BMC Plant Biology

RESEARCH

Open Access

Gibberellin-3 induced dormancy and suppression of flower bud formation in pitaya (*Hylocereus polyrhizus*)

Kamran Shah[1], Xiaoyue Zhu[1], Tiantian Zhang[2], Jiayi Chen[1], Jiaxuan Chen[1] and Yonghua Qin[1*]

Abstract

Background Flowering is a complex, finely regulated process involving multiple phytohormones and transcription factors. However, flowering regulation in pitaya (*Hylocereus polyrhizus*) remains largely unexamined. This study addresses this gap by investigating gibberellin-3 (GA3) effects on flower bud (FB) development in pitaya. Our findings reveal that GA3 application induces significant bud dormancy and suppresses FB formation, highlighting GA3's role in modulating flowering in this species.

Results GA3 application during peak flowering period significantly altered hormone levels, reducing auxin (AUX), cytokinin (CTK) active forms dihydrozeatin riboside (dhZR), zeatin riboside (ZR), N6-isopentenyladenosine (iPA), and brassinosteroid (BR), while increasing jasmonic acid (JA), GA3, and gibberellin-4 (GA4) levels, with abscisic acid (ABA) levels remaining unchanged compared to control. Conversely, FB formation was associated with increased levels of AUX, dhZR, ZR, iPA, ABA, and JA, and decreased GA3 and GA4 levels. Transcriptomic analysis revealed batches of differentially expressed genes (DEGs) associated with phytohormone signal transduction, aligning with observed hormone changes. Notably, except four *CONSTANS-like* (*CO*) (*HU06G02633*, *HU10G00019*, *HU04G00234*, and *HU02G01458*), all other *CO* genes were preferentially active in GA3-treated buds. GA3 treatment inhibited genes linked to the ABC model (*AP1*, *AP2*, *MADS-box*, *AGL*, *SPL*) and floral identity genes (*LFY*, *FT*), favoring dormancy and clean sweep of FB formation.

Conclusion These findings underscore the potential of GA3 as a powerful modulator of flowering and bud dormancy in pitaya. By elucidating the hormonal and genetic responses to GA3 treatment, this study contributes to our understanding of flowering regulation in pitaya and highlights the significant impact of GA3 on bud developmental pathways.

Keywords Dormancy, Flowering, Gibberellin, Phytohormone, Pitaya

*Correspondence:
Yonghua Qin
qinyh@scau.edu.cn
Full list of author information is available at the end of the article

Background

Phytohormones play both promotive and inhibitory roles in the flowering process, with hormones like AUX, CTK, and JA often promoting floral initiation, while ABA and ethylene (ETH) can inhibit it under certain conditions. However, GA3 demonstrates an unpredictable role; while it promotes flowering in annual plants species like *Arabidopsis thaliana* [1] and radish [2], it suppresses flowering in others, including *Citrus unshiu* [3], *Mangifera indica* [4] at 250 ppm, *Prunus avium* [5] at 200 mg/l, and *Malus domestica* [6] at 300 mg/l. Decreased GA levels or altered GA-CTK ratios may facilitate the transition to reproductive growth [7, 8]. Zhang et al. [9] revealed that GA acts as a negative regulator of flowering in *Jasminum sambac*. Guardiola et al. [10] found that *Citrus sinensis* and *Citrus unshiu* from November until bud sprouting exposure to GA, resulted in a significant inhibition of flowering. In *Vitis vinifera*, pre-flowering 7 mg/l GA3 application can decrease bunch compactness, improve fruit quality, but reduce flowering [11]. These findings collectively indicate the detrimental role of GA in the flowering process across different plant species.

Plant growth refers to the increase in size, mass, or volume over time due to continuous cell division, elongation, and differentiation. The modes of growth can differ significantly, with vegetative growth primarily involving leaves, stems, and roots, while reproductive growth involves the formation of flowers, fruits, and seeds, which are essential for fruit production and species continuation [12]. Abnormal growth in plants is when things don't grow like they should because of genetic mutations, environmental stressors, or pathogen infections. A precise metabolic and hormonal configuration triggers each growth type, leading to distinct developmental outcomes [8]. Among these stages, flowering is one of the most important and complex growth phases, crucial for fruit production and plant survival.

Pitaya, a tropical, climbing fruit from the genus *Hylocereus* (Cactaceae), thrives as a long-day plant loaded with betalains, antioxidants, vitamins, fiber, and minerals [13]. The plant faces challenges with prolonged dormant buds and limited FB formation. GA are recognized for their dual role in both promoting and suppressing flowering, but their specific impact on flowering in cacti, especially pitaya, remains unexplored. This study aimed to uncover the genetic changes by which GA3 induces dormancy and inhibits flowering, as well as to identify the specific genes involved in these processes, providing insights into the vegetative phase transition in pitaya.

Material and methods

Experimental site and climatic condition

This experiment was conducted in the germplasm and resource garden for pitaya at South China Agricultural University, located in Guangzhou, China (23.158′ N, 113.361′ E). Guangzhou features a subtropical climate characterized by mild winters and long, hot, humid summers that are often rainy and muggy. Detailed climatic data for each month is displayed in Supplementary Table S1.

Experimental design and sampling

A total of 80 equal sized, 60 six-year-old pitaya (*H. polyrhizus*) grown in trellis system at the pitaya germplasm and resource garden were selected. Initial control bud samples were collected on July 6th before treatment of GA3. Then, trellis of plants was divided in two groups. Gibberellin-3 (GA3; $C_{19}H_{22}O_6$, CAS# 77–06-5, molecular weight 346.37 g/mol, 90% purity) was purchased from Yuanye Biotech. China. A 100 mg quantity was weighed using an Ohaus digital scale (OHAUS Scale Corporation, Florham Park, NJ, United States) and dissolved in 97% ethanol, followed by the addition of distilled water to achieve the final volume. One group was treated with 100 mg/l GA3 on July 15th, representing the GA3 group, while the other group was treated with distilled water, representing FB group. Sample was collected from both GA3-treated and FB on July 22nd, 2023. The samples were promptly transported on ice to the laboratory. Upon arrival, any extra green and dead brown tissues were removed from around the buds using a sterile razor. Next, the buds were finely ground into a powder using a rapid grinder (Model: Tissue-lyser-24, Shanghai Jingxin Industrial Development Co., Ltd., China) to prepare them for phytohormone analysis. The remaining samples were then stored at −80 °C for future RNA sequencing.

Plant hormone quantification

Three replications of initial control, GA3, and FB buds ground samples were precisely weighted 100 mg in a 2 ml centrifuge tube. To this, 1 ml of ethyl acetate was added, the mixture was subjected to shaking at 2000 rpm for 10 min. Subsequently, the samples underwent centrifugation at 12,000 rpm for 10 min, and the supernatant was carefully aspirated into a fresh 2 ml centrifuge tube. Next, a nitrogen blower was used to evaporate organic solvent. Post-evaporation, 200 µl of 50% methanol was introduced to the dried samples and the mixture was again centrifuged at 13,000 rpm for 15 min. The resulting liquid was filtered through a 0.22 µm organic filter membrane, and 100 µl of the filtered supernatant was carefully transferred into a sample vial in preparation for chromatographic analysis. For quantification, authentic reference

standards of AUX (Yuanye Biotech, CAS# 87–51-4), dhZR (Yuanye Biotech, CAS# 22,663–55-4), ZR (Yuanye Biotech, CAS# 6025–53-2), iPA (Yuanye Biotech, CAS# 7724–76-7), BR (Yuanye Biotech, CAS# 457,603–63-3), ABA (Yuanye Biotech, CAS# 21293–29-8), JA (Yuanye Biotech, CAS# 6894–38-8), GA3 (Yuanye Biotech, CAS# 77–06-5), and GA4 (Yuanye Biotech, CAS# 468–44-0), were employed. These hormones were analyzed using an Agilent 1290 Infinity II-6470 system (Agilent Technology, USA), equipped with a ZORBAX Eclipse Plus C18 column (1.8 µm, 2.1×50 mm; Agilent Technologies). The mobile phase was composed of (A) 0.1% (v/v) formic acid and (B) methanol, applying a gradient program that included an isocratic phase of 75% A and 25% B for 0–0.8 min, followed by a linear gradient from 75 to 25% A and 25% to 75% B from 0.8–1.5 min, and concluding with a linear gradient from 25 to 75% A and 75% to 25% B from 1.5–2.5 min, with a flow rate of 0.3 ml/min. A sample volume of 5 µl was injected for analysis. GA3 and GA4 were detected using an electrospray ionization source in the negative ionization mode, while AUX was detected in the positive mode. The dynamic multiple reaction monitoring was optimized for each targeted metabolite based on the corresponding authentic standards. The instrumental settings were a capillary voltage of 3500 V, nebulizer gas pressure of 45 psi, sheath gas flow rate of 11 L/min, and a column oven temperature of 300 °C. The quantification of the hormones was based on the analysis of the extracted ion chromatograms and comparison of the peak positions with those of the standard solutions.

