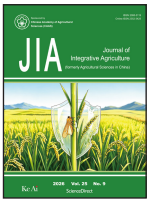
Journal of Integrative Agriculture 2026, 25(9): 3656–3672

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Unraveling the molecular and metabolic mechanisms of 6-BA-mediated dormancy release in Xianheng 01 apple rootstock

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Highlights

- 6-BA induces bud dormancy release through modulation of the cytokinin–ABA balance.
- A total of 7,009 DEGs were identified in response to 6-BA, with *A-ARR8* upregulated and ABA-related genes downregulated.
- A shift from phenylpropanoid/flavonoid pathways to ABC transporter-mediated processes facilitates bud break.

Abstract

Bud dormancy is a key adaptive strategy in perennial plants, enabling them to survive adverse environmental conditions. However, it poses challenges in crop cultivation, especially in fruit crops such as apple, in which synchronized bud break is crucial for consistent growth and yield. The synthetic cytokinin 6-benzylaminopurine (6-BA) promotes dormancy release; however, its molecular and metabolic mechanisms remain largely unclear. This study investigates dormancy release in nursery-grown Xianheng 01 apple rootstocks through integrated transcriptomic, metabolomic, and hormonal analyses. Dormant buds were treated with 6-BA, with morphological, biochemical, and molecular profiling performed over 30 d. 6-BA treatment increased plant height and leaf emergence by increasing the levels of cytokinins (DHZR, IPA) and decreasing the level of abscisic acid (ABA). Transcriptomics analysis identified 7,009 differentially expressed genes (DEGs) in response to 6-BA treatment. The cytokinin-responsive gene *A-ARR8* exhibited a distinct expression pattern, being upregulated at 1, 3, and 6 d post-treatment but downregulated at 11 d. In contrast, ABA-related genes *SnRK2a/b*, *PP2C*, and *ABF3*, were consistently downregulated throughout the treatment period. Metabolomic analysis identified 2,053 metabolites, showing early-phase dominance of phenylpropanoids, and flavonoids, followed by a shift towards ABC transporter-mediated nutrient mobilization. Conjoint analysis highlighted the coordinated activation of secondary metabolite biosynthesis and cytokinin signaling. These results demonstrate that 6-BA induces dormancy release through cytokinin–ABA antagonism and phased metabolic reprogramming from stress defense to growth promotion. Our findings provide a comprehensive framework for optimizing dormancy management in apple cultivation and highlight 6-BA as an effective agrochemical for improving temperate fruit production.

Keywords: bud dormancy, 6-benzylaminopurine, apple rootstock, cytokinin signaling, multi-omics analysis, temperate fruit production

Received 30 April 2025; Received in revised form 9 April 2026; Accepted 13 April 2026; Available online 2 June 2026
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doi: 10.1016/j.jia.2026.06.001

1. Introduction

Apple (*Malus domestica*) is one of the most important fruit crops, recognized for its excellent nutritional content and widespread consumer demand. The successful cultivation of apple trees in temperate regions is strongly dependent on the regulation of bud dormancy (González and Hadley 2023). Dormancy, a survival mechanism developed to endure adverse situations, ensures that bud growth is coordinated with environmental cues such as photoperiod and temperature. However, delayed or inadequate dormancy release can result in irregular bud break, poor flowering, and reduced yield, offering a significant challenge for farmers (Kumar and Singh 2016; González *et al.* 2023).

Dormancy in perennial plants is categorized into endodormancy, ecodormancy, and paradormancy. In particular, endodormancy is regulated internally by genetic and hormonal factors, even under favorable environmental conditions. This dormancy is particularly important for temperate fruit crops, including apples (Fadón *et al.* 2020).

Apple primarily experiences endodormancy, which is broken when chilling requirements are met. Failure to meet these chilling requirements can lead to incomplete or delayed dormancy release, resulting in irregular bud break and reduced yield (Dennis *et al.* 2003). In temperate trees, this cold requirement regulates a system in which a group of genes called dormancy-associated mad box (DAM) act as switches and cease growth until long chilling periods turn them off (Zhao *et al.* 2023). However, the molecular mechanisms that regulate dormancy in major subtropical and tropical tree crops like mango or lychee remain largely unknown. In these species, environmental factors, such as water/drought stress or temperature changes, initiate bud break, but specific metabolic and genetic pathways involved are still poorly characterized. This knowledge gap makes it challenging to create effective methods for uniform bud break in subtropical regions, a problem that is now becoming common with climate change.

In these dormancy regulatory networks, phytohormones act as central integrators of both environmental and internal signals. Dormancy onset and release are finely regulated processes involving intricate interactions of environmental signals, genetic pathways, and phytohormones (Yamane *et al.* 2021). Previous research has focused on the participation of phytohormones such as gibberellins (GAs) and abscisic acid (ABA) in dormancy regulation. However, the specific contributions of cytokinins, particularly 6-benzylaminopurine (6-BA), remain unexplored in dormancy release of apple buds (Spíchal *et al.* 2012).

Synthetic cytokinin, especially 6-BA, is frequently used in horticulture due to its ability to stimulate cell division and delay senescence. In apple trees, 6-BA has demonstrated potential in breaking bud dormancy by altering hormonal balance and boosting metabolic activity (González *et al.* 2023; Milyaev *et al.* 2024).

Cytokinins regulate dormancy release with the interaction of other phytohormones, particularly GA and ABA. GA is known to promote cellular elongation and division, facilitating bud growth, while ABA acts as a growth suppressant, maintaining dormancy by inhibiting cell cycle progression. Cytokinins enhance GA biosynthesis and signaling while inhibiting ABA pathways, resulting in an environment favorable to dormancy release (Wen *et al.* 2016; Kitamura *et al.* 2019).

Furthermore, using metabolomics and transcriptomics to explore cytokinin-mediated dormancy release in apple buds is a unique technique for understanding these processes at a molecular level. Transcriptomics detects dynamic changes in gene expression, which helps to identify transcriptional changes and regulatory networks linked with dormancy. Metabolomics enables the comprehensive characterization of metabolites, providing insights into the metabolic mechanisms involved during dormancy release (González *et al.* 2023). Integrating these technologies allows researchers to explore the interplay between metabolic and genetic factors that drive physiological changes in plants (Xu *et al.* 2023). Previous studies documented dormancy transitions in other woody perennials, such as grapevine, peach, and sweet cherry (Serrano *et al.* 2017). Understanding the molecular and biochemical mechanisms of dormancy release can help in developing targeted treatments, such as synthetic cytokinins, to mitigate challenges and ensure consistent productivity under changing environmental conditions. Although 6-BA is commonly used in horticulture, its molecular and metabolic role in dormancy release of subtropical apple cultivation remains poorly understood. Previous studies have focused on ABA and GA crosstalk, but the regulation of cytokinin and its hormonal interactions remains unexplored. Moreover, an integrated transcriptomic and metabolomic investigation of 6-BA-induced dormancy release in apple rootstocks has not yet been conducted.

To bridge these knowledge gaps, this study combines metabolomics and transcriptomics approaches to uncover the molecular basis of 6-BA-induced dormancy release in apples. How do dormant buds respond after 6-BA treatment? How do endogenous hormones and genes respond to 6-BA treatments in apple rootstock during bud dormancy break? To understand these questions, we have treated Xianheng 01 apple rootstock nursery plants with 6-BA and compared them with the control. Samples were taken at different stages for multi-omics analysis. Our findings will provide unique insight into the metabolic and transcriptional networks involved in dormancy release with practical implications for increasing apple yield and tackling dormancy-related challenges in horticulture.

2. Materials and methods

2.1. Plant material selection and treatment application

The experiment was performed using Xianheng 01 apple rootstock nursery plants using their dormant buds as study material. The apple rootstocks were sourced from a commercial nursery and grown under controlled conditions (temperature (27±1)°C/(21±1)°C, relative humidity 60–70%) at the Yangling Substation, Northwest Agriculture and Forestry University, Shaanxi Province, China. At the time of treatment, the buds were in a fully dormant state, as confirmed by morphological characteristics typical of dormancy. To break

bud dormancy, 6-BA was applied at a concentration of 0.48 g L^{-1}. The solution was prepared by dissolving 6-BA in 50 mL double distilled water (ddH_2O), with 1 mol L^{-1} NaOH added dropwise until 6-BA was fully dissolved in water. Then ddH_2O was added to make the final volume of the solution up to 1 L. For the experiment, a total of 60 plastic trays and 50 plants in each tray containing uniform plant sizes were randomly assigned to two treatment groups. Thirty trays were subjected to 6-BA treatment and 30 trays were kept as a control treated with ddH_2O.

2.2. Morphological characterization

Thirty days after the application of 6-BA and control treatments, morphological characterization of the apple rootstock buds was conducted to assess the effects of 6-BA on breaking bud dormancy. Plant samples from both treated and control groups (a group of 10 buds from different plants/replication) were examined, and photographs were taken to document any morphological changes: plant height and the number of newly emerged leaves after treatment application were measured. Height was measured from the base of the plant to the top of the tallest shoot, while the number of new leaves was recorded as the total count of newly emerged leaves on the plant.

2.3. Transcriptome sequencing

The shoot bud samples were taken (three replications) for RNA sequencing at 1, 3, 6, and 11 d after 6-BA treatment. TRIzol Reagent Kit (Invitrogen, Carlsbad, CA, USA) was used for RNA extraction from each sample. RNA integrity was measured using a 2100 Bioanalyzer (Agilent Technologies) and verified by RNase-free agarose gel electrophoresis. cDNA was synthesized from mRNA by reverse transcription using a reverse transcription kit (NEB #7530, New England Biolabs, Ipswich, MA, USA). After purifying double-stranded cDNA, performing end repair, and amplification with PCR, cDNA libraries were sequenced by using a NovaSeq 6000 platform (Illumina Co., San Diego, CA, USA) at Gene Denovo Biotechnology Co. (Guangzhou, China).

2.4. Functional enrichment analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses were performed to identify DEGs that were significantly enriched in metabolic pathways and GO terms as compared to the whole transcriptome background at a Bonferroni-corrected *P*-value≤0.05 (Aickin and Gensler 1996). Then KOBAS and Goat tools were performed for the KEGG pathway and the GO functional enrichment analysis (Xie *et al.* 2011).

