architecture, and resistance to pests and diseases (Pratiwi et al., 2020). Conventional selection is primarily based on vegetative traits and bunch analysis, but such approaches are time-consuming and may not reliably predict oil quality or biochemical potential (Azahra & Ernayunita, 2023).

A promising strategy is to complement phenotypic evaluation with molecular and biochemical indicators. Key biosynthetic pathways strongly influence oil yield and metabolite profiles, including fatty acid synthesis regulated by acetyl-CoA carboxylase (*ACCase*) and carotenoid biosynthesis mediated by phytoene synthase (*PSY*) (Tranbarger et al., 2011; Wan Sulaiman et al., 2011). To describe the relationship between key traits and their underlying molecular physiology, we also

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measured the expression of *ACCase* and *PSY* to evaluate the potential of these oil palm ortets.

Based on this gap, the present study preliminarily investigates the oil and metabolite content of several candidate ortets. This integrative approach provides a scientific basis for identifying elite parent material to support efficient clonal propagation through tissue culture. This research aimed at determining chemical parameters to assess the initial potential of candidate ortets, including oil quality and metabolite content of oil palm leaves. Furthermore, this research also aims at evaluating how *ACCase* and *PSY* gene expression levels relate to oil yield and β-carotene content in the selected ortets, supporting the overall assessment of the ortet's potential. Therefore, this preliminary study aims at applying this integrated approach on a limited set of candidate ortets to identify promising individuals and establish proof-of-concept. The findings from this initial investigation will guide and inform a larger, more comprehensive study currently underway on a broader population.

## Materials and Methods

### *Plant materials*

Plant materials consisted of fruits and leaves collected from Tenera oil palms planted in 2010 at the Indonesian Oil Palm Research Institute (IOPRI), Bogor Unit, Ciomas, Bogor, Indonesia. Sampling was conducted under uniform field conditions. Mature fruits were harvested at a comparable stage of ripeness. Leaves were collected from the same position on the frond.

In this study, the candidate ortet is identified by a unique code (e.g., 3.7, 4.3, 5.5, etc). All fruits and leaves analyzed were collected from the same coded candidates. Mature fruits harvested from each candidate were used for CPO quality assessment, including oil yield, FFA content, and β-carotene concentration. Leaves (17th fronds) from the corresponding candidates were analyzed for total chlorophyll and total phenolic content. Based on the combined biochemical screening results, three candidate ortets with comparatively favorable parameters were selected for further molecular analysis. Young fruits harvested from these candidates were subsequently used to evaluate the expression levels of *ACCase* and *PSY* genes.

### *Determination of metabolite contents of CPO*

Metabolite contents of CPO analyzed in this study include oil yield, FFA content, and β-carotene. CPO yield was measured using the modified method of Priyatama et al. (2023), with modifications based on the amount of sample and solvents used. The mesocarp (fruit flesh) was separated from the seeds and cut into small pieces. The dried samples were ground after overnight drying (±16 hours) in an oven. A 5 g sample was wrapped in filter paper and placed in a Soxhlet tube. The Soxhlet apparatus was assembled with a condenser and a fat flask containing 100 mL of petroleum benzene. Extraction was performed at 100°C and terminated when the solvent in the Soxhlet tube appeared clear. The fat flask was removed and dried in an oven at 100°C for 1 hour. The flask was reweighed to determine oil yield. Free fatty acid content in CPO was determined by the titrimetric method based on SNI 01-2901-2006 (AOCS, 2004). Two grams of CPO was dissolved in 50 mL of ethanol and heated to 40°C. Phenolphthalein (PP) was added as an indicator and titrated with 0.1 N NaOH. β-carotene was extracted from 0.1 g of CPO with 25 mL of *n*-hexane. β-carotene absorbance was measured at λ 446 nm (Sarip et al., 2023).

### *Determination of chlorophyll contents*

Chlorophyll content was measured using the method of Singh et al. (2019). Chlorophyll was extracted from 0.1 g of palm leaf with 10 mL of 80% acetone. Chlorophyll absorbance was measured at λ 645 and 663 nm. The colorimetric assay for phenolic compounds was based on the Folin-Ciocalteu (FC) method (Leng et al., 2017) that involved phenolic extraction, pure phenolic compound (gallic acid) calibration, and absorbance measurement after color development. Phenolics were extracted from 0.2 g of dried palm leaves with 20 mL of methanol. A 1000 ppm gallic acid standard solution was diluted to 50, 75, 100, 150, and 200 ppm. Sample filtrate/standard solution (0.1 mL) was mixed with 0.5 mL FC reagent and 5 mL distilled water, vortexed, and left for 6 min. The mixture was reacted with 1.5 mL 20% $Na_2CO_3$ and adjusted to 10 mL with distilled water. Each reaction mixture was incubated for 2 hours before measuring absorbance at λ 765 nm.

