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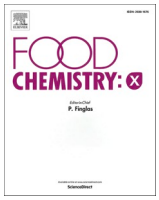

Mechanisms of the response of apple fruit to postharvest compression damage analyzed by integrated transcriptome and metabolome

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ABSTRACT

Apple fruit is susceptible to compression damage within the postharvest supply chain given its thin peels and brittle texture, which can result in decay and deterioration and have a substantial impact on its marketability and competitiveness. Thorough bioinformatics investigations are lacking on postharvest compression damage stress-induced alterations in genes and metabolic regulatory networks in fruits. In the present study, a comprehensive analysis of both the transcriptome and metabolome was conducted on 'Red Fuji' apples experiencing compression-induced damage. During the storage after damage has occurred, the gene expression of *MdOFUT19*, *MdWRKY48*, *MdCBP60E*, *MdCYP450* and *MdSM-like* of the damaged apples was consistently higher than that of the control group. The damaged apples also had higher contents of some metabolites such as procyanidin A1, Dl-2-Aminooctanoic acid, 5-*O*-p-Coumaroyl shikimic acid and 5,7-Dihydroxy-3′,4′,5′-trimethoxyflavone. Analysis of genes and metabolites with distinct expressions on the common annotation pathway suggested that the fruit may respond to compression stress by promoting volatile ester and lignin synthesis. The above results can deepen the comprehension of the response mechanisms in apple fruits undergoing compression-induced damage.

1. Introduction

The supply and consumption of quality fruit is an important indicator of a country's social development and standard of living. With the continuous improvement of living standards and consumption capacity, fresh and diversified fruits of high quality are preferred by consumers. However, fruit is susceptible to mechanical damage stress during the postharvest supply chain, which is an important factor in fruit spoilage and deterioration (Yang et al., 2022). Mechanical damage is a common and persistent problem encountered in fruit production and storage, which can result in significant economic losses for farmers, producers, and retailers. Mechanical damage can be caused by balanced inward forces at various locations on the fruit, such as compression caused by the picker's hand, branches, ladders, railings, crates, etc. The unreasonable height of the carton may also cause mechanical damage to the fruit due to compression (Opara & Fadiji, 2018). Among the various types of mechanical damage, compression mechanical damage is particularly prevalent, often caused by the impact of packing or handling equipment in the postharvest supply chain.

Mechanical damage can result in physical and physiological changes in fruit tissues, leading to decreased firmness, accelerated respiration, and increased susceptibility to decay and microbial contamination, further accelerating the spread of rotten fruit reducing the shelf-life of the fruit, and ultimately causing huge economic losses to agricultural production (Li & Thomas, 2014; Shen et al., 2021). Meanwhile, mechanical damage can cause defects in fruit appearance, seriously affecting the fruit's market value and competitiveness (Fernando et al., 2019). Presently, research on postharvest mechanical damage in fruits predominantly centers on quantifying physiological and biochemical parameters of the fruit, alongside designing protective and damage-mitigating packaging technologies for the logistics industry.

Transcriptomics has proven to be a valuable methodology for exploring gene expression and transcriptional regulation at the mRNA level. This approach enables researchers to closely examine cellular

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phenotypes and functions. Transcriptomics has been extensively used across various fields of postharvest fruit research, including investigations into molecular mechanisms responding to non-biological stress (Yang et al., 2023). As transcriptomics focuses on mRNA-level data, metabolomics has emerged as the next step to provide a crucial widening of the lens of systems biology (Li et al., 2022). While transcriptomics reveals transcriptional changes, examining metabolomics allows researchers to connect these transcriptional fluctuations to the functional output of metabolic pathways. This metabolic perspective is vitally important for scrutinizing non-biological stress response mechanisms in fruits (Shen et al., 2021). The integration of transcriptomic and metabolomic analyses in fruit is expected to become more prevalent with advances in high-throughput omics technologies. The joint profiling of transcripts and metabolites will enable more holistic elucidation of the biological processes governing fruit development, ripening, quality attributes, and stress resilience through connecting gene expression patterns with metabolic pathways. Concurrently, improved curated databases and multi-species comparative analyses will be crucial for bioinformatic interpretation, extracting predictive biomarkers from such large-scale multi-omics datasets, and revealing signatures associated with desired fruit quality traits and stress adaptations. Overall, multi-omics integration coupled with sophisticated bioinformatics will be integral to harnessing these comprehensive profiles for gaining new insights into the genetic regulation of fruit biology.

Secondary metabolites are intimately connected with the nutritional quality, flavor, color, and storage capacity of plants (Liu et al., 2022). The presence of compounds like flavonoids, alkaloids, terpenes, tannins, and phenols contributes to their diverse biological activity. Their accumulation can enhance the nutritional quality, flavor, color, and shelf life of agricultural products (Peng & Fu, 2023). Certain amino acids, flavonoids, aromatics, and some terpenes also influence the flavor of plants. Furthermore, secondary metabolites play a crucial role in plant defense against abiotic stress. For example, flavonoids are being increasingly investigated for their role in resisting drought, pests, and UV radiation (Rai et al., 2023), while anthocyanins have been found to exhibit antibacterial and antioxidant properties (Lu et al., 2021). Besides, a focused examination of the synthesis and utilization of secondary metabolites induced by mechanical damage of fruits could provide novel insights and evidence to enhance shock-absorbing and damage-reducing technologies for logistics processes and improve the postharvest nutritional quality of fruits. Nevertheless, a systematic investigation of the underlying metabolic and molecular network responsible for deterioration in physiological characteristics due to mechanical damage is currently lacking. Moreover, the metabolites and genes associated with the stress response mechanism to mechanical damage remain unclear.