2.5. Metabolomics analysis

About 100 mg of fresh samples from each treatment were homogenized in liquid nitrogen. The resultant homogenate was reconstituted in pre-chilled 80% methanol and 0.1% formic acid by thorough vortexing. Then after 5 min incubation on ice, the samples were centrifuged at 15,000 r min^{-1} at 4°C for 20 min. The collected supernatant was then diluted with LC-MS grade water to achieve a final concentration of 53% methanol. Subsequently, it was transferred to a fresh tube and centrifuged at 15,000 r min^{-1} at 4°C for 20 min. Ultimately, the sample was injected into the LC-MS/MS system using an ExionLC™ AD system (SCIEX) coupled with a QTRAP® 6500+ mass spectrometer (SCIEX) at Genedenovo (Guangzhou, China). Multiple reaction monitoring (MRM) was performed using a self-constructed in-house database containing over 2,500 substances. The chromatographic peak area represents the relative abundance of each corresponding substance. We exported all the chromatographic peak area integration data (without units) to obtain quantitative and qualitative results of metabolites. Q3 was used for metabolite quantification, while DP (declustering potential), Q1, Q3, RT (retention time), and CE (collision energy) were used for the identification of metabolites. HPLC-MS/MS generated data files were processed with SCIEX OS Version 1.4 for peak integration and correction. Metabolites with a VIP (variable importance in projection) score of ≥1 and a *P*-value<0.05 were identified as differentially accumulated metabolites (DAMs).

2.6. Conjoint analysis of the transcriptomic and metabolomic

All DEGs and DAMs from the comparisons of control *vs.* 6-BA were aligned with the KEGG database. Pathways that showed significant enrichment (*P*-value<0.05) among both the DEGs and the DAMs were selected and visualized on a pathway diagram using Adobe Illustrator v. 2023.

2.7. Quantitative real-time PCR assays

qRT-PCR analysis was performed following the method outlined by Fan *et al*. (2016). Total RNA was extracted from apple buds treated with 6-BA and the control groups. Gene expression levels were quantified using SYBR Green. Relative expression level was calculated by $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001). Quantitative analysis used the apple *ACTIN* gene as a control for normalization (Tahir *et al.* 2023). All the primer sequences of genes are listed in Appendix A.

2.8. Measurement of endogenous hormone

The levels of seven major endogenous hormones, indole-3-acetic acid (IAA), methyl jasmonate (MeJA), ABA, zeatin riboside (ZR), dihydrozeatin riboside (DHZR), isopentenyl adenosine (IPA), and gibberellic acid 3 (GA_3), were measured at 1, 3, 6, and 11 d in both groups (treated and control) by the enzyme-linked immunosorbent assay (ELISA) method (Dobrev and Kamınek 2002). Monoclonal antibodies targeting each hormone were sourced from the Center of Plant Growth Regulator, China Agricultural University. Three biological replicates (group of 10 buds from different plants per replication) per time point were analyzed for both groups. The methodology outlined by Wang *et al.* (2020) was used for hormone extraction and purification.

2.9. Statistical analysis

Data were analyzed using the Statistics 8.1 software (Tallahassee, FL 32317, USA), with analysis of variance (ANOVA). Differences among treatment means were

assessed using the least significant difference (LSD) test at $P<0.05$. GraphPad Prism version 10 for Windows GraphPad software (San Diego, California, USA) was used for graphical presentation.

3. Results

3.1. Effect of 6-BA on morphology of Xianheng 01 apple rootstock

The 6-BA application significantly affected the morphology of Xianheng 01 apple rootstock nursery plants. Treated plants exhibited visible improvements in plant height and leaf development compared to the control group. Morphological differences between the groups were apparent, with control plants exhibiting stunted growth and smaller leaves. In contrast, the treated plants displayed vigorous growth and expanded, healthy leaves (Fig. 1-A). The average increase in plant height was higher in the treated group, with a mean increase of approximately 3 cm. In comparison, the control group showed a modest increase of around 1 cm (Fig. 1-B).

In addition to promoting height, 6-BA treatment also enhanced leaf formation. The treated group showed a significantly higher number of new leaves per plant, with an average of 4 leaves compared to fewer than two leaves in the control group (Fig. 1-C). These results highlight the role of 6-BA in promoting vegetative growth in Xianheng 01 apple rootstocks.

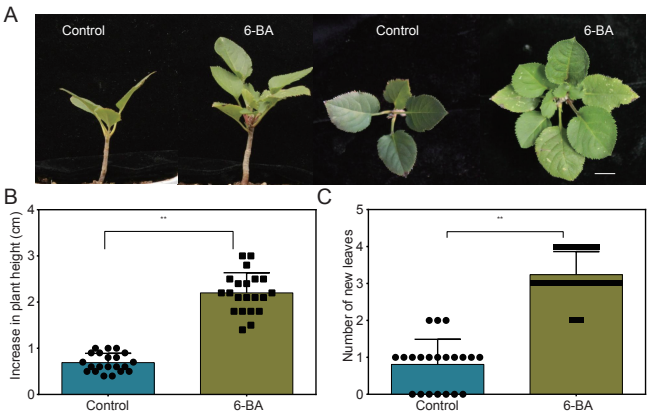

Fig. 1 Morphological responses of Xianheng 01 apple rootstock to 6-BA treatment after 30 d of application. A, representative morphology of control and 6-BA treated plants (side and top views); scale bar=1 cm. B, increase in plant height. C, number of new leaves. Error bars represent the average values±SD (n=3 biological replicates). Asterisks (**) denote significant differences at $P<0.01$.

3.2. Measurement of endogenous hormones

The endogenous levels of different hormones, including IAA, MeJA, ABA, ZR, DHZR, IPA, and GA_3, were measured at various time points (1, 3, 6, and 11 d) during dormancy break in Xianheng 01 rootstock. The levels of DHZR peaked on 1 d in 6-BA-treated samples and showed a 2-fold increase as compared to the control. Although the level slightly declined by 11 d, it remains significantly higher than those in the control group. Similarly, IPA levels were consistently higher in the treated samples, with higher concentrations across all time points. The level of ZR at 1 d was higher in the control group as compared to the treated samples, while the 11 d treated samples exhibited a higher concentration of ZR as compared to the control group. The concentration level of MeJA was recorded more in treated samples at 1 and 11 d, while its value was significantly higher in the control group at 6 d, as shown in the graph (Fig. 2).

The results demonstrated that the concentration of IAA in the control group was higher on 1 d and continued to rise at 3 and 6 d. However, by 11 d, its concentration decreased in the control group compared to the treated samples. Similarly, the ABA level in the control group remained consistently high at 1 and 3 d as compared to the treated samples, while its level became significantly low at 6 and 11 d in comparison with the control group. The GA_3 levels were significantly higher in the control as compared to the 6-BA-treated group at 1 and 3 d

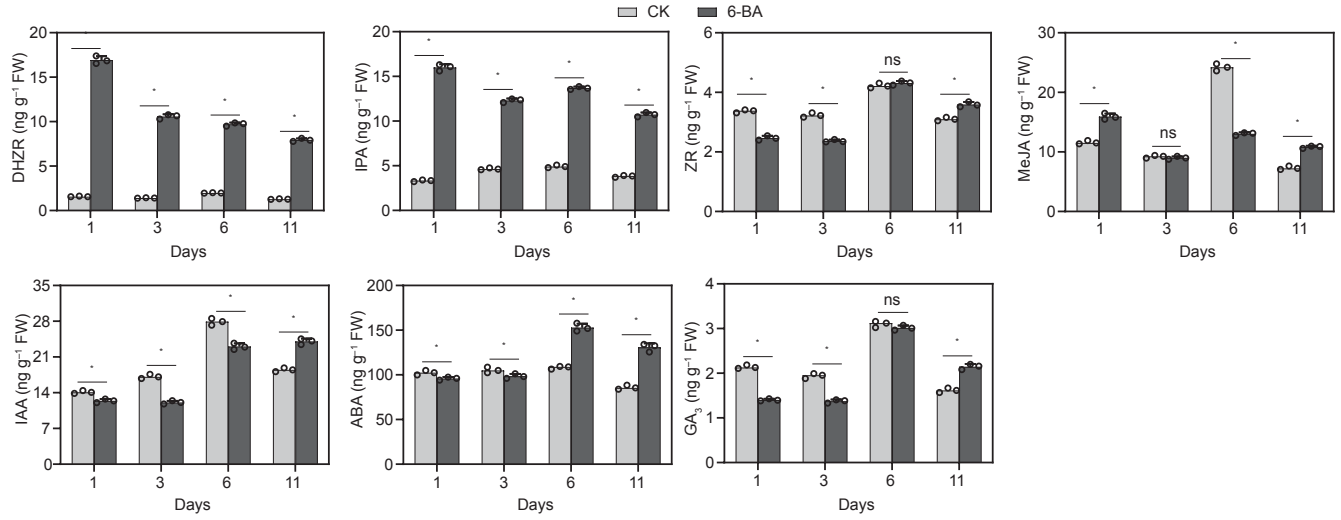

Fig. 2 Effects of 6-BA treatment (0.48 g L^{-1}) on endogenous hormone contents during dormancy break in Xianheng 01 apple rootstock. Endogenous levels of DHZR, IPA, ZR, MeJA, IAA, ABA, and GA_3 were measured at various time points (1, 3, 6, and 11 d). Error bars refer to the average value±SD (n=3 biological replicates). ns, $P>0.05$ and *, $P<0.05$.

post-treatment. However, by 6 d, there was no significant difference observed between the treated and control groups. Notably, at 11 d, the GA_3 level was significantly higher in the treated group than in the control (Fig. 2).

3.3. RNA-sequencing analysis

A comprehensive transcriptomic analysis was performed to investigate the molecular mechanisms underlying the effects of 6-BA on bud dormancy release in apple rootstock. RNA sequencing was performed on the sprouting buds of treated and control plants at different intervals: 1 d (A *vs.* B), 3 d (C *vs.* D), 6 d (E *vs.* F), and 11 d (G *vs.* H) to understand the molecular mechanism of dormancy release in Xianheng 01 apple rootstock. In this A, C, E, and G represent the control groups, and B, D, F, and H represent the 6-BA-treated groups. A total of 41.47–43.39 million raw reads were obtained. The percentages of Q20 and Q30 were 99.30% and 97.50%, respectively. The percentage of GC ranged from 45.92% to 46.61% (Appendix B). In all 22 libraries, the total reads ranged from 20,735,365 to 21,699,412 bp. Uniquely mapped reads ranged from 91.48% to 93.04%, while multiple mapped and unmapped reads ranged from 3.64% to 4.76% and 2.99% to 4.16%, respectively. Detailed information is present in Appendix C. Principal component analysis (PCA) graph was generated for both groups, revealing distinct differences that highlight stage-specific changes in gene expression associated with dormancy (Fig. 3-A). A boxplot displaying the expression of all genes in both control and 6-BA-treated groups was created, highlighting the general distribution and range of gene expression (Fig. 3-B). The density plot illustrates the distribution of gene expression levels (Fig. 3-C). The density curves for all samples are largely overlapping, suggesting a consistent global gene expression profile across the different treatment groups. Correlations between samples are shown in Appendix D. Total expressed genes were 30,854, 30,945, 30,952, and 30,679 in each comparison group A *vs.* B, C *vs.* D, E *vs.* F, and G *vs.* H, respectively.

