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Unraveling the response of secondary metabolites to cold tolerance in oil palm by integration of physiology and metabolomic analyses

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Abstract

Background Oil palm (*Elaeis guineensis*), a tropical crop, is highly sensitive to temperature fluctuations, with low temperatures significantly limiting its growth, development, and geographical distribution. Understanding the adaptive mechanisms of oil palm under low-temperature stress is essential for developing cold-tolerant varieties. This study focused on analyzing the physiological and metabolomic responses of annual thin-shell oil palm seedlings to low-temperature exposure (8 °C) for different time periods: 0 h (CK), 0.5 h (CD05), 1 h (CD1), 2 h (CD2), 4 h (CD4), and 8 h (CD8).

Results Physiological analysis showed a significant increase in the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase (POD), highlighting the activation of oxidative stress defense mechanisms. Concurrently, elevated relative conductivity, indicated cell membrane damage, a common consequence of cold-induced oxidative stress. Metabolomic profiling using LC-MS/MS revealed significant changes in metabolite composition, with differential metabolites predominately enriched in key metabolic pathways such as arginine and proline metabolism, glycine, serine, and threonine metabolism, plant hormone biosynthesis, and flavonoid biosynthesis pathways. Notable metabolites such as citric acid, L-aspartic acid, L-tryptophan, and vitexin showed significant accumulation, indicating their roles in enhancing cold tolerance through improved antioxidant defenses, promoting osmoregulation, and stabilizing cellular structures. Correlation analysis further emphasized the importance of flavonoids and plant hormones in the cold stress response. In particular, vitexin, isovitexin, and apigenin 6-C-glucoside were significantly enriched, suggesting their contribution to antioxidant and stress signaling networks. Furthermore, metabolites involved in amino acid metabolism, including L-glutamic acid, sarcosine, and proline, were

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upregulated, supporting enhanced protein synthesis and cellular repair under stress. This metabolic reprogramming correlated with physiological improvements, as evidenced by increased relative conductivity and post cold exposure growth recovery.

Conclusion This study provides critical insights into the physiological and metabolic adaptations of oil palm to cold stress, emphasizing the significant role of secondary metabolites—such as flavonoids, amino acids, and plant hormones—in enhancing cold tolerance. Theses metabolites contribute to oxidative stress protection, osmotic regulation, and cell wall stabilization enabling the plant to better withstand with low temperature condition. The findings provide a strong foundation for molecular research and breeding initiatives aimed at developing cold tolerant oil palm varieties, a crop of siginificant economic value. By combining metabolomic profiling with physiological analyses, provides a holistic understanding of the adaptive mechanisms in oil palm under cold stress. This integrated approach identifies key metabolic pathways that can be targeted in breeding programs to enhance cold resilience, paving the way for improved crop performance in challenging environments.

Keywords Oil palm, Low temperature stress, Physiology, Metabolomics, Cold tolerance

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a significant tropical oil crop, cultivated primarily in regions such as Southeast Asia, Africa, Brazil, and Central America [1–2], where its growth thrives under optimal temperatures ranging from 24 to 28 °C [3]. However, temperatures below 20 °C can adversely affect its growth, with development potentially halting at 12 °C [4]. In subtropical regions, winter-induced low temperatures present significant challenges to oil palm growth, fruit development, and oil production [5], severely limiting its productivity. Low-temperature stress is a critical abiotic factor that disrupts plant growth, causing rapid and profound changes in morphological, physiological, and molecular processes [6-7]. Such stress triggers the production of reactive oxygen species (ROS), leading to cellular damage and dysfunction [8-9]. To counter these effects, plants activate defense mechanisms including antioxidant enzyme systems and the accumulation of cryoprotectants like proline and soluble sugars [10].

Although significant progress has been made in understanding cold tolerance in oil palm through physiological, biochemical, and genetic studies, the integration of physiological and metabolomic perspectives remains relatively under-explored. Advances in metabolomic technologies, particularly high-throughput approaches, have enabled the detailed analysis of stress-induced changes in metabolic profiles [11]. Applications of metabolomics in other crops, such as bell pepper, grape, and wheat, have highlighted the importance of key pathways like energy metabolism, hormone signaling, and amino acid biosynthesis in cold stress responses [12–15]. For instance, studies in tobacco have revealed differences in energy metabolism and hormone metabolism between cold-tolerant and cold-sensitive varieties [16]. Similarly, research on kiwifruit has shown that nucleotide metabolism and phenolic acid metabolism pathways play a role in regulating frost resistance [17]. In wheat, differentially expressed metabolites (DEMs) under low temperature were significantly enriched in abscisic acid/jasmonic acid (ABA/JA) signaling and proline biosynthesis pathways [18]. Additionally studies on *Taxux chinensis* have revealed the role of starch, sucrose, and amino acid metabolism in regulating low-temperature stress responses [19]. Furthermore, research on loquat fruit, has demonstrated that organic acids, sugars, phenols, and cell wall metabolism, are critical during refrigeration [20].These studies underscore the potential of metabolomic tools to identify core metabolites and regulatory mechanisms that contribute to cold tolerance in plants, providing valuable insights for improving stress resilience in crops like oil palm.

In this study, we utilized physiological assessments and LC-MS/MS-based metabolomic analysis to investigate the responses of oil palm seedlings to low-temperature stress across different exposure durations. The research aimed to evaluate physiological changes, identify differentially accumulated metabolites (DAMs) and associated pathways, and elucidate the molecular mechanisms underlying oil palm's cold stress responses. The results offer foundational insights into the adaptive strategies of oil palm under low temperature conditions and provide a theoretical framework for the development of cold-tolerant varieties, which is crucial for enhancing the crop's resilience and productivity in sub tropical regions.

Materials and methods

Plant materials

Thin-shell oil palm seedlings were obtained from the Wenchang National Tropical Palm Germplasm Nursery (110.8°E, 19.6°N) in Hainan Province, China. These seedlings were cultivated in controlled conditions at the Hainan Coconut Research Institute and selected for uniformity in growth for experimental purposes.

Experimental design and stress treatment

To examine the physiological and metabolomic responses of oil palm seedlings to low-temperature stress, a controlled low-temperature treatment regimen was implemented. Seedlings were divided into a control group (CK), maintained at optimal growth temperatures, and experimental groups (CD), exposed to 8 °C for varying durations: 0.5, 1, 2, 4, and 8 h. Each treatment group included three biological replicates, with each replicate consisted of one plant, resulting in a total of 18 plants (3 replicates × 6 time points). Leaves from three plants within each treatment group were pooled to form a mixed sample, ensuring sufficient material for analysis.

Sample collection and storage

Functional leaves from the same age and position on each plant were collected for analysis. Samples were immediately frozen in liquid nitrogen and stored at − 80 °C until further use for physiological characterization and metabolomic analysis.

Physiological measurements

Antioxidant enzyme assays

Superoxide dismutase (SOD) activity

SOD activity was quantified using the nitroblue tetrazolium (NBT) reduction method. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 0.075 mM NBT, and 2 μM riboflavin. The reduction of NBT by superoxide radicals was measured spectrophotometrically at 560 nm under light exposure.

Peroxidase (POD) activity

POD activity was determined by monitoring the oxidation of guaiacol in the presence of hydrogen peroxide (H2O2). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, and 10 mM H2O2. The increase in absorbance at 470 nm was recorded over 5 min period.

Relative water content (RWC)

Leaf RWC was calculated as follows:

$$RWC\,(\%) = FW - TW/TW - DW \times 100$$

where FW is the fresh weight, DW is the dry weight (after oven-drying at 80 °C for 48 h), and TW is the turgid weight (after soaking leaves in distilled water for 4 h at room temperature).

Malondialdehyde (MDA) content

Lipid peroxidation levels were estimated by measuring MDA content using the thiobarbituric acid (TBA) reaction. Leaf samples were homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 g for 10 min. The supernatant was mixed with 0.5% TBA in 20% TCA, boiled at 95 °C for 30 min, and cooled rapidly. Absorbance was measured at 532 nm and corrected for non-specific turbidity at 600 nm.

Metabolite extraction and analysis

Leaf samples (100 mg) were ground into a fine powder in liquid nitrogen. Metabolites were extracted using 80% methanol and water. The samples were vortexed, incubated on ice, and centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was collected, concentrated under vacuum, and reconstituted in 80% methanol for analysis.

The extracted metabolites were analyzed using an ExionLC ultra-performance liquid chromatography (UPLC) system coupled to a SCIEX QTRAP 6500 + mass spectrometer. Chromatographic separation was achieved on an XSelect HSS T3 column (2.5 μm, 2.1 mm × 150 mm). Data were acquired in both positive and negative ionization modes using Analyst 1.6.3 software, and metabolite identifications were made by referencing a database of known plant metabolites. Differentially accumulated metabolites (DAMs) were identified using orthogonal partial least squares discriminant analysis (OPLS-DA), with significance thresholds of VIP ≥ 1 and fold change ≥ 2 or ≤ 0.5.