RNA quantification and qualification

The pitaya buds were subjected to extraction of the total RNA with three biological replications of each GA3 resembling GA and FB using the RNAprep Pure Plant Kit (Tiangen, Beijing, China) as per the manual provided by the manufacturer. The purity, concentration, and integrity of the RNA sample were determined by NanoDrop, Qubit 2.0, and the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA).

Library construction and RNA deep sequencing

A total measure of 1 µg RNA per example was utilized as input material for the RNA sample preparations. Sequencing libraries were generated utilizing NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations, and index codes were included to attribute sequences to each example. mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext. First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase, and second strand cDNA synthesis was then carried out using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends through exonuclease/polymerase activities. After adenylation of the 3′ ends of DNA fragments, NEBNext Adaptor with a hairpin loop structure was ligated to prepare for hybridization. To select cDNA fragments of preferentially 240 bp in length, the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 µl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by 5 min at 95 °C before PCR. PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers, and Index (X) Primer. Finally, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

Transcriptome analysis using reference genome-based reads mapping

The raw data was initially processed through custom Perl scripts to extract clean reads by removing adapters, poly-N, and low-quality sequences. These clean reads were then aligned to the pitaya genome (http://pitayagenomic.com/) sequence using Hisat2 tools soft. Gene expression levels were determined based on FPKM values (fragments per kilobase of exon per million fragments mapped) using the Cufflinks software [14].

Identification of differential gene expression

The differential expression analysis of GA and FB was conducted using the DESeq2 software. The P values obtained were adjusted using Benjamini and Hochberg's method to control the false discovery rate. Genes with an adjusted P-value less than 0.01 were identified as DEG. Additionally, the KOBAS software [15] was employed to assess the statistical enrichment of these DEGs in KEGG pathways.

Sequence annotation

Genes were compared against various protein databases using BLASTX, including the National Center for Biotechnology Information (NCBI) GenBank database, NCBI non-redundant protein sequences (Nr), Pfam (Protein family), Swiss-Prot (a manually annotated and reviewed protein sequence database), and KO (KEGG Ortholog database) with a cut-off E-value of 10^{-5}. Genes were selected based on the best BLAST hit (highest score) and were associated with their protein functional annotation.

Quantitative real-time PCR validation (qRT-PCR)

For qRT-qPCR validation, twelve genes were selected from the DEGs list, four from *CO* and 8 from key flowering genes involved in photoperiodism, ABC model, and floral identity, all encoding enzymes or components critical in flowering signaling. Primers for the qRT-PCR were designed using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (accessed on June 8, 2024), (Supplementary Table S2). Quantitative real-time PCR analyses were conducted using the CFX384 Real-Time System (C1000 Touch Thermal Cycler series, Bio-Rad, Irvine, CA, USA) and the RealUniversal Color PreMix (SYBR Green) from TIANGEN (Beijing, China). The pitaya *ACTIN* gene (*HU07G00802*) served as the internal standard. Expression levels of the selected genes were quantified in three replications using the $2^{-\Delta\Delta C}$T method [16].

Statistical analysis

Author's performed statistical analysis of raw data, and applied an ordinary one-way ANOVA with Šídák's multiple comparisons test, comparing each group cell mean with the other groups cell mean using Graphad Prism 10 for macOS (Version 10.1.1) (Graphpad Software, San Diego, California, United States of America, www.graphpad.com). Results were presented as mean ± standard deviations (n = number of repeats), with significance values as follows: *$p < 0.05$; * *$p < 0.01$; * **$p < 0.001$; * ** *$p < 0.0001$; and non-significant (ns) ($p > 0.05$).

Results

Flowering phenotype between GA3-treated and untreated during FB induction

The phenotypes of GA3-treated and control groups resembling FB were observed during the FB induction period and at full bloom. Figure 1 shows that control buds were developing into FB, but GA3 exposure during this critical stage disrupted flowering signals and shifted metabolism to vegetative and abnormal growth. Control plants underwent normal flowering, while GA3-treated buds resembled abnormal growth (Fig. 1A). GA3 triggered vegetative growth, reverting floral meristems back to vegetative state, resembling floral reversion (Fig. 1B). Figure 1C and D show zero flowering and year-round dormancy induced by GA3. Figure 1E and F display normal FB formation, 1G indicates normal FB growth, and 1H displays full bloom in FB group. These findings indicate that GA3 application inhibited FB formation and induced dormancy in pitaya. The observed hazardous effects of GA3 suggested significant alterations in hormonal balance and genetic mutations suppressing FB formation.

Alteration in hormone levels between initial control, GA3-treated and FB group

The analysis revealed that in the GA3-treated group, the levels of AUX, ZR, dhZR, iPA, and BR were downregulated, while the levels of JA, GA3, and GA4 were upregulated compared to the initial control. The ABA content was observed to be significantly similar between the initial control and GA3-treated groups (Fig. 2). In the FB group, the levels of AUX, dhZR, iPA, ABA, and JA were upregulated, while GA3 and GA4 levels were downregulated compared to the initial control (Fig. 2). When comparing the GA3-treated group to the FB group, the levels of AUX, ZR, dhZR, iPA, BR, and ABA were upregulated in the FB group, while the levels of GA3 and GA4 were downregulated. The JA levels remained unchanged between the two groups (Fig. 2).

Fig. 1 Phenotype of application of gibberellin-3 induced dormancy and control (FB) group. **A**-**D** represent GA-treated plants phenotype. **E**–**H** denote control (FB) group