3.4. Identification of DEGs

To identify responsive DEGs for dormancy release, the statistical software R was used to analyze gene expression levels and detect DEGs, facilitating the study of various processes underlying 6-BA-treated apple rootstock. The analysis identified a total of 7,009 DEGs in all pairwise comparisons, with 2,976 (2,038 upregulated and 938 downregulated), 3,437 (1,969 upregulated and

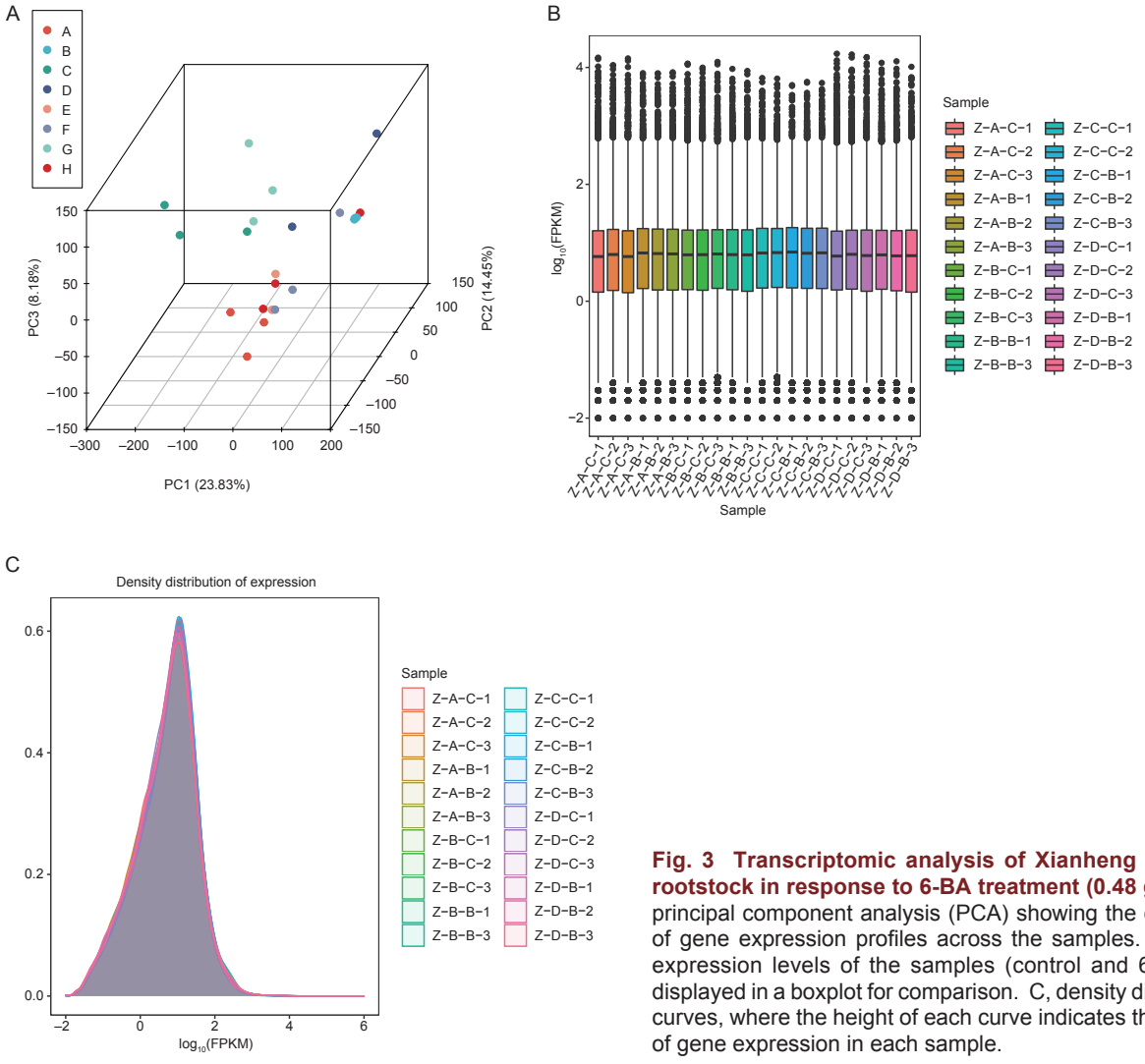

Fig. 3 Transcriptomic analysis of Xianheng 01 apple rootstock in response to 6-BA treatment (0.48 g L^{-1}). A, principal component analysis (PCA) showing the clustering of gene expression profiles across the samples. B, gene expression levels of the samples (control and 6-BA) are displayed in a boxplot for comparison. C, density distribution curves, where the height of each curve indicates the density of gene expression in each sample.

1,468 downregulated), 157 (45 upregulated and 112 downregulated), and 439 (187 upregulated and 252 downregulated) (Fig. 4).

3.5. Functional enrichment analyses of DEGs

KEGG and GO enrichment analyses were conducted to identify significantly enriched biological processes and functions among DEGs. The DEGs were categorized into three major GO categories: biological process, cellular component, and molecular function. GO analysis indicated that cell cycle, developmental process, nucleus, intracellular organelle, DNA binding, protein binding and response to stimulus were the most enriched in all comparison groups (Appendix E). KEGG pathway analysis revealed significant enrichment of several pathways, including MAPK signaling, plant hormone and signal transduction, ribosome, biosynthesis of secondary metabolites, protein processing in endoplasmic reticulum and plant pathogen interaction in all pairwise comparisons (Fig. 5). The KEGG pathway circle diagram identified five major categories, such as cellular processes, environmental information processing, genetic information processing, metabolism, and organismal systems. Notably, the metabolism and organismal systems categories contained the most significant proportion of DEGs (Appendix F).

3.6. Expression analysis of hormone signaling-related DEGs

KEGG pathway analysis identified hormone signaling-related genes that encode receptors and response factors. Comprehensive explanations are provided below:

Expression analysis of auxin-related DEGs The expression profile of auxin-related DEGs from all pairwise combinations is present in this section. Representative DEGs include the auxin-responsive GH3 family protein (GH3), IAA, auxin-responsive factor (ARF), small auxin up RNA (SAUR), SAUR-like auxin-responsive protein family, and AUX/IAA transcriptional regulator family protein. In the A *vs.* B group, a total of 31 DEGs were present, of which all the *SAUR* genes were downregulated except *SAUR1* and *SAUR4*. The *GH3b* and *GH3c* were also downregulated, while all the remaining other genes, including *IAA*, *ARF*, and *AUX/IAA*, were upregulated.

In the C *vs.* D group, a total of 22 genes were found, of which only 6, *AUX/IAA8*, *AUX/IAA17*, *GH3c*, *GH3F*, *SAUR8*, and *SAUR9*, were downregulated, and the others were upregulated. In the E *vs.* F group, only *GH3g* was found with downregulated expression. In G *vs.* H, there was no gene present (Fig. 6-A).

Expression analysis of cytokinin-related DEGs This analysis summarizes the expression patterns of cytokinin-related DEGs from all combinations. Representative DEGs include the histidine-containing phosphotransmitter (APH), CHASE domain-containing histidine kinase protein (CRE), histidine kinase (APH), and response regulator (ARR). In the A *vs.* B group, a total of 18 DEGs were identified, of which only two *B-ARR1* and *B-ARR4* genes were downregulated. The remaining others, including *CRE2a*, *CRE3b*, and *APH1*, were upregulated. In the C *vs.* D group, there were 15 DEGs, of which only two DEGs, *CRE1d*, and CRE1e, were downregulated. In comparison, 13 others, including, *APH2*, *ARR3*, and *ARR10*, were upregulated. In the E *vs.* F, only *B-ARR7* gene showed downregulation, while *A-ARR1*, *A-ARR3*, *A-ARR6*, *A-ARR7*, *A-ARR8,* and *A-ARR9,* were observed in upregulation. In the G *vs.* H, only two genes, *B-ARR8* and *A-ARR8,* were present, which showed downregulation (Fig. 6-B). *A-ARR8* showed a different response as compared to the other DEGs because its expression at 1, 3, and 6 d remained upregulated.

Expression analysis of GA-related DEGs The DEGs related to expression analyses for GA signaling include gibberellin receptor *GID1* members, DELLA family members and transcription factor (TF). In A *vs.* B comparison, 19 DEGs were found, including *GID1c*, *DELLA7*, *TF1*, *TF2* *GID1a*, *DELLA1*, *DELLA10*, and *TF4*. From these, 15 genes were shown to be upregulated, while 4 genes were observed in downregulation. In the C *vs.* D 4 genes, *GID1d*, *DELLA6*, *DELLA8*, and TF4 were upregulated, and all others showed downregulation. In E *vs.* F, only two genes, *DELLA4* and *TF2,* were found upregulated and downregulated, respectively. In G *vs.* H only one gene, *DELLA9,* was found in upregulation (Fig. 6-C).

Expression analysis of ABA-related DEGs The expression patterns of ABA-related DEGs include protein phosphatase 2C (PP2C), SNF1-related protein kinase 2 (SnRK2), and basic leucine zipper transcription factors (ABF). The analysis indicated that in comparison to A *vs.* B, there were two genes present *ABF1*, and *SnRK2a*, which showed upregulation and downregulation, respectively. In the C *vs.* D, a total of 10 DEGs were present, and all were downregulated. In E *vs.* F, only one gene, *SnRK2b* was present, which showed downregulation, and not a single gene was found in the group G *vs.* H (Fig. 7-A).

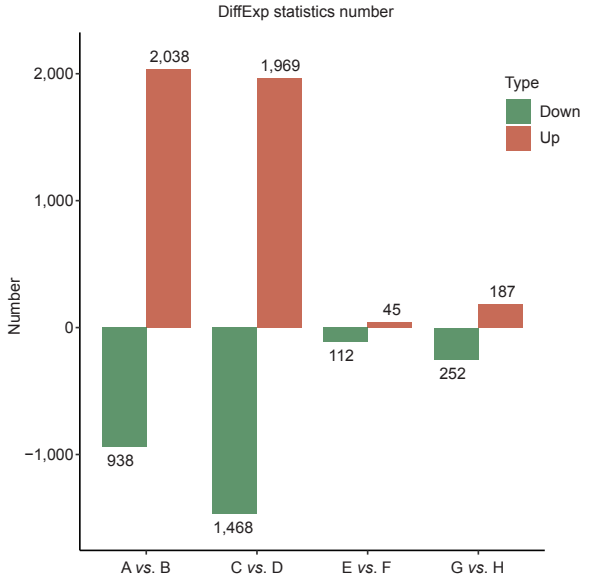

Fig. 4 Differentially expressed genes (DEGs) identified in Xianheng 01 apple rootstock following 6-BA treatment (0.48 g L^{-1}). Numbers of upregulated and downregulated DEGs in each pairwise comparison at 1 d (A *vs.* B), 3 d (C *vs.* D), 6 d (E *vs.* F), and 11 d (G *vs.* H). A, C, E, and G represent the control groups, and B, D, F, and H represent the 6-BA-treated groups.

