Metabolomic profiling unravels divergent adaptive responses of oil palm (*Elaeis guineensis*) seedlings to drought stress under shaded and unshaded conditions

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Abstract. *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: 2404-2414.* This research takes a unique approach by investigating the metabolomic responses of oil palm (*Elaeis guineensis* Jacq.) seedlings to drought stress under both shaded and unshaded conditions. Drought stress poses a significant threat to oil palm cultivation, with profound implications for global palm oil production. Our study aims to unravel the adaptive mechanisms of oil palm seedlings through metabolomic profiling, specifically focusing on amino acids and isoprenoids. The methodology involved collecting samples at distinct time points, including normal conditions, 7 days after drought stress (7 DAT), and 14 days after drought stress (14 DAT). Gas Chromatography-Mass Spectrometry (GC-MS) was used for comprehensive metabolite analysis. The results unveiled substantial changes in metabolite profiles, particularly in amino acids and isoprenoids, indicating the plant's concerted efforts to maintain biochemical homeostasis and vitality during drought stress. Cluster analysis revealed distinct metabolic responses between shaded and unshaded conditions, suggesting an initial conservative response followed by subsequent adaptive measures over time. The fold-change analysis identified key compounds such as Proline, Methyl Palmitate, and octadecylamine, which are crucial for the plant's adaptation to drought conditions. ROC analysis further confirmed Proline and D-Glucuronic Acid Amide as potential biomarkers for distinguishing plant responses to drought stress. This comprehensive metabolomic investigation provides valuable insights into the adaptive strategies employed by oil palm seedlings, thereby offering practical implications for developing sustainable cultivation practices and mitigating drought stress in oil palm cultivation.

Keywords: Drought stress, GCMS analysis, metabolomic profiling, oil palm seedlings, plant stress physiology

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) plays a pivotal role in the global vegetable oil industry, primarily contributing to palm oil production worldwide. Particularly in Indonesia, the oil palm industry occupies a significant position in the plantation sector, driving economic growth and agricultural advancement. Nonetheless, this sector faces challenges, notably environmental disruptions like the El Niño phenomenon. Historically, this climatic event has disrupted rainfall patterns across Indonesia, resulting in prolonged drought periods and heightened air temperatures in specific regions (IRFC 2023). The combination of elevated temperatures and arid climatic conditions accelerates water evaporation from both soil and plants, exacerbating vulnerability to drought (Zeng et al. 2023). These environmental constraints significantly hinder plant growth and productivity, underscoring the urgent need for a comprehensive understanding of their impact on oil palm cultivation (Arora 2019).

Amidst these challenges, exploring the potential of light filtration emerges as a compelling avenue of research.

Light filtration, achieved through shading, establishes a stable microenvironment by reducing the intensity of light reaching the plants. Understanding the divergent effects of direct sunlight exposure (unshaded) versus light-filtering protection (shaded) becomes crucial in unraveling plant growth dynamics and responses to drought stress. Consequently, deeper investigation into the factors influencing the growth and productivity of oil palm plants, particularly under drought stress conditions, becomes imperative. Several studies on the shading effect of black shade nets have been extensively conducted to mitigate sunlight exposure (Buthelezi et al. 2016; Chang et al. 2016; Sivakumar et al. 2017). Low irradiation levels induce changes in plant morphological and chemical features, resulting in broader and thinner leaves, less dense canopy, and modulation of phytochemical content (Bonasia et al. 2017; Caruso et al. 2019).

As formidable environmental stressor, drought profoundly affects plant growth and development, particularly in oil palm cultivation. Young oil palm seedlings, inherently vulnerable to drought stress, encounter impediments in growth and developmental processes. In response to drought stress, plants orchestrate multifaceted array of morphological,

physiological, and biochemical adaptations to mitigate the adverse effects (Ali-Dinar et al. 2023). Researchers have pursued various scientific approaches encompassing physio-biochemical and molecular strategies. These approaches aim to uncover the underlying mechanisms governing plant responses to drought stress and to identify potential targets for enhancing drought tolerance. Such efforts have been crucial in unraveling the intricate regulatory networks governing plant responses to drought stress and identifying key genes, proteins, and metabolites involved in drought tolerance mechanisms. Plant resilience against drought stress lies in metabolic regulation, a fundamental process that coordinates the synthesis, degradation, and utilization of primary and secondary metabolites as responses to environmental signals (Wahab et al. 2022; Liu et al. 2023).

Secondary metabolites, including phenolic compounds, terpenoids, and flavonoids, emerge as discernible markers of plant responses to environmental stressors. Profiling these secondary metabolites provides a comprehensive snapshot of metabolic alterations during drought stress (Patel et al. 2020). For instance, across various plant species, such as peanuts, rice, and soybeans, the accumulation of raffinose and galactinol under water deficit conditions has been documented (Selvaraj et al. 2017; Salvi et al. 2020). In peanuts specifically, significant metabolites like pentitol, phytol, and xylonic acid exhibit marked responsiveness to drought (Gundaraniya et al. 2020). Salvi et al. (2020) revealed the impact of water deficiency on seven metabolic pathways, including galactose metabolism, starch, and sucrose metabolism, among others.