### *cDNA preparation*

Total RNA was isolated using the GeneJET RNA Purification Kit (Thermo Scientific, 2025). The concentration and purity of the isolated RNA were measured using a NanoDrop™ 2000 Spectrophotometer. For cDNA synthesis, 1 µg of total RNA was reverse-transcribed using ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Toyobo). PCR amplification was performed using MyTaq HS Red Mix (Bioline) in a 10 µL reaction containing 5 µL 2× master mix, 0.4 µM of each primer listed in Table 1, and 1 µL cDNA template. The amplification was conducted under the following conditions: initial denaturation at 95°C for 1 min; 35 cycles of 95°C for 15 s, 55–60°C for 15 s,

and 72°C for 30 s; and a final extension at 72°C for 5 min. PCR products were resolved on 1% agarose gel.

*Real-time PCR (RT-PCR) setup*

Quantitative real-time PCR (qRT-PCR) was performed using SensiFAST™ SYBR® Hi-ROX Kit (Bioline) in [x system]. Each 10 µL reaction contained 5 µL 2× master mix, 0.4 µM of each primer described previously, 1 µL cDNA template, and nuclease-free water. The amplification conditions were: 95°C for 2 min, followed by 40 cycles of 95 °C for 10 s, 60°C for 10 s, and 72°C for 15 s. Melting curve analysis was subsequently performed to confirm primer specificity, with the following steps: 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. Each sample was analyzed in three technical replicates and three biological replicates. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001), where Ct values of target genes were normalized against the housekeeping gene (actin) to obtain ΔCt. The ΔΔCt values were calculated relative to the mean ΔCt of all samples. Amplification efficiency was assumed to be comparable between target and reference genes.

## Results and Discussion

*CPO quality: oil yield and FFA content*

The oil yield of ten candidate ortets ranged from 77% to 83% (w/w) based on mesocarp dry weight (Figure 1). Candidate 3.7 recorded the highest yield at 83.56%, approximately 2–6% higher than the other candidates. Similarly, candidate 5.7 exhibited a comparatively high yield of 81.64%. These elevated CPO yields highlight both candidates for further evaluation. The yields reported here are higher than those obtained by Sarip et al. (2023), who recorded ~75% using Soxhlet extraction, yet align with Hasibuan (2020), who reported 80–84% yields from boiled and mechanically pressed fresh fruit. This consistency suggests that the extraction protocol applied in the present study is robust, with minor differences likely reflecting intrinsic variation in fruit physiology rather than methodological artifacts.

Table 1. Primer pairs used in RT-qPCR

| Gene | Primer sequence | Gene function |
|---|---|---|
| *ACCase* | 5'-GAAGCACCWTYCCTGCMYT<br>5'-CKHGTTGGRGCCAYACRAT | Fatty acid biosynthesis |
| *PSY* | 5'-TCCGACTGTGGCTTTCTTCT<br>5'-AGTCACACCAGCCTCTGCTT | β-Carotene biosynthesis |
| *Actin* | 5'-TGCTGATCGTATGAGCAAGGAAA<br>5'-GAAATCCACATCTGCTGGAAGGT | Housekeeping gene |

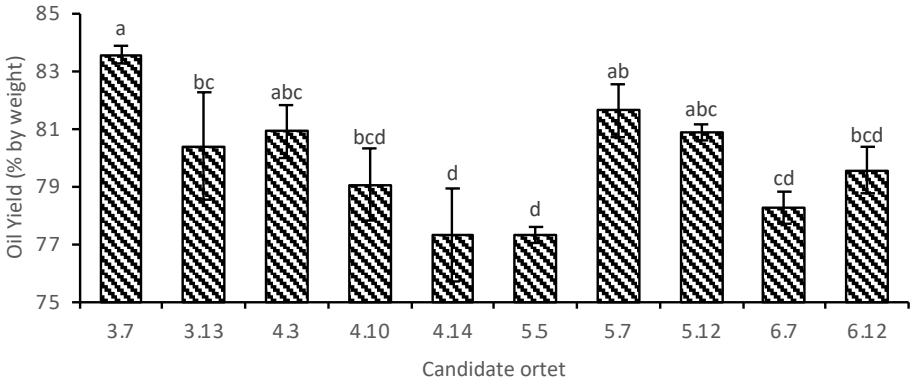


Figure 1. CPO yield from extraction. Data are presented as mean ± standard error (SE) (n = 3). ANOVA showed a $p < 0.05$. Different letters indicate significant differences.

