

Multi-omics Analysis Reveals the Mechanism of Yam Bulbil Sprouting in Response to Growth Regulators

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Abstract

Background

Bulbil sprouting is the cornerstone of asexual propagation in yam (*Dioscorea polystachya*). However, gibberellic acid (GA₃), a classic growth-promoting hormone, paradoxically suppresses this process through an unknown mechanism.

Results

Through an integrated multi-omics approach encompassing physiological phenotyping, transcriptomic profiling, and metabolomic analysis, we uncovered two diametrically opposed regulatory regimes. GA₃ inhibits sprouting by establishing a "high GA—low GID1 receptor—high DELLA" signal-desensitization state, which systemically represses growth-promoting hormone networks (auxin, cytokinin, ethylene, and jasmonates) and dampens primary energy metabolism. In stark contrast, paclobutrazol (PP₃₃₃) promotes sprouting via a "low GA—high GID1 receptor—stable DELLA" signal-hypersensitization state, which orchestrates the coordinated activation of multiple pro-sprouting pathways, including those mediated by ethylene, jasmonic acid, cytokinins, and brassinosteroids.

Conclusions

Our findings delineate the phytohormone crosstalk network governing yam bulbil sprouting, provide a theoretical foundation for precise sprouting management in yam cultivation, and expand the conceptual understanding of GA signaling functional plasticity beyond conventional model systems.

1 Introduction

The germination of plant buds and seeds is finely orchestrated by phytohormonal networks, among which gibberellin (GA) plays a central and extensively elucidated role. In model plants such as *Arabidopsis* and rice, the canonical GA signaling cascade is initiated when GA binds to its receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1). This interaction triggers the proteasomal degradation of DELLA transcriptional repressors, thereby relieving DELLA-mediated suppression of germination-associated genes and ultimately facilitating dormancy release and germination initiation [1–4]. Within this pathway, beyond the receptor GID1, DELLA proteins function not only as central repressors of GA signaling but also as key signaling hubs that integrate crosstalk between GA and other hormones, including jasmonic acid, auxin, and ethylene, thereby broadly coordinating plant growth and development [5]. The conserved GA-GID1-DELLA core signaling module is evolutionarily highly conserved, and its molecular mechanisms and functions have been validated across diverse plant species [6–10]. Transcriptomic and other approaches have revealed that GA orchestrates global gene expression reprogramming during germination through this module, involving key processes such as energy

metabolism, cell cycle regulation, and hormonal crosstalk^[1, 4, 6], providing a classical framework for understanding the molecular network of germination.

However, the modified stems—including tubers and bulbils—of yam (*Dioscorea opposita*) exhibit a unique pattern of GA responses. In stark contrast to the canonical role of GA in promoting germination in model plants, exogenous GA₃ promotes tuber (storage stem) growth yet paradoxically inhibits bulbil sprouting in yam, whereas the GA biosynthesis inhibitor paclobutrazol (PP₃₃₃) significantly enhances bulbil sprouting^[11–13]. This paradoxical response raises a fundamental mechanistic question: how is the GA signaling pathway rewired to operate in reverse during this specific developmental process, and what molecular basis underlies this inversion? Previous studies have provided initial clues. The core GA receptor genes in yam (e.g., multiple GID1 homologs) have been cloned and characterized^[14]. Our earlier work demonstrated that DoGID1A expression increases during bulbil sprouting and is further up-regulated by PP₃₃₃ treatment^[15]. Studies on DELLA proteins—another essential component of the GA signaling module—have shown that GA₃ promotes yam tuber growth via a DELLA-dependent pathway^[13]; that DoDELLA proteins (e.g., GAI2) integrate gibberellin and ethylene signaling^[16]; and that yam DELLA proteins (e.g., DoDELLA1) can modulate plant development by fine-tuning endogenous GA levels^[17]. Nevertheless, it remains largely unknown how the GID1-DELLA module is precisely regulated at the transcriptional and post-translational levels during bulbil sprouting, whether its functional state undergoes a fundamental switch to accommodate this 'reverse' response, and how such a switch orchestrates global reprogramming of the transcriptome and metabolome.

- To systematically dissect the molecular mechanisms underlying the opposing effects of GA₃ and PP₃₃₃ on yam bulbil sprouting, we integrated phenotypic, transcriptomic, and metabolomic analyses. We hypothesized that GA₃ and PP₃₃₃ oppositely remodel the functional state of the core GA signaling module (GID1-DELLA). Specifically, we sought to address two central questions: (i) Does GA₃ inhibit sprouting by establishing a "high GA—low GID1 receptor—high DELLA" state that desensitizes signaling and broadly suppresses pro-sprouting hormone networks? (ii) Conversely, does PP₃₃₃ promote sprouting by creating a "low GA—high GID1 receptor—stable DELLA" state that hypersensitizes signaling and coordinately activates pro-sprouting pathways, including those mediated by ethylene and cytokinins?
- To test this hypothesis, we defined three specific objectives: (1) to characterize the dynamic interplay among GA levels, GID1 expression, and DELLA accumulation under GA₃ and PP₃₃₃ treatments, and to determine whether these patterns constitute a "signal-desensitization" versus "signal-hypersensitization" molecular switch; (2) to reveal how this core switch drives global reprogramming of hormone interaction networks and metabolic pathways; and (3) to construct a comprehensive model of multi-hormone synergistic regulation governing yam bulbil sprouting. By achieving these objectives, this study not only aims to uncover the molecular basis of this unique biological phenomenon but also to provide a direct theoretical foundation for precision sprouting management in yam agriculture, thereby advancing our mechanistic understanding of reproductive physiology and signaling regulation in *Dioscorea* species.

2 Results

2.1 Effects of Plant Growth Regulators on Yam Bulbil Sprouting

Treatment with 100 mg/L gibberellic acid (GA_3) and 40 mg/L paclobutrazol (PP_{333}) significantly affected the sprouting rate of yam bulbils ($P < 0.05$) (Fig. 1A). In the water-treated control group (CK), bulbils began to sprout on day 6, and the sprouting rate gradually increased, reaching 22.72% by day 15. In contrast, GA_3 treatment exhibited a strong inhibitory effect on sprouting, with an extremely low sprouting rate throughout the 15-day observation period; only a trace amount (0.32%) of sprouting was observed on day 15, which was significantly different from both the CK and PP_{333} treatment groups. By comparison, PP_{333} treatment significantly promoted bulbil sprouting, with sprouting rates at each time point from day 6 onward being significantly higher than those of the CK, and continuously increasing over time, reaching 28.32% by day 15 (Fig. 1B).

2.2 Transcriptome Sequencing, Gene Annotation, and Differential Expression Analysis

To elucidate the molecular basis underlying the phenotypic differences described above, we performed transcriptome sequencing on yam bulbils treated with water (C), gibberellic acid (G), and paclobutrazol (P). A total of 297.25 Gb of high-quality clean data was obtained from all samples, which were assembled into 35,655 non-redundant unigenes with an average length of 1,165 bp. Among these, 27,497 unigenes (77.1%) were successfully annotated in public databases. Functional classification revealed that the annotated genes were mainly enriched in categories such as translation, ribosomal structure, post-translational modification, and signal transduction.

Differential expression gene (DEG) analysis revealed that the two treatments induced completely distinct transcriptional reprogramming patterns. PP_{333} treatment induced a rapid transcriptional activation, with 567 DEGs detected at the early stage (3 days post-treatment, C3 vs P3), of which 70.9% (402 genes) were up-regulated. In contrast, GA_3 treatment exhibited a delayed but persistent transcriptional repression. At the mid-term (6 days post-treatment, C6 vs G6), GA_3 induced 631 DEGs, with 60.1% (379 genes) being down-regulated. This repression became even more pronounced at the later stage (9 days post-treatment, C9 vs G9), where the number of DEGs increased sharply to 1,052, and down-regulated genes still accounted for the majority (577 genes, 54.9%). These data confirm, at the genomic level, that the promoting and inhibiting sprouting phenotypes correspond to two opposite global gene expression programs — ‘activation’ versus ‘repression’ — and that the inhibitory effect of GA_3 is persistent and cumulative.

2.2.1 KEGG pathway enrichment analysis of DEGs under GA_3 treatment

KEGG pathway enrichment analysis further revealed the key mechanisms by which GA₃ inhibited sprouting. Notably, the plant hormone signal transduction pathway was the most significantly enriched at both time points of GA₃ treatment (C6 vs G6 and C9 vs G9), suggesting that disruption of the hormone signaling network was the central event in GA₃-mediated sprouting inhibition.

At 6 days post-treatment (C6 vs G6), KEGG enrichment analysis showed that plant hormone signal transduction was the top-ranked significantly enriched pathway and contained the highest number of DEGs (Fig. 2A). Within this pathway, we identified 6 up-regulated and 13 down-regulated transcripts. Functional annotation indicated that these DEGs were primarily associated with signal transduction mechanisms, transcription, defense mechanisms, or unknown functions. Notably, among the up-regulated genes, three (TRINITY_DN3377_c1_g1, TRINITY_DN6047_c0_g1, TRINITY_DN9904_c0_g1) encoded DELLA proteins, and one (TRINITY_DN4547_c0_g1, an ETHYLENE INSENSITIVE 3-like homolog) was ethylene-related. The 13 down-regulated genes included multiple MYB transcription factors, auxin-responsive proteins (IAA10, IAA25-like), gibberellin receptors (TRINITY_DN9410_c0_g1, TRINITY_DN9505_c0_g1), and signal transduction response regulators. This expression pattern—characterized by DELLA accumulation and suppression of GA receptor, auxin, and cytokinin signaling components—indicated that GA₃ treatment induced substantial remodeling of the hormone signaling network as early as day 6.

By 9 days post-treatment (C9 vs G9), KEGG enrichment analysis again placed plant hormone signal transduction as the most significantly enriched pathway (Fig. 2C), which consistented with the result at day 6. The starch and sucrose metabolism pathway also remained significantly enriched, suggesting persistent perturbation of energy metabolism under GA₃ treatment. Interaction network analysis (Fig. 2D) revealed complex crosstalk between GA signaling and other hormone pathways, including auxin, ethylene, and jasmonic acid. Among hormone-related DEGs, 11 were up-regulated and 17 were down-regulated. Critically, the DELLA-encoding SLR1 genes (TRINITY_DN3377_c1_g1, TRINITY_DN6047_c0_g1) remained significantly up-regulated, while multiple gibberellin receptor genes (TRINITY_DN6677_c0_g1, TRINITY_DN9410_c0_g1, TRINITY_DN9505_c0_g1) were consistently down-regulated. This persistent "high DELLA–low receptor" expression pattern constituted the core molecular switch that effectively maintained sprouting suppression. Moreover, auxin response factors showed differential regulation, while genes encoding ethylene receptor 2 (TRINITY_DN1467_c1_g1) and a jasmonic acid-amido synthetase JAR1 homolog (TRINITY_DN9234_c0_g1) were down-regulated, indicating that by day 9, not only the perception of GA but also the signal transmission of other pro-sprouting hormones was broadly inhibited.

2.2.2 KEGG pathway enrichment analysis of DEGs under PP₃₃₃ treatment

In sharp contrast to the GA₃ treatment, PP₃₃₃ treatment induced a molecular response dominated by transcriptional activation that actively promoted sprouting.

At 6 days post-treatment (C6 vs P6), KEGG enrichment analysis showed that glutathione metabolism, photosynthesis, and plant hormone signal transduction were among the top five significantly enriched pathways (Fig. 3A). Among these, glutathione metabolism was the most significantly enriched pathway, indicating that PP₃₃₃ treatment caused oxidative stress in the bulbils and activated the antioxidant defense system. Within the plant hormone signal transduction pathway (Fig. 3B), 8 genes were up-regulated and 9 were down-regulated. Notably, one gibberellin receptor gene (TRINITY_DN6677_c0_g1) was significantly up-regulated; meanwhile, both ethylene receptor 2 (TRINITY_DN1467_c1_g1) and ethylene response sensor 1 (TRINITY_DN3325_c0_g1) were up-regulated, while the negative regulator ETHYLENE INSENSITIVE 3-like 1 (TRINITY_DN4547_c0_g1) was significantly down-regulated. This expression pattern of 'receptor up-regulation – negative regulator down-regulation' indicated strong activation of the ethylene signaling pathway, which was an important hallmark of the pro-sprouting state induced by PP₃₃₃.

