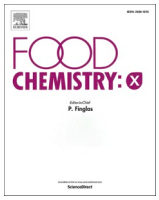
Food Chemistry: X 23 (2024) 101619

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Unraveling the metabolic profile regulation of camellia oilseeds under insect and heat stress: Insights into functional effects and mechanistic basis

Qingyang Li [a,b,1], Wei Zhu [c,1], Shiman Sun [a], Maokai Cui [a], Wei Zhang [a], Jinping Shu [a], Runhong Mo [a], Fubin Tang [a], Yirong Guo [b], Yihua Liu [a,*]

[a] Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang 311400, PR China
[b] Institute of Pesticide and Environmental Toxicology, Key Laboratory of Biology of Crop Pathogens and Insects of Zhejiang Province, Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Zhejiang University, Hangzhou, Zhejiang 310058, PR China
[c] Key Laboratory of Precision Diagnosis and Treatment for Hepatobiliary and Pancreatic Tumor of Zhejiang Province, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, 310009, PR China

ARTICLE INFO

Keywords:
Camellia oilseed
Metabolome
Insect stress
Heat stress
Function

ABSTRACT

There is very little information on the impacts of pre/post-harvest stresses on oilseeds. Individual and combined insect (pre-harvest) and heat stress (post-harvest) impacts on the metabolic profile of camellia oilseeds (COs) were investigated using a combination of widely-targeted metabolomics and network pharmacology. A total of 1875 metabolites were identified. In response to individual and combined stresses, 169 (insect),149 (heat), and 21 (insect + heat) metabolites were screened as differential metabolic markers (DEMs), Terpenoids, phenolic acids, and flavonoids are the most impacted metabolite species, accounting for almost 49% of total DEMs. Then network pharmacological analysis identifies 98 key active ingredients (AIs) in CO. A single stress may induce CO to impede cardiovascular system function, but the combined stress induced AI-promoting effects of CO in the urinary system. The individual and combined perturbed biological mechanisms were related to the flavonoid biosynthesis and the biosynthesis of various plant secondary metabolites pathway, respectively.

1. Introduction

Oil extracted from Camellia oilseeds (COs), also known as 'eastern olive oil', is a popular edible oil in China, India, Japan, and Korea (Guo et al., 2022). CO, which is high in monounsaturated fatty acids, has gained popularity in worldwide markets in recent years due to its nutritional and medicinal properties (T. Shi, Wu, Jin, & Wang, 2020). COs contain abundant bioactive compounds, including phytosterol polyphenols, fat-soluble vitamins, and other useful elements (T. Shi et al., 2020). These bioactive compounds possess various anti-disease activities, such as cardiovascular and tumor prevention due to their pharmacological features, which include antioxidant benefits (Hu, Zhang, Xing, Yu, & Chen, 2022). Previous research focused on the precise compositions and functions of some specific components (mostly fatty acids) in CO (T. Shi et al., 2020) and the influencing factors (He, Wu, Liu, Huang, & Zhang, 2022; M. Wang et al., 2022), however the comprehensive profiles of CO's metabolome remain unclear.

Apart from pre-harvest insect stresses, oilseeds are also susceptible to post-harvest heat stresses, particularly during the processing stage (Oliete, Lubbers, Fournier, Jeandroz, & Saurel, 2022; Xu et al., 2022; D. Zhu, Guan, Fan, Sun, & Wang, 2022). The functional health components of oilseeds are also often destroyed by these insect/heat stresses, which lessens the oilseeds' health benefits and economic value (Memoli et al., 2017; Valencic, Butinar, Podgornik, & Bucar-Miklavcic, 2021). Reports of this detrimental effect have concentrated on the main primary metabolites, which include amino acids, organic acids, and sugars (Anzano, Bonanomi, Mazzoleni, & Lanzotti, 2022). According to a review, changes in amino acid levels have been linked to stresses on important European crops such as wheat, maize and barley (Kopecka, Kameniarova, Cerny, Brzobohaty, & Novak, 2023). The application of metabolomics technology has increased the finding of secondary metabolite responses to stresses in recent years (Silva et al., 2021; D. Zhu et al., 2022). Secondary metabolites such as phenols, flavonoids, and terpenoids play essential roles in plant defenses against stresses such as UV-B radiation, low temperatures, salt, and heavy metal stress via ROS detoxification (Zhuang et al., 2023). Numerous studies have also

* Corresponding author.
E-mail address: liuyh@caf.ac.cn (Y. Liu).
[1] Contributed to this article equally and are co-first authors.

https://doi.org/10.1016/j.fochx.2024.101619
Received 21 March 2024; Received in revised form 3 June 2024; Accepted 2 July 2024
Available online 3 July 2024

demonstrated that some flavonoid compounds act as phytoalexins against pathogenic bacteria, fungi, and nematodes (Zhuang et al., 2023). However, in comparison to cereals and fruits, little research has been conducted to investigate the function of metabolite alterations against stress in oilseeds utilizing the metabolomics technique (Li et al., 2022; Silva et al., 2021).

Previous stress studies, with or without the use of metabolomics techniques, have placed more emphasis on the response of primary/secondary metabolites to stresses (Li et al., 2022; Silva et al., 2021; D. Zhu et al., 2022). However, there is little information on the corresponding changes in primary and secondary metabolite function (physiology and health) induced by these complex effects (Li et al., 2023; Xia et al., 2023). More importantly, because drying is the most important step in the postharvest processing of oilseeds, the effects of heat stress cannot be ignored, as evidenced by reports of different drying temperature stresses in oilseeds such as soybean (D. Zhu et al., 2022), walnut (Li et al., 2023), and peanut (Klevorn & Dean, 2018).

While oilseed crops subjected to insect stress remain susceptible to heat stress during the post-harvest drying phase, the need to establish effective control measures for this combined stress effect in the food production chain has not been thoroughly characterized. Herein, we conducted a metabolomic profiling experiment to investigate the effects of insect and heat stresses on the quality of CO. We also used bioinformatics analyses such as network pharmacology to understand how these stresses impact the health functions of CO. Our aims were to: (1) examine the effects of insect and heat stresses on CO; (2) explore the combined transmission effects of insect and heat stresses in the food production chain; and (3) assess the potential health-related consequences of the effects. This study will not only contribute to a more thorough understanding of the effects of controlled insect/heat stress on COs composition and health functions but will also provide new insights into the combined effects induced by multiple stresses in the food production chain, emphasizing the importance of effective control measures in food control research.

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade methanol (MeOH) and acetonitrile (ACN), which were purchased from Merck in Hangzhou, China. Formic acid (FA) was purchased from Merck in Aladdin, China. Other solvents were of analytical grade and were purchased from Shanghai GuoYao Chemical Reagents (Shanghai, China).

2.2. Preparation of camellia oilseeds samples

The material for this study was obtained from the *Camellia oleifera* Abel Experimental Base in Qingtian County, Lishui City, Zhejiang Province (120.3814378E, 28.20055022 N), and the variety was 'Changlin No. 4'. All samples were taken from 9- to 11-yearold trees of the base during mature stage. About 20 kg of camellia fruits with the same degree of dehiscence (to ensure the same degree of maturity) were randomly collected from the collection sites. Camellia oilseeds were divided into non-infested oilseeds of non-infested fruit, non-infested oilseeds of infested fruit, and infested oilseeds based on the presence of insect-dug holes on the shells of camellia fruits and seed shells.

After manually removing the fruit shells, the three types of camellia oilseeds were divided into two sections and dried by freeze-drying and hot-air drying until the moisture content of the camellia oilseeds was between 6 and 8%. Six different camellia oilseeds were obtained (Fig. S1): CLA: non-infested oilseeds of non-infested fruit under freeze-drying; CLB: non-infested oilseeds of infested fruit under freeze-drying; CLC: infested oilseeds under freeze-drying; CHA: non-infested oilseeds of non-infested fruit under hot-air drying; CHB: non-infested oilseeds of infested fruit under hot-air drying; CHC: infested oilseeds under hot-air drying. Following that, the oilseed shells were manually cracked, and the oleaginous seeds were ground to a powder under liquid nitrogen and stored at −80 °C until analyzed.