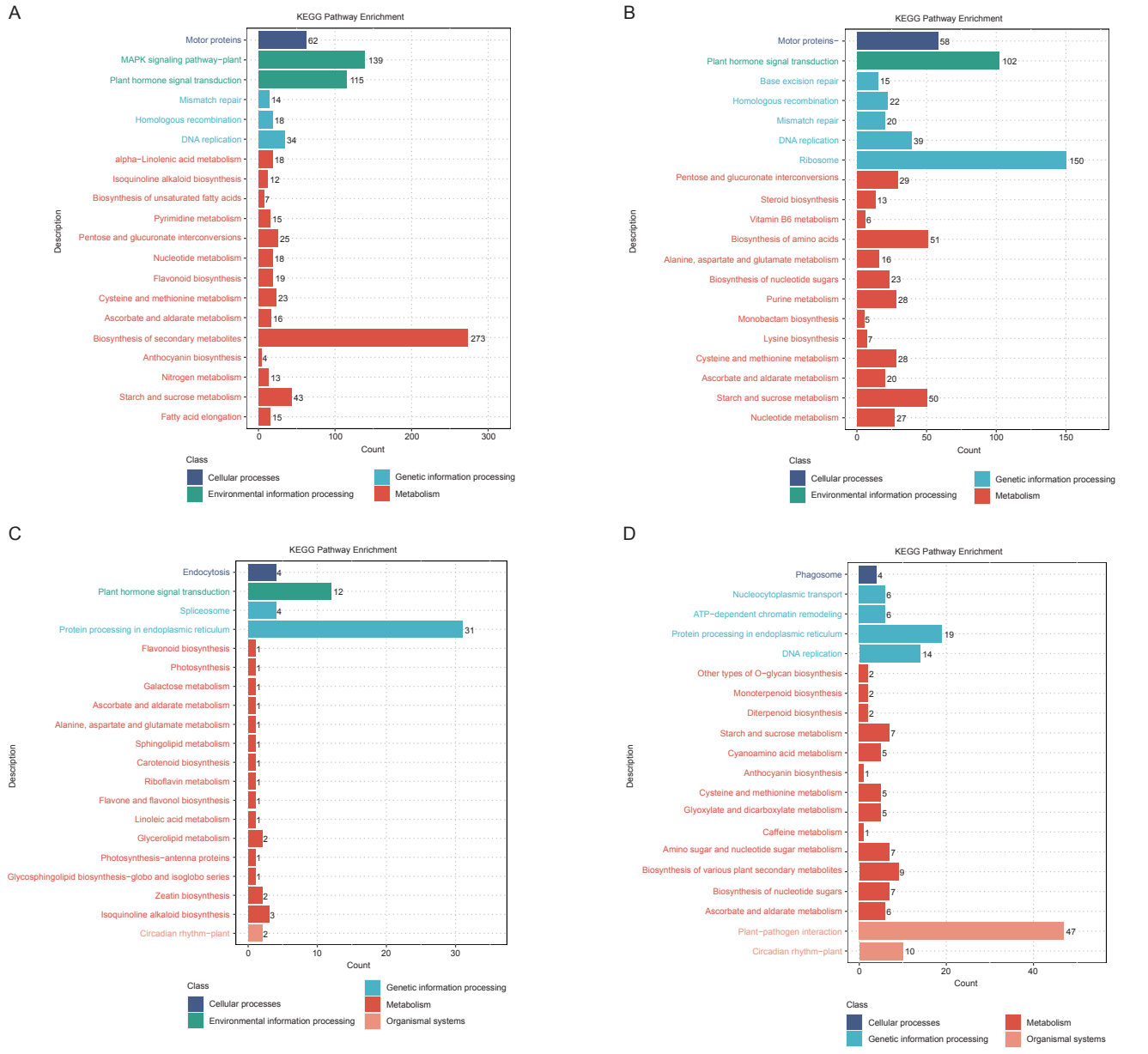

Fig. 5 KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in Xianheng 01 apple rootstock following 6-BA treatment (0.48 g L^{-1}). The top enriched KEGG pathways identified in pairwise comparisons between control and 6-BA-treated samples at A, 1 d (A *vs.* B); B, 3 d (C *vs.* D); C, 6 d (E *vs.* F); and D, 11 d (G *vs.* H) after treatment. The *x*-axis indicates the number of DEGs enriched in each pathway, and the *y*-axis shows the pathway names. Bar colors represent different KEGG functional categories. A, C, E, and G denote control samples, whereas B, D, F, and H denote 6-BA-treated samples.

Expression analysis of BR-related DEGs The expression pattern of BR-related DEGs was observed in all comparison groups. Genes include xyloglucan endotransglycosylase 6 (TCH6), Xyloglucan endotransglucosylase/ hydrolase family protein (TCH), BRI1 kinase inhibitor 1 (BRI1) and CYCLIN D3 (CYCD3). In comparison A *vs.* B, there were a total of 22 DEGs found, of which *BRI1a*, *BRI1b*, *BRI1c*, *BRI1e*, *BAK1a*, *BAK1c*, *BAK1e*, and *BAK1h*, were downregulated, and the remaining 14 genes were upregulated. In the C *vs.* D comparison, a total of 30 DEGs were observed, among which 16 were downregulated and 14 were upregulated. In the E *vs.* F, *BAK1q* and *BAK1p* genes were found upregulated and downregulated, respectively. In the comparison G *vs.* H, *BAK1r* was upregulated, while *TCH4e* and *TCH4e* were downregulated (Fig. 7-B).

Expression analysis of ethylene-related DEGs This portion presented the expression pattern of ethylene-related DEGs. Genes for ethylene include ethylene receptor protein (ETR1) and ethylene response factor 1 (ERF1). A *vs.* B had only three genes, *ETR1*, *ERF1/2a*, and *ERF1/2b,* which showed downregulation, and C *vs.* D had a total of two genes *ERF1/2a* and *ERF1/2b,* which also showed downregulation Appendix G.

Expression analysis of JA-related DEGs This section presents the expression pattern of JA-related DEGs, which include jasmonate-ZIM domain protein 3 (JAZ3), jasmonate-

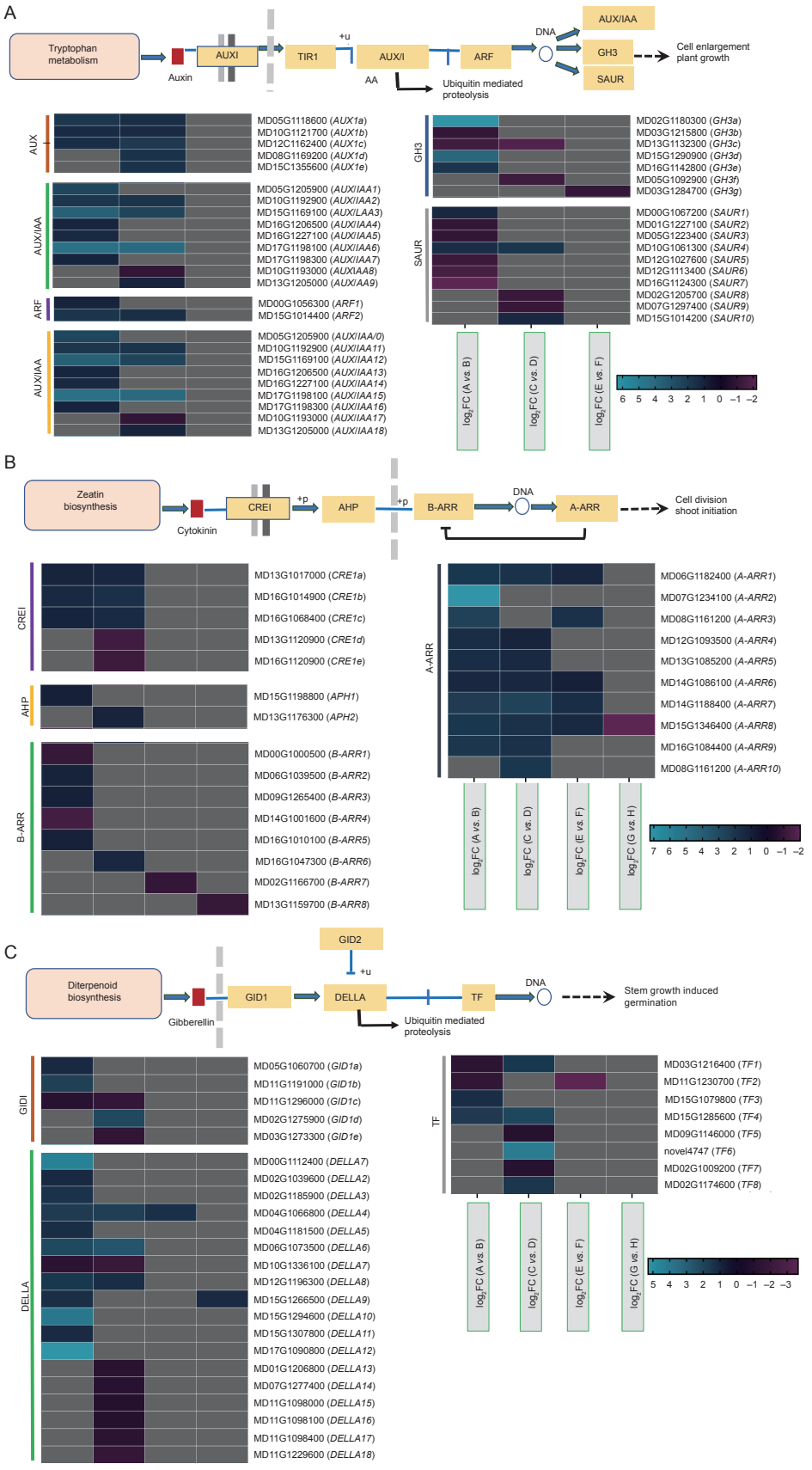

Fig. 6 Expression profiles of differentially expressed genes (DEGs) involved in AUX, CK, and GA signaling pathways. A, heatmap of AUX-related DEGs involved in signaling pathways. B, heatmap of CK-related DEGs involved in signaling pathways. C, heatmap of GA-related DEGs involved in signaling pathway. The log_2FC values of DEGs identified in each pairwise comparison. The color gradient represents the gene expression levels, while grey colour indicate no DEGs were identified. +u, ubiquitination modification; +p, phosphorylation modification.

amino synthease (JAR1), and MYC transcription factors (MYC). In the A *vs.* B all 18 genes, including *JAR1a*, *JAZ3*, *MYC2b*, *MYC2i*, and *MYC2n*, were upregulated, and no gene was observed in downregulation. In the C *vs.* D group all the present genes were upregulated except two genes, *MYC2m* and *JAZ4*, which showed downregulation. No gene was found in E *vs.* F comparison, while G *vs.* H had two *JAR1e* and *MYC2n* genes in downregulation (Appendix G).