The concentration of FFA is a critical indicator of CPO degradation and overall oil quality. Across the ten candidate ortets, FFA content ranged from 5% to 9% (w/w) (Figure 2). Candidate 4.14 exhibited the highest FFA level, whereas candidates 5.12 and 5.7 recorded the lowest. These values exceeded the maximum threshold established by SNI 2901-2021 (≤5%). Nonetheless, the measured range is consistent with previous studies. Igile et al. (2023) reported FFA contents between 2.44% and 12.76%, while Japir et al. (2017) documented values of 8.7% and 3.8% across two oil palm fruit types. Similarly, Ekwenye (2010) observed varietal differences, with FFA levels ranging from 4.46% to 7.33% in Dura and from 6.02% to 14.76% Tenera, indicating a genetic predisposition toward higher FFA accumulation in the latter.

Free fatty acids are produced through lipase-catalyzed hydrolysis of triacylglycerols (Mahesar et al., 2016; Shidiq et al., 2022). Its accumulation is influenced by multiple factors, including storage duration, processing temperature, and fruit moisture content. Compared to triacylglycerols, FFAs are more prone to oxidation, which promotes rancidity and reduces oil quality (Fokou et al., 2009). Hudori (2016) further demonstrated that mesocarp storage conditions substantially influence FFA levels, with prolonged storage leading to marked increases. In extreme cases, FFA concentrations can reach 20% (Ekwenye, 2010), underscoring the susceptibility of CPO to post-harvest degradation. Similarly, Japir et al. (2017) reported that high temperatures accelerate triacylglycerol breakdown, while fermentation processes involving lipolytic enzymes also enhance triacylglyceride hydrolysis (Fokou et al., 2009).

*Metabolite content: β-carotene, chlorophyll, and phenolic compounds*

Secondary metabolites in plants are broadly classified into terpenoids, phenolic compounds, and nitrogen-containing compounds (Anulika et al., 2016). In this study, we focused on β-carotene, chlorophyll, and phenolic compounds as key indicators of oil palm physiological status and CPO quality.

β-carotene ($C_{40}H_{56}$) was detected in concentrations ranging from 200 to 450 mg $kg^{-1}$ CPO (Figure 3). Candidate 6.12 contained the highest level (440 mg $kg^{-1}$) of β-carotene, consistent with reported ranges of 200–500 ppm (Tai & Brunner, 2019) and 305–506 ppm (Hasibuan, 2020). Freshly processed fruit often contains higher levels (542–868 ppm) (Silalahi, 2022). Beyond its role as a natural pigment, β-carotene functions as an antioxidant and is enzymatically converted to retinol (vitamin A) by β-carotene dioxygenase (Sari & Mayasari, 2018), linking its accumulation to both nutritional and industrial value.

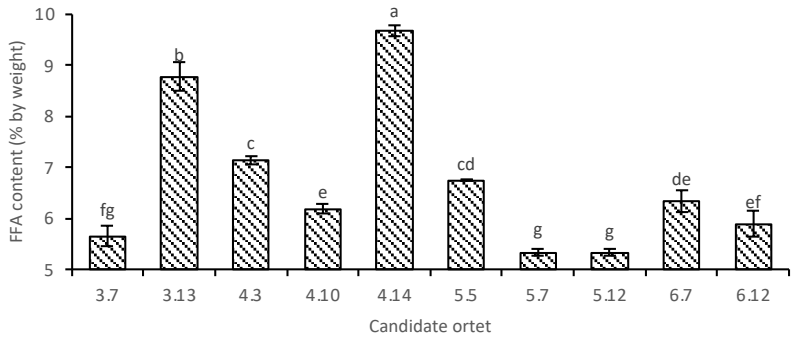


Figure 2. FFA content of CPO. Data are presented as mean ± standard error (SE) (n = 3). ANOVA showed a $p < 0.05$. Different letters indicate significant differences.

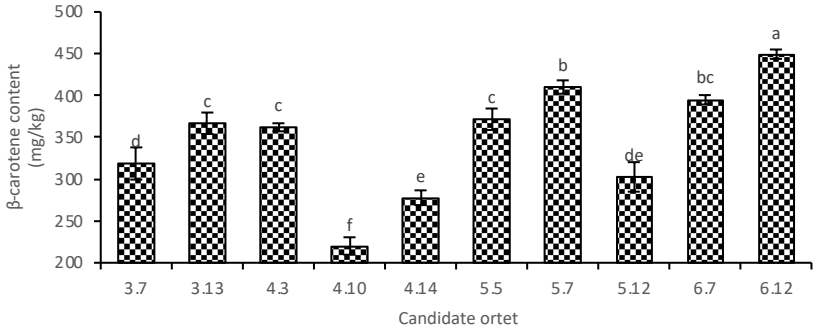


Figure 3. β-carotene content of CPO. Data are presented as mean ± standard error (SE) (n = 3). ANOVA showed a $p < 0.05$. Different letters indicate significant differences.