2.3. Sample extraction

The seed samples were ground to powder form using a Retsch MM 400 grinder (Verder Scientific, Shanghai, China), for 1.5 min at 30 Hz. A 50 mg portion of the sample powder was extracted with 1200 μL of 70% methanol in water contain 2-chlorophenylalanine (purity: 98%) that had been previously cooled to −20 °C. The mixture was vortexed every 30 s for 30 min. This procedure was repeated 6 times. The samples were then centrifuged (Centrifuge 5424R, Eppendorf, Germany) at 12,000 rpm for 3 min. The supernatant was filtered through a 0.22 μm membrane (microfiltration membrane, JINTENG, Tianjin, China) into an autosampler vial for UPLC-MS/MS analysis.

2.4. UPLC conditions

The sample extracts were analyzed using a UPLC-ESI-MS/MS system (UPLC, ExionLC™ AD; MS, Applied Biosystems 6500 Q TRAP). The analytical conditions were as follows: Column: Agilent SB-C18 (1.8 μm, 2.1 mm × 100 mm); Mobile phase: Solvent A, pure water with 0.1% formic acid; Solvent B, acetonitrile with 0.1% formic acid; Gradient program: The specific elution procedure settings can be found in Shen et al. (Shen et al., 2024). Column oven temperature: 40 °C; Injection volume: 2 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

2.5. ESI-q trap-MS/MS

The ESI source operation parameters were as follows: Source temperature: 500 °C; Ion spray voltage (IS): 5500 V (positive ion mode) / -4500 V (negative ion mode); Ion source gas I (GSI), gas II (GSII), curtain gas (CUR): 50, 60, and 25 psi, respectively.

Collision-activated dissociation (CAD): High; QQQ scans were acquired as MRM (Multiple Reaction Monitoring) experiments. Collision gas: Nitrogen, set to medium.

DP (declustering potential) and CE (collision energy) for individual MRM transitions were optimized. A specific set of MRM transitions was monitored for each period based on the eluted metabolites within that period.

2.6. Identification of active ingredients (AIs) in COs by bioinformatics analysis

The compounds with potential bioactive activity were searched for Traditional Chinese Medicine Database and Analysis Platform (TCMSP) with integrated criterion of oral bioavailability (OB) ≥ 25% and druglike (DL) ≥ 0.07 (Li et al., 2023).

2.7. Data analysis

Statistical significance of all data was determined by SPSS22.0. In order to visualize group separation and find significantly changed metabolites, principal component analysis (PCA) and OPLS-DA was applied. The differential metabolic markers (DEMs) were determined based on Student's *t*-test with a permutation-based FDR q-value of 0.1 and S0 (Harel et al., 2019). The identified metabolites were annotated using KEGG compound database, and then mapped to the KEGG pathway database. The data visualization was performed using the Metware Cloud (https://cloud.metware.cn), Cytoscape_v3.10.1, and Origin2019. Based on the data of the AIs in the samples, further perturbed metabolic pathway analyses were performed using MetaboAnalyst 6.0.

3. Results

3.1. Widely targeted metabolomic profiling of COs

A widely targeted metabolite analysis was conducted for comprehensive metabolic profiling of COs using UPLC-MS/MS. According to Fig. 1A, whereas 1875 metabolites were identified and classified into at least 54 unique groups (most of them fell into just 7 categories). The primary metabolites of these are amino acids and derivatives (8.21%), lipids (7.20%), and nucleotides and derivatives (4.48%). Phenolics (45.07%), alkaloids (8.00%), terpenoids (6.40%), lignans (2.56%), and coumarins (1.65%) are the most abundant secondary metabolites. The most abundant metabolite, 1805, was found in CHA; in contrast, metabolites from CHC, CLC, CHB, CLA and CLB were found in 1802, 1800, 1797, 1796, and 1785, respectively. Despite the discovery of 1670 metabolites across all groups (Fig. 1B), their abundance varied. These findings suggest that the metabolic profiles of the six group samples differed significantly.

3.2. Characterization of COs metabolome in response to insect stress

3.2.1. Identification of metabolite markers of response to insect stress

To examine the metabolite composition of COs between the insect stress treatment groups, metabolite data were submitted to multivariate analysis. The two principal's components (PC1, 44.32%, PC2, 18.82%; Fig. S2 A) well divided the three sample groups (CLA, CLB, and CLC), showing a significant difference in the metabolites between the groups. The OPLS-DA model can filter out orthogonal variables in metabolites that are irrelevant to the classification variables to enhance group differentiation and discover differential metabolites. Similarly, OPLS-DA demonstrated a distinct separation of these metabolite groups (Fig. S2B, C, D). As a result, a variable importance in the projection (VIP) > 1 was utilized to identify 800, 990, and 1007 differentially metabolites (DE) in the CLA vs CLB, CLA vs CLC and CLB vs CLC groups, respectively. This suggests that insect stress has a considerable impact on the metabolome profile of COs.

Furthermore, to screen for differential metabolic markers (DEMs), a more stringent principle of Student's *t*-test; permutation-based FDR q value <0.1, S0 $= 0.1$ was used. There were 50 DEMs discovered between CLA and CLB (Fig. 2A), 169 DEMs discovered between CLA and CLC (Fig. 2B), and 174 DEMs discovered between CLB and CLC (Fig. 2C). The Venn-UpSet diagram demonstrates that the various comparison groups share and have separate DEMs (Fig. 2D). Obviously, there were 110 common DEMs (45% of total DEMs) in CLA vs CLC and CLB vs CLC, indicating that insect damage had a consistent influence on the majority of the differential metabolites in two healthy oilseeds. These DEMs were predominantly focused on amino acids and derivatives (8), free fatty acids (10), organic acids (10), phenolic acids (18) and triterpenes (24).

3.2.2. Enrichment metabolic pathways analysis

The KEGG database was used to annotate and conduct enrichment analysis of the common differential metabolites among three types of oilseeds in order to investigate the physiological processes associated with DEMs. The DEMs between CLA and CLB were significantly ($p < 0.05$) enriched in diterpenoid biosynthesis as well as cysteine and methionine metabolism (Fig. 2E). The DEMs of CLA vs CLC were significantly ($p < 0.05$) involved in lipid metabolic (Linoleic acid metabolism, alpha-Linolenic acid metabolism) (Fig. 2F). In addition to these metabolic pathways, it was discovered that the DEMs was significantly enriched in pathways of glutathione metabolism and lysine degradation in CLB vs. CLC group (Fig. 2G). These pathway enrichment analyses imply that the insect has an impact on the amino acid metabolism of healthy seeds within infested fruit.

3.3. Characterization of COs metabolome in response to heat stress

3.3.1. Identification of metabolite markers of response to heat stress

To investigate the effect of drying temperature on metabolic profiles in oilseeds during processing, the metabolic profiles of three types of oilseeds was compared under freeze-drying (CL) and hot-air drying (CH). The OPLS-DA analysis first revealed that the temperature effect was evident for either type of oilseed, and the contribution of the sum of the first two principal components of the three types of oilseeds was >60% (Fig. S3). Based on the stringency of VIP > 1, heat stress induced considerably fewer DEs (835: CLA vs CHA, 854: CLB vs CHB, 881: CLC vs CHC) than insect stress (990: CLA vs CLC).

A total of 297 DEMs were identified between CL and CH groups using the more stringent principle (Fig. 3A, B, C), including 138, 149, and 107 DEMs that upregulate in CHA, CHB, and CHC, respectively, and 11, 31, and 18 DEMs that downregulate in CHA, CHB, and CHC, respectively. According to the Venn-UpSet diagram (Fig. 3D), the different comparison groups share 41 common DEMs (primarily 13 amino acids and derivatives, 6 phenolic acids, and 6 nucleotides and derivatives). In addition, when only the common DEMs of CLA vs CHA and CLB vs CHB were considered, up to 89 DEMs were discovered. This suggests that the effect of heat stress on metabolome profiles in both types of healthy seeds were equivalent. It is worth emphasizing that, unlike the DEMs induced by insect stress, which are secondary metabolites like terpenes and phenolic acids. On both types of healthy seeds, the primary metabolites were the focus of 89 common DEMs induced by heat stress.