Concurrently, the study of biological mechanisms related to fruit mechanical damage stress response can contribute to the development of new varieties with improved quality and storage properties. Saeed et al. (2014) identified multiple small-effect quantitative trait loci controlling the browning of pear fruit skin caused by frictional mechanical force, which provides a theoretical foundation for selecting genotypes with low or no friction sensitivity during early breeding cycles. Additionally, the rapid recognition of fruit mechanical damage information during the postharvest logistics process is vital for the early detection of fruit quality deterioration and improvement of subsequent preservation and logistics strategies. Earlier research has suggested that fruit mechanical damage induces the synthesis and accumulation of volatile compounds (Lin et al., 2021; Yan et al., 2020), and the perception of volatile compounds is not limited by logistics packaging or dark environments, thereby potentially serving as an important indicator for evaluating whether fruits have undergone mechanical damage. Integrated transcriptome and metabolome analysis has been utilized in studies to investigate the regulatory mechanisms of non-biological factors on fruits and crops (Ke et al., 2022; Tang et al., 2022). However, the identification of target volatile compounds related to fruit mechanical damage and their associated synthesis biology remains a critical knowledge gap. Consequently, there is an urgent need to investigate the biological mechanisms underlying fruit postharvest mechanical damage stress response and screen potential characteristic volatile compounds and their synthesis-related genes induced by mechanical damage of fruits.

In the present study, resistance mechanism of apple fruits undergoing compression damage was investigated using a combined analysis of transcriptome and metabolome data, candidate defense compounds and genes were screened, and preliminary multiple metabolic pathway resistance mechanisms related to mechanical damage stress were proposed. Our hypothesis posits that the resilience of apples against compression damage correlates with the biosynthesis of volatile esters and lignin.

2. Samples and methods

2.1. Apple samples

Samples of 'Red Fuji' apple (*Malus* × *domestica* Borkh.) were procured from an adjacent supermarket. Before transportation to the research laboratory, the apple fruits were carefully chosen based on uniform dimensions and coloration, comparable maturity status (commercially ripe), absence of physical damage, and no visible indications of insect infestation or disease.

2.2. Inducing compression damage in apple fruit

The compression damage of apple fruits was induced using a texture analyzer (TA-XT2i plus, UK) outfitted with a 100 mm aluminum disk probe. Apple fruits were axially compressed with a load of 130 N for a duration of 60 s. The regions of the apple fruit subjected to compressive force and the non-compressed segments of the same fruit were designated as the compression-treated sample (CD) and the non-compressed control sample (CK_{CD}). Fifteen samples were randomly chosen from each designated sampling location and partitioned into three distinct biological replicates, with each biological replicate containing five fruits. Following the compression test, the fruits were kept at a temperature of 20 °C and subsequently sampled at 0 d (d0CD, $d0CK_{CD}$), 2 d (d2CD, $d2CK_{CD}$), 6 d (d6CD, $d6CK_{CD}$), 24 d (d24CD, $d24CK_{CD}$), and 60 d (d60CD, $d60CK_{CD}$) after the compression treatment. After each sampling, the fruit flesh samples were placed at −80 °C.

2.3. Transcriptomic profiling and analysis

2.3.1. RNA extraction

The protocol for RNA extraction was modified based on Kiefer et al. (2000). Suspend 0.5 g of pulverized fruit pulp in 4 mL of preheated CTAB/β-mercaptoethanol (80 μL) solution at 65 °C, vortex and homogenize thoroughly, then incubate in a 65 °C water bath for 5 min to induce lysis. Subsequently supplement with chloroform/isoamyl alcohol, perform two phases of extraction, centrifuge, and collect the supernatant. Add 1/5 vol of 12 M lithium chloride to the supernatant solution and leave it overnight (greater than12 h) at 4 °C. The next day, after 30 min of high-speed centrifugation at 4 °C, discard the supernatant, add 400 μL of pre-warmed SSTE at 65 °C to dissolve the precipitate, and aspirate repeatedly. Add 400 μL of chloroform/isoamyl alcohol and extract once, then centrifuge at high speed at 4 °C to remove the supernatant. Then add 2 times the volume of anhydrous ethanol prechilled at −20 °C to the supernatant, mix upside down, and place at −80 °C for 30 min to precipitate the RNA. Subsequently, the precipitate was gathered through high-speed centrifugation at 4 °C. The resultant precipitate underwent a 10-minute drying period in a fume hood, followed by the addition of 20 μL of DEPC water to facilitate the dissolution of the precipitate, resulting in the acquisition of RNA samples. The electrophoresis results showed that the band brightness ratio of the upper and

lower bands was 2:1, and the OD_{260}/OD_{280} was between 1.8 and 2.0. It indicated that the total RNA samples were structurally intact and of high purity, and could be used for further analysis.

2.3.2. Transcriptomic analysis

Transcriptomic sequencing of RNA extracted from compression-treated and control apple fruit at 0, 2, 6, 24, and 60 days of storage was carried out by Beijing Biomarker Technologies Co., Ltd. utilizing the Illumina Hiseq sequencing platform, with three distinct biological replicates per sample from each sampling point. The downstream data were preprocessed to filter out low quality data to obtain clean reads. Subsequently, transcripts were constructed by consulting the apple genome using the *Malus* × *domestica* Genome V1.0 database as a reference (https://www.rosaceae.org/species/malus/malus_x_domestica). The details of data processing were referred to Yang et al. (2023).

2.4. Metabolomic profiling and analysis

Fruit samples at 6 days of storage were selected for extensive targeted metabolomic profiling (Wuhan Metwell Biotechnology Co., Ltd.). Pulp samples were lyophilized under vacuum conditions and then pulverized into a powdered form (30 Hz, 1.5 min) employing a grinder (MM400, Retsch). The powdered sample was dissolved in aqueous methanol (70%), and then subjected to overnight extraction at 4 °C. Then, the samples underwent centrifugation at 10,000 g for 10 min. Before ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) profiling, the supernatant was collected via aspiration and then filtered through a microporous membrane. The parameters for UPLC-MS/MS were referred to Yang et al. (2023).

2.5. Statistical analysis

Principal component analysis (PCA) was executed through the intrinsic statistical function prcomp in the R software. The generation of figures was achieved using the R software ComplexHeatmap toolkit and Origin Pro 2020 (OriginLab Corp., Northampton, MA). ANOVA was conducted employing the SPSS 17.0 software (IBM, New York, NY), and statistically significant distinctions were denoted as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

3. Results and discussion

3.1. Impact of compression-induced damage on the transcriptome

3.1.1. Examination of the comprehensive attributes of the transcriptome

Transcriptome analysis was performed on 30 apple fruit samples using RNA-Seq technology. 224.26 Gb of clean data was acquired, with the proportion of Q30 bases exceeding 92.21%. The clean data from each sample were aligned against the specified reference genome for sequence comparison, and the alignment efficiency ranged from 65.79% to 72.12%. The statistical analysis of compression damage on the quality of apple fruit transcriptome sequencing is shown in Table S1 (Supplementary Material).

