

Physiological and transcriptomic analysis reveal the regulation of IBA-induced adventitious root formation in three stone fruit rootstock cuttings

Fan Zhang

CAAS Gansu Academy of Agricultural Sciences Institute of fruit and flower research

Jiaxuan Ren

CAAS Gansu Academy of Agricultural Sciences Institute of fruit and flower research

Chenbing Wang

Wangchb7109@163.com

CAAS Gansu Academy of Agricultural Sciences Institute of fruit and flower research

Research Article

Keywords: Endogenous hormone, Physiological and biochemical, hardwood cuttings, indole-3-butyric acid, adventitious root, transcriptome analysis, *Prunus persica*

Posted Date: June 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9961111/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Adventitious root (AR) formation is a major limiting factor for the cutting propagation of peach rootstocks, and IBA-induced rooting is a complex biological process orchestrated by the crosstalk between exogenous and endogenous signaling pathways. In this study, three continuous-cropping tolerant peach clonal rootstocks, including 'GF677', 'Cadaman', and 'Zhongtao Kangzhen No.1', were used to explore the physiological and molecular regulatory mechanisms of exogenous IBA on ARs development. The results showed that the optimal IBA concentration varied among rootstocks: 300 mg·L⁻¹ for 'GF677' (91% rooting rate), 800 mg·L⁻¹ for 'Cadaman' (81% rooting rate), and 500 mg·L⁻¹ for 'Zhongtao Kangzhen No.1' with the highest rooting rate of 95%. Exogenous IBA significantly enhanced the activities of POD and IAAO in cuttings. The maximum IAA/CTK ratio was observed at 21 d in 'GF677', 28 d in 'Cadaman', and 35 d in 'Zhongtao Kangzhen No.1'. The MDA accumulation dynamics differed distinctly among rootstocks, indicating diverse temporal responses of membrane lipid peroxidation and oxidative stress during rooting. Correlation analysis revealed a significantly negative correlation between TN and TK, while IAA was extremely positively correlated with CTK, and MDA was positively correlated with both hormones. A total of 9,170 DEGs were identified via transcriptome sequencing at the key rooting stages, with 'Zhongtao Kangzhen No.1' exhibiting the most prominent transcriptional response. GO and KEGG analyses demonstrated significant enrichment of plant hormone signal transduction pathways, in which the auxin signaling pathway played a dominant role. Numerous hormone-related transcription factor families, including RLK-Pelle_DLSV, MYB, and NAC, were identified. This study systematically clarifies the regulatory mechanism of IBA-induced adventitious rooting in peach rootstocks and provides a theoretical basis for the genetic improvement of peach cutting propagation.

1. Introduction

The shortage of self-rooted peach rootstocks in China is mainly caused by imperfect seedling propagation techniques for rootstock seedlings [1]. Solving the bottlenecks in clonal propagation of peach rootstocks and optimizing the improved variety breeding system are essential for the development of modern peach industry, and will also advance the research on fruit tree developmental biology. Traditional clonal propagation methods for fruit trees, including grafting, division, tissue culture and layering, are restricted by complicated operations, high costs, long propagation cycles or poor genetic stability [2, 3]. By contrast, cutting propagation features simple operation and low cost. It can fully retain the superior genetic traits of mother plants with high genetic purity, and is more suitable for large-scale seedling production, thus serving as an ideal approach for clonal propagation of fruit trees [4, 5]. Adventitious root (AR) formation is a prerequisite for the successful propagation of peach cuttings [6]. After years of exploration, the green cutting technology for peach has been well developed [7]. Nevertheless, hardwood cutting using dormant branches exhibits markedly lower rooting capacity than green cutting [8]. At present, systematic researches on the endogenous and exogenous regulatory mechanisms, physiological response patterns and molecular regulatory networks underlying AR formation in peach hardwood cuttings are still inadequate.

Numerous studies have demonstrated that phytohormones exert remarkable effects on the formation and development of ARs. Among them, auxin, especially indole-3-acetic acid (IAA), plays the most prominent role [9]. The elevation of endogenous IAA content is critical for root primordium differentiation at the early stage of AR induction [10]. As the major native auxin in plants, IAA functions importantly in rooting regulation. However, the synthetic auxin indole-3-butyric acid (IBA) shows better performance in improving root quality. Exogenous application of IBA is widely adopted to induce AR initiation in hard-to-root plant species including peach [6]. Previous research has revealed that IBA treatment can trigger dynamic changes in endogenous hormone homeostasis during AR formation in five *Prunus* rootstocks [11]. In addition, ethylene is also involved in AR formation, and it can promote adventitious rooting in *Rheum rhabarbarum* and petunia [12, 13]. Auxin and ethylene jointly regulate and facilitate AR formation in apple rootstocks [14]. Genes of the ethylene-responsive AP2/ERF transcription factor family are significantly upregulated during AR induction in poplar [15]. Moreover, a relatively high ratio of IAA to cytokinin (CTK) is conducive to AR formation [6].

Benefiting from the development and application of RNA sequencing (RNA-seq) technology, the research on AR formation has shifted from anatomical and physiological analysis to molecular exploration [16]. Transcriptomic analyses have verified that plant hormone signaling pathways dominate the regulatory network of AR development. As reported by *An Haishan et al.* [17], exogenous IBA regulates AR formation by disturbing endogenous hormone balance and modulating the expression of genes related to plant hormone signal transduction. Another RNA-seq study on adventitious rooting of mung bean seedlings indicated that IBA promoted AR regeneration and development in tea plants via regulating differentially expressed genes (DEGs) associated with hormone signaling and 345 transcription factor (TF) genes [18]. Currently, most studies on the molecular mechanisms of AR formation focus on model plants such as *Arabidopsis thaliana* and rice [19, 20], while relevant researches on fruit trees remain limited.

In this study, hardwood cuttings of three continuous-cropping tolerant peach clonal rootstocks, namely 'GF677', 'Cadaman' and 'Zhongtao Kangzhen No.1', were used as experimental materials. The basal parts of cuttings were treated with IBA at different concentrations to screen the optimal concentration for each rootstock. We systematically analyzed the dynamic changes of physiological and biochemical characteristics, nutrient accumulation and endogenous hormones during AR formation, and identified the key rooting stages mediated by the IAA/CTK ratio. Furthermore, transcriptome sequencing was performed to screen DEGs related to AR formation in different rootstocks. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analyses were conducted, and hormone-related genes as well as transcription factors were further investigated. The results of this study will provide theoretical support and practical reference for clonal propagation of peach rootstocks, and also benefit the cultivation and research of other fruit tree species.

2. Materials and methods

Plant material and sample preparation

The experiment was conducted in mid-December 2024 at Gansu Academy of Agricultural Sciences. The hardwood cuttings (dormant branches) of three peach clonal rootstocks, namely 'GF677', 'Zhongtao Kangzhen No.1' and 'Cadaman', were used as plant materials. 'GF677' (*Prunus amygdalus* × *P. persica*) is a peach rootstock hybrid developed in France in the 1960s, which is a widely used peach rootstock with resistance to chlorosis and continuous-cropping disease [21]. The virus-free clonal seedlings of 'GF677' adopted in this research were originally introduced from Italy to the Sichuan Academy of Agricultural Sciences in 2003 and preserved by vegetative propagation in the experimental orchard of the Gansu Academy of Agricultural Sciences. This rootstock presents strong grafting compatibility with peach and nectarine cultivars, and possesses excellent tolerances to iron chlorosis in calcareous alkaline soil, continuous cropping stress and drought. Its superior genetic traits can only be maintained via vegetative propagation. Currently, large-scale vegetative propagation of 'GF677' has been widely applied for the renovation of aged and continuous-cropping peach orchards in France, Italy, Spain, Turkey and other countries. 'Zhongtao Kangzhen No.1' is a clonal peach rootstock with vigorous tree growth and semi-open canopy. It exhibits strong resistance to peach replant disease and southern root-knot nematode, as well as high tolerance to drought and poor soil conditions. This rootstock has good grafting affinity with various peach cultivars and can produce uniform and robust seedlings. The virus-free clonal seedlings of 'Zhongtao Kangzhen No.1' used in this study were introduced from the germplasm nursery of Zhengzhou Fruit Research Institute, CAAS, and preserved via vegetative propagation in the experimental orchard of the Gansu Academy of Agricultural Sciences. 'Cadaman' (*Prunus davidiana* × *P. persica*) is another clonal resistant rootstock with prominent resistance to continuous cropping stress and drought. 'Cadaman' was selected in Hungary and distributed under license from INRA-France.

Healthy and disease-free segments collected from the middle part of current-year branches with a diameter of 0.45–0.75 cm were selected as cuttings. The basal 5 cm of each cutting was treated with IBA at different concentrations, and then the cuttings were inserted into pre-disinfected seedbeds with a size of 1.5 m × 20 m. The growth medium was composed of perlite, vermiculite and peat at a volume ratio of 2:1:1, with a thickness of 20 cm. The cuttings were planted at a spacing of 5 cm × 5 cm, and the cutting depth accounted for two-thirds of the total length of each cutting. Electric heating wires with a diameter of 14 mm and a spacing of 9 cm were laid beneath the growth medium. One temperature controller was configured for every 100 m heating wire, which operated at 220 V voltage and 35 W power. Continuous heating was applied to keep the medium temperature at 25 ± 1 °C. Ventilation and cooling measures were implemented timely to ensure the air temperature in the greenhouse did not exceed 20 °C during the whole experimental period. A 24-hour automatic atomization system was equipped to maintain humidity during rooting. The atomization frequency was adjusted according to ambient weather conditions: on sunny and hot days, atomization was performed for 6 seconds every 20 minutes; at night, atomization lasted for 10 seconds every 50 minutes. The relative humidity of the medium was maintained above 80% throughout the experiment. Decayed and diseased cuttings were removed promptly, and disinfection treatment was carried out every 7 days. Daily management measures were adjusted appropriately according to actual growth conditions during the rooting stage.

Experimental Design

Screening of optimal Hormone concentration

A total of 11 treatments were established in this experiment, including a water control (CK) and ten IBA concentration groups: 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, 500 mg/L, 600 mg/L, 700 mg/L, 800 mg/L, 900 mg/L and 1000 mg/L. The cuttings were divided into 11 groups with 100 cuttings per group, and each treatment was set up with three biological replicates. Prior to hormone treatment, the basal portion of cuttings was immersed in 800-fold diluted carbendazim solution for 3 min. Afterwards, the cuttings were rinsed with running water and dried with gauze. Subsequently, the basal 5 cm of each cutting was soaked in the corresponding IBA solution for 20 s, and all cuttings were planted on the same day. At 50 days after cutting, the rooting rate was investigated, and root length was measured using a vernier caliper. The optimal IBA concentration for hardwood cutting of each rootstock was screened out for subsequent cutting experiments. When the IAA/CTK ratio reached the maximum value, phloem tissues were collected from the basal 2 cm of cuttings for transcriptome analysis.

Analysis of physiological and biochemical changes during cutting rooting

A new cutting experiment was carried out using the optimal exogenous IBA concentration screened above, with the same plant materials and cultivation management as described previously. The sample label was designated in the format of 'Cultivar-Treatment Day-Treatment Group'. For instance, the control sample of 'GF677' collected on day 0 was marked as GF677-0 d-CK. The analytical-grade IBA used in this study was purchased from Sigma Company.

Determination of indicators and methods

Sample preparation and determination procedures

Selected branches were cut into cuttings with a length of 20–25 cm. The base of each cutting was trimmed into a 40–45° oblique cut, and the phloem within 2 cm above the oblique surface was peeled off. The top end of each cutting was cut flat and daubed with medical petrolatum to prevent water loss. After cuttage, the growth status of cuttings was observed regularly. Samples were collected every 7 days for the determination of enzyme activities, endogenous hormone contents and nutrient components until day 35 post-cutting. For each sampling, the phloem tissues within the basal 2 cm of cuttings were rapidly stripped, fully mixed and divided into three groups. All samples were frozen in liquid nitrogen and subsequently stored in an ultra-low temperature refrigerator at -80 °C. The activities of indoleacetic acid oxidase (IAAO) and peroxidase (POD) were determined according to previously described methods [22]. The contents of total nitrogen (TN), total phosphorus (TP) and total potassium (TK) were measured following the methods reported in relevant studies [23]. Endogenous IAA and CTK were measured using established protocols [6].

