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A Metabolomics Approach to Identify Fertilizer-Efficient Oil Palm (Elaeis guineensis) Genotypes for Marginal Land Cultivation

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Abstract

The cultivation of oil palm on marginal lands is constrained by poor soil fertility, leading to a heavy reliance on costly fertilizers. Developing genotypes with high nutrient use efficiency is therefore critical for sustainable agriculture. This study employed a metabolomics approach to evaluate the performance of 10 oil palm genotypes under fertilized and non-fertilized conditions. Through analysis of biomass allocation and metabolite profiles, we identified genotype BGA103 as exceptionally resilient. Under nutrient-deficient conditions, BGA103 maintained a high shoot-to-root ratio and exhibited a stable metabolite profile, indicating efficient nutrient utilization. Genotypes BGA102, BGA107, and BGA109 also showed adaptive potential, though to a lesser extent. Key metabolic pathways, including steroid and fatty acid biosynthesis, were significantly influenced by fertilization, while biomarkers like Tocopherol and Neophytadiene were identified as robust indicators of nutrient status. These findings provide a strong biochemical basis for selecting and breeding elite oil palm varieties tailored for low-input agriculture on marginal lands, paving the way for more cost-effective and environmentally friendly production systems

INTRODUCTION

As the world's largest palm oil producer, Indonesia's economy heavily relies on the oil palm (Elaeis guineensis Jacq.) industry, which supplies global food, cosmetic, and bioenergy markets (MPOB, 2020). To meet rising global demand and maintain economic competitiveness, enhancing plantation productivity and efficiency is a national priority. However, this ambition faces a significant obstacle: the increasing use of marginal lands for cultivation. These lands, characterized by low soil fertility, demand intensive fertilization to achieve viable yields, creating substantial economic and environmental pressures. With fertilizer costs accounting for up to 60% of total operational expenses, strategies to reduce this dependency are urgently needed for the industry's long-term sustainability (Khatiwada et al., 2018; Zuhdi et al., 2021).

In response to various challenges faced in the field, several innovations and technologies have been introduced in the palm oil industry (Murphy et al., 2021). The primary aim is to increase productivity and maximize the utilization of available resources. However, beyond merely increasing production yields, sustainability efforts are also a major focus. This is reflected in the implemented policies, which aim not only to reduce negative environmental impacts but also to enhance social welfare in the vicinity of oil palm plantations (Tscharntke et al., 2012; Abubakar et al., 2023).

One of the main challenges faced by oil palm plantation companies in Indonesia is the management of marginal lands (Fairhurst and Griffiths, 2014). Marginal lands, often characterized by low or poor soil fertility, pose a significant constraint to efforts to increase productivity and efficiency in plantations (Ahmadzai H et al., 2023). Suboptimal soil characteristics on marginal lands, such as limited nutrient availability and poor soil structure, hinder the growth of oil palm plants. These plants require optimal soil conditions for healthy growth, including sufficient nutrient availability and adequate soil structure for optimal root growth (Paramananthan, 2013). In practice, managing marginal lands requires extra efforts,

especially in terms of intensive fertilizer application to achieve optimal growth of oil palm plants (Hidayat et al., 2023). Intensive fertilization is necessary to compensate for the low nutrient availability in marginal soils and ensure that plants receive the necessary nutrients for optimal growth (Fairhurst and Griffiths, 2014).

The high cost of fertilization in the Indonesian oil palm industry is a significant economic aspect that demands serious attention. Fertilization, as a crucial stage in crop management, not only requires significant financial investment but also affects the overall profitability of companies. According to recent research, the cost of fertilization can account for 30–60% of the total operational costs of oil palm plantation companies (Saleh et al., 2018; USDA, 2021). This significant cost percentage indicates that fertilization has substantial economic impacts and significantly drains the financial resources of companies (Goh et al., 2016). Therefore, fertilizer strategies tailored to the specific needs of plants and soil conditions become essential in optimizing operational cost efficiency.

In efforts to address the challenge of high fertilization costs, companies in the oil palm plantation sector have begun directing their efforts towards developing oil palm varieties resistant to low nutrient conditions. These varieties, known as "stay green," stand out for their ability to continue growing and producing well even under limited nutrient conditions. The concept of "stay green" refers to plants that maintain green leaves and actively perform photosynthesis for as long as possible, even under nutrient stress conditions (Kamal et al., 2019). Research has shown that "stay green" genotypes have better adaptations to environments with low nutrient resources (Jaegglia et al., 2017; Zhang et al., 2019). These plants tend to be more efficient in utilizing available nutrients and have internal mechanisms to cope with nutrient deficiencies. The "stay green" concept reflects the physiological and genetic adaptations of plants to unfavorable environmental conditions. The mechanisms involved in this adaptation include hormonal regulation, carbohydrate metabolism, and plant stress responses. Thus, the use of "stay green" varieties can reduce dependence on intensive fertilization, which in turn can lower overall production costs (Christopher et al., 2016; Antonietta et al., 2016; Riache et al., 2023).

To enhance the sustainability of the Indonesian oil palm industry, reducing the significant financial burden of fertilization is critical. This research focuses on identifying nutrient-efficient "stay-green" oil palm varieties as a potential solution. We evaluated 10 genotypes under both fertilized and non-fertilized conditions using a metabolomics approach. This approach allows for a holistic characterization of metabolic compounds and pathways involved in plant adaptation to low-nutrient stress (Kumar et al., 2017; Roychowdhury et al., 2023; Baker et al., 2023). While the "stay-green" concept is promising, its underlying biochemical mechanisms in oil palm remain poorly understood. Our study's novelty is the application of non-targeted metabolomics to dissect this metabolic reprogramming. This moves beyond traditional methods by providing a biochemical fingerprint of resilience, which can significantly accelerate the development of elite, cost-effective oil palm cultivars

MATERIAL AND METHODS

Plant Material and Growth Conditions

This study was conducted at the main nursery of Bumitama Gunajaya Agro in the Pundu Region, Central Kalimantan, Indonesia (1.9954°S, 113.0607°E). The 12 genotypes used in this study (BGA100-BGA111) are part of Bumitama Gunajaya Agro's proprietary collection, derived from selected crosses of Dura x Pisifera populations known for their varying responses to environmental conditions. The seedlings were individually planted in polybags and maintained under standard nursery conditions for 36 weeks before evaluation. All plant materials originated from cultivated sources, and no wild specimens were collected. Since the plant materials are part of the company’s proprietary breeding program, no additional permits were required.

Experimental Design and Treatments

The experiment was arranged in a nested design with three replications. The main treatment factor consisted of two fertilization levels: fertilized and non-fertilized. The nested factor within each fertilization level was the 12 oil palm genotypes. Each experimental unit (genotype within a treatment level) consisted of 10 plants, ensuring robust data collection. The fertilization treatment was applied according to standard nursery practices, which included the application of NPK 12-12-12 fertilizer at a rate of 50 grams per plant and Kieserite at 25 grams per plant. The non-fertilized group received no nutrient supplementation throughout the experimental period.

