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# Transcriptome and metabolite conjoint analysis reveals the seed dormancy release process of perilla

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**Seed dormancy is a common physiological phenomenon during storage which has a great impact on timely germination of seeds. An in-depth analysis of the physiological and molecular mechanisms of perilla seed dormancy release is of great significance for cultivating high-vigor perilla varieties. We used gibberellin A3-soaked seeds (GA), natural dormancy-release seeds (CK) and water-soaked seeds (WA) to study the changes in the transcriptome and metabolome of dormancy release. The germination test revealed that the optimum concentration of gibberellin A3 for releasing dormancy from perilla seeds was 200 mg/L. The results revealed that plant hormone signal transduction, starch and sucrose metabolism and citric acid cycle were significantly enriched metabolic pathways closely related to seed dormancy release. Perilla seeds release their dormancy by enhancing the expression of GID1, PIF3, SnRK2, IAA, ARR-A, GH3, MKK4_5, otsB, GN1_2_3, glgC, WAXY, inhibiting the expression of DELLA, PP2C, glga, bglX, and GN4, and regulating the content of gibberellin A4, abscisic acid, auxin, sucrose, maltose, trehalose, and α-D-glucose 1-phosphate. Auxin plays an important role in breaking perilla seed dormancy and promoting seed germination. The energy required for breaking seed dormancy and germination of perilla seeds is mainly provided through sucrose metabolism. Citric acid cycle (TCA cycle) is the main energy supply transformation pathway for seed germination.**

**Keywords** Perilla frutescent, Seed dormancy, Transcriptome, Metabolome, Gibberellin A3, Dormancy release

Seed dormancy refers to a biological phenomenon that a complete seed with vital signs fails to complete germination under the best conditions[1]. It is an adaptive mechanism to adjust the time and place of seed germination, and can effectively adjust the spatial and temporal distribution of seed germination[2,3] is also a survival strategy for plants to survive under seasonal adverse weather conditions[4,5]. Although seed dormancy can prevent germination, it is conducive to storage and seed vigor after harvest[6], but this state is not conducive to crop production and planting. Understanding the dormancy mechanism, the method of dormancy release, and to explore the regulatory transformation of crop seeds from dormancy to germination have the great significance for agricultural production[7].

Dormancy and germination are regulated by various environmental factors and endogenous factors (such as plant hormones)[8,9]. Plant hormones play a crucial role in regulating seed dormancy and germination[10]. Abscisic acid (ABA) and gibberellin (GA) are the main hormones that act antagonistically in regulating seed germination and dormancy[11]. ABA induces the establishment and maintenance of seed dormancy during seed maturation, thus inhibiting seed germination, GA is a positive regulator of seed dormancy release and germination[12]. The spatiotemporal balance of ABA and GA plays an important role in seed biology, when the ratio of ABA/GA is high, it is beneficial for dormancy, but when the ratio is low, it is the opposite. Their interaction in metabolism and signal transduction determines the final dormancy and germination state of seeds[13]. The early sensing and signal transmission of GA involves three main components: GID1 (GA receptor) and GID2 (F-box) proteins are positive regulators[14], while DELLA proteins are negative regulators. GID1 can interact with DELLA protein to form GA GID1-DELLA complex in the presence of GAs, and then the complex is degraded, resulting in the activation of GA signal pathway[15]. In the process of GA-mediated seed germination promotion, GA3ox1 and GA3ox2 are two key genes regulating GA synthesis[16,17], and GA2ox oxidase promotes GA degradation[18]. As in the case of GAs, early ABA perception and signal transmission involve three main components: ABA receptor

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(PYR/PYL/RCAR) protein and SnRK2 protein kinase, which are positive regulators of the pathway, and PP2Cs protein phosphatase is negative regulators. ABA-binding receptors can bind and inhibit PP2Cs, which in turn allows phosphorylation and activation of SnRK2s[19]. Activated downstream targets of SnRK2s phosphorylation, such as ABI5 and other members of AREB/ABFs b-ZIP transcription factor (TF) family, to activate ABA response in plants[20]. Other plant hormones have been confirmed to be involved in regulating seed dormancy and germination[21–24], Some studies found that the expression of GA and auxin related DEG was significantly higher in germinated seeds, and the expression of DEG related to ABA signal transduction was significantly downregulated. Both GA and auxin can promote germination to break seed dormancy, while ABA can promote seed dormancy[25]. Ethylene (ETH), brassinolide (BR) and cytokinin (CTK) also participate in the regulation of seed dormancy and germination through direct or indirect ABA or GA signal pathway ETH, BR and CTK are also involved in seed dormancy and germination regulation through direct or indirect involvement in ABA or GA signaling pathways[26–28].

During the process from dormancy to germination of seeds, in addition to plant hormone signal transduction pathway, carbohydrate metabolism pathway is also activated[29], Carbohydrates and proteins are mobilized during seed germination to provide energy and substrate for seedling development[30]. Starch is a polysaccharide stored in seeds, which can be converted into reducing sugars (such as maltose and glucose) and plays an important role in seed germination. During seed germination, the active substances induce α-Amylase gene expression, then α-Amylase syntheses[31,32] to hydrolyze stored starch[33], and organic acids and flavonoids may participate in the acidification of endosperm tissue, releasing stored starch, sucrose, etc. into metabolism and affecting seed germination[34,35]. It is reported that sucrose may be an intermediate product of plant metabolism. The reduction of sucrose decomposition leads to the reduction of glucose and fructose levels and inhibits seed germination[36]. Exogenous GA4 may play an important role in dormancy release by changing the abundance of metabolites such as galactose, glyoxylic acid, dicarboxylic acid, starch and sucrose metabolism[37].Therefore, the seed from dormancy to germination is a complex process, which is affected by genes and metabolites.