Pathway enrichment analysis

Pathway enrichment of DAMs was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to identify key metabolic pathways associated with cold stress responses. DAMs were annotated with KEGG compound IDs based on their chemical structure and known roles in plant metabolism, using fold change values and thresholds (VIP ≥ 1, fold change ≥ 2 or ≤ 0.5, and $p \leq 0.05$) to filter significant metabolites. The KEGG Mapper tool was employed for pathway mapping to visualize the involvement of DAMs in biological processes, with the hypergeometric test calculating the statistical significance of pathway enrichment. Top pathways identified included arginine and proline metabolism, glycine, serine, and threonine metabolism, flavonoid and flavonol biosynthesis, plant hormone biosynthesis, and 2-oxocarboxylic acid metabolism, prioritized based on impact scores from topological analysis. Visualizations such as bubble charts and KEGG pathway diagrams highlighted enriched pathways and DAM abundance. This comprehensive approach enabled insights into the critical biochemical mechanisms underlying oil palm's adaptation to low-temperature stress. Pathway enrichment of DAMs was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Enrichment analysis identified key metabolic pathways associated with cold stress responses.

Statistical analysis

Data were analyzed using IBM SPSS Statistics (version 21). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to determine significant differences ($p \leq 0.05$) between treatments. Correlation analysis was conducted using Pearson's regression model to identify relationships between physiological indicators and key metabolites.

Result

Morphological and growth responses of oil palm seedlings to Low-Temperature stress

Figure 1 illustrates the progressive effects of low-temperature stress on oil palm seedlings, highlighting visible morphological and growth responses. The whole-seedling responses, show that control plants maintain strong growth with well-developed leaves, while stressed seedlings exhibit progressive reductions in leaf expansion, stunting, wilting, and necrosis, particularly under higher stress levels (CD4 and CD8) (Fig. 1A). Leaf samples reveal that control (CK) plants exhibit healthy, uniformly green leaves, while stress treatments (CD05, CD1, CD2, CD4, and CD8) lead to progressively severe chlorosis, streaking, and necrosis, indicating escalating cellular damage (Fig. 1B). These results demonstrate that low-temperature stress significantly disruptsphysiological functions and growth in oil palm, with severity intensifying as stress levels increase. The findings emphasize the need for further physiological and metabolomic studies to uncover stress tolerance mechanisms and develop strategies for mitigating cold-induced damage.

Classification of cold damage in oil palm

All plants were placed under low temperature stress and then placed under natural conditions to resume growth for one month, and then the symptoms of cold damage (wilting, drying, dry and rotten leaves) of oil palm leaves were observed every day, and the cold damage grades were divided into three grades according to the observation results (Table 1).

Physiological and antioxidative responses of oil palm seedlings under low-temperature stress

The study examines the physiological and antioxidative responses of oil palm seedlings subjected to low-temperature stress, with stress severity increasing from CK (control) to CD8 (Fig. 1C-G). Relative electrolyte leakage (REL) progressively increases across treatments, indicating membrane destabilization and damage, with the highest levels observed in CD8. Malondialdehyde (MDA) content, a marker of lipid peroxidation, also rises significantly, reflecting enhanced oxidative stress under severe conditions. Proline content shows a substantial increase, peaking at CD8, emphasizing its role as an osmoprotectant in alleviating stress-induced cellular damage. Peroxidase (POD) activity increases initially, reaching a peak at CD1, but subsequently declines under higher stress levels, suggesting potential enzyme saturation or reduced efficient under prolonged stress. Similarly, superoxide dismutase (SOD) activity increases under mild stress (CD05 and CD1) but decreases under severe conditions (CD4 and CD8), indicating its critical role in ROS scavenging during early stages of stress. These findings demonstrate the disruption of cellular integrity, oxidative balance, and the activation of adaptive responses in oil palm seedlings under low-temperature stress, providing insights into their physiological and antioxidative mechanisms for stress tolerance.

Hormonal changes under cold stress

In response to cold stress, abscisic acid (ABA) levels in oil palm exhibited a significant increase,, particularly during the initial stages of stress exposure (CD05 and CD1). This elevation is consistent ABA's established role in stress response mechanisms, including the promotion of stomatal closure and the accumulation of osmolytes, such as proline, which contribute to the maintenance of and stress mitigation. The data show that ABA concentrations rise from 1.2 μg/g (CD05) to 2.2 μg/g (CD8), with a pronounced increase observed in the early stages (CD05 to CD2), indicating a strong hormonal response to cold (Fig. 1H). On the other hand, gibberellins (GA) showed a marked decrease during the later stages of cold treatment (CD4 and CD8), which suggests a reduction in growth-related processes and an energy conservation strategy. GA concentrations dropped from 0.8 μg/g (CD05) to 0.3 μg/g (CD8), indicating a suppression of growth processes as the plant redirects resources toward stress adaptation. The suppression of GA during cold stress supports the hypothesis that the plant prioritize survival over growth under adverse condition. The observed dynamics in ABA and GA are consistent with the findings in other plants species, where ABA acts as a stress hormone, enhancing tolerance mechanisms, while reduced GA levels facilitates the reallocation of resources from growth to survival [18, 31]. The interplay between ABA and GA in oil palm under cold stress thus appears to regulate the balance between stress adaptation and the trade-off between growth and survival, highlighting the complex hormonal coordination underlying plant responses to environmental stress.

Metabolomic profiles

In this study, a total of 1121 metabolites were identified in oil palm leaves, which were catagorized into 21 distinct subclasses based on their chemical characteristics (Supplementary Table 1) To ensure data consistency and comparability, metabolite content was normalized, and a

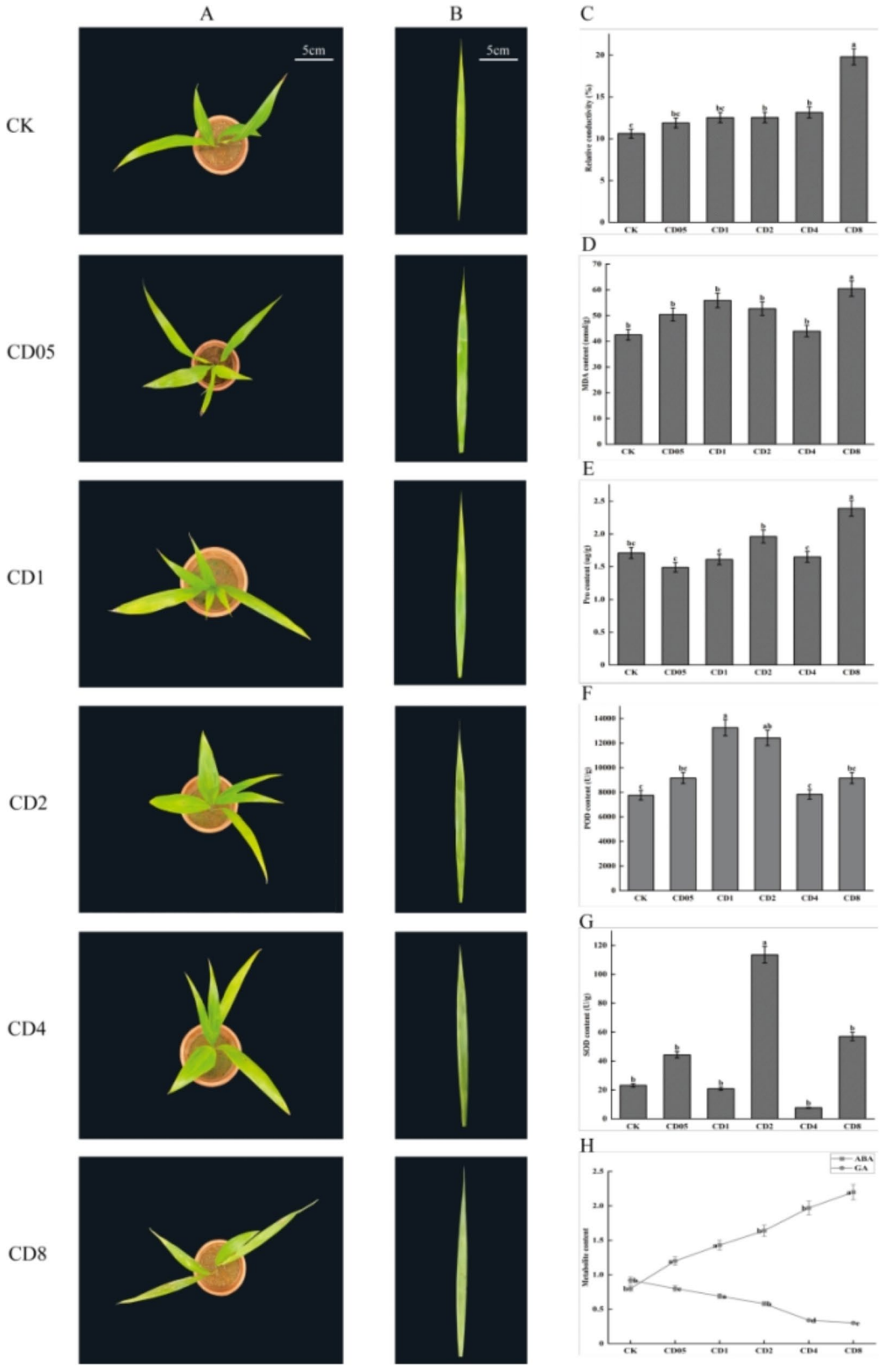

Fig. 1 Morphological and physiological characteristics of oil palm seedlings under chilling stress. oil palm seedlings exposed to low temperature 8 °C for 8 h. **(A)** Morphological traits of whole plant. **(B)** Morphological traits of leaves. **(C)** Relative conductivity. **(D)** MDA content. **(E)** Pro content c. **(F)** POD content. **(E)** SOD content. **(H)** Hormonal content