3.7. Validation of DEGs using qRT-PCR

To verify the accuracy of the RNA-seq data, 12 DEGs related to CK, AUX, GA, and BR were selected for the qRT-PCR for expression analysis. Interestingly, relative expression patterns of all DEGs determined from RT-qPCR closely resembled those observed from RNA-seq data (Fig. 8), except for the *BAK1L* gene, which showed upregulation in RNA-seq data but in RT-qPCR showed downregulation. Thus, the data derived from RNA-seq is reliable.

3.8. Metabolomics analyses of 6-BA-treated apple rootstock across different stages

Metabolomics profiling was conducted to assess the biochemical changes induced by 6-BA treatment during dormancy release in apple rootstock. A total of 2,053 DAMs were detected and categorized into 12 superclasses, 64 classes, and 230 subclasses (Fig. 9-A). The pie chart represents the distribution of various superclasses based on their relative abundance. The 12 superclasses of metabolites contain alkaloids, amino acids and peptides, benzenoids, organic oxygen compounds, carbohydrates, lipids, fatty acids, and lipids like molecules, phenylepropanoids and polyketides, shikimates and phenylpropanoid, polyketides and terpenoids. Among all these

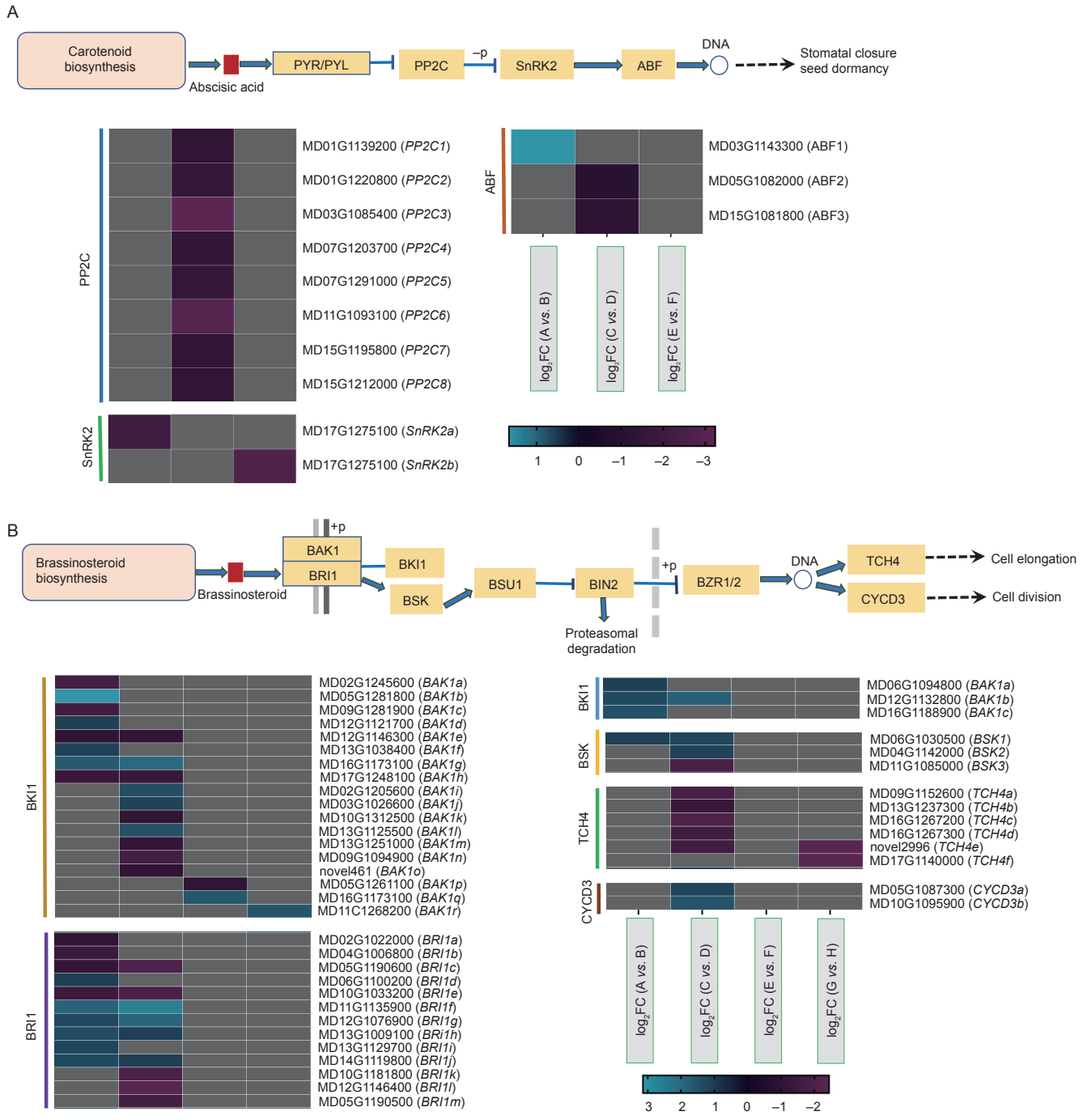

Fig. 7 Expression profiles of differentially expressed genes (DEGs) involved in ABA and BR signaling pathways. A, heatmap of ABA-related DEGs involved in signaling pathways. B, heatmap of BR-related DEGs involved in signaling pathways. The log_2FC values of DEGs identified in each pairwise comparison. The color gradient represents the gene expression levels, while grey colour indicate no DEGs were identified. A, C, E, and G denote control samples, whereas B, D, F, and H denote 6-BA-treated samples. –p, dephosphorylation modification; +p, phosphorylation modification.

superclasses shikimates and phenylpropanoid, others, and terpenoids 32.197%, 23.624% and 16.464%, respectively, were found in high percentages as compared to the other 9 superclasses (Fig. 9-B). There were 962 DAMs identified in all pairwise comparisons. In the A *vs.* B, 231 DAMs (191 upregulated and 40 downregulated); C *vs.* D, 208 DAMs (147 upregulated and 61 downregulated); E *vs.* F, 301 DAMs (197 upregulated and 104 downregulated); and G *vs.* H 222 DAMs (83 upregulated and 139 downregulated) were found, as shown in the Venn diagram (Fig. 9-C). The PCA plot showed PC1 and PC2 cumulatively contributed 19.9% and 10% of the variation, respectively (Fig. 9-D).

A k-means clustering analysis further illustrates the grouping of samples based on their metabolic profiles. The plot revealed nine different clusters, indicating groups of samples with varying numbers and similar metabolic

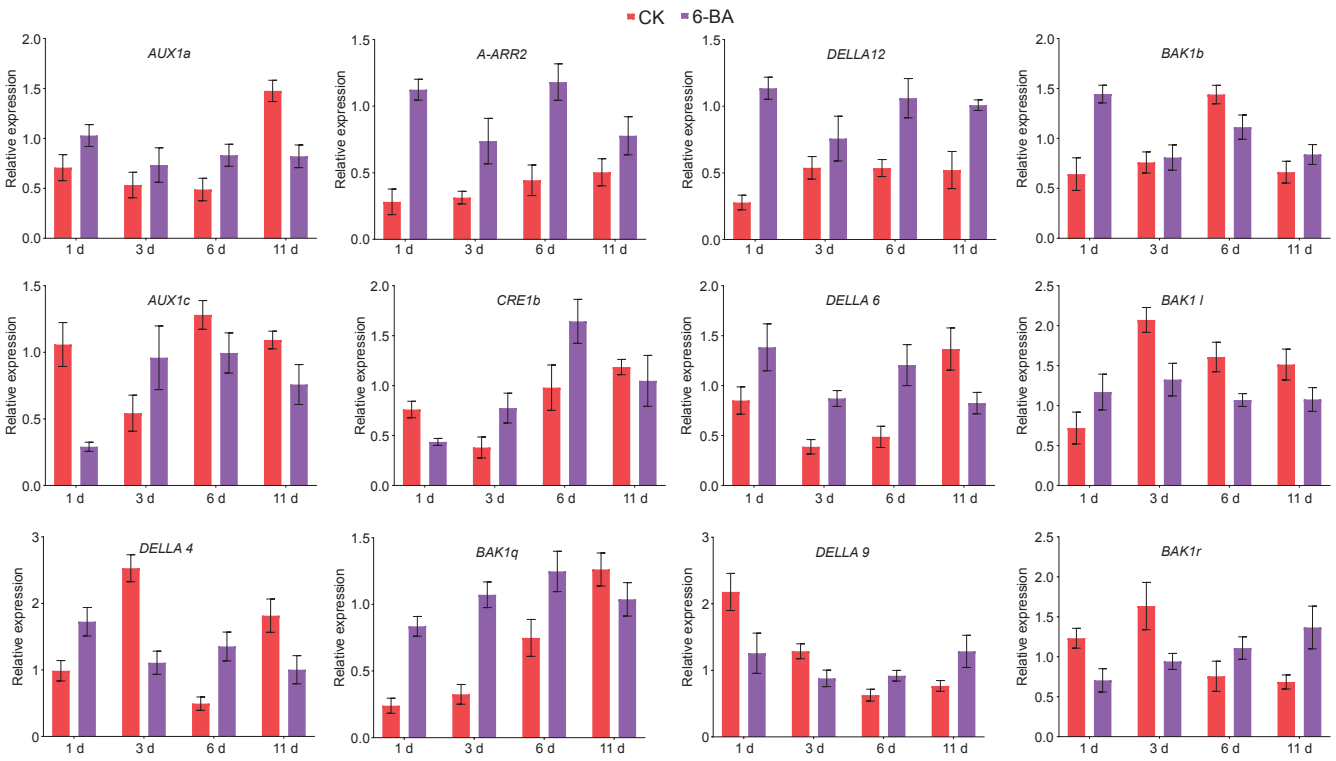

Fig. 8 Validation of RNA-sequence data by qRT-PCR analyses. Relative expression levels of selected differentially expressed genes (DEGs) were determined by qRT-PCR in control and 6-BA-treated samples at 1, 3, 6, and 11 d after treatment. Data are presented as mean±SD (*n*=3 biological replicates).

patterns in each cluster in treated and control samples from A to H. This clustering supports the observation that dormancy release is associated with significant shifts in the metabolome, with samples from different stages of dormancy release grouping into different clusters (Fig. 9-E).

3.9. Functional enrichment analysis of the KEGG metabolic pathway for DAMs

KEGG functional annotation analysis for the DAMs in different stages, followed by pathway analysis to further understand their biological functions. Enrichment analysis identified a total of 15 pathways in A *vs.* B. Significantly enriched pathways were flavonoid biosynthesis, biosynthesis of secondary metabolites, isoflavonoid and anthocyanin biosynthesis (Fig. 10-A). While enriched pathways in C *vs.* D were metabolic pathways, flavonoid biosynthesis, and ABC transporters (Fig. 10-B). In E *vs.* F, the significantly enriched pathways were flavonoid biosynthesis, biosynthesis of secondary metabolites, biosynthesis of amino acids, and biosynthesis of co-factors (Fig. 10-C). In G *vs.* H, highly enriched pathways were biosynthesis of secondary metabolites and flavonoid biosynthesis. Overall, in all comparisons, the highly enriched pathways were biosynthesis of secondary metabolites (70%) and flavonoid biosynthesis (80%), as shown in Fig. 10-D.