PCA calculations were performed on the transcriptome data between compression and control groups, and the results are shown in Fig. 1A, where principal component 1 (PC1) accounted for 87.2% of the variance and principal component 2 (PC2) accounted for 6.8% of the variance. At the level of PC1, $d2CK_{CD}$ could be clearly distinguished from d2CD and $d6CK_{CD}$ from d6CD, while the compression and control groups at other storage times points could not be clearly distinguished at the level of PC1. The results indicated that on the day following mechanical damage caused by compression, all types of samples exhibited difficulty in differentiation. However, clear separation between samples was observed at 2 d and 6 d. The differentiation became less apparent at the time points of 24 d and 60 d.

3.1.2. Identification and analysis of differentially expressed genes (DEGs)

The fold change (FC) signifies the expression ratio between compression and control groups. False discovery rate (FDR) was obtained by calibrating the *p*-value for significance of differences (*p*-value). FC ≥ 2 and FDR < 0.01 were used as screening conditions to screen for DEGs. The statistics of the number of DEGs in apple fruits by compression damage are shown in Table S2 (Supplementary Material). The number of DEGs during storage exhibited a pattern of initial increase followed by subsequent decrease after the fruit was subjected to mechanical damage caused by compression. At 0 d, the number of DEGs was 505, and the number of DEGs increased at 2 d and 6 d, while the number of DEGs decreased significantly again at 24 d and 60 d, which aligns with the outcomes obtained from the PCA analysis in section 3.1.1.

The Venn diagram of DEGs caused by compression damage during storage are shown in Fig. 1B. Among all DEGs, five genes exhibited presence at every storage time in the compression-treated and control groups. These genes included rockulose glycosyltransferase (O-fucosyltransferase 19, *MdOFUT19*, *MDP0000250895*), calmodulin-binding protein *MdCBP60E* (*MDP0000235112*), phytochrome P450 (Cytochrome P450, *MdCYP450*, *MDP0000490696*), squalene monooxygenase *MdSM-like* (Squalene monooxygenase, *MDP0000746652*), and *MdWRKY48* (WRKY transcription factor 48, *MDP0000146390*).

3.1.3. Elucidation of functional annotations for DEGs

The DEGs were functionally annotated in eight different databases and the statistics of the number of annotated DEGs are shown in Table S3 (Supplementary Material). A total of 499 (0 d), 1792 (2 d), 1737 (6 d), 387 (24 d) and 103 (60 d) DEGs were subjected to annotation in eight databases.

3.1.4. Analysis of GO annotation and enrichment for DEGs

Fig. 1C, 1E, 1G, 1I, and 1 K illustrate the results of GO analysis conducted on all DEGs to examine their functions in fruits under compression and in the control group. The GO annotation classification pattern of DEGs in compressed fruits at all 5 storage time points was similar, and the DEGs in the biological process classification were mainly associated with cellular process, single-organism process and metabolic process. The three major subgroups of the cellular component classification are cell, cell part, and organelle. The main subcategories of molecular function are binding and catalytic activity.

3.1.5. Analysis of annotation and enrichment of KEGG for DEGs

KEGG enrichment analysis of DEGs was performed and the 20 most enriched metabolic pathways were demonstrated (Fig. 1D, 1F, 1H, 1 J, 1L). Upon reaching the 0-d time point, DEGs exhibited enrichment in the pathways of plant-pathogen interaction, sesquiterpenoid and triterpenoid biosynthesis, and steroid biosynthesis. Upon reaching the 2-d time point, DEGs prominently exhibited enrichment in the pathways of photosynthesis-antenna proteins, terpenoid backbone biosynthesis, and ribosome. Upon reaching the 6-d time point, DEGs prominently exhibited enrichment in the pathways of amino sugar and nucleotide sugar, terpenoid backbone, and steroid metabolism. DEGs prominently exhibited enrichment in the pathways of plant-pathogen interaction, photosynthesis-tactin, and photosynthesis at 24 d. At 60 d, DEGs prominently exhibited enrichment in the pathways of phenylalanine, tyrosine, tryptophan biosynthesis, as well as cyanoamino acid metabolism (*p*-value < 0.05).

3.2. Impact of compression-induced damage on the metabolome

3.2.1. PCA analysis and evaluation of repeat correlations

PCA analysis was used to evaluate the variability of metabolites between the compression and control groups. The cumulative contributions to variance for PC1 and PC2 amounted to 38.22% and 23.51% (Fig. 2A). The PCA analysis could clearly differentiate samples in the