Gas Chromatography-Mass Spectrometry (GCMS) emerges as an indispensable analytical tool, offering intricate insights into metabolite composition (Choudhury et al. 2022). Its application in metabolomic studies facilitates a deeper understanding of metabolite dynamics during drought stress (Gundaraniya et al. 2020). Metabolic profiling is a potent means to unravel the molecular intricacies of stress response mechanisms, thereby informing breeding programs and refining cultivation techniques. This research investigates secondary metabolites pivotal in the oil palm's response to drought and elucidates the plant's adaptation mechanisms to this stress. Through metabolomic profiling, the study aims to unravel the divergent adaptive responses of oil palm seedlings to drought stress under shaded and unshaded conditions, thereby enriching our understanding of plant-environment interactions and informing sustainable agricultural practices to pose climate change challenges.

MATERIALS AND METHODS

Plant material and treatment conditions

The experiment was conducted within the controlled environment of the Bumitama Seed Production greenhouse, situated in the Pundu Region, Cempaga Hulu, East Kotawaringin, Central Kalimantan, Indonesia. The statistics used are Completely Randomized Design (CRD). The shading condition was implemented using a 60% shade net that filtered sunlight, while the unshaded condition represented plants exposed to direct sunlight. Samples were

obtained at three distinct time intervals: under standard conditions (control), 7 days post-drought stress (7 DAT), and 14 days post-drought stress (14 DAT), with three replications for each condition. Leaves were selected as the experimental samples due to their sensitivity to drought stress (Yavas et al. 2024). The oil palm seedlings utilized in this study consisted of clone seeds propagated through tissue culture, obtained from PT Bumitama Gunajaya Agro, and were chronologically 3 months old following the acclimatization process.

Sample extraction for untargeted metabolomics analysis using GC-MS

The extraction method followed Halim et al. (2019) with minor adjustments. A total of 10 g of sample leaves from each treatment were carefully dried in an oven for 36 hours to remove moisture content. Following drying, the leaves were finely ground and then macerated with absolute methanol for 5 days to facilitate extraction. Ultrasonication was employed for 60 minutes at a temperature of 60°C to enhance the extraction process. The resulting extracts were subsequently suspended in 200 mL of maceration extract. Finally, the purified extracts were meticulously prepared for analysis using Agilent Technologies 7890A/G3440A 5975C inert/G3171A for GC-MS, ensuring accurate identification of compounds present.

Data processing and statistical analysis

The data generated from the GCMS analysis were validated and cleaned to ensure quality and accuracy. Compound identification was conducted using the ChEBI (Degtyarenko et al. 2007), PubChem (Bolton et al. 2008), and ChemSpider (Pence and Williams 2010) databases. The data were then subjected to statistical analysis, including univariate and multivariate analysis, Partial Least Squares-Discriminant Analysis (PLS-DA), and calculation of Variable Importance in Projection (VIP) Score to identify the contribution of metabolites to group differences. Subsequently, pathway analysis was performed to understand metabolite changes in the context of metabolic pathways. Receiver Operating Characteristic (ROC) analysis using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) was conducted to assess the potential of specific compounds as key biomarkers for distinguishing treatment groups.

RESULTS AND DISCUSSION

Temporal metabolite shifts and adaptive responses in oil palm seedlings

This study conducted a metabolomic analysis using Gas Chromatography-Mass Spectrometry (GC-MS) on oil palm seedlings subjected to two treatments: drought stress with shade and drought stress without shade. The results revealed significant changes in the pattern of metabolite profiles, particularly in amino acids and isoprenoids (Figures 1.A and 1.B).

Drought stress challenges plants and triggers fundamental changes in their metabolic systems (Ali et al. 2017; Anjum et al. 2017; Hussain et al. 2018). This transformation

encompasses noteworthy processes in the biosynthesis and transport of primary and secondary metabolites (Ma et al. 2020). Primary metabolites, including sugars, polyols, amino acids, and lipids, constitute the foundation for plant cell development (Salam et al. 2023). Beyond structural elements, these primary metabolites are involved in crucial processes such as cellular growth, photosynthesis, and respiration (Patel et al. 2020). Amino acids, as the fundamental building blocks of proteins, and isoprenoids, a group of organic compounds encompassing terpenoids and steroids, play a crucial role in plants' adaptive response to drought stress (Hildebrandt 2018).

The increase in amino acids and isoprenoid levels indicates the plant's effort to maintain vitality and biochemical balance under unfavorable environmental conditions. The heatmap (Figures 2.A and 2.B) visually represents the relative changes in metabolite levels among treatment conditions and during the drought period. Oil palm plants exhibited a stable metabolite profile under normal conditions, but fluctuations were observed in some specific metabolites during drought. Phytol, lupeol, squalene, and alpha-tocopherol were consistent compounds across all treatment levels but with noticeable concentration changes between the control group and 14 days after treatment. This indicates the formation of specific compounds due to the interaction between drought stress and light intensity received by the plant. During drought stress, visual heat map analysis demonstrates substantial shifts in metabolite profiles during drought periods. Following previous research, these findings indicate that drought stress significantly impacts metabolite

expression in plants, particularly focusing on amino acids, sugars, and their derivatives (Khan et al. 2019).
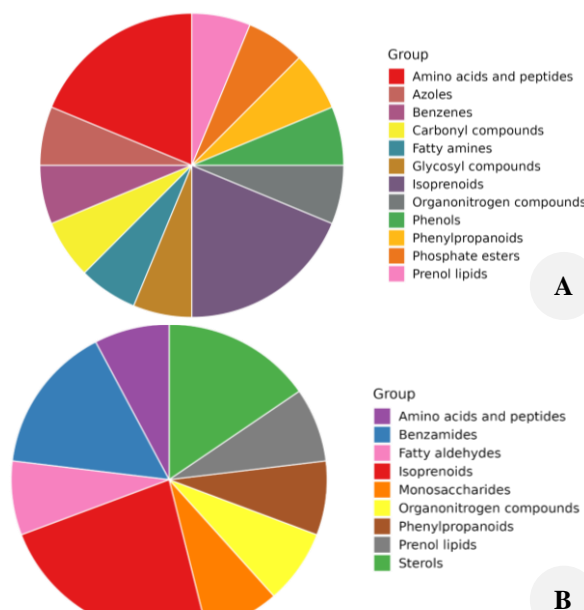