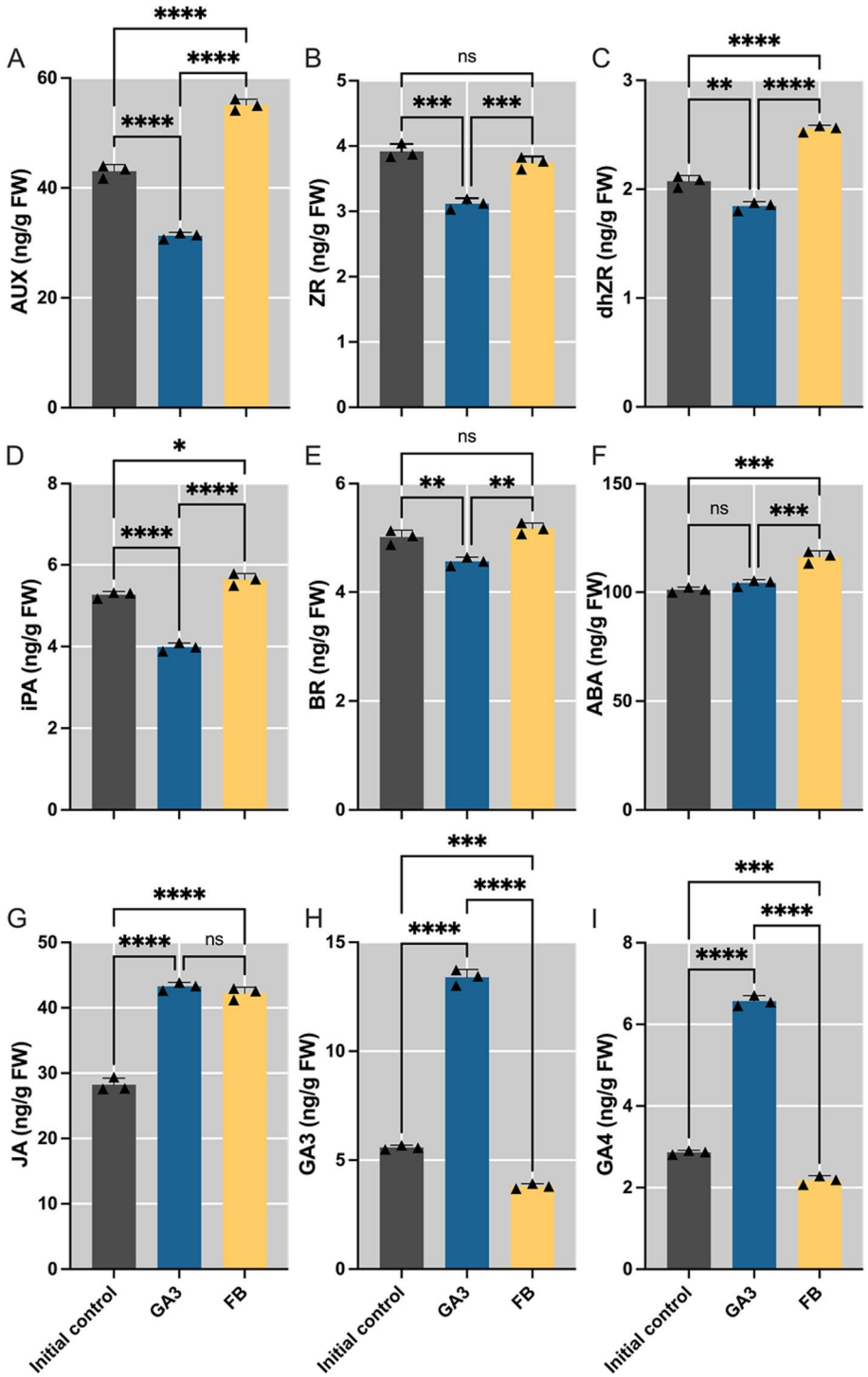

Fig. 2 Dynamic variations in hormone levels in *Hylocereus polyrhizus* buds in initial control, GA3-treated and FB group. **A** Auxin, **B** Zeatin riboside, **C** Dihydrozeatin riboside, **D** N6-isopentenyladenosine, **E** Brassinosteroid, **F** Abscisic acid, **G** Jasmonic acid, **H** Gibberellin-3, and **I** Gibberellin-4. Data are presented as mean ± SD for three replicates ($n = 3$). Small triangles represent the distribution of data for each biological replicate. Significance levels are denoted as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; nonsignificant differences are indicated by 'ns' ($p > 0.05$)

Analysis of DEGs

To investigate the molecular mechanisms of dormancy and FB suppression induced by GA3, and to elucidate the associated gene expression patterns, buds from GA3-treated and FB groups were subjected to transcriptome sequencing, each in three replicates for data reliability. Clean read counts per library ranged from 32.35 and 41.61 million. Q20 and Q30 percentages were above 97% and 94%, respectively, with GC content higher than 48% in all libraries (Supplementary Table S3). Uniquely mapped reads to the pitaya genome varied from 19.51% to 45.97%, with total reads, mapped reads, and multiple mapped read ratios at 64.7–83.22 million, 20.81%–48.99%, and 1.30%–3.02%, respectively. Positive and negative read maps were 11.32%–26.49% and 11.32%–26.48% (Supplementary Table S4). The Venn diagram (Fig. 3A) illustrates GA3's impact on bud dormancy and flowering inhibition. The left circle shows 1,397 GA-specific elements involved in dormancy or non-flowering processes, while the right circle shows 1,305 FB-specific elements crucial for flowering. The overlap of 18,559 elements indicates significant interaction between GA and FB-related factors. The principal component analysis (PCA) diagram (Fig. 3B) highlights variance in samples treated with FB and GA. PC1 captures 64.33% of the variance, distinguishing GA3-treated samples from FB samples, while PC2 captures 11.29%. FB samples (red squares) cluster tightly, indicating high similarity, whereas GA samples (blue circles) spread along PC1, showing significant variance and differentiation from FB samples, reflecting GA-induced changes in gene expression and behavior. The DEG counts figure (Fig. 3C) compares gene expression between FB and GA conditions. The blue bar shows 6877 total DEGs, with 3215 genes downregulated (green bar) and 3662 genes upregulated (orange bar) in GA-treated buds. Downregulated genes are likely involved in promoting flowering, while upregulated genes are associated with bud dormancy and inhibition of flowering, indicating GA's profound impact on molecular mechanisms. KEGG pathway enrichment analysis (Fig. 3D) reveals that upregulated genes in GA vs. FB are primarily involved in phytohormone signal transduction pathways. These findings suggest that bud dormancy and FB induction in pitaya are driven by hormonal modulation and associated gene activity.

Regulation of DEGs related to phytohormones

The interaction between hormone components and flowering has been demonstrated in earlier research. For example, salicylic acid (SA) application induces flowering by increasing the expression of AUX-related genes and inhibiting the GA-related genes [8]. CTK also plays a positive role in flowering [17]. To determine phytohormone-related gene expression causing dormancy in pitaya under GA3 treatment, GA-treated and FB groups were further screened for responses to pitaya hormone-related DEGs to map heatmaps (Fig. 4). Results showed that the expression patterns of ABA, AUX, BR, CTK, ETH, GA, JA, and SA exhibited varying changes following GA3 treatment, indicating that GA3 induces major metabolic shifts in phytohormones synthesis and regulation, leading to strong dormancy.

Hormone's level correlated with the gene expression levels of hormone biosynthesis transcripts in pitaya

Figure 5 displays a comprehensive analysis of gene expression patterns related to various hormone signaling pathways in pitaya. In AUX signaling pathway, *HU03G00380, HU01G00047, HU02G01009, NewGene_6105, HU08G01003, HU04G00334, HU04G00084, HU02G01245, HU10G01877, HU02G01250, HU06G00395, HU01G01860, HU01G02696, HU04G01929, HU03G00626, HU01G00718, HU01G00565, HU01G00825, HU03G00685, HU05G02286, HU04G00084, HU02G01245, HU10G01877,* and *HU02G01250* were found differentially expressed among GA-treated and FB group which are feedback regulation genes and modulate AUX signaling (Fig. 5A; Supplementary Table S5). In CTK signaling pathway, *HU11G00040, HU03G01134, HU08G01627, HU03G01727, HU10G00370, HU02G00513, HU07G00841* were differentially expressed among GA-treated and FB group reflecting CTK signal transduction (Fig. 5B; Supplementary Table S6). In GA signaling pathway, *HU06G00986, HU06G00988, HU05G01983, HU06G00358, HU02G03005, HU04G00148, HU05G02278, HU02G01108, HU01G02373, HU04G01551, HU01G02692, HU02G00788, HU02G00791,* and *HU03G02152* were differentially expressed among GA-treated and FB group reflecting GA signal transduction (Fig. 5C; Supplementary Table S7). In ABA signaling pathway, *HU11G00494, HU06G02610, HU06G00546, HU05G00054, HU08G01036* were differentially expressed among GA-treated and FB group reflecting ABA signal transduction (Fig. 5D; Supplementary Table S8). In BR signaling pathway, *HU08G02255, HU10G01229, HU07G00803, HU07G02367, HU11G01227, HU03G02803, HU09G01162, HU05G02102, HU11G01253, HU04G01421, HU04G01106, HU02G02234, HU10G00131, HU02G00304, HU06G02429, HU02G02625, HU05G00524, HU07G01084, HU02G01615, HU07G02210, NewGene_369, HU03G01206, HU01G00165, HU03G00243, HU06G00405* were differentially expressed among GA-treated and FB group reflecting BR signal transduction (Fig. 5E; Supplementary Table S9). In JA signaling pathway *HU10G01498, HU03G01802, HU11G01791,*