Table 1

Cold injury level	Cold damage symptoms
Level 1	The canopy leaves and lance leaves had no symptoms of cold damage; The growth point is not damaged, and the growth can be restored after 1 ~ 2 weeks.
Level 2	Some leaves in the crown were damaged, and the leaf margins and tips of some leaflets in the affected leaves fade green to yellow; The leaves of the guns are dry; The growth point is not damaged, and the growth can be restored after 1 ~ 2 months
Level 3	All the leaves in the crown were damaged, and all the leaflets in 1 ~ 3 of the affected leaves were damaged (the leaflets dried up and turned brown or the rest were brown except for the main vein and its vicinity, and the leaf margins and tips of some leaflets in the remaining leaves fade green to yellow; The leaves of the guns are dry; The growth point is not damaged, and the growth can be restored after 2 ~ 3 months

hierarchical clustering heat map (Fig. 2A) and principal component analysis (Fig. 2B) were constructed. These analyses were visualized the metabolomic profile of oil palm seedlings exposed to different cold-treatment time points, revealing substantial differences across the six cryo-treatment stages. To identify differentially accumulated metabolites (DAMs), a combined threshold of VIP values (VIP ≥ 1) and fold change (Fold Change ≥ 2 or ≤ 0.5), was applied, allowing for the identification of the most significantly altered metabolites under cold stress. The VIP plots (Fig. 2C) further elucidated the expression trends of DAMs across each comparison group, providing insights into the dynamic metabolite responses induced by the cold treatments (Supplementary Fig. 1).

Differential metabolite expression and venn analysis of oil palm responses to cold stress

The metabolic responses of oil palm to cold stress were assessed through the analysis of metabolite changes across different treatment stages. Figure 2D shows the number of upregulated (red) and downregulated (blue) metabolites under each condition. From the control (CK) to the initial treatment (CD05), 34 metabolites were upregulated, while 77 were downregulated, indicating a suppression of metabolic activity. Between CD05 and CD1, 70 metabolites were upregulated and 25 downregulated, suggesting an activation phase. A moderate metabolic reprogramming was observed between CD1 and CD2 with 37 metabolites upregulated and 53 downregulated. More pronounced suppression occurred from CD2 to CD4, where 20 metabolites were upregulated and 67 downregulated. Finally, from CD4 to CD8, 56 metabolites were upregulated and 30 downregulated, suggesting partial recovery (Supplementary Table 2). These findings underscore the dynamic and complex nature of metabolic responses in oil palm under cold stress, with distinct phases of suppression, activation, and recovery.

The Venn diagram (Fig. 2E) illustrates the distribution of metabolites in oil palm across five comparative groups. Each group contains unique metabolite with. 15 in CK-vs-CD05, 19 in CD1-vs-CD2, and 10 in CD4-vs-CD8. Shared metabolites such as 7 between CK-vs-CD05 and CD05-vs-CD1 or 9 among the intermediate groups, indicate active metabolic pathways across multiple stages. Notably, 15 metabolites are common to all five groups, suggesting their role in core adaptive pathways under cold stress.

Metabolite correlation analysis under cold stress conditions

The heatmap presents a correlation matrix of metabolites involved in the oil palm's response to cold stress, with correlation coefficients ranging from −1 to +1, as indicated by the color gradient (Fig. 3A). Positive correlations (red) indicate metabolites that increase or decrease in tandem, often reflecting shared biochemical pathways or complementary functional roles, In contrast, negative correlations (blue) suggest opposing metabolic trends, highlighting potential trade-offs during stress adaptation. Clusters of metabolites, such as amino acids (e.g., Phenylalanine, Arginine, L-Ornithine) and energy-related compounds (e.g., D-Fructose 6-phosphate), demonstrate their coordinated roles in critical processes, including amino acid metabolism, energy production, and cellular repair. Conversely, metabolites associated with flavonoid synthesis, show distinct correlations pattern, emphasizing their role in mitigating oxidative stress under cold conditions. Central metabolites like Arginine and Phenylalanine emerge as key regulators, with their strong correlations suggesting pivotal roles in metabolic adjustments during stress. This analysis reinforces the importance of pathways like flavonoid biosynthesis and amino acid metabolism in oil palm's cold stress response, providing valuable insights into the metabolomic mechanisms undelying stress tolerance.

Dynamic changes in key differential metabolite levels under low-temperature stress in oil palm

The Table 2 presents the relative contents of key differential metabolites in oil palm seedlings under six cryo-treatment time points: 0 h (CK), 0.5 h (CD05), 1 h (CD1), 2 h (CD2), 4 h (CD4), and 8 h (CD8). Metabolite levels exhibit distinct patterns, highlighting both early and late responses to cold stress. Sugars and sugar phosphates such as D-Fructose 6-phosphate show a significant increase during cold exposure, reflecting their roles in stress signaling and carbohydrate metabolism. Amino acids such as L-Tryptophan display pronounced

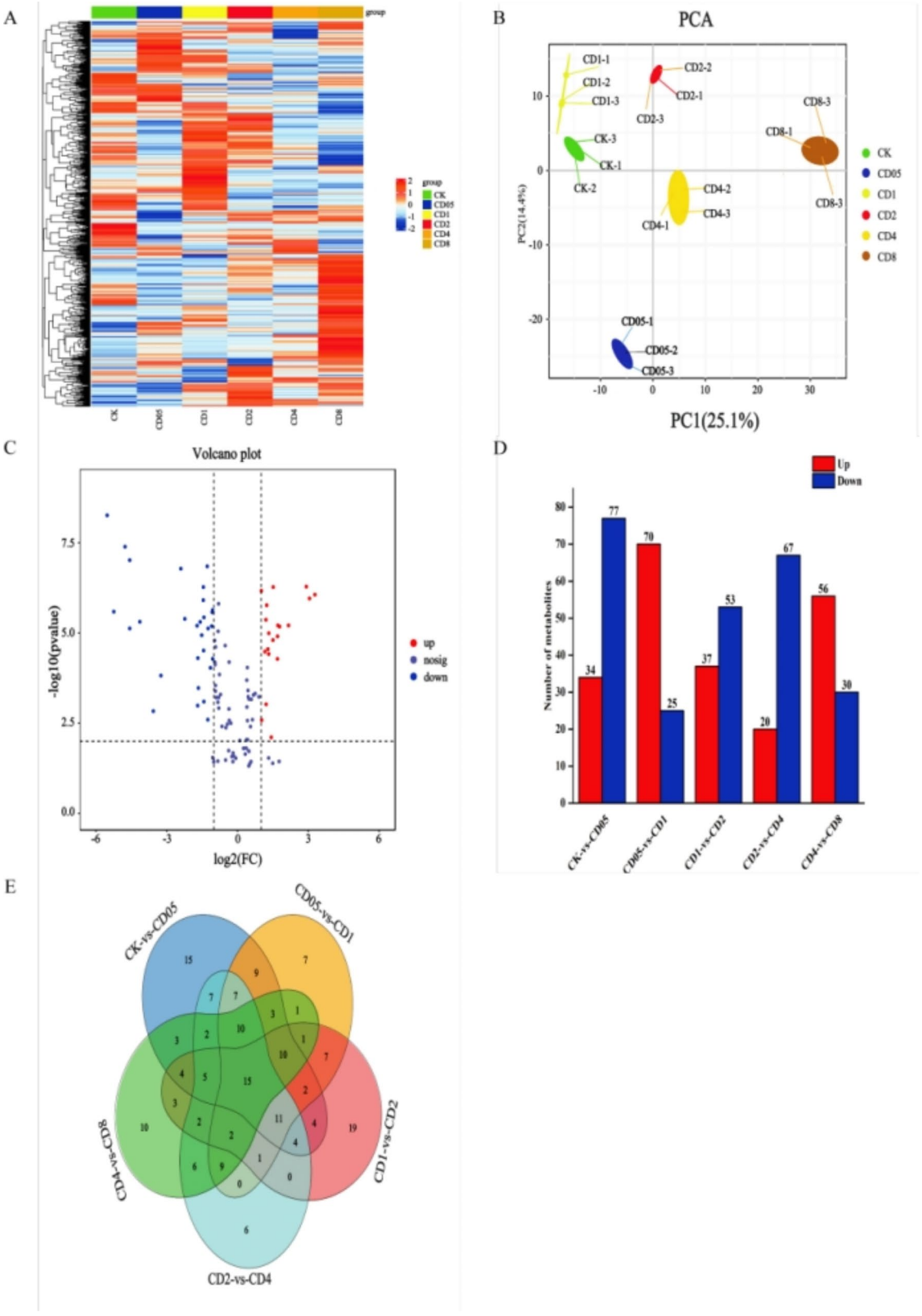

Fig. 2 (See legend on next page.)