Figure 1. Illustrations of the compound groups detected: A. In leaves subjected to drought stress + shaded conditions; B. In plants subjected to drought stress + unshaded conditions
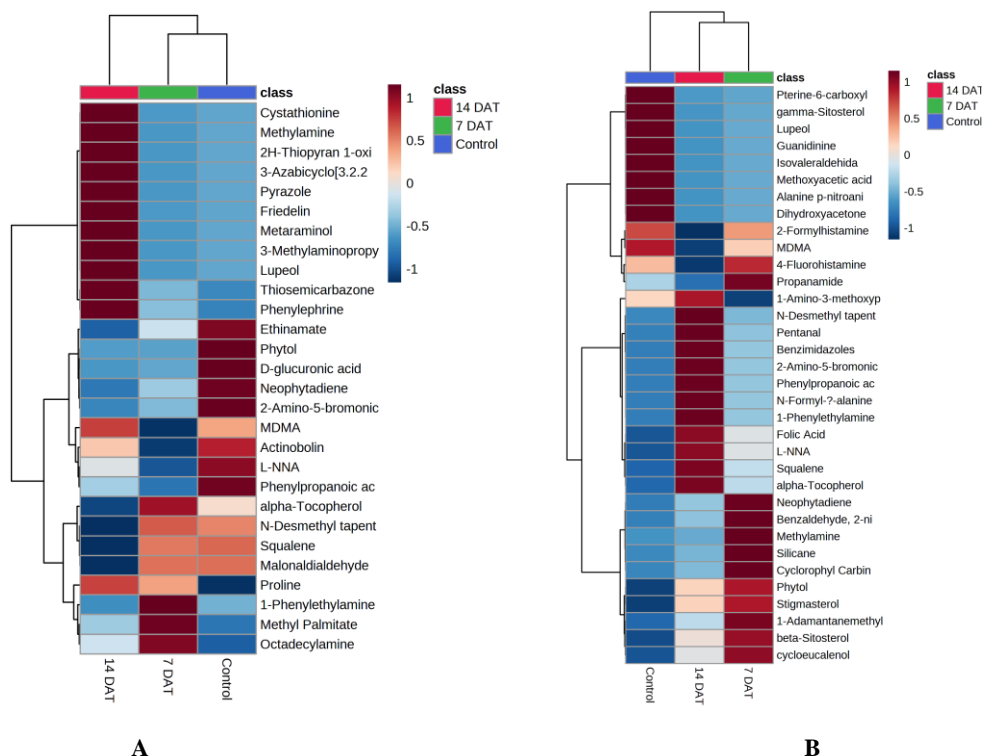

Figure 2. Presentation of the heatmap and clustering of detected compounds: A. In plants subjected to drought stress + shaded conditions; B. In leaves subjected to drought stress + unshaded conditions

Cluster analysis revealed significant patterns in the metabolomic response of plants to drought at different stages and treatments. In the drought stress with shade treatment, normal conditions and 7 DAT formed a cluster with similar metabolite profiles, indicating a resemblance in the metabolic response under these conditions. Conversely, 7 DAT and 14 DAT formed a similar cluster in the drought stress without shade treatment, reflecting the plant's early adaptation to drought stress under low light intensity conditions (Figures 2.A and 2.B). Further adjustments or changes in metabolite pathways occurred in response to sustained drought stress. Biologically, these findings provide insights into plant adaptation strategies changes to drought over time. It can be observed that there is an initial conservative response phase, followed by further changes where the plant responds more intensively to prolonged drought stress (Yang et al. 2021)

The diversity of metabolites involved in the response to drought stress plays a central role in aiding plants to adapt and recover from the multifaceted impacts of such stress (Kumar et al. 2021). This indicates that during drought stress, plants significantly alter the production and distribution of metabolites as a response strategy to intense environmental pressure (Seleiman et al. 2021). Therefore, this research

highlights the complexity of plant adaptation mechanisms to drought, demonstrating how changes in both primary and secondary metabolites are crucial to maintaining plant cells' survival and physiological balance.