RNA-seq

In this experiment, cuttings of 'GF677', 'Cadaman' and 'Zhongtao Kangzhen No.1' were treated with the optimal IBA concentration. Samples were collected at 21 days, 28 days and 35 days after treatment for

the three rootstocks respectively, and then delivered to Biomarker Technologies Corporation for transcriptome sequencing and bioinformatics analysis. Each treatment group contained three biological replicates.

Total RNA was extracted using the CTAB-PBIOZOL method combined with ethanol precipitation. After the purity, concentration and integrity of extracted total RNA were verified to meet the requirements, cDNA libraries were constructed. Eukaryotic mRNA was enriched using magnetic beads coupled with Oligo (dT). The captured mRNA was randomly fragmented with Fragmentation Buffer. Taking the fragmented mRNA as templates, the first-strand and second-strand cDNA were synthesized sequentially, followed by cDNA purification. The purified double-stranded cDNA was subjected to end repair, 3'-adenylation and sequencing adapter ligation. AMPure XP beads were used for fragment size selection, and the final cDNA libraries were obtained via PCR amplification. Upon completion of library construction, the concentration of each library was preliminarily quantified using a Qubit 3.0 Fluorometer, and the insert size was detected with a Qsep400 High-Resolution Fragment Analyzer. Qualified libraries were subjected to paired-end 150 bp (PE150) sequencing on a high-throughput sequencing platform. Raw sequencing data were analyzed using the bioinformatics pipelines integrated on BMK Cloud (www.biocloud.net) developed by Biomarker Technologies. DEGs were screened based on gene read counts of all samples. For groups with biological replicates, DEG analysis was performed using DESeq2 [24]. Genes with Fold Change ≥ 2 and FDR < 0.01 were defined as differentially expressed genes. Finally, functional annotation of the identified DEGs was conducted against multiple public databases.

3. Results

Effects of exogenous hormone (IBA) concentrations on adventitious rooting of continuous-cropping tolerant clonal rootstock cuttings

As shown in Fig. 1, exogenous IBA treatments at varying concentrations significantly regulated the rooting percentage and adventitious root elongation of cuttings from three rootstock genotypes, which exhibited differential responses to IBA concentration gradients (Fig. 1a). The highest rooting percentage of 'GF677' reached 91% under $300 \text{ mg}\cdot\text{L}^{-1}$ IBA treatment. For 'Cadaman', the maximum rooting rate of 81% was observed at an IBA concentration of $800 \text{ mg}\cdot\text{L}^{-1}$. Notably, 'Zhongtao Kangzhen No.1' exhibited the optimal rooting performance among the three rootstocks, with a maximum rooting percentage of 95% at $500 \text{ mg}\cdot\text{L}^{-1}$ IBA. The variation tendency of average adventitious root length was consistent with that of rooting percentage across three rootstocks (Fig. 1b). Specifically, the average adventitious root length of 'GF677' peaked at 15.2 cm with $300 \text{ mg}\cdot\text{L}^{-1}$ IBA application. 'Cadaman' attained an average root length of 13.2 cm when treated with $800 \text{ mg}\cdot\text{L}^{-1}$ IBA. Treatment with $500 \text{ mg}\cdot\text{L}^{-1}$ IBA yielded the maximum average root length of 18.5 cm in 'Zhongtao Kangzhen No.1', exceeding the values recorded for the other two rootstock cultivars.

Changes in major nutrients of cuttings during adventitious root formation

Endogenous nutrients are crucial for adventitious root formation in cuttings. The contents of TN, TP, and TK varied across three rootstock genotypes, IBA treatment (CT) and control (CK) groups during the rooting process. The N content of 'GF677' CT gradually decreased in the first 21 days and peaked at $23.12 \text{ mg}\cdot\text{g}^{-1}$ on day 28, and its CK group always had lower N levels. 'Cadaman' CT showed a fluctuating upward trend in N content, while its CK exhibited higher N accumulation before 14 d and lower levels thereafter. For 'Zhongtao Kangzhen No.1', CT and CK groups presented "up-down-up-down" and "up-down-up" N variation patterns, respectively, with both reaching the maximum values at 21 d ($29.13 \text{ mg}\cdot\text{g}^{-1}$ and $28.61 \text{ mg}\cdot\text{g}^{-1}$) (Fig. 2a). 'GF677' CT showed an up-down-up trend with a peak of $3.10 \text{ mg}\cdot\text{g}^{-1}$ at 28 d, whereas its CK increased first and then decreased, peaking at $2.61 \text{ mg}\cdot\text{g}^{-1}$ on day 14. Both 'Cadaman' and 'Zhongtao Kangzhen No.1' showed a unimodal TP variation. For 'Cadaman', CT reached the maximum TP ($2.57 \text{ mg}\cdot\text{g}^{-1}$) at 14 d, and CK peaked at $2.40 \text{ mg}\cdot\text{g}^{-1}$ at 21 d. For 'Zhongtao Kangzhen No.1', the highest TP content of CT ($2.61 \text{ mg}\cdot\text{g}^{-1}$) appeared at 28 d, while CK peaked at 21 d ($2.40 \text{ mg}\cdot\text{g}^{-1}$) (Fig. 2b). 'GF677' TK increased before 21 d and then decreased in the CT group, while the CK group showed an up-down-up fluctuation. 'Cadaman' CT had a steadily rising TK level that peaked at $32.89 \text{ mg}\cdot\text{g}^{-1}$ on day 28 and then declined, whereas its CK showed irregular fluctuations throughout the experiment. For 'Zhongtao Kangzhen No.1', TK contents of both groups slightly decreased at the initial stage and remained stable in the later rooting period (Fig. 2c).

Changes in endogenous hormone contents of cuttings during adventitious root formation

Endogenous hormone levels significantly regulate adventitious root formation. The contents of IAA and CTK, as well as the IAA/CTK ratio, varied among three peach rootstock genotypes and between different treatments over the cutting period (Fig. 3). The IAA contents of all CT groups increased first and then decreased (Fig. 3a). The IAA content of 'GF677' CT peaked at $94.73 \text{ ng}\cdot\text{g}^{-1}$ on day 21, and its CK group maintained a lower IAA level throughout the experiment. Similarly, the maximum IAA content of 'Cadaman' CT was $91.39 \text{ ng}\cdot\text{g}^{-1}$ at 21 d, with its CK value consistently lower than that of the CT group. For 'Zhongtao Kangzhen No.1', CT treatment reached the highest IAA content of $86.35 \text{ ng}\cdot\text{g}^{-1}$ at 21 d, while its CK group showed an up-down-up fluctuation and obtained a maximum value of $60.48 \text{ ng}\cdot\text{g}^{-1}$ at 14 d. The CTK contents of the three rootstocks under CT treatment exhibited a unimodal trend, all peaking at 21 d. The maximum CTK values of 'GF677', 'Cadaman', and 'Zhongtao Kangzhen No.1' were $403.11 \text{ ng}\cdot\text{g}^{-1}$, $396.09 \text{ ng}\cdot\text{g}^{-1}$, and $470.18 \text{ ng}\cdot\text{g}^{-1}$, respectively. The CK groups of 'GF677' and 'Cadaman' showed a fluctuating down-up-down-up pattern and reached their highest CTK contents at 28 d ($283.29 \text{ ng}\cdot\text{g}^{-1}$ and $290.45 \text{ ng}\cdot\text{g}^{-1}$). The CK group of 'Zhongtao Kangzhen No.1' presented a down-up-down trend and peaked at $304.76 \text{ ng}\cdot\text{g}^{-1}$ on day 14. In general, CTK contents in CT groups increased before 21 d and declined thereafter, with distinct differences among treatments (Fig. 3b). Hormone ratio analysis showed that the IAA/CTK values of all treatments were lower than 1 during the entire rooting process. The IAA/CTK ratio of 'GF677' fluctuated irregularly under both CK and CT treatments. The CT groups of 'Cadaman' and 'Zhongtao Kangzhen No.1' shared an up-down-up ratio trend and peaked at day 28 and day 35, respectively. The IAA/CTK ratio of 'GF677' CT peaked at 21 d, while that of 'Zhongtao Kangzhen

No.1' CK achieved the highest level at 35 d (Fig. 3c). According to these peak dates, phloem tissues at the basal 2 cm of cuttings were sampled for transcriptome analysis: sampling was conducted on day 21 for 'GF677', day 28 for 'Cadaman', and day 35 for 'Zhongtao Kangzhen No.1'.

Changes in Oxidase Activities, MDA and Soluble Sugar Contents of Cuttings during Adventitious Root Formation

Dynamic variations in POD, IAAO activities, malondialdehyde (MDA) and soluble sugar contents differed across three peach rootstock cultivars and between CK and CT with prolonged cutting culture. The POD activity of 'GF677' CT fluctuated throughout the experiment and peaked at $4655 \text{ U}\cdot\text{g}^{-1}$ on day 7, while its CK declined firstly and then increased. For 'Cadaman', CT showed fluctuating POD levels: POD was lower than CK before day 28, rose afterwards and reached the maximum of $5041.95 \text{ U}\cdot\text{g}^{-1}$ at day 35. POD activity of 'Zhongtao Kangzhen No.1' CT was consistently lower than CK, and CK achieved its highest value ($4177.09 \text{ U}\cdot\text{g}^{-1}$) on day 35 (Fig. 4a). For IAAO activity, both CT and CK of 'GF677' decreased initially and increased later, with peak values of $266.70 \text{ U}\cdot\text{g}^{-1}$ and $842.62 \text{ U}\cdot\text{g}^{-1}$ at day 35, respectively. 'Cadaman' CT presented a down-up-down pattern and peaked at $525.46 \text{ U}\cdot\text{g}^{-1}$ on day 28, whereas its CK reached the maximum IAAO of $919.30 \text{ U}\cdot\text{g}^{-1}$ at day 35. The IAAO activities of 'Zhongtao Kangzhen No.1' (CT and CK) increased gradually and both arrived at $920.30 \text{ U}\cdot\text{g}^{-1}$ on day 35 (Fig. 4b).

MDA contents varied with cultivars and treatments. MDA of 'GF677' CK remained higher than CT before day 28; CT increased thereafter and obtained the peak of $74.07 \text{ nmol}\cdot\text{g}^{-1}$ at day 35. MDA of 'Cadaman' (CK and CT) changed in a down-up-down trend: CK had higher MDA before day 7, while CT exceeded CK after that. 'Zhongtao Kangzhen No.1' CT displayed an up-down-up MDA fluctuation with the peak of $107.79 \text{ nmol}\cdot\text{g}^{-1}$ on day 14, and its CK reached the highest MDA ($97.05 \text{ nmol}\cdot\text{g}^{-1}$) at day 35 (Fig. 4c). Soluble sugar of 'GF677' (CT and CK) decreased first then increased, and the maximum content ($26.45 \text{ mg}\cdot\text{g}^{-1}$) occurred at day 0. Both CT and CK of 'Cadaman' followed an up-down-up tendency and peaked on day 35 at $27.78 \text{ mg}\cdot\text{g}^{-1}$ and $30.12 \text{ mg}\cdot\text{g}^{-1}$ separately. Soluble sugar of 'Zhongtao Kangzhen No.1' also varied as up-down-up; CK contained more soluble sugar than CT before day 21 and was surpassed later, with respective peaks of $23.32 \text{ mg}\cdot\text{g}^{-1}$ (CT) and $28.63 \text{ mg}\cdot\text{g}^{-1}$ (CK) on day 7 (Fig. 4d).