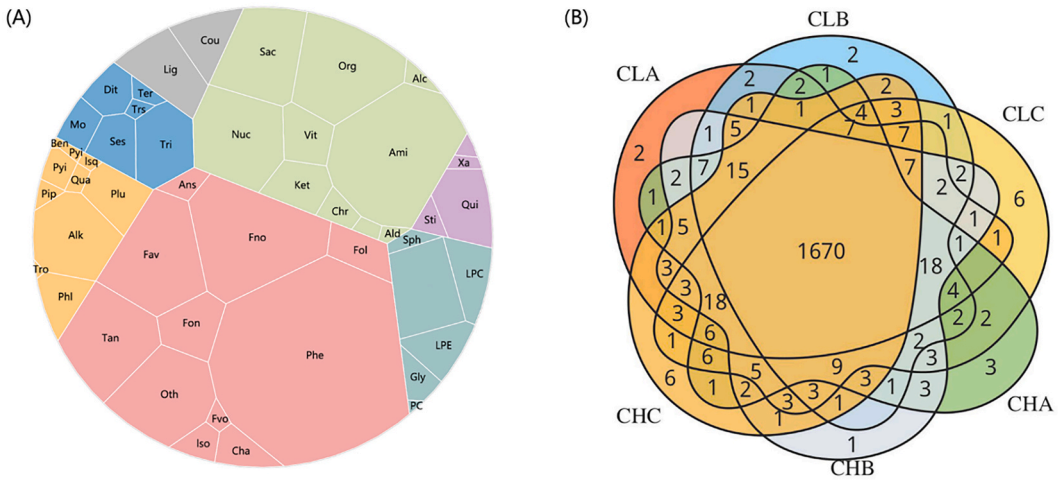


Fig. 1. (A) The categories of metabolites in camellia oilseeds. (B) Petalograms of metabolic profiles for six types of camellia oilseeds.

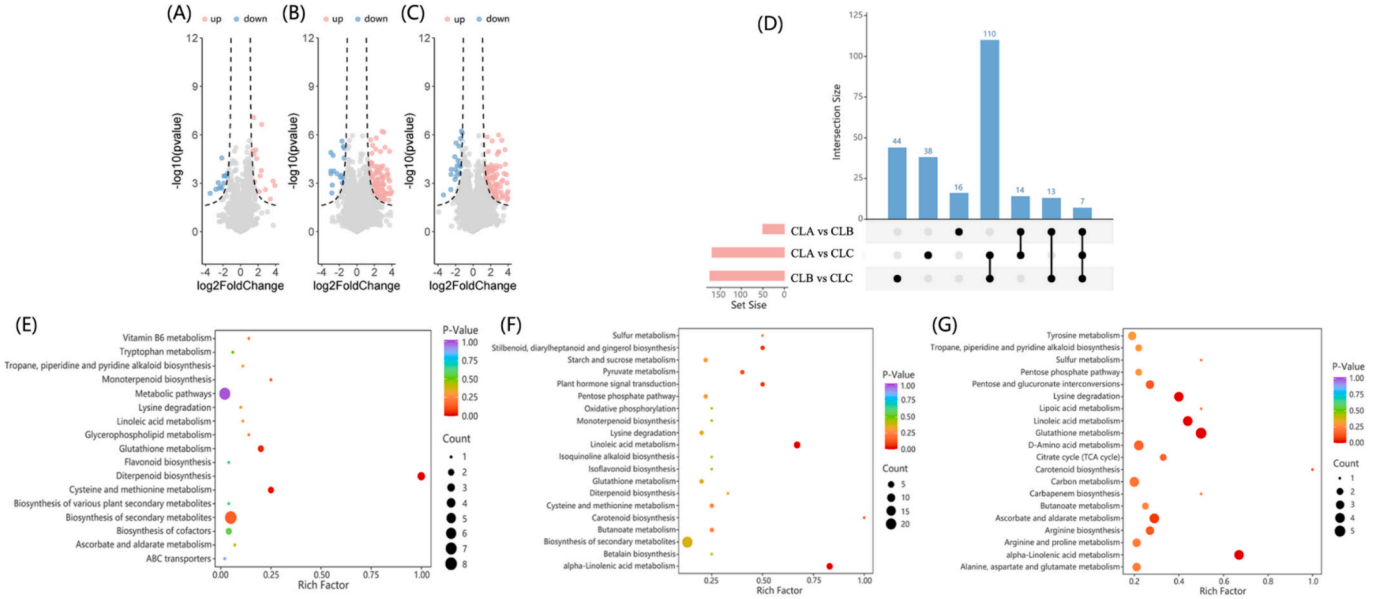


Fig. 2. (A) A volcano plot of the results of a Student's *t*-test comparing CLA and CLB (FDR q value <0.1, S0 = 0.1). (B) A volcano plot of the results of a Student's t-test comparing CLA and CLC (FDR q value <0.1, S0 = 0.1). (C) A volcano plot of the results of a Student's t-test comparing CLB and CLC (FDR q value <0.1, S0 = 0.1). (D) The Venn-UpSet diagram of DEMs among insect stress groups. (E) Metabolic pathway diagram for differences in CLA and CLB group. (F) Metabolic pathway diagram for differences in CLA and CLC group. (G) Metabolic pathway diagram for differences in CLB and CLC group. Note: For plot E, F, G: The bubble size represents the number of different metabolites involved in this pathway, and the bubble color represents the *p*-value of this metabolic pathway.

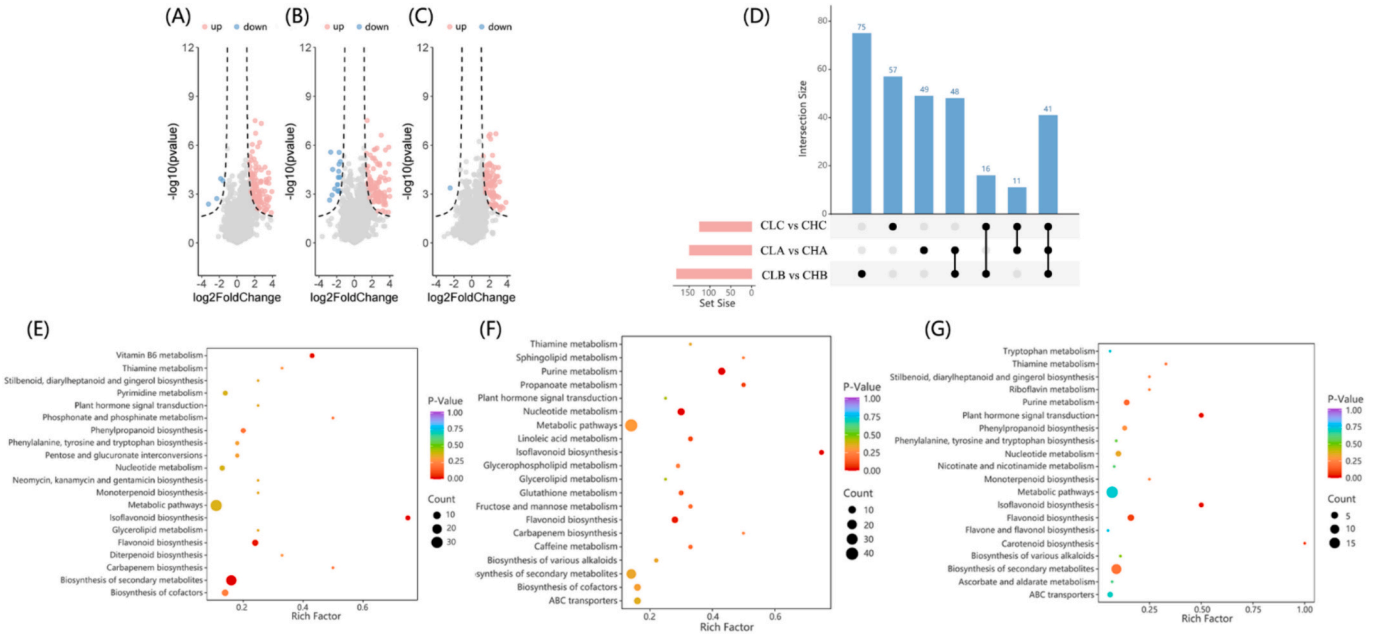


Fig. 3. (A) A volcano plot of the results of a Student's t-test comparing CLA and CHA (FDR q value <0.1, S0 = 0.1). (B) A volcano plot of the results of a Student's t-test comparing CLB and CHB (FDR q value <0.1, S0 = 0.1). (C) A volcano plot of the results of a Student's t-test comparing CLC and CHC (FDR q value <0.1, S0 = 0.1). (D) The Venn-UpSet diagram of DEMs among heat stress groups. (E) Metabolic pathway diagram for differences in CLA and CHA group. (F) Metabolic pathway diagram for differences in CLB and CHB group. (G) Metabolic pathway diagram for differences in CLC and CHC group. Note: For plot E, F, G: The bubble size represents the number of different metabolites involved in this pathway, and the bubble color represents the p-value of this metabolic pathway.