Perilla [*Perilla frutescens (L.) Britt.*] is an annual herb of Perilla in Labiatae[38]. The content of α-linolenic acid can reach 65% in perilla seed oil[39], which is one of the species with the highest α-linolenic acid content in terrestrial plants. The leaves and stems of Perilla frutescens contain flavonoids, rosmarinic acid, volatile oils and other chemicals[40,41]. The fresh and tender leaves of perilla are deeply loved by people in China, South Korea, Japan and other countries as vegetables[42,43]. The nutritional and functional characteristics of Perilla frutescens have important development value in medicine, industry, food and other fields[44]. Under natural conditions, the dormancy period of Perilla frutescens seeds lasts 5–7 months, and their dormancy characteristics are the primary cause of the low germination rate and uneven germination, which significantly restricts the field cultivation and breeding processes of Perilla. To date, there have been no reports on the dormancy characteristics of Perilla seeds or the molecular mechanisms underlying their dormancy.

In this study, we established a method for relieving seed dormancy by investigating the effects of gibberellic acid (GA3) on dormancy breaking and germination. Additionally, we conducted transcriptomic and metabolomic analyses to identify genes and metabolites associated with the dormancy and germination processes of Perilla frutescens seeds, thereby elucidating the molecular mechanisms governing the transition from dormancy to germination. This research provides valuable reference material for subsequent studies on dormancy-related functional genes and offers insights into artificially regulating seed germination and shortening the dormancy period. The findings hold significant theoretical and practical implications for Perilla frutescens breeding programs and field production practices.

## Results

### Effects of GA on the germination rate, germination index and germination potential of perilla seeds

Compared with the water soaked seeds groups, all five concentrations of the gibberellin A3 (GA3) solution broke perilla seed dormancy and significantly promoted perilla seed germination in the GA3 soaked seeds groups (Fig. 1). The germination time of Perilla seeds first decreased but then increased with increasing GA3 concentration, and the germination quantity of Perilla seeds first increased but then decreased with increasing GA3 concentration. The shortest germination time of the seeds treated with the GA3 solution occurred on the second day, and the germination time of the other treatments was 4 or 5 days. The seeds of the water soaked seeds group hardly germinated. The germination rate, germination index and germination potential of the Perilla seeds after treatment with the GA3 solution were greater than the untreated seeds, and the best results were achieved after treatment with a GA3 concentration of 200 mg/L, with 73.33%, 14.56% and 88.66% greater than the water soaked seeds group.

### Transcriptome analysis of the dormancy release process of perilla seeds

RNA-Seq analysis was performed on the seed samples to investigate the regulatory genes underlying the dormancy changes in seeds induced by gibberellin A3-soaked seeds (GA), with natural dormancy-release seeds (CK) and water-soaked seeds (WA) serving as controls. All samples generated 63.68 Gb of valid bases with Q30 values ranging from 91.83 ~ 95.76%, and the mean GC content was 48.52% (Table 1). A total of 413,329,746 clean reads were obtained from the three comparison groups, the reads were then mapped to the reference genome sequence of the tetraploid cultivation variety Perilla frutescens[45]. The alignment ratio of the reads ranged from 65.06 to 69.77% among the nine libraries, and 82.21–92.66% of the reads were uniquely mapped.

Through KEGG analysis, a total of 13,926 Unigenes were annotated to metabolic pathways, which can be categorized into five major classes and 18 subcategories (Fig. 2Ai). Specifically, these Unigenes were distributed as follows across the major classes: Cellular Processes: 816 Unigenes, Environmental Information Processing: 2037 Unigenes, Metabolism: 11,337 Unigenes, Genetic Information Processing: 4210 Unigenes, Organismal Systems:

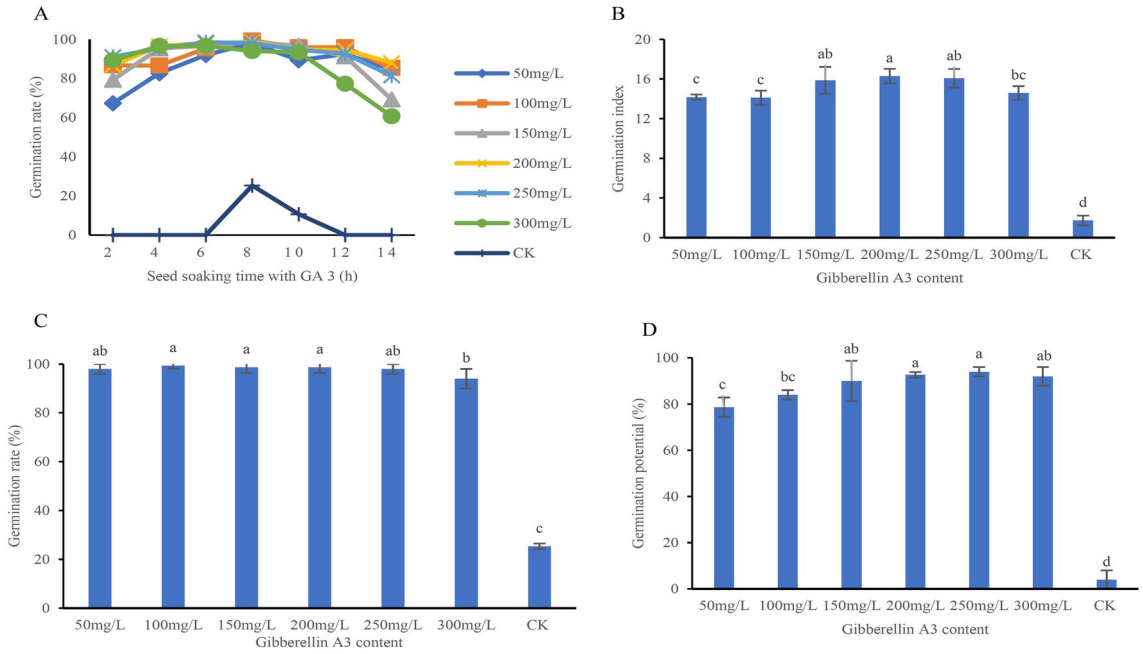


**Fig. 1**. Effects of different concentrations of GA3 and soaking times on the perilla seeds. (**A**) Effects of different concentrations of GA3 and soaking times on the germination rate of perilla. (**B**) Germination index. (**C**) Germination rate. (**D**) Germination potential. Different letters (a–d) on the bar plots indicate significant difference at $p < 0.05$ using one-way analysis of variance with Duncan's multiple-range test.