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Fig. 2 Metabolic analysis of oil plam under cold stress **(A)** Cluster heat map of six experimental groups, CK (control), CD05, CD1, CD2, CD4, and CD8(Experimental group) Each row represents a metabolite, while columns correspond to the different experimental conditions. The color gradient reflects normalized abundance, where red signifies upregulation, blue indicates downregulation, and white denotes intermediate levels relative to the control (CK). **(B)** PCA Analysis. **(C)** Differential metabolite volcanic map. **(D)** The number of DAMs in oil palm tenera at different development experimental groups. **(E)** Venn diagram of the number of DAMs in various comparisons (CK-vs-CD05, CD05-vs-CD1, CD1-vs-CD2, CD2-vs-CD4 and CD4-vs-CD8)

fluctuations, with notable accumulation at later stage (e.g., L-Tryptophan at 8 h), suggesting their involvement in nitrogen metabolism, osmoprotection, and secondary metabolite synthesis. Organic acids like Phenylalanine Acid exhibit dynamic changes, with initial declines followed by recovery at later stage, highlighting its roles in energy pathways and stress adaptation. Flavonoid derivative compounds such as 4'-O-Glucosylvitexin and Chrysoeriol 7-O-hexoside show temporal variation, consistent with their antioxidant and protective functions unders stress. Statistical analyses (superscripts) reveal significant differences across time points. Metabolites with highly variability, such as Dl-Indole-3-lactic acid demonstrating active participation in cold stress responses, while stable metabolites like L-Dihydroorotic Acid suggest a consistent role in maintaining cellular homeostasis. Overall, these results illustrate the complex metabolic shifts in oil palm under cold stress. Early responses focus on immediate energy production and stress signaling, while and later stages emphasize recovery processes and the biosynthesis of secondary metabolites, underscoring the plant's adaptive strategies to low temperature stress.

Hierarchical clustering heatmap of metabolite profiles across experimental groups under low temperature

The heatmap analysis illustates the metabolomic response of oil palm to low-temperature stress, exhibit variations in metabolite abundance across different experimental conditions (Fig. 3B). Rows represent individual metabolites, while columns correspond to treatment groups, including the control (CK) and stress conditions (CD05, CD1, CD2, CD4, CD8). A gradient color scale used to depict metabolite levels, with red indicating high abundance, blue representing low levels, and white representing baseline. Hierarchical clustering reveals shared patterns, such as similar trends for L-Dihydroorotic acid and 4'-O-Glucosylvitexin, suggesting common regulatory pathways. Notable findings include elevated L-Phenylalanine acid levels in CD05 and CD1, reflecting its potential role in stress adaptation, and consistently low levels of D-Fructose 6-Phosphate, implying suppressed glycolytic activity under cold stress. The control group (CK) serves as a baseline for comparison, while outliers like L-Tryptophan exhibit distinct abundance trends, indicating specific metabolic responses. These results reveal significant shifts in amino acid metabolism, energy related pathways, and stress-related metabolites, providing insights into the physiological and metabolic mechanisms underlying cold stress tolerance in oil palm.

Time-Series clustering of metabolites in oil palm under low-temperature stress

The time-series clustering analysis of metabolites in oil palm under low-temperature stress, reveals four distinct clusters based on standardized expression profiles across six cryo-treatment time points: CK (control), CD05 (0.5 h), CD1 (1 h), CD2 (2 h), CD4 (4 h), and CD8 (8 h) (Fig. 3C-F). Class 1, comprising 51 metabolites, shows a gradual increase, peaking at CD4 before stabilizing or slightly declining, These metabolites are likely involved in mid-phase responses aimed at maintaining cellular homeostasis during prolonged cold stress. Class 2, consisting of 27 metabolites, exhibits a delayed response, with levels peaking at CD8, likely associated with late-phase adaptation mechanisms such as repair processes or biosynthesis of protective compounds. Class 3 includes 40 metabolites with a rapid early rise (CD05 and CD1), followed by stabilization or a decline, representing early response metabolites involved in immediate stress mitigation, such as antioxidant defense or osmotic adjustment. Class 4, the smallest group with 13 metabolites, shows a moderate increase up to CD4, followed by a sharp rise at CD8, indicating roles in both sustained and late-phase cold adaptation. Thin gray lines depict individual metabolite trajectories, while thick black lines represent average trends for each cluster, highlighting the temporal coordination of metabolite responses to facilitate oil palm adaptation to low-temperature stress.

Impact of low stress treatments on the relative concentrations of 21 key metabolites in oil palm (tenera variety)

Low-temperature stress induces significant changes in the metabolome of oil palm (Tenera variety), as evidenced by the relative concentrations of 21 key metabolites following an 8-hour exposure to varying stress treatments. The study revealed distinct metabolic shifts, indicating complex adaptive responses of oil palm to cold stress. For instance, metabolites such as Dl-Indole-3-lactic acid and 6-Aminocaproic acid exhibited significantly higher concentrations in treatments CD05 and CD8, suggesting their potential protective role in mitigating cold-induced damage. The accumulation of 6-Aminocaproic acid, a compound known to be involved in stress responses, especially under more prolonged cold

stress, indicates an enhanced defensive mechanism in these treatments. Conversely, metabolites associated with membrane structure and lipid metabolism, such as LysoPC 18:1 (2n isomer) and LPC (1-acyl 18:1), were found at significantly lower concentrations in treatments like CD1 and CD2. This suggests that cold stress compromises membrane integrity, possibly impairing lipid signaling and disrupting normal cellular functions. Additionally, secondary metabolites, including Chrysoeriol derivatives and Tricin 5-O-hexoside, exhibited altered concentrations, particularly in treatments CD2 and CD4. These metabolites, often involved in antioxidant activity, may contribute to mitigating oxidative damage caused by cold exposure. The study also observed variations in lipid-related compounds like MAG (18:3) and 2-alpha-Linolenoyl-glycerol, which play important roles in membrane stability and signaling under stress conditions. The data suggests that cold stress triggers complex metabolic responses, involving both primary metabolism (lipid and phospholipid regulation) and secondary metabolism (flavonoids and stress-related compounds), highlighting potential targets for improving cold tolerance in oil palm. Overall, the study contribute valuable insights into the biochemical impacts of cold stress on oil palm, contributing to deeper understanding of its adaptive mechanisms. These findings could inform breeding strategies aimed at developing more resilient oil palm varieties capable of withstand fluctuating environmental conditions.

KEGG enrichment analysis of dams

KEGG enrichment analysis of differential accumulated metabolites (DAMs) in oil palm leaves under low-temperature stress identified 74 key metabolic pathways involved in the plant's response to cold stress across different treatment times (Supplementary Table 3). The top 20 most enriched pathways in each comparison group were selected based on enrichment and topological analysis (Fig. 4), revealing co-enriched pathways such as 2-Oxocarboxylic acid metabolism, Arginine and proline metabolism, Glycine, serine, and threonine metabolism, Alanine, aspartate, and glutamate metabolism, Aminoacyl-tRNA biosynthesis, Arginine biosynthesis, Biosynthesis of alkaloids derived from ornithine, lysine, and nicotinic acid, and Biosynthesis of amino acids across the five comparisons. Additionally, Lysine biosynthesis and the Citrate cycle (TCA cycle) were co-enriched in the CK-vs-CD05 comparison, while the CD1-vs-CD2 comparison showed a greater number of co-enriched pathways, including butanoate metabolism, flavone and flavonol biosynthesis, and insulin resistance. In contrast, the CD4-vs-CD8 comparison had fewer enriched pathways, such as biosynthesis of plant hormones, phenylalanine, tyrosine, and tryptophan biosynthesis, and biosynthesis of alkaloids derived from the shikimate pathway (Fig. 4; Table 3). Notably, 2-Oxocarboxylic acid metabolism was consistently co-enriched, and pathways like Arginine and proline metabolism (ko00330), Flavone and flavonol biosynthesis (ko00944), Biosynthesis of plant hormones (ko01070), and Glycine, serine, and threonine metabolism (ko00260) emerged as the most frequently co-enriched, highlighting their critical roles in cold stress adaptation. Significant enrichment was also observed in pathways such as arginine and proline metabolism (KO00330), which supports osmoprotection and stress mitigation; glycine, serine, and threonine metabolism (KO00260), which maintains amino acid balance; plant hormone biosynthesis (KO01070), which regulates growth and stress responses; flavonoid and flavonol biosynthesis (KO00944), which enhances antioxidant capacity to counteract oxidative stress; and 2-oxocarboxylic acid metabolism (KO01210), which underscores the importance of amino acid metabolism and energy production under stress. Collectively, these findings demonstrate that oil palm employs a broad spectrum of metabolic pathways—ranging from amino acid metabolism to secondary metabolism and hormone signaling—to enhance its tolerance to low-temperature stress, enabling comprehensive metabolic reprogramming that ensures adaptation and survival under challenging environmental conditions.