40.45% of these were amino acids and their derivatives, followed by nucleotides and their derivatives and phenolic acids, both of which had 11 species.

3.3.2. *Enrichment metabolic pathways analysis*

Pathway enrichment analyses revealed that these DEMs were primarily significantly enriched in secondary metabolic pathways. This suggests that heat stress affects metabolic pathways in oilseeds differently than insect stress does. Isoflavonoid biosynthesis was enhanced by heat stress on all three types of oilseeds (with the different healthy conditions). Furthermore, DEMs produced by the temperature effect on both types of healthy seeds were enriched in flavonoid biosynthesis pathways. Importantly, when only the effect of heat stress on non-infested oilseeds from non-infested fruits (Type A) was considered, the biosynthesis of secondary metabolites and vitamin B6 metabolism pathways were significantly ($p < 0.05$) enriched (Fig. 3E, F).

Plant hormone signal transduction was only found to be enriched in the CLC and CHC metabolic pathways (Fig. 3G).

4. Discussions

4.1. Chain transmission response of DEMs in COs to two stresses

COs, as a key oilseed crop, has been extensively studied, with a specific emphasis on examining its core components such as fatty acid content using methods such as GC-FID (G. Zhu et al., 2024). Our previous work used lipidomic to investigate the lipid composition of oil derived from COs and how it changes in response to insect stress (Li et al., 2022). Furthermore, researchers discovered 73 phenolic compounds in camellia seed cake using UPLC-QTOF-MS (Hong et al., 2019). Exploring the total material makeup of COs provides a more comprehensive understanding of the metabolic changes that occur in COs under stress, as well as the chemical basis for oil's health-promoting qualities. A recent study utilized UPLC-MS/MS and HS-SPME/GC–MS to conduct a metabolomic analysis of healthy mature COs from Hainan and Liangguang provinces, resulting in a rich dataset on the metabolic composition of 1057 species (Xia et al., 2023). In the current study, 1875 metabolites were identified in COs using UPLC-MS/MS. This study not only discovered a larger number of metabolites, including tannins, terpenoids, and alkaloids (by >60%), but it also revealed previously unknown subclasses such as quinones.

It has been well established that plants regulate secondary metabolites, particularly phenolics, in response to insect stress (Ren et al., 2023; Shen et al., 2024; Zhuang et al., 2023). According to one study, the metabolite types that changed >2-fold in coconut as a result of *Ceratocystis paradoxa*-induced stress were primarily phenolic acids, which accounted for 15.98% of the DEMs, with the phenolic acids 4-hydroxybenzoic acid and protocatechualdehyde having the highest multiplicity of change exceeding 500 (Shen et al., 2024). Similar findings were reported in the current study, where the proportion of phenolic acids with

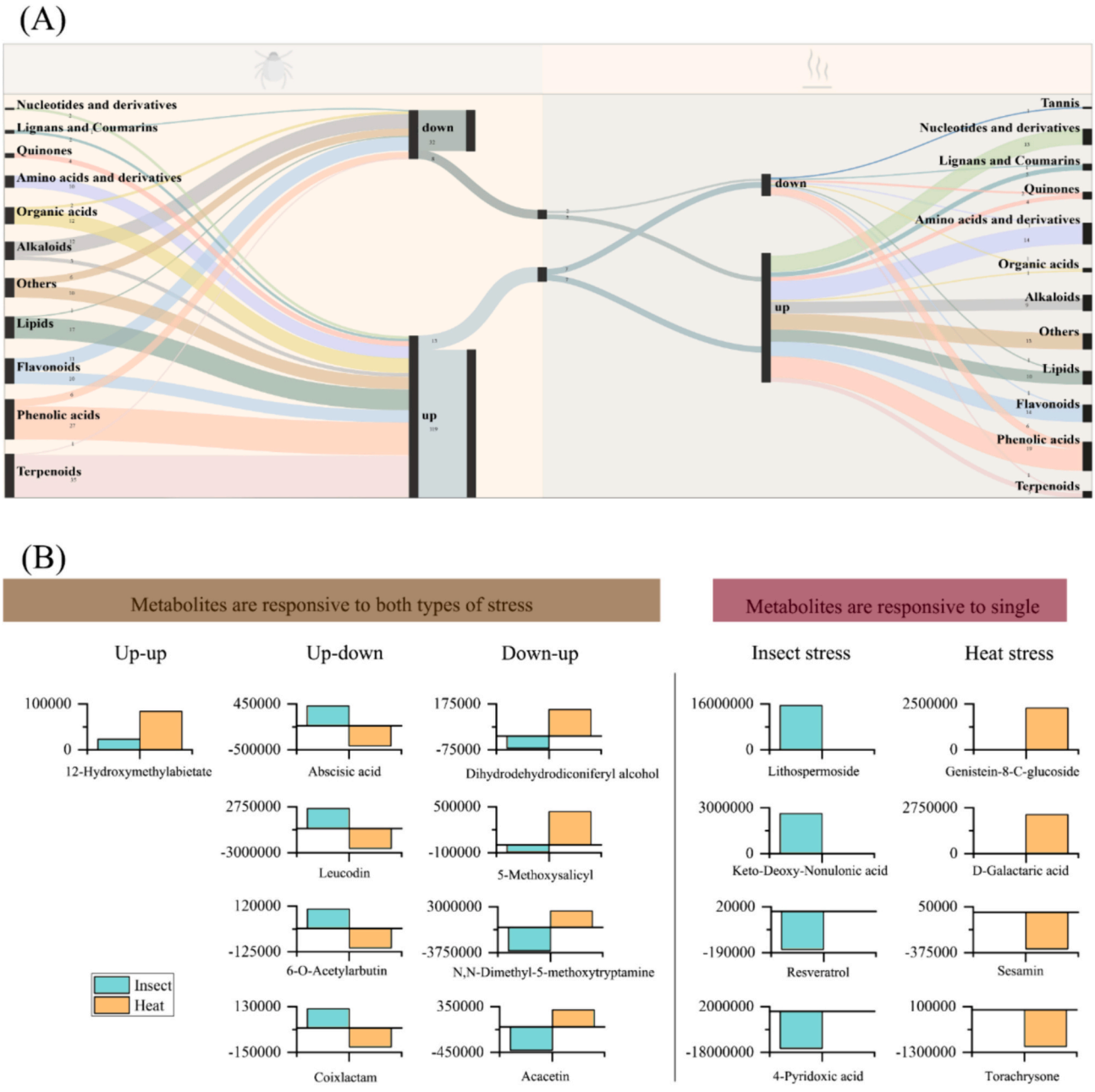


Fig. 4. (A) The Sankey diagram of the transition of DEMs from insect stress to heat stress. (B) Column plots of DEMs responses to insect stress and heat stress.

>2-fold change following insect stress reached 19.53%, with 5-o-p-coumaroylquinic acid and androsin being the most altered phenolic acids. In comparison, the percentage of terpenes in our study's DEMs was significantly higher, topping 20%. In addition, our investigation revealed that insect stress changes primary metabolites such as lipids and amino acid derivatives. Notably, during insect stress, levels of 15 (*R*)-hydroxyoleic acid and 17-hydroxylinolenic acid were up-regulated by >20-fold.