| Sample id | Total reads | Clean_reads | Total giga bases | q30 rate | gc content | Aligned to genes | Uniquely_mapped |
|---|---|---|---|---|---|---|---|
| CK1 | 53758126 | 49828886 | 8.12G | 93.94% | 48.80% | 65.08% | 82.21% |
| CK2 | 48301512 | 47398690 | 7.29G | 95.76% | 47.27% | 65.84% | 83.82% |
| CK3 | 61536390 | 60391154 | 9.29G | 95.68% | 47.36% | 65.06% | 82.22% |
| WA1 | 42169896 | 41553966 | 6.33G | 93.14% | 48.96% | 69.10% | 92.08% |
| WA2 | 45397624 | 44639006 | 6.81G | 93.13% | 49.23% | 69.14% | 92.66% |
| WA3 | 39220396 | 38671542 | 5.88G | 93.39% | 49.67% | 69.27% | 91.57% |
| GA1 | 45412274 | 44655306 | 6.81G | 92.99% | 48.62% | 69.77% | 92.36% |
| GA2 | 45075298 | 44386284 | 6.76G | 93.22% | 48.49% | 69.48% | 90.91% |
| GA3 | 42623950 | 41804912 | 6.39G | 91.83% | 48.27% | 68.99% | 91.36% |

**Table 1**. Valid data evaluation statistics.

1709 Unigenes. Within these metabolic pathways, 4,952 Unigenes were further annotated to 15 Carbohydrate metabolism and 3 Signal transduction (Fig. 2Aii). The most highly annotated Carbohydrate metabolism pathways included: Starch and sucrose metabolism (ko00500, 517 Unigenes), Pentose and glucuronate interconversions (ko00040, 388 Unigenes), glycolysis/gluconeogenesis (ko00010, 353 Unigenes), Amino sugar and nucleotide sugar metabolism (ko00520, 319 Unigenes). The most highly annotated Signal transduction pathways was Plant hormone signal transduction (ko04075, 973 Unigenes). This analysis provides insights into the potential metabolic pathways and the involvement of unigenes in these pathways in dormancy release of perilla seed. Additionally, based on transcriptome data and KEGG pathway analysis, the biosynthesis pathways of hormone, such as GA4, ABA, and IAA, were inferred.

The criteria of $|\log2(\text{fold change})| \geq 1$ and $\text{padj} \leq 0.05$ were used to screen for differentially expressed genes (DEGs) (Fig. 2Bi). Across all comparison groups (WA vs GA, WA vs CK, CK vs GA), a total of 1,129 DEGs were identified (Fig. 2Bii). Among these, the highest number of DEGs was observed in the comparison between WA vs CK (5466 upregulated and 4831 downregulated), while the lowest number of DEGs was found in the comparison between WA vs GA (3138 upregulated and 2088 downregulated). To validate the biological functions of the DEGs that affect the dormancy release of perilla seeds under $GA_3$ treatment, KEGG enrichment analysis was conducted on the DEGs. Compared to WA and GA, the top 6 enriched pathways with DEGs were Plant-pathogen interaction (175 genes), Plant hormone signal transduction (175 genes), MAPK signaling pathway-plant (96 genes), Starch and sucrose metabolism (95 genes), Protein processing in endoplasmic reticulum (90 genes) and Glycolysis /Gluconeogenesis (65 genes) (Fig. 2Ci). Between CK and GA, most DGEs were enriched in Plant-pathogen interaction (285 genes), Plant hormone signal transduction (244 genes), Protein processing