Chlorophyll, the primary pigment in photosynthesis, exists in two forms in higher plants: chlorophyll a (dark green) and chlorophyll b (light green) (Holidi et al., 2016; Mustafa et al., 2015). Chlorophyll a serves as the main electron donor in reaction centers, while chlorophyll b expands light-harvesting efficiency (Mulero et al., 2022). Total chlorophyll in fresh oil palm leaves ranged from 1.4 to 1.7 mg $g^{-1}$ (Figure 4), consistent with previous reports of 1–1.5 mg $g^{-1}$ (Manoh et al., 2021). The chlorophyll a/b ratio varied between 2.5 and 4.5, within the normal physiological range (Sonobe et al., 2020). Notably, candidate 6.7 exhibited a high a/b ratio, suggesting growth under high light intensity, whereas candidate 3.7 displayed the lowest, consistent with shaded conditions (Zhang et al., 2020). Variations in chlorophyll concentration and a/b ratios highlight how environmental factors, including temperature and rainfall, modulate photosynthetic efficiency and, ultimately, productivity (Djs et al., 2020).

Phenolic compounds, characterized by hydroxyl groups attached to aromatic rings, are another major class of secondary metabolites with key roles in plant defense (Mahardani & Yuanita, 2021). Here, total phenolic content in dried leaves ranged from 200 to 450 mg $kg^{-1}$ (Figure 5), aligning with previous findings of 50–300 ppm (Safa et al., 2024) and 200–500 ppm (Hasan & Manan, 2020). Phenolics are synthesized in response to environmental stress (Hanin & Pratiwi, 2017) and function as antioxidants by donating hydrogen atoms to neutralize reactive oxygen species (Andrés et al., 2023). They also protect against UV-B radiation and DNA damage (Kuljarusnont et al., 2024), suggesting that elevated levels reflect both genetic predisposition and environmental stress adaptation.

Within our limited population, integrating metabolite and oil quality profiles reveals three promising candidate ortets. Candidate 3.7 combined high oil yield with elevated phenolic content, indicating superior stress tolerance and stability. Candidate 6.12 contained the highest β-carotene concentration, conferring added nutritional and antioxidant value. Meanwhile, candidate 5.7 exhibited low FFA levels and favorable chlorophyll profiles, traits associated with improved oil quality and photosynthetic capacity. However, the synergistic roles of phenolics (stress defense), chlorophyll (photosynthetic efficiency), and β-carotene (nutritional and oxidative protection) suggest a multifaceted contribution to CPO productivity and stability (Djs et al., 2020; Fokou et al., 2009; Hanin & Pratiwi, 2017).

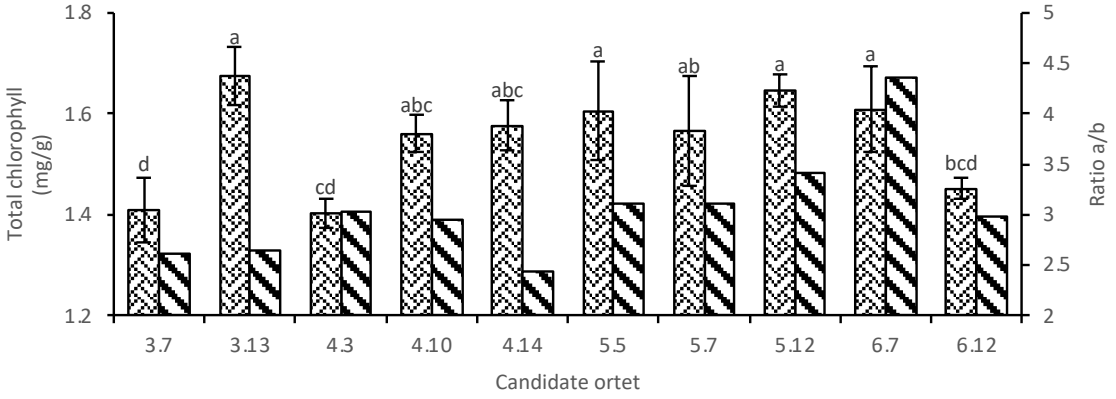


Figure 4. Total chlorophyll content and chlorophyll a/b ratio of oil palm leaves. Data are presented as mean ± standard error (SE) (n = 3). The ANOVA test yielded a $p < 0.05$. Different letters indicate a significant difference.

Table 2. Expression levels of actin target genes

| Gene | Candidate Ortet | Expression level |
|---|---|---|
| *ACCase* | 3.7 | Down-regulated |
| | 5.7 | Down-regulated |
| | 6.12 | Down-regulated |
| *PSY* | 3.7 | Down-regulated |
| | 5.7 | Down-regulated |
| | 6.12 | Down-regulated |

*Expression of the metabolite-related genes in fresh fruit bunches*

The relative expression levels of the *acetyl-CoA carboxylase* (*ACCase*) and *phytoene synthase* (*PSY*) genes, normalized to actin, ranged from 0.02–0.3 and 0.3–0.9, respectively (Figure 6). The comparatively low expression of *ACCase* indicates strong transcriptional repression in the mesocarp tissue analyzed (Table 2). Although *PSY* expression was also relatively low, its higher magnitude compared to *ACCase* suggests preferential activation of carotenoid biosynthesis over fatty acid biosynthesis in this tissue at the time of sampling.