By 9 days post-treatment (C9 vs P9), the plant hormone signal transduction pathway remained the core enriched category (Fig. 3C). Compared with the earlier stage, pathways associated with cell wall lignification and stress resistance, such as phenylpropanoid biosynthesis, became increasingly enriched, suggesting that structural changes related to pericarp rupture and organ elongation had begun during sprouting. Among the hormone-related DEGs, 11 were up-regulated and only 2 were down-regulated (Fig. 3D). The gene encoding chitin-inducible gibberellin-responsive protein 1 (TRINITY_DN16379_c0_g1) was significantly up-regulated, indicating that despite the PP₃₃₃-mediated inhibition of endogenous GA synthesis, the downstream GA response system had become derepressed or hypersensitive. The gene (TRINITY_DN6211_c0_g1) encoding ETHYLENE-INSENSITIVE 2 (EIN2), a core positive regulator of ethylene signaling, was also significantly up-regulated, providing direct evidence for sustained activation of the ethylene pathway. Meanwhile, the gene (TRINITY_DN7983_c0_g1) encoding IAA-amido synthetase GH3.8, which may reduce active auxin levels by conjugating free IAA, was up-regulated, while the gene (TRINITY_DN2401_c4_g1) encoding the cell elongation positive regulator auxin-responsive protein SAUR32 was down-regulated. Furthermore, the gene (TRINITY_DN2401_c4_g1) encoding a BZR1 homolog, a key transcription factor in brassinosteroid signaling, was up-regulated, suggesting that brassinosteroid signaling acted synergistically with GA and ethylene pathways to collectively promote bulbil sprouting.

Note

(A) C6 vs P6 KEGG classification diagram of differentially expressed genes; (B) KEGG pathway enrichment plot of differentially expressed genes in C6 vs P6; (C) C9 vs P9 KEGG classification diagram of differentially expressed genes; (D) KEGG pathway enrichment plot of differentially expressed genes in C9 vs P9;

2.2.3 Expression analysis of key genes under different treatments

In sharp contrast to the transcriptional suppression pattern induced by GA₃ treatment, PP₃₃₃ treatment triggered a completely opposite regulatory effect on the core components of the GA signaling pathway. To validate this conclusion, we used qRT-PCR to detect the relative expression levels of GA receptor genes (*DoGID1A*, *DoGID1B*, *DoGID1B2*), DELLA protein genes (DN3377, DN6047), and CIGR1 family genes (DN16379, DN7859) at 9 days post-treatment. The results showed that the two treatments exerted significant and opposite regulatory effects on the expression of these genes (Fig. 4), directly confirming the two opposing states of ‘signal desensitization’ and ‘signal hypersensitization’ at the molecular level.

At the level of GA signal perception, GA₃ treatment significantly suppressed the expression of all GA receptor genes (Fig. 4-A). Compared with the control group, the expression levels of *DoGID1A*, *DoGID1B*, and *DoGID1B2* were significantly down-regulated (decreased by 75.6%, 93.8%, and 52.6%, respectively, $P < 0.05$). In contrast, PP₃₃₃ treatment significantly induced the expression of *DoGID1A* and *DoGID1B* (increased by 118.6% and 67.3%, respectively, $P < 0.05$), although *DoGID1B2* was significantly down-regulated (decreased by 34.4%, $P < 0.05$). This opposite pattern— ‘broad receptor down-regulation under GA₃ treatment’ versus ‘major receptor up-regulation under PP₃₃₃ treatment’—established the molecular basis for the difference in GA signal perception capacity between the two treatments.

At the level of GA signal repression, the DELLA-encoding genes DN3377 and DN6047 were significantly up-regulated under GA₃ treatment (increased by 188.8% and 325.1%, respectively), and the up-regulation magnitudes were significantly higher than those under PP₃₃₃ treatment (increased by 29.0% and 144.9%, respectively) (Fig. 4-B). Notably, although DELLA genes also showed an up-regulation trend under PP₃₃₃ treatment, the fold changes were much lower than those under GA₃ treatment, indicating that GA₃ treatment established a strong ‘high DELLA’ inhibitory state, whereas PP₃₃₃ treatment maintained a relatively ‘stable DELLA’ state. This opposite pattern— ‘broad receptor down-regulation + strong repressor up-regulation’ versus ‘major receptor up-regulation + moderate repressor up-regulation’—directly confirmed the ‘signal desensitization’ and ‘signal hypersensitization’ molecular switch states induced by GA₃ treatment and PP₃₃₃ treatment, respectively.

It was worth noting that the CIGR1 family genes DN16379 and DN7859 exhibited extremely significant up-regulation under PP₃₃₃ treatment; under GA₃ treatment, although these two genes were also significantly up-regulated, the up-regulation magnitudes were much lower than those under PP₃₃₃ treatment (Fig. 4-C). As a crosstalk node between stress response and GA signaling, the strong activation of CIGR1 suggested that PP₃₃₃ treatment might establish a ‘signal hypersensitization’ state that rendered the bulbils highly sensitive to trace amounts of GA, thereby preparing for post-sprouting growth.

In summary, the expression patterns of key genes at day 9 revealed the opposite regulation of the GA signaling pathway by GA₃ and PP₃₃₃ at the molecular level: GA₃ caused signal desensitization by suppressing receptor genes and enhancing inhibitory factor gene; PP₃₃₃ established a signal hypersensitization state by activating major receptor genes and the CIGR1 family genes, thereby laying the foundation for sprouting initiation.

Note

(A) Relative expression levels of GA receptor genes (DoGID1A, DoGID1B, DoGID1B2) ; (B) Relative expression levels of DELLA protein genes (DN3377, DN6047) ; (C) Relative expression levels of CIGR1 family genes (DN16379, DN7859). Different lowercase letters indicate significant differences among treatments ($P < 0.05$).

2.3 Endogenous hormone profiles of yam bulbils in response to GA₃ and PP₃₃₃ treatments

To characterize the endogenous hormone profiles under different treatments, principal component analysis (PCA) and hierarchical clustering analysis were performed. The PCA score plot revealed a clear separation among the control (C), GA₃-treated (G), and PP₃₃₃-treated (P) groups at each time point, with tight clustering of biological replicates, indicating high data reliability (Fig. 5B). The heatmap further demonstrated distinct hormone accumulation patterns among the three groups (Fig. 5A).

Gibberellins (GAs)

Compared with the control, GA₃ treatment significantly increased the levels of GA₁ and GA₃, with the most pronounced accumulation observed at 9 days post-treatment (G9). In contrast, at the same time point, PP₃₃₃ treatment resulted in decreased GA₁ and GA₃ levels (Fig. 5A).

Cytokinins (CTKs)

In the control group, levels of the active cytokinin trans-zeatin (tZ) and its precursor increased over time. GA₃ treatment strongly suppressed tZ accumulation while up-regulating the less active cis-zeatin. Under PP₃₃₃ treatment, tZ levels were slightly lower than those in the control but remained significantly higher than those in the GA₃-treated group, and cis-zeatin was not detected.

Auxin (IAA)

At the early stage (P9), the IAA content in the PP₃₃₃-treated group was lower than in the control, and a higher proportion of IAA existed in its methylated (inactive) form. In contrast, the GA₃-treated group maintained relatively high IAA levels, with the active form being predominant at the same stage.

Absciscic acid (ABA)

Under PP₃₃₃ treatment, ABA content showed a sustained decrease throughout the experimental period. In contrast, ABA levels in the GA₃-treated group remained higher than those in the control at day 9 and further increased by day 15.

Other hormones

At the early stage (P9), PP₃₃₃ treatment significantly up-regulated strigolactone, whereas salicylic acid levels showed no obvious change compared with the control. GA₃ treatment also up-regulated strigolactone but down-regulated salicylic acid. In addition, both PP₃₃₃ and GA₃ treatments led to decreased levels of the ethylene precursor ACC.

Together, these results demonstrate that GA₃ and PP₃₃₃ treatments induce distinct and opposite endogenous hormone profiles in yam bulbils. Specifically, PP₃₃₃ treatment is associated with a hormone environment characterized by low GA and ABA levels, a moderate tZ/IAA ratio, and activation of strigolactone, whereas GA₃ treatment is associated with high GA, ABA, and IAA levels together with suppression of salicylic acid.

Note

(A) Hierarchical clustering analysis of all sample metabolites; (B) PCA analysis of all samples

2.3.1 KEGG pathway enrichment analysis of differential metabolites

To investigate the metabolic basis of GA₃-induced sprouting inhibition, KEGG enrichment analysis was performed on differential metabolites on day 9 (G9 vs C9) and day 15 (G15 vs C9) (Fig. 6A, B). At G9, the zeatin biosynthesis pathway was significantly enriched, with down-regulation of the active cytokinin trans-zeatin and its related metabolites. At G15, the diterpenoid biosynthesis pathway (for GA synthesis) emerged as a core enriched category, where endogenous GA₁, GA₄, and GA₇ were down-regulated. Concurrently, the tryptophan metabolism pathway (for auxin synthesis) was also enriched, with altered levels of the active auxin IAA. In addition, the ABA and SA pathways were enriched at both time points.

For PP₃₃₃-treated samples, KEGG enrichment analysis was performed on day 9 (P9 vs C9) and day 15 (P15 vs C9) (Fig. 6C, D). At P9, the diterpenoid biosynthesis and tryptophan metabolism pathways were significantly enriched, endogenous GA₁, GA₃, and GA₄ were downregulated, and metabolites related to IAA and ABA were also affected at this early stage. At P15, the diterpenoid biosynthesis pathway remained significantly enriched, with sustained down-regulation of GA₃, GA₄, and GA₇. Importantly, the plant hormone signal transduction pathway became central in the network at this stage, linking multiple hormone metabolites including trans-zeatin (tZ), ABA, SA, and IAA. Notably, tZ appeared as a key node in the P15 network, with its levels elevated compared to the control.

Note

(A) C9-G9 KEGG enrichment network diagram; (B) C9-G15 KEGG enrichment network diagram; (C) C9-P9 KEGG enrichment network diagram; (D) C9-P15 KEGG enrichment network diagram;

2.4 Expression patterns of DoGID1A, DoGID1B, and DoGID1B2 genes

To further validate the opposite transcriptional reprogramming patterns induced by GA₃ and PP₃₃₃, the expression dynamics of three gibberellin receptor genes—*DoGID1A*, *DoGID1B*, and *DoGID1B2*—were examined across the time course (0, 3, 6, 9, 12, and 15 days post-treatment) using qRT-PCR (Fig. 7).

Under GA₃ treatment, the expression levels of all three receptor genes were consistently suppressed throughout the experimental period. Compared with the initial level (day 0), *DoGID1A* expression remained significantly lower at all time points, with its minimum observed at day 9 (0.25-fold of the day-0 level). *DoGID1B* expression reached its lowest level at day 3 (0.13-fold) and then stabilized between 0.21- and 0.25-fold thereafter. Similarly, *DoGID1B2* expression was persistently suppressed by GA₃ treatment (Fig. 7).

In contrast, PP₃₃₃ treatment significantly induced the expression of *DoGID1A* and *DoGID1B2*. *DoGID1A* expression was gradually increased over time, reaching 4.96-fold of the day-0 level by day 15, and differed significantly from the control at all time points. *DoGID1B2* expression was up-regulated in a bimodal pattern, with the first peak (4.69-fold) at day 6 and the second (3.62-fold) at day 12. In contrast, *DoGID1B* expression was initially suppressed to 0.20-fold at day 3, peaked at 1.36-fold at day 9, and decreased again to 0.46-fold by day 15 (Fig. 7).

Together with the expression patterns of DELLA and CIGR1 genes described in Section 2.2.3, these results demonstrated that GA₃ and PP₃₃₃ induce opposite expression profiles of GA signaling components at the transcriptional level. Specifically, GA₃ treatment caused persistent down-regulation of GA receptor genes, whereas PP₃₃₃ treatment up-regulates major receptor genes (*DoGID1A* and *DoGID1B2*) while maintaining relatively stable DELLA levels (as shown in Section 2.2.3).

Note

Values are means ± SD of three biological replicates. Different lowercase letters indicate significant differences among treatments at the same time point (P < 0.05).