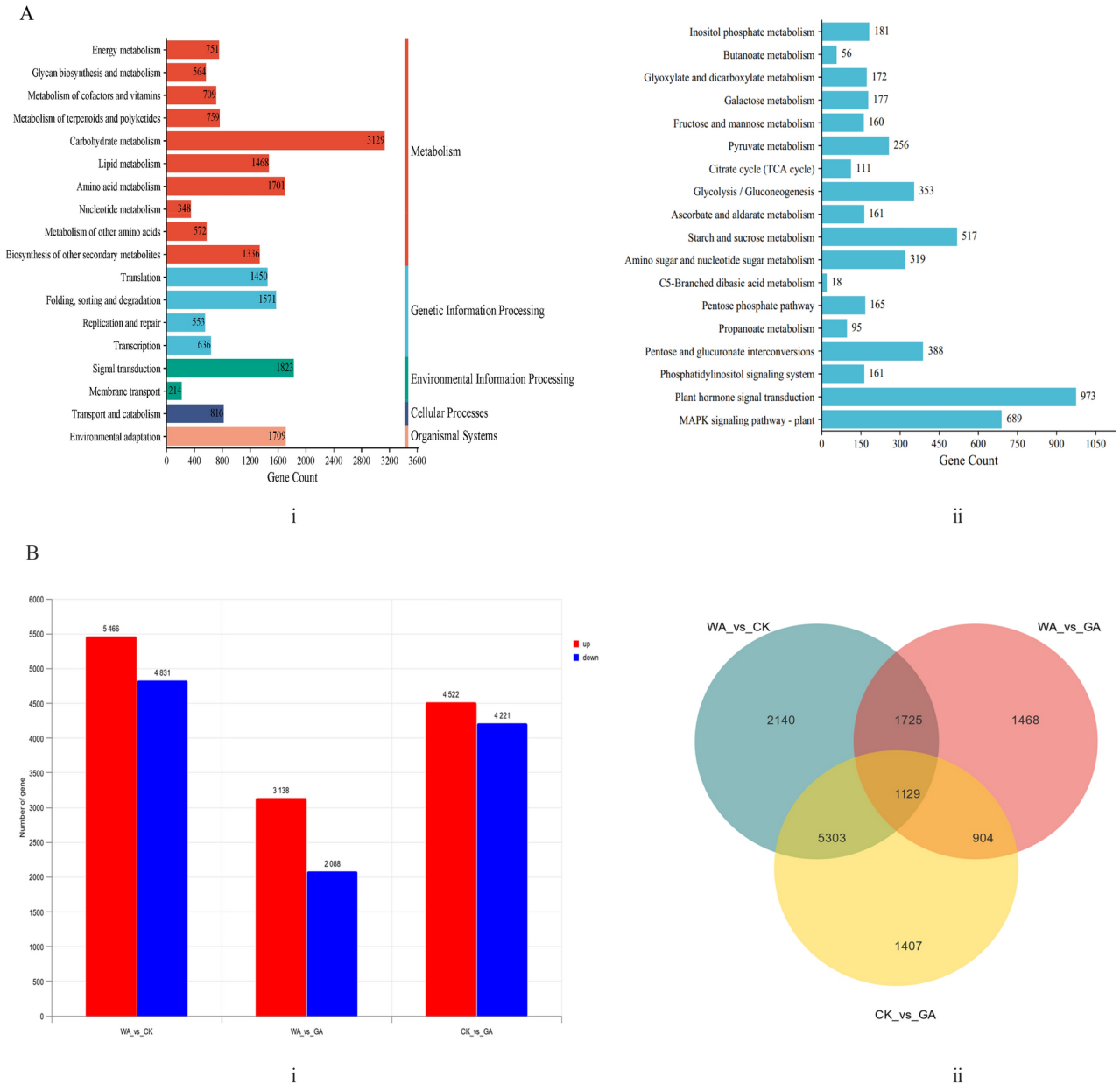


**Fig. 2**. (**A**) KEGG annotation results. (i) KEGG metabolic pathway classification. (ii) Pathways involved in carbon metabolism and signal transduction. (**B**) Identification and expression analysis of the differentially expressed genes (DEGs) and KEGG enrichment of DEGs. (i) The number of DEGs among the three comparison groups. (ii) Venn diagram of DEGs among the three comparison groups. (**C**) KEGG pathway enrichment analysis. (i) The first 20 enriched KEGG pathway of DEGs under WA vs GA. (ii) The first 20 enriched KEGG pathway of DEGs under CK vs GA. (iii) The first 20 enriched KEGG pathway of DEGs under WA vs CK.

in endoplasmic reticulum (158 genes), MAPK signaling pathway-plant (157 genes), Ribosome (138 genes) and Starch and sucrose metabolism (133 genes) (Fig. 2Cii). In the comparison between WA and CK, the top 5 enriched pathways were Plant-pathogen interaction, Plant hormone signal transduction, Ribosome, MAPK signaling pathway-plant, Protein processing in endoplasmic reticulum and Starch and sucrose metabolism (Fig. 2Ciii). These findings indicate that signal transduction and carbohydrate metabolism play important roles in the dormancy release and germination process of perilla seeds.

## Verification of DEGs by qRT-PCR

We chose to perform quantitative real-time polymerase chain reaction (qRT-PCR) analysis on a selection of genes exhibiting markedly differential expression levels to corroborate the precision of our transcriptome dataset. The specific primers for these genes (Supplemental Table 1) were designed with Primer 6.0 software. The results of the qRT-PCR validation (Supplemental File 1) revealed that the expression trends of the 14 genes were