Among the candidate ortets, the expression patterns of *ACCase* and *PSY* aligned with the observed metabolite accumulation. Candidate 3.7 exhibited the highest *ACCase* expression, approximately twofold above the mean level, and correspondingly produced the highest CPO yield. Similarly, candidate 6.12 showed the strongest *PSY* expression, ~1.2-fold higher than average, which was consistent with it containing the greatest β-carotene concentration. The physiological traits of candidate 5.7 were not strongly aligned with the expression of either target gene, which may reflect technical variation in RNA quality or pipetting inconsistencies, as noted in standard qPCR reliability studies (Kozera & Rapacz, 2013).

ACCase catalyzes the committed step of fatty acid biosynthesis, the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA (Wu & Huang, 2020). In oil palm mesocarp, the enzyme exists in a prokaryotic-type multienzyme complex, a complex comprising biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP), and carboxyltransferase (CT) (Shen et al., 2024). The reaction proceeds in two sequential steps: (i) carboxylation of biotin bound to the BCCP domain, and (ii) transfer of the carboxyl group to acetyl-CoA by the CT domain to yield malonyl-CoA (Milke & Marienhagen, 2020). Thus, the observed variation in *ACCase* expression among ortets provides a plausible biochemical explanation for the differences in oil accumulation.

By contrast, PSY functions as the first committed enzyme in carotenoid biosynthesis (Zhou et al., 2022). The higher *PSY* expression in candidate ortet 6.12 offers a mechanistic insight into the enhanced β-carotene accumulation observed. Together, these findings describe a direct molecular relationship between the expression of these specific biosynthetic genes and the accumulation of their end products in these selected candidates. This correlation provides a physiological context for the observed phenotypic variation within these candidate ortets and underscores the value of an integrated approach for the preliminary characterization of ortet potential.

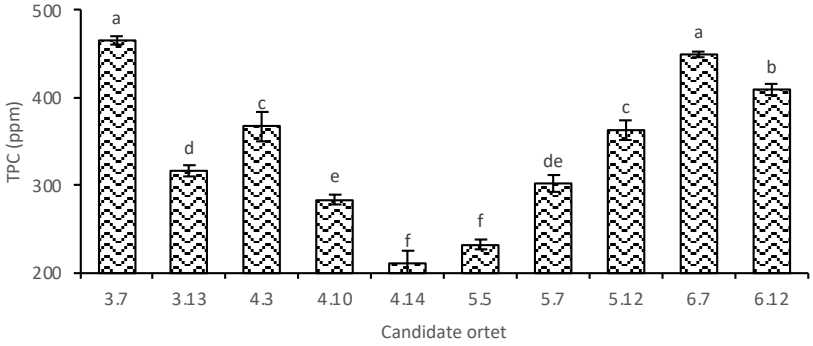


Figure 5. Total phenolic content (TPC) of oil palm leaves. Data are presented as mean ± standard error (SE) (n = 3). The ANOVA test yielded a p-value < 0.05. Different letters indicate a significant difference.

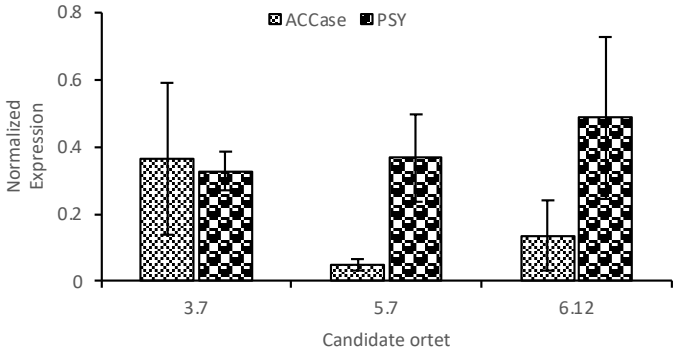


Figure 6. Expression of *ACCase* and *PSY* genes in oil palm fruit

Together, these findings describe a concurrent relationship between the expression of these specific biosynthetic genes and the accumulation of their end products in these selected ortets. The alignment of high *ACCase* expression with high oil yield in candidate ortet 3.7, and high *PSY* expression with high β-carotene in candidate 6.12, provides a physiological context for the observed phenotypic variation and underscores the value of an integrated approach for characterizing ortet potential. However, given the limited sample size of this initial investigation, the identified candidates are promising preliminary rather than definitive elite selections. A more comprehensive study on a larger population is currently ongoing to further investigate these relationships and identify robust selection markers.