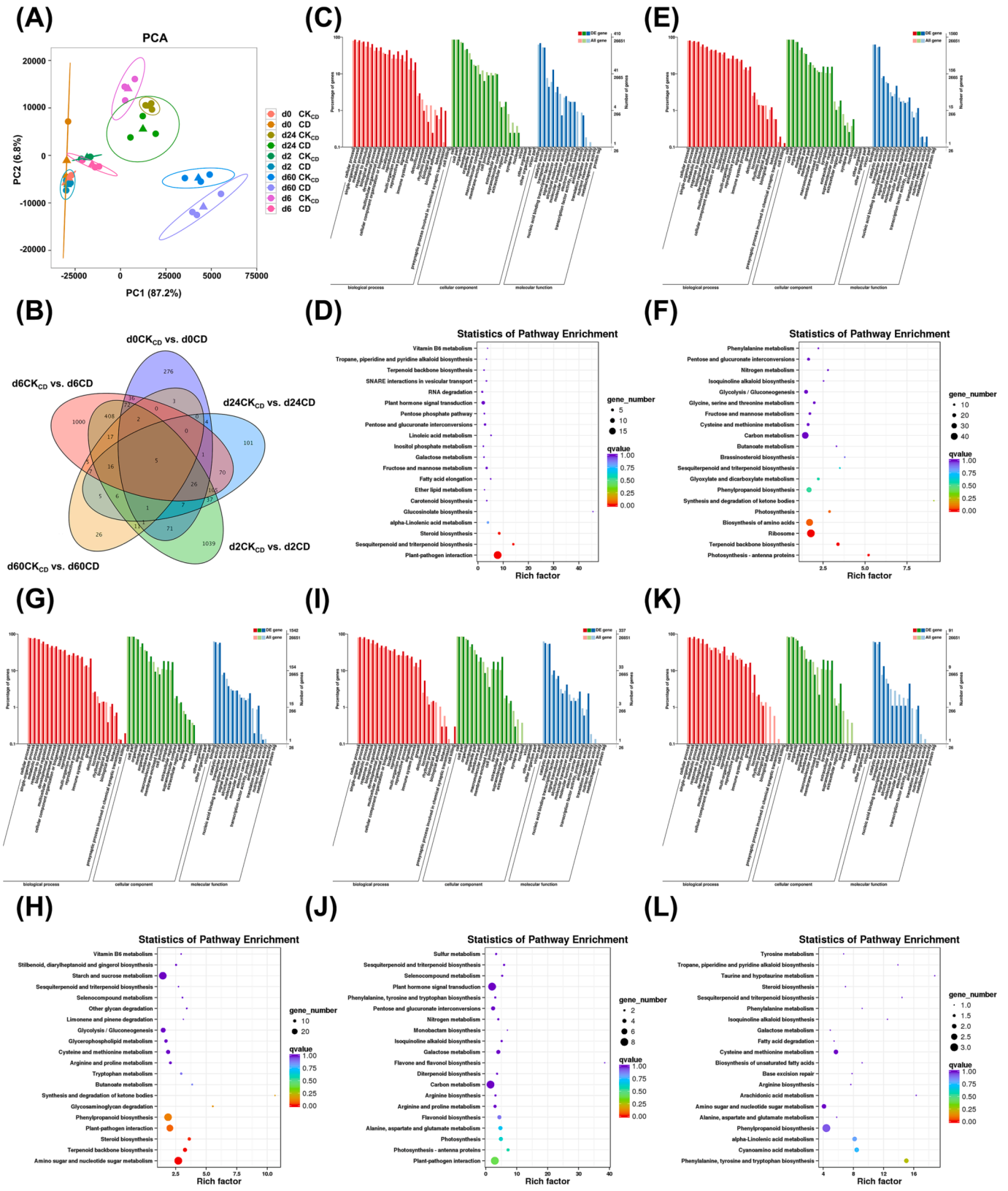

Fig. 1. The influence of compression damage on the transcriptome of apples. (A) PCA analysis for transcriptome data. (B) Venn diagrams for DEGs. (C, E, G, I, K) GO enrichment for DEGs at 5 time points (0, 2, 6, 24, 60 d). (D, F, H, J, L) KEGG enrichment for DEGs at 5 time points (0, 2, 6, 24, 60 d). CD: Compression damage group; CK_{CD}: Control group.