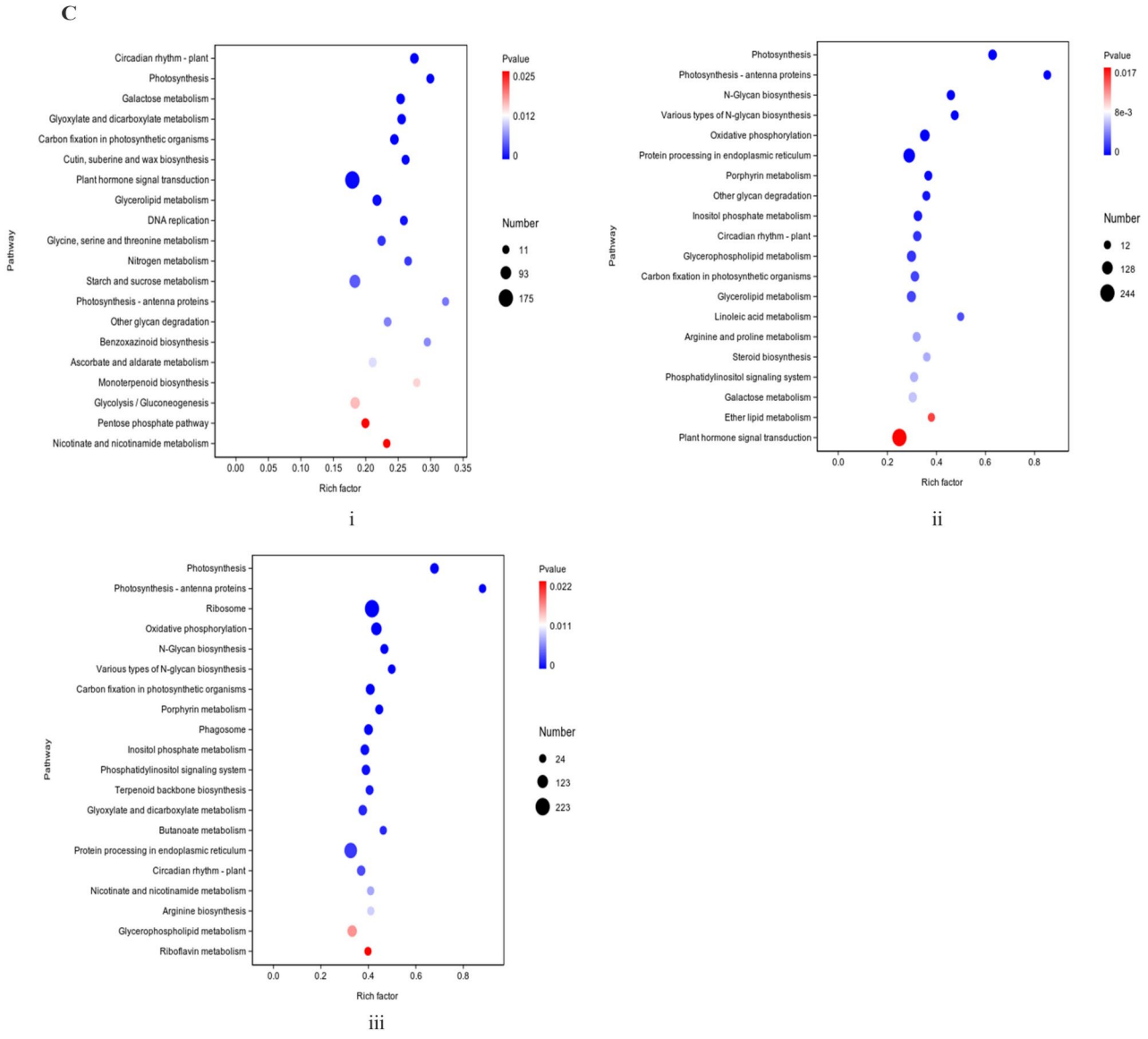


**Figure 2.** (continued)

consistent with the transcriptome sequencing results, indicating that the RNA-seq data are reliable and can be used for in-depth analysis.

## Metabolic analysis of the three treatment seeds

Metabolome sequencing technology (LC–MS) was performed on the seed samples to investigate the metabolic mechanism underlying the dormancy changes in seeds induced by GA3-soaked seeds (GA), with natural dormancy-release seeds (CK) and water-soaked seeds (WA) serving as controls. PCA was used to assess the levels of metabolites (Fig. 3A). PC1 effectively distinguished between the natural dormancy-release seeds and soaked seeds, whereas PC2 distinguished between the GA3-soaked and water-soaked, with PC1 accounting for 58.9% and PC2 accounting for 12.7%. On the basis of the above analysis, we believe that there should be significant differences in metabolites among the three comparison groups. A total of 2741 metabolites were detected across all samples. Metabolites with FDR < 0.05 and VIP ≥ 1 were chosen as differentially abundant metabolites (DIMs) for further analysis. We identified 272 (80 upregulated and 192 downregulated), 992 (507 upregulated and 485 downregulated) and 1037 (498 upregulated and 539 downregulated) DIMs in WA versus GA, WA versus CK, and CK versus GA, respectively (Fig. 3B). A Venn diagram analysis was also conducted to uncover the overlap between different comparison groups (Fig. 3C). A total of 144 metabolites exhibited stage-specific expression patterns. These common differentially abundant metabolites may play key roles in regulating seed dormancy release.

Comparative analysis of the three comparison groups revealed a significant difference in metabolites, and the significantly enriched kegg pathways of (WA vs GA) group included biosynthesis of amino acids, ABC transporters, citrate cycle (TCA cycle), arginine biosynthesis, and metabolic pathways(Fig. 3D); the significantly