Correlations of nutrients, hormones and physiological traits of peach cuttings

As shown in Fig. 5, linear correlations existed among various physiological and rooting-related indexes of three peach rootstock cuttings. TN was positively correlated with TP, IAA and IAAO, and negatively correlated with TK at significant or extremely significant levels. TP displayed positive correlations with TK, IAA, CTK and IAAO. An extremely significant positive correlation was detected between IAA and CTK, whereas IAA was negatively correlated with most other detected parameters. In addition, MDA was significantly positively correlated with both IAA and CTK. The above findings suggested that the extremely positive association between IAA and CTK served as the core driving factor facilitating adventitious root initiation. Positive correlations of TN and TP with IAA indicated that nitrogen and

phosphorus metabolism provided essential material basis for root primordium formation. Furthermore, significant positive correlations between MDA and two key hormones revealed that mild oxidative stress at the early rooting stage was not a destructive injury; instead, it coordinated with endogenous hormonal signals to activate root primordium development.

Sequencing data quality control of reference genomes and evaluation of transcriptome sequencing samples

The phloem tissues of cuttings from three peach rootstock cultivars ('GF677', 'Cadaman' and 'Zhongtao Kangzhen No.1') were used as experimental materials. Two experimental groups were established for each cultivar: an optimal-concentration IBA treatment group and a blank control without exogenous hormone application. Tissue sampling for transcriptome sequencing was performed at 21 d post-treatment for 'GF677', 28 d post-treatment for 'Cadaman', and 35 d post-treatment for 'Zhongtao Kangzhen No.1', respectively.

In total, 18 independent cDNA libraries derived from control and IBA-treated samples were sequenced on the Illumina HiSeq2000 platform. After rigorous quality filtering of raw sequencing reads, a total of 122.44 Gb of high-quality clean data was obtained. The Q30 ratio across all samples ranged from 97.57% to 98.13%, and the GC content varied between 45.36% and 45.80%. Both sequencing quality and base composition were qualified for subsequent bioinformatic analyses, with detailed sequencing statistics summarized in **Table S1**.

Venn analysis, principal component analysis (PCA) and pairwise Pearson correlation analysis were performed on genome-wide expression data to characterize the transcriptional composition and experimental repeatability of phloem samples from three peach rootstocks under control and exogenous IBA treatments. Venn analysis identified a total of 14,634 ubiquitously expressed genes across all six sample groups, which constituted the core constitutive transcriptional repertoire under different experimental conditions. The number of cultivar- and treatment-specific genes ranged from 130 to 272; Z-35d-T possessed the maximum unique transcripts (272), while Z-35d-C contained the fewest (130). Such variation indicated that exogenous IBA induced abundant genotype-specific transcriptional activation in 'Zhongtao Kangzhen No.1' at 35 days post-treatment, conferring a more pronounced transcriptional response to auxin stimulation (**Figure S1a**). PCA partitioned global transcriptional variance into two primary axes: PC1 and PC2 accounted for 25.74% and 20.25% of total expression variation, respectively, with a cumulative explanatory rate of 45.99%. Obvious spatial separation was observed among samples derived from distinct rootstock genotypes and IBA treatments, demonstrating that both cultivar genotype and exogenous IBA application substantially remodeled the genome-wide transcriptional landscape of cutting phloem tissues (**Figure S1b**). All pairwise Pearson correlation coefficients ranged from 0.8 to 1.0 (**Figure S1c**). Near-perfect correlation among biological replicates guaranteed favorable intra-group repeatability. Despite slight reduction of correlation across distinct groups, all samples maintained generally high relatedness, which verified robust library construction and

high-quality sequencing data for subsequent identification of differentially expressed genes and functional enrichment.

Analysis of DEGs between treatment and control groups

Comparative transcriptome screening was performed between IBA-treated and untreated control samples, and DEGs were obtained from all three peach rootstock groups. For G-21d-C_vs_G-21d-T, 1025 genes were significantly up-regulated and 1235 genes were down-regulated. The comparison K-28d-C_vs_K-28d-T yielded 1644 up-regulated and 1737 down-regulated DEGs, while Z-35d-C_vs_Z-35d-T had 2164 up-regulated and 1365 down-regulated transcripts. Among all tested cultivars, 'Zhongtao Kangzhen No.1' displayed the most intensive transcriptional upregulation upon exogenous IBA induction. A total of 523 common DEGs were shared across the three pairwise comparison sets, implying that these conserved genes underwent persistent and significant transcriptional fluctuation during IBA-triggered adventitious root formation.

GO and KEGG enrichment analysis of DEGs

To elucidate the molecular mechanisms underlying the IBA-induced adventitious rooting of three peach rootstocks, GO and KEGG enrichment analyses were performed on DEGs from the three comparative groups. GO enrichment analysis categorized the DEGs into three main domains: biological process (BP), cellular component (CC), and molecular function (MF) (Fig. 7). Within the BP category, terms related to cellular process, metabolic process, stress response, multicellular organism development, and interspecies interaction were prominently enriched across all groups. In the CC domain, cellular anatomical entity, intracellular region, and protein-containing complex were the most significantly enriched components. Within the MF category, binding and catalytic activity exhibited the highest enrichment levels. Notably, the Z-35d comparison group showed significantly higher overall abundance of enriched genes across all three GO classifications than the G-21d and K-28d groups.

Unlike GO annotation, KEGG pathway enrichment displayed distinct patterns among the three comparisons. For G-21d-C_vs_G-21d-T, enriched pathways included terpenoid biosynthesis, glutathione metabolism, and carbohydrate secondary metabolism. In the K-28d-C_vs_K-28d-T comparison, rooting-related pathways, the plant MAPK signaling pathway, and phenylpropanoid biosynthesis were significantly enriched. For Z-35d-C_vs_Z-35d-T, flavonoid biosynthesis, photosynthesis-antenna proteins, and plant hormone signal transduction were among the most significantly enriched pathways (Fig. 8). Collectively, these results indicate that the majority of DEGs under IBA treatment were associated with secondary metabolite biosynthesis pathways, suggesting their strong involvement in organic matter metabolism. Importantly, after comparing the number of enriched genes in each pathway, plant hormone signal transduction contained the highest number of DEGs, further demonstrating that hormone signaling cascades were strongly activated by IBA application. This finding is consistent with previous studies on adventitious root formation, reinforcing that plant hormone signal transduction represents a key focus for future mechanistic investigations.

Analysis of hormone signaling pathways among differentially expressed genes

As described above, genes associated with plant hormone signaling pathways were significantly enriched in three peach rootstocks after IBA treatment. Further analysis revealed that exogenous IBA mediated adventitious root formation of peach rootstock cuttings by regulating multiple plant hormone signaling pathways, and obvious differences in gene expression responses were observed among different genotypes (Fig. 9). The auxin pathway acted as the core responsive pathway. The auxin transporter gene *AUX1* (*Prupe.1G503100*) was up-regulated to 2.11-fold in IBA-treated 'Zhongtao Kangzhen No.1'. Most members of the auxin repressor *AUX/IAA* family were induced, and *Prupe.3G074800* was increased to 1.49-fold, 1.50-fold and 2.49-fold in 'GF677', 'Cadaman' and 'Zhongtao Kangzhen No.1', respectively. The *GH3* gene responsible for auxin homeostasis exhibited the strongest response; the expression of its representative gene *Prupe.8G137900* was elevated by 4.99-fold, 7.79-fold and 30.80-fold in the three rootstocks (Fig. 9a). The gibberellin pathway showed a distinct expression pattern. The receptor gene *GID1* was down-regulated in partial rootstocks, while most negative regulator *DELLA* genes were up-regulated, resulting in remarkable changes in overall pathway activity (Fig. 9b). Key genes in the ethylene pathway were only slightly up-regulated, with a much weaker response than the auxin pathway (Fig. 9c). Genes encoding receptors, kinases and cell cycle regulators in the brassinosteroid pathway were markedly up-regulated under IBA induction, which promoted cell division and root primordium differentiation (Fig. 9d). Genes of the salicylic acid pathway, including *TGA* and *PR-1*, showed negligible expression changes, indicating that this pathway did not play a major role in IBA-induced adventitious rooting (Fig. 9e). In conclusion, the auxin signaling pathway is central to adventitious root formation, and gibberellin, ethylene and brassinosteroid pathways participate in this biological process synergistically. Moreover, 'Zhongtao Kangzhen No.1' displayed a significantly stronger transcriptional response to IBA than the other two rootstocks.

Transcription factor analysis of DEGs

Transcription factor annotation was performed on differentially expressed genes (DEGs) of three peach rootstocks before and after IBA treatment, and a total of 3096 valid annotation entries were obtained. The statistical results revealed that these DEGs belonged to diverse families of transcription factors, transcriptional regulators and protein kinases. The RLK-Pelle_DLSV family had the largest number of genes (158), followed by the MYB (122) and NAC (115) transcription factor families (Fig. 10). Typical hormone-related transcriptional regulatory families including FAR1, C2H2, bHLH, AP2/ERF and WRKY were also significantly enriched (Fig. 10). Previous studies have demonstrated that most transcription factors and regulatory factors identified in this study are involved in plant hormone signaling pathways, which is consistent with former researches. This result verifies that DEGs play vital roles in the regulatory network of plant hormones.

4. Discussion

As one of the most effective auxin-type plant growth regulators for promoting cutting rooting, IBA exerts concentration-dependent effects on adventitious root formation. Low concentrations of IBA present a weak rooting-promoting capacity, while excessive high concentrations inhibit rooting and impair root quality [25]. Adventitious root formation and growth consume large quantities of nutrients and energy, among which carbohydrates and nitrogen are indispensable substances for root regeneration [26]. In this study, the dynamic changes of TN, TP and TK contents in cuttings of three peach rootstocks were investigated during adventitious root formation under IBA treatment. The results showed that the optimal IBA concentration significantly regulated the metabolism and translocation of TN, TP and TK in cuttings, thereby providing essential nutritional support for the initiation and development of adventitious roots. Distinct strategies of nutrient metabolism among different cultivars were one of the internal causes leading to differential rooting capacity. Under the optimal IBA concentration, obvious nitrogen accumulation was observed at the basal part of cuttings of all three peach rootstocks during the late rooting stage (21–28 d). The TN content of ‘Zhongtao Kangzhen No.1’ was significantly higher than that of the other two rootstocks at 21 d after treatment. It indicated that exogenous IBA acted as a signal to activate nitrogen mobilization and redistribution in cuttings, which was consistent with previous findings [27]. From 21 d to 35 d post-treatment, TP and TK contents increased to peak values and then decreased markedly. For ‘GF677’, TP and TK contents reached the maximum levels at 21–28 d, and a positive correlation was detected between the two indicators. These results were in agreement with previously reported conclusions [28]. The differential responses of the three peach rootstocks to the same IBA concentration fully demonstrated that their nutrient metabolism patterns were determined by inherent genetic characteristics.

Adventitious root formation of cuttings is a complex biological process synergistically regulated by multiple endogenous phytohormones [29, 30]. The regulatory effect of exogenous auxin is achieved by breaking the original endogenous hormone balance and establishing a new hormone profile favorable for root primordium initiation and development. IBA bidirectionally modulates the biosynthesis of endogenous IAA to match the hormone demand at different rooting stages, so as to optimize rooting performance [31]. The accumulation and redistribution of IAA serve as the primary signal for rooting initiation [32]. Study had shown that plant growth regulators could effectively promote endogenous IAA synthesis in cuttings and exert a distinct positive effect on root primordium induction and adventitious root formation [33]. In this study, IAA contents in both treatment and control groups of the three peach rootstocks increased within the first 21 days. The ‘GF677’ cuttings treated with IBA had the highest IAA level at 21 d. It is speculated that exogenous IBA may enhance endogenous IAA signaling by inhibiting IAA degradation or accelerating its synthesis, and further activate the expression of downstream genes related to cell dedifferentiation and cell cycle reinitiation, which lays a molecular foundation for root primordium formation. IAA contents declined significantly in the late stage, which was probably because IAA was gradually oxidized during callus and root primordium development, and its content further decreased after adventitious roots fully formed. Low concentration of CTK facilitates the differentiation of adventitious roots [34]. In this study, CTK contents increased gradually at the early rooting stage (0–21 d) and showed a positive correlation with IAA content, which might be associated with their

synergistic effect on promoting cell division. After 21 d, CTK contents of all three rootstocks decreased generally, which relieved the inhibitory effect of CTK on root differentiation and elongation. Consequently, the IAA/CTK ratio shifted toward a state conducive to root differentiation.