Each treatment revealed approximately 81.8% (Figure 3.A) and 81.5% (Figure 3.B) of the dataset variation, with drought stress with shade slightly higher. Although this difference is small, this interpretation suggests that drought stress with shade has a slightly more dominant effect on controlling variability in the dataset. The VIP score results reflect the relative contribution of metabolites to the separation of treatment groups, with drought stress with shade affecting 17 metabolites while drought stress without shade affecting 21 metabolites (Figures 3.C and 3.D). Variables with high VIP scores significantly contributed to metabolite stability under normal conditions. At 7 days of drought, high VIP scores reflected early changes in response to drought stress, and at 14 days of drought, high VIP scores reflected further metabolite changes. The decrease in VIP scores for some metabolites at 14 days of drought may indicate plant adaptation, while the increase in VIP scores for specific metabolites may indicate a more intensive metabolite response at this stage.
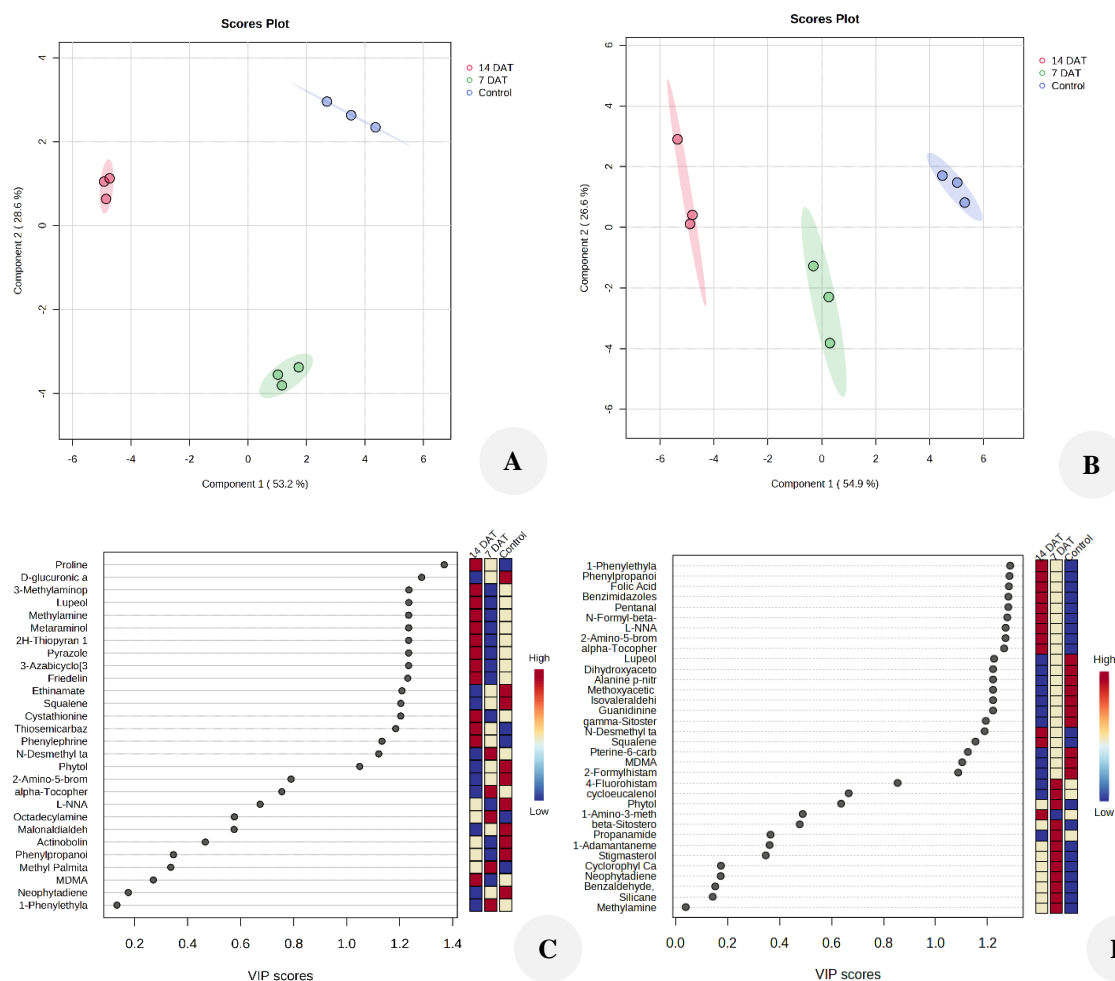

Figure 3. Displays of the PLSDA (Partial Least Squares Discriminant Analysis) scores and VIP scores of compounds detected: A-C: In leaves subjected to drought stress + shaded conditions; B-D: In plants subjected to drought stress + unshaded conditions

Comprehensive analysis of changes in compound expression

Fold change analysis illustrates the relative changes in compound expression in response to treatment. Under drought stress with shade, 13 upregulated and 4 down-regulated compounds were observed. Drought stress without shade resulted in 17 upregulated and 9 downregulated compounds; Tables 1 and 2 detail the upregulated and downregulated compounds in each treatment. For example, under drought stress with shade, there was a significant increase in the expression of compounds like Methyl Palmitate, octadecylamine, Proline, Cystathionine, Friedelin, and Lupeol. In contrast, certain compounds experienced a decrease in expression.

Comprehensive analysis in this research illustrates that dominant compounds, especially those derived from amino acids and isoprenoids, act not only as essential components for protein formation but also as raw materials in synthesizing photo-synthetic pigments and cell membrane constituents. Amino acids, playing a crucial role in protein synthesis and plant cellular functions (Ahanger et al. 2018), not only function as an osmolyte to maintain cellular osmotic balance but also serve as scavengers of Reactive Oxygen Species (ROS) generated during drought stress (Zandi and Schnug 2022). Aromatic amino acids, such as phenylalanine, tryptophan, and tyrosine, play a dual role as precursor secondary metabolites supporting overall plant growth (Maeda and Dudareva 2012).