Biomass Measurement and Sample Collection

After 36 weeks of treatment, plant samples were harvested for analysis. The plants were carefully separated into roots and shoots. To determine the dry weight (DW), the root and shoot samples were dried in an oven at 60°C for 36 hours until a constant weight was achieved. The shoot-to-root dry weight ratio was subsequently calculated. For metabolomic analysis, 10 grams of fully expanded leaves were collected from each treatment unit.

Metabolite Extraction and GC-MS Analysis

Metabolite extraction followed the protocol described by Halim et al. (2019) and Adrian et al. (2024). The collected leaf samples were oven-dried and finely ground. The ground powder was then macerated in absolute methanol for five days to facilitate compound extraction. To improve extraction efficiency, the mixture was subjected to ultrasonication for 60 minutes at 60°C. The resulting crude extracts were filtered and prepared for analysis. Profiling was performed using a Gas Chromatography-Mass Spectrometry (GC-MS) system (Agilent Technologies 7890A/G3440A 5975C). The separation was achieved using an HP-5ms non-polar capillary column (30 m × 0.25 mm, 0.25 µm film thickness), which is suitable for analyzing a wide range of semi-volatile compounds

Data Processing and Statistical Analysis

The raw data from the GC-MS analysis was validated and cleaned to ensure accuracy. Compound identification was performed by cross-referencing the mass spectra with major chemical databases, including ChEBI, PubChem, and ChemSpider. Biomass data were analyzed using Duncan's Multiple Range Test ($\alpha = 0.05$) to determine significant differences among treatments. The processed metabolomic data were subjected to multivariate statistical analysis, including heatmap clustering. To evaluate the discriminatory power of potential biomarkers, a Receiver Operating Characteristic (ROC) curve analysis was conducted using MetaboAnalyst 5.0.

RESULTS AND DISCUSSION

Biomass Allocation: Root and Shoot Dry Weight Analysis

The allocation of biomass between roots and shoots offers crucial insights into a plant's overall health and resource utilization strategy. As illustrated in the boxplot analysis in Fig. 1, fertilization consistently increased both root dry weight (DW Roots) and shoot dry weight (DW Shoots). This response is attributed to the enhanced availability of essential nutrients like nitrogen, phosphorus, and potassium, which are critical for robust root development and, consequently, more effective water and nutrient uptake (Marschner 2012; Fageria and Moreira 2011; De la Peña et al., 2023, 2024). The improved nutrient status, in turn, supports greater photosynthetic activity and biomass production in the shoots (Taiz and Zeiger, 2020). The lack of these nutrients, conversely, limits plant growth and resilience to environmental stress (Mareri et al., 2022).

Notably, the analysis revealed significant genotypic variation in response to fertilization. Several genotypes, including BGA102, BGA107, BGA104, and BGA109, maintained stable root dry weights under both fertilized and unfertilized conditions, suggesting they are promising candidates for cultivation in minimal-input systems. For shoot dry weight, however, only genotype BGA111 exhibited similar stability across treatments.

The shoot-to-root dry weight ratio (DW_SR), a key indicator of resource allocation efficiency, provided further distinction among genotypes. According to Duncan's Multiple Range Test shown in Fig. 1, genotype BGA104 displayed the highest DW_SR (approximately 5.41) under fertilization, highlighting its excellent responsiveness to nutrient inputs. However, for identifying genotypes adapted to nutrient-limited conditions, performance without fertilization is paramount. In this context, genotype BGA103 emerged as a strong performer, achieving a high DW_SR of approximately 2.75 even without fertilizer.

The superior performance of BGA103 under minimal fertilization is highly significant, indicating its potential adaptation to nutrient-poor soils and a high degree of nutrient utilization efficiency (Marschner 2012; dos Santos et al., 2020). Plants tolerant to low-nutrient stress often possess more effective root systems for nutrient acquisition and advanced physiological or biochemical mechanisms that sustain

productivity through efficient photosynthesis and internal resource allocation (Li et al., 2016; Ali et al., 2018; Iqbal et al., 2019, 2023; Lai et al., 2024). The strong performance of BGA103 suggests it possesses these advantageous traits, making it a valuable genetic resource for developing resilient and efficient cultivars.

Fertilization Alters the Distribution and Chemical Composition of Metabolites

A comparative analysis of metabolite profiles reveals the profound impact of nutrient availability on the metabolic strategy of oil palm. The investigation highlights distinct patterns in both the distribution and chemical nature of compounds produced under fertilized and unfertilized conditions.

An examination of the metabolite distribution using Venn diagrams, as illustrated in Fig. 2, shows three distinct groups. First, a strikingly high proportion of metabolites, ranging from 44.00–61.76%, was detected exclusively under unfertilized conditions. This suggests that nutrient deficiency triggers a significant metabolic reprogramming, prompting the production of specific compounds that likely play a crucial role in adaptation and survival under stress (Pandey et al., 2021; Kouame et al., 2024). Second, a smaller but distinct set of metabolites (10.52–32.35%) was unique to fertilized conditions, indicating that adequate nutrition induces specific pathways that contribute to optimal growth and development (Reshi et al., 2023). Finally, a core group of overlapping metabolites (17.64–33.33%) was present in both treatments, representing essential "housekeeping" pathways that remain consistently active regardless of nutrient status (Cadena-Zamudio et al., 2023).

Further analysis of the chemical composition, shown in Fig. 3, clarifies these metabolic priorities. Under fertilized conditions, the categories of "Lipids and lipid-like molecules" and "Organonitrogen compounds" were more dominant. This reflects a metabolic shift towards producing molecules essential for cellular functions and energy storage, and it directly corresponds to higher nitrogen availability for the biosynthesis of crucial building blocks like amino acids, proteins, and nucleotides (Zayed et al., 2023). Additionally, distinct differences in the profiles of "Benzenoids" and "Organic acids and derivatives" were observed. These shifts underscore the breadth of metabolic adjustments, influencing pathways related to plant defense, pollinator attraction, and primary energy metabolism (Picazo-Aragonés et al., 2020). Collectively, the changes in compound composition demonstrate that fertilization enhances the synthesis of a diverse array of bioactive molecules that support overall plant health and productivity.

Clustering of Secondary Metabolite Variations Among Different Oil Palm Genotypes Under Fertilization Treatments

Hierarchical clustering analysis, visualized as a heatmap in Fig. 4, reveals significant genotype-by-fertilization interactions that shape the secondary metabolome of oil palm. The analysis clearly segregates the genotypes into two distinct groups based on their metabolic response to nutrient

availability. The first cluster contains genotypes BGA106, BGA107, and BGA108. A key feature of this group is that for each genotype, the fertilized and unfertilized samples cluster closely together. This indicates that their secondary metabolite profiles are remarkably stable and not significantly impacted by the fertilization treatment. This finding highlights the critical role of genetic background in mediating plant responses, aligning with previous research showing that responses to fertilization are strongly dependent on the specific genotype (Hirel et al., 2007; Francis et al., 2023).