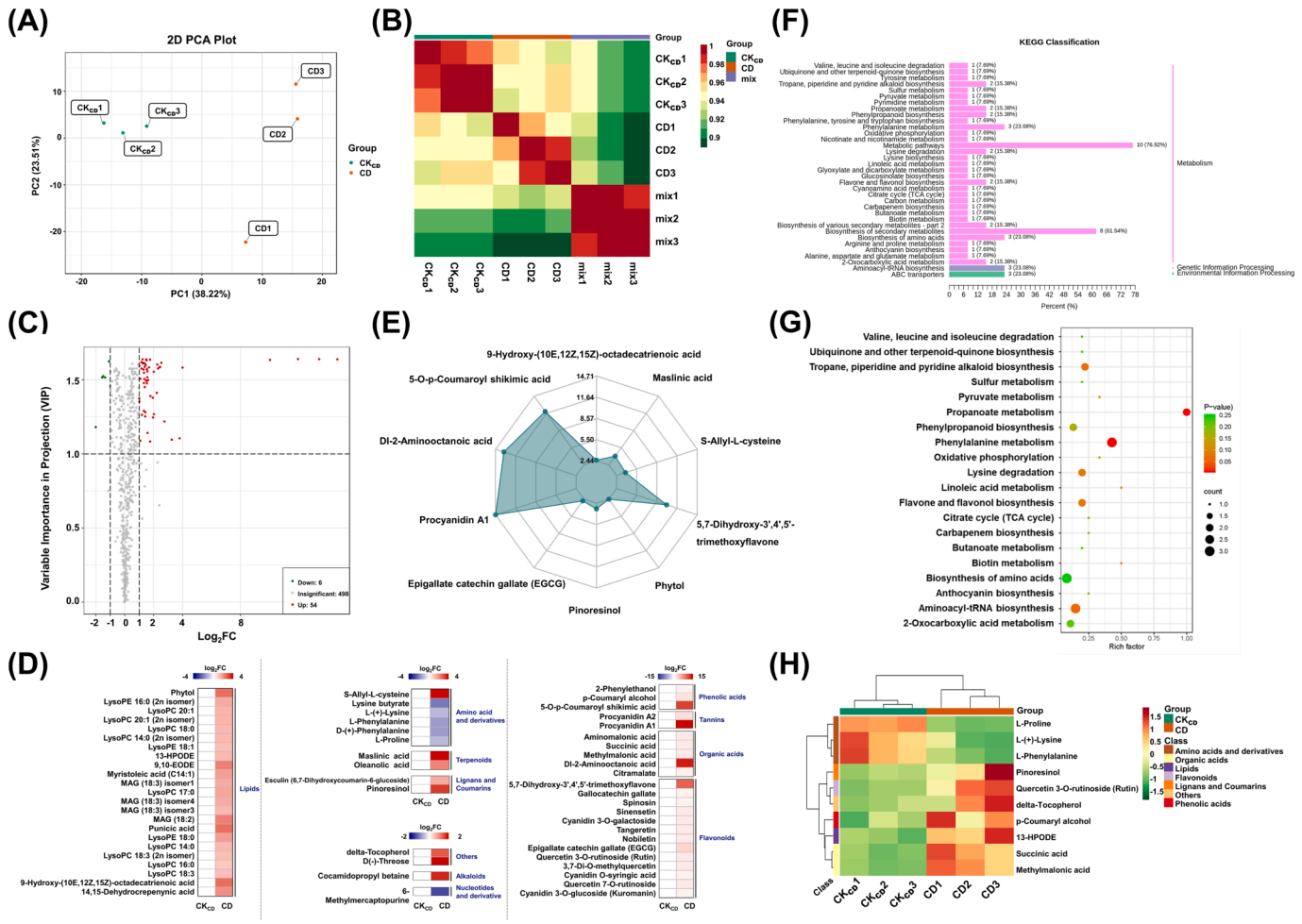

Fig. 2. The influence of compression damage on the metabolome of apples. (A) PCA score plot of metabolite data. (B) Correlation heat map. (C) Volcano plot of DEMs. (D) Classification of DEMs. (E) Radar chart of DEMs. (F) KEGG classification of DEMs. (G) KEGG enrichment of DEMs. (H) KEGG heatmap of DEMs. CD: Compression damage group; CK_{CD}: Control group.

compression damage group and control group.

Pearson correlation coefficients were utilized to calculate correlations among replicates of samples (Fig. 2B). The outcomes demonstrated a substantial degree of data consistency across the three biological replicates. The correlation coefficients among samples within each group surpassed those observed between the two groups, indicating the elevated reliability of the data.

3.2.2. *Identification for differentially expressed metabolites (DEMs)*

Apple fruit samples were subjected to extensive targeted metabolomics analysis, resulting in the detection of 558 metabolites. The findings indicated that 60 metabolites of fruit were significantly affected by mechanical damage caused by compression, including 54 metabolites that were up-regulated and 6 metabolites that were up-regulated. The specific information of each differential metabolite is shown in Table S4 (Supplementary Material). Volcano plot analysis was further performed on the 60 DEMs to visualize the change patterns and expression level differences of metabolites between the compressed and control group of fruits, and the results are shown in Fig. 2C.

3.2.3. *Categorization for DEMs*

Differential multiplicities of DEMs were transformed and classified using the obtained Log_2FC for mapping (Fig. 2D), including 23 lipids, 13 flavonoids, 6 amino acids and their derivatives, 5 organic acids, 3 phenolic acids, 2 lignan and coumarin metabolites, 2 terpenoids, 2 tannins, 2 other metabolites, 1 nucleotide and its derivatives, and 1 alkaloid metabolite.

The radar plot (Fig. 2E) was plotted to visualize the ten most significant metabolites with the highest difference multiplicity, which included procyanidin A1, DDL-2-aminooctanoic acid, 5-*O*-p-coumaroyl shikimic acid, maslinic acid, S-allyl-L-cysteine, 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone, pinoresinol, epigallate catechin gallate (EGCG), 9-hydroxy-(10E,12Z,15Z)- octadecatrienoic acid, and phytol.

3.2.4. *Analysis of annotation and enrichment of KEGG for DEMs*

Fig. 2F and Fig. 2G show the KEGG classification map and the enrichment analysis map of the 60 DEMs. The 20 most enriched metabolic pathways primarily encompassed various secondary metabolites, namely (1) the biosynthesis of other secondary metabolites, including flavone and flavonol, carbapenem, tropane, phenylpropanoid, piperidine and pyridine alkaloid, (2) amino acid metabolism, including phenylalanine metabolism, lysine degradation, valine, leucine and isoleucine degradation, etc.; (3) carbohydrate metabolism, namely the citric acid cycle (TCA cycle), the metabolism of propanoate, pyruvate, and butanoate, etc.; (4) energy metabolism, including oxidative phosphorylation, sulfur metabolism, etc.; (5) cofactors and vitamins metabolism, including biotin metabolism, ubiquinone and other terpenoid-quinone biosynthesis, etc.; (6) global and overview maps, namely 2-oxocarboxylic acid metabolism, amino acids biosynthesis, etc.; (7) translation, including aminoacyl-tRNA biosynthesis, etc.; (8) lipid metabolism, including linoleic acid metabolism. Furthermore, the results of KEGG enrichment plots revealed significant enrichment in two

metabolic pathways, namely the metabolism of phenylalanine and propionate.

3.2.5. *KEGG annotation information clustering for DEMs*

Based on annotation information of DEMs in KEGG pathways, KEGG pathways containing at least five DEMs were selected, and all DEMs annotated to these pathways were analyzed by clustering (Fig. 2H) to investigate the variation patterns of DEMs in important metabolic pathways. Fig. 2h shows that these DEMs mainly include L-proline, L-(+)-lysine, L-phenylalanine, pinoresinol, quercetin 3-*O*-rutinoside (rutin), delta-tocopherol, p-coumaryl alcohol, 13-HPODE, succinic acid and methylmalonic acid. Among them, the relative contents of three