The top 5 compounds, derived from the fold change analysis with a higher threshold (5.0), were identified as potential key markers (Figures 4.A and 4.B). Proline, an amino acid crucial in environmental stress response, exhibited increased levels, indicating its role in osmoregulation and cell protection against drought stress (Meena et al. 2019; Yang et al. 2021; Li et al. 2024). Methyl palmitate and octadecyl amine were implicated in cellular membrane protection and the synthesis of lipophilic compounds crucial for drought adaptation (Ullah et al. 2022). In addition, Glucuronic Acid Amide, known for its involvement in detoxification mechanisms (Bhagavan and Ha 2015), displayed decreased expression during drought stress with shade. This suggested reduced detoxification activity during stress, signifying the plant's allocation of resources to other adaptive responses. Propanamide potentially plays a role in osmotic pressure regulation (Oloyede et al. 2020), a vital mechanism helping plants maintain cell water balance during drought. Likewise, Benzaldehyde, 2-nitro-, and diamino methylidenhydra-zone might be involved in adaptive signaling that regulates gene expression and specific metabolic pathways to cope with environmental stress. Furthermore, the compound N-Desmethyl tapentadol is indicated to play a role in regulating specific metabolic pathways as part of the plant's adaptation mechanism to drought. Conversely, the downregulated expression of Pterine-6-carboxylic acid was considered a strategy for the plant to optimize its limited resources and adjust metabolism to meet changing environmental demands (Haghighi et al. 2022).

Proteins like proline, commonly found in stress conditions, along with the accumulation of amino acids such as glutamate, arginine, histidine, isoleucine, and tryptophan, show an increase in rice and wheat plants during drought periods (Degenkolbe et al. 2013; Do et al. 2013; Hein et al. 2016; Yadav et al. 2019). These findings reflect the adaptive response of plants to drought stress by enhancing the production of compounds that support survival and growth. Conversely, several studies have reported a decrease in amino acid levels during drought stress in certain types of oil-producing plants, such as soybean and oil tea (Silvente et al. 2012; Qu et al. 2019), indicating specific adjustments in metabolite regulation as a response to environmental pressure.

Table 1. Enumerates the compounds regulated under the drought stress + shaded treatment
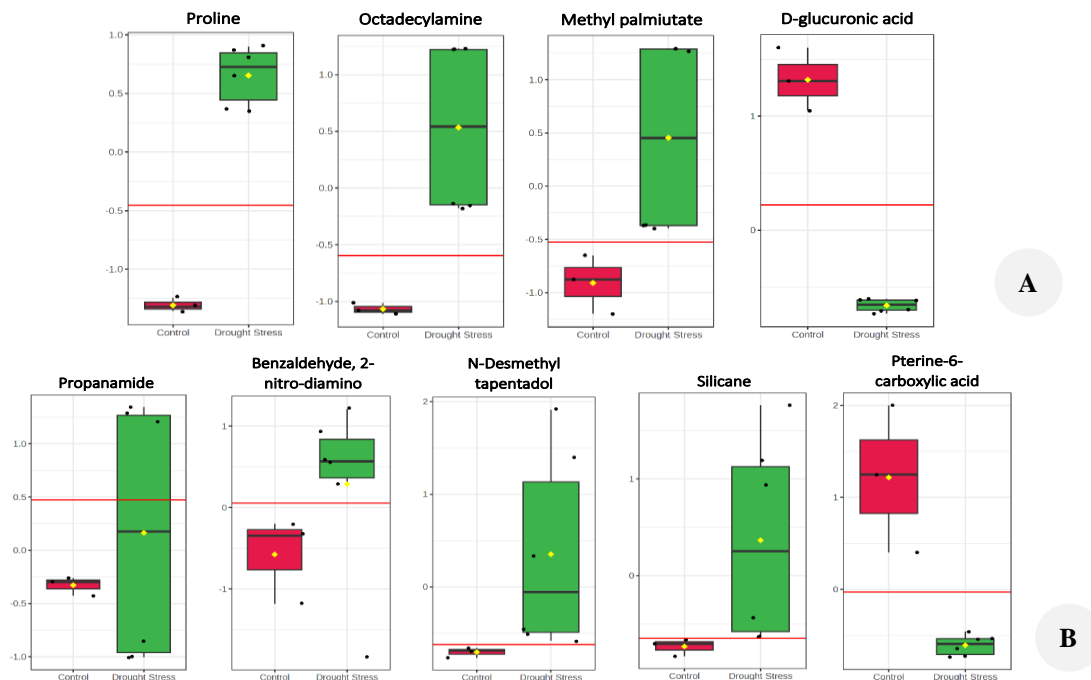
Compound	Group of biosynthesis	Fold change	Log2(FC)	Regulation
Methyl Palmitate	Fatty Acid	8.0762	3.0137	Upregulated
Octadecylamine	Fatty Acid	7.4545	2.8981	Upregulated
Proline	Amino Acid	6.4075	2.6798	Upregulated
Cystathionine	Amino Acid	4.3382	2.1171	Upregulated
Friedelin	Terpenoid	3.1290	1.6457	Upregulated
3-Methylaminopropylamine	Amine	2.7717	1.4708	Upregulated
Lupeol	Terpenoid	2.7688	1.4692	Upregulated
1-Phenylethylamine	Alkaloid	2.7549	1.4620	Upregulated
Metaraminol	Amine	2.6870	1.4260	Upregulated
3-Azabicyclo [3.2.2] nonane	Alkaloid	2.5695	1.3615	Upregulated
Methylamine	Amine	2.5695	1.3615	Upregulated
2H-Thiopyran 1-oxide	Unknown	2.5695	1.3615	Upregulated
Pyrazole	Unknown	2.5695	1.3615	Upregulated
N-Desmethyl tapentadol	Alkaloid	0.4898	1.0295	Upregulated
2-Amino-5-bromonicotinamide	Amino Acid	0.3831	-1.3840	Downregulated
Actinobolin	Aminoglycoside	0.2482	-2.0102	Downregulated
D-glucuronic acid amide	Carbohydrate	0.1227	-3.0262	Downregulated