3.10. Integration of transcriptomic and metabolomic analysis

An integrated analysis of transcriptomic and metabolomics data was conducted to explore the relationship between metabolite accumulation and gene expression. Most enriched pathways in A *vs.* B were biosynthesis of secondary metabolites, phenylpropanoid biosynthesis, and flavonoid biosynthesis. Starch and sucrose metabolism, plant hormone signal transduction, and nucleotide metabolism were found enriched in C *vs.* D. isoquinoline alkaloid biosynthesis, biosynthesis of secondary metabolites, and flavonoid biosynthesis were enriched pathways in comparison E *vs.* F, while in G *vs.* H, flavone and flavonol biosynthesis, ascorbate and aldarate metabolism, and biosynthesis of secondary metabolites were highly enriched pathways. (Fig. 11-A–D).

The circular correlation plot depicts the conjoint analysis of transcriptomic and metabolomic data to clarify the molecular mechanisms underlying dormancy release in apple rootstock. In the figure, genes and metabolites are classified as upregulated, down-regulated and showing no difference based on their response to 6-BA (Fig. 12-A–D).

4. Discussion

Bud dormancy is a crucial adaptive strategy in perennial plants, allowing them to endure harsh environmental conditions and ensuring coordinated growth initiation in the spring (Cooke *et al.* 2012; Fennell *et al.* 2014; Cai *et al.* 2024). However, the molecular mechanisms underlying dormancy release, especially in apple rootstocks, are not yet fully understood (Rohde and Bhalerao 2007; Cai *et al.* 2024). Recent advances suggest that phytohormones,

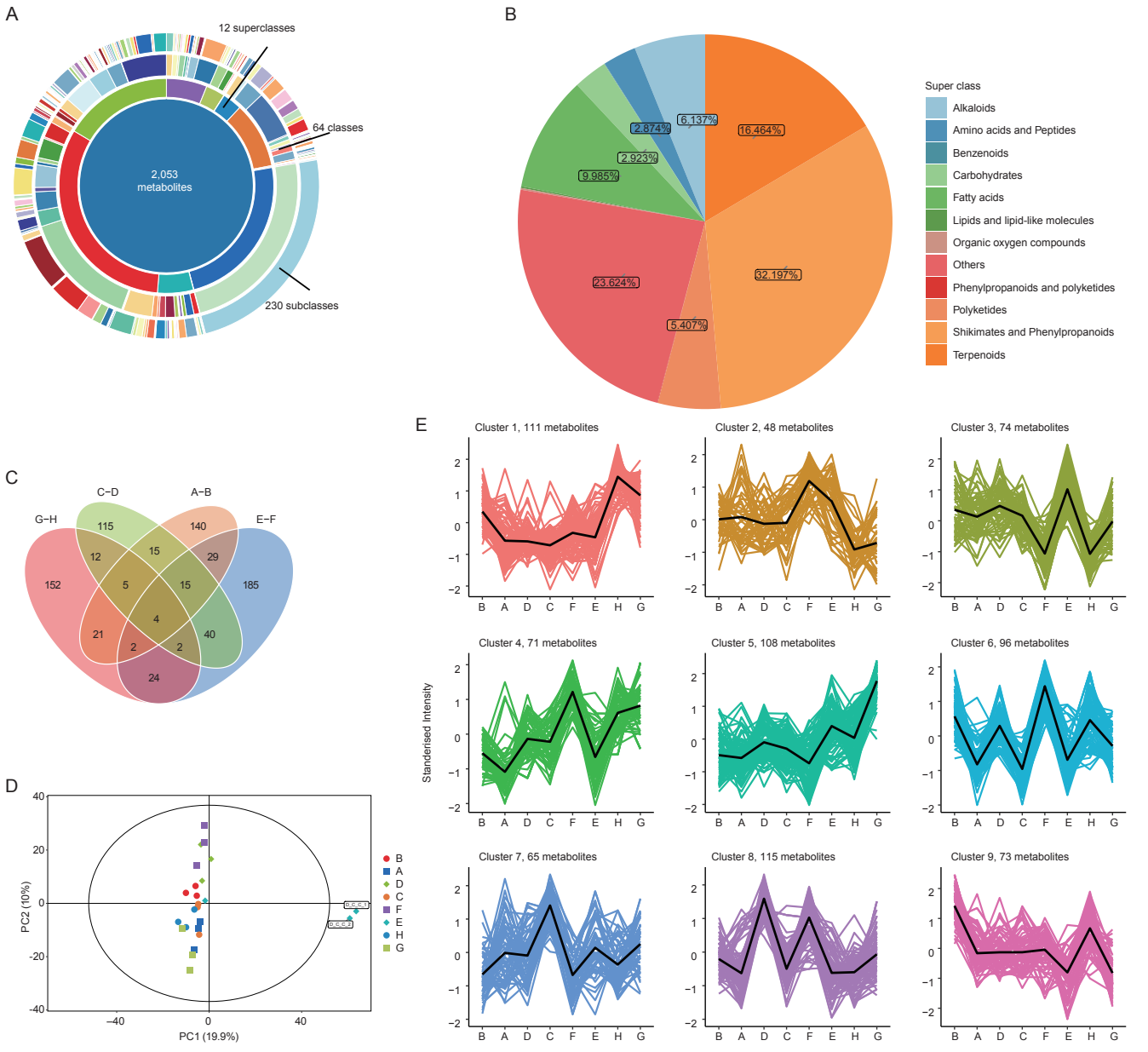

Fig. 9 Metabolomic profiling of Xianheng 01 apple rootstock following 6-BA treatment (0.48 g L^{-1}). A, classification of the identified metabolites into superclasses, classes, and subclasses. B, the pie chart represents the distribution of various superclasses based on their relative abundance. C, a Venn diagram illustrates the overlap of metabolites across all group stages of 1, 3, 6, and 11 d. D, PCA score label plot. E, k-means clustering analysis across control and 6-BA-treated samples at different time points. A, C, E, and G denote control samples, whereas B, D, F, and H denote 6-BA-treated samples.

particularly cytokinins and ABA, act as central regulators of dormancy transitions, though their crosstalk with transcriptomic networks remains poorly characterized in woody species (Chen *et al.* 2023; Singh and Roychoudhury 2023). The interplay between hormonal changes and transcriptomic regulation plays a critical role in bud dormancy release. The endogenous levels of key hormones, including IAA, ZR, cytokinins (DHZR and IPA), ABA, MeJA, and GA_3, were measured at different comparison groups (1, 3, 6, and 11 d) during dormancy break in Xianheng 01 apple rootstock. Cytokinins are well-known for regulating cell division and shoot development, and their application has been shown to promote bud break in several plant species (Ramírez *et al.* 2008; Wang *et al.* 2021). Results showed that 6-BA treatment significantly promotes vegetative growth; treated plants exhibited a 3 cm increase in height and an average of 4 new leaves per plant, compared to 1 cm and fewer leaves in the control group. These morphological changes underscore the potential of 6-BA as an effective strategy for enhancing dormancy management in apple cultivation (Ríos *et al.* 2014; Li *et al.* 2019). At the molecular level, transcriptomic analysis identified significant changes in gene expression profiles at four different comparison groups.

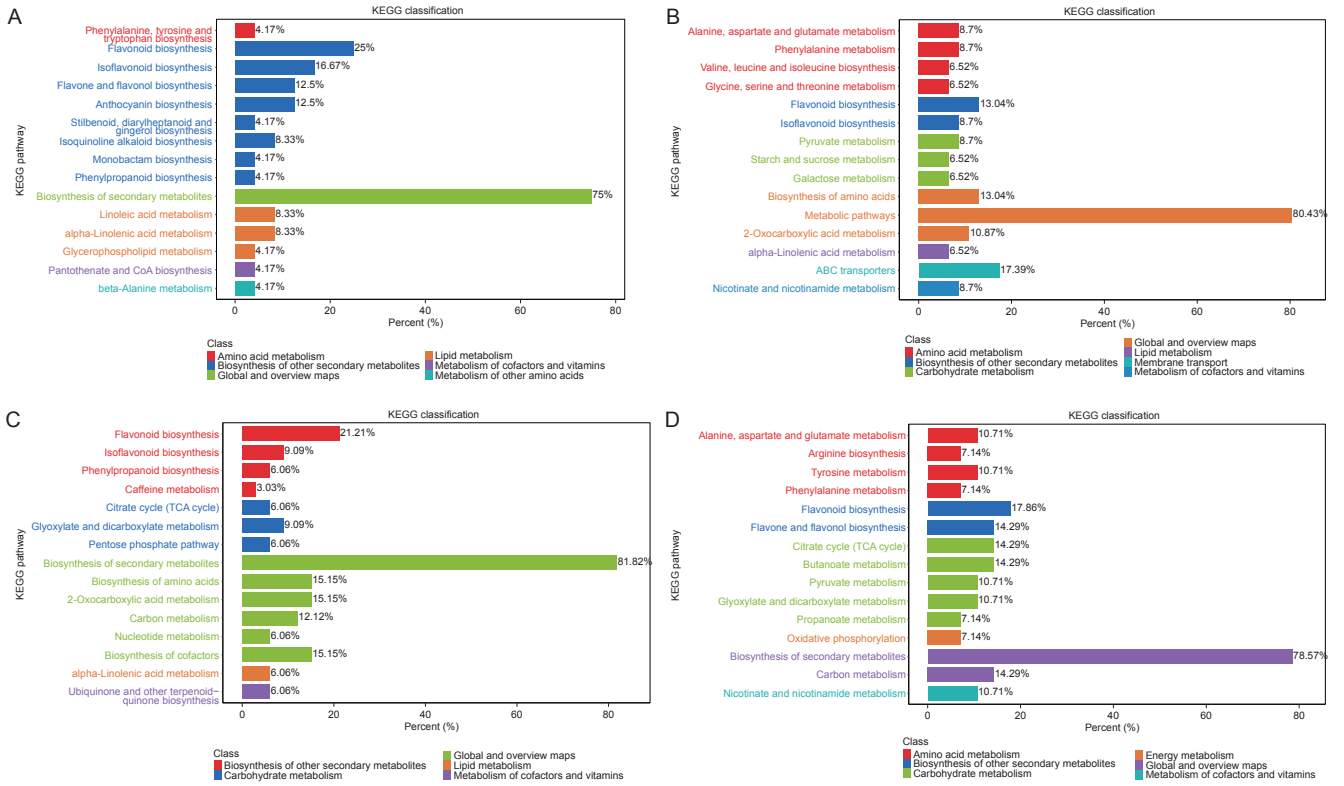

Fig. 10 KEGG pathway classification of differential metabolites. KEGG classification of differential metabolites identified in pairwise comparisons between control and 6-BA-treated samples at A, 1 d (A *vs.* B); B, 3 d (C *vs.* D); C, 6 d (E *vs.* F); and D, 11 d (G *vs.* H) after treatment. Here, A, C, E, and G represent the control groups, and B, D, F, and H represent the 6-BA-treated groups.