In contrast, the second, larger cluster, which includes prominent genotypes like BGA103 and BGA102, demonstrates a clear and consistent separation between samples from fertilized and unfertilized conditions. This shows that these genotypes exhibit a high degree of metabolic plasticity, where fertilization acts as a potent environmental cue that significantly affects their secondary metabolite production. Furthermore, the column dendrogram, which clusters individual metabolites, reveals that specific groups of compounds are co-expressed. The coordinated accumulation of these metabolite clusters suggests they are likely governed by common biosynthetic or regulatory pathways. Understanding this variability is critical, as it underscores the need to move beyond generalized recommendations and toward developing efficient, genotype-specific fertilization strategies to optimize plant health and productivity.

Analyzing Discriminatory Biomarkers: ROC Curve Analysis

To identify metabolites that can effectively discriminate between fertilized and unfertilized conditions, a Receiver Operating Characteristic (ROC) curve analysis was performed, with the results for two key compounds presented in Fig. 5. This analysis assesses the diagnostic accuracy of each potential biomarker by quantifying its Area Under the Curve (AUC).

Tocopherol emerged as an outstanding biomarker with an exceptionally high AUC of **0.992** (95% Confidence Interval: 0.973–1.000), indicating a near-perfect ability to distinguish between the two treatment groups. A specific coordinate on the curve demonstrates that 90% sensitivity (True Positive Rate) can be achieved at a very low 7.7% False Positive Rate (1-Specificity). The accompanying box plot visually confirms this separation, illustrating significantly higher and more consistent levels of Tocopherol in fertilized samples. This finding is biologically consistent, as nutrient availability is known to significantly influence Tocopherol accumulation, which in turn enhances the plant's tolerance to stress (Gosh et al., 2020).

Neophytadiene also proved to be a strong biomarker, yielding an AUC of **0.924** (95% CI: 0.845–0.970), which demonstrates good discriminatory power. However, its performance was slightly lower than Tocopherol's, achieving 90% sensitivity with a higher False Positive Rate of 23.0%. The corresponding box plot showed a similar trend of significantly elevated levels in fertilized plants. This result aligns with findings that fertilization regimes can alter secondary metabolite profiles, including compounds like Neophytadiene that play a role in plant defense mechanisms (Adeosun et al., 2017). Both compounds serve as reliable indicators, but Tocopherol's superior accuracy makes it a premier candidate for assessing plant nutritional status.

Fertilization Reprograms Metabolic Pathways in Oil Palm

Metabolic pathway analysis provides a systemic view of how fertilization fundamentally reprograms the cellular processes in oil palm. The analysis, visualized in Fig. 6, reveals a clear functional trade-off, where the plant shifts its metabolic resources from a "defense and survival" mode under nutrient deficiency to a "growth and production" mode when nutrients are abundant.

Under fertilized conditions, a significant enhancement was observed in biosynthetic pathways crucial for growth and oil production. The most prominently up-regulated pathways included 'Steroid biosynthesis,' 'Unsaturated fatty acid biosynthesis,' and 'Fatty acid biosynthesis.' The activation of these pathways indicates that providing adequate nutrition directly fuels the core metabolic machinery responsible for generating lipids for new cell membranes, signaling molecules, and the precursors for oil, which is the primary economic product (Alzain et al., 2023).

In contrast, under the stress of unfertilized conditions, the plant's metabolic priorities pivoted towards resilience and defense. This was evidenced by the significant up-regulation of 'Monobactam biosynthesis,' a pathway known for producing antimicrobial compounds, suggesting a heightened state of defense readiness. Additionally, adjustments in pathways like amino acid and sulfur metabolism were noted, likely reflecting a strategy to conserve and recycle scarce resources for essential functions.

Despite this defensive posture, the analysis showed that certain core pathways, such as 'Fatty acid biosynthesis,' remained active even in the absence of fertilization. This demonstrates the plant's inherent resilience and its capacity to sustain vital functions even in suboptimal environments, a cornerstone of plant survival strategies (Marschner, 2012). Understanding this metabolic reprogramming is critical for developing advanced fertilization strategies that not only maximize yield but also support the plant's natural defense systems, leading to healthier and more productive plantations.

Limitations of the Study

This study has several limitations inherent to its **metabolomics-focused design**. First, as our primary goal was to characterize the metabolic signatures of nutrient efficiency, the physiological assessment relied mainly on biomass allocation. We acknowledge that future studies would be significantly strengthened by integrating our metabolic findings with a broader range of vegetative parameters (e.g., plant height, LAI) to establish a more direct link between specific metabolites and whole-plant agronomic performance. Second, this study did not include soil or tissue nutrient analysis. While our metabolomic data strongly indicates differential responses, incorporating direct nutrient measurements in future work would provide a definitive mechanistic link between nutrient uptake and the observed metabolic shifts.

CONCLUSION

This study identifies the oil palm genotype BGA103 as a superior candidate for cultivation in nutrient-limited environments. This genotype exhibits stay-green characteristics, a high shoot-to-root ratio, and

the ability to consistently maintain biomass productivity without fertilizer application. This physiological resilience is underpinned by a distinct metabolic strategy, characterized by the activation of unique defense-related pathways under nutrient stress. While other genotypes such as BGA102, BGA107, and BGA109 also showed adaptive potential, BGA103 consistently demonstrated superior nutrient utilization efficiency. Furthermore, this research validates Tocopherol (AUC = 0.992) as a near-perfect biomarker for assessing the plant's nutritional status. Collectively, these findings provide both a valuable genetic asset (BGA103) for future breeding programs and a robust diagnostic tool (Tocopherol) to accelerate the development of more sustainable and fertilizer-efficient oil palm varieties

Abbreviations

AUC Area Under the Curve

DW Dry Weight

DW_SR Shoot-to-Root Dry Weight Ratio

GC-MS Gas Chromatography-Mass Spectrometry

NPK Nitrogen, Phosphorus, and Potassium

ROC Receiver Operating Characteristic

Declarations

Ethics approval and consent to participate

The collection and use of oil palm (Elaeis guineensis) materials complied with national and institutional guidelines. All plant materials originated from cultivated sources and were obtained from Bumitama Gunajaya Agro’s proprietary breeding program in Central Kalimantan, Indonesia (coordinates: 1.9954°S, 113.0607°E). No wild specimens were collected, and no specific permits were required as the materials are part of the company’s proprietary collection.