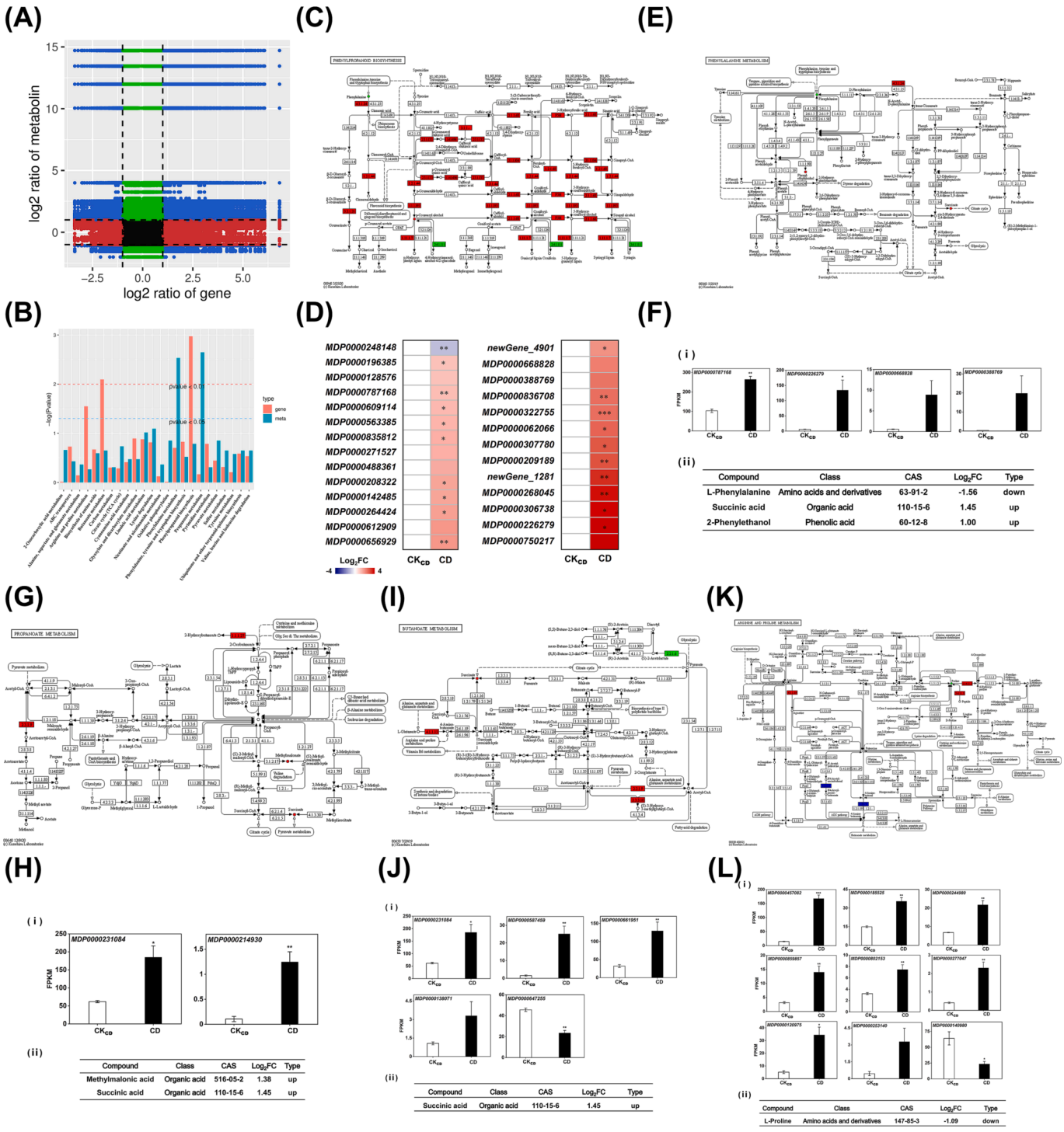

Fig. 3. Synergistic analysis of the transcriptome and metabolome of apples subjected to compression damage. (A) Ninequadrants of DEMs and DEGs. (B) *p*-value of KEGG of DEMs and DEGs. (C) Phenylpropanoid, (E) Phenylalanine, (G) Propanoate, (I) Butanoate, and (K) Arginine and proline metabolism for DEGs and DEMs. The red markers, green and blue markers indicate up-regulation of DEGs/DEMs, down-regulation of DEGs/DEMs, and up- /down-regulation of DEGs/DEMs, respectively. (D) Expression data of DEGs related to phenylpropanoid metabolism pathway. (F, H, J, L) Expression of DEGs (i) and DEMs (ii) associated with (F) phenylalanine, (H) propanoate, (J) butanoate, and (L) arginine and proline metabolism pathway. CD: Compression damage group; CK_{CD}: Control group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

amino acids and their derivatives, such as L-proline, L-(+)-lysine and L-phenylalanine, exhibited a decrease within the compression group. Meanwhile, the remaining seven metabolites demonstrated higher levels in the compression group.

3.3. Synergistic analysis of the transcriptome and metabolome

3.3.1. Investigation of the correlation between transcriptome and metabolome data

Fig. 3A depicts a nine-quadrant graphical representation, illustrating the prevalence of DEGs and DEMs that exhibited notable differential expression and possessed Pearson correlation coefficients exceeding 0.8. Genes and metabolites of quadrants 3 and 7 showed consistent differential expression patterns, suggesting that the changes of metabolites located in quadrants 3 and 7 might be subject to positive regulation by genes. Moreover, additional investigation revealed that 63 DEMs were positively regulated by 1653 DEGs, indicating that they might be related to the response to the stress of compression damage of apple fruit. Genes and metabolites of quadrants 1 and 9 showed opposite differential expression patterns, suggesting that alterations of these metabolites may be subject to negative regulation by genes located in same quadrants. Besides, 63 DEMs were negatively regulated by 1389 DEGs, indicating a possible response to compression damage of apple fruit. Quadrants 2, 4, 6, and 8 indicated no changes in metabolites and up- or down-regulation of genes, or no changes in genes and up- or down-regulation of metabolites.

3.3.2. Simultaneous enrichment analysis for DEGs and DEMs

Fig. 3B displays the degree of enrichment observed in metabolic pathways housing both DEGs and DEMs. The findings indicated a notable enrichment on pathways related to phenylpropane biosynthesis, metabolism of phenylalanine, propionate, butyrate, arginine and proline (p-value < 0.05).

3.3.3. Expression of DEGs and DEMs of the phenylpropanoid biosynthesis pathway

The KEGG map of the phenylpropanoid metabolic pathway was used as a model to mark the DEGs and DEMs, and the expression changes were observed (Fig. 3C). Fig. 3D illustrates the expression levels of the 27 DEGs. Within the DEGs, some of the genes were considerably upregulated after compression damage, including *MdCOMT* (caffeic acid 3-*O*-methyltransferase, *MDP0000208322*, *MDP0000656929*), *MdCCoAOMT* (caffeoyl-CoA O-methyltransferase 1, *MDP0000226279*), *MdPAL* (phenylalanine ammonia-lyase, *MDP0000787168*), *MdCCR* (cinnamoyl-CoA reductase, *MDP0000268045*, *MDP0000322755*), *MdCAD* (probable mannitol dehydrogenase, *MDP0000609114*), *MdCYP450 84A1* (cytochrome P450 84A1, *MDP0000563385*), *MdCYP450 98A2* (cytochrome P450 98A2, *MDP0000836708*), *MdXyl* (beta-xylosidase/alpha-L-arabinofuranosidase, *MDP0000062066*, *MDP0000196385*), *MdPER16* (peroxidase 16, *MDP0000142485*), *MdPNC1* (cationic peroxidase 1, *MDP0000209189*), MdHST (shikimate O-hydroxycinnamoyltransferase, *MDP0000264424*, *MDP0000307780*), *MdBGLU47* (beta-glucosidase 47, *MDP0000306738*), *MdCSE* (caffeoyl shikimate esterase, *MDP0000835812*), *newGene 4901* (aldehyde dehydrogenase family 2 member C4), *newGene 1281* (peroxidase 52), etc. *MdGT5* (anthocyanidin 3-O-glucosyltransferase 5, *MDP0000248148*) was considerably downregulated after compression damage. Details of the two DEMs are provided in Table S5. The content of p-coumaryl alcohol increased after compression damage, whereas the content of L-phenylalanine decreased. Additional correlation analysis demonstrated a significant positive correlation between *MdCAD* and p-coumaryl alcohol, with a Pearson correlation coefficient of 0.929***.