Interestingly, secondary metabolites revealed to be more vulnerable to heat stress than primary metabolites, accounting for >55% of the DEMs. In this group, phenolic acids alone accounted for almost 20% of the DEMs. This suggests that the plant's response to heat stress is predominantly mediated by the modification of secondary metabolic pathways, particularly those involved in the formation of phenolic acid. Several researchers indicated that metabolites associated with primary metabolism-related pathways respond primarily in foods like soybean (D. Zhu et al., 2022), walnut (Wang et al., 2022), and peanut (Klevorn & Dean, 2018). It was reported the DEMs between heat stressed and normal soybean were mainly lipids, peptides, nucleic acids, and amino acids (D. Zhu et al., 2022). The amino acid metabolic pathway produced 12 of the 16 newly produced metabolites in peanut after heat stress (Klevorn & Dean, 2018). The present study discovered that the major primary metabolites of CO in response to heat stress were amino acids and their derivatives, as well as lipids, and that practically all of them showed up-regulation, with a multiplicity of up-regulation ranging from 2.50 to 14.62.

The chain dynamic transfer effect of insect stress can occur at the temperature stress link as the food travels from cultivation to processing. Nevertheless, there is a scarcity of research that examines the combined effects of various stresses in this transmission process. A Sankey diagram illustrates the dynamic transfer of metabolites in response to two stresses by CO throughout the production chain (Fig. 4A). Furthermore, we may divide the metabolites into two groups based on their response to the two stresses (Fig. 4B). A total of 21 metabolites in this study showed a significant ($p < 0.05$) response to both stresses across the production chain. Only 12-Hydroxymethylabietate showed consistent alterations following both stresses, increasing 6.88-fold under insect stress and 4.05-fold with additional heat stress. When exposed to the combined stresses, other metabolites altered in opposing ways. Metabolites such as leucodin, abscisic acid, and stilbostemin B exhibited an “up-down” tendency. In both single-factor stress groups (insect or heat), these metabolites differed significantly ($p < 0.5$) from the control. However, following the combined impact of two stresses, they were no longer substantially different ($p > 0.5$) from the control. Other metabolites, such as acetoacetic acid, syringic acid, and 5-methoxysalicylic acid, on the other hand, demonstrated a “down-up” tendency, with levels after two stress transmissions ranging from 0.54 to 4.75 of the control values. Moreover, a higher number of metabolites showed a significant response to only one of the stresses. After insect stress, 4-pyridoxic acid was down-regulated >500-fold, but there was no additional substantial change in its content following heat stress. Correspondingly, sesamin did not change significantly after insect stress, but was screened as DEMs following heat stress, suggesting that this class of metabolites is more sensitive to heat stress.

4.2. The impact of two stress on the functions of COs

The health-related benefits of CO in previous paper are centered on urinary disease (Prasad, Srilatha, Sailaja, Raju, & Jayasree, 2016; Zeng et al., 2020), cardiovascular system disease (Huang et al., 2023; H. Shi, Zhou, He, Wang, & Zhou, 2022), nervous system disease (Guo et al., 2022; Weng, Chen, Li, & Yen, 2020) and digestive system disease (Cheng et al., 2014; Lee, Tung, Wu, Tu, & Yen, 2018; Wang, Tung, Chen, Lee, & Yen, 2019) (Fig. 5A). The present study performed a functional search and identification of the 1875 metabolites detected using the TMSCP database and organized them with the combination of the experimental literature on the health effects of CO. The key AIs in CO were screened by selection criterion of OB ≥ 25% and DL ≥ 0.07 (Li et al., 2023), which would be useful in determining the chemical foundation of CO's health promoting function. Then 98 metabolites were identified as AI in CO, with 29 flavonoids, 12 terpenoids, 11 lipids, and 10 phenolic acids. This indicates that the principal chemical metabolites in CO that support

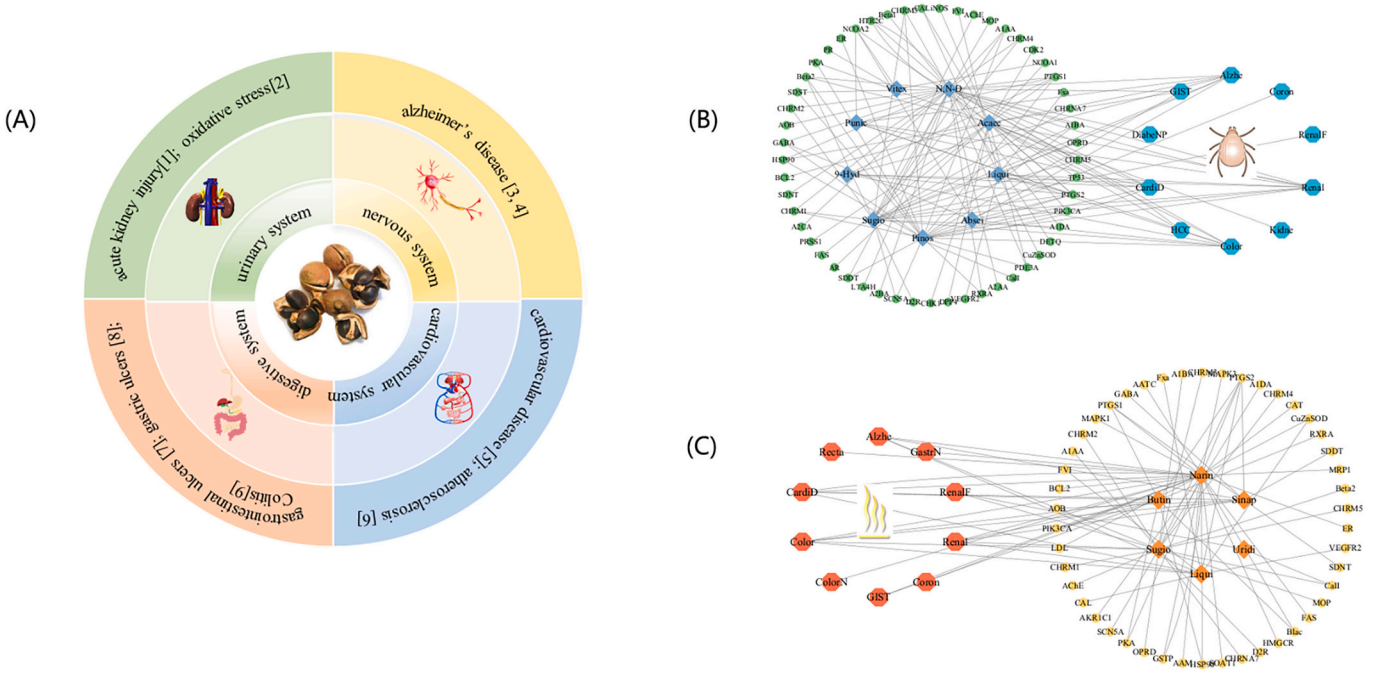


Fig. 5. (A) The main systemic diseases associated with CO. [1] (Zeng et al., 2020), [2] (Prasad et al., 2016), [3] (Guo et al., 2022), [4] (Weng et al., 2020), [5] (H. Shi et al., 2022), [6] (Huang et al., 2023), [7] (Cheng et al., 2014), [8] (Wang et al., 2019), [9] (Lee et al., 2018). (B) Pharmacological maps of the network related to differential AIs under insect stress. (C) Pharmacological maps of the network related to differential AIs under heat stress.

human health are the secondary metabolic. Other primary metabolites, especially lipids, are also crucial in health promotion.

An additional mechanism was found by building a pharmacological network based on the relationship between DEMs and four typical human systemic diseases (Fig. 5B, C). Acacetin and *N*,*N*-dimethyl-5-methoxytryptamine are DEMs that are found in both insect and heat stress. The target of this action includes PTGS2, PTGS1, and HSP90.