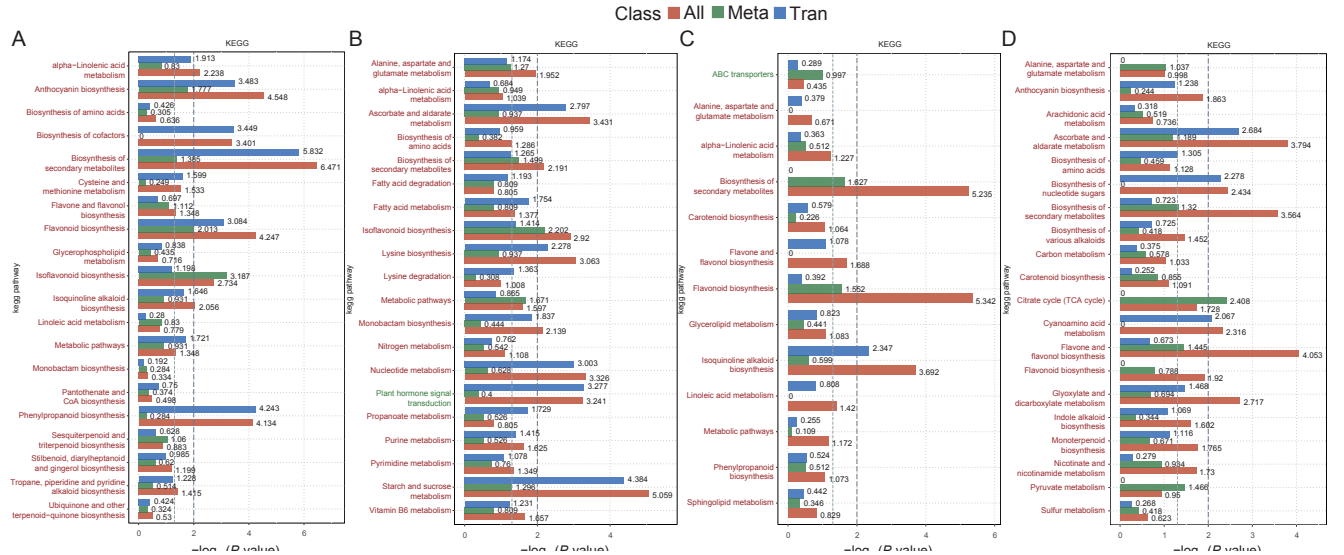

Fig. 11 Integrated transcriptomic and metabolomic KEGG pathway analysis. KEGG pathways enriched by differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) in pairwise comparisons between control and 6-BA-treated samples at A, 1 d (A *vs.* B); B, 3 d (C *vs.* D); C, 6 d (E *vs.* F); and D, 11 d (G *vs.* H) after treatment. The *y*-axis shows the enriched KEGG pathways, whereas the *x*-axis represents the enrichment significance $-\log_{10}(P\text{-value})$. Different colors indicate genes, metabolites, and pathways identified through integrated transcriptomic and metabolomic analyses. A, C, E, and G denote control samples, whereas B, D, F, and H denote 6-BA-treated samples.

4.1. Effect of hormonal content and related DEGs on bud dormancy break

The application of 6-BA induced significant changes in both hormonal levels and gene expression profiles in Xianheng 01 apple rootstock nursery plants, providing a comprehensive understanding of the molecular mechanisms underlying

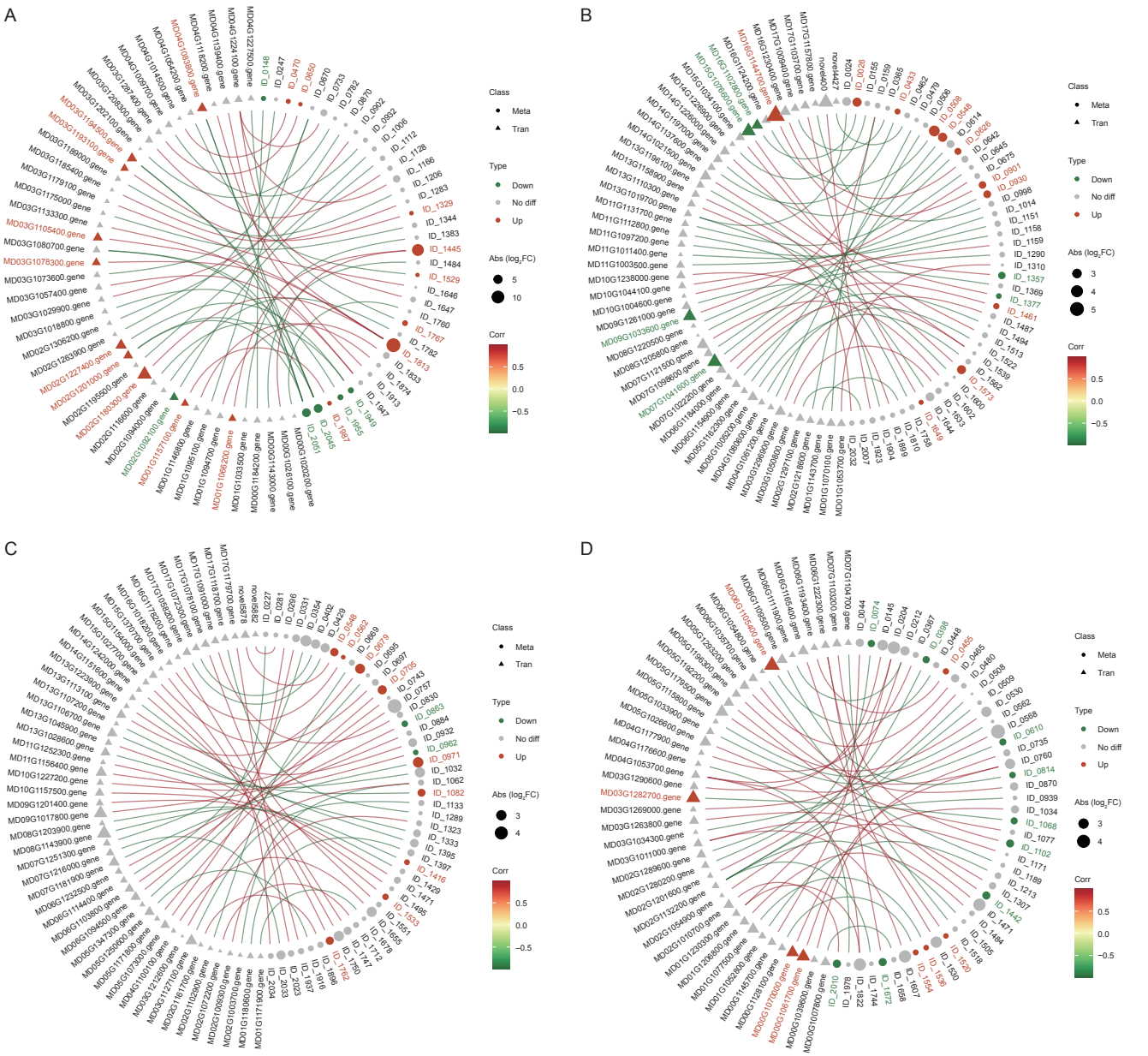

Fig. 12 Integrated transcriptomic and metabolomic correlation analysis. Circular correlation plots show associations between differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) in pairwise comparisons between control and 6-BA-treated samples at A, 1 d (A *vs.* B); B, 3 d (C *vs.* D); C, 6 d (E *vs.* F); and D, 11 d (G *vs.* H) after treatment. Genes and metabolites are categorized as upregulated, downregulated, or unchanged (no diff). Symbol size represents the absolute $\log_2$fold-change. Connecting lines indicate correlations between genes and metabolites, with red and green lines representing positive and negative correlations, respectively. A, C, E, and G denote control samples, whereas B, D, F, and H denote 6-BA-treated samples.

dormancy release. Cytokinins play an important role in breaking bud dormancy by activating cell cycle progression and antagonizing dormancy-promoting signals. In this study, endogenous hormone analysis revealed that cytokinin levels, including DHZR and IPA, peaked on 1 d in treated samples and remained elevated throughout the experiment, correlating with the upregulation of *A-ARR* genes such as *A-ARR1*, *A-ARR7*, *A-ARR8*, and *A-ARR9,* and downregulation of *B-ARR1* and *B-ARR4* in treated samples. 6-BA treatment induced a rapid increase in bioactive cytokinins, correlating with the upregulation of A-type *ARR* genes (*A-ARR1/7/8/9*) and downregulation of B-type *ARRs* (*B-ARR1/4*). The changes in gene expression we observed can be mapped directly onto the core signaling pathways for cytokinin and ABA (Figs. 6-B, 7-A). Cytokinin signaling begins when 6-BA is recognized by receptors (*CRE1*). This initiates a signal relay, mediated by histidine phosphotransfer proteins (*AHPs*) leads to the phosphorylation and activation of B-type *ARR* (*B-ARR*) transcription factors. The sustained increase in A-type ARR genes (*A-ARR1/7/8/9*) is a direct outcome of this signaling and a well-known cytokinin response. These genes then promote growth by triggering cell division and shoot development. At the same time as the cytokinin pathway was activated, the ABA signaling pathway was being

suppressed. In the ABA pathway, the hormone binds to *PYR/PYL* receptors, which block the activity of *PP2C* proteins. With *PP2Cs* inactive, *SnRK2* kinases are free to activate *ABF* transcription factors, which then turn on genes that maintain dormancy. The results showed that key components of the ABA pathway, including *SnRK2a/b* and *ABF1*, were significantly downregulated. This coordinated activation of the cytokinin pathway and suppression of the ABA pathway reveals the core molecular mechanism behind the cytokinin–ABA antagonism that drives dormancy release.

Recent studies demonstrate that 6-BA releases dormancy through cytokinin-mediated ABA suppression, where activation of *A-ARRs* simultaneously downregulates ABA biosynthesis genes, enhances ABA catabolism, and inhibits ABA signaling components, collectively shifting the hormonal balance toward bud break (Corot *et al.* 2017; Milyaev *et al.* 2022).