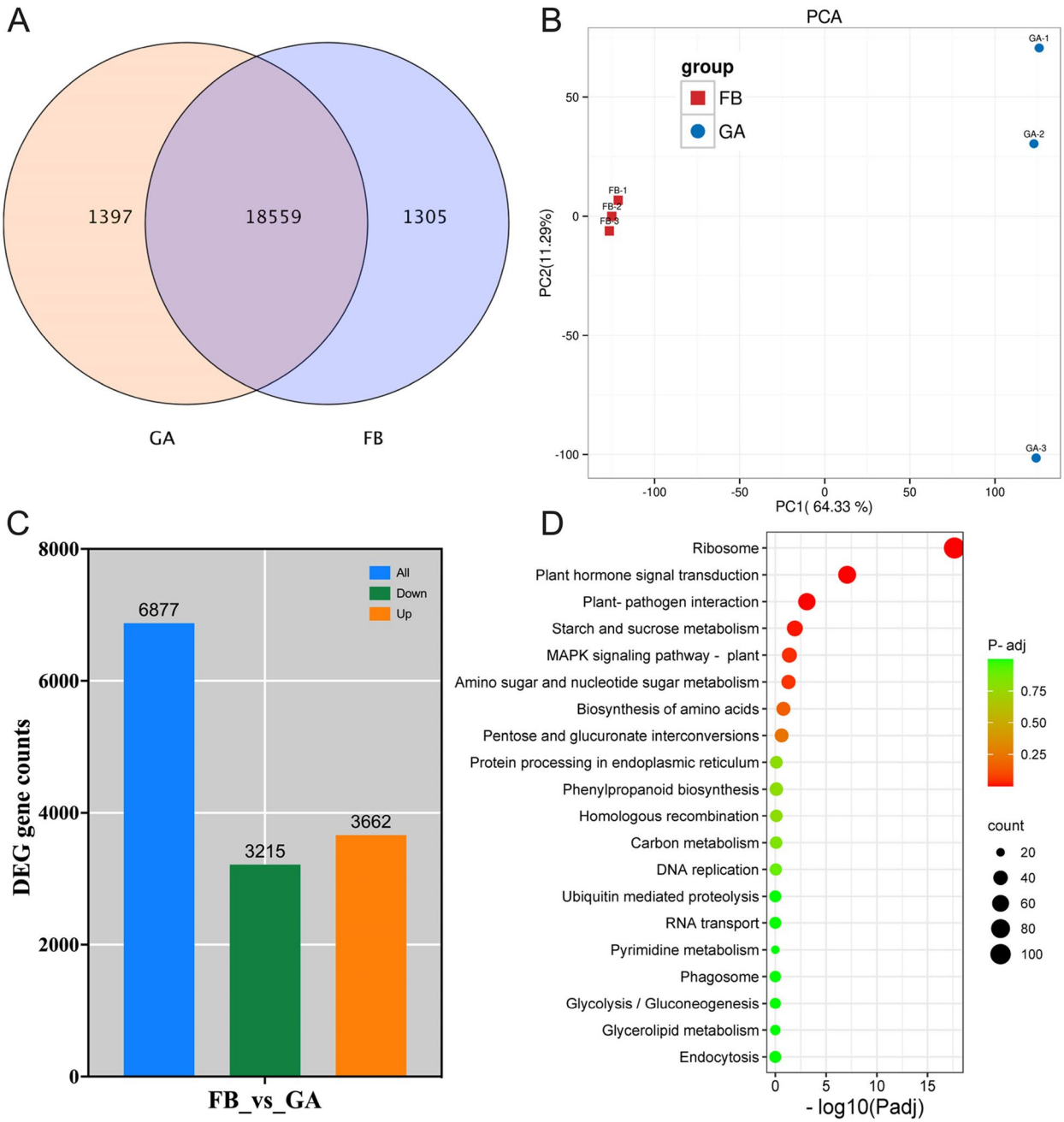

Fig. 3 Multifaceted analyses of gene expression in *Hylocereus polyrhizus* buds between GA3-treated and FB group. **A** Venn diagram showing intersecting and unique DEGs between GA3-treated and FB group, **B** PCA plot highlighting gene expression variability between GA3-treated and FB group, **C** DEGs distribution with the X-axis indicating comparison groups and the Y-axis showing gene counts: blue bars for total regulated genes, green bars for downregulated genes, and orange bars for upregulated genes, **D** KEGG pathway enrichment bubble plot of DEGs between GA3-treated and FB group. Biological pathways are on the Y-axis; -log10(Padj) values on the X-axis. Dot color reflects enrichment significance (P-adj), and size indicates the number of DEGs

HU06G01887, HU06G01888, HU03G00470, HU07G00114, and *HU04G00514* were differentially expressed among GA-treated and FB group reflecting JA signal transduction (Fig. 5F; Supplementary Table S10).

Regulation of CO related genes

CO genes are central to photoperiodism, typically regulated with light to regulate FB formation. GA3 treatment during peak long days induced year-round dormancy,

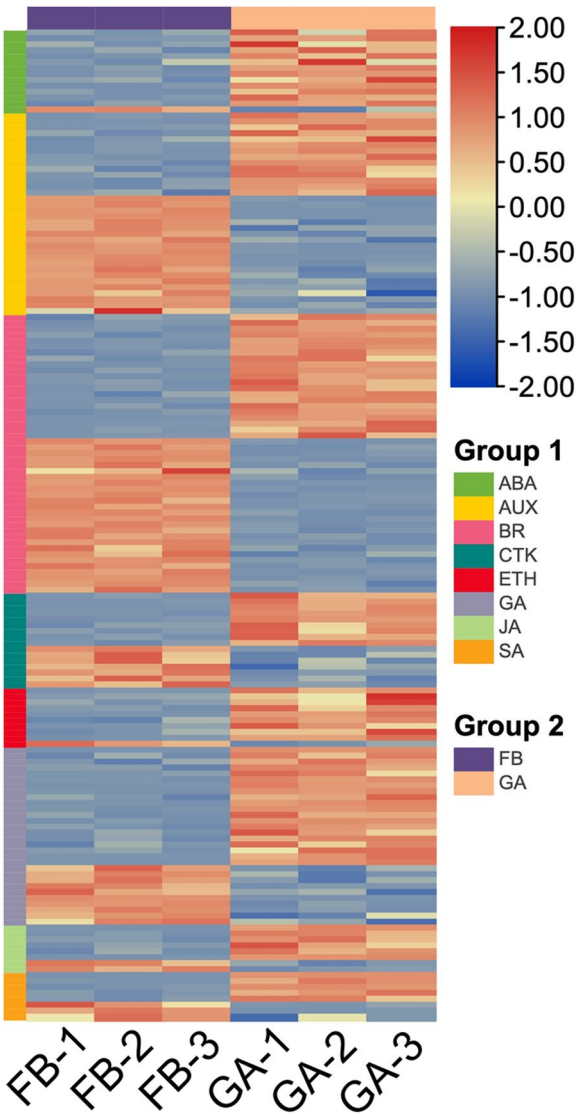

Fig. 4 Changes in the expression of phytohormone-related DEGs at GA3-treated and FB group in pitaya buds. Group 1 represent the temporal changes in gene expression related to phytohormone metabolism and signaling (ABA, AUX, BR, CTK, ETH, GA, JA, SA). Group 2 represents GA-treated and FB group

exploring gibberellin's role under these conditions. Four *CO* genes, *HU06G02633*, *HU10G00019*, *HU04G00234*, and *HU02G01458*, were upregulated in the FB group. In contrast, *HU03G02031, NewGene_4046, HU07G02338, HU02G00626, HU10G00653, HU05G00776, HU08G00074, HU01G01161, HU09G00845, HU01G01234, HU07G01374, HU02G01005, HU06G01458, HU02G02309, HU02G00012, HU06G01339, HU09G00352, HU10G00611, HU09G01186, HU09G00431, HU04G01185, HU06G00744, HU05G01805, HU02G03425, HU03G02630, HU09G00144, HU02G03306, HU11G00602* were upregulated in response to the GA3-treatment (Fig. 6; Supplementary Table S11).

Clean swept of structural floral genes by GA3

In this study, several key structural genes were strongly inhibited by GA3 treatment and preferentially upregulated in the FB group, primarily involving ABC model genes. These genes include *APETALA1* (*AP1)*: *HU04G01466*, *HU02G00417*; *APETALA2* (*AP2)*: *HU09G00807*, *HU05G01919*; *MADS-box*: *HU05G01774* (*MADS2*), *HU05G00903* (*MADS9*); *AGAMOUS*: *HU08G01892* (*AGL2*), *HU01G02169* (*AGL6*), *HU02G00039* (*AGL9*); *SPL*: *HU08G00500* (*SPL4*), *HU10G00465* (*SPL6*), *HU07G00586* (*SPL8*), *HU09G01807* (*SPL9*), *HU02G00038* (*SPL13*); *LEAFY*: *HU04G01377* (*LFY*); *FLOWERING LOCUS T* (*FT*): *HU03G01546* (Fig. 7; Supplementary Table S12).

qRT-PCR validation

Given that GA exposure adversely affects FB formation by altering phytohormone synthesis and photoperiodism, leading to dormancy in pitaya, we verified selected gene expression using qRT-PCR. We selected one gene each from AUX, CTK, GA, and ABA pathways, four from photoperiodism, six from ABC model of flower development and two from floral identity genes for validation. The results showed that the expression patterns were consistent with transcriptome data trends (Fig. 8).

Discussion

Dynamic changes in flowering rate

The study investigated the impact of GA3 treatment on drastic dormancy in pitaya compared with an untreated FB group. GA3 application during the critical differentiation stage inhibited flowering, redirecting growth towards vegetative and abnormal. Initially, GA3-treated buds appeared as FBs but reverted to vegetative growth, showing deformity, abnormality, or bud drop, likely due to hormonal imbalances and nutritional deficiencies [18]. In contrast, the control group showed normal FB formation and blooming. These findings suggest that GA3 induce dormancy and inhibit FB formation in pitaya (Fig. 1).

Hormone level alterations

Hormonal analysis revealed significant differences between initial control, GA3-treated, and FB groups. GA3 modifies hormone balances, make possibilities to interacting with key TFs (*CO*, *PIF*) and flowering genes (*AP1, 2; MADS2, 9; AGL2, 6, 9; SPL4, 6, 8, 9, 13; LFY, FT*) to favor dormancy over flowering (Fig. 2).