These targets are of interest due to their involvement in a variety of clinical processes, including liver cancer and neurodegenerative disorders (Soni, Meshram, Lawal, Patel, & Mahady, 2021). Insect stress down-regulated both compounds, especially *N*,*N*-dimethyl-5-methoxytryptamine, which was down-regulated >200-fold. This suggests that insect stress has reduced their significance in digestive system diseases, especially hepatocellular carcinoma, and nervous system diseases. However, heat stress in the subsequent drying phase up-regulated their levels to >65% of their original content, allowing some CO to be recovered in the above-mentioned disease functions. However, pinostrobin, which acts primarily on cardiovascular system diseases especially coronary atherosclerosis, only responds down to insect stress. This response reduces CO's therapeutic effect in cardiovascular system diseases. AIs such as abscisic acid and punicic acid, which are produced specifically in response to insect stress, act mainly in diseases such as rectal tumours and colorectal tumours via targets such as PKA, PR and NCOA2. These AIs were significantly up-regulated after insect stress, suggesting that insect stress even increased the pharmacological activity of COs in the digestive and urinary systems. Heat stress resulted in the

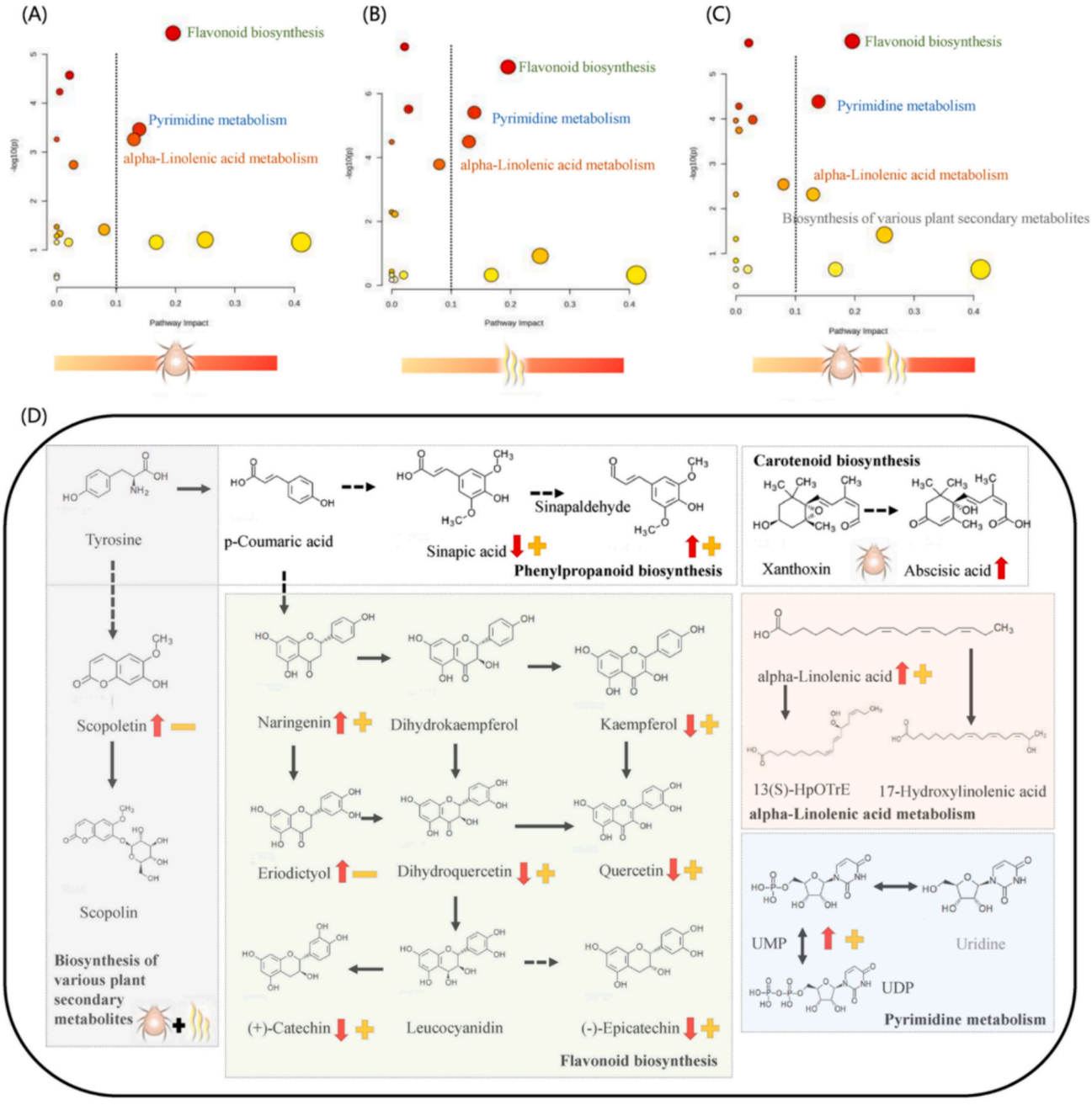


Fig. 6. (A) Biological pathways disturbed by insect stress. (B) Biological pathways disturbed by heat stress. (C) Biological pathways disturbed by the combined stresses of insect and heat. (D) The network of AIs-related metabolic pathways. Note: Red arrows up or down indicate up- or down-regulation of the metabolite under insect stress. Plus and minus indicates that the metabolite is up- or down-regulated under heat stress. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

production of particular AIs, primarily naringenin, butin, and liquiritin. These AIs are more effective in stomach and intestine diseases. These actives were likewise up-regulated following heat stress, suggesting that heat stress aided the generation of AIs in COs.

4.3. Perturbed biological mechanism of the two stresses

Changes in metabolite enrichment reflect the inhibition or activation of certain metabolic pathways, which also represent a perturbed biological mechanism of various stresses. Many previous studies have concentrated on finding DEMs in order to better understand the metabolic pathways involved in plant physiological responses (Li et al., 2023; Ren et al., 2023; Shen et al., 2024). However, there is a scarcity of information on the mechanisms associated with the effects of perturbed important AIs, and there are significant knowledge gaps of the mechanisms of the combined effects of multiple stresses, which can help us better understand the effects of stress on plant function or provide useful information for plant exploitation and utilization. The present study used MetaboAnalyst 6.0 to investigate the metabolic perturbation pathways associated with 98 AIs with pharmacological effects (Fig. 6A, B, C). A smaller *p*-value, a greater impact value implies a greater degree of disturbance (Hao et al., 2023). Under the screening conditions of $p < 0.05$ and impact >0.1, the results of biopathway analyses revealed that insect stress significantly ($p < 0.05$) induced perturbations in three biopathways, including flavonoid biosynthesis, pyrimidine metabolism and alpha-Linolenic acid metabolism. Interestingly, these three pathways are also the most disrupted by heat stress. Flavonoid biosynthesis has been discovered to be considerably disrupted metabolic pathways in various oily plants when subjected to heat stress or insect stress, including soybean (D. Zhu et al., 2022), coconut (Shen et al., 2024), and chestnut rose (Ren et al., 2023). However, previous research has not evaluated the impact of insect and heat stress on the flavonoid biosynthesis pathway at the same time. The findings of this study provide novel evidence suggesting that the flavonoid metabolic pathway is prominently disrupted as the primary secondary metabolic pathway in oilseed crops, experiencing both insect and heat stresses.

Naringenin, an upstream metabolite in the flavonoid metabolic pathway that was influenced by insect stress and heat stress, was up-regulated 1.33-fold and 7.48-fold, respectively. Then, naringenin may be catalyzed by naringenin 3-dioxygenase and flavonoid 3′,5′-hydroxylase to generate dihykaempferol and dihydroquercetin (Zhuang et al., 2023). Dihydroflavonols are then converted to flavonols such as kaempferol, quercetin, and myricetin, which may be accelerated by flavonol synthase (FLS) (Baba & Ashraf, 2019). Delivery via the flavonoid metabolic pathway inhibited the downstream metabolites catechin and epicatechin by >13% in both cases (Fig. 6D).