Oxidases play important roles in regulating adventitious root formation of cuttings [35]. *BRITO et al.* [36] reported that POD activity was closely linked to adventitious root formation, with two distinct activity peaks appearing at the root induction and expression stages, respectively. In this study, POD activity in 'Cadaman' and 'Zhongtao Kangzhen No.1' decreased firstly from 0 d to 7 d, then fluctuated and finally peaked at the late rooting stage. The dynamic variation and peak value of POD activity at the late stage were highly synchronized with the breakthrough and elongation of root primordia. At this stage, high POD activity mainly functions in catalyzing cell wall lignification and strengthening, providing mechanical support and defense barriers for newly formed roots. By contrast, POD activity of 'GF677' reached the maximum at the early induction stage (7 d) and then declined in a fluctuating manner, which was consistent with previous research results. Plant growth regulators can increase the activities of key rooting-related enzymes such as POD and indoleacetic acid oxidase (IAAO), and thereby promote adventitious root formation in cuttings [37]. In this study, IAAO activity of the three rootstocks presented a trend of initial decrease followed by continuous increase, and reached the peak during 28–35 d. The reduction of IAAO activity in the early stage reduced IAA catabolism, maintaining a high IAA concentration at the cutting base. The subsequent rise of IAAO activity during 28–35 d occurred when root primordia had fully formed and were ready to break through the cortex. Apart from the dynamic changes of key enzyme activities, the synergism of carbon source supply and oxidative stress signals is also a crucial prerequisite for adventitious root formation [38–40]. As the core substance for carbon skeleton construction and energy supply, soluble sugar in 'GF677' peaked at 7 d during the early rooting induction stage, while that in 'Cadaman' and 'Zhongtao Kangzhen No.1' reached the maximum at 35 d when root primordia broke through the cortex. Such genotypic differences reflected diverse strategies of carbon source mobilization and utilization among rootstocks, though all of them could satisfy the energy demand for root primordium initiation and elongation. MDA is a typical marker of membrane lipid peroxidation [41]. The peak time of MDA content also exhibited obvious genotypic specificity: MDA content of 'GF677' peaked at the late rooting stage (35 d), while that of 'Cadaman' and 'Zhongtao Kangzhen No.1' reached the maximum at the early stage (14 d). Combined with its significant positive correlation with IAA and CTK, MDA accumulation should not be simply regarded as a sign of cell damage. Instead, it is more likely a constructive oxidative signal derived from vigorous metabolic activities. Moderate oxidative stress can activate specific signaling pathways and cooperate with the hormone regulatory network to jointly drive root primordium breakthrough and morphogenesis.

Transcriptome sequencing at key rooting stages further revealed the molecular regulatory network of IBA-induced adventitious rooting in peach rootstocks. High-quality sequencing data and excellent biological repeatability confirmed the reliability of transcriptome results. A total of 9,170 DEGs were identified in the three comparison groups, and 'Zhongtao Kangzhen No.1' had the largest number of DEGs and the most intense transcriptional response, which was consistent with its superior rooting performance at the physiological level. GO functional annotation showed that DEGs were mainly

enriched in cellular metabolism, stress response, and catalytic activity, indicating that IBA induced widespread reprogramming of physiological metabolism and stress adaptation in cuttings [42]. KEGG enrichment analysis further proved that plant hormone signal transduction was the most dominant pathway during rooting, which has been verified in multiple woody plant rooting studies [42, 43]. Transcriptomic analysis of hormone-related genes in peach rootstock cuttings identified differentially expressed genes associated with five phytohormone signaling pathways, including auxin, gibberellin, ethylene, brassinosteroid, and salicylic acid. This finding indicates that these phytohormones participate in this complex rooting process through distinct regulatory mechanisms and at different magnitudes. Auxin signaling pathway played a core regulatory role: key genes such as *AUX1*, *AUX/IAA*, and *GH3* were significantly up-regulated, especially the auxin homeostasis gene *Prupe.8G137900* showed ultra-high fold change expression in the three rootstocks, demonstrating that exogenous IBA mainly relies on activating auxin signal transduction and reconstructing endogenous auxin homeostasis to promote rooting [44]. This finding is consistent with previous studies confirming that IAA acts as a core regulator of adventitious root formation in cuttings [45]. Meanwhile, gibberellin, ethylene, and brassinosteroid signaling pathways synergistically participated in the rooting process.

Transcription factor annotation further enriched the molecular regulatory mechanism of peach rooting. Within the same species, the MYB family gene MYB77 may modulate lateral root quantity via interaction with ARF7 [46]. In *Arabidopsis thaliana*, the transcription factor NAC1 is specifically induced by wounding in leaf explants and facilitates root tip regeneration [47]. AP2/ERF family genes mediate ethylene-auxin crosstalk to regulate adventitious root initiation [48]. The massive enrichment of these transcription factors in this study further illustrated that IBA regulates the complex transcriptional network of hormone signal transduction and stress adaptation through multiple transcription factor families, thereby controlling the rooting difference of different peach rootstocks. Combined with physiological and transcriptomic results, this study confirmed that genotypic differences in peach rootstock rooting are determined by the comprehensive effect of nutrient metabolism, hormone homeostasis, antioxidant response, and hormone signal transcriptional regulation.

5. Conclusion

In conclusion, this study systematically clarified the physiological and molecular mechanism of exogenous IBA regulating hardwood cutting rooting of three anti-replanting peach rootstocks, and determined the optimal IBA concentration and key rooting time nodes for each rootstock. It also revealed that auxin signal pathway dominates the rooting regulation, and multi-hormone synergism and transcription factor-mediated transcriptional reprogramming jointly drive adventitious root formation. In future research, key candidate genes and core transcription factors screened in this study can be functionally verified, which will further improve the molecular regulatory model of peach rootstock rooting and provide valuable genetic resources and theoretical basis for the breeding and efficient clonal propagation of high-quality peach rootstocks.

Abbreviations

adventitious roots (AR)

Indole-3-butyric acid (IBA)

Cytokinin (CTK)

Gene Ontology (GO)

Kyoto Encyclopedia of Genes and Genomes (KEGG)

Fragments Per Kilobase Million (FPKM)

differentially expressed genes (DEGs)

GRETCHEN HAGEN3 (GH3)

indoleacetic acid oxidase (IAAO)

peroxidase (POD)

total nitrogen (TN)

principal component analysis (PCA)

total phosphorus (TP)

indole-3-acetic acid (IAA)

total potassium (TK)

malondialdehyde (MDA)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data Availability Statement:

The RNA-seq data generated in this study were submitted to the NCBI SRA (<https://www.ncbi.nlm.nih.gov/sra/>) database under accession no. PRJNA1402745.

Competing interests

The authors declare no competing interests.

Funding: This research was supported with projects the National Natural Science Foundation of China Project (No. 32360717) and the Modern Agricultural Industry Technology System Construction Special Project (No. CARS 30-1-6).

Authors' contributions

C.W. conceived and designed the overall research framework, undertook project supervision, and was responsible for funding acquisition and resource support throughout the research. F.Z. organized the whole experimental arrangement, conducted all experimental operations, sample testing and relevant experimental verification, and ensured the smooth progress and reproducibility of the research work. J.R. completed the manuscript writing, sorted out and analyzed all experimental data, summarized the research results, and polished the logical content of the full text. All authors reviewed, revised, and approved the final version of the manuscript.

Acknowledgements

Not applicable.

References

1. Musacchi S. Optimization of fruit tree propagation techniques and agronomical performances in the nursery. *Acta Hort* 2024(1413):165–84.
2. Franzon RC, Goncalves RDS, Correa Antunes LE, Bassols Raseira MDC, Trevisan R. Surinam cherry propagation by means of grafting. *REVISTA BRASILEIRA DE FRUTICULTURA*. 2008;30(2):4.
3. Goyali JC, Igamberdiev AU, Debnath SC. Propagation Methods Affect Fruit Morphology and Antioxidant Properties but Maintain Clonal Fidelity in Lowbush Blueberry. *HortScience: publication Am Soc Hortic Sci*. 2015;50(6):888–96.
4. Sharma Y, Singh S, Thakur N. Clonal propagation in apple. 2013.
5. Upadysheva G, Yastrebkova N. VAS. Improving propagation efficiency of clonal rootstocks of stone fruit crops using a technology of green cuttings [plum and cherry]. 2011.
6. Zhang F, Ren J, Wang C. Multi-omics analysis reveals the mechanism of adventitious roots formation in peach green branch cuttings. *Front Plant Sci*. 2025;16:1740503.
7. Zhang F, Ren J, Wang C. Multi-omics analysis reveals the mechanism of adventitious roots formation in peach green branch cuttings. *Frontiers in plant science* 2026.

8. Tsipouridis C, Thomidis T, Isaakidis A. Rooting of peach hardwood and semi-hardwood cuttings. *Aust J Exp Agric*. 2003;43(11):1363–8.
9. Wang Y, Pang D, Ruan L, Liang J, Zhang Q, Qian Y, Zhang Y, Bai P, Wu L, Cheng H, et al. Integrated transcriptome and hormonal analysis of naphthalene acetic acid-induced adventitious root formation of tea cuttings (*Camellia sinensis*). *BMC Plant Biol*. 2022;22(1):319.
10. Kawai Y. Changes in Endogenous IAA during Rooting of Hardwood Cuttings of Grape, 'Muscat Bailey A' with and without a Bud. *Engei Gakkai Zasshi*. 1996;65(1):33–9.
11. Justamante MS, Mhimdi M, Molina-Pérez M, Albacete A, Moreno M, Mataix I, Pérez-Pérez JM. Effects of Auxin (Indole-3-butyric Acid) on Adventitious Root Formation in Peach-Based Prunus Rootstocks. *Plants (Basel, Switzerland)* 2022, 11(7).
12. Wojtania A, Waligórski P, Markiewicz M. Involvement of Ethylene in Adventitious Root Formation of Red-Stalked Rhubarb In Vitro. *Int J Mol Sci* 2025, 26(19).
13. Denq RY, Fridovich I. Formation of endonuclease III-sensitive sites as a consequence of oxygen radical attack on DNA. *Free Radic Biol Med*. 1989;6(2):123–9.
14. Bai T, Dong Z, Zheng X, Song S, Jiao J, Wang M, Song C. Auxin and Its Interaction With Ethylene Control Adventitious Root Formation and Development in Apple Rootstock. *Front Plant Sci*. 2020;11:574881.
15. Trupiano D, Yordanov Y, Regan S, Meilan R, Tschaplinski T, Scippa GS, Busov V. Identification, characterization of an AP2/ERF transcription factor that promotes adventitious, lateral root formation in *Populus*. *Planta*. 2013;238(2):271–82.
16. Luo C, Liu X, Zheng Y, Dai X, Tang X, Zhang T, Zhang X. Physiological and transcriptomic analysis reveal the regulation of adventitious root formation in *Cinnamomum parthenoxylon* cuttings. *BMC Plant Biol*. 2024;24(1):1217.
17. An H, Zhang J, Xu F, Jiang S, Zhang X. Transcriptomic profiling and discovery of key genes involved in adventitious root formation from green cuttings of highbush blueberry (*Vaccinium corymbosum* L). *BMC Plant Biol*. 2020;20(1):182.
18. Li SW, Shi RF, Leng Y, Zhou Y. Transcriptomic analysis reveals the gene expression profile that specifically responds to IBA during adventitious rooting in mung bean seedlings. *BMC Genomics*. 2016;17:43.
19. Liu P, Zhang S, Wang X, Du Y, He Q, Zhang Y, Shen L, Hu H, Zhang G, Li X. Adventitious Root Formation in Cuttings: Insights from *Arabidopsis* and Prospects for Woody Plants. *Biomolecules* 2025, 15(8).
20. Tripathi DK, Rai P, Guerriero G, Sharma S, Corpas FJ, Singh VP. Silicon induces adventitious root formation in rice under arsenate stress with involvement of nitric oxide and indole-3-acetic acid. *J Exp Bot*. 2021;72(12):4457–71.
21. Song HY, Zhao K, Wang Q, Jiang GL, Sun SX, Li J, Tu MY, Wang LL, He CY, Wang T, et al. Mechanism for efficient nitrogen utilization by peach rootstock GF677 (*Prunus amygdalus* × *Prunus persica*) in alkaline orchard soils. *Front Plant Sci*. 2025;16:1699942.