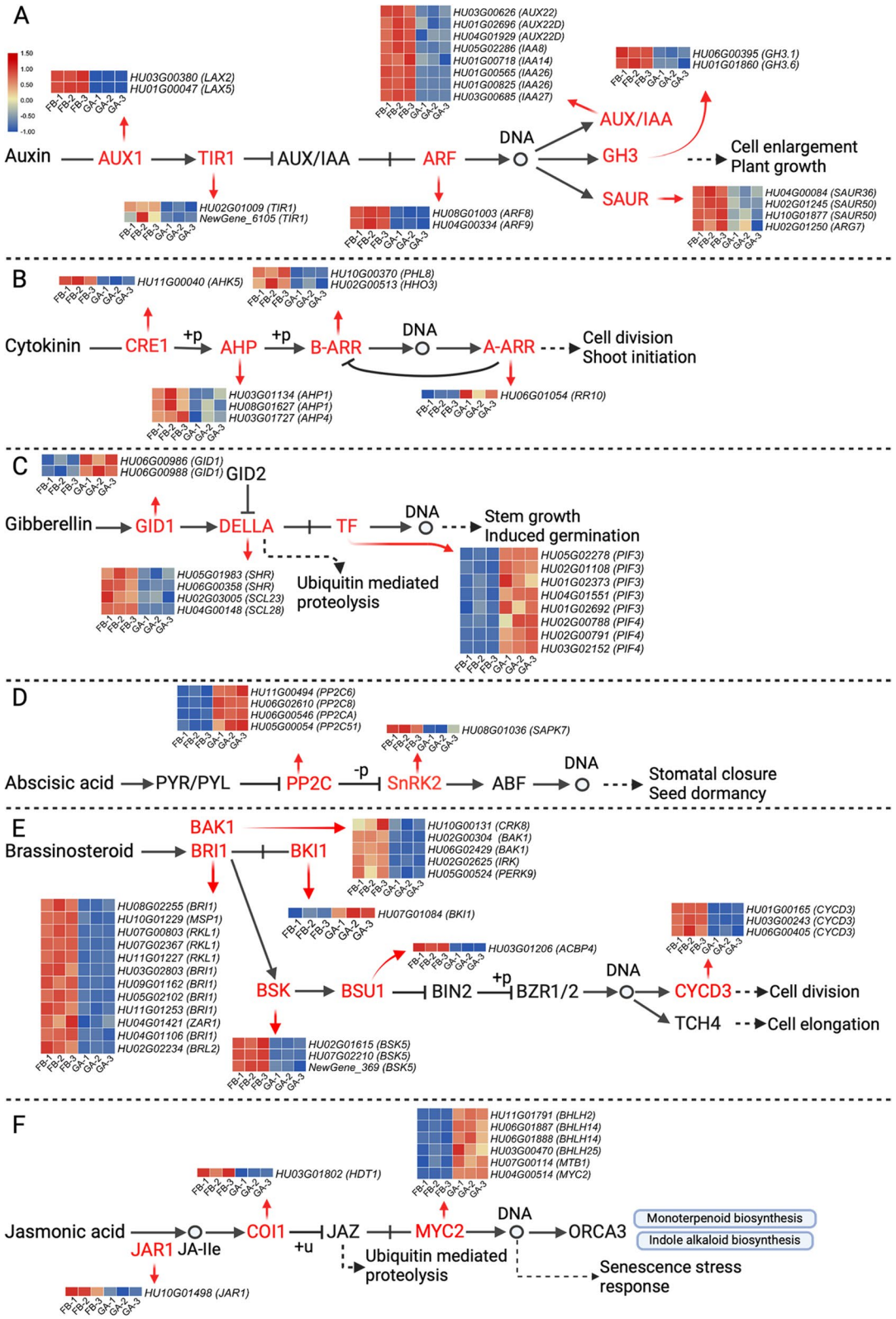

Fig. 5 Changes in expression of genes related to the pitaya hormone pathway at GA3-treated and FB group. Red fonts indicate upregulated gene, and the heatmap shows the expression of relevant genes in the phytohormone signaling pathway that regulates compound synthesis

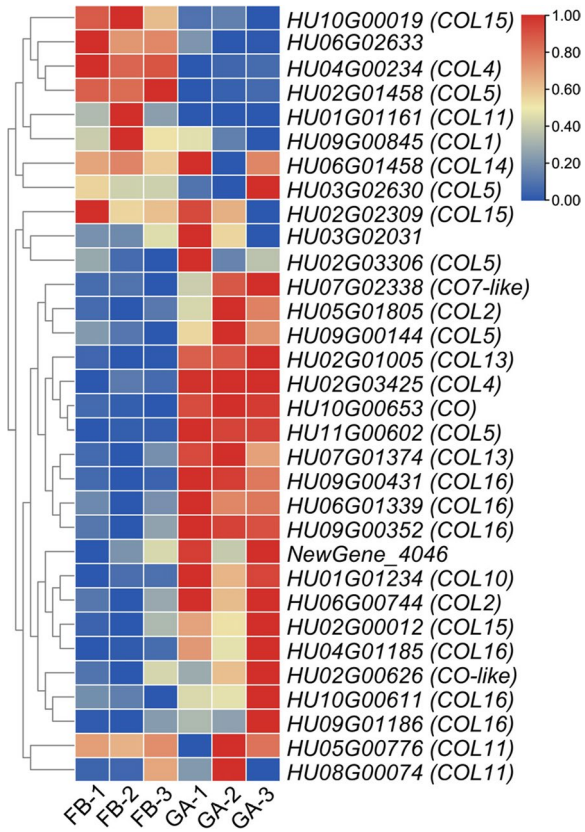

Fig. 6 Changes in *CO* gene expression in GA3-treated and FB group. Red indicates upregulated and blue indicates down regulated *CO* genes in pitaya buds

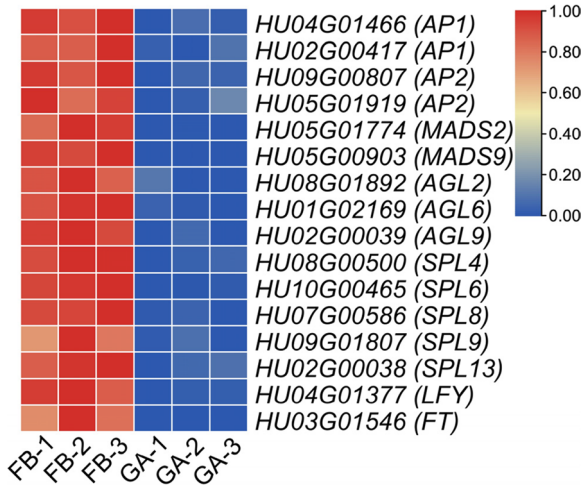

Fig. 7 Changes in key floral genes expression in GA3-treated pitaya buds. Red indicates upregulated and blue indicates downregulated genes

GA-treated pitaya plants showed increased GA and JA levels and decreased AUX, CTK, and BR levels. In contrast, the FB group had increased AUX, CTK, ABA, JA, and decreased GA levels, indicate that GA exposure disrupts hormone balance. Previous studies show that differences in CTK, ABA and GA levels distinguish profusely and weakly flowering varieties of *Malus domestica* [19]. During flowering induction period, increased AUX and ABA levels and decreased GA levels suggest that hormonal control of flowering [17]. Application of SA, can positively affect AUX while negatively impacting GA biosynthesis and signaling, thereby regulating flowering [8].

Alterations in hormone signaling

Phytohormones are crucial internal factors governing plant development throughout their life cycle. In model plants, phytohormone pathways interact in complex networks with additive, antagonistic, and synergistic effects [20]. In current study, significant shifts in phytohormone signal transduction pathway were observed (Fig. 4), suggesting that it greatly governs the flowering process of pitaya. AUX is synthesized from the tryptophan through a series of biochemical reactions. *AUX1* facilitates AUX uptake into the cell, while the auxin receptor complex *TIR1* binds to auxin, promoting *AUX/IAA* degradation via the ubiquitin–proteasome pathway. *AUX/IAA* proteins modulate auxin signaling through feedback regulation. *ARF* proteins are TFs regulating the expression of auxin-responsive genes like *AUX/IAA*, *GH3*, and *SAUR* , which promote cell enlargement and plant growth [21, 22]. In current results, AUX levels were downregulated in GA-treated while upregulated in FB group (Fig. 2A). This is supported by the downregulation of *AUX1* (*HU03G00380*, *HU01G00047*), *TIR1* (*HU02G01009*, *NewGene_6105*), *ARF* (*HU08G01003*, *HU04G00334*), *AUX/IAA* (*HU01G02696*, *HU04G01929*, *HU03G00626*, *HU01G00718*, *HU01G00565*, *HU01G00825*, *HU03G00685*, *HU05G02286*), *GH3* (*HU06G00395*, *HU01G01860*), and *SAUR* (*HU04G00084*, *HU02G01245*, *HU10G01877*, *HU02G01250*) genes by GA-treatment. This indicates that increased AUX levels and signaling are required for FB formation in pitaya, which were pronounced in the FB group but inhibited by GA-treatment (Fig. 5A). CTK, a class of plant hormones, promotes cell division and shoot formation. CTK binds to its receptor *CRE1* on the cell membrane, causing *CRE1* to autophosphorylate (+p) and transfers the phosphate group to *AHP*. *AHP* then transfers the phosphate group to *B-ARR* , which binds to DNA after phosphorylated, and initiates the expression of cytokinin-responsive genes. This signaling pathway ultimately promotes cell division and shoot initiation, crucial for FB induction. *A-ARRs*