Clinical Trial

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

A.A. designed the study, wrote the manuscript. **M.A.** analyzed the data and wrote the manuscript. **B.N., R.W., S.D.T.** provided technical assistance. All authors read and approved the final manuscript

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References

1. Abubakar, A., Kasim, S., Ishak, M.Y. (2023). Maximizing oil palm yield: Innovative replanting strategies for sustainable productivity. *Journal of Environmental & Earth Sciences*, 5(2), 61-75. doi: 10.30564/jees.v5i2.5904
2. Adrian, M., Wulandari, R., Sembiring, E., Natawijaya, A. (2024). Metabolomic profiling unravels divergent adaptive responses of oil palm (*Elaeis guineensis*) seedlings to drought stress under shaded and unshaded conditions. *Biodiversitas*. 25(6), 2404-2414.
3. Adeosun, T. E., Ogunwande, I. A., Avoseh, O. N., Raji, I. P., Lawal, O. A. (2017). Composition and anti-inflammatory activity of essential oil of Jatropha curcas. *Natural Product Communications*. 12, 439-440.
4. Ahmadzai, H., Tutundjian, S., Dale, D., Brathwaite, R., Lidder, P., Selvaraju, R., Malhotra, A., Boerger, V., Elouafi, I. (2022). *Marginal lands: potential for agricultural development, food security and poverty reduction*. Rome: FAO. doi: 10.4060/cc2838en.
5. Ali, J., Jewel, Z. A., Mahender, A., Anandan, A., Hernandez, J., Li, Z. (2018). Molecular genetics and breeding for nutrient use efficiency in rice. *International Journal of Molecular Sciences*. 19(6), 1762. doi: 10.3390/ijms19061762.
6. Alzain, M. N., Loutfy, N., Aboelkassem, A. (2023). Effects of different kinds of fertilizers on the vegetative growth, antioxidative defense system and mineral properties of sunflower plants. *Sustainability*. 15(13), 10072. doi: 10.3390/su151310072.

7. Antonietta, M., Acciaresi, H. A., Guiamet, J. J. (2016). Responses to N deficiency in stay green and non-stay green Argentinean hybrids of maize*. Journal of Agronomy and Crop Science*. 202, 231-242. doi: 10.1111/jac.12136.
8. Baker, C. R., Patel-Tupper, D., Cole, B. J., Ching, L. G., Dautermann, O., Kelikian, A. C., Allison, C., Pedraza, J., Sievert, J., Bilbao, A., Lee, J. Y., Kim, Y. M., Kyle, J. E., Bloodsworth, K. J., Paurus, V., Hixson, K. K., Hutmacher, R., Dahlberg, J., Lemaux, P. G., Niyogi, K. K. (2023). Metabolomic, photoprotective, and photosynthetic acclimatory responses to post-flowering drought in sorghum. *Plant Direct*. 7(11), e545. doi: 10.1002/pld3.545.
9. Bolton, E.E., Wang, Y., Thiessen, P.A., Bryant, S.H. 2008. Integrated platform of small molecules and niological activities. *Ann Rep Comput Chem4*: 217-241.doi: 10.1016/S1574-1400(08)00012-1.
10. Cadena-Zamudio, J. D., Monribot-Villanueva, J. L., Pérez-Torres, C. A., Alatorre-Cobos, F., Guerrero-Analco, J. A., Ibarra-Laclette, E. (2023). Non-targeted metabolomic analysis of Arabidopsis thaliana (L.) heynh: metabolic adaptive responses to stress caused by N starvation. *Metabolites*. 13(9), 1021. doi: 10.3390/metabo13091021.
11. Christopher, J. T., Christopher, M. J., Borrell, A. K., Fletcher, S., Chenu, K. (2016). Stay-green traits to improve wheat adaptation in well-watered and water-limited environments. *Journal ofExperimental Botany*. 67(17), 5159-5172. doi: 10.1093/jxb/erw276.
12. De la Peña, M., Ruiz-Romero, R., Castro-Arza, L. I., Romero, H. M. (2024). Changes in the root architecture of oil palm seedlings in response to nitrogen starvation. *Agronomy*. 14, 409. doi: 10.3390/agronomy14030409.
13. De la Peña, M., Ruiz-Romero, R., Romero, H. M. (2023). Nitrogen use efficiency in oil palm seedlings: unraveling the untapped potential of elevated external ammonium supply. *Plants*. 12, 2819. doi: 10.3390/plants12152819.
14. Degtyarenko, K., de Matos, P., Ennis, M., Hastings, J., Zbinden, M., McNaught, A., Alcántara, R., Darsow, M., Guedj, M., Ashburner, M. 2007. ChEBI: A database and ontology for chemical entities of biological interest. *Nucleic Acids Res.* 36: D344-D350. doi: 10.1093/nar/gkm791.
15. dos Santos, V. R., Costa, L. C., Rocha, A. M., Gomes, C., Santos, M., Rabelo, F. H., Prado, R. D. (2020). Biomass accumulation, extraction and nutrient use efficiency by cover crops. *Research Society and Development*. 9(10), e9969109433. doi: 10.33448/rsd-v9i10.9433.
16. Fageria, N. K., Moreira, A. (2011). The role of mineral nutrition on root growth of crop plants. *Advances in Agronomy*. 110, 251-331. doi: 10.1016/B978-0-12-385531-2.00004-9.
17. Fairhurst, T. H., Griffiths, W. (2014). No tillage, residue management and replanting. In Achieving sustainable cultivation of oil palm. Cambridge, UK: Burleigh Dodds Science Publishing. pp. 1-24.
18. Francis, B., Aravindakumar, C. T., Brewer, P. B., Simon, S. (2023). Plant nutrient stress adaptation: A prospect for fertilizer limited agriculture. *Environmental and Experimental Botany*. 213, 105431. doi: 10.1016/j.envexpbot.2023.105431.
19. Ghosh, U. K., Hossain, M. S., Islam, M. N., Khan, M. A. R. (2022). *Role of tocopherol in conferring abiotic stress tolerance in plants. In Antioxidant Defense in Plants: Molecular Basis of Regulation,*

Singapore, Singapore: Springer Nature. pp. 215-233.