2.5 Multi-omics Integrative Analysis

2.5.1 Multi-omics comparative analysis of GA₃ and PP₃₃₃ responses

To systematically compare the molecular responses to by GA₃ or PP₃₃₃, we performed integrated KEGG enrichment analysis using both differentially expressed genes (DEGs) and differential metabolites (DAMs). Plant hormone signal transduction was the most significantly enriched pathway under both treatments, indicating that hormone crosstalk was the central mechanism regulating bulbil sprouting (Fig. 8A, B).

Gene–metabolite correlation networks revealed distinct interaction patterns between GA₃ treatment and PP₃₃₃ treatment. Under GA₃ treatment (C9 vs G9), the network was predominantly centered on

endogenous GA metabolites (GA₁ and GA₄), with fewer connections to other hormone pathways (Fig. 8C). Under PP₃₃₃ treatment (C9 vs P9), a more interconnected network was observed. Hub genes including gene_DRNTG_04343, gene_DRNTG_00753, gene_DRNTG_28624, and gene_DRNTG_08055 were connected to multiple hormone metabolites, namely trans-zeatin, GA₁, abscisic acid, IAA, and GA₄ (Fig. 8D).

Correlation analysis between DEGs and endogenous hormones further supported these network differences. Under GA₃ treatment (C9 vs G9), multiple hormones, including ABA, GA₃, and IAA, showed significant correlations with large numbers of DEGs. For example, ABA was correlated with more than 500 DEGs (Fig. 8E). Under PP₃₃₃ treatment (C9 vs P9), only specific GA components (GA₄, GA₇) and trans-zeatin (tZ) exhibited strong and balanced correlations with DEGs, whereas other hormones showed weaker associations with DEGs (Fig. 8F).

2.5.2 Opposing regulatory modes revealed by pathway and WGCNA module integration

To systematically dissect the regulatory mechanisms of GA₃ and PP₃₃₃, we performed multi-omics integration at both the pathway and WGCNA module levels.

Pathway-level integration revealed that plant hormone signal transduction was the most significantly enriched pathway common to both omics layers under both GA₃ and PP₃₃₃ treatments (GA₃: DEGs $P = 1.6 \times 10^{-6}$, DAMs $P = 4.2 \times 10^{-3}$; PP₃₃₃: DEGs $P = 5.3 \times 10^{-7}$, DAMs enrichment 5/7) (Table S2), indicating that hormonal network reprogramming constitutes the central event governing sprouting regulation.

WGCNA-based module-level integration further unveiled opposing regulatory patterns between the two treatments. In the GA₃ treatment group (C9d vs G9d), correlation analysis between the metabolite module ME_{grey} (comprising 28 hormone-related metabolites, Table S1) and gene modules (Table S3) revealed that modules including GE_{lightcyan1} ($r = -0.996$, $P = 2.74 \times 10^{-5}$) and GE_{magenta1} ($r = -0.952$, $P = 3.38 \times 10^{-3}$) were significantly suppressed, whereas GE_{indianred3} ($r = 0.984$, $P = 3.61 \times 10^{-4}$) was activated. GA₃ treatment induced a “high GA—low receptor—high DELLA” signal desensitization state, broadly repressing pro-sprouting transcriptional programs. Single-gene-to-hormone correlation analysis showed that ABA correlated with over 500 DEGs, indicating a broadly disrupted hormone-gene network.

In contrast, in the PP₃₃₃ treatment group (C9d vs P9d) (Table S3), modules including GE_{grey60} ($r = 0.953$, $P = 3.27 \times 10^{-3}$) and GE_{bisque4} ($r = 0.941$, $P = 5.17 \times 10^{-3}$) exhibited significant positive correlations with ME_{grey} and were enriched in CIGR1 family genes (gene_DRNTG_16379, gene_DRNTG_07859), indicating that the downstream GA response system had become hypersensitized. Correlation analysis revealed coordinated associations only between specific hormones (GA₄, GA₇, tZ) and DEGs, suggesting that PP₃₃₃ established a highly coordinated hormone-gene network.

Collectively, the integrative analysis uncovered two diametrically opposed regulatory modes: GA₃ drives decoupling between the transcriptome and metabolome through signal desensitization, whereas PP₃₃₃

establishes a synergistically activated pro-sprouting network through signal hypersensitization.

3 Discussion

3.1 Two opposing modes of regulation of yam bulbil sproutin by GA₃ and PP₃₃₃

This study reveals the regulatory mechanism by which GA₃ and PP₃₃₃ exert opposite effects on yam bulbil sprouting through two opposing regulatory modes. The core of this mechanism lies in two antagonistic phytohormone signaling patterns: GA₃ establishes a 'High GA–Low Receptor–High DELLA' signal desensitization mode, whereas PP₃₃₃ establishes a 'Low GA–High Receptor–Stable DELLA' signal hypersensitization mode. This finding not only confirms the central role of the GID1-DELLA module in mediating GA signaling in yam bulbils but also reveals the functional plasticity of this module in response to exogenous growth regulators^[18–19].

The observation that exogenous GA₃ inhibits rather than promotes yam bulbil sprouting is consistent with previous reports in *Dioscorea* species. Notably, Okagami and Tanno (1971) first reported that GA induces dormancy in yam bulbils^[20], and Okagami (1979)^[21] further demonstrated that GA₃ treatment suppresses sprouting in several herbaceous plants, including yam. Additionally, Tanno et al.^[12] showed that GA₁, GA₃, and GA₄ suppress sprouting in non-dormant bulbils of Chinese yam in a concentration-dependent manner, while GA biosynthesis inhibitors such as uniconazole and prohexadione promote sprouting. These findings contrast with the classical role of GA in promoting seed germination and bud break in most plants^[22–23], suggesting that the GA signaling machinery in yam bulbils operates under a unique regulatory logic.

Our transcriptomic and metabolomic data provide a mechanistic explanation for this phenomenon. We find that exogenous GA₃ triggers a negative-feedback response that down-regulates GID1 receptor expression while up-regulating DELLA accumulation, leading to signal desensitization and broad suppression of growth-promoting hormone networks. In contrast, PP₃₃₃, a GA biosynthesis inhibitor widely used to retard excessive shoot elongation in crops^[24], significantly promotes sprouting in yam bulbils, consistent with previous observations^[11]. Our data show that PP₃₃₃ treatment reduces endogenous GA levels while up-regulating GID1 receptor expression and maintaining relatively stable DELLA levels. This 'hypersensitization' mode enables the bulbils to perceive and respond to minimal GA signals, thereby creating a suitable hormonal environment for sprouting initiation.

3.2 GA₃ treatment triggered signal desensitization and broad suppression of pro-sprouting networks

After GA₃ treatment, the plant hormone signal transduction pathway was the most significantly enriched KEGG category at both day 6 and day 9, indicating that disruption of the hormone signaling network was a central event in GA₃-mediated sprouting inhibition^[25]. At day 6 (C6 vs G6), we observed up-regulation of DELLA-encoding genes (three SLR1 homologs) and down-regulation of multiple GA receptor genes

(GID1), auxin-responsive proteins (IAA10, IAA25-like), and cytokinin response regulators. This expression pattern—characterized by DELLA accumulation and suppression of GA receptor, auxin, and cytokinin signaling components—indicated Our finding that GA₃ induces DELLA accumulation is consistent with the well-established role of DELLA as a repressor of GA signaling ^[1].

By day 9 (C9 vs G9), this suppression network became more extensive and sustained. The DELLA-encoding SLR1 genes remained significantly up-regulated, while multiple GA receptor genes were consistently down-regulated. This persistent ‘high DELLA–low receptor’ expression pattern constituted a core molecular switch that effectively maintained sprouting suppression. Moreover, genes encoding ethylene receptor 2 and a jasmonic acid-amido synthetase JAR1 homolog were down-regulated, indicating that by day 9, not only GA perception but also the signaling of other pro-sprouting hormones was broadly inhibited. In the present study, we observed that by day 9, not only GA perception but also the signaling of other pro-sprouting hormones was broadly inhibited (Fig. 4C). This pattern of widespread suppression is consistent with previous reports that ethylene and jasmonate signaling can be coordinately down-regulated under certain conditions ^[26–27]. The starch and sucrose metabolism pathway also remained significantly enriched, suggesting persistent perturbation of energy metabolism under GA₃ treatment ^[28–30].

Our qRT-PCR results further validated these findings. After GA₃ treatment, DoGID1A, DoGID1B, and DoGID1B2 were consistently suppressed, with expression levels significantly lower than the initial level at all time points. This pattern was consistent with previous reports that exogenous GA application reduces GID1 expression ^[31–33] indicating a conserved negative-feedback mechanism ^[34].

3.3 PP₃₃₃ treatment established a hypersensitized state and coordinately activated pro-sprouting pathways

In sharp contrast to GA₃ treatment, PP₃₃₃ treatment induced a transcriptional activation-dominant response. At day 6 (C6 vs P6), glutathione metabolism was the most significantly enriched pathway, indicating that PP₃₃₃ treatment caused oxidative stress in the bulbils and activated the antioxidant defense system ^[35]—an early response that may prime the tissue for subsequent sprouting. Within the plant hormone signal transduction pathway, we observed up-regulation of a GA receptor gene (TRINITY_DN6677_c0_g1), ethylene receptor 2 (ETR2), and ethylene response sensor 1 (ERS1), along with down-regulation of the negative regulator EIN3-like1. This pattern of ethylene signaling component regulation—receptor up-regulation combined with repressor down-regulation—is characteristic of ethylene pathway activation, which has been shown to promote sprouting in various plant species (Tamar A, 2023; Viet M T N, 2024). This “up-regulation of receptor – down-regulation of negative regulator” pattern indicated strong activation of the ethylene signaling pathway ^[36].

By day 9 (C9 vs P9), the plant hormone signal transduction pathway remained the core enriched category. The gene encoding chitin-inducible gibberellin-responsive protein 1 was significantly up-regulated, indicating that despite PP₃₃₃-mediated inhibition of endogenous GA biosynthesis, the

downstream GA response system had become derepressed or hypersensitized^[37]. The gene encoding ETHYLENE-INSENSITIVE 2 (EIN2), a central positive regulator of ethylene signaling, was also up-regulated, providing direct evidence for sustained activation of the ethylene pathway^[38].

The coordinated activation of ethylene signaling by PP₃₃₃ treatment was particularly noteworthy. Our results showed up-regulation of ethylene receptors (ETR2, ERS1) and down-regulation of the negative regulator EIN3-like1, accompanied by increased ACC levels. This pattern of ethylene pathway activation aligns with recent findings in yam tuber development, where this ethylene pathway activation mode, in which DoDELLA-GAI2 was proven to integrate GA and ethylene signals, was consistent with findings in yam tuber growing^[39–40]. Combining our observation that DELLA genes are stably expressed under PP₃₃₃ treatment (with up-regulation but at much lower levels than under GA₃ treatment), together with previous findings^[19, 41], it can be concluded that DELLA-ethylene crosstalk may represent a conserved mechanism in Dioscorea species, operating in both bulbil development and tuber growth contexts.

Furthermore, the elevation of trans-zeatin levels and the maintenance of relatively low IAA levels by PP₃₃₃ treatment resulted in a substantially increased CTK/IAA ratio. This hormonal context is known to favor the initiation of cell division^[42–44]. Our findings were consistent with previous observations that yam bulbil growth requires adequate levels of cytokinins^[45], and indicated that as reported in various plant systems, reshaping the cytokinin-auxin balance to create a cellular environment conducive to cell division is a key strategy for promoting organ regeneration and sprouting^[44]. Consistently, our data indicate that PP₃₃₃ promotes sprouting not merely by inhibiting GA biosynthesis but also by actively reshaping this balance^[44].

The up-regulation of a BZR1 homolog, a key transcription factor in brassinosteroid signaling, further suggests that brassinosteroid signaling acts synergistically with GA and ethylene pathways to collectively promote bulbil sprouting, a mode of multi-hormone synergistic regulation that has been documented in other plant species^[46].

3.4 Integrated multi-omics analysis revealed distinct hormone–gene interaction networks

Gene–metabolite correlation networks revealed fundamentally different interaction patterns between the two treatments. After PP₃₃₃ treatment (C9 vs P9), a highly integrated network was observed, in which hub genes such as gene_DRNTG_04343 and gene_DRNTG_00753 connected multiple hormone metabolites, including trans-zeatin, GA₁, abscisic acid, IAA, and GA₄ (Fig. 8D). This multi-hormone integrated network contrasted sharply with the GA₃-induced network, which was predominantly centered on GA₁ and GA₄ with limited connections to other hormone pathways (Fig. 8C).