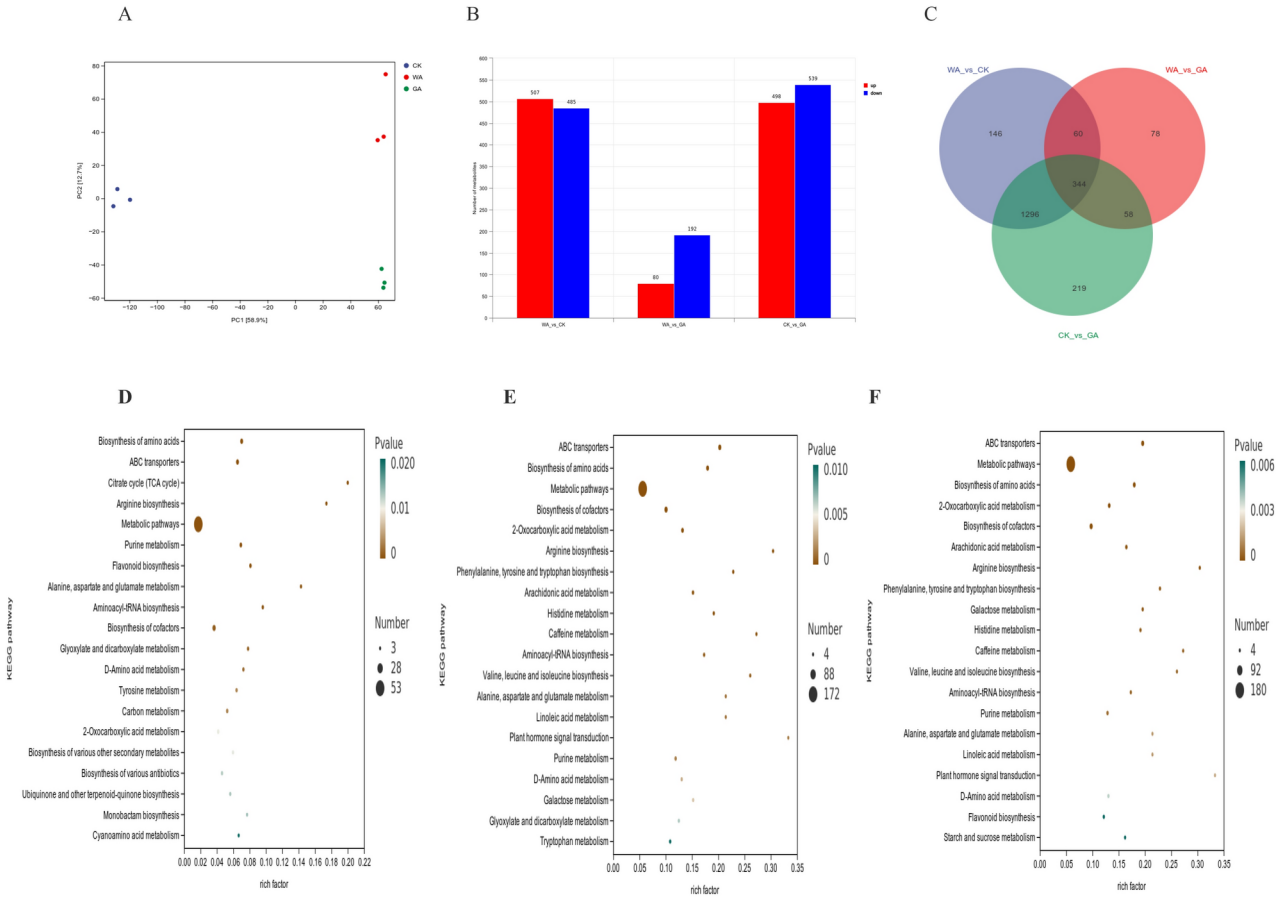


**Fig. 3**. Dynamic metabolomic changes involved in the dormancy release regulation of Perilla frutescens seeds. (**A**) PCA of metabolites in perilla seeds between groups. (**B**) The number of DIMs among the three comparison groups (**C**) Venn diagram of differential metabolites (DIMs) between the three comparison groups. (**D**) KEGG functional enrichment analysis of DIMs between WA vs GA groups. (**E**) KEGG functional enrichment analysis of DIMs between WA vs CK groups. (**F**) KEGG functional enrichment analysis of DIMs between CK vs GA groups.

enriched kegg pathways of (WA vs CK) group included ABC transporters, biosynthesis of amino acids, metabolic pathways, biosynthesis of cofactors, and 2-oxocarboxylic acid metabolism(Fig. 3E); and the significantly enriched kegg pathways of (CK vs WA) group included ABC transporters, metabolic pathways, biosynthesis of amino acids, 2-oxocarboxylic acid metabolism, and biosynthesis of cofactors (Fig. 3F).

## Association analysis of the metabolomic and transcriptomic data

To investigate the relationships between the genes and metabolites involved in the dormancy release of perilla seeds, DIMs and DEGs of the three comparison groups (WA versus GA, WA versus CK, and CK versus GA) were mapped to the KEGG database. There were 123, 191, and 197 comapped pathways in WA versus GA, WA versus CK, and CK versus GA, respectively. Among these comapped pathways, ABC transporters, carbon fixation in photosynthetic organisms, citrate cycle (TCA cycle), glyoxylate and dicarboxylate metabolism, etc., were significantly enriched with DIMs and DEGs between WA and GA (Fig. 4A), ABC transporters, oxidative phosphorylation, arginine biosynthesis, porphyrin metabolism, etc., were significantly enriched with DIMs and DEGs between WA and CK (Fig. 4B). Photosynthesis, ABC transporters, oxidative phosphorylation, arachidonic acid metabolism, etc., were significantly enriched with DIMs and DEGs between CK and GA (Fig. 4C). We thus detected common pathways enriched by seed dormancy release, involving plant hormone signal transduction, starch and sucrose metabolism, and citrate cycle (TCA cycle). The three pathways involved a total of 17 DIMs and 67 DEGs (Fig. 4D, Figs. 5, 6), which may have significantly changed during dormancy release and may play key roles in this process.

## Analysis of the correlation between transcriptome and metabolic based on the three pathways

Through comprehensive analysis of the common DEGs and DIMs involved in the three comparison groups, to model the synthetic and regulatory characteristics of DIMs and DEGs, subnetworks were constructed based on the three pathways to determine transcript-metabolite correlations. Only correlation pairs with correlation