20. Goh, K. J., Hardter, R., Fairhurst, T. H. (2016). Enhancing nutrient use efficiency in oil palm cultivation: Improving fertilizer management through best management practices. *Oil Palm Industry Economic Journal*. 16(2), 1-12.
21. Hidayat, F., Yudhistira, Y., Desika Putri Pane, R., Sapalina, F., Listia, E., Amalia, R., Muhayat, M., Winarna, W. (2023). Biofertilizer application to enhance the oil palm growth and productivity. *Jurnal Penelitian Kelapa Sawit*. 31(2), 96-107. doi: 10.22302/iopri.jur.jpks.v31i2.226.
22. Hirel, B., Le Gouis, J., Ney, B., Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany*. 58(9), 2369-2387. doi: 10.1093/jxb/erm097.
23. Iqbal, A., Gui, H., Zhang, H., Wang, X., Pang, N., Dong, Q., Song, M. (2019). Genotypic variation in cotton genotypes for phosphorus-use efficiency. *Agronomy*. 9, 689. doi: 10.3390/agronomy9110689.
24. Iqbal, A., Qiang, D., Xiangru, W., Huioing, G., Hengheng, Z., Ziling, Z., Meizhen, S. (2023). Phosphorus and carbohydrate metabolism contributes to low phosphorus tolerance in cotton. *BMC Plant Biology*. 23, 97. doi: 10.1186/s12870-023-04100-6.
25. Jaegglia, B. G., Mortlockb, M. Y., Borrell, A. (2017). Bigger is not always better: Reducing leaf area helps stay-green sorghum use soil water more slowly. *Environmental and Experimental Botany*. 138, 119-129. doi: 10.1016/j.envexpbot.2017.03.002.
26. Kamal, N. M., Alnor Gorafi, Y. S., Abdelrahman, M., Abdellatef, E., Tsujimoto, H. (2019). Stay-green trait: a prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. *International Journal of Molecular Sciences*. 20(23), 5837. doi: 10.3390/ijms20235837.
27. Khatiwada, D., Palmén, C., Silveira, S. (2018). Evaluating the palm oil demand in Indonesia: production trends, yields, and emerging issues. *Biofuels.* 12(2), 135-147. doi: 10.1080/17597269.2018.1461520.
28. Kouame, K. P., Agrahari, R. K., Konjengbam, N. S., Koyama, H., Kobayashi, Y. (2024). Ability of nutrient management and molecular physiology advancements to overcome abiotic stress: A study on sub-Saharan African crops. *Agriculture.* 14, 285. doi: 10.3390/agriculture14020285.
29. Kumar, R., Bohra, A., Pandey, A. K., Pandey, M. K., Kumar, A. (2017). Metabolomics for plant improvement: status and prospects. *Frontiers in Plant Science*. 8, 1302. doi: 10.3389/fpls.2017.01302.
30. Lai, Z., Zhang, H., Ding, X., Liao, Z., Zhang, C., Yu, J., Pei, S., Dou, Z., Li, Z., Fan, J. (2024). Ridge-furrow film mulch with nitrogen fertilization improves grain yield of dryland maize by promoting root growth, plant nitrogen uptake and remobilization. *Soil and Tillage Research.* 241, 106118. doi: 10.1016/j.still.2024.106118.
31. Li, X., Zeng, R., Liao, H. (2016). Improving crop nutrient efficiency through root architecture modifications. *Journal of Integrative Plant Biology*. 58, 193-202.

32. Mareri, L., Parrotta, L., Cai, G. (2022). Environmental stress and plants. *International Journal of Molecular Sciences*. 23(10), 5416. doi: 10.3390/ijms23105416.
33. Marschner, H. (2012). *Marschner's Mineral Nutrition of Higher Plants*. California, US: Academic Press, pp. 100-204.
34. Marschner, H. (2012). *Marschner's Mineral Nutrition of Higher Plants*. California, US: Academic Press, pp. 100-204.
35. Malaysian Palm Oil Board (MPOB). (2020). *Malaysian Palm Oil Statistics 2020*. Kuala Lumpur,Malaysia: MPOB.
36. Murphy, D. J., Goggin, K., Paterson, R. R. M. (2021). Oil palm in the 2020s and beyond: challenges and solutions. *CABI Agriculture and Bioscience*. 2, 39. doi: 10.1186/s43170-021-00058-3.
37. Pandey, R., Vengavasi, K., Hawkesford, M. J. (2021). Plant adaptation to nutrient stress. *Plant Physiology Reports*. 26, 583-586. doi: 10.1007/s40502-021-00636-7.
38. Paramananthan, S. (2013). Managing marginal soils for sustainable growth of oil palms in the tropics. *Journal of Oil Palm and the Environment*. 4(1), 1-16. doi: 10.5366/jope.2013.1.
39. Pence, H.E., Williams, A. 2010. ChemSpider: An online chemical informationresource. *J Chem Educ.* 87(11): 1123-1124. DOI: 10.1021/ed100697w
40. Picazo-Aragonés, J., Terrab, A., Balao, F. (2020). Plant volatile organic compounds evolution: Transcriptional regulation, epigenetics and polyploidy. *International Journal of Molecular Sciences*. 21(23), 8956. doi: 10.3390/ijms21238956.
41. Reshi, Z. A., Ahmad, W., Lukatkin, A. S., Javed, S. B. (2023). From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and in vitro approaches. *Metabolites*. 13(8), 895. doi: 10.3390/metabo13080895. PMID: 37623839; PMCID: PMC10456650.
42. Riache, M., Djemel, A., Revilla, P., Malvar, R. A., Mefti, M. (2023). Genetic analyses of stay green for tolerance to water stress and nitrogen deficiency in Algerian Saharan maize populations. *Euphytica*. 219, 66. doi: 10.1007/s10681-023-03193-2.
43. Roychowdhury, R., Das, S. P., Gupta, A., Parihar, P., Chandrasekhar, K., Sarker, U., Kumar, A., Ramrao, D. P., Sudhakar, C. (2023). Multi-omics pipeline and omics-integration approach to decipher plant's abiotic stress tolerance responses. *Genes.* 14(6), 1281. doi: 10.3390/genes14061281.
44. Saleh, S., Bagja, B., Suhawa, T. A., Widyapratami, H. (2018). Intensification by smallholder farmers is key to achieving Indonesia’s palm oil targets. Washington, United States: World Resources Institute. https://www.wri.org/insights/intensificationsmallholder-farmers-key-achieving-indonesias-palm-oil-targets.
45. Taiz, L., Zeiger, E. (2020). *Plant Physiology and Development*. Massachusetts, US: Sinauer Associates, pp. 90-120.
46. United States Department of Agriculture (USDA). (2021). *Indonesia Oil Seeds and Product Annual Report*. https://www.fas.usda.gov/data/indonesiaoilseeds-and-products-annual-5.