Arginine and proline metabolic pathways

The temporal dynamics of key metabolites in the arginine and proline metabolic pathways of oil palm leaves under low-temperature stress (Fig. 5a). Six major metabolites—arginine, D-proline, γ-aminobutyric acid (GABA), ornithine, N-feruloylputrescine, and L-hydroxyproline—were significantly enriched. Arginine, D-proline, and GABA showed significant accumulation during the early phase (0.5–1 h), suggesting their roles in the initial stress response, likely through stabilizing cellular components and contributing to osmotic regulation. In contrast, N-feruloylputrescine and L-glutamic acid were enriched at later time points (4–8 h), indicating their involvement in long-term adaptation processes such as stress signaling and nitrogen metabolism. The differential timing of metabolite accumulation reveals a multi-phase metabolic response. Early-stage enrichment focuses on osmoprotection and antioxidant defense, while later responses emphasize stress signaling and metabolic regulation, collectively enhancing the plants's tolerance cold stress.

Glycine, serine, and threonine metabolic pathways

The enrichment of key metabolites in the glycine, serine, and threonine metabolic pathways of oil palm leaves under low-temperature stress reveals significant insights into plants adaptive mechanisms. Six metabolites—D-serine, L-tryptophan, L-aspartic acid,

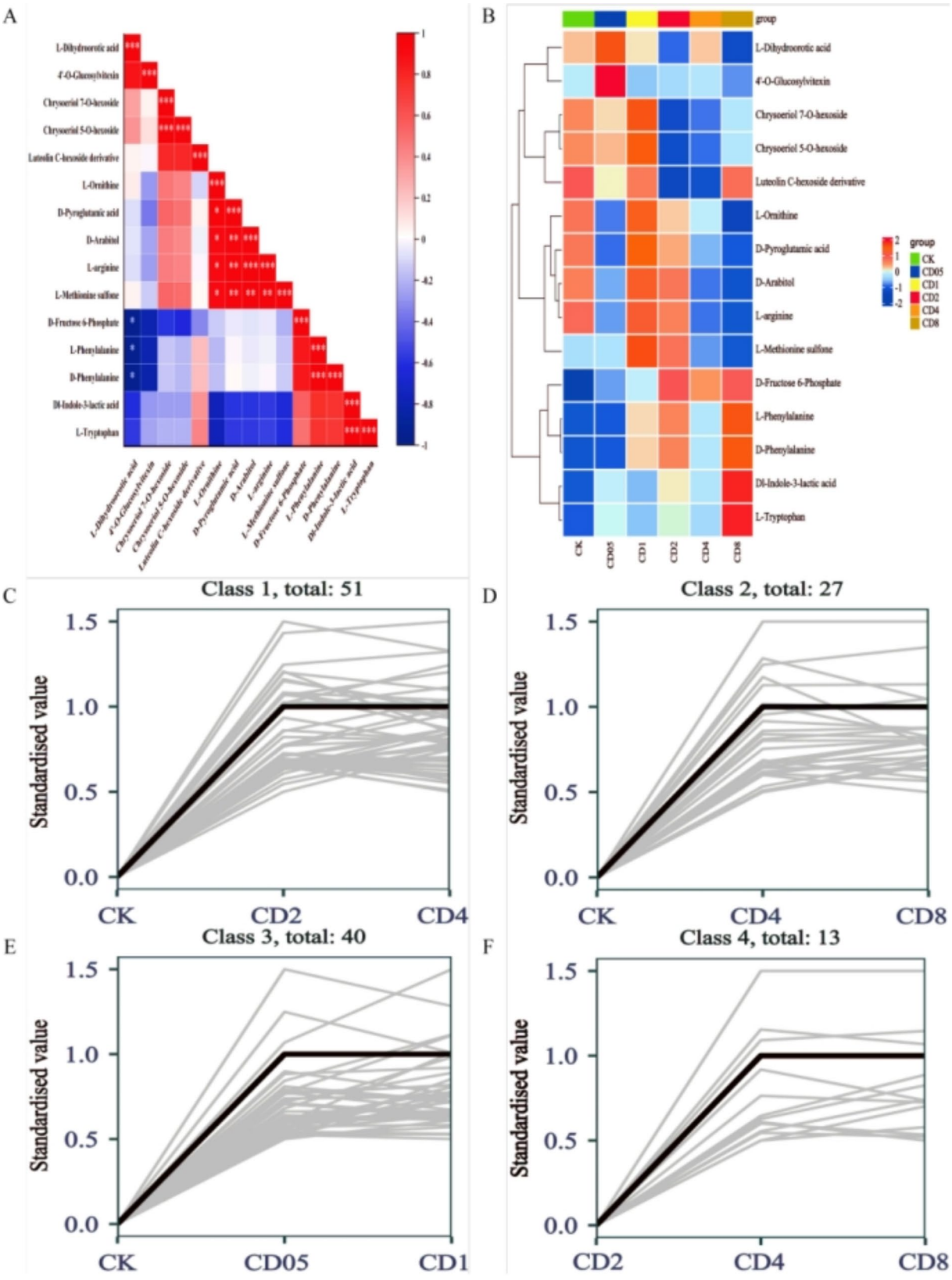

Fig. 3 (See legend on next page.)

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Fig. 3 Metabolic analysis of oil plam under cold stress. **(A)** Heat Map of Metabolites Clustering in Tenera Oil Palm Under Cold Stress During Different experimental Periods ($n = 15$). **(B)** Heatmap illustrates the normalized abundance of metabolites across six experimental groups (CK, CD05, CD1, CD2, CD4, and CD8). Each row corresponds to a metabolite, and each column represents a group. The color gradient reflects metabolite abundance levels, with red indicating high levels, blue indicating low levels, and white representing intermediate levels ($n = 15$). (**C**-**F**) Time-series clustering of metabolites in oil palm under low-temperature stress. The clusters represent groups of metabolites exhibiting temporal expression patterns during the stress response. Each line within a cluster represents a metabolite's expression profile, while the bold line indicates the average trend for the cluster

N,N-dimethylglycine, sarcosine, and betaine—were significantly enriched in at least three comparison groups, indicating their central role in the plant's stress response (Fig. 5b). Notably, the abundance of these metabolites showed distinct temporal changes: L-tryptophan, most enriched between 4 and 8 h, suggesting its involvement in late-stage adaptation, potential related to protein synthesis or stress response regulation. In contrast, L-aspartic acid was enriched early (0–0.5 h), reflecting its potential role in immediate metabolic adjustments or amino acid biosynthesis under cold stress. Sarcosine showed a significant increase at 1–2 h, indicating its potential role in methylation processes and protein regulation during stress. The temporal variation in metabolite accumulation highlights dynamic nature of plant's metabolic response. Different pathways were activated at specific time points enabling the plant to adapt cold stress effectively. Early responses focus on immediate metabolic adjustments, while later stages emphasize long-term adaptation and stress mitigation, collectively supporting the plant's survival under low-temperature conditions.

2-oxocarboxylic acid metabolic pathway

The 2-oxocarboxylic acid metabolic pathway plays a critical role in the response of oil palm leaves' to low-temperature stress, as demonstrated by the significant enrichment of four key metabolites: L-leucine, L-glutamic acid, L-tryptophan, and L-phenylalanine (Fig. 5c). These metabolites serve as pivotal intermediates in amino acid biosynthesis and energy metabolism. Under cold stress, their abundance exhibited distinct temporal patterns, reflecting the pathway's dynamic involvement in stress adaptation. L-phenylalanine and L-glutamic acid levels increased significantly between 1 and 2 h, suggesting their early involvement in enhancing protein synthesis and nitrogen metabolism. Similarly, the contents of L-leucine and L-tryptophan were significantly elevated between 4 and 8 h, indicating their role in sustaining energy production and secondary metabolite biosynthesis during later stages of stress. This temporal modulation of metabolite levels highlights the importance of 2-oxocarboxylic acid metabolic pathway in coordinating cellular metabolism and energy demands. By dynamically regualting these processes, the pathway enhances the plant's resilience to low temperature ensuring adapation under challenging environmental conditions.