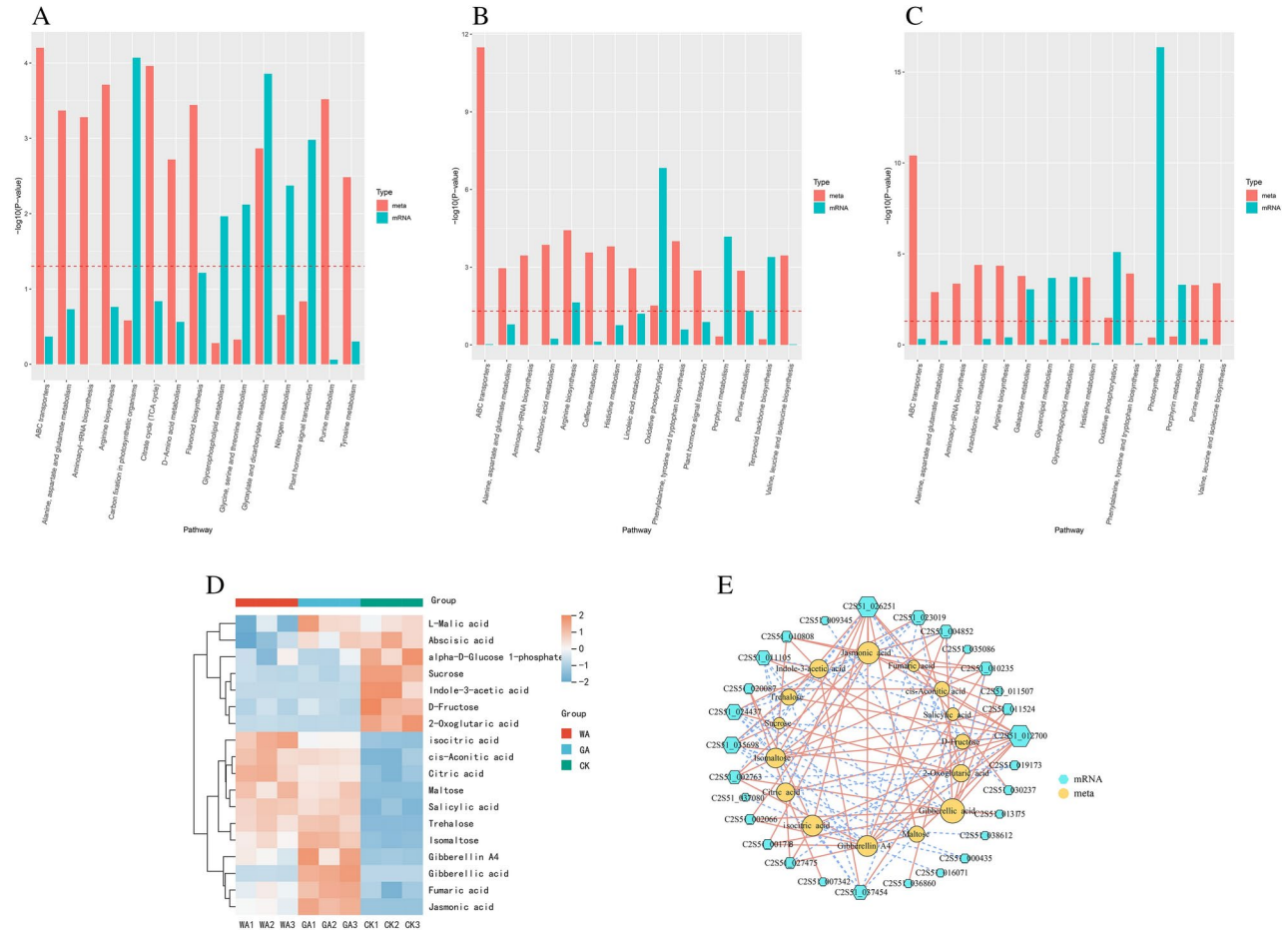


**Fig. 4**. Association Analysis of the Metabolomic and Transcriptomic Data. (**A**)The significantly enriched signaling pathways associated with highly correlated DEGs and DMs between WA and GA. (**B**) The significantly enriched signaling pathways associated with highly correlated DEGs and DMs between WA and CK. (**C**) The significantly enriched signaling pathways associated with highly correlated DEGs and DMs between CK and GA. (**D**) Hierarchy clustering of the key DIMs specifically overexpressed in different groups. (**E**) Correlation co-expression network of differential genes (metabolites). The solid brown line represents positive correlation, the dashed blue line represents negative correlation.

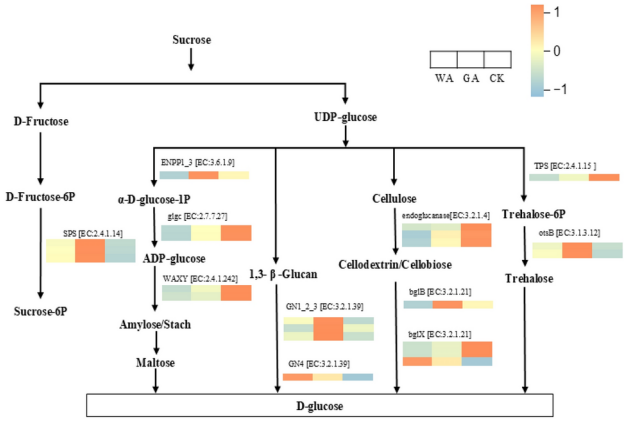


**Fig. 5**. Starch and sucrose pathway analysis. The metabolic pathway was based on the KEGG database. The heatmap represents the gene expression levels in WA, GA, and CK.

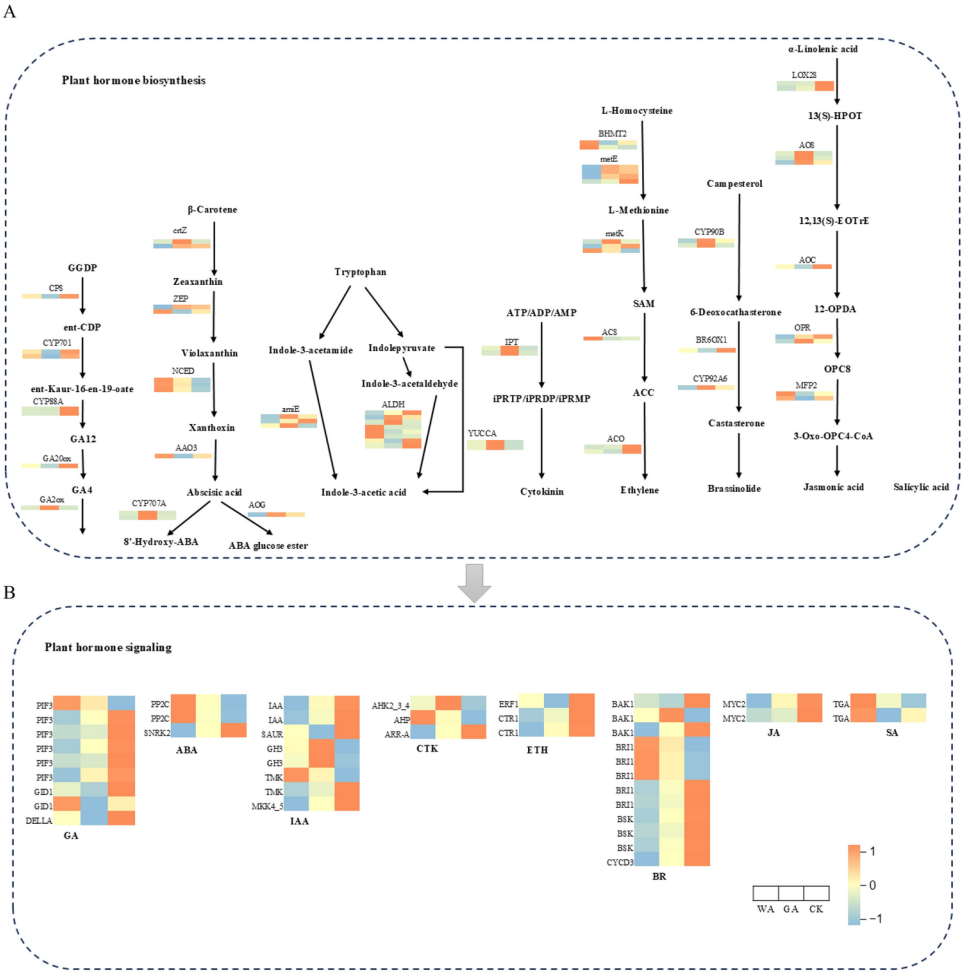


**Fig. 6**. The hormone synthesis and transduction pathway, expression levels are indicated by the heatmap of groups. (**A**) Expression heatmaps of the DEGs involved in plant hormone biosynthesis. (**B**) Expression heatmaps of the DEGs involved in plant hormone signaling.