Correlation analysis between DEGs and endogenous hormones further supported these network differences. After GA₃ treatment (C9 vs G9), multiple hormones (ABA, GA₃, IAA) showed significant correlations with large numbers of DEGs—for example, ABA was correlated with more than 500 DEGs.

This indicated a broad but poorly coordinated transcriptional response to GA₃ treatment. After PP₃₃₃ treatment (C9 vs P9), only specific GA components (GA₄, GA₇) and trans-zeatin exhibited strong and balanced correlations with DEGs, indicating the establishment of a coordinated and specific hormone–gene interaction network favorable for sprouting.

The expression patterns of three GA receptor genes—*DoGID1A*, *DoGID1B*, and *DoGID1B2*—further supported these conclusions. PP₃₃₃ treatment significantly induced the expression of *DoGID1A* and *DoGID1B2*, while GA₃ treatment suppressed all three receptors. We speculated that under conditions of inhibited GA biosynthesis, the expression of gibberellin receptor genes was upregulated to maximize the perception and utilization of residual GA signals, thereby initiating necessary downstream physiological processes including sprouting. This phenomenon is similar to the compensatory up-regulation of receptors observed in previous studies^[18–19, 47], suggesting that the GA signaling pathway might maintain basic signal transduction by enhancing receptors expression when they were inhibited.

3.5 Two working models for the opposing regulation of yam bulbil sprouting

Based on our integrated transcriptomic and metabolomic data, we proposed two working models for the opposing regulation of yam bulbil sprouting by GA₃ and PP₃₃₃, respectively.

After GA₃ treatment, high exogenous GA₃ level triggered a negative-feedback loop that down-regulated expression of GID1 genes and stabilizes DELLA repressors. The accumulated DELLA proteins then broadly suppressed, which is reported by other researchers^[19, 48], ultimately blocking sprouting.

After PP₃₃₃ treatment, the inhibition of GA biosynthesis is compensated by up-regulation of GID1 gene expression and maintenance of stable DELLA levels, resulting in a hypersensitive GA signaling state. This state permits the coordinated activation of multiple pro-sprouting pathways, as evidenced by our observations: up-regulation of ethylene signaling components (ETR2/ERS1) and down-regulation of the negative regulator EIN3-like1, elevated trans-zeatin levels, and increased expression of the brassinosteroid signaling transcription factor BZR1. Such multi-pathway synergistic activation has been reported to promote growth and development in various plant species^[26, 38, 46]. The increased CTK/IAA ratio further creates a hormonal environment conducive to cell division, a relationship that is well established in plant cell cycle regulation^[44]. Together, these coordinated events drive cell division and elongation at the sprouting site.

The two models once again emphasized the reported role of the GID1-DELLA module as a molecular switch that integrates multiple hormonal signals^[49–52] and pointed out the core role of the GID1-DELLA module as a molecular switch determining sprouting outcomes. Our findings provided a theoretical foundation for precise sprouting control in yam cultivation and offered insights into the functional plasticity of GA signaling in non-model crops.

3.6 Limitations and future directions

Despite the systematic elucidation of two opposing regulatory modes governing GA₃- and PP₃₃₃-mediated yam bulbil sprouting through integrated transcriptomic, metabolomic, and physiological data, several limitations in the present study should be acknowledged and warrant further investigation.

First, the proposed molecular models are primarily derived from correlative analyses of transcriptomic and metabolomic data, along with qRT-PCR validation, and lack direct pharmacological or genetic functional validation. For instance, we hypothesized that the inhibitory effect of GA₃ could be partially rescued by activating ethylene or cytokinin pathways. However, we did not perform co-treatment experiments with GA₃ plus ACC (an ethylene precursor) or 6-BA (a cytokinin analog) to directly test this antagonistic relationship—an experimental strategy widely employed to dissect hormone crosstalk [53]. Similarly, we did not confirm whether activation of the ethylene pathway following PP₃₃₃ treatment is necessary for its sprouting-promoting effect. This would require co-treatment with an ethylene inhibitor such as 1-MCP, a well-established approach for blocking ethylene signaling [54]. Such pharmacological interventions would provide critical causal evidence to complement our current correlative findings.

Second, the temporal resolution of our transcriptomic and metabolomic sampling may be insufficient to capture the earliest molecular events following hormone treatment. We monitored gene expression and metabolite dynamics at 3, 6, 9, 12, and 15 days post-treatment and observed that down-regulation of GID1 receptor genes and up-regulation of DELLA genes under GA₃ treatment were detectable as early as day 3. However, the negative-feedback response of the GA signaling pathway may be initiated much earlier—potentially within hours (e.g., 1–2 hours post-treatment). Without earlier sampling time points, we could not distinguish between direct primary responses and secondary downstream effects. Future studies incorporating a finer temporal series (e.g., 0.5, 1, 2, 4, 8, and 24 hours after treatment) would be necessary to dissect the onset timing and hierarchical ordering of GA signal desensitization.

Third, the functional roles of the key hub genes identified in this study, including gene_DRNTG_04343 and the GA receptor genes (DoGID1A, DoGID1B, DoGID1B2), remain to be experimentally verified. While our multi-omics analyses pinpointed these genes as central nodes in the regulatory networks, their actual contributions to sprouting regulation await validation through gain-of-function or loss-of-function approaches, such as virus-induced gene silencing (VIGS), RNA interference (RNAi), or heterologous overexpression in model systems like *Nicotiana benthamiana* or *Arabidopsis* [53]. Furthermore, the biochemical mechanism underlying the proposed molecular switch—specifically, the physical interaction between GID1 and DELLA proteins under different hormonal contexts—has not been directly tested. Co-immunoprecipitation (Co-IP) or yeast two-hybrid (Y2H) assays would help determine whether GA₃ and PP₃₃₃ treatments differentially affect GID1-DELLA complex formation, which is the core event governing GA signaling output [49–52].

Fourth, our study focused primarily on transcriptional and metabolic responses, leaving post-translational regulation largely unexplored. DELLA protein stability is known to be regulated by the ubiquitin-proteasome pathway upon GA perception, a process that cannot be captured by transcriptome analysis alone. Future investigations should examine DELLA protein accumulation and degradation

dynamics using immunoblotting, as well as the phosphorylation status of downstream signaling components, to provide a more complete picture of the signaling switch at the protein level.

Fifth, while we observed cultivar- and tissue-specific responses in yam bulbils, it remains unclear whether the two regulatory modes identified here are conserved across other yam varieties or applicable to other asexual reproductive organs, such as tubers or rhizomes, in related species. Comparative studies across multiple genotypes and developmental contexts would help establish the generalizability of our findings.

Despite these limitations, the two opposing regulatory modes proposed herein are highly self-consistent, grounded in rigorous multi-omics data across multiple time points, and supported by strong concordance between qRT-PCR validation and transcriptomic profiles. The consistency across independent analytical platforms provides a solid evidential foundation for our conclusions. Future functional validation experiments—alongside higher-resolution temporal and protein-level analyses—will further refine this regulatory network and offer more direct targets for precision sprouting control in yam bulbils.

4 Materials and Methods

4.1 Plant Materials

The 'Niuwei' yam landrace used in this study was sourced from Lufeng County, Yunnan Province, China.

4.2 Experimental Methods

4.2.1 Recording of Bulbil Sprouting Following Paclobutrazol and Gibberellin Treatments

Yam bulbils at the sprouting initiation stage (characterized by visible radicle protrusion) and of uniform size were selected. The experiment comprised three treatments: distilled water (control, CK), 100 mg/L gibberellic acid (GA₃), and 40 mg/L paclobutrazol (PP₃₃₃). For each treatment, 50 bulbils were used, with three biological replicates. After soaking for 24 hours, the bulbils were planted in seedling trays. The emergence of shoots from the soil was defined as the sprouting criterion, and sprouting was recorded daily. The sprouting rate was calculated as follows:

Sprouting rate (%) = (Number of sprouted bulbils / Total number of bulbils tested) × 100%.

4.2.2 Transcriptome Sequencing and Analysis

4.2.2.1 Plant Treatments and Sampling

Bulbils at the sprouting initiation stage were soaked in 100 mg/L GA₃ or 40 mg/L PP₃₃₃ solutions for 24 hours, with distilled water used as the control (CK). Following treatment, the bulbils were incubated at

25°C. A total of 400 bulbils per treatment were used, with three replicates. Sampling was conducted on days 0, 3, 6, 9, 12, and 15 post-treatment. Approximately 0.5 g of epidermal tissue from the basal end of each bulbil was collected, immediately flash-frozen in liquid nitrogen, and stored at – 80°C for subsequent transcriptome sequencing.

4.2.2.2 Transcriptome Sequencing

Transcriptome sequencing was performed by Beijing Biomarker Technologies Co., Ltd. The raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1483274. The data will be released on March 31, 2027 (embargoed until publication).

4.2.2.3 Transcriptome Data Analysis

The sequencing data underwent library quality assessment, differential expression analysis, gene functional annotation, and functional enrichment analysis.

4.2.2.4 Gene Functional Annotation and Differential Expression Analysis

Genes with a P-value < 0.01 and |fold change| ≥ 1.5 were defined as differentially expressed genes (DEGs).

4.2.3 Metabolome Sequencing and Analysis

4.2.3.1 Sample Preparation for Endogenous Hormone Quantification

The treatment and sampling procedures for endogenous hormone quantification were the same as described in Section 4.2.2.1. Briefly, 0.5 g of epidermal tissue from the bulbil base was collected on days 0, 3, 6, 9, 12, and 15 post-treatment, flash-frozen in liquid nitrogen, and stored at – 80°C until analysis.

4.2.3.2 Metabolome Data Analysis

The determination of endogenous hormone levels was entrusted to Beijing Baimaike Biotechnology Co., Ltd. The raw sequencing data have been submitted to the NCBI Sequence Read Archive (SRA) database under BioProject accession number PRJNA1483274. The data will be publicly released on March 31, 2027 (after manuscript publication).

4.2.3.3 Metabolome Data Analysis

After quality control, libraries were constructed and preliminarily quantified. Clean data were assembled to construct a Unigene database for the species. Subsequent analyses included library quality evaluation, differential metabolite screening, and metabolic pathway enrichment analysis.

4.2.4 Expression Analysis of Key Genes in the GA Signaling Pathway

The treatments were the same as those described in Section 4.2.2.1. Samples were collected on days 0 (pre-treatment baseline), 3, 6, 9, 12, and 15 post-treatment. Approximately 0.5 g of epidermal tissue from the bulbil base was collected, flash-frozen in liquid nitrogen, and stored at -80°C . Pre-treatment samples (L0) served as the baseline control. The expression profiles of DoGID1A, DoGID1B, and DoGID1B2 were analyzed using quantitative real-time PCR (qPCR), with the yam CHC (Clathrin Heavy Chain) gene used as an internal reference. Each sample included three biological replicates, and data were analyzed using the $2^{-\Delta\Delta\text{CT}}$ method.

4.2.4.1 Total RNA Extraction and cDNA Synthesis

Total RNA was extracted using the Eastep® Super Total RNA Extraction Kit, and cDNA was synthesized using the Mighty Script™ First-Strand cDNA Synthesis Master Mix (with gDNA removal), following the manufacturers' instructions.

4.2.4.2 Quantitative Real-Time PCR (qPCR)

The qPCR reaction was performed using the 2× Q3 SYBR® Master Mix (Universal) (TOLOBIO) according to the manufacturer's protocol. The primers used are listed in Table 8. Each sample included three independent biological replicates.