22. Li XM, He XY, Zhang LH, Chen W, Chen Q. Influence of elevated CO and O on IAA, IAA oxidase and peroxidase in the leaves of ginkgo trees. *Biol Plant*. 2009;53(2):p339–342.
23. Yuansong X, Futian P, Zhuqing, Dang, Xiaomei, Jiang, Jianghong, Zhang. Influence of rhizosphere ventilation on soil nutrient status, root architecture and the growth of young peach trees. *Soil Sci Plant Nutr*. 2015;61(5):775–87.
24. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
25. Loconsole D, Cristiano G, De Lucia B. Image Analysis of Adventitious Root Quality in Wild Sage and Glossy Abelia Cuttings after Application of Different Indole-3-Butyric Acid Concentrations. *Plants (Basel Switzerland)* 2022, 11(3).
26. Shao F, Wang S, Huang W, Liu Z. Effects of IBA on the rooting of branch cuttings of Chinese jujube (*Zizyphus jujuba* Mill.) and changes to nutrients and endogenous hormones. *J Forestry Res* 2018.
27. Shufu D, Lailiang C, Scagel CF, Fuchigami LH. Nitrogen mobilization, nitrogen uptake and growth of cuttings obtained from poplar stock plants grown in different N regimes and sprayed with urea in autumn. *Tree Physiol* 2004(3):355.
28. Murthy HN, Dandin VS, Paek KY. Tools for biotechnological production of useful phytochemicals from adventitious root cultures. *Phytochem Rev*. 2016;15(1):129–45.
29. Tahir MM, Chen S, Ma X, Li S, Zhang X, Shao Y, Shalmani A, Zhao C, Bao L, Zhang D. Transcriptome analysis reveals the promotive effect of potassium by hormones and sugar signaling pathways during adventitious roots formation in the apple rootstock. *Plant Physiol Biochem*. 2021;165:123–36.
30. Kunc P, Medic A, Grohar MC, Mrak T, Osterc G. Physiological maturation and hormonal profiles associated with rooting performance and root system architecture of leafy cuttings in *Prunus subhirtella* 'Autumnalis'. *BMC Plant Biol* 2026, 26(1).
31. Chen S, Liu J, Zhang J, Jiang L, Liu Q, Zhou X, Li P, Chen J, Chen Y, Yang Y. Effect of exogenous IBA on root morphology and endogenous hormone Metabolic pathways in castor seedlings under Pb/Zn stress. *Plant Physiol Biochem*. 2025;219:109365.
32. Woodward AW, Bartel B. Auxin: regulation, action, and interaction. *Ann Botany*. 2005;95(5):707–35.
33. Wu Q, Du M, Wu J, Wang N, Wang B, Li F, Tian X, Li Z. Mepiquat chloride promotes cotton lateral root formation by modulating plant hormone homeostasis. *BMC Plant Biol*. 2019;19(1):573.
34. Liao C. Advances on Internal Influence Factors of Adventitious Rooting of Forest Trees. *森林科学*(*Forest Science*); 2017.
35. Qin R, Zhao Q, Gu C, Wang C, Zhang L, Zhang H. Analysis of oxidase activity and transcriptomic changes related to cutting propagation of hybrid larch. *Sci Rep*. 2023;13(1):1354.
36. Brito BP, Jori F, Dwarka R, Maree FF, Heath L, Perez AM. Transmission of Foot-and-Mouth Disease SAT2 Viruses at the Wildlife-Livestock Interface of Two Major Transfrontier Conservation Areas in Southern Africa. *Front Microbiol*. 2016;7:528.

37. Zhou X, Li R, Shen H, Yang L. Effect of Exogenous Plant Growth Regulators and Rejuvenation Measures on the Endogenous Hormone and Enzyme Activity Responses of *Acer mono Maxim* in Cuttage Rooting. *Int J Mol Sci* 2023, 24(15).
38. Druege U, Zerche S, Kadner R. Nitrogen- and storage-affected carbohydrate partitioning in high-light-adapted *Pelargonium* cuttings in relation to survival and adventitious root formation under low light. *Ann Botany*. 2004;94(6):831–42.
39. Tewari RK, Hahn EJ, Paek KY. Modulation of copper toxicity-induced oxidative damage by nitric oxide supply in the adventitious roots of *Panax ginseng*. *Plant Cell Rep*. 2008;27(1):171–81.
40. Striker GG, Yamauchi T, Mollard FPO. Maize physiology under waterlogging: shoot and root adaptive responses to mitigate low oxygen stress. *J Exp Bot* 2025.
41. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Anal Biochem*. 2017;524:13–30.
42. El-Banna MF, Farag NBB, Massoud HY, Kasem MM. Exogenous IBA stimulated adventitious root formation of *Zanthoxylum beecheyanum* K. Koch stem cutting: Histo-physiological and phytohormonal investigation. *Plant Physiol Biochem*. 2023;197:107639.
43. Wang S, Wu H, Zhang Y, Sun G, Qian W, Qu F, Zhang X, Hu J. Transcriptome Reveals the Regulation of Exogenous Auxin Inducing Rooting of Non-Rooting Callus of Tea Cuttings. *Int J Mol Sci* 2024, 25(15).
44. Lei C, Fan S, Li K, Meng Y, Mao J, Han M, Zhao C, Bao L, Zhang D. iTRAQ-Based Proteomic Analysis Reveals Potential Regulation Networks of IBA-Induced Adventitious Root Formation in Apple. *Int J Mol Sci* 2018, 19(3).
45. Pacurar DI, Perrone I, Bellini C. Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiol Plant*. 2014;151(1):83–96.
46. Shin R, Burch AY, Huppert KA, Tiwari SB, Murphy AS, Guilfoyle TJ, Schachtman DP. The Arabidopsis transcription factor MYB77 modulates auxin signal transduction. *Plant Cell*. 2007;19(8):2440–53.
47. Chen X, Cheng J, Chen L, Zhang G, Huang H, Zhang Y, Xu L. Auxin-Independent NAC Pathway Acts in Response to Explant-Specific Wounding and Promotes Root Tip Emergence during de Novo Root Organogenesis in Arabidopsis. *Plant Physiol*. 2016;170(4):2136–45.
48. Kitomi Y, Ito H, Hobo T, Aya K, Kitano H, Inukai Y. The auxin responsive AP2/ERF transcription factor CROWN ROOTLESS5 is involved in crown root initiation in rice through the induction of OsRR1, a type-A response regulator of cytokinin signaling. *Plant journal: cell Mol biology*. 2011;67(3):472–84.

Figures

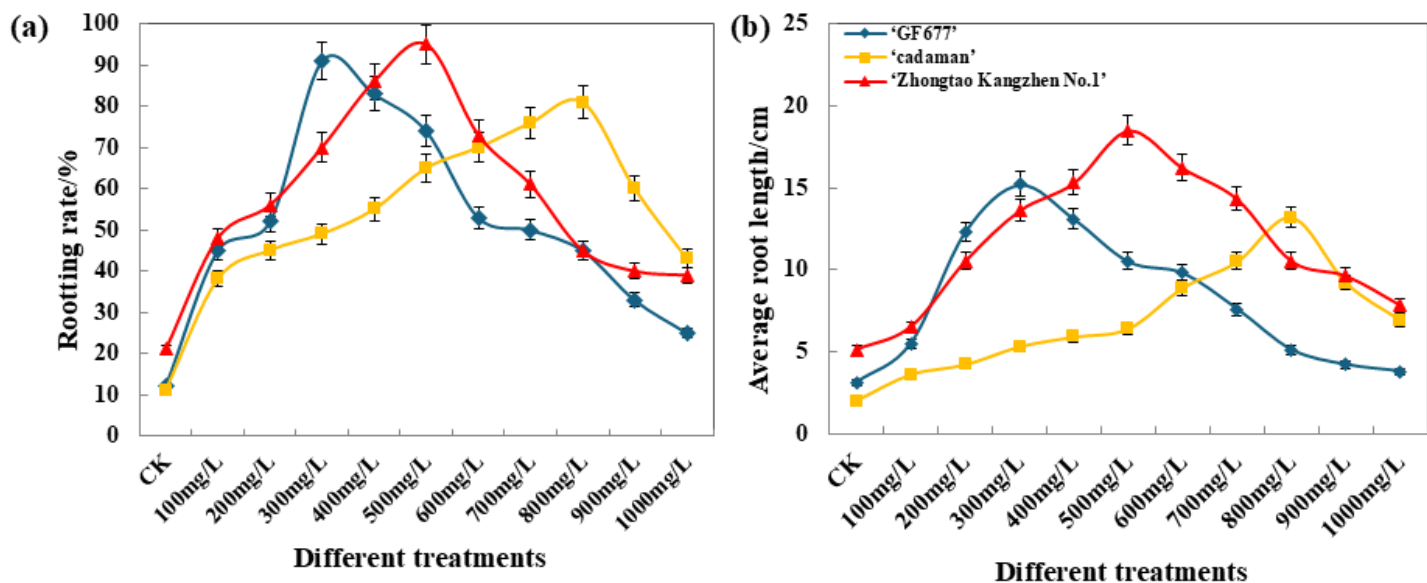


Figure 1

Effects of exogenous IBA concentrations on rooting percentage (a) and average adventitious root length (b) of cuttings from three peach rootstock cultivars ('GF677', 'Cadaman' and 'Zhongtao Kangzhen No.1'). CK represents the control treatment without IBA application; the gradient concentrations of IBA ranged from 100 mg·L⁻¹ to 1000 mg·L⁻¹. Vertical error bars denote the standard error of the mean.