## Conclusion

This study establishes a foundational framework for selecting elite oil palm ortets by integrating phenotypic screening with molecular profiling. Within our tested population, candidate ortets 3.7, 5.7, and 6.12 emerged as superior candidates. The mechanistic consistency between *ACCase* expression and oil yield in candidate 3.7, and between *PSY* expression and β-carotene content in candidate 6.12, underscores the utility of this approach. While these results are promising, they represent a critical first step; future work must expand to a larger population and translate this proof-of-concept into validated, robust selection criteria for tissue culture applications.

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## References

Andrés, C. M. C., Pérez de la Lastra, J. M., Juan, C. A., Plou, F. J., & Pérez-Lebeña, E. (2023). Polyphenols as antioxidant/pro-oxidant compounds and donors of reducing species: Relationship with human antioxidant metabolism. *Processes*, *11*(9), 2771. https://doi.org/10.3390/pr11092771.

Anulika, N. P., Ignatius, E. O., Raymond, E. S., Osasere, O.-I., & Abiola, A. H. (2016). The chemistry of natural product: Plant secondary metabolites. *International Journal of Technology Enhancements and Emerging Engineering Research*, *4*(8), 1–8.

Azahra, P. S., & Ernayunita. (2023). Upaya meminimalkan abnormalitas pada klon kelapa Sawit. *WARTA PPKS*, *28*(1), 55–62. https://doi.org/10.22302/iopri.war.warta.v28i1.102

Baharuddin, B., Isnaini, M., & Solihin, M. D. (2025). Improving the productivity of Cavendish bananas through tissue culture technology for the welfare of farmers. *Jurnal IPTEK Bagi Masyarakat, 5*(2), 264–276. https://doi.org/10.55537/j-ibm.v5i2.1389

Bioline Reagents Ltd. (n.d.). MyTaq™ HS Red Mix. https://www.bioline.com/mytaq-hs-red-mix.html.

Bioline Reagents Ltd. (n.d.). SensiFAST™ SYBR® Hi-ROX kit. https://www.bioline.com/sensifast-sybr-hi-rox-kit.html.

Djs, A. J., Febrianto, E. B., & Sinambela, B. M. T. (2020). Analisa jumlah klorofil daun terhadap produksi buah kelapa sawit (*Elaeis guineensis* Jacq.) pada dataran tinggi di Kebun Bah Birung Ulu PTPN IV Sumatera Utara. *Jurnal Agro Estate*, *4*(1), 51–58.

Ekwenye, U. (2010). Chemical characteristics of palm oil biodeterioration. *Biokemistri*, *18*(2), 141–149. https://doi.org/10.4314/biokem.v18i2.56415

FAOSTAT. (2023). Agricultural production statistics 2000–2021. In *FAOSTAT Analytical Brief 79*. Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/cc3751en

Fokou, E., Achu, M. B., Kansci, G., Ponka, R., Fotso, M., Tchiégang, C., & Tchouanguep, F. M. (2009). Chemical properties of some *Cucurbitaceae* oils from Cameroon. *Pakistan Journal of Nutrition*, *8*(9), 1325–1334. https://doi.org/10.3923/pjn.2009.1325.1334

Hambali, H., & Muzdalifah, N. (2020). Processing, characteristics, and potential application of red palm oil in food and non-food products. *Indonesian Journal of Oil Palm Research*, *3*(2), 1–11. https://doi.org/10.35876/ijop.v3i2.47

Hanin, N. N. F., & Pratiwi, R. (2017). Kandungan fenolik, flavonoid dan aktivitas antioksidan ekstrak daun paku laut (*Acrostichum aureum* L.) fertil dan steril di Kawasan Mangrove Kulon Progo, Yogyakarta. *Journal of Tropical Biodiversity and Biotechnology*, *2*, 51–56.

https://doi.org/10.22146/jtbb.29819

Hasan, H., & Manan, F. A. (2020). Total phenolic and flavonoid content of *Elaeis guineensis* leaves treated with different amount of nitrogen-potassium fertilizer. *International Journal of Life Sciences and Biotechnology*, *3*(2), 156–163. https://doi.org/10.38001/ijlsb.699527

Hasibuan, H. A. (2020). Penentuan rendemen, mutu dan komposisi kimia minyak sawit dan minyak inti sawit tandan buah segar bervariasi kematangan sebagai dasar untuk penetapan standar kematangan panen. *Jurnal Penelitian Kelapa Sawit*, *28*(3), 123–132. https://doi.org/10.22302/iopri.jur.jpks.v28i3.106

Holidi, Safriyani, E., Warjiyanto, & Sutejo. (2016). Pertumbuhan bibit kelapa sawit pada tanah gambut berbagai ketinggian genangan. *Ilmu Pertanian*, *18*(3), 135–140. https://doi.org/10.22146/ipas.5433