Note: Fold change threshold = 2.0

Table 2. Enumerates of the compounds regulated under the drought stress + unshaded treatment

Compound	Group of biosynthesis	Fold change	log2(FC)	Regulation
Propanamide	Amide	14.7000	3.8777	Upregulated
Benzaldehyde, 2-nitro-, diamino methyli- denhydrazone	Aldehyde	13.2550	3.7284	Upregulated
N-Desmethyl tapentadol	Alkaloid	11.5280	3.5271	Upregulated
Silicane	Terpenoid	7.2692	2.8618	Upregulated
Folic Acid	Amino Acid	5.1955	2.3773	Upregulated
2-Amino-5-bromonicotinamide	Amino Acid	5.1281	2.3584	Upregulated
Phenylpropanoid acid	Phenol	4.9895	2.3189	Upregulated
Cyclorophyl Carbinol	Unknown	4.7872	2.2592	Upregulated
N-Formyl-beta-alanine	Amino Acid	4.7460	2.2467	Upregulated
L-NNA	Amino Acid	4.7233	2.2398	Upregulated
Pentanal	Aldehyde	4.7141	2.2370	Upregulated
Benzimidazoles	Unknown	4.6336	2.2121	Upregulated
1-Phenylethylamine	Alkaloid	4.3222	2.1118	Upregulated
1-Adamantanemethylamine	Amine	2.3621	1.2401	Upregulated
alpha-Tocopherol	Terpenoid	2.2805	1.1894	Upregulated
Squalene	Terpenoid	2.1267	1.0886	Upregulated
Phytol	Terpenoid	2.0092	1.0066	Upregulated
MDMA	Unknown	0.4811	-1.0557	Downregulated
Methoxyacetic acid	Unknown	0.2995	-1.7396	Downregulated
Guanidinine	Amino Acid	0.2995	-1.7396	Downregulated
Alanine p-nitroanilide	Amino Acid	0.2995	-1.7396	Downregulated
Dihydroxyacetone	Carbohydrate	0.2995	-1.7396	Downregulated
Isovaleraldehida	Fatty Acid	0.2995	-1.7396	Downregulated
Lupeol	Terpenoid	0.2916	-1.7780	Downregulated
gamma-Sitosterol	Terpenoid	0.2361	-2.0827	Downregulated
Pterine-6-carboxylic acid	Co-Enzyme	0.0756	-3.7249	Downregulated

Note: Fold change threshold = 2.0

Figure 4. Portrays of the boxplot results of fold-change analysis with a threshold of 5.0 related to compound expression under: A. Drought stress + shaded conditions; B. Drought stress + unshaded conditions treatments

Therefore, expanding beyond amino acids, these observations enter the realm of various types of sugars and their derivatives identified during drought periods; in line with previous research on crops such as soybeans found

that myoinositol and maltose as drought stress biomarkers indicate the complexity of these metabolites' involvement in sugar and amino acid biosynthesis processes (Guo et al. 2018; Vital et al. 2022). It is important to note that sugar

formation becomes a strategic mechanism for plants facing drought stress (Kaur et al. 2021). Limited photosynthesis leads to the remobilization of stored starch to generate energy, sugars, and their derivatives (MacNeill et al. 2017; Thalmann and Santelia 2017). Other studies highlight an increase in sugar levels such as glucose, sucrose, fructose, ribose, raffinose, eritronate, and xylonate, with the presence of pyruvate as an intermediate in glycolysis during drought stress (De Miguel et al. 2016; Mukherjee et al. 2019; Wu et al. 2023).

Moreover, the study on other plants, such as rice and others, shows a significant decrease in sucrose sugar levels during drought stress, depicting variations in metabolite responses among plant species (Li et al. 2015). These findings reflect the specific adaptation of plants to drought stress conditions; the sugar profiles and their derivatives shifts illustrate the crucial role of metabolite regulation in responding to changing environmental conditions. Furthermore, a thorough understanding of the role of each type of sugar and its derivatives can provide a more comprehensive insight into plant adaptation strategies to drought stress.

Receiver Operating Characteristic (ROC)

Receiver Operating Characteristic (ROC) Analysis, as depicted in Figures 5.A and 5.B, assesses the strength of compounds as key biomarkers for treatment. Proline and D-glucuronic acid Amide exhibited perfect Area Under the Curve (AUC) values of 1, signifying highly effective differentiation between positive and negative response groups. Pterin-6-carboxylic acid also had a perfect AUC value under drought stress without shade treatment. These compounds hold potential as biomarkers in determining the response to drought stress. ROC Analysis is a commonly used method for evaluating the performance of biomarkers or diagnostic tests. In this research, ROC is employed to assess how well specific compounds can differentiate between groups experiencing a positive response and those with a negative response to drought stress treatment (Figure 5).

In Figures 5.A and 5.B, the ROC curves compare the tested biomarkers' sensitivity (true positive rate) and specificity (true negative rate). In this case, Proline and D-glucuronic acid Amide showed an AUC value of 1, the highest achievable value. A perfect AUC indicates that these biomarkers have a highly effective ability to distinguish between groups experiencing drought stress and those that are not. Pter-in-6-carboxylic acid also stood out with a perfect AUC value, indicating that this compound also has the potential to be a strong biomarker in determining the response to drought stress, especially under shade-free conditions. This conclusion is supported by the fact that the AUC reflects the accuracy level of the biomarker, and a perfect value characterizes the biomarker's ability to provide highly accurate results.