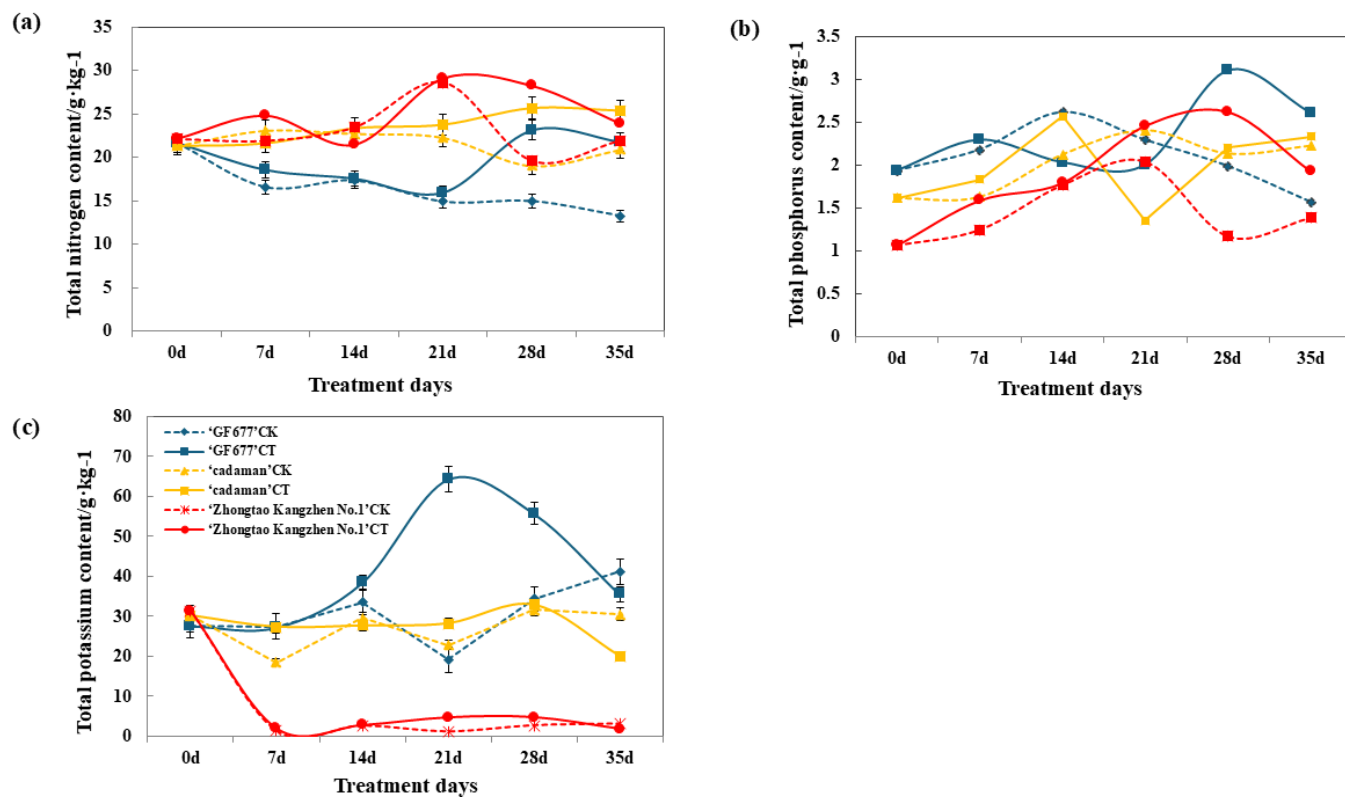


Figure 2

Dynamic changes in total nitrogen (a), total phosphorus (b), and total potassium (c) contents of three peach rootstock cuttings under control (CK) and IBA treatment (CT) during adventitious root formation. Data represent the measured nutrient contents at different treatment time points (0, 7, 14, 21, 28, 35d).

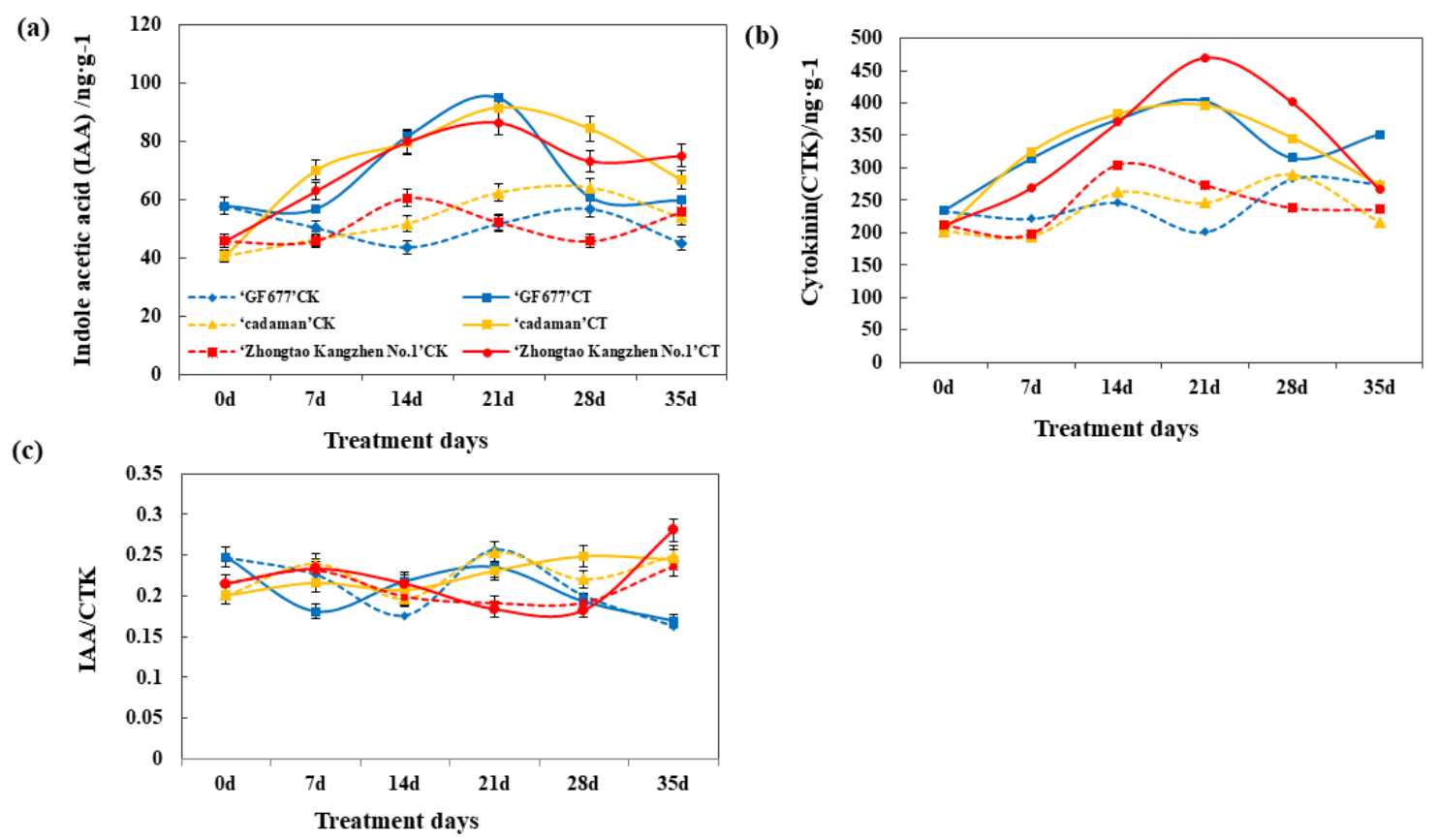


Figure 3

Dynamic changes in IAA (a), CTK (b) contents and IAA/CTK ratio (c) of three peach rootstock cuttings under control (CK) and IBA treatment (CT) at different treatment time points (0, 7, 14, 21, 28, 35d) during adventitious root formation.

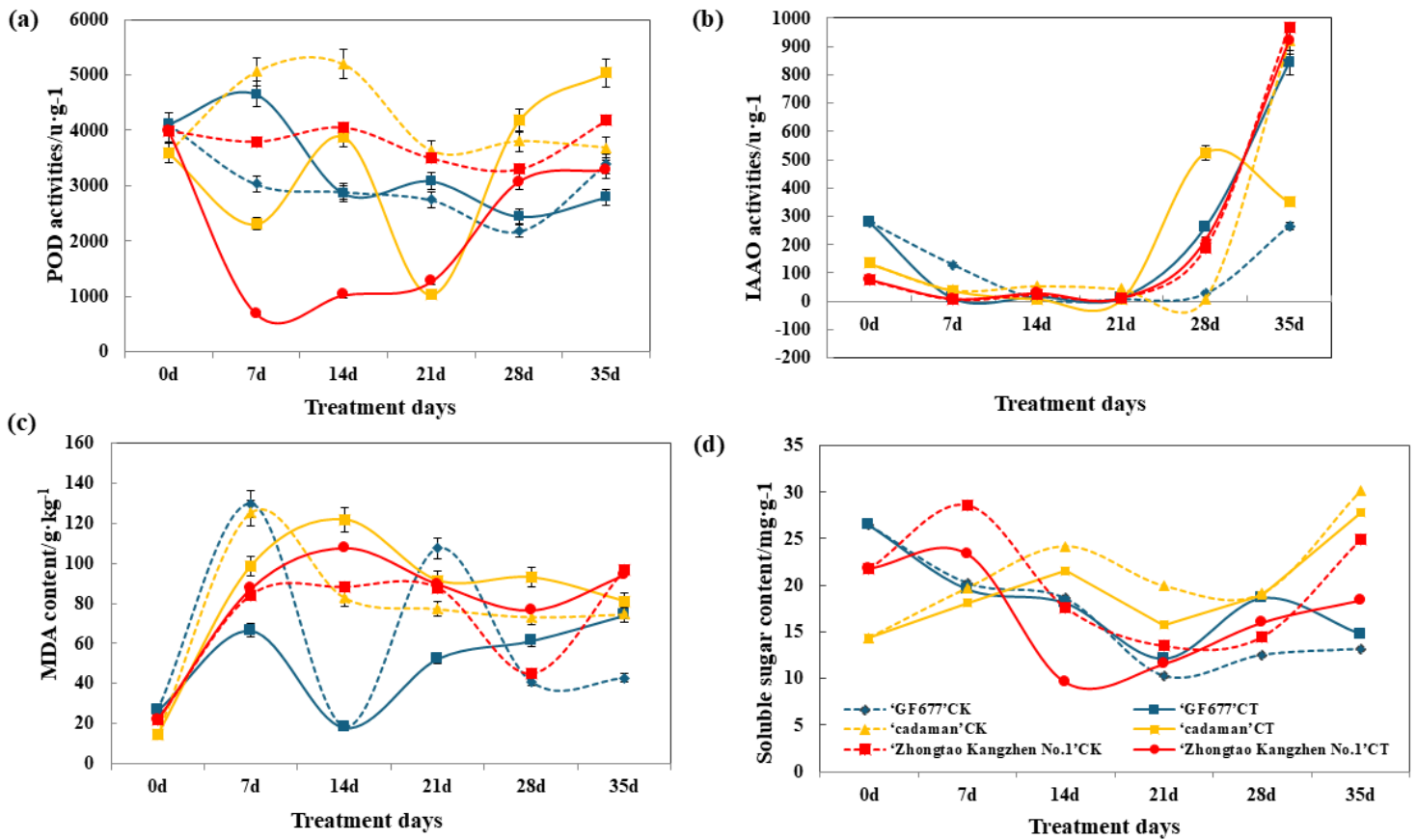


Figure 4

Dynamic variations in peroxidase (POD) activity (a), indoleacetic acid oxidase (IAAO) activity (b), malondialdehyde (MDA) content (c) and soluble sugar content (d) of cuttings from three peach rootstock cultivars ('GF677', 'Cadaman' and 'Zhongtao Kangzhen No.1') under the blank control (CK) and exogenous hormone treatment (CT). Samples were collected at successive treatment days of 0, 7, 14, 21, 28 and 35 throughout the whole adventitious root formation period; vertical error bars represent standard error of the mean.