The unique expression of *A-ARR8* (upregulated during 1 and 6 d then downregulated at 11 d) in treated plants indicates its role in the modification of cytokinin signaling during early dormancy release, similar to findings in apple, where *ARR* genes play an important role in dormancy break by stimulating cytokinin signaling (Cattani *et al.* 2020). This reflects the activation of cytokinin signaling pathways, where increased cytokinin levels promote the expression of positive regulators (*A-ARR* genes) and suppress negative regulators (*B-ARR* genes), ultimately enhancing cell division and shoot development (To *et al.* 2004; Kieber and Schaller 2014; Zubo and Schaller 2020). This distinct temporal pattern of *A-ARR8* suggests that it serves as a molecular switch initially amplifying cytokinin signals during dormancy release before facilitating growth. This aligns with the simultaneous hormonal shifts where sustained high cytokinin (DHZR/IPA) maintains *A-ARR* activation, progressive ABA suppression (declining *ABF1/SnRK2a/b*) removes dormancy restrictions, and late GA_3 elevation promotes cell cycle progression. This regulation aligns with the observations in apple rootstocks where cytokinin-driven dormancy release requires coordinated ABA decline and GA activation (Tan *et al.* 2018). Similar cytokinin–ABA crosstalk has been documented in apple regeneration studies, where cytokinin-activated *ARRs* directly inhibited ABA pathway genes (Liu *et al.* 2023).

The IAA level was higher in the control group on 1 and 3 d. However, it increased significantly in treated samples by 11 d, aligning with the upregulation of auxin-related genes (*ARF* and *AUX/IAA*) in treated samples and downregulation of *SAUR* genes (e.g., *SAUR1* and *SAUR4*) in the control group. This alignment reflects the activation of auxin signaling pathways, where increased IAA levels promote the expression of auxin-responsive genes (*IAA*, *ARF*, and *AUX/IAA*) and repress *SAUR* genes, ultimately enhancing cell elongation and division. These findings are consistent with previous studies demonstrating the role of auxin in growth promotion (Liscum and Reed 2002; Mueller-Roeber and Balazadeh 2014) and the repression of *SAUR* genes by auxin to regulate cell expansion (Spartz *et al.* 2014). ABA levels, which were lower in the treated samples at 1 and 3 d, increased significantly at 6 and 11 d, supported by the downregulation of ABA-related genes (*ABF1*, *SnRK2a*, and *SnRK2b*), indicating a suppression of dormancy promoting signals. The suppression of *SnRK2a/b* (key kinases in ABA signal transduction) relates to the findings, where *SnRK2* silencing reduced dormancy duration (Zheng *et al.* 2024). This reflects the antagonistic relationship between cytokinin and ABA, where 6-BA treatment reduces ABA levels and downregulates ABA signaling genes to promote dormancy release and line up the previously reported role of ABA in dormancy regulation (Cutler *et al.* 2010) and the antagonistic interaction between cytokinin and ABA in promoting bud break (Zheng *et al.* 2015).

GA_3 levels were higher in the control group at 1 and 3 d but increased significantly in treated samples by 11 d, accompanied by the downregulation of *DELLA* genes (e.g., *DELLA1*, *DELLA7*, and *DELLA10*) in the control. DELLA proteins are repressors of GA signaling; when GA levels rise, DELLA proteins are degraded, releasing their repression and enhancing GA activity. This matches previous literatures (Achard *et al.* 2006; Sun *et al.* 2011) confirming that 6-BA treatment likely enhances GA biosynthesis or signaling, leading to dormancy release. MeJA levels were higher in treated samples at 1 and 11 d but higher in the control group at 6 d, correlating with the upregulation of jasmonic acid signaling genes (*JAZ3*, *JAR1*, and *MYC2*), particularly in the A *vs.* B and C *vs.* D groups, indicating a role in stress adaptation during dormancy release (Kazan and Manners 2013; Wasternack and Hause 2013). These findings highlight the complex interplay between hormonal changes and transcriptomic regulation, providing new insights into the mechanisms of 6-BA-mediated dormancy release and offering potential targets for improving dormancy management in apple cultivation.

4.2. Metabolomic changes during dormancy release

Metabolomic profiling revealed significant biochemical changes induced by 6-BA treatment during dormancy release in Xianheng 01 apple rootstock nursery plants. A total of 2,053 metabolites were identified and categorized into 12 superclasses, with shikimates and phenylpropanoids (32.197%), others (23.624%), and terpenoids (16.464%) being the most abundant. These findings highlight the central role of secondary metabolites in mediating dormancy release, consistent with previous studies showing that phenylpropanoids and terpenoids are critical for stress responses and growth regulation in plants (Vogt *et al.* 2010; Dudareva *et al.* 2013; Salam *et al.* 2023).

The shifting pattern of DAMs over time showed upregulation of metabolites in early stages, followed by downregulation in later stages. This pattern suggests that 6-BA initially activates metabolic pathways to prime the buds for growth, followed by a stabilization phase as dormancy release progresses. This biphasic response parallels findings in grapevine, where cytokinin-induced metabolites peaked during bud swelling and declined after bud break (Böttcher *et al.* 2015). Similar metabolic reprogramming has been reported in other woody perennials during bud break, where cytokinins play a key role in mobilizing resources for growth (Liu and Sherif 2019). KEGG pathway analysis identified

the biosynthesis of secondary metabolites and flavonoid biosynthesis as the most significantly enriched pathways. The predominance of phenylpropanoid metabolites suggests its dual role in both providing structural components for cell wall remodeling during bud swelling and serving as precursors for protective compounds. Flavonoids, known for their roles in stress mitigation and growth regulation, were particularly prominent, suggesting their involvement in 6-BA-mediated dormancy release (Agati *et al.* 2012). Notably, the temporal dynamics of flavonoid accumulation (early upregulation followed by stabilization) correlate precisely with the phased transition from dormancy maintenance to active growth, implying stage-specific functions, initial stress defense, followed by growth promotion. Additionally, pathways, such as ABC transporters and amino acid biosynthesis were enriched, indicating enhanced nutrient mobilization and metabolic activity in response to 6-BA treatment (Kang *et al.* 2010). This aligns up with the concurrent enrichment of amino acid biosynthesis pathways, which reflects increased nitrogen assimilation for protein synthesis, a prerequisite for meristem reactivation. These findings collectively demonstrate that 6-BA regulates dormancy release not only through hormonal signaling but also *via* coordinated metabolic reprogramming and are parallel with a previous report that cytokinins enhance secondary metabolite production and nutrient transport to support bud growth (Sakakibara *et al.* 2006).

The upregulation of phenylpropanoids and flavonoids, coupled with the downregulation of specific metabolites in later stages, suggests a finely tuned metabolic reprogramming during dormancy release. This pattern suggests phenylpropanoids initially provide both structural components (lignin precursors for cell wall expansion) and antioxidant protection during bud reactivation, while flavonoids likely mitigate oxidative stress associated with metabolic reactivation. This aligns with the known role of phenylpropanoids in lignin biosynthesis and cell wall remodeling, which are essential for bud swelling and shoot emergence (Vanholme *et al*. 2010). Furthermore, the enrichment of terpenoid pathways highlights their potential role in signaling and stress adaptation during dormancy release (Tholl *et al.* 2015).

4.3. Integration of transcriptomic and metabolomic data

The integration of transcriptomic and metabolomic data provided a comprehensive understanding of the molecular mechanisms underlying 6-BA-mediated dormancy release in Xianheng 01 apple rootstock.

The combined analysis revealed significant enrichment of pathways, such as biosynthesis of secondary metabolites, phenylpropanoid biosynthesis, flavonoid biosynthesis, and plant hormone signal transduction across all stages of dormancy release. These pathways were consistently highlighted in both transcriptomic and metabolomic datasets, underscoring their critical roles in the dormancy release process. The biosynthesis of secondary metabolites emerged as a central pathway, with high enrichment in both transcriptomic and metabolomic data. This pathway includes the production of phenylpropanoids and flavonoids, which are known to play essential roles in stress responses, cell wall remodeling, and growth regulation (Vogt *et al.* 2010; Agati *et al.* 2012). The upregulation of genes and metabolites associated with these pathways suggests that 6-BA treatment enhances secondary metabolite production, facilitating bud break and shoot development. Similarly, the enrichment of flavonoid biosynthesis aligns with previous studies showing that flavonoids act as antioxidants and signaling molecules during stress adaptation and growth transitions (Agati *et al.* 2012; Liu and Sherif 2019). The enrichment of plant hormone signal transduction pathways highlights the crosstalk between cytokinins, auxins, and other hormones in regulating dormancy release (Zheng *et al.* 2015). This crosstalk ensures a coordinated response to 6-BA treatment, where growth-promoting hormones (cytokinins, auxins, and gibberellins) are activated, while dormancy-promoting hormones (ABA) are suppressed. Additionally, hormones related to stress-adaptation pathways (MeJA) are upregulated to support the transition from dormancy to active growth. The circular correlation plots further elucidated the relationships between transcriptomic and metabolomic changes. Positive correlations between specific genes and metabolites suggest coordinated regulation, where genes and metabolites are co-regulated in the same direction, while negative correlations indicate inverse relationships, potentially reflecting feedback mechanisms or resource reallocation. This reallocation of resources is consistent with the role of cytokinins in mobilizing energy reserves to promote bud growth (Sakakibara *et al.* 2006). These integrated multi-omics analyses provide a comprehensive molecular view of 6-BA-driven dormancy release and determine key regulatory factors and metabolites that can be targeted for better dormancy control in apple orchards.

5. Conclusion

This study elucidates the complex mechanisms by which 6-BA releases dormancy in Xianheng 01 apple rootstock through integrated multi-omics analysis. Key findings reveal that 6-BA treatment promotes vegetative growth, evidenced by significant increases in plant height and leaf formation, reprograms hormonal networks, with early cytokinin (*DHZR/IPA*) surges activating *A-ARRs* (particularly *A-ARR8* expression) while suppressing ABA signaling (*SnRK2a/b* downregulation), and induces metabolic shifts, where phased phenylpropanoid/flavonoid accumulation precedes nutrient mobilization *via* ABC transporters. The coordinated upregulation of secondary metabolite biosynthesis (flavonoids and terpenoids) and downregulation of *DELLA* repressors highlight 6-BA's dual role in stress defense and growth promotion. Crucially, our multi-omics integration reveals that dormancy release requires synchronized resource allocation; early investment in protective compounds transitions to energy-intensive growth processes, mediated by cytokinin-ABA antagonism. These findings advance the understanding of dormancy regulation in woody perennials and establish 6-BA as a potent agrochemical for dormancy

management, with potential applications in temperate fruit cultivation. Despite this, these findings also significantly highlight the importance of 6-BA use in apple orchards to overcome inadequate winter chilling, a challenge associated with climate change in temperate regions. Thus, targeted application of 6-BA may provide farmers a practical strategy to promote uniform bud break and ensure consistent yield under inadequate chilling conditions.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (32372657, 32372675), the Breeding of New Apple Rootstock Cultivar Xianheng 01, Xi'an Xianheng Agricultural Technology Co., Ltd., Shaanxi, China, the National Key Research and Development Project of China (2023YFD2301002), the Science and Technology Major Project of Xinjiang Production and Construction Corps, China (2023AB077), the Chinese Universities Scientific Fund (2452023005), the China Apple Research System (CARS-27), the Cyrus Tang Foundation, USA, the Fundamental Research Funds for the Central Universities, China, and the Shandong Provincial Natural Science Foundation, China (ZR2024MC117).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2026.06.001

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