Hudori, M. (2016). Perencanaan kebutuhan kendaraan angkutan tandan buah segar di perkebunan kelapa sawit. *Industrial Engineering Journal*, *5*(1), 23–28. https://doi.org/10.53912/iejm.v5i1.147

Igile, G. O., Iwara, A. I., Ekpe, O. O., Essien, N. M., & Egbung, G. E. (2023). Quality evaluation of low free fatty acid and high free fatty acid crude palm oil and variation of total fatty matter and fatty acid composition in Nigerian palm oil. *African Journal of Biotechnology*, *22*(10), 247–256. https://doi.org/10.5897/ajb2020.17181

Japir, A. A. W., Salimon, J., Derawi, D., Bahadi, M., Al-Shuja'A, S., & Yusop, M. R. (2017). Physicochemical characteristics of high free fatty acid crude palm oil. *OCL*, *24*(5), D506. https://doi.org/10.1051/ocl/2017033

Kozera, B., & Rapacz, M. (2013). Reference genes in real-time PCR. *Journal of Applied Genetics*, *54*(4), 391–406. https://doi.org/10.1007/s13353-013-0173-x

Kuljarusnont, S., Iwakami, S., Iwashina, T., & Tungmunnithum, D. (2024). Flavonoids and other phenolic compounds for physiological roles, plant species delimitation, and medical benefits: A promising view. *Molecules*, *29*(22), 1–15. https://doi.org/10.3390/molecules29225351

Kushairi, A., Tarmizi, A., Ong-Abdullah, M., Kamal, R. S., Zamzuri, I., Ooi, S. E., & Rajanaidu, N. (2010). Production, performance and advances in oil palm tissue culture. *International Seminar on Advances in Oil Palm Tissue Culture*, 1–23.

Leng, L. Y., Nadzrin, Shaari, A. R., Norawanis, A. R., & Khor, C. Y. (2017). Antioxidant capacity and total phenolic content of fresh, oven-dried and stir-fried tamarind leaves. *Current Research in Nutrition and Food Science*, *5*(3), 282–287. https://doi.org/10.12944/CRNFSJ.5.3.13

Mahardani, O. T., & Yuanita, L. (2021). Efek metode pengolahan dan penyimpanan terhadap kadar senyawa fenolik dan aktivitas antioksidan. *Unesa Journal of Chemistry*, *10*(1), 64–78. https://doi.org/10.26740/ujc.v10n1.p64-78

Mahesar, S. A., Sherazi, S. T. H., Khaskheli, A. R., Kandhro, A. A., & Sirajuddin. (2016). Analytical approaches for free fatty acids assessment in oils and fats. *Analytical Methods*, *6*(14), 4956–4963. https://doi.org/10.1039/C4AY00344F

Manoh, H. P., Yanti, Y., & Toruan-Mathius, N. (2021). Growth and development of oil palm shoots under different light qualities. *Biotropia*, *28*(1), 21–28. https://doi.org/10.11598/btb.2021.28.1.903

Milke, L., & Marienhagen, J. (2020). Engineering intracellular malonyl-CoA availability in microbial hosts and its impact on polyketide and fatty acid synthesis. *Applied Microbiology and Biotechnology*, *104*(14), 6057–6065. https://doi.org/10.1007/s00253-020-10643-7

Mulero, G., Bacher, H., Kleiner, U., Peleg, Z., & Herrmann, I. (2022). Spectral estimation of in vivo wheat chlorophyll a/b ratio under contrasting water availabilities. *Remote Sensing*, *14*(11), 2585. https://doi.org/10.3390/rs14112585

Mustafa, N., Ya'acob, N., Latif, Z. A., & Yusof, A. L. (2015). Quantification of oil palm tree leaf pigment (chlorophyll a) concentration based on their age. *Jurnal Teknologi*, *75*(11), 129–134. https://doi.org/10.11113/jt.v75.5341

Mustafa, R. (2022). Pengaruh harga CPO (*crude palm oil*) di global market terhadap harga minyak goreng di pasar domestik. *SIBATIK JOURNAL*, *1*(8), 1565–1574. https://doi.org/10.54443/sibatik.v1i8.209.

Pratiwi, D. R., Wening, S., Supena, N., Setiowati, R. D., & Yenni, Y. (2020). Kultur jaringan kelapa sawit: tantangan dan peluangnya. *Warta PPKS*, *25*(1), 1–10.