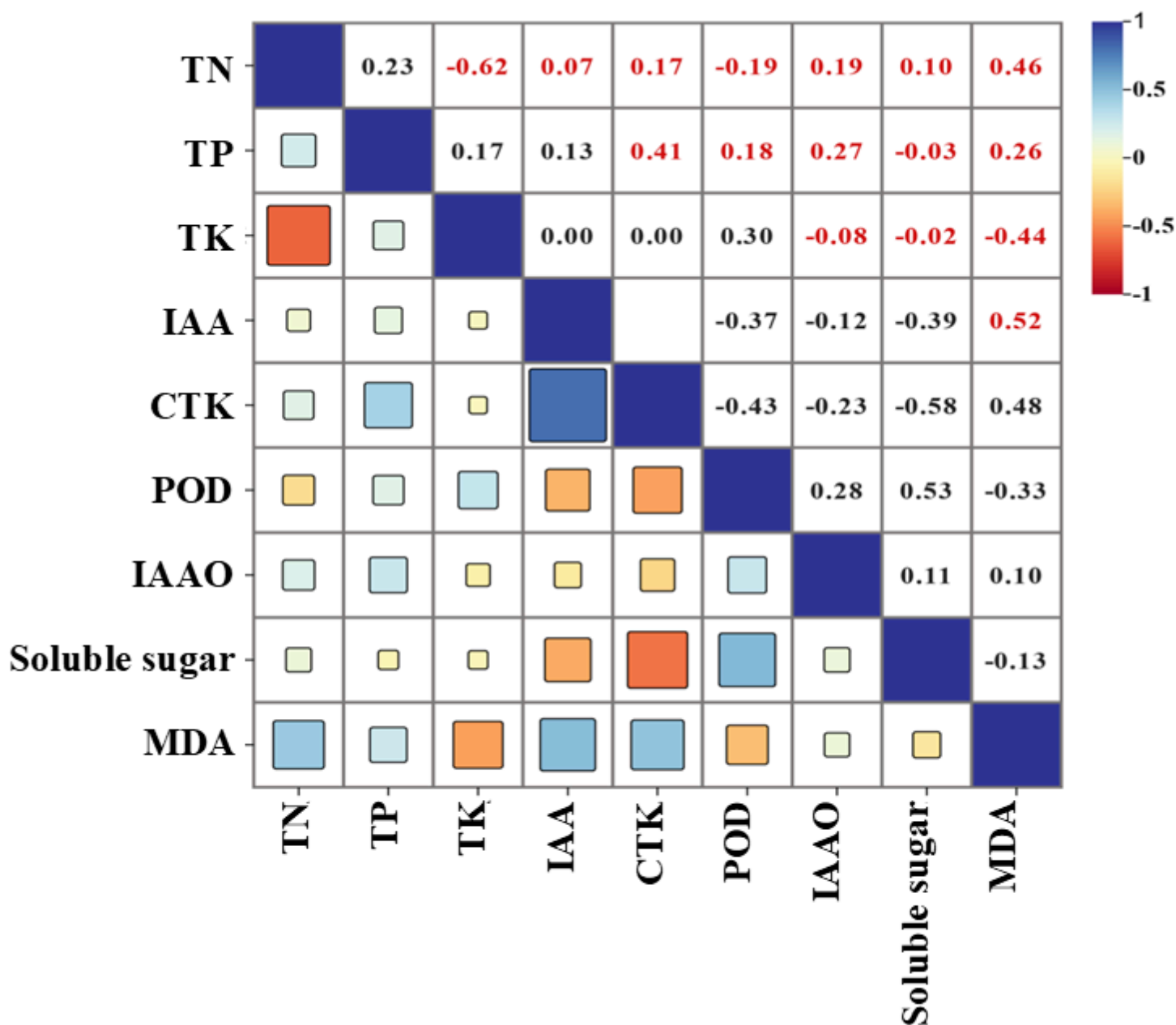


Figure 5

Pearson correlation heatmap of nutritional, hormonal and physiological parameters in cuttings of three peach rootstock cultivars during adventitious root formation. TN, total nitrogen; TP, total phosphorus; TK, total potassium; IAA, indole-3-acetic acid; CTK, cytokinin; POD, peroxidase; IAAO, indoleacetic acid oxidase; Soluble sugar, soluble sugar content; MDA, malondialdehyde content. Blue color represents positive correlation, red color represents negative correlation, and numerical values in each cell stand for corresponding correlation coefficients between paired indicators.

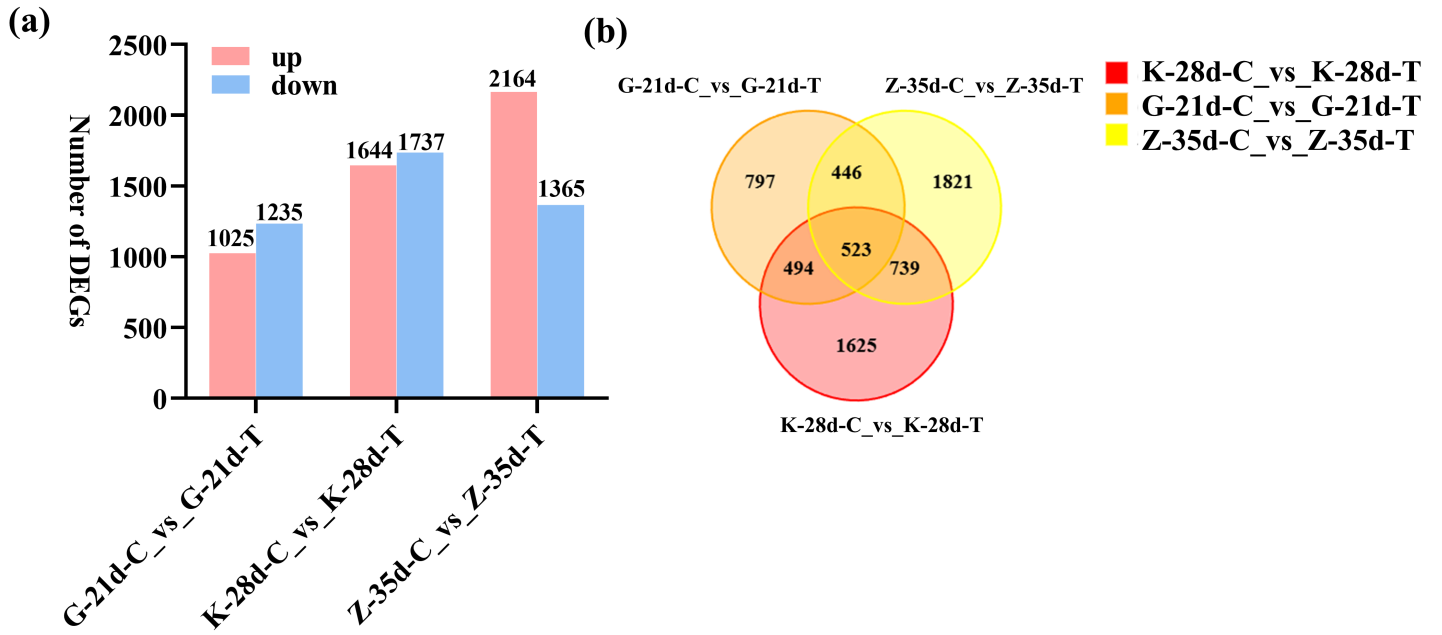


Figure 6

Statistics of differentially expressed genes from three comparative groups. (a) Bar chart quantifying the numbers of up- and down-regulated DEGs in each comparison; (b) Venn diagram illustrating shared and unique DEGs among three cultivar comparison sets. G: 'GF677', K: 'Cadaman', Z: 'Zhongtao Kangzhen No.1'; C = control, T = IBA treatment.

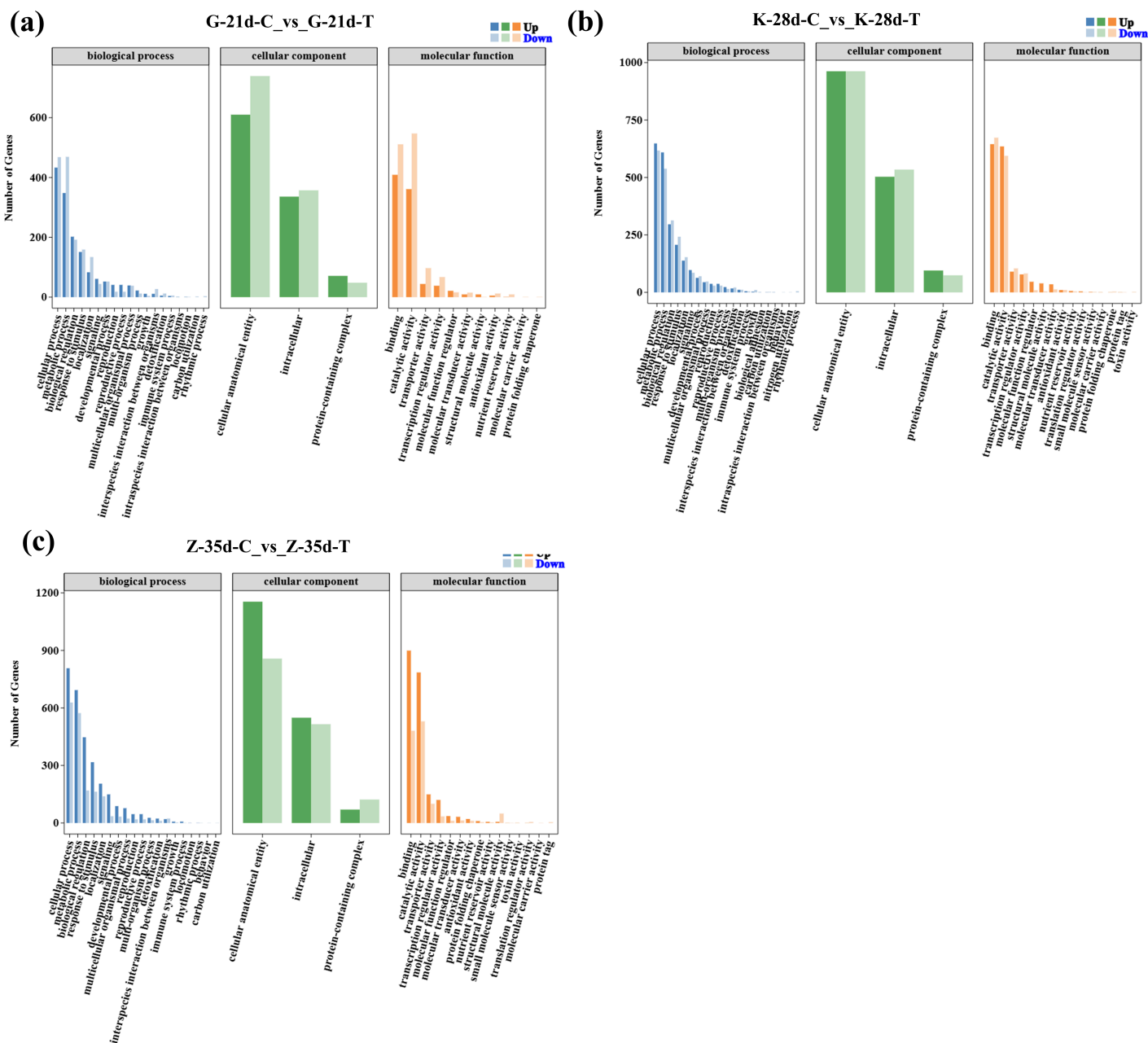


Figure 7

Gene Ontology (GO) functional classification of differentially expressed genes (DEGs) in three peach rootstock cuttings under IBA treatment. (a) GO classification of DEGs in G-21d-C_vs_G-21d-T. (b) GO classification of DEGs in K-28d-C_vs_K-28d-T. (c) GO classification of DEGs in Z-35d-C_vs_Z-35d-T. The bar charts display the number of genes assigned to three main GO categories: biological process, cellular component, and molecular function. G: 'GF677', K: 'Cadaman', Z: 'Zhongtao Kangzhen No.1'; C = untreated control, T = IBA treatment.