The phenylpropanoid biosynthesis occurs at the intersection of the flavonoid biosynthesis and biosynthesis of various plant secondary metabolites (Fig. 6D). All three are metabolic pathways that have been significantly perturbed under the two stresses. As an upstream metabolite of naringenin, the phenylpropanoid biosynthesis of p-coumaric acid is involved in the control of the flavonoid metabolic pathway.

Interestingly, in our study, the biosynthesis of various plant secondary metabolites pathway ($p < 0.05$, impact value = 0.25), which has received less attention in previous studies, was revealed to be the metabolic pathway most perturbed by the combined effect of the two stresses. This implies that after a sequence of two stresses, the flavonoid metabolic pathway, which was initially the most affected by a single stress, is overtaken by the pathway for the biosynthesis of various plant secondary metabolites. This shift can be attributed to the fact that scopoletin was up-regulated 6-fold under pest stress but down-regulated 200-fold during heating stress. This shift in metabolic pathways also highlights the need for a fresh and more comprehensive assessment of the combined impact of various stresses in the food production chain.

Furthermore, insect stress selectively altered the carotenoid metabolic pathway, resulting in an increase in abscisic acid (ABA) concentration. ABA signaling has the ability to stimulate ethylene production or interact with ethylene signaling, altering fruit ripening processes (Chidley et al., 2017). This suggested that increased ABA accumulation in the flavonoid synthesis pathway, caused by insect stress, may stimulate COs ripening. And we hypothesized that the early dropping of infested fruits seen in infested CO forests could be related to this, but more research is needed. Although the single or combined effects of both stresses on CO were mostly in the secondary metabolic pathways, alpha-Linolenic acid metabolism in the primary metabolic pathway was also severely affected as a typical oilseed. The stimulatory effect is mostly on α-linolenic acid, which promotes the process of lipid oxidation.

5. Conclusion

The impact of pre-harvest insect stress and post-harvest heat stress on COs has been extensively studied using targeted metabolomics and network pharmacology. Using UPLC-MS/MS, we identified 1875 metabolites (the majority of which fell into 7 categories) in COs under different treatments. In response to insect, heat, and insect + heat stress, OPLS-DA analysis revealed 169, 149, and 21 metabolites as differential metabolites markers (DEMs). Among the DEMs, 5-(2-hydroxypropyl)-3H-2-benzofuran-1-on, Eriodictyol-7-O-glucoside, and 5-(2-hydroxypropyl)-3H-2-benzofuran-1-one is the most sensitive to stress alone and combined stress. A pharmacological network associating DEMs with four typical human systemic diseases was constructed. Acacetin and *N*, *N*-dimethyl-5-methoxytryptamine were downregulated by insect stress, lowering their participation in digestive and other systemic illnesses. Heat stress, on the other hand, mostly had a favorable regulating impact on COs. The diverse metabolic pathways that individual and combined stresses influence help explain the disparities in their effects. This study's findings provide new evidence that the flavonoid metabolic pathway as the main perturbed secondary metabolic pathway in oilseed crops exposed to both insect and heat stresses. While the biosynthesis of various plant secondary metabolites became the most affected pathway under combined stresses. This study provides a comprehensive understanding of the chemical composition and health-promoting components in COs. These discoveries aid in the development and exploration of the CO industry, allowing for its further expansion and exploitation.

CRediT authorship contribution statement

Qingyang Li: Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Wei Zhu**: Writing – original draft, Visualization, Software. **Shiman Sun:** Visualization, Formal analysis, Data curation. **Maokai Cui:** Validation, Methodology, Investigation. **Wei Zhang:** Validation, Methodology, Investigation. **Jinping Shu:** Validation, Methodology, Investigation. **Runhong Mo:** Investigation, Formal analysis. **Fubin Tang:** Validation, Methodology. **Yirong Guo:** Investigation, Conceptualization. **Yihua Liu:** Writing – review & editing, Visualization, Software, Conceptualization.

Declaration of competing interest

All authors confirm that this article's contents have no conflict of interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the "Pioneer" and "Leading Goose" R&D Program of Zhejiang (2023C02034, 2023C02045).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101619.

References

Anzano, A., Bonanomi, G., Mazzoleni, S., & Lanzotti, V. (2022). Plant metabolomics in biotic and abiotic stress: A critical overview. *Phytochemistry Reviews, 21*(2), 503–524. https://doi.org/10.1007/s11101-021-09786-w

Baba, S. A., & Ashraf, N. (2019). Functional characterization of flavonoid 3′-hydroxylase, CsF3′H, from *Crocus sativus* L: Insights into substrate specificity and role in abiotic stress. *Archives of Biochemistry and Biophysics, 667*, 70–78. https://doi.org/10.1016/j.abb.2019.04.012

Cheng, Y.-T., Wu, S.-L., Ho, C.-Y., Huang, S.-M., Cheng, C.-L., & Yen, G.-C. (2014). Beneficial effects of camellia oil (*Camellia oleifera* Abel.) on ketoprofen-induced gastrointestinal mucosal damage through upregulation of HO-1 and VEGF. *Journal of Agricultural and Food Chemistry, 62*(3), 642–650. https://doi.org/10.1021/jf404614k

Chidley, H. G., Deshpande, A. B., Oak, P. S., Pujari, K. H., Giri, A. P., & Gupta, V. S. (2017). Effect of postharvest ethylene treatment on sugar content, glycosidase activity and its gene expression in mango fruit. *Journal of the Science of Food and Agriculture, 97*(5), 1630–1639. https://doi.org/10.1002/jsfa.7912

Guo, P., Zeng, M., Cao, B., Liu, M., Zhang, Y., Jia, J., Zhang, Q., Zhang, B., Wang, R., Xiong, W., Zheng, X., & Feng, W. (2022). Camellia oil improves Aβ25-35-induced memory impairment by regulating the composition of the gut microbiota and lipid metabolism in mice. *Journal of Functional Foods, 96*. https://doi.org/10.1016/j.jff.2022.105214

Hao, J., Tan, J., Zhang, Y., Wang, S., Zhang, X., Wang, Z., & Li, J. (2023). Metabolomics reveals the molecular mechanism of sewage sludge-derived nutrients and biostimulants stimulating resistance enhancement and the redistribution of carbon and nitrogen metabolism in pakchoi cabbage. *Science of the Total Environment, 891*. https://doi.org/10.1016/j.scitotenv.2023.164330

Harel, M., Ortenberg, R., Varanasi, S. K., Mangalhara, K. C., Mardamshina, M., Markovits, E., … Geiger, T. (2019). Proteomics of melanoma response to immunotherapy reveals mitochondrial dependence. *Cell, 179*(1), 236–+. https://doi.org/10.1016/j.cell.2019.08.012

He, J., Wu, X., Liu, J., Huang, Y., & Zhang, J. (2022). Comprehensive evaluation of quality of *Camellia semiserrata* seed oils from various harvest dates. *Journal of Oleo Science, 71*(9), 1275–1287. https://doi.org/10.5650/jos.ess22114

Hong, C., Chang, C., Zhang, H., Jin, Q., Wu, G., & Wang, X. (2019). Identification and characterization of polyphenols in different varieties of *Camellia oleifera* seed cakes by UPLC-QTOF-MS. *Food Research International, 126*. https://doi.org/10.1016/j.foodres.2019.108614

Hu, Q., Zhang, J., Xing, R., Yu, N., & Chen, Y. (2022). Integration of lipidomics and metabolomics for the authentication of camellia oil by ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry coupled with chemometrics. *Food Chemistry, 373*. https://doi.org/10.1016/j.foodchem.2021.131534

Huang, T., Jiang, J., Cao, Y., Huang, J., Zhang, F., & Cui, G. (2023). Camellia oil (*Camellia oleifera* Abel.) treatment improves high-fat diet-induced atherosclerosis in apolipoprotein E (ApoE)−/− mice. *Bioscience of Microbiota Food and Health, 42*(1), 56–64. https://doi.org/10.12938/bmfh.2022-005

Klevorn, C. M., & Dean, L. L. (2018). A metabolomics-based approach identifies changes in the small molecular weight compound composition of the peanut as a result of dry-roasting. *Food Chemistry, 240*, 1193–1200. https://doi.org/10.1016/j.foodchem.2017.08.058