Biosynthetic pathways of plant hormones

The study highlights the enrichment of five key metabolites—L-aspartic acid, L-phenylalanine, citric acid, isocitrate, and phosphoenolpyruvate—in the biosynthetic pathways of plant hormones under low-temperature stress (Fig. 5d). These metabolites are essential for the synthesis of hormones that regulate plant growth and stress response. Notably, citric acid and isocitrate metabolites showed significant increases within the first 0.5 h of cold stress, suggesting an early response in the plant's metabolic reprogramming. As key components of the tricarboxylic acid (TCA) cycle, which plays a central role in energy production and provide intermediates for hormone biosynthesis. The L-phenylalanine enriched at 8 h, this metabolites is a precursor to several plant hormones, including auxins and phenolic compounds suggesting its involvement in later stages of stress adaption. The accumulation of these metabolites suggests that the plant dynamically adjusts its metabolic pathways, particularly those related to energy production and hormone regulation, to enhance cold stress tolerance and maintain growth under adverse conditions.

Flavonoid and flavonol biosynthesis pathways

The flavonoid and flavonol biosynthesis pathways in oil palm leaves exhibit significant enrichment of four key metabolites—vitexin, isovitexin, apigenin 6-C-glucoside, and vitexin-2-O-rhamnoside—under low-temperature stress as illustrated in Fig. 5e. These metabolites, which are important flavonoids and flavonols, play a vital role in antioxidant defense and stress protection. The significant increase in their levels between 4 and 8 h of low-temperature stress suggests their involvement in the later stages of the plant's adaptive response. Flavonoids and flavonols, such as those identified in the study, are well known for their ability to neutralize reactive oxygen species (ROS) and mitigate oxidative damage caused by environmental stresses like cold. The temporal increase in these metabolites highlights the plant's strategy to enhance its antioxidant capacity and improve stress resilience by accumulating secondary metabolites, particularly during the later phases of the stress response.

Low-temperature stress triggred the activation of several key amino acid metabolism pathways in oil palm, resulting in a significant increase in amino acids such as L-tryptophan, L-glutamic acid, L-aspartic acid, and sarcosine. These amino acids likely instrumental in

Table 2 Relative concentration of 21 key metabolites, after low temperature stress treatments for 8 h

Key differential metabolites	Key differential metabolites content at different periods					
	CK	CD05	CD1	CD2	CD4	CD8
L-Dihydroorotic acid	111.17 ± 2.25a	101.4 ± 2.82b	81.45 ± 6.06c	67.5 ± 0.88d	82.84 ± 1.13c	62.92 ± 0.8d
4′-O-Glucosylvitexin	55.77 ± 0.98b	94.18 ± 3.85a	32.59 ± 0.89d	36.86 ± 0.4c	39.99 ± 0.9c	26.78 ± 0.31e
Chrysoeriol 7-O-hexoside	11.09 ± 0.43b	6.95 ± 0.19c	11.85 ± 0.13a	2.33 ± 0.07f	3.48 ± 0.13e	5.72 ± 0.09d
Chrysoeriol 5-O-hexoside	10.4 ± 0.35b	6.99 ± 0.37c	11.02 ± 0.22a	2.11 ± 0.07f	3.17 ± 0.12e	5.39 ± 0.23d
Luteolin C-hexoside derivative	20.84 ± 0.74a	11.12 ± 0.25c	14.06 ± 0.85b	3.7 ± 0.16d	4.74 ± 0.13d	14.68 ± 2.63b
L-Ornithine	9.96 ± 0.14a	2.18 ± 0.13d	9.76 ± 0.25a	5.73 ± 0.11b	4.64 ± 0.17c	0.42 ± 0.05e
D-Pyroglutamic acid	36.62 ± 6.08a	4.87 ± 0.21c	38.94 ± 5.59a	22.74 ± 2.33b	8.83 ± 1.5c	3.09 ± 0.05c
D-Arabitol	132.97 ± 11.3a	36.98 ± 1.66c	133.27 ± 6.73a	103.35 ± 3.3b	27.01 ± 1.27c	14.02 ± 4.06d
L-arginine	62.6 ± 2.44a	11.75 ± 0.24d	56.8 ± 5.02b	42.21 ± 2.73c	8.29 ± 0.27de	3.55 ± 0.59e
L-Methionine sulfone	17.3 ± 0.39c	13.17 ± 0.33d	27.17 ± 2.7a	20.58 ± 0.3b	9.68 ± 0.38e	7.29 ± 0.27e
D-Fructose 6-Phosphate	16.7 ± 0.87e	22.69 ± 1.6d	29.99 ± 0.82c	42.3 ± 0.95a	36.26 ± 1.6b	41.84 ± 1.57a
L-Phenylalanine	25.05 ± 0.18e	19.97 ± 0.11f	42.86 ± 0.9c	51.71 ± 0.62	35.83 ± 0.7db	71.5 ± 1.12a
D-Phenylalanine	26.94 ± 0.14e	22.04 ± 0.67f	47.34 ± 1.03c	56.09 ± 1.09b	39.23 ± 0.51d	76.41 ± 2.82a
Dl-Indole-3-lactic acid	11.84 ± 0.05f	35.64 ± 1.11c	21.58 ± 0.25e	40.71 ± 1.23b	31.36 ± 0.54d	89.45 ± 1.88a
L-Tryptophan	5.51 ± 0.18e	18.54 ± 0.5b	10.51 ± 0.14d	19.25 ± 0.47b	13.13 ± 0.43c	45.73 ± 1.16a
MAG (18:3)	10.36 ± 0.08d	11.09 ± 0.24c	12.75 ± 0.31a	11.7 ± 0.18b	10.45 ± 0.32d	8.11 ± 0.17e
LysoPC 18:1 (2n isomer)	4.58 ± 0.09a	2.2 ± 0.02c	1.88 ± 0.04d	2.21 ± 0.08c	2.24 ± 0.08c	3.77 ± 0.06b
LPC(1-acyl 18:1)	3.84 ± 0.03a	1.79 ± 0.05c	1.51 ± 0.05d	1.76 ± 0.11c	1.83 ± 0.06c	3.08 ± 0.07b
2-alpha-Linolenoyl-glycerol	14.05 ± 0.24bc	10.39 ± 0.35e	15.83 ± 1.07a	14.94 ± 0.58ab	12.25 ± 0.38d	13.15 ± 0.46 cd
Tricin 5-O-hexoside	15.94 ± 0.38c	21.63 ± 0.87b	23.6 ± 1.33a	6 ± 0.26e	8.28 ± 0.14d	7.47 ± 0.05de
6-Aminocaproic acid	2.84 ± 0.11c	1.76 ± 0.23d	4.67 ± 1.07b	6.08 ± 0.07a	4.71 ± 0.2b	6.71 ± 0.06a

These major metabolites were selected using Student's t test ($P < 0.05$), VIP value > 1.0, and ratio > 2.0 or < 0.5. Each treatment had three biological replicates

promoting plant growth and enhancing cold resistance by supporting essential cellular functions and metabolic processes. Additionally, the accumulation of metabolites like citric acid, isocitrate, and phosphoenolpyruvate in the plant hormone biosynthesis pathway helps in regulating the tricarboxylic acid (TCA) cycle, enhancing overall metabolic activity and improving the plant's ability to withstand low temperatures. Moreover, the significant increase in metabolites related to flavonoid and flavonol biosynthesis, including vitexin, isovitexin, apigenin 6-C-glucoside, and vitexin-2-O-rhamnoside, suggests that these secondary metabolites play a significant role in strengthening the plant's antioxidant defense system. This metabolic reprogramming enables the plant to effectively manage oxidative stress and enhance cold tolerance, ultimately improving its ability to survive and adapt to low-temperature conditions.

Discussion

This study explored the physiological and metabolomic responses of oil palm to low-temperature stress, focusing on mechanisms that enhance cold tolerance. The integration of physiological measurements and metabolomic analyses, the research revealed crucial insights into how oil palm adapts to adverse conditions.