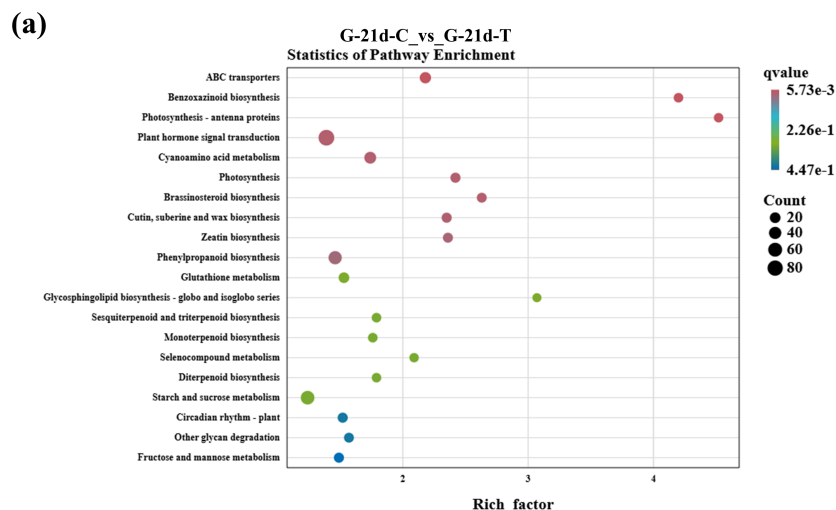
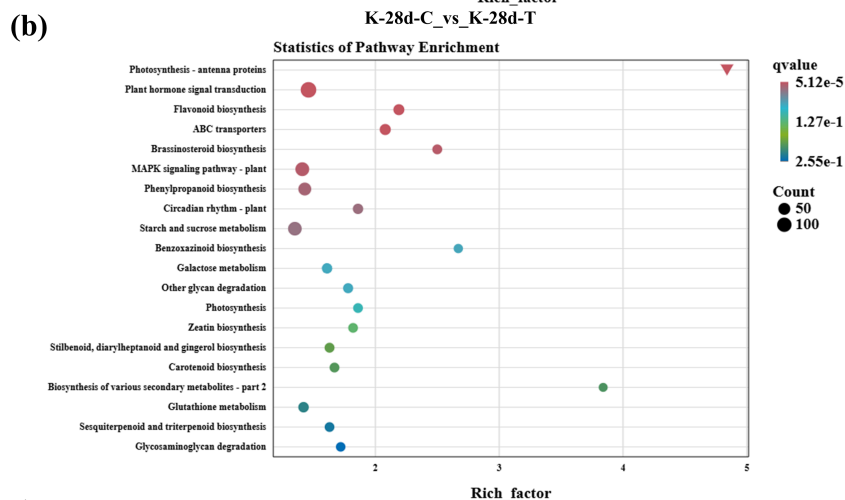
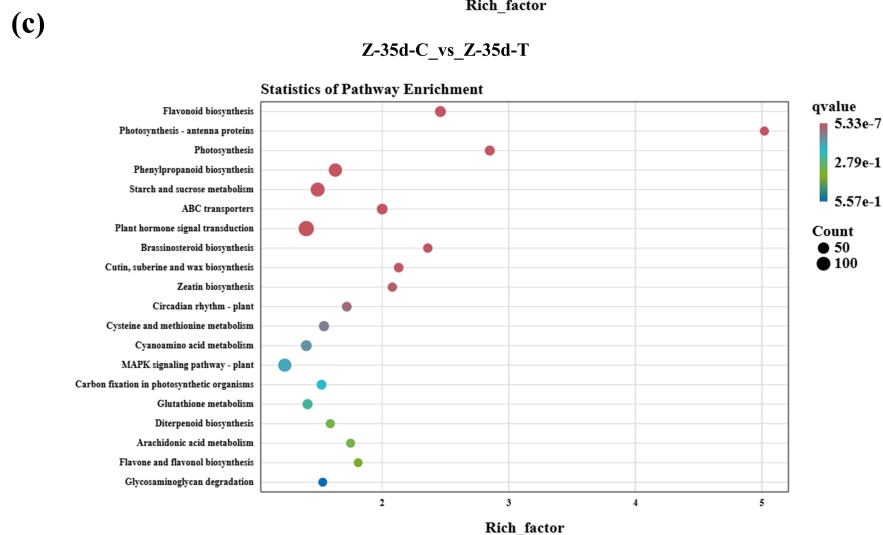


Figure 8

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs) in three peach rootstock cuttings under IBA treatment. (a) KEGG enrichment bubble plot of DEGs in G-21d-C_vs_G-21d-T. (b) KEGG enrichment bubble plot of DEGs in K-28d-C_vs_K-28d-T. (c) KEGG enrichment bubble plot of DEGs in Z-35d-C_vs_Z-35d-T. The horizontal axis represents the enrichment factor (Rich factor), and the vertical axis lists the enriched pathway names. The color

gradient of the bubbles indicates the q-value (corrected P-value), where redder colors represent higher statistical significance (smaller q-value). The size of each bubble corresponds to the number of DEGs enriched in the pathway. Symbols (triangles or circles) denote the regulatory patterns of genes within each pathway (up-regulated, down-regulated, or both). G: 'GF677', K: 'Cadaman', Z: 'Zhongtao Kangzhen No.1'; C = untreated control, T = IBA treatment.

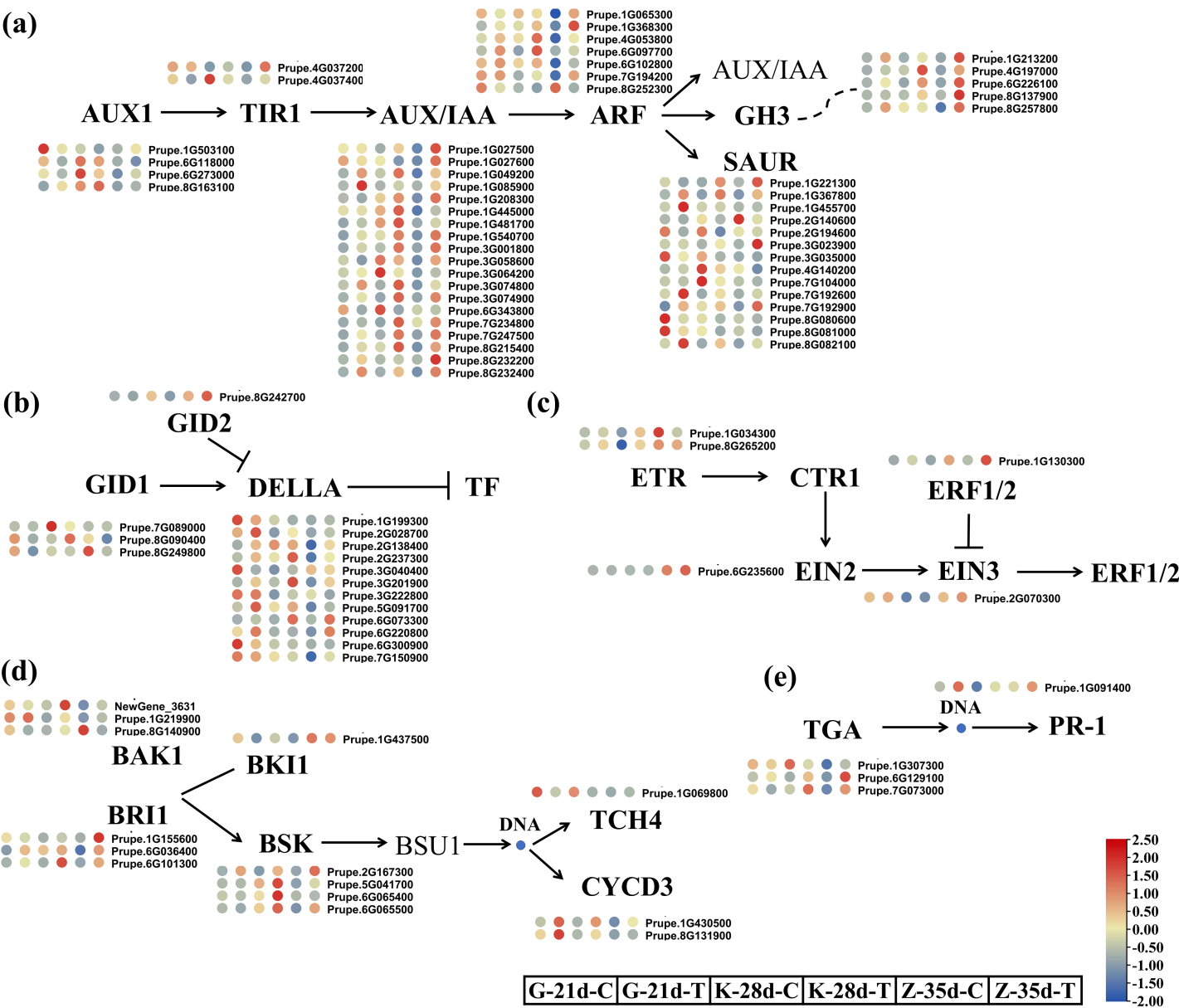


Figure 9

Expression patterns of key differentially expressed genes involved in plant hormone signaling pathways in three peach rootstock cuttings under IBA treatment. (a) Auxin signaling pathway; (b) Gibberellin signaling pathway; (c) Ethylene signaling pathway; (d) Brassinosteroid signaling pathway; (e) Salicylic acid signaling pathway. The color gradient of heatmap blocks represents the fold change of gene expression (red indicates up-regulation, blue indicates down-regulation, with more intense colors

representing greater magnitude of change). Columns correspond to control and IBA-treated samples of the three rootstocks (G-21d-C/T: ‘GF677’; K-28d-C/T: ‘Cadaman’; Z-35d-C/T: ‘Zhongtao Kangzhen No.1’), and rows represent key genes with their gene IDs in each pathway.

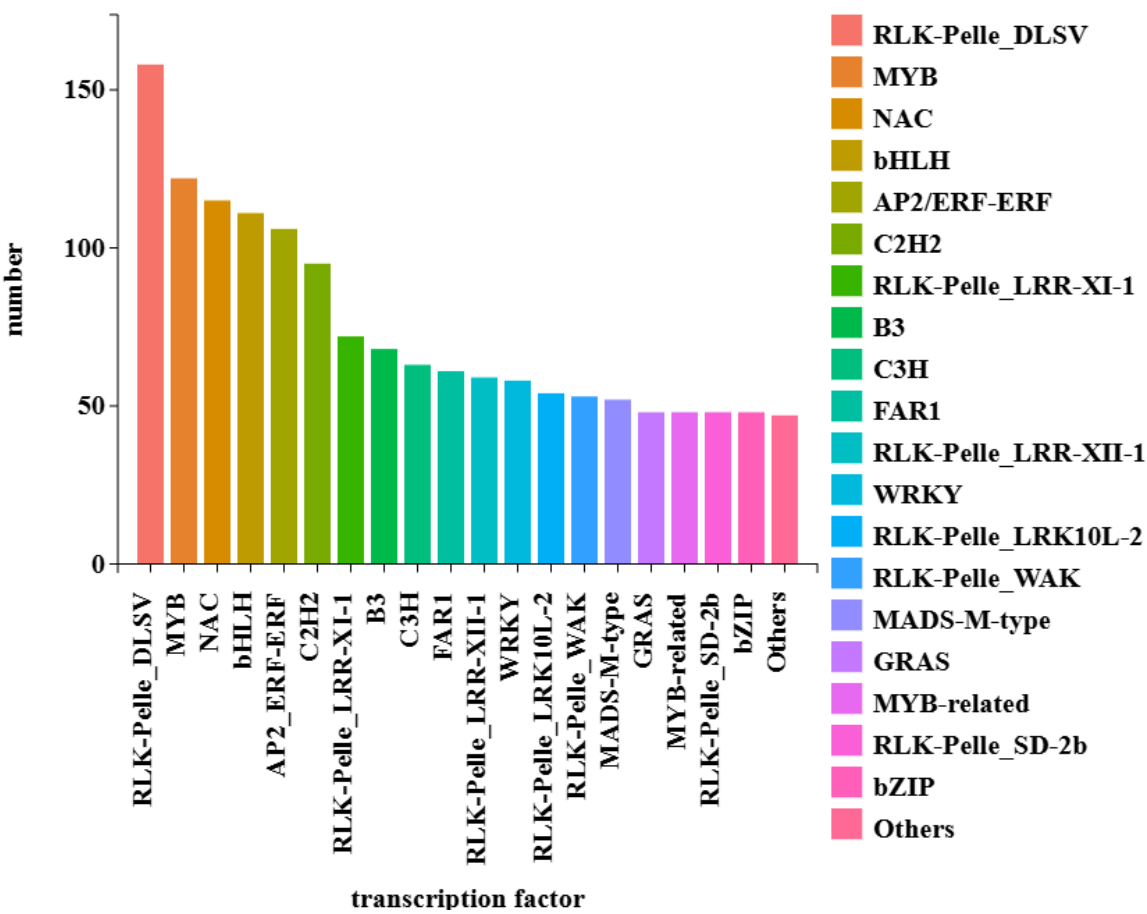


Figure 10

Distribution of transcription factor families of differentially expressed genes in peach rootstocks before and after IBA treatment. The x-axis represents different transcription factor families, and the y-axis represents the number of genes annotated to each family.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigureS1.png](#)
- [TableS1StatisticsofSequencingData.xlsx](#)