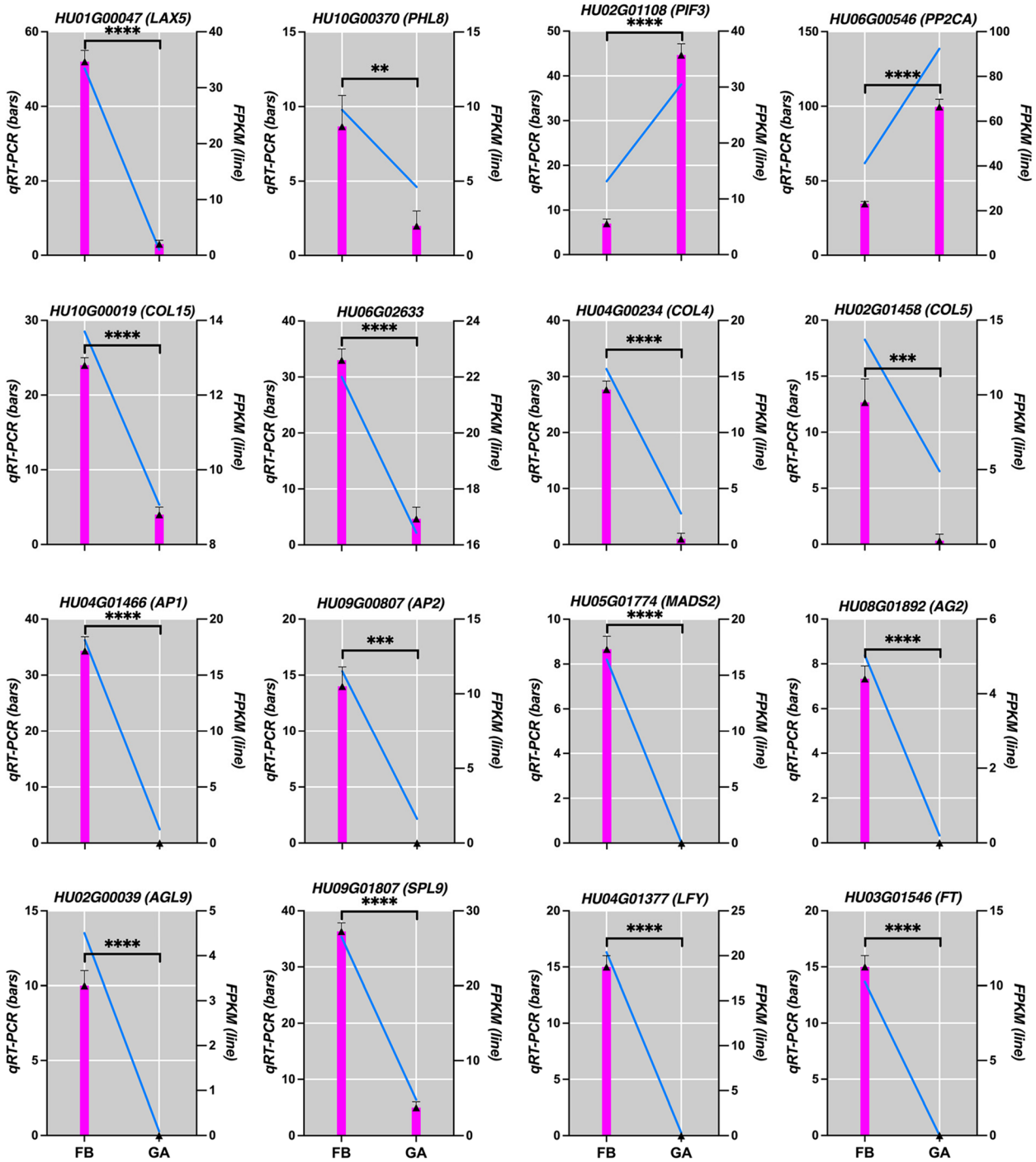

Fig. 8 Changes in phytohormone, photoperiodism, ABC model of flower development, floral identity genes in GA3-treated and FB pitaya buds. Bars indicate gene expression values detected by qRT-PCR, while lines represent transcriptome sequencing values. Data are presented as mean ± SD for three replicates ($n = 3$). Significant differences were determined by t-tests using Graphad Prism 10 for macOS (Version 10.1.1) and denoted as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; nonsignificant differences are indicated by 'ns' ($p > 0.05$)

provide negative feedback by inhibiting *B-ARR*s, ensuring tight regulation and preventing excessive responses [23]. In current results, CTK levels were downregulated in GA-treated while upregulated in FB group (Fig. 2B-D). This is supported by the downregulation of *CRE1* (*HU11G00040*), *AHP* (*HU03G01134*, *HU08G01627*,

HU03G01727), *B-ARR* (*HU10G00370*, *HU02G00513*), while downregulation of *A-ARR* (*HU06G01054*) genes by GA-treatment. This indicates that increased CTK levels and signaling are required for FB formation in pitaya, which were pronounced in the FB group but inhibited by GA-treatment (Fig. 5B). GA act as signaling molecules that bind to their receptor, *GID1*, and interact with *DELLA*, the negative regulators of GA signaling. The degradation of *DELLA* releases TFs, which then activate the expression of gibberellin-responsive genes, promoting stem growth and germination, crucial for vegetative growth [24, 25]. In current results, GA3 and GA4 levels were upregulated in GA-treated and downregulated in the FB group (Fig. 2H, I). This is supported by the upregulation of *GID1* (*HU06G00986, HU06G00988*), *TF* (*HU05G02278, HU02G01108, HU01G02373, HU04G01551, HU01G02692, HU02G00788, HU02G00791, HU03G02152*), and downregulation of *DELLA* (*HU05G01983, HU06G00358, HU02G03005, HU04G00148*) genes by GA-treatment. This indicates that increased GA levels block FB formation, while decreased GA levels facilitate the FB formation in pitaya, with GA signaling being more pronounced in GA-treated plants but inhibited in the FB group (Fig. 5C). Additionally, GA inhibits *DELLA,* which directly binds to *CO* through its CCT domain and prevent *CO* from binding to the *FT* promoter and thus downregulating *FT* [26, 27]. Notably, GA3 application upregulated TFs belonging to the *PIF* gene family, while their downregulation is required to regulate *CO* genes for photoperiod-induced flowering [28]. Carotenoids are precursors for ABA synthesis. ABA binds to *PYR/PYL* receptors, inhibiting *PP2C*. This inhibition activates *SnRK2*, which phosphorylates and activates *ABF*, leading to the expression of genes responsible for stomatal closure and seed dormancy [29, 30]. In current results, ABA levels were upregulated in FB groups compared to the initial control and GA-treated (Fig. 2F). This is supported by the downregulation of *PP2C* (*HU11G00494*, *HU06G02610*, *HU06G00546*, *HU05G00054*) and upregulation of *SnRK2* (*HU08G01036*) genes in FB group. This indicates that increased ABA levels facilitate FB formation in pitaya and that ABA signaling was more pronounced in the FB group but remained unchanged with GA-treatment (Fig. 5D). BR bind to receptor kinases *BRI1* and *BAK1*, initiating downstream signaling. Activated *BRI1* phosphorylates BSK, which then activate *BSU1*. *BSU1* deactivates the kinase *BIN2* through dephosphorylation, leading to *BIN2* degradation. In the absence of BRs, *BIN2* phosphorylates and inhibits *BZR1/2*, TFs for BR responses. When BRs are present, dephosphorylated *BZR1/2* move to the nucleus to regulate BR-responsive genes, including *CYCD3*, which is involved in cell division [31, 32]. In current results, BR levels were downregulated in GA-treated compared to the initial control and FB group (Fig. 2E). This is supported by the downregulation of *BRI1* (*HU08G02255, HU10G01229, HU07G00803, HU07G02367, HU11G01227, HU03G02803, HU09G01162, HU05G02102, HU11G01253, HU04G01421, HU04G01106, HU02G02234*), *BAK1* (*HU10G00131*, *HU02G00304*, *HU06G02429*, *HU02G02625*, *HU05G00524*) *BSK* (*HU02G01615*, *HU07G02210*, *NewGene_369*) *BSU1* (*HU03G01206*), *CYCD3* (*HU01G00165*, *HU03G00243*, *HU06G00405*) and upregulation of *BKI1* (*HU07G01084*) genes in GA-treated. This indicates that decreased BR levels may induce dormancy, while increased BR levels facilitates FB formation in pitaya. BR signaling was more pronounced in the FB group but inhibited by GA-treatment (Fig. 5E). JA is produced from α-linolenic acid and converted to its active form, jasmonoyl-isoleucine (JA-Ile), by the enzyme *JAR1*. JA-Ile binds to *COI1*, leading to the ubiquitination and degradation of *Jasmonate ZIM-domain* (*JAZ*) protein repressors. This release allows the TF *MYC2* to activate JA-responsive genes, such as ORCA3, which mediate processes like senescence and stress responses [33]. In the current results, JA levels were upregulated in both GA-treated and FB groups compared to the initial control (Fig. 2G). This is supported by the upregulation of *JAR1* (*HU10G01498*), *COI1* (*HU03G01802*) genes in FB group and upregulation of *MYC2* (*HU11G01791*, *HU06G01887*, *HU06G01888*, *HU03G00470*, *HU07G00114*, *HU04G00514*) genes in GA-treated. This indicates that increased JA levels might be support general growth, whether vegetative or floral, in pitaya. JA signaling was more pronounced in the both GA-treated and FB group (Fig. 5F). The current study provides multidimensional insights and signaling possibilities for identified phytohormones interacting with TFs and floral genes after GA3 exposure in pitaya-inducing dormancy.