47. Zayed, O., Hewedy, O. A., Abdelmoteleb, A., Ali, M., Youssef, M. S., Roumia, A. F., Seymour, D., Yuan, Z. C. (2023). Nitrogen journey in plants: From uptake to metabolism, stress response, and microbe interaction. *Biomolecules.* 13(10), 1443. doi: 10.3390/biom13101443.
48. Zhang, J., Fengler, K. A., John, L., Hemert, V., Gupta, R., Mongar, N., Sun, J., Allen, W. B., Wang, Y., Weers, B. (2019). Identification and characterization of a novel stay-green QTL that increases yield in maize. *Plant Biotechnology Journal.* 17, 2272-2285. doi: 10.1111/pbi.13139.
49. Zuhdi, D. A. F., Abdullah, M. F., Suliswanto, M. S. W., Wahyudi, S. T. (2021). The competitiveness of Indonesian crude palm oil in international market. *Jurnal Ekonomi Pembangunan*. 19(1), 111-124

Figures

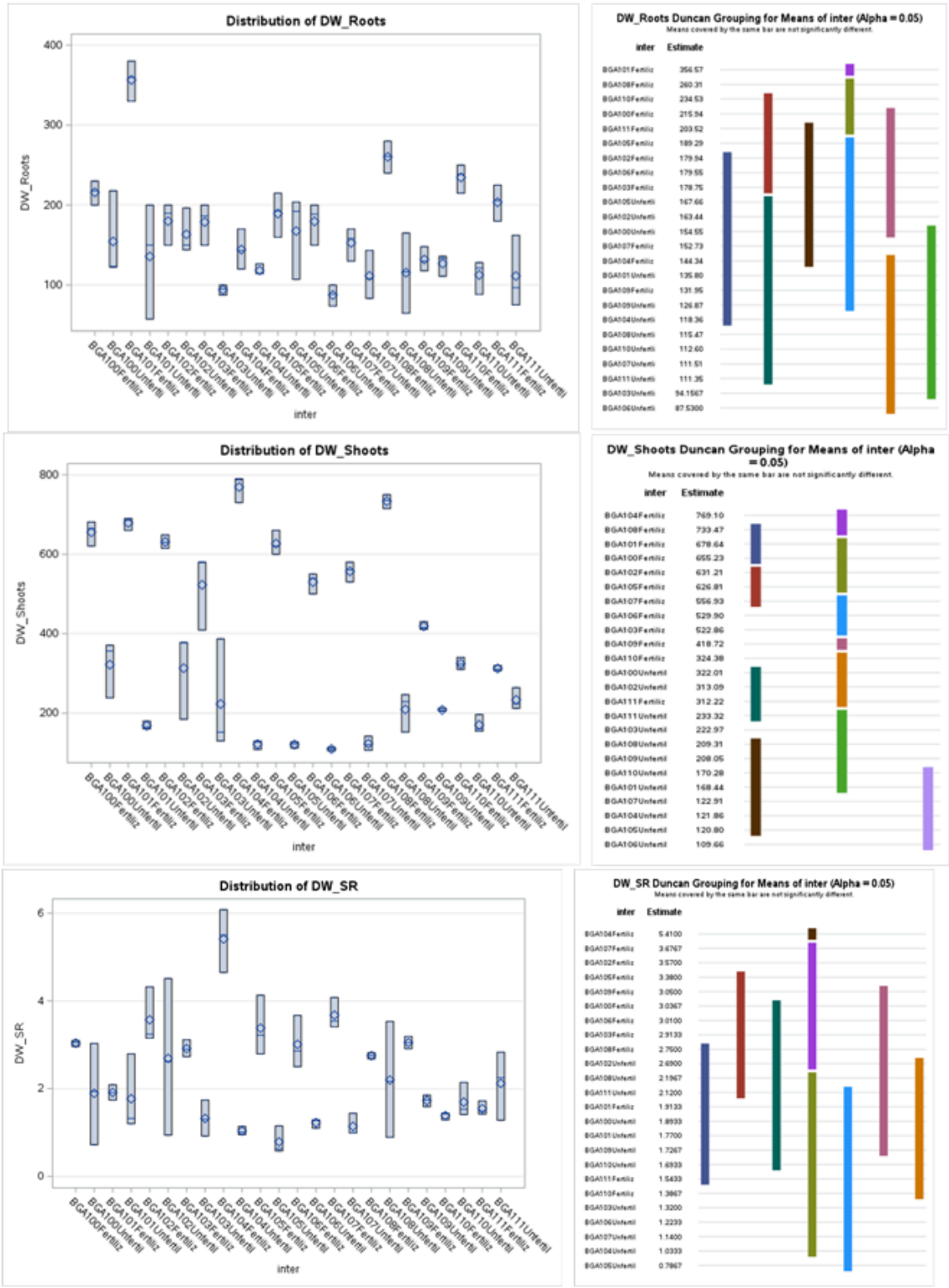


Figure 1

Boxplot Analysis of Biomass Allocation (Root and Shoot Dry Weight), with Duncan's Multiple Range Test for Significant Differences