Physiological adaptations to low-temperature stress

The study found that the activity of superoxide dismutase (SOD) and peroxidase (POD) increased under low-temperature stress, suggests that these antioxidant enzymes play a central role in mitigating reactive oxygen species (ROS) accumulation. Similar results have been reported in crops like rice and maize, where enhanced antioxidant enzyme activity was essential for combating oxidative damage caused by cold stress [21–22]. ROS, which are produced during stress, can harm cellular structures, but elevated antioxidant enzyme activity helps maintain cellular balance. Additionally, the levels of proline (Pro) increased over time, reflecting its function as an osmoprotectant and its role in stabilizing proteins and membranes under stress conditions. This finding is consistent with previous result on plant like, wheat, and Arabidopsis, where proline accumulation correlated with enhanced stress tolerance and facilitated osmotic adjustment and ROS scavenging under cold conditions [23]. Interestingly, malondialdehyde (MDA) levels initially decreased and later increased, indicating a pattern of dynamic membrane lipid peroxidation during stress progression. The initial decrease may indicate effective ROS scavenging and membrane stabilization by antioxidant systems during the initial phase of stress. However, the subsequent increase in MDA levels suggests that prolonged cold stress eventually overwhelms these protective mechanisms, leading to increased oxidative damage and lipid peroxidation. The gradual increase in relative conductivity over extended stress periods further emphasize the impact of cold stress on membrane stability. These findings demonstrate that oil palm seedlings

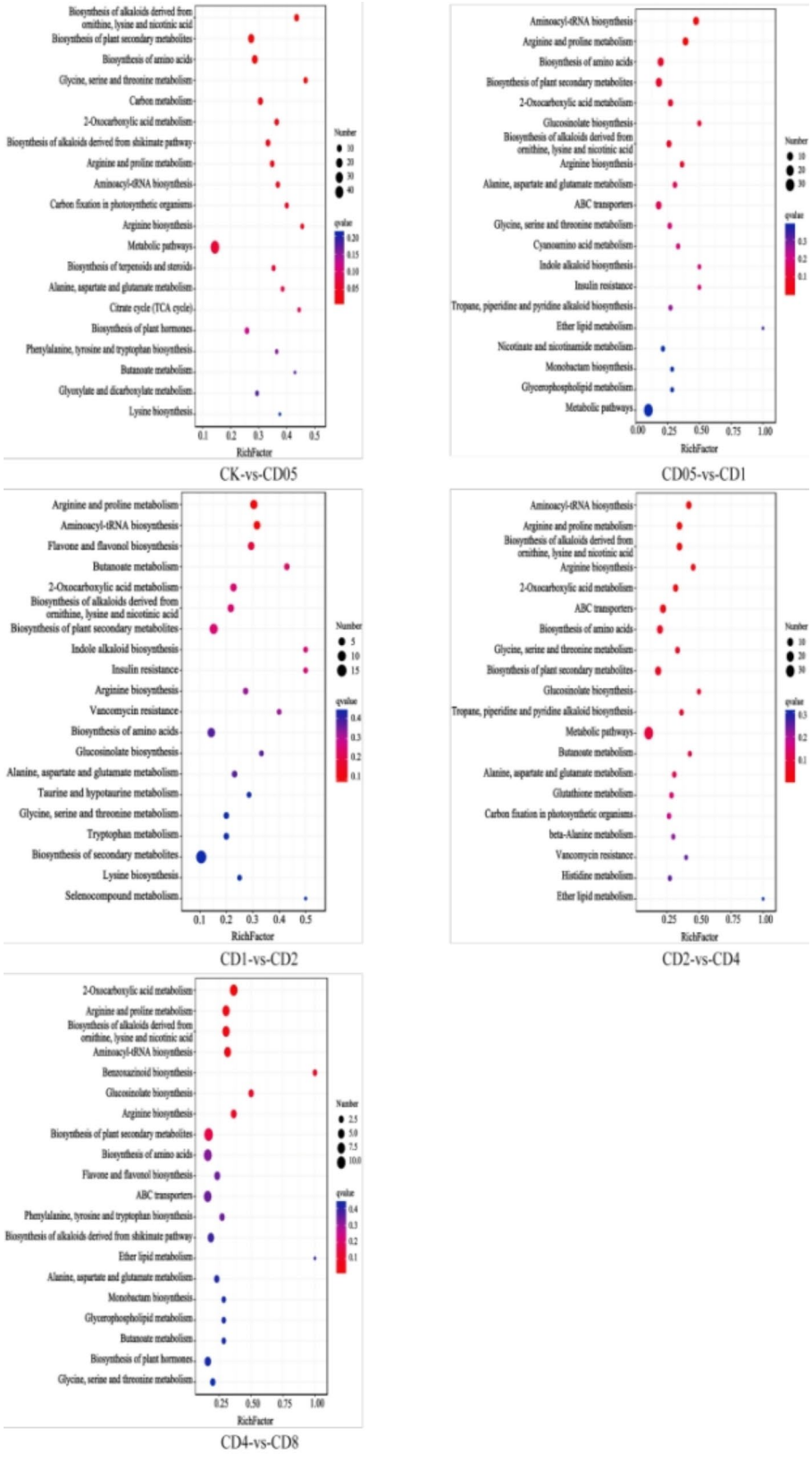

Fig. 4 KEGG pathway enrichment analysis of DAM in different comparison groups

Table 3 Coenriched pathways of dams in oil palm under chilling stress

Comparison	KEGG ID	Pathways
Total	ko01210	2-Oxocarboxylic acid metabolism
	ko00250	Alanine, aspartate and glutamate metabolism
	ko00970	Aminoacyl-tRNA biosynthesis
	ko00330	Arginine and proline metabolism
	ko00220	Arginine biosynthesis
	ko01064	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid
	ko01230	Biosynthesis of amino acids
	ko00260	Glycine, serine and threonine metabolism
CK-vs-CD05	ko00300	Lysine biosynthesis
	ko00020	Citrate cycle (TCA cycle)
C05-vs-CD1	ko02010	ABC transporters
	ko00966	Glucosinolate biosynthesis
CD1-vs-CD2	ko00650	Butanoate metabolism
	ko00944	Flavone and flavonol biosynthesis
	ko04931	Insulin resistance
CD2-vs-CD4	ko00565	Ether lipid metabolism
	ko00960	Tropane, piperidine and pyridine alkaloid biosynthesis
	ko01502	Vancomycin resistance
	ko00710	Carbon fixation in photosynthetic organisms
CD4-vs-CD8	ko00400	Phenylalanine, tyrosine and tryptophan biosynthesis
	ko01063	Biosynthesis of alkaloids derived from shikimate pathway
	ko01070	Biosynthesis of plant hormones

possess an intrinsic capacity to adjust their physiological responses to survive under low temperatures conditions.

Metabolomic insights into cold tolerance

The metabolomic analysis revealed 469 differentially accumulated metabolites (DAMs) across different treatment durations, providing a comprehensive overview of the metabolic changes in response to cold stress. These DAMs were enriched in several key metabolic pathways, including amino acid metabolism, flavonoid biosynthesis, and plant hormone biosynthesis—each of which plays a critical role in stress adaptation and resilience to cold.

Amino acid metabolism in cold tolerance in oil palm

The metabolomic analysis of oil palm revealed significant enrichment of metabolites involved in arginine and proline metabolism, as well as glycine, serine, and threonine metabolism, emphasizing the vital role of amino acids in cold stress adaptation. In oil palm, amino acids such as L-aspartic acid, L-tryptophan, and L-glutamic acid were found to contribute to cellular repair and ROS scavenging. These amino acids serve as precursors for essential metabolites that help maintain cellular integrity under cold stress. Similar metabolic responses have been documented in other cold-stressed crops, including wheat, kiwifruit, and rice, where the accumulation of these amino acids has been associated with improved cold tolerance and reduced oxidative damage [17, 24]. Proline, in particular, exhibited significant accumulation in oil palm, reinforcing its crucial role in allevating cold stress. As a compatible solute, proline plays a key role in maintaining osmotic balance within cells during freezing conditions, protecting cellular structures from damage caused by ice formation. Additionally, proline act as an effective ROS scavenger, neutralizing harmful free radicals generated during cold stress, thereby protecting cellular components like proteins and membranes. It also stabilizes proteins and cell membranes, ensuring proper folding and preventing denaturation under low temperatures.These mechanisms collectively enhance the plant's ability to withstand and adapt to cold stress conditions.

In oil palm, amino acid metabolism not only helps maintain osmotic balance but also plays an important role in cellular defense mechanisms. The regulation of pathways such as the shikimate pathway contributes to the synthesis of additional metabolites involved in stress responses. For example, L-tryptophan, a precursor of auxins, may modulate stress-related signaling pathways, further enhancing cold tolerance by influencing growth inhibition and activating defense mechanisms. Overall, the metabolic pathways related to amino acids in oil palm are essential for sustaining cellular functions and improving the plant's resilience to prolonged cold exposure.

Flavonoid biosynthesis in cold stress adaptation in oil palm

Flavonoids such as vitexin, isovitexin, and apigenin 6-C-glucoside were found to accumulate significantly in oil palm under cold stress, indicating their crucial role

Fig. 5 (See legend on next page.)

(See figure on previous page.)