In line with the research conducted by Qian et al. (2022); Salsinha et al. (2022); and Khaled et al. (2024) these findings imply that Proline, D-glucuronic acid Amide, and Pterine-6-carboxylic acid are highly reliable biomarkers for identifying plant responses to drought stress. ROC analysis provides a robust scientific basis for the potential application of these biomarkers in monitoring and managing plant resilience to unfavorable environmental conditions.

A significant increase in proline levels as an amino acid and a synchronous decrease in glucuronate amine levels as a sugar derivative emerge as a characteristic response to drought stress in this study. These observations highlight the roles of osmoregulation and cellular protection against the adverse effects of drought stress. Additional compounds, such as octadecylamine, identified as an amine compound, contribute positively to the plant's response to stress. Increased activity in specific pathways reflects metabolite regulation associated with plant adaptation to drought and shading conditions, revealing changes in sugar processing and amino acid metabolism during stress treatment.

Comparative metabolic pathway: An enrichment analysis

Enrichment analysis, as illustrated in Figure 6, provides a comprehensive understanding of the differences in metabolite groups and their concentrations between the treatments of drought stress with shade and drought stress without shade. This analytical approach helps identify specific metabolic pathways that are significantly influenced by variations in environmental conditions. This study revealed the enrichment analysis focused on metabolite groups and their associated pathways, as well as significant differences in metabolite concentrations between the two treatment conditions.

The concentration changes indicate how the plant's metabolism responds differently to the presence or absence of shade during drought stress. Under the drought stress with shade treatment, several pathways exhibited heightened activity. Notable among them were pentose and glucuronate interconversions, ascorbate and aldarate metabolism, inositol phosphate metabolism, arginine and proline metabolism, and amino acid tRNA biosynthesis. When subjected to shade, these pathways play a vital role in the adaptive response of oil palm seedlings to drought stress. For instance, arginine and proline metabolism are involved in stress tolerance mechanisms, and alterations in these pathways suggest the plant's effort to cope with the combined stress of drought and shade.