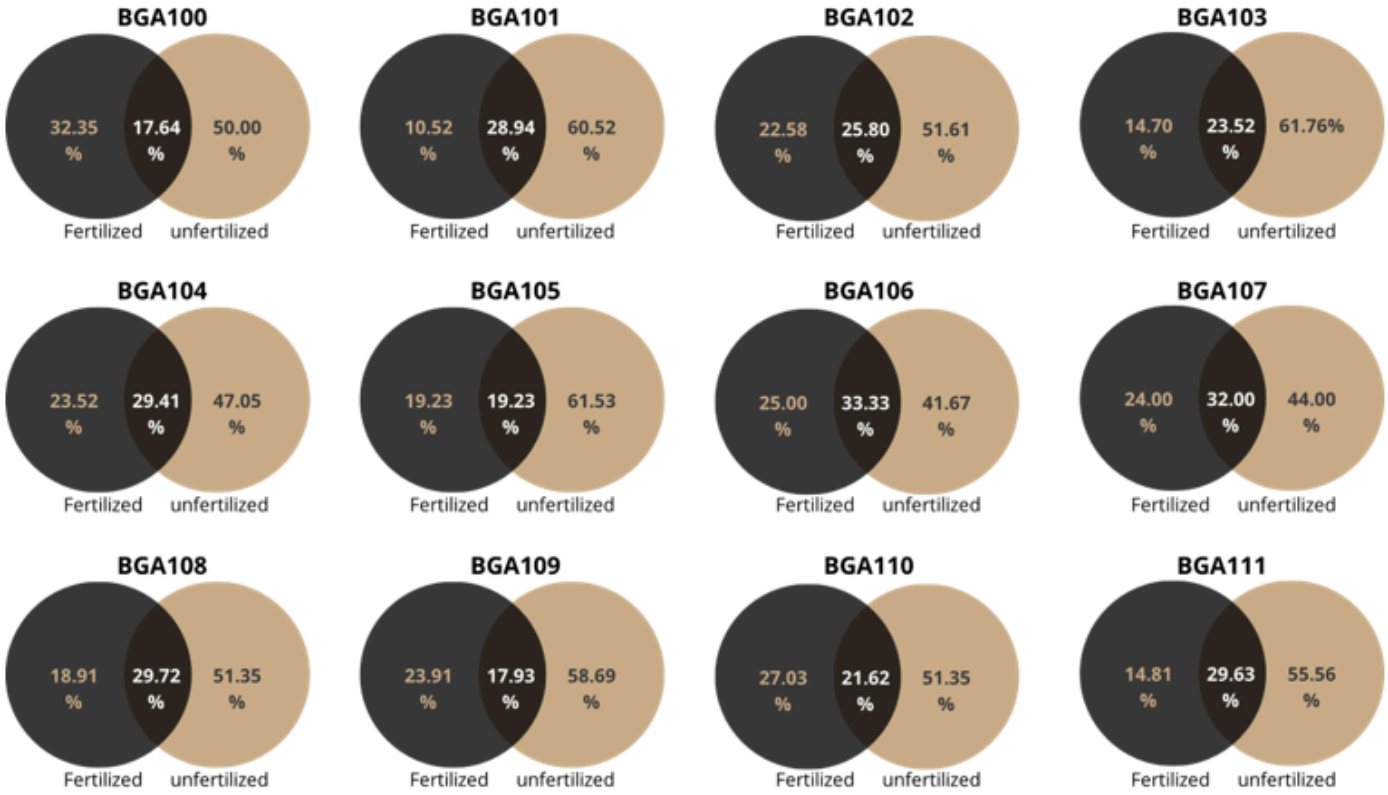


Figure 2

illustrates the comparative Venn diagram of metabolite profiles in fertilized and unfertilized conditions across 12 oil palm genotypes

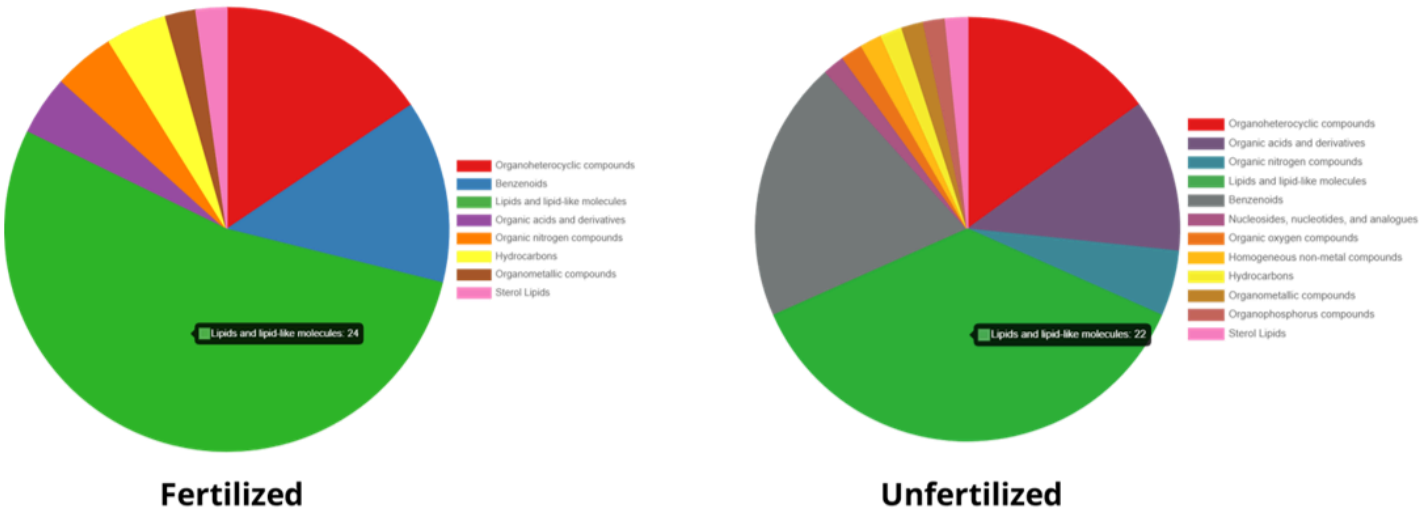


Figure 3

depicts the composition of compound types in fertilized and unfertilized conditions

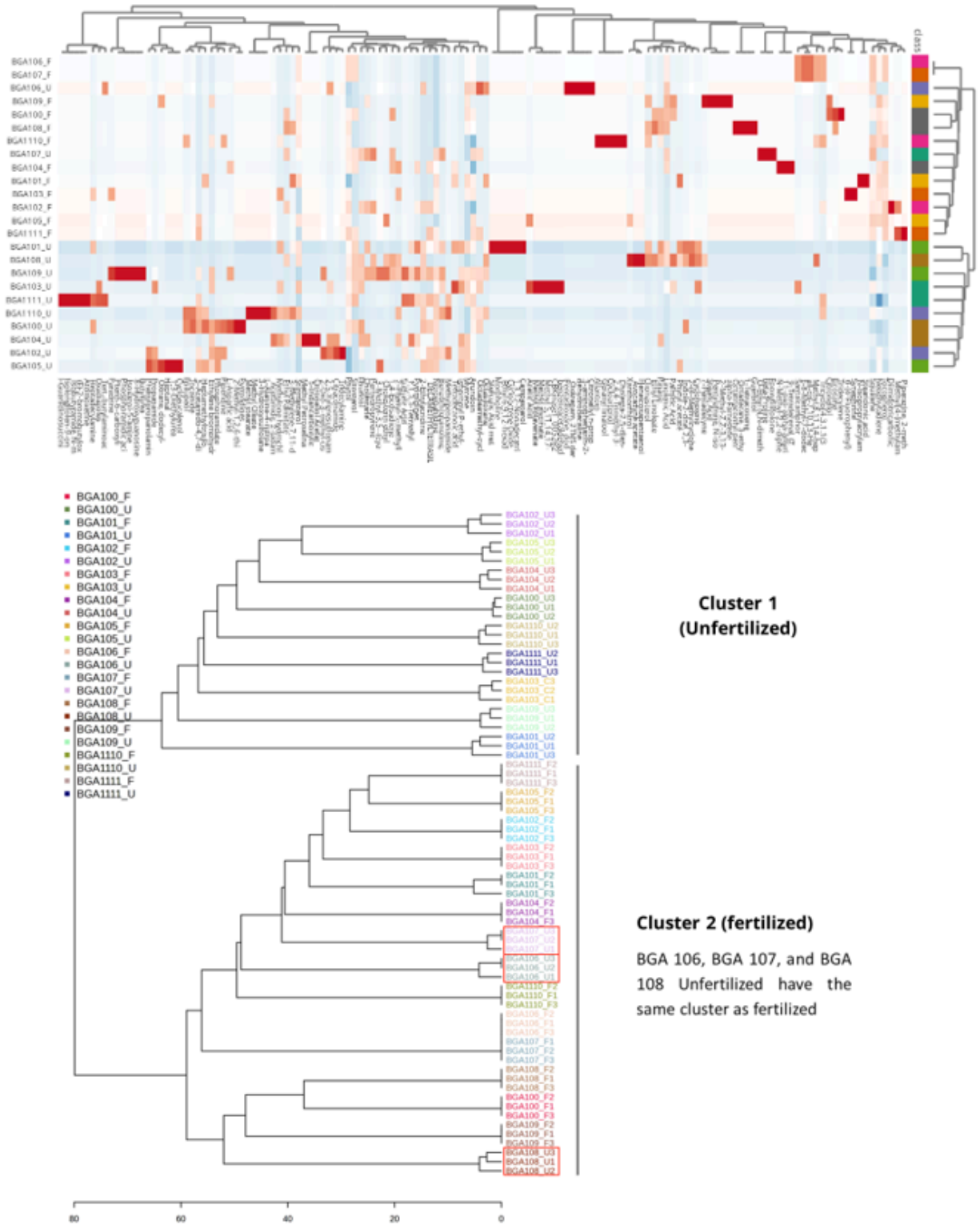


Figure 4

illustrates the heatmap and clustering analysis results for the metabolite profiles in fertilized and unfertilized conditions across 12 oil palm genotype.