Table 8
Primer sequences

Primer usage	Sequence (5'→3')
CHC	F:GGTGAAGTGTATGAGGCGG R:GCATCCACACAAGCAAAGCA
DoGID1A-qPCR	F:TTCCACCAACCTCCTCGTC R:CAGGGATAACGGTGCTCAGG
DoGID1B-qPCR	F:TGCTGCCGCCACCTCTAACA R:CTCCGACGGTGACCTACATTGC
DoGID1B2-qPCR	F:TCATCTGCCAACACTGCCATCT R:CGGAAGTATCGCCAGCAAGGAA
DELLA(DN3377)	F:GAGAGAGGAAGAGGATGCGG R:GCACGGGTTGGTCCCATCAG
DELLA(DN6047)	F:CGACGGTGTTATAGTGACAGTATC R:GCGAAGATGGAGCAGCTTGA
CIGR1(DN16379)	F:GGCACAACATCTACAAAGCCCTC R:GATATCCGCATGTAGTGGAGCAG
DN7859	F:GCCTACATTTCTCCGGCCAG R:CACGTTCTCAAGGGCATGTG

4.3 Statistical Analysis

All data were organized using Microsoft Office Excel 2010. Statistical analyses were performed using IBM SPSS Statistics 27 software. One-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test was used for multiple comparisons, and a P-value < 0.05 was considered statistically significant. Figures were generated using Origin 2018 software.

5 Conclusions

This study elucidates the molecular mechanisms by which exogenous GA₃ and PP₃₃₃ regulate yam bulbil sprouting. The core of this mechanism lies in two antagonistic phytohormone signaling patterns:

GA₃ treatment establishes a “High GA–Low GID1 receptor–High DELLA” signal desensitization mode, leading to GA signal desensitization. Acting as a central repression hub, the accumulated DELLA proteins coordinately suppress multiple growth-promoting hormone pathways (including auxin, cytokinin, ethylene, and jasmonic acid), while also disrupting energy supply routes such as starch and sucrose

metabolism. Sprouting of bulbils is thus inhibited by the collapse of the entire growth-promoting hormone network.

In contrast, PP₃₃₃ establishes a “Low GA–High GID1 receptor–Stable DELLA” signal hypersensitization mode, thereby enhancing GA signal transduction efficiency. This is accompanied by activation of the ethylene and jasmonic acid signaling pathways, whose synergistic crosstalk promotes sprouting. Furthermore, PP₃₃₃ elevates cytokinin levels to form a high CTK/IAA ratio, establishing a hormonal context conducive to cell division, while up-regulation of the brassinosteroid signaling transcription factor BZR1 provides direct driving force for cell division and elongation. Collectively, these PP₃₃₃-induced regulatory events establish a coordinated hormonal and transcriptional network that effectively drives yam bulbil sprouting.

Our findings provide a theoretical foundation for precise sprouting control in yam cultivation and offer insights into the functional plasticity of GA signaling in non-model crops. Future research should focus on functional validation of key hub genes and investigate the crosstalk mechanisms between GA signaling and defense pathways at the protein level.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The raw transcriptomic and metabolomic sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1483274 and will be publicly released on March 31, 2027.

Competing interests: The authors declare no competing interests.

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Authors' contributions: YC and SSX conceived and designed the research, performed experiments, analyzed data, and wrote the manuscript. YJL and YBD contributed to sample collection and data curation. SH performed validation and formal analysis. WHL supervised the project, acquired funding, and revised the manuscript. All authors read and approved the final manuscript.

References

1. Sun T, Gubler F, MOLECULAR MECHANISM OF, GIBBERELLIN SIGNALING IN PLANTS[J]. *Annu Rev Plant Biol.* 2004;55(1):197–223.
2. Tai-Ping S. Gibberellin metabolism, perception and signaling pathways in Arabidopsis.[J]. *arabidopsis book.* 2008;6:e103.
3. Miyako U, Motoyuki A, Masatoshi N, et al. GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin.[J]. *Nature.* 2005;437(7059):693–8.
4. Ueguchi-Tanaka M, Nakajima M, Motoyuki A, et al. Gibberellin Receptor and Its Role in Gibberellin Signaling in Plants[J]. *Annu Rev Plant Biol.* 2007;58(0):183–98.
5. Lorenzo A, Jun K, Haggag A, et al. Functional analysis of cotton orthologs of GA signal transduction factors GID1 and SLR1.[J]. *Plant Mol Biol.* 2008;68(1–2):1–16.
6. Li J, Gao H, Jiang J, et al. Overexpression of the *Galega orientalis* gibberellin receptor improves biomass production in transgenic tobacco[J]. *Plant Physiol Biochem.* 2013;73:1–6.
7. Liu Y, Zhang YQ, Liu GC, et al. Cloning and expression of gibberellin receptor genes in *Brassica napus*. *J Northwest Bot.* 2011;31(5):868–74. (in Chinese with English abstract).
8. Song Y, Zhang YM, Wu SJ, et al. Cloning and expression analysis of the gibberellin receptor gene MdGID1a and its promoter from a spur-type apple cultivar. *Acta Horticulturae Sinica.* 2013;40(11):2237–44. (in Chinese with English abstract).
9. Li XL, Guo XP, Shen YY, et al. Preliminary identification of a gibberellin–deficient short–male–inflorescence mutant in chestnut and expression analysis of CmGID1 gene. *Acta Horticulturae Sinica.* 2011;38(7):1251–8. (in Chinese with English abstract).
10. Wang XC, Wu WM, Fang JG, et al. Isolation, subcellular localization and expression analysis of the grape gibberellin receptor gene VvGID1A. *Acta Horticulturae Sinica.* 2013;40(5):839–48. (in Chinese with English abstract).
11. Long WH, Wang Q, Xiao GL, et al. Changes in endogenous hormone contents of yam bulbils during dormancy and the effect of paclobutrazol on dormancy breaking. *Southwest China J Agricultural Sci.* 2013;26(5):1996–2000. (in Chinese with English abstract).
12. Tanno N, Yokota T, Abe M, et al. Promotive and inhibitory effect of uniconazole and prohexadione on the sprouting of bulbils of Chinese yam, *Dioscorea opposita*. *Plant Growth Regul.* 1995;16(2):129–34.
13. Zhou Y, Li Y, Gong M, et al. Regulatory mechanism of GA3 on tuber growth by DELLA-dependent pathway in yam (*Dioscorea opposita*)[J]. *Plant Mol Biol.* 2021;106(4):433–48.
14. Hong WL, Sheng SX, ,Gui JM et al. CLONING AND CHARACTERIZATION OF TWO GIBBERELLIN RECEPTOR GENES IN YAM (*DIOSCOREA OPPOSITA*)[J].*PAKISTAN JOURNAL OF BOTANY*,2021,53(5):1717–25.
15. Long WH, Meng JG, Xu SS, et al. Cloning and expression analysis of the gibberellin receptor gene DoGID1A in yam. *J Agricultural Biotechnol.* 2019;27(11):1933–41. (in Chinese with English abstract).

16. Ge M, Zhang Y, **ng Y, et al. DoDELLA-GAI2 Integrates Gibberellin and Ethylene Signaling to Regulate Chinese Yam (*Dioscorea opposita*) Tuber Development[J]. *Biology*. 2025;14(6):635.
17. Zhou Y, Li Y, Huang J, et al. DoDELLA1, a DELLA protein from *Dioscorea opposita*, regulates the growth and development in transgenic tobacco by controlling gibberellin level[J]. *Plant Growth Regul*. 2022;97(3):571–83.
18. N B,N A, Molecular. GA pathways as conserved integrators for adaptive responses.[J].*Plant biology* (Stuttgart, Germany),2023,25(5).
19. Sarwar R, Zhu KM, Jiang T, et al. DELLAs directed gibberellins responses orchestrate crop development: A brief review[J]. *Crop Sci*. 2023;63(1):28.
20. Okagami N, Tanno N. Gibberellin-induced dormancy in bulbils of *Dioscorea*. *Planta* 101:91–4.
21. Okagami N. al. Dormancy in bulbils of several herbaceous plants: Effects of photoperiod, light, temperature, oxygen and gibberellic acid. *J Plant Res* 92:39–58.
22. Song SQ, Liu J, Xu HH, Liu X, Huang H. ABA metabolism and signaling and their molecular mechanism regulating seed dormancy and germination[J]. *Scientia Agricultura Sinica*. 2020;53(5):857–73. (in Chinese with English abstract).
23. Zuo Y, Xu YH. Research progress on the mechanism of GA and ABA in seed germination[J]. *Mol Plant Breed*. 2021;19(18):6221–6. (in Chinese with English abstract).
24. Hedden P, Graebe JE. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. *J Plant Growth Regul*. 1985;4:111–22.
25. Waadt R, Sella CA, Hsu PK et al. Plant hormone regulation of abiotic stress responses (May, 10.1038/s41580-022-00479-6, 2022)[J].*Nature reviews: molecular cell biology*, 2022(7):23.
26. Tamar A, Sebastian S, ,Dikla N et al. A Role for Ethylene Signaling and Biosynthesis in Regulating and Accelerating CO₂ - and ABA-mediated Stomatal Movements in *Arabidopsis*. [J].*The New phytologist*,2023,238(6):2460–75.
27. Kou X, Zhou J, ,Wu EC, et al. The interplay between ABA/ethylene and NAC TFs in tomato fruit ripening: a review[J]. *Plant Mol Biol*. 2021;106(3):1–16.
28. Jean-Baptiste D, Cyril A, ,Anis L et al. Seed quality and carbon primary metabolism.[J].*Plant, cell & environment*,2019,42(10):2776–2788.
29. Environmental Botany; Data from University of Angers Provide New Insights into Environmental Botany. (Seed Quality and Carbon Primary Metabolism)[J].*Ecology Environment & Conservation*,2019.
30. L B,H R, R R et al. Seed development and differentiation: a role for metabolic regulation.[J].*Plant biology* (Stuttgart, Germany),2004,6(4):375 – 86.
31. Aleman L, Kitamura J, Abdel-Mageed H, et al. Functional analysis of cotton orthologs of GA signal transduction factors GID1 and SLR1[J]. *Plant Mol Biol*. 2008;68(1–2):1.

32. Du H, Chang Y, Huang F, et al. GID1 modulates stomatal response and submergence tolerance involving abscisic acid and gibberellic acid signaling in rice[J]. *Journal of Integrative Plant Biology*; 2015.
33. Sun TP. The Molecular Mechanism and Evolution of the GA–GID1–DELLA Signaling Module in Plants[J].*Current biology*. Volume 21. CB; 2011. pp. R338–45. 910.1016/j.cub.2011.02.036.
34. Ahmad F, Kamal A, Singh A, et al. Seed priming with gibberellic acid induces high salinity tolerance in *Pisum sativum* through antioxidant system, secondary metabolites and upregulation of antiporter genes[J]. *.Plant Biology*; 2020.
35. Zhu B et al. Transcriptome analysis of glutathione response: RNA-Seq provides insights into balance between antioxidant response and glucosinolate metabolism.*Antioxidants* 11.7 (2022): 1322.
36. Ada L, Gerhard L. Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination.[J].*Plant cell reports*,2012,31(2):253 – 70.
37. Kovi M, Rao et al. Candidacy of a chitin-inducible gibberellin-responsive gene for a major locus affecting plant height in rice that is closely linked to Green Revolution gene *sd1*.*Theoretical and applied genetics* 123.5 (2011): 705–14.
38. Viet MT, N, Thi D T T, Bram PDV et al. The impact of growing season on the ethylene biosynthesis and signaling pathways of a heat tolerant tomato during off-vine postharvest ripening[J].*Postharvest Biology and Technology*,2024,207.
39. Gallego-Bartolome J, Minguet EG, Grau-Enguix F, et al. Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in *Arabidopsis*[J]. *Proc Natl Acad Sci USA*. 2012;109(33):13446–51.
40. Yongqi P, Jintong L, Bishu Q, et al. Aquaporin AtTIP5;1 as an essential target of gibberellins promotes hypocotyl cell elongation in *Arabidopsis thaliana* under excess boron stress[J]. *Funct Plant Biol*. 2018;45(3):305.
41. Sabir AI, Hu X, Khan I et al. Regulatory Mechanisms of Bud Dormancy: Environmental, Hormonal, and Genetic Perspectives[J]. *Int J Mol Sci* 2025,26(6):2517–2517.
42. Tianyi H, Juan D, Chujia C et al. Dysregulation of transfer RNA-derived small RNAs that regulate cell activity and its related signaling pathways in human cancers.[J].*Current gene therapy*,2023,23(4):291–303. 10.2174/1566523223666230601102506
43. Wenxue L, Yujing Q, Xiujuan C et al. Cell division cycle associated 8 promotes the growth and inhibits the apoptosis of endometrial cancer cells by regulating cell cycle and P53/Rb signaling pathway.[J].*American journal of translational research*,2023,15(6):3864–81.
44. Wanyu XNL, Tingting Z et al. Substance metabolism, IAA and CTK signaling pathways regulating the origin of embryogenic callus during dedifferentiation and redifferentiation of cucumber cotyledon nodes[J].*Scientia Horticulturae*,2022,293.
45. Tang WF, Xu SS, Duan YB, Long WH. Cloning and expression analysis of *DolPTs* gene in yam during bulbil sprouting[J]. *Acta Agriculturae Boreali-Sinica*. 2022;37(1):27–34. (in Chinese with English abstract).