Priyatama, D. B., Priyambada, & Supriyanto, G. (2023). *Analisis rendemen minyak kelapa sawit (CPO) berdasarkan tingkat kematangan buah di PT. Bumitama Gunajaya Agro (Karya Bakti Agro Sejahtera)*. Jurnal Online Mahasiswa (JOM), 1(3). https://jurnal.instiperjogja.ac.id/

index.php/JOM/article/view/807/604

Safa, P., Ismail, P., Noor, S., & Manan, N. A. (2024). Inhibitory effects of oil palm leaf extract on osteoclastogenesis in RAW 246.7 macrophages. *JOBIMB*, *12*(2), 24–32. https://doi.org/10.54987/jobimb.v12i2.1014

Sari, A. E., & Mayasari, E. (2018). Analisa beta karoten pada sayuran lokal di Indonesia. *Jurnal Mitra Kesehatan*, *1*(1), 16–19. https://doi.org/10.47522/jmk.v1i1.5

Sarip, M. S. M., Morad, N. A., Nawi, M. A. H. M., Aziz, M. K. T. A., & Jaapar, S. Z. imah S. (2023). B-carotene enrichment in crude palm oil using subcritical water extraction and its relationship with the solubility. *Food Chemistry Advances*, *2*, 1–8. https://doi.org/10.1016/j.focha.2023.100245

Shen, J., Wu, W., Wang, K., Wu, J., Liu, B., Li, C., Gong, Z., Hong, X., & Fang, H. (2024). Chloroflexus aurantiacus acetyl-CoA carboxylase evolves fused biotin carboxylase and biotin carboxyl carrier protein to complete carboxylation activity. *Molecular and Cellular Biology*, *15*, e03414-23. https://doi.org/10.1128/mbio.03414-23

Shidiq, M., Lestari, W., & Saragih, S. H. Y. (2022). Crude palm oil (CPO) quality analyze of *Elaeis guineensis* at palm oil mill PT. Sinar Pandawa, Labuhanbatu Regency (Based on free fatty acid levels, water content, and impurities). *Jurnal Pembelajaran Dan Biologi Nukleus*, *8*(2), 386–398. https://doi.org/10.36987/jpbn.v8i2.2705

Silalahi, M. (2022). Penggunaan spektrofotometri untuk pengujian kadar beta karoten CPO (*crude palm oil*) di laboratorium kimia analisa. *Jurnal Ilmiah Teknik Industri Prima*, *5*(2), 26–30.

Singh, C., Chauhan, N., Gupta, S., Rani, A., & Tomar, S. (2019). Analysis of chlorophyll content in *Adiantum capillus-veneris* L. growing in different habitats in Doon Valley and nearby areas. *International Journal of Botany Studies*, *4*(1), 116–118.

Sonobe, R., Yamashita, H., Mihara, H., Morita, A., & Ikka, T. (2020). Estimation of leaf chlorophyll a, b and carotenoid contents and their ratios using hyperspectral reflectance. *Remote Sensing*, *12*(19), 1–19. https://doi.org/10.3390/rs12193265

Tai, H. P., & Brunner, G. (2019). Extraction of oil and minor compounds from oil palm fruit with supercritical carbon dioxide. *Processes*, *7*(2), 1–10. https://doi.org/10.3390/pr7020107

Thermo Fisher Scientific. (2025). GeneJET RNA purification kit user guide. Thermo Fisher Scientific. https://documents.thermofisher.com/TFS-Asset/LSG/manuals/MAN0012664_GeneJET_RNA_Purification_UG.pdf

Toyobo Co., Ltd. (n.d.). ReverTra Ace™ qPCR RT kit & master mix. https://www.toyobo-global.com/products/lifescience/products/cdna_001/index.html

Tranbarger, T. J., Dussert, S., Joët, T., Argout, X., Summo, M., Champion, A., Cros, D., Omore, A., Nouy, B., & Morcillo, F. (2011). Regulatory mechanisms underlying oil palm fruit mesocarp development and ripening. *Plant Physiology, 156*(2), 564–584. https://doi.org/10.1104/pp.111.175141.

Wan Sulaiman, W. N. S., Abd Rasid, O., & Ghulam Kadir, A. P. (2012). *Phytoene synthase gene from oil palm (Elaeis oleifera) for modification of carotenoid content* (MPOB Information Series No. 513). Malaysian Palm Oil Board.

Wu, X., & Huang, T. (2020). Recent development in acetyl-CoA carboxylase inhibitors and their potential as novel drugs. *Future Medicinal Chemistry*, *12*(6), 533–561. https://doi.org/10.4155/fmc-2019-0312

Zhang, Y., Li, Y., Wang, R., Xu, L., Li, M., Liu, Z., Wu, Z., Zhang, J., Yu, G., & He, N. (2020). Spatial variation of leaf chlorophyll in northern hemisphere grasslands. *Frontiers in Plant Science*, *11*, 1244. https://doi.org/10.3389/fpls.2020.01244

Zhou, X., Rao, S., Wrightstone, E., Sun, T., Lui, A. C. W., Welsch, R., & Li, L. (2022). Phytoene synthase: The key rate-limiting enzyme of carotenoid biosynthesis in plants. *Frontiers in Plant Science*, *13*(April), 884720. https://doi.org/10.3389/fpls.2022.884720