Dynamic alterations in photoperiodism

In pitaya, light supplementation is considered the primary regulator of FB formation [34, 35]. However, current findings contradict this, demonstrating that phytohormones, not light, trigger and control flowering. While some hormones are beneficial, GA3 has drastic effects on flowering in pitaya. Current study showed that GA3 treatment during long light days strongly inhibited flowering. Apart from four *CO* genes (*HU06G02633*, *HU10G00019*, *HU04G00234*, and *HU02G01458*), all other *CO* genes were upregulated in response to GA3 treatment (Fig. 6). This suggests two possibilities: first, the light-regulated *CO* genes other than four mentioned are upregulated, yet no flowering was observed under

GA3 treatment. Second, GA3 inhibits these four key *CO* genes, preventing FB formation. This indicates that light-regulated pathways alone may not overcome dormancy when gibberellins are involved. The specific inhibition of these four *CO* genes by GA3 suggests a regulatory mechanism of GA3 induced dormancy. The inhibition of these genes could be a strategic point where GA3 exerts its effect, thereby preventing the transition from vegetative to reproductive growth. The study highlights the complex interplay between light signaling and gibberellin pathways in pitaya dormancy, emphasizing the importance of these four *CO* genes as potential regulators in dormancy.

Clean swept of floral gene

In this study, GA3 application in pitaya significantly impacted the expression of key floral genes involved in FB formation, thus causing dormancy. Several critical genes were downregulated in the GA3-treated compared to the FB group. ABC model of flower development (*AP1*, *AP2*, *MADS-box*, *AGL*, *SPL*) genes and other floral identity genes (*LFY*, *FT*) were significantly downregulated by GA3, indicating their role in clean swept of flowering and induce strong bud dormancy (Fig. 7). The upregulation of these genes in the FB group underscores their importance in floral organ identity and development [36]. *MADS-box* genes (*MADS2* and *MADS9*) are well-documented for their regulatory roles in flowering [37, 38]. Similarly, *AGL* genes (*AGL2*, *AGL6*, *AGL9*) specify reproductive organ identity [39]. *SPL* genes (*SPL4*, *SPL6*, *SPL8*, *SPL9*, and *SPL13*), play significant roles in the timing of floral transition and meristem identity [40]. *LFY* and *FT* are critical for promoting flowering and integrating various floral inductive signals [41, 42]. Increased *LFY* activity reduces GA levels, thus regulate flowering [1]. GA3 treatment appears to exert a clean sweep effect on these floral genes, effectively inhibiting their expression and induced hazardous dormancy. This suppression indicates that GA3 promotes dormancy at the expense of flowering, reinforcing the role of gibberellins in maintaining dormancy [43].

GA3 is widely used as plant growth regulator in agriculture; however, its hazardous effects on flowering in pitaya are very severe. The current study indicated that even during the peak flowering period, GA3 induced year-round dormancy by disrupting genes linked to phytohormones, *CO*, ABC model of flower development, and floral identity (Fig. 9). By identifying these molecular determinants, multiple approaches can be designed in future breeding research to tackle these challenges.

Conclusion

This study underscores the potent role of GA3 in inducing dormancy. GA3 disrupting the biological processes critical for the continuation of the pitaya species by inducing dormancy. Our findings demonstrate that GA3 at 100 mg/L induces bud dormancy and suppresses FB formation in pitaya (*H. polyrhizus*) by altering phytohormone levels and genes expression. GA3 application during peak flowering periods led to reduced AUX, CTK, and BR levels, while increasing JA, GA3, and GA4 levels. Transcriptomic analysis revealed DEGs involved in phytohormone signal transduction, with notable changes in *CO* and inhibition of genes related to ABC model of flower development and floral identity genes. These results highlight the GA3 induces strong year-round dormancy by disrupting plant hormone balances, *CO* and ABC model of flower development and floral identity genes in pitaya.

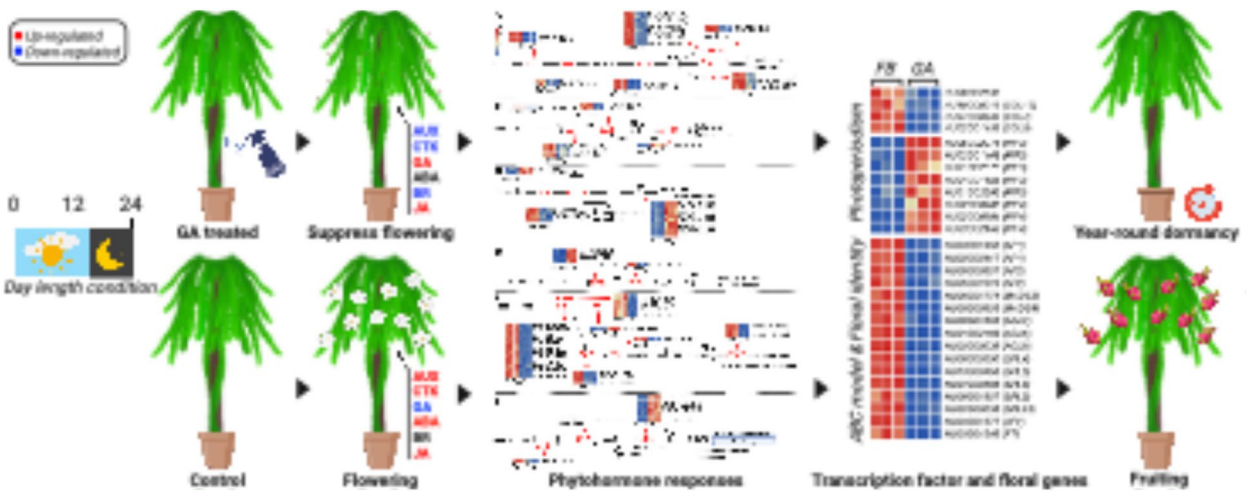

Fig. 9 Schematic illustration of GA3 induced alterations in phytohormone, photoperiod, ABC model and floral identity associated genes during the flowering period and leading to year-round dormancy

Abbreviations

GA3	Gibberellin-3
FB	Flower bud
AUX	Auxin
CTK	Cytokinin
dhZR	Dihydrozeatin riboside
ZR	Zeatin riboside
iPA	N6-isopentenyladenosine
BR	Brassinosteroid
JA	Jasmonic acid
GA4	Gibberellin-4
ABA	Abscisic acid
DEGs	Differentially expressed genes
CO	*CONSTANS-like*
NCBI	National Center for Biotechnology Information
SA	Salicylic acid
ETH	Ethylene

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05880-1.

Supplementary Material 1: Table S1. Monthly meteorological statistics of experimental site. Table S2. Primers used in this study. Table S3. Summary of sequencing data for the clean reads in each sample and every replicate. Red and green values represent highest and lowest value, respectively in each column. Table S4. Summary of the sequencing data in each sample and every replicate. Red and green values represent highest and lowest value in each column. Table S5. Differentially expressed genes involved in auxin pathway. Table S6. Differentially expressed genes involved in cytokinin pathway. Table S7. Differentially expressed genes involved in gibberellin pathway. Table S8. Differentially expressed genes involved in abscisic acid pathway. Table S9. Differentially expressed genes involved in brassinosteroid pathway. Table S10. Differentially expressed genes involved in jasmonic acid pathway. Table S11. Differentially expressed genes involved in *CONSTANS-Like* pathway. Table S12. Differentially expressed genes involved in ABC model of flower development and floral identity gene pathway.