46. Cao X, Wei Y, Shen B et al. Interaction of the Transcription Factors BES1/BZR1 in Plant Growth and Stress Response[J]. *Int J Mol Sci* 2024,25(13):6836–6836.
47. Zhang S, Zhang D, Fan S et al. Effect of exogenous GA 3 and its inhibitor paclobutrazol on floral formation, endogenous hormones, and flowering-associated genes in ‘Fuji’ apple (*Malus domestica* Borkh.)[J]. *Plant Physiology and Biochemistry*, 2016, 107: 178-186.
48. Zhou Y, Chen J, Hu Y, et al. Comprehensive Survey of the DELLA Transcription Factors in Cucumber and Functional Characterization of CsDELLA1 in Regulation of GA-Mediated Growth and Development[J]. *J Plant Growth Regul.* 2024;43(8):2802–16.
49. Murase K, Hlirano Y, Sun TP et al. Gibberellin-induced DELLA recognition by the gibberellin receptor GID1[J]. *Nature*, 2008(7221):456.
50. Jun, Cheng. Mengmeng, et al. A single nucleotide mutation in GID1c disrupts its interaction with DELLA1 and causes a GA-insensitive dwarf phenotype in peach.[J]. *Plant Biotechnology Journal*; 2019.
51. Hauvermale AL, Ariizumi T, Steber CM. The roles of the GA receptors GID1a, GID1b, and GID1c in sly1-independent GA signaling[J]. Volume 9. *Plant signaling & behavior*; 2014. p. e28030. 2.
52. Fukazawa J, Ito T, Kamiya Y, et al. Binding of GID1 to DELLAs promotes dissociation of GAF1 from DELLA in GA dependent manner[J]. Volume 10. *Plant Signaling & Behavior*; 2015. 10.
53. Carrera E, Ruiz-Rivero O, Peres LEP, et al. Characterization of the procera Tomato Mutant Shows Novel Functions of the SIDEELLA Protein in the Control of Flower Morphology, Cell Division and Expansion, and the Auxin-Signaling Pathway. during Fruit-Set and Development[J]. *PLANT PHYSIOLOGY*; 2012.
54. Ogasawara T, Sakamura K. Inactivation of the CTD phosphatase-like gene OsCPL1 enhances the development of the abscission layer and seed shattering in rice.[J]. *plant journal*, 2010.
55. Wenhui ZPL, Zhennan Z et al. Genome-Wide Identification of Auxin-Responsive GH3 Gene Family in *Saccharum* and the Expression of ScGH3-1 in Stress Response[J]. *Int J Mol Sci* 2022,23(21):12750–12750.

Figures

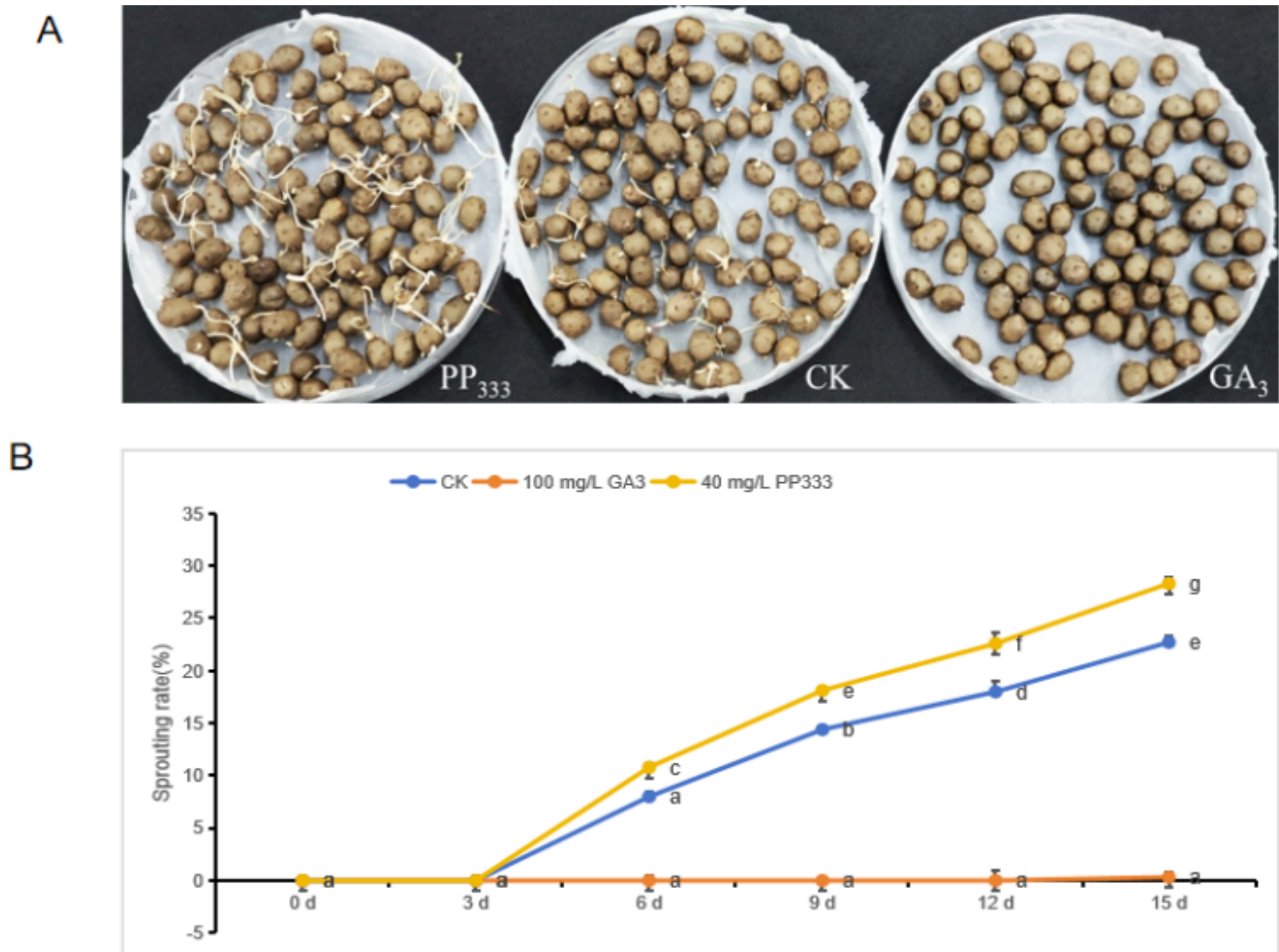


Figure 1

Effect of Growth Regulators on the Sprouting of Bulbils

Note: (A) Sprouting status of bulbils treated with different plant growth regulators; (B) Sprouting rate of the treated bulbils. Different lowercase letters indicate significant differences at the $P < 0.05$ level.

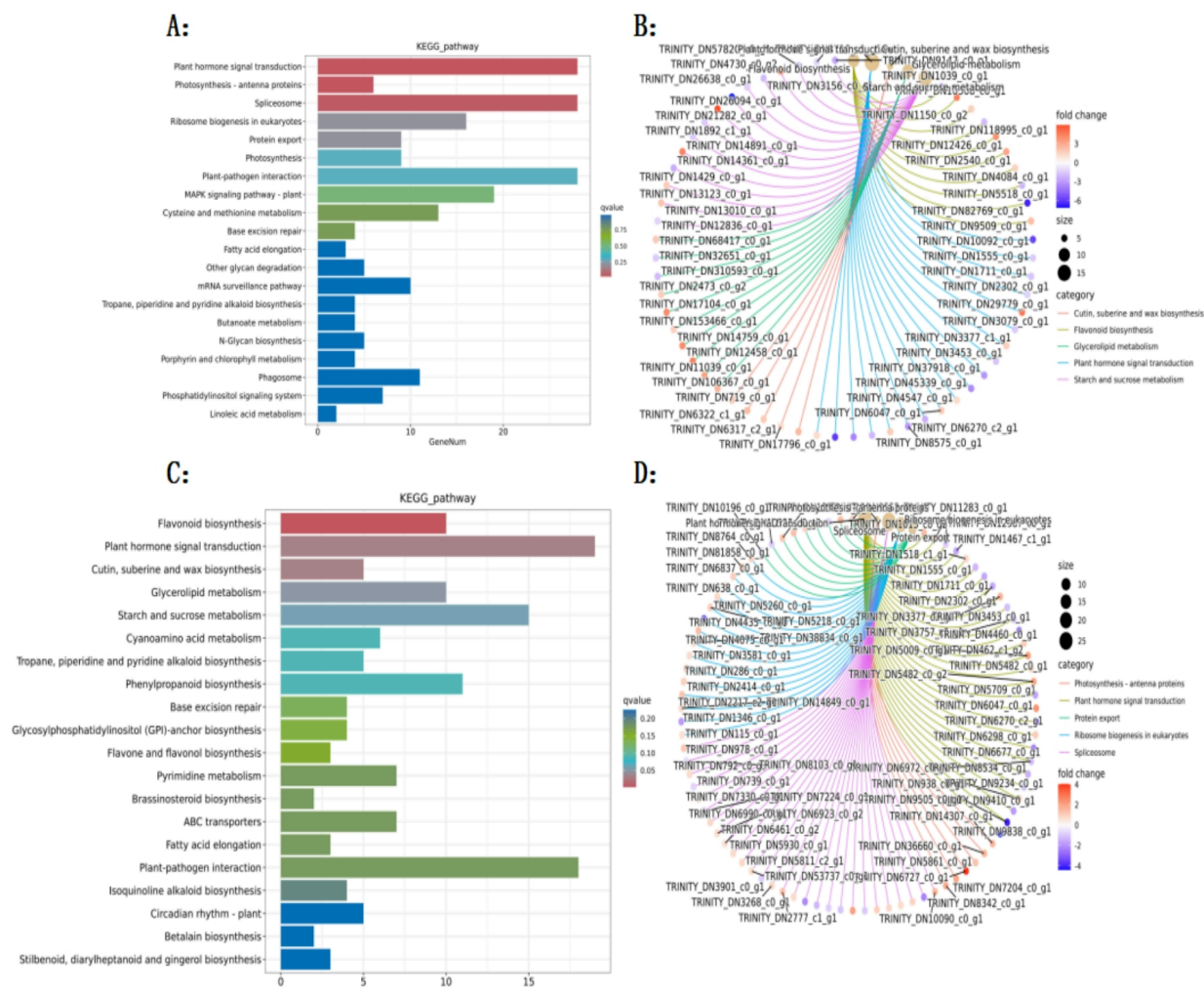


Figure 2

KEGG pathway enrichment analysis of differentially expressed genes under gibberellin treatment Note: (A) C6 vs G6 KEGG classification diagram of differentially expressed genes; (B) KEGG pathway enrichment plot of C6 vs G6 differentially expressed genes; (C) C9 vs G9 KEGG classification diagram of differentially expressed genes; (D) KEGG pathway enrichment plot of C9 vs G9 differentially expressed genes;