3.3.4. Expression of DEGs and DEMs of the phenylalanine biosynthesis pathway

The KEGG map of the phenylalanine metabolic pathway was used as a model to mark the DEGs and DEMs and the expression changes were observed (Fig. 3E). The phenylalanine metabolic pathway was annotated with 4 DEGs and 3 DEMs. Fig. 3F (i) presents the expression levels of the 4 DEGs. *MdCCoAOMT* (*MDP0000226279*) and *MdPAL* (*MDP0000787168*) exhibited significant up-regulation after compression damage. Details of 3 DEMs are provided in Fig. 3F (ii). The content of succinic acid and 2-phenylethanol increased after compression damage, while the content of L-phenylalanine decreased. Additional correlation analysis found that *MdPAL* was significantly and positively correlated with phenylethanol (0.934***) and succinic acid (0.942***), and *MdCCoAOMT* exhibited a significant and positive correlation with succinic acid (0.987***).

3.3.5. Expression of DEGs and DEMs of the propanoate biosynthesis pathway

The KEGG map of the propanoate metabolic pathway was used as a model to annotate the DEGs and DEMs, and the expression changes were observed (Fig. 3G). The phenylalanine metabolic pathway was annotated with 2 DEGs and 2 DEMs. Fig. 3H (i) presents the expression levels of 2 DEGs, in which *MdAAT* (Acetyl-CoA acetyltransferase, *MDP0000231084*) and *MdL-LDH* (L-lactate dehydrogenase, *MDP0000214930*) were significantly upregulated after being subjected to compression mechanical injury. Details of the 2 DEMs are provided in Fig. 3H (ii), showing that the content of methylmalonic acid and succinic acid were increased after compression damage. Additional correlation analysis showed that *MdAATI* exhibited a significant and positive correlation with succinic acid (0.96***) and methylmalonic acid (0.953***).

3.3.6. Expression of DEGs and DEMs of the butanoate biosynthesis pathway

The KEGG map of the butanoate metabolic pathway was used as a model to mark the DEGs and DEMs and the expression changes were observed (Fig. 3I). The butanoate metabolic pathway was annotated with 5 DEGs and 1 DEM. Fig. 3J (i) presents the expression levels of 5 DEGs, among which *MdAAT*, *MdGAD2* (glutamate decarboxylase 2, *MDP0000587459*) and *MdHMGS* (hydroxymethylglutaryl-CoA synthase, *MDP0000661951*) exhibited significant up-regulation after compression damage. *MdVAT* (acetolactate synthase small subunit, *MDP0000647255*) was significantly downregulated after compression damage. Details of 1 DEM are provided in Fig. 3J (ii), in which succinate content was increased after compression damage. Additional correlation analysis showed that succinate exhibited a significant and positive correlation with *MdAAT* (0.960***) and *MdVAT* (0.993***), while a significant negative correlation with *MdGAD2* (-0.992***).

3.3.7. Expression of DEGs and DEMs of arginine and proline biosynthesis pathway

The KEGG map of arginine and proline metabolic pathway was used as a model to mark the DEGs and DEMs and the expression changes were observed (Fig. 3K). The pathway of arginine and proline was annotated with 9 DEGs and 1 DEM. Fig. 3L (i) illustrates the expression levels of 9 DEGs. *MdADC* (arginine decarboxylase, *MDP0000120975*, *MDP0000457082*), *MdPIP* (proline iminopeptidase, *MDP0000277047*), *MdP4H3* (prolyl 4-hydroxylase 3, *MDP0000802153*), *MdP4H7* (prolyl 4-hydroxylase 7, *MDP0000244980*), *MdP4H10* (prolyl 4-hydroxylase 10, *MDP0000185525*), *MdALDH2B4* (aldehyde dehydrogenase family 2 member B4, *MDP0000859857*) exhibited significant up-regulation after compression damage. *MdALDH2B4* (*MDP0000140980*) was significantly downregulated after compression damage. Details of 1 DEM are provided in Fig. 3L (ii), indicating a decrease in the L-proline content following compression damage.

4. Discussion

Secondary metabolites exert a significant role in modulating fruit

flavor and pigmentation, improving nutritional quality, and resisting stresses such as pests and diseases, temperature extremes, drought, and radiation. Fruit flavonoid accumulation reduces oxidative damage caused by the accumulation of reactive oxygen species induced by UV-B radiation stress, while increasing resistance to pathogenic bacteria (Agati et al., 2013; Ruiz et al., 2016). Flavanones and anthocyanins promote pollen and seed dispersal by influencing the coloration of plant flowers and fruits, while their synthetic accumulation also helps plants to resist stress and provides antioxidant properties (Shen et al., 2022; Wu et al., 2022). Proanthocyanidins, also known as condensed tannins, have antibacterial activity and antioxidant capacity against food-borne bacteria (Lu et al., 2021). Studies have shown that synthetically accumulated tetrahydro-β-carboline alkaloids in fruits can act as antioxidants and free radical scavengers (Maity et al., 2019). In addition, terpenoids such as carotenoids (Geng et al., 2021), nerolidol (Chen et al., 2020), and limonene (Lee et al., 2015) also play an active role in plant defense against adverse stresses.