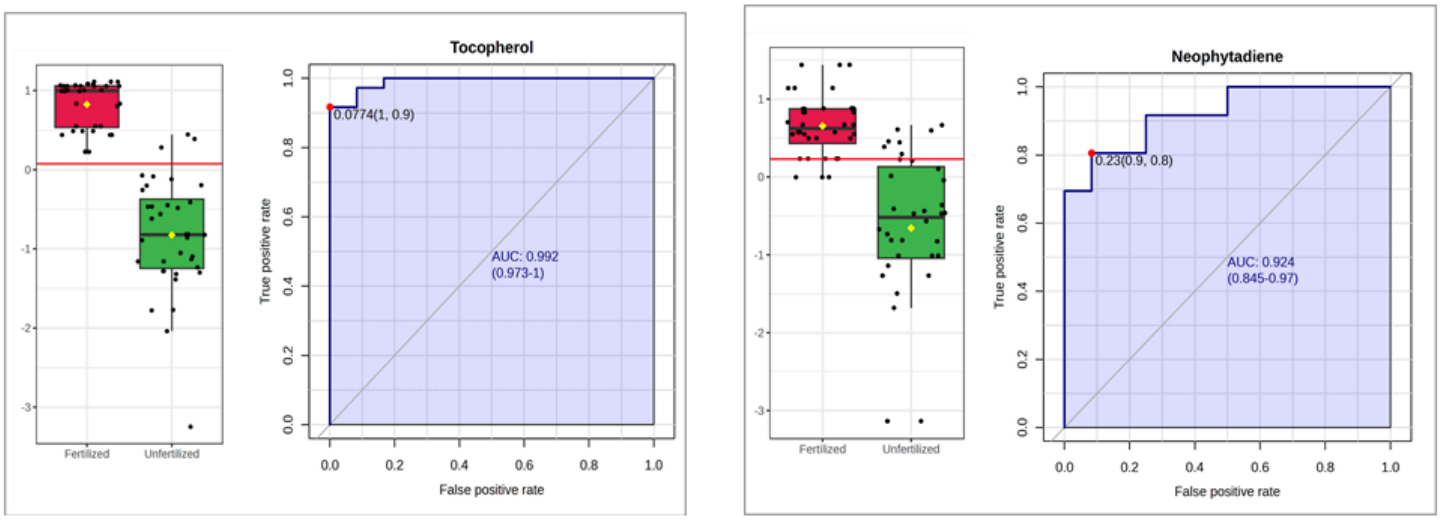


Figure 5

ROC Analysis for Tocopherol and Neophytadiene as Biomarker Candidates under Fertilized and Unfertilized Conditions

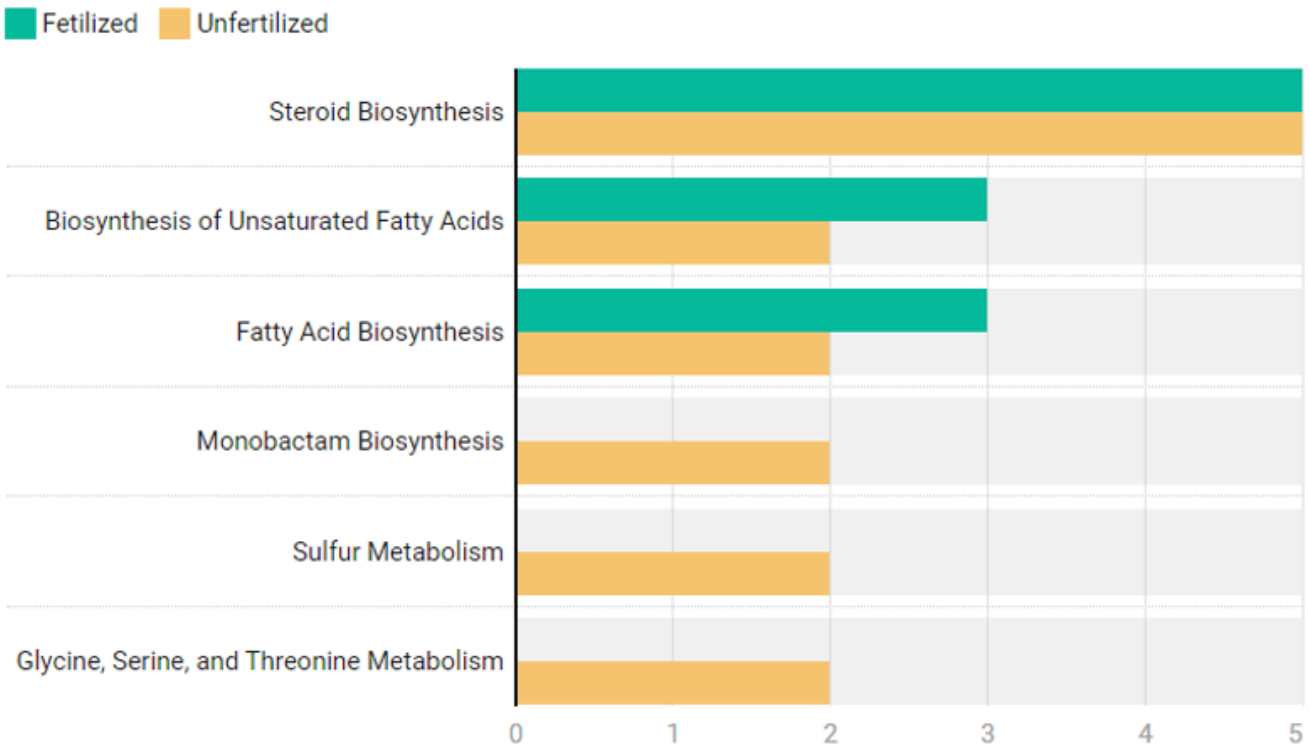


Figure 6

Pathway Analysis under Fertilized and Unfertilized Conditions