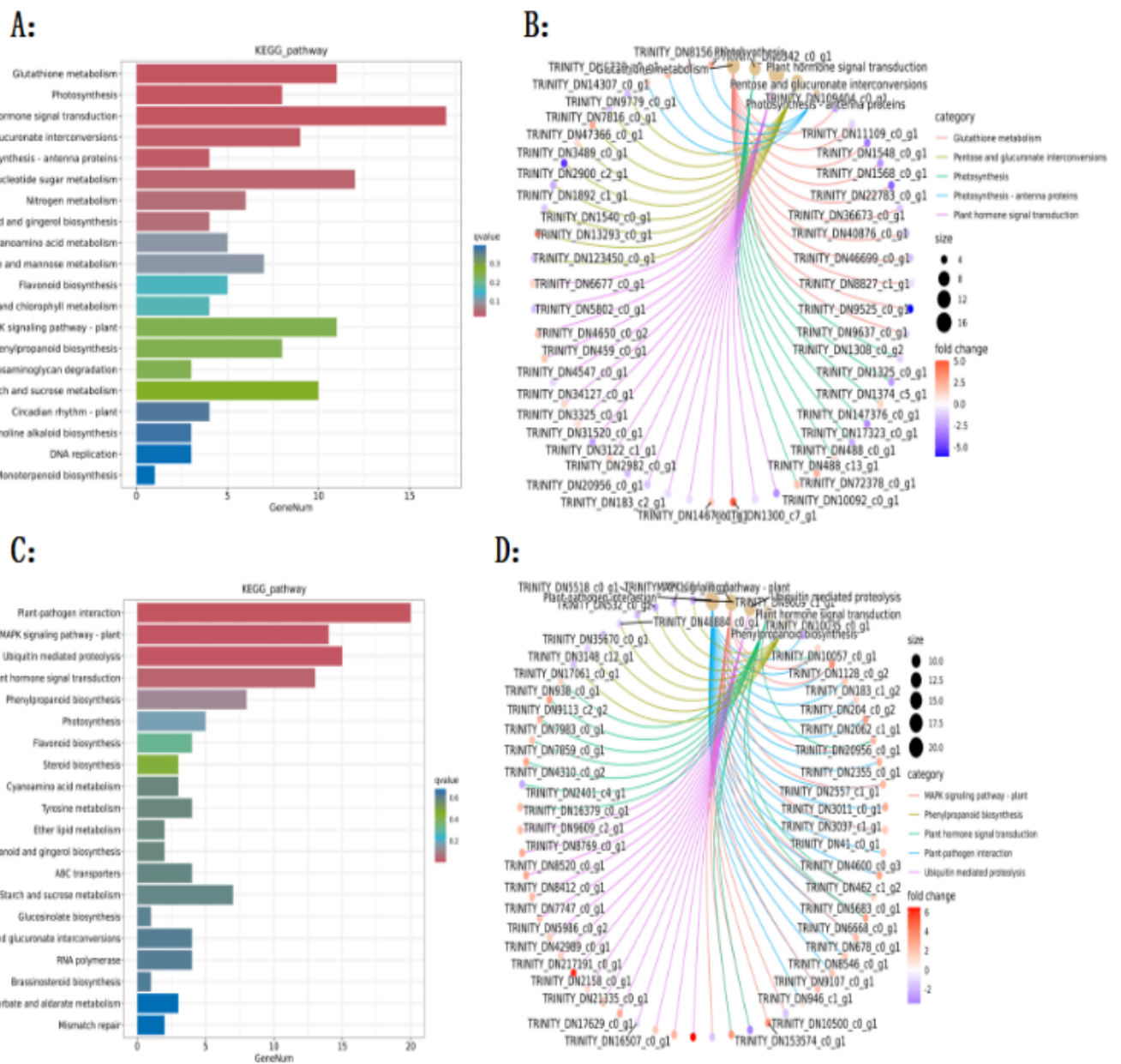


Figure 3

KEGG pathway enrichment analysis of differentially expressed genes under polyoxin M treatment

Note:(A) C6 vs P6 KEGG classification diagram of differentially expressed genes; (B) KEGG pathway enrichment plot of differentially expressed genes in C6 vs P6; (C) C9 vs P9 KEGG classification diagram of differentially expressed genes; (D) KEGG pathway enrichment plot of differentially expressed genes in C9 vs P9;

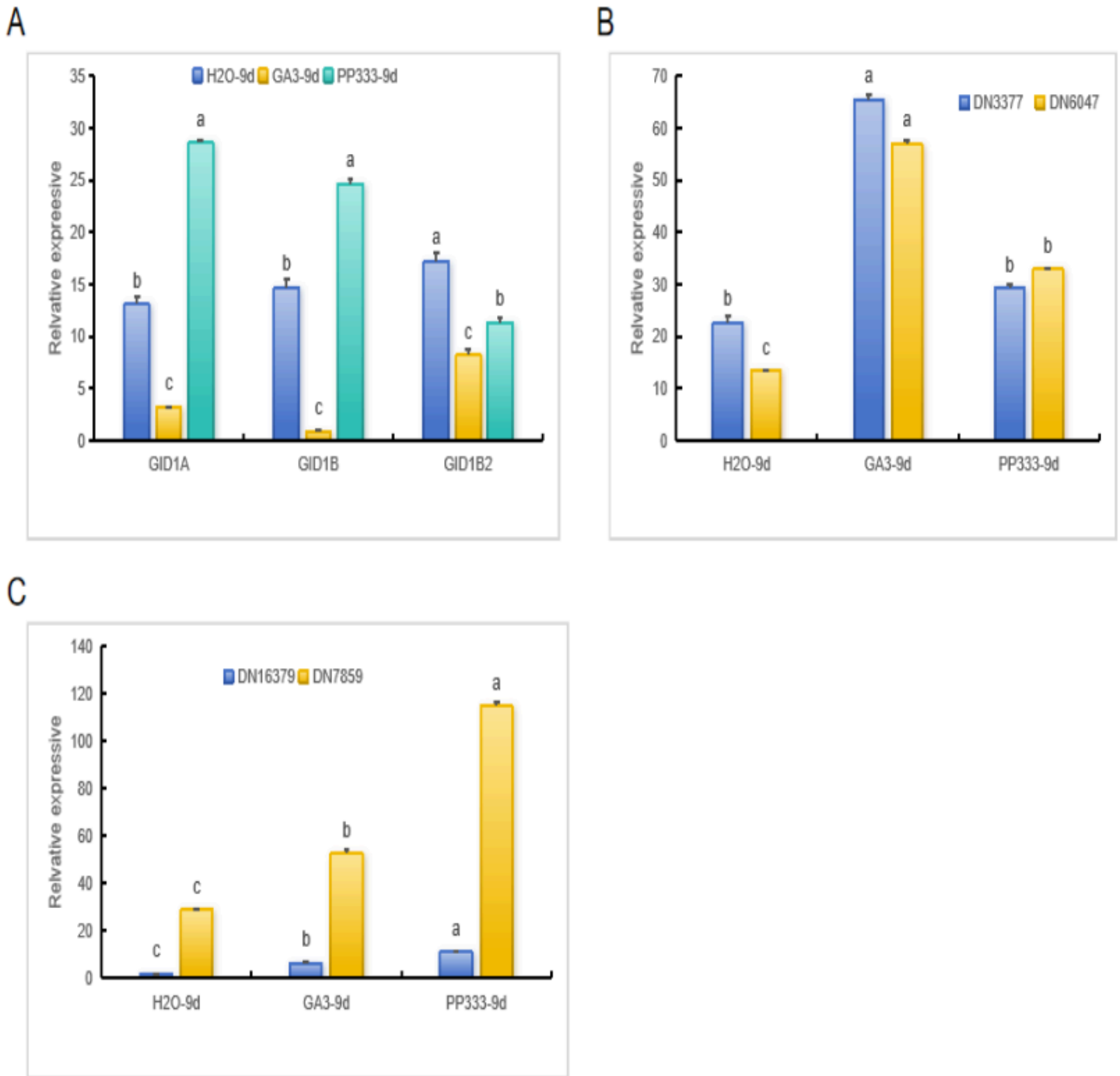


Figure 4

Expression analysis of key genes in the GA signaling pathway under GA₃ treatment or PP₃₃₃ treatment.

Note: (A) Relative expression levels of GA receptor genes (DoGID1A, DoGID1B, DoGID1B2) ; (B) Relative expression levels of DELLA protein genes (DN3377, DN6047) ; (C) Relative expression levels of CIGR1 family genes (DN16379, DN7859). Different lowercase letters indicate significant differences among treatments ($P < 0.05$).

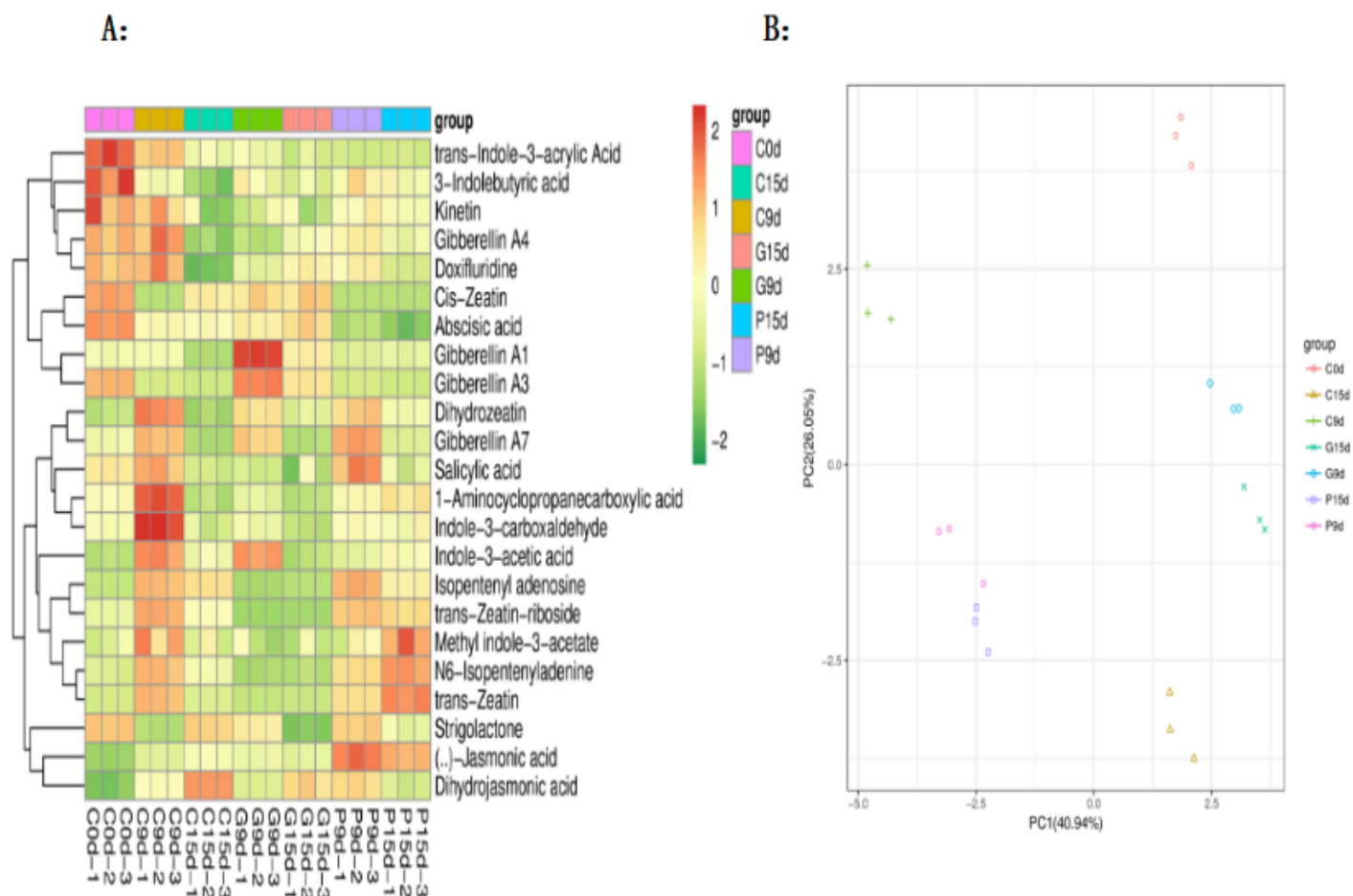


Figure 5

Analysis of variation of all sample metabolite data

Note:(A) Hierarchical clustering analysis of all sample metabolites; (B) PCA analysis of all samples