In the present study, compression damage to fruits promoted the accumulation of a large number of secondary metabolites, including flavonoids (centaureidin-3-*O*-glucoside, quercetin-7-*O*-glucoside, centaureidin-*O*-butyric acid, 3,7-di-*O*-methylquercetin, quercetin-3-*O*-rutinoside, epigallocatechin gallate, trichothecene, orangiferin, centaureidin galactoside (cucurbitacin, spinosin, epigallocatechin gallate and 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone), terpenoids (oleanolic acid), alkaloids (cocamidopropyl betaine), tannins (proanthocyanidin A1, proanthocyanidin A2), lignans and coumarins (epipetiol, qinpi methanin). The synthetic accumulation of these substances may contribute to the fruit's ability to withstand mechanical damage caused by compression.

Transcriptome and metabolite association analysis in apple fruit identified 27 DEGs and 2 DEMs that were simultaneously annotated within the biosynthesis pathway of benzylpropane (p-value < 0.01). The phenylpropane metabolic pathway holds significant prominence in plants, serving as a pivotal contributor to plant growth, development, and interactions with the environment. The phenylpropanoid metabolism starts with phenylalanine and undergoes a series of enzymatic reactions to derive more than 8000 secondary metabolites. The initial reactions of the phenylalanine metabolic pathway are catalyzed by phenylalanine deaminase (PAL), cinnamic acid-4-hydroxylase (C4H), and p-coumarate/coenzyme A ligase (4CL) to form p-coumaroyl coenzyme A, which provides precursors for several downstream branches of the metabolic pathway. The lignin pathway constitutes a vital branch within the phenylpropane metabolism. Lignin, ranking as the Earth's second most prevalent polymer, holds substantial significance and accumulates mainly in plant secondary cell walls, where it provides mechanical support to plants while participating in duct formation and transporting water and minerals (Zhao, 2016). In addition, lignin is involved in biological processes such as resistance to invasion by pathogenic bacteria, resistance to feeding by herbivores, and defense against abiotic stresses (Cesarino, 2019; Xie et al., 2018). It has been shown that *PAL* in tobacco (Silva et al., 2018), *CAD2* and *CAD3* in melon (Liu et al., 2020), and *4CL7* in cotton are upregulated in expression under drought stress and accumulate lignin, thus contributing to plant resistance to drought stress. The expression levels of *PAL* and *CAD* were upregulated under cold stress in miscanthus (Domon et al., 2013) and loquat (Zhang et al., 2020), resulting in lignin accumulation. White birch (Hu et al., 2019) and apple (Chen et al., 2019) promoted lignin accumulation, secondary cell wall thickening and thus enhanced salt tolerance by upregulating the expression levels of *C4H*, *C3H*, *CAD*, *F5H*, *HCT*, *4CL*, *COMT*, *CCR* and *CCoAOMT*. Bunsiri et al. (2012) found that increased hardness of the mangosteen pericarp after mechanical damage by impact was associated with increased activity of enzymes required for lignin biosynthesis. Among them, the enzyme activities of PAL, POD and CAD increased within 15 min after the onset of mechanical damage by impact and then decreased (Bunsiri et al., 2012). In the present study, it was found that key genes of the lignin synthesis pathway, including *MdPAL*, *MdCCoAOMT*, *MdCCR*, *MdCOMT*, and *MdCAD*, were significantly up-regulated in apple fruits after compression damage. The content of L-phenylalanine, an upstream product of the lignin synthesis pathway, decreased, while the content of p-coumaryl alcohol, a downstream product, increased. Therefore, it is hypothesized that apple fruits may respond to mechanical damage stress by synthesizing lignin.

Among the metabolic pathways in which DEGs and DEMs are co-enriched under compression damage stress in apple fruit, a total of one differential gene in the propanoate metabolism and butanoate metabolism pathways is *MdAAT*, which is significantly upregulated. Alcohol acyltransferase (AAT) serves as a vital enzyme in the ultimate rate-limiting phase of volatile ester biosynthesis, exerting a noteworthy influence on the synthesis of volatile (Cao et al., 2021; Yang et al., 2020). Functional studies of AAT in volatile ester biosynthesis have been carried out in papaya (Balbontín et al., 2010), kiwi (Günther et al., 2011), peach (Yang et al., 2020), and apple (Liu et al., 2023) fruits. Koeda et al. (2023) purified and characterized AAT genes from pepper fruit to evaluate the potential role of AAT in fruit flavor development. Therefore, it is hypothesized that apple fruit in this study may respond to the compression damage stress by inducing the expression of *MdAAT*, a vital gene of the lipoxygenase (LOX) pathway, to promote the synthesis of volatile esters.

5. Conclusion

In the present study, resistance mechanism of apple fruits subjected to compression damage was investigated using transcriptomic and metabolomic analyses. After the damage has occurred, the gene expressions of *MdOFUT19*, *MdWRKY48*, *MdCBP60E*, *MdCYP450* and *MdSM-like* were found to be consistently higher than those of the control during storage. Metabolites such as proanthocyanidin A1 and DL-2-aminosuberic acid, were also found to be differentially higher and upregulated after mechanical damage to the fruit. DEGs and DEMs were annotated with 5 metabolic pathways, among which *MdAAT* was upregulated in several pathways related to volatile ester biosynthesis. Meanwhile, the expression of key genes (*MdPAL* and *MdCCoAOMT*) and metabolites of the lignin synthesis pathway was upregulated, suggesting that fruits may respond to mechanical damage stress by accumulating lignin. In conclusion, the apple fruits employ multiple metabolic pathways as a resistance mechanism in response to compression damage. Findings of the present study provide more biological knowledge about the potential mechanism of resistance of apple fruit to compression damage, which will help to develop more effective strategies to reduce compression damage and to breed fruit varieties with greater resistance to compression damage.

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CRediT authorship contribution statement

Zhichao Yang: Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Menghua Lin:** Investigation, Formal analysis, Data curation, Writing – original draft. **Xiangzheng Yang:** Investigation, Formal analysis, Data curation. **Changqing Zhu:** Investigation, Formal analysis, Data curation. **Di Wu:** Supervision, Funding acquisition, Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. **Kunsong Chen:** Supervision, Funding acquisition, Conceptualization, Methodology, Validation.

Declaration of Competing 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

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100972.

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