Supplementary Material 2

Authors' contributions

KS, YQ contributed to the conception of study. KS, XZ collected the plant material. KS, TZ, Jiayi Chen performed the experiment. KS, Jiaxuan Chen performed the data analyses and wrote the manuscript. KS, YQ helped revise the paper with constructive discussions. All authors read and approved the manuscript.

Funding

This research was funded by the Provincial Rural Revitalization Strategy Special Project of Guangdong in 2024 (2024-NPY-00–030), the National Natural Science Foundation of China (31972367), and the Science and Technology Program of Guangzhou (202002020060) and Yangjiang (yangketong[2021]50).

Data availability

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request. Sequences have been deposited in NCBI Sequence Read Archive under project PRJNA1143693.

Declarations

Ethics approval and consent to participate

The experiments did not involve endangered or protected species. The data collection of plant materials and laboratory experiments were carried out with permission of related institution, and complied with national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture and Rural Affairs, College of Horticulture, South China Agricultural University, Guangzhou 510642, China. [2]Department of Ecology, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China.

Received: 14 August 2024 Accepted: 26 November 2024
Published online: 13 January 2025

References

1. Yamaguchi N, et al. Gibberellin acts positively then negatively to control onset of flower formation in Arabidopsis. Science. 2014;344(6184):638–41.
2. Jung H, et al. Gibberellin promotes bolting and flowering via the floral integrators RsFT and RsSOC1-1 under marginal vernalization in radish. Plants. 2020;9(5):594.
3. Koshita Y, et al. Involvement of endogenous plant hormones (IAA, ABA, GAs) in leaves and flower bud formation of satsuma mandarin (*Citrus unshiu* Marc.). Sci Hortic. 1999;79(3–4):185–94.
4. Nakagawa M, et al. Isolation and expression analysis of *FLOWERING LOCUS T-like* and gibberellin metabolism genes in biennial-bearing mango trees. Sci Hortic. 2012;139:108–17.
5. Lenahan OM, Whiting MD, Elfving DC. Gibberellic acid inhibits floral bud induction and improves 'Bing'sweet cherry fruit quality. HortScience. 2006;41(3):654–9.
6. Zhang S, et al. Effect of exogenous GA3 and its inhibitor paclobutrazol on floral formation, endogenous hormones, and flowering-associated genes in 'Fuji'apple (*Malus domestica* Borkh.). Plant Physiol Biochem. 2016;107:178–86.
7. Fan S, et al. Mediation of flower induction by gibberellin and its inhibitor paclobutrazol: mRNA and miRNA integration comprises complex regulatory cross-talk in apple. Plant Cell Physiol. 2018;59(11):2288–307.
8. Shah K, et al. Transcriptome analysis reveals dual action of salicylic acid application in the induction of flowering in *Malus domestica*. Plant Sci. 2022;324:111433.
9. Zhang H, et al. Role of gibberellin and its three GID1 receptors in *Jasminum sambac* stem elongation and flowering. Planta. 2022;255:1–16.
10. Guardiola J, Monerri C, Agusti M. The inhibitory effect of gibberellic acid on flowering in *Citrus*. Physiol Plant. 1982;55(2):136–42.
11. Xie S, et al. Effects of gibberellin applications before flowering on the phenotype, ripening, and flavonoid compounds of Syrah grape berries. J Sci Food Agric. 2022;102(13):6100–11.
12. Shah K, et al. Regulation of flowering time by improving leaf health markers and expansion by salicylic acid treatment: a new approach to induce flowering in *Malus domestica*. Front Plant Sci. 2021:1141.
13. Shah K, et al. Pitaya nutrition, biology, and biotechnology: A review. Int J Mol Sci. 2023;24(18):13986.
14. Trapnell C, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28(5):511–5.
15. Mao X, et al. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. Bioinformatics. 2005;21(19):3787–93.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$T method. Methods. 2001;25(4):402–8.
17. Xing L, et al. Transcription profiles reveal sugar and hormone signaling pathways mediating flower induction in apple (*Malus domestica* Borkh.). Plant Cell Physiol. 2015;56(10):2052–68.
18. Sun K, et al. DNA demethylation induces tree peony flowering with a low deformity rate compared to gibberellin by inducing PsFT expression under forcing culture conditions. Int J Mol Sci. 2022;23(12):6632.

19. Chen X, et al. Comparative RNA-sequencing-based transcriptome profiling of buds from profusely flowering 'Qinguan' and weakly flowering 'Nagafu no. 2' apple varieties reveals novel insights into the regulatory mechanisms underlying floral induction. BMC Plant Biol. 2018;18(1):1–21.
20. Altmann M, et al. Extensive signal integration by the phytohormone protein network. Nature. 2020;583(7815):271–6.
21. Leyser O. Auxin signaling. Plant Physiol. 2018;176(1):465–79.
22. Quint M, Gray WM. Auxin signaling. Curr Opin Plant Biol. 2006;9(5):448–53.
23. Kieber JJ, Schaller GE, Cytokinin signaling in plant development. Development. 2018;145(4):dev149344.
24. Hartweck LM. Gibberellin signaling. Planta. 2008;229(1):1–13.
25. Ueguchi-Tanaka M, et al. Gibberellin receptor and its role in gibberellin signaling in plants. Annu Rev Plant Biol. 2007;58:183–98.
26. Wang H, et al. The DELLA-CONSTANS transcription factor cascade integrates gibberellic acid and photoperiod signaling to regulate flowering. Plant Physiol. 2016;172(1):479–88.
27. Xu F, et al. DELLA proteins physically interact with CONSTANS to regulate flowering under long days in Arabidopsis. FEBS Lett. 2016;590(4):541–9.
28. Park E, Kim Y, Choi G. Phytochrome B requires PIF degradation and sequestration to induce light responses across a wide range of light conditions. Plant Cell. 2018;30(6):1277–92.
29. Chen K, et al. Abscisic acid dynamics, signaling, and functions in plants. J Integr Plant Biol. 2020;62(1):25–54.
30. Hirayama T, Umezawa T. The PP2C–SnRK2 complex: the central regulator of an abscisic acid signaling pathway. Plant Signal Behav. 2010;5(2):160–3.
31. Wang Z, et al. Brassinosteroid signaling network and regulation of photomorphogenesis. Annu Rev Genet. 2012;46:701–24.
32. Yang C, et al. The mechanisms of brassinosteroids' action: from signal transduction to plant development. Mol Plant. 2011;4(4):588–600.
33. Ruan J, et al. Jasmonic acid signaling pathway in plants. Int J Mol Sci. 2019;20(10):2479.
34. Wang M, et al. Light supplementation in pitaya orchards induces pitaya flowering in winter by promoting phytohormone biosynthesis. Int J Mol Sci. 2024;25(9):4794.
35. Xiong R, et al. Transcriptomic analysis of flower induction for long-day pitaya by supplementary lighting in short-day winter season. BMC Genomics. 2020;21(1):1–17.
36. Wils CR, Kaufmann K. Gene-regulatory networks controlling inflorescence and flower development in *Arabidopsis thaliana*. Biochim Biophys Acta (BBA)-Gene Regulatory Mechanisms. 2017;1860(1):95–105.
37. Smaczniak C, et al. Developmental and evolutionary diversity of plant MADS-domain factors: insights from recent studies. Development. 2012;139(17):3081–98.
38. Gramzow L, Theissen G. A hitchhiker's guide to the MADS world of plants. Genome Biol. 2010;11:1–11.
39. Stellari GM, Jaramillo MA, Kramer EM. Evolution of the APETALA3 and PISTILLATA lineages of MADS-Box–containing genes in the basal angiosperms. Mol Biol Evol. 2004;21(3):506–19.
40. Preston JC, Hileman LC. Functional evolution in the plant SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) gene family. Front Plant Sci. 2013;4:80.
41. Zuo X, et al. FLOWERING LOCUS T1 and TERMINAL FLOWER1 regulatory networks mediate flowering initiation in apple. Plant Physiol. 2024:kiae086.
42. Hiraoka K, et al. The florigen genes FT and TSF modulate lateral shoot outgrowth in *Arabidopsis thaliana*. Plant Cell Physiol. 2013;54(3):352–68.
43. Hedden P. The current status of research on gibberellin biosynthesis. Plant Cell Physiol. 2020;61(11):1832–49.

Publisher's Note