Kopecka, R., Kameniarova, M., Cerny, M., Brzobohaty, B., & Novak, J. (2023). Abiotic stress in crop production. *International Journal of Molecular Sciences, 24*(7). https://doi.org/10.3390/ijms24076603

Lee, W.-T., Tung, Y.-T., Wu, C.-C., Tu, P.-S., & Yen, G.-C. (2018). Camellia oil (*Camellia oleifera* Abel.) modifies the composition of gut microbiota and alleviates acetic acid-induced colitis in rats. *Journal of Agricultural and Food Chemistry, 66*(28), 7384–7392. https://doi.org/10.1021/acs.jafc.8b02166

Li, Q., Wang, R., Sun, S., Shen, D., Mo, R., & Liu, Y. (2023). Comparison of different drying technologies for walnut (*Juglans regia* L.) pellicles: Changes from phenolic composition, antioxidant activity to potential application. *Food Chemistry-X, 20*. https://doi.org/10.1016/j.fochx.2023.101037

Li, Q., Zhang, W., Shen, D., Li, Z., Shu, J., & Liu, Y. (2022). Comprehensive lipidomics analysis reveals the changes in lipid profile of camellia oil affected by insect damage. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.993334

Memoli, A., Albanese, D., Esti, M., Lombardelli, C., Crescitelli, A., Di Matteo, M., & Benucci, I. (2017). Effect of bug damage and mold contamination on fatty acids and sterols of hazelnut oil. *European Food Research and Technology, 243*(4), 651–658. https://doi.org/10.1007/s00217-016-2778-x

Oliete, B., Lubbers, S., Fournier, C., Jeandroz, S., & Saurel, R. (2022). Effect of biotic stress on the presence of secondary metabolites in field pea grains. *Journal of the Science of Food and Agriculture, 102*(11), 4942–4948. https://doi.org/10.1002/jsfa.11861

Prasad, W. L. N. V. V., Srilatha, C., Sailaja, N., Raju, N. K. B., & Jayasree, N. (2016). Amelioration of gamma-hexachlorocyclohexane (Lindane) induced renal toxicity by *Camellia sinensis* in Wistar rats. *Veterinary world, 9*(11), 1331–1337. https://doi.org/10.14202/vetworld.2016.1331-1337

Ren, T., Li, B., Xu, F., Chen, Z., Lu, M., & Tan, S. (2023). Research on the effect of oriental fruit moth feeding on the quality degradation of chestnut rose juice based on metabolomics. *Molecules, 28*(20). https://doi.org/10.3390/molecules28207170

Shen, X., Niu, X., Yang, Y., Yang, D., Li, J., Yu, F., Sun, X., & Meng, X. (2024). Widely targeted metabolomics combined with E-tongue and E-nose reveal dynamic changes of tender coconut water in responses to the infection of *Ceratocystis paradoxa*. *Food Chemistry, 439*. https://doi.org/10.1016/j.foodchem.2023.138035

Shi, H., Zhou, X., He, X., Wang, R., & Zhou, W. (2022). Camellia oil enhances plasma antioxidant metabolism and improves plasma lipid metabolism in high-fat diet-fed rats. *Natural Product Communications, 17*(3). https://doi.org/10.1177/1934578x221081368

Shi, T., Wu, G., Jin, Q., & Wang, X. (2020). Camellia oil authentication: A comparative analysis and recent analytical techniques developed for its assessment. A review. *Trends in Food Science & Technology, 97*, 88–99. https://doi.org/10.1016/j.tifs.2020.01.005

Silva, E., Belinato, J. R., Porto, C., Nunes, E., Guimaraes, F., Meyer, M. C., & Pilau, E. J. (2021). Soybean metabolomics based in mass spectrometry: Decoding the plant's signaling and defense responses under biotic stress. *Journal of Agricultural and Food Chemistry, 69*(26), 7257–7267. https://doi.org/10.1021/acs.jafc.0c07758

Soni, K. K., Meshram, D., Lawal, T. O., Patel, U., & Mahady, G. B. (2021). Fractions of *Boswellia Serrata* suppress LTA4, LTC4, cyclooxygenase-2 activities and mRNA in HL-60 cells and reduce lung inflammation in BALB/c mice. *Current Drug Discovery Technologies, 18*(1), 95–104. https://doi.org/10.2174/1570163817666200127112928

Valencic, V., Butinar, B., Podgornik, M., & Bucar-Miklavcic, M. (2021). The effect of olive fruit fly *Bactrocera oleae* (Rossi) infestation on certain chemical parameters of produced olive oils. *Molecules, 26*(1). https://doi.org/10.3390/molecules26010095

Wang, M., Wan, Y., Liu, T., Zeng, X., Liang, X., Wu, X., & Fu, G. (2022). Effect of refining degree on the quality changes and lipid xxidation of camellia (*Camellia oleifera*) oil during heating. *Foods, 11*(15). https://doi.org/10.3390/foods11152232

Wang, P., Zhong, L., Yang, H., Zhu, F., Hou, X., Wu, C., Zhang, R., & Cheng, Y. (2022). Comparative analysis of antioxidant activities between dried and fresh walnut kernels by metabolomic approaches. *Lwt-Food Science and Technology, 155*. https://doi.org/10.1016/j.lwt.2021.112875

Wang, R.-Y., Tung, Y.-T., Chen, S.-Y., Lee, Y.-L., & Yen, G.-C. (2019). Protective effects of camellia oil (*Camellia brevistyla*) against indomethacin-induced gastrointestinal mucosal damage *in vitro* and *in vivo*. *Journal of Functional Foods, 62*. https://doi.org/10.1016/j.jff.2019.103539

Weng, M.-H., Chen, S.-Y., Li, Z.-Y., & Yen, G.-C. (2020). Camellia oil alleviates the progression of Alzheimer's disease in aluminum chloride -treated rats. *Free Radical Biology and Medicine, 152*, 411–421. https://doi.org/10.1016/j.freeradbiomed.2020.04.004

Xia, T., Xiong, Z., Sun, X., Chen, J., Wang, C., Chen, Y., & Zheng, D. (2023). Metabolomic profiles and health-promoting functions of *Camellia drupifera* mature-seeds were revealed relate to their geographical origins using comparative metabolomic analysis and network pharmacology approach. *Food Chemistry, 426*. https://doi.org/10.1016/j.foodchem.2023.136619

Xu, B., Hu, W., Gao, M., Zhao, W., Wang, Y., & Zhou, Z. (2022). Effects of elevated air temperature coupling with soil drought on carbohydrate metabolism and oil synthesis during cottonseed development. *Physiologia Plantarum, 174*(1). https://doi.org/10.1111/ppl.13643

Zeng, M., Li, M., Zhang, B., Li, B., Kan, Y., Zheng, X., & Feng, W. (2020). Camellia oil inhibits oxidative stress and inflammatory response to ameliorate LPS-induced acute kidney injury via downregulation of TLR4-mediated activation of the NF-κB/AP-1/IRF3 and NLRP3 pathways. *Journal of Functional Foods, 68*. https://doi.org/10.1016/j.jff.2020.103908

Zhu, D., Guan, D., Fan, B., Sun, Y., & Wang, F. (2022). Untargeted mass spectrometry-based metabolomics approach unveils molecular changes in heat-damaged and normal soybean. *Lwt-Food Science and Technology, 171*. https://doi.org/10.1016/j.lwt.2022.114136

Zhu, G., Lei, D., Xie, Y., Zhang, Y., Shi, J., & Liu, Y. (2024). Effects of postharvest piling up in bulk on qualities of Camellia oleifera seeds. *Journal of Stored Products Research, 106*. https://doi.org/10.1016/j.jspr.2024.102291

Zhuang, W.-B., Li, Y.-H., Shu, X.-C., Pu, Y.-T., Wang, X.-J., Wang, T., & Wang, Z. (2023). The classification, molecular structure and biological biosynthesis of flavonoids, and their roles in biotic and abiotic stresses. *Molecules, 28*(8). https://doi.org/10.3390/molecules28083599