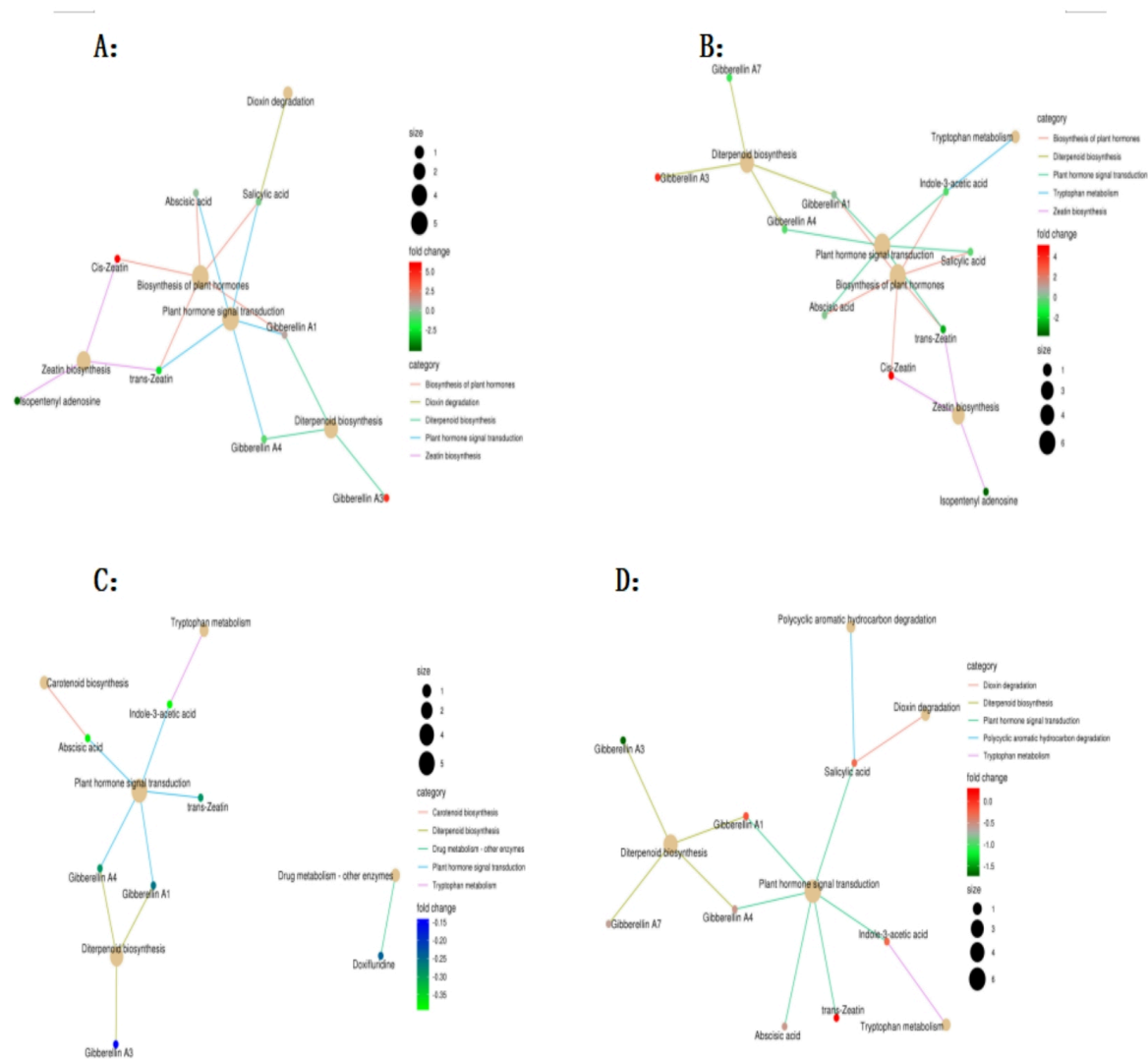


Figure 6

KEGG pathway enrichment network diagram of different metabolites after different treatments

Note:(A) C9-G9 KEGG enrichment network diagram; (B) C9-G15 KEGG enrichment network diagram; (C) C9-P9 KEGG enrichment network diagram; (D) C9-P15 KEGG enrichment network diagram;

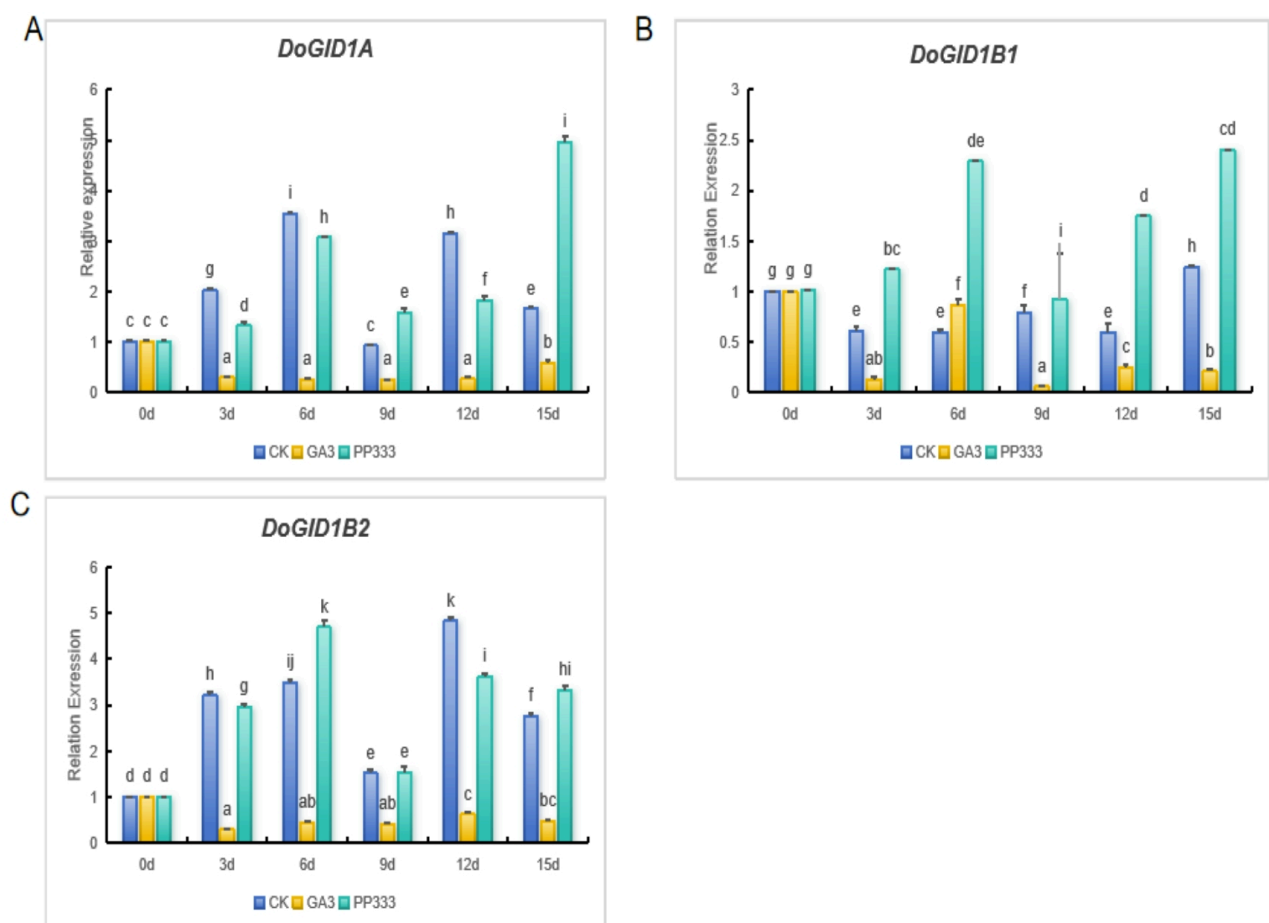


Figure 7

Relative expression of *DoGID1A*, *DoGID1B*, and *DoGID1B2* genes in bulbils treated with GA₃ or PP₃₃₃

Note: Values are means $\pm$ SD of three biological replicates. Different lowercase letters indicate significant differences among treatments at the same time point ($P < 0.05$).

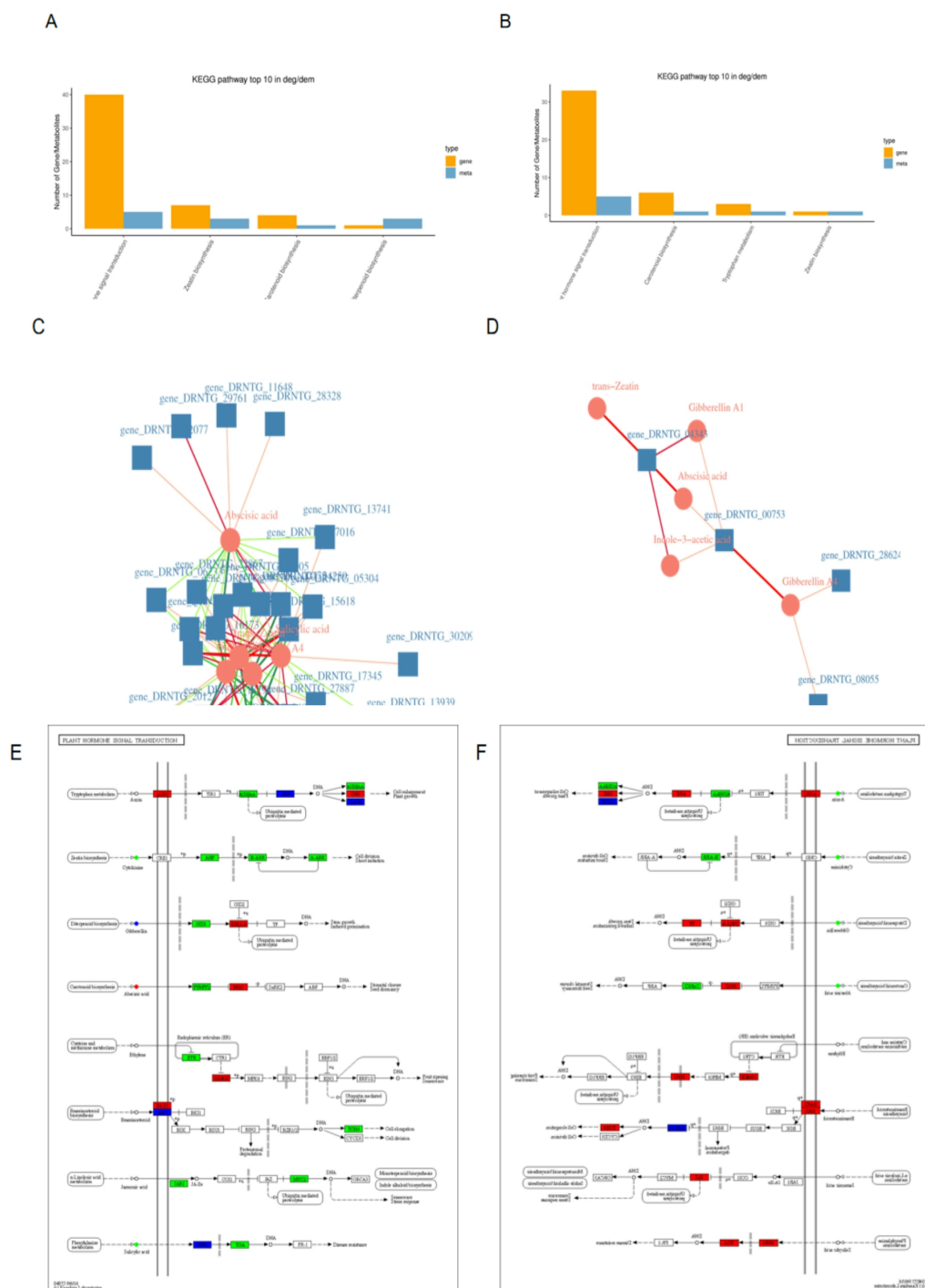


Figure 8

Integrated transcriptomic and metabolomic analysis of GA_3 and PP_{333} and hormone–gene correlation characteristics.

Noto:(A) KEGG pathway enrichment of DEGs and DEMs under GA_3 treatment.

(C) Gene–metabolite correlation network under GA₃ treatment (C9 vs G9).

(D) Gene–metabolite correlation network under PP₃₃₃ treatment (C9 vs P9). Nodes: circles = metabolites, squares = genes. Hub genes (gene_DRNTG_04343, gene_DRNTG_00753, gene_DRNTG_28624, gene_DRNTG_08055) are connected to trans-zeatin, GA₁, abscisic acid, IAA, and GA₄.

(E) Correlations between hormones and DEGs under GA₃ treatment (C9 vs G9).

(F) Correlations between hormones and DEGs under PP₃₃₃ treatment (C9 vs P9).

Supplementary Files

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- [SuppTable.docx](#)