Conversely, different set of pathways showed prominence under the drought stress without shade treatment. These included pathways related to alanine, aspartate, and glutamate metabolism; selenocompound metabolism; glycerolipid metabolism; steroid biosynthesis; and amino acid tRNA biosynthesis. The heightened activity in these pathways implies distinct metabolic adjustments in response to drought stress without the ameliorating effect of shade. The increased activity in these pathways has also been observed in other studies such as those by Ackah et al. (2021), Neto et al. (2021), and Yin et al. (2024), further confirming typical metabolic adaptations in response to drought stress without the mitigating effect of shading. For example, glycerolipid metabolism is crucial for membrane stability (Lavell and Benning 2019), and its increased prominence suggests the plant's adaptation to maintain membrane integrity under more challenging conditions.
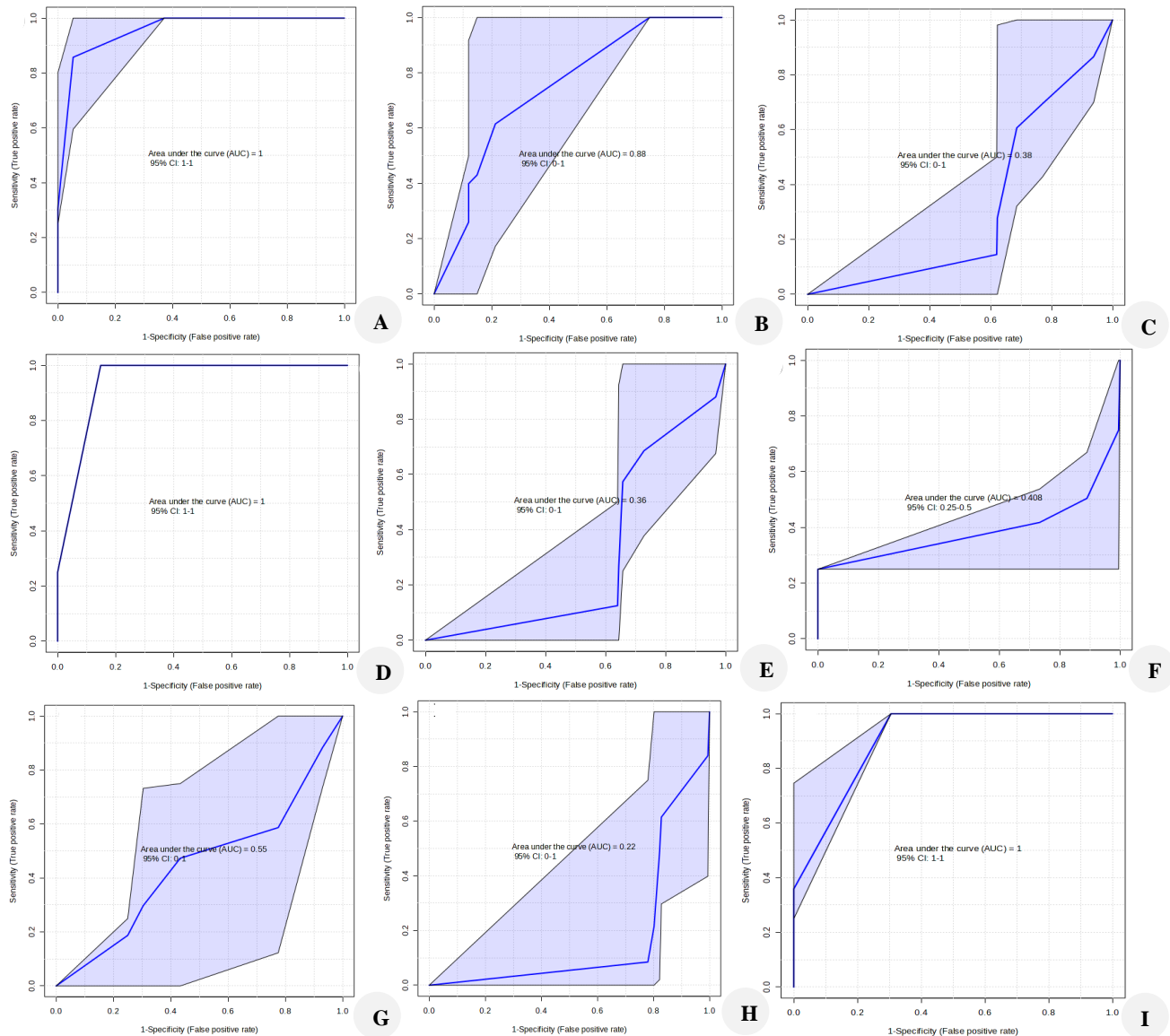
Figure 5. Demonstrates the ROC analysis results with a threshold of 5.0 related to compound expression: A. Proline; B. Methyl Palmitate; C. Octadecylamine; D. D-glucuronic acid; E. Pro-panamide; F. Benzoaldehyde, 2-nitro-, diamino methylidenhydrazone; G. N-desmethyl tapentadol; H. Silicane; I. Pterine-6-carboxylic acid
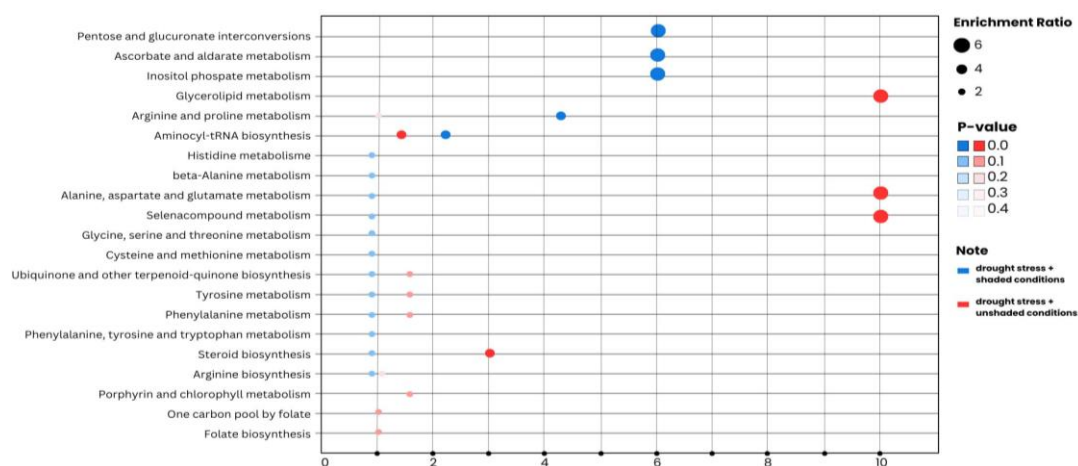

Figure 6. Show various enrichment analyses related to compound expression under drought stress + shaded conditions and drought stress + unshaded conditions treatments

These findings underscore the complexity of plant metabolic responses to different environmental stressors, emphasizing the importance of understanding specific pathways that contribute to adaptation. The enriched pathways identified in this analysis provide valuable insights into oil palm seedlings' biochemical strategies to navigate the challenges of varying light conditions during drought stress.

This study provides a deeper understanding of how plants respond to drought stress by detailing the role of each metabolite and investigating their interactions. Primary metabolites, with their primary roles in basic cellular processes, become the focal point in plant efforts to sustain vitality and functionality under extreme environmental pressure (Salam et al. 2023). Furthermore, understanding the role of secondary metabolites, such as isoprenoid compounds and aromatic amino acids, provides a more comprehensive picture of plant strategies to pose drought stress challenges (Diniz et al. 2020).

In conclusion, metabolomic analysis of oil palm seedlings subjected to two drought treatments, with shading and without shading, has yielded significant insights into the changes in metabolite profiles, particularly focusing on amino acids and isoprenoids. Utilizing heatmap, clustering, and PLS-DA analyses, our findings reveal divergent adaptive responses between the two treatments, characterized by an initial conservative response phase followed by subsequent changes over time. Identifying specific compounds, such as Proline, Methyl Palmitate, and octadecylamine, as key elements in plant adaptation to drought underscores their crucial roles in maintaining biochemical balance and cellular vitality. Fold-change analysis and ROC validation affirm the potential of certain compounds, particularly Proline and D-glucuronic acid Amide, as effective biomarkers capable of distinguishing nuanced plant responses to drought. Furthermore, enrichment pathway analysis elucidates distinct metabolite regulations in drought conditions with and without shading, emphasizing the pivotal roles of specific metabolic pathways in facilitating plant adaptation to environmental stress.

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