Fig. 5 Main metabolic pathways of oil palm plants under low temperature stress at different treatment times. **(a)** Differentially accumulated metabolites (DAMs) involved in the arginine and proline metabolism pathways under low temperature treatment. **(b)** DAMs involved in the glycine, serine, and threonine metabolism pathways under low temperature treatment. **(c)** DAMs involved in the 2-oxocarboxylic acid metabolism pathway under low temperature treatment. **(d)** DAMs involved in the biosynthesis pathway of plant hormones under low temperature treatment. **(e)** DAMs involved in the flavonoid and flavonol biosynthesis pathways under low temperature treatment. The yellow ellipses indicate differential metabolites with varying content, while the white ellipses represent differential metabolites with the same content. The rectangles represent the differential metabolites and are divided into five comparison groups: CK-vs-CD05, CD05-vs-CD1, CD1-vs-CD2, CD2-vs-CD4, CD4-vs-CD8 (from left to right). The color scale of the rectangles indicates the degree of metabolite accumulation or depletion under low temperature treatment, with intensity reflecting the extent of change

in oxidative stress resistance. These flavonoids exhibit potent antioxidant properties, which help counteract oxidative damage by scavenging reactive oxygen species (ROS) generated during cold exposure. Consistent with this, previous studies on tea plants, flavonoids were shown to be induced and expressed under low temperature stress, and may be involved in the response to low temperature stress, and thereby enhancing the cold tolerance [25]. The upregulation of these metabolites may serve as a protective mechanism against low-temperature stress, with increased flavonoid biosynthesis potentially act as a key defense strategy for oil palm. These secondary metabolites contribute to stabilizing cell membranes, preventing lipid peroxidation, and enhancing the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase. Together, these mechanisms strengthen the plant's ability to defend against oxidative stress and improve its resilience to cold conditions.

The role of flavonoids in oil palm under cold stress extends to membrane stabilization, a critical factor for maintaining cellular function under low temperatures. Membrane fluidity is particularly important in cold conditions, and flavonoids help preserve membrane integrity by protecting cellular components from damage. This membrane-stabilizing effect, coupled with enhanced antioxidant enzyme activity, significantly contributes to improving cold tolerance and overall plant resilience.By preventing lipid peroxidation and supporting the structural integrity of cell membranes, flavonoids play a vital role in enabling oil palm to withstand and adapt to cold stress conditions.

Additionally, the dynamic changes in flavonoid levels across different treatment durations in oil palm suggest their dual role in cold stress adaptation. During the initial phase of cold exposure, flavonoids may act as part of the early stress response, rapidly scavenging ROS and stabilizing membranes to mitigate immediate damage. As cold stress persists, flavonoids accumulation becomes more pronounced, supporting prolonged defense mechanisms, and maintaining cellular integrity over extended periods of exposure. This temporal regulation of flavonoid biosynthesis highlights their importance in enhancing oil palm's ability to survive under prolonged cold stress. The result were consistent with previous studies showing that flavonoid levels vary depending on the intensity and duration of low temperature stress [26]. For instance, flavonoid content has been observed to increase with the duration of cold stress [27]. Additionally, low temperature stress treatment has been shown to enhance the flavonoid biosynthesis pathway at the metabolic level in seedlings [17]. Similarly, Wu et al. found that the KEGG pathway was enriched in plant hormone signaling, phenylpropanoid, and flavonoid biosynthesis in camellia under low temperature stress [28]. In oil palm, flavonoid accumulation plays a multifaceted role in cold stress adaptation. It helps protect against oxidative damage, stabilizes cellular membranes, and support antioxidant defense systems, ensuring that the plant can maintain its physiological functions and resist damage despite prolonged low temperature stress. Therefore, metabolomic analysis in this study reveals that the dynamic changes in flavonoid levels are the key factor contribute to oil palm's resilience under low-temperature stress conditions.

Plant hormone biosynthesis

In oil palm, abscisic acid (ABA) and gibberellins (GA) serve as pivotal regulators of stress responses, particularly under environmental challenges such as drought, cold, or salinity. These hormones are integral to complex signaling networks that help the plant balance stress tolerance with growth and development. Studies have shown that *Arabidopsis thaliana* and rice exhibit a significant increase in ABA content in leaves under low temperatures [29]. ABA is primarily recognized as a stress hormone, promoting stomatatal closure to prevent water loss and inducing the accumulation of osmolytes (e.g., proline, sugars) to maintain cell turgor under stress conditions [30]. Beyond stress tolerance, ABA also regulates oil biosynthesis during fruit development, influencing the accumulation of lipids and fatty acids that are essential for palm oil production. The dynamic regulation of ABA is crucial for managing the plant's trade-off between activating stress responses and sustaining growth, ensuring survival under harsh conditions. Conversely, GA plays a key role in promoting growth and cell elongation, However during prolonged stress, GA levels are downregulated, which helps conserve energy by limiting growth and redirecting resources toward survival mechanisms. Metabolome analysis showed that ABA levels were upregulated during the initial 0–8 h of low temperature stress, suggesting that the early phase of the cold exposure may trigger ABA production In cotton, increased in

ABA levels contributes for adaptation to low temperature stress [31] Similarly, in Arabidopsis, increased ABA levels were associated with enhanced cold tolerance [32].These findings are consistent with the current study, suggesting that increased levels of ABA may enhance the cold tolerance in oil palm. In addition to ABA,, gibberellins play a significant role in various physiological processes such as light signal transduction, the accumulation of non-structural carbohydrates and proteins, and the induction of calcium-dependent protein kinase gene expression [33]. GA also enhances the stability of plant cell membranes and improve the water-retention capacity of leaves [34]. The homeostasis of active GA content in plants is regulated by core components of the GA signaling pathway, such as DELLA proteins (e.g., RGA, GAI, RGL1/2/3), the F-box protein SLEEPY1, and the soluble GA receptor protein GID1 [35–36]. In Arabidopsis, GA content typically decreases under low temperature stress, but the accumulation of DELLA protein can enhance plant tolerance to cold stress [37]. Furthermore, the DELLA protein also help inhibit the production of ROS induced by abiotic stress, thereby improving the plant's recovery ability [38]. In oil palm, GA plays an important role in fruit development and oil production. Reductions in GA under stress conditions may modulate fruit maturation and oil biosynthesis, contributing to the plant's ability to redistribute energy. The suppression of GA during stress emphasizes the plant's strategy of prioritizing survival over growth, conserving resources and managing energy more efficiently. In this study, GA expression levels showed a decreasing trend during the 0–8 h treatment period. speculated that GA content in oil palm may be negatively correlated with prolonged low temperature stress [39–40]. The balance between ABA and GA is essential for coordinating stress responses and growth. While ABA promotes stress adaptation, GA typically supports growth and development, with their opposing yet complementary actions creating a dynamic equilibrium that enables oil palm to survive and adapt to fluctuating environmental conditions. This hormonal balance is particularly significant in oil palm, where environmental stress may influence both the yield and quality of palm oil. Understanding the interaction between ABA and GA could provide valuable insights into enhancing stress tolerance and optimizing oil production efficiency.

Conclusion

This research provides a foundational framework for understanding the molecular and physiological responses of oil palm to low-temperature stress. By identifying key metabolites and pathways, the research highlights potential targets for developing cold-tolerant oil palm varieties through genetic engineering or biotechnological approaches. The cold-tolerance mechanisms in oil palm are complex and highly integrated, involving critical metabolites such as citric acid, L-tryptophan, and vitexin, as well as the upregulation of amino acids and flavonoids. These components work in concert with hormonal regulation to fine-tune metabolic processes, enabling the plant to mitigate cold stress through ROS, detoxification, membrane stabilization, and energy conservation. The identification of the 2-oxocarboxylic acid metabolism as a potentially unique pathway for oil palm under cold stress is a novel finding that warrants further exploration. This comprehensive insight into the cold-tolerance mechanisms of oil palm provides a valuable foundation for developing strategies to enhance its resilience in cooler climates. By leveraging these findings, it may be possible to improve the productivity and sustainability of oil palm cultivation in regions prone to low-temperature stress, ultimately supporting global agricultural demands.

Abbreviations

SOD	Superoxide Dismutase
POD	Peroxidase
REL	Relative electrolyte leakage
MDA	Malondialdehyde
ABA	Abscisic Acid
GA	Gibberellins
ROS	Reactive oxygen species
GABA	γ-aminobutyric acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06292-5.

Supplementary Material 1: Fig. 1. VIP score plots show the trends and expression levels of differentially accumulated metabolites (DAMs) for five control groups.

Supplementary Material 2: Table 1. All sample data and detailed information of the twenty-one categories metabolites.

Supplementary Material 3: Table 2. Five comparison groups up-down DAMs statistics.

Supplementary Material 4: Table 3. Five comparison groups the KEGG pathways of DAMs enrichment degree.

Author contributions

YS, XL (Xiaoyu liu), MH, MR, WX conducted the experiments and performed the data analysis. WL and HC organized and supervised the overall project. XL (Xinyu Li) guided the analysis of experimental data. YS Writing– Original Draft. JJJM Writing– Review & Editing the manuscript. All authors contributed to the article and approved the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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