coefficients > 0.9 were included in the analysis. In the network, 76 pairs showed a positive correlation, and 50 pairs were negatively correlated (Fig. 4E). The network revealed 34 DEGs and 16 DIMs with rich correlations. Among the metabolites, Indole-3-acetic acid, Gibberellic acid, Gibberellin A4, D-Fructose, and Trehalose were located in the center of the network. Genes such as SNRK2 (C2S51_017090), PIF3 (C2S51_019173), GID1 (C2S51_0230 19), PP2C (C2S51_027475, C2S51_032686), and C2S51_030237 (TMK) have been predicted to be correlated with the presence of these metabolites.

## Key pathways analysis

By investigating the common DEGs and DIMs among the three comparison groups, based on the WA vs GA comparison group, we analyzed the key metabolic pathways of perilla seed dormancy release caused by GA3.

A range of plant hormones play crucial roles in maintaining and releasing dormancy in perilla seeds. Comparative analysis between the WA and GA groups revealed no significant differences in the levels of abscisic acid, cytokinine, ethylene, and brassinolide; however, notable changes were observed in the concentrations of salicylic acid, jasmonic acid, indole-3-acetic acid, gibberellic acid, and gibberellin A4. In total, 35 critical structural gene families involved in hormone signal transduction pathways were identified; 22 gene families exhibited continuous upregulation (accounting for 62.86%), while 13 gene families exhibited continuous downregulation (accounting for 37.14%)(Fig. 6B).

The increase in the GA3 content promotes the synthesis of GA4, elevated the content of GA4, which aligns with the findings from the metabolomics analysis. 2 DEGs encoding gibberellin receptor GID1 (GID1) were downregulated, while 1 DEG encoding DELLA protein (DELLA) also was downregulated. Conversely, 5 of 6 DEGs encoding phytochrome-interacting factor 3 (PIF3) showed significantly enhanced expression. GID1 plays a regulatory role in GA signaling, its downregulation diminishes the efficacy of GA signaling. But the content of GA4 increased, downregulated of the DEG encoding DELLA proteins, and the upregulated of PIF3 regulatory genes promote GA signal transduction, thereby GA playing a positive role in seed dormancy release. Abscisic acid (ABA) is a key hormone responsible for maintaining seed dormancy and inhibiting germination. 2 DEGs encoding protein phosphatase 2C (PP2C) were downregulated, 1 DEG encoding serine/threonine-protein

kinase SRK2 (SnRK2) was upregulated. The downregulation of PP2C weakens ABA signal transduction, the upregulation of SnRK2 effectively counteracts the original effects of ABA, thereby diminishing its control over seed dormancy. IAA content increased significantly after GA3-soaked seeds, and the unigenes screened for involvement in the IAA metabolic pathway included The expression levels of DEGs encoding auxin-responsive protein IAA (IAA), auxin responsive GH3 gene family (GH3), SAUR family protein (SAUR), mitogen-activated protein kinase kinase 4/5 (MKK4_5) and receptor protein kinase TMK (TMK) were significantly upregulated except for 1 DEG encoding SAUR family protein and 1 DEG encoding receptor protein kinase TMK. The signaling of IAA were significantly increased by the exogenous application of GA3, suggesting that IAA promotes the dormancy release of perilla seeds.

The DEGs associated with CTK signaling transduction were upregulated except for the DEGs encoding histidine-containing phosphotransfer peotein (AHP). The DEGs encoding cyclin D3, plant (CYCD3) and BR-signaling kinase (BSK) were upregulated, encoding brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1) and protein brassinosteroid insensitive 1 (BRI1) had different expression patterns in BR signaling transduction. ETH signaling transduction related the DEGs encoding ethylene-responsive transcription factor 1 (ERF1) were downregulated and encoding serine/threonine-protein kinase CTR1 (CTR1) were upregulated. JA signaling transduction related 2 DEGs encoding transcription factor MYC2 (MYC2) were upregulated. The SA content increased in GA3-soaked seeds, and the DEGs regulating SA signaling were downregulated.

Starch and sucrose metabolism can provide energy and a carbon skeleton for biosynthesis during seed germination, therefore, we mapped the energy metabolism pathways associated with the dormancy release of perilla seeds. (Fig. 5). The contents of sucrose, D-(−)-fructose, isomaltose, α,α-trehalose, and α- D-glucose 1-phosphate increased, and the content of maltose decreased. After GA3-soaked seeds, 1 DEG encoding ectonucleotide pyrophosphatase/phosphodiesterase family member 1/3 (ENPP1_3), 3 DEGs encoding glucose-1-phosphate adenylyltransferase (glgC), and 2 DEGs encoding granule-bound starch synthase (WAXY) were significantly upregulated to promote amylose synthesis and indirectly promote starch synthesis, and the genes regulating beta-amylase [EC:3.2.1.2] enzymes for the conversion of starch to Maltose were significantly upregulated to promote the synthesis of Maltose. Maltose was converted into D-glucose, which was consistent with the decrease in maltose content after GA3-soaked seeds. Sucrose was converted to UDP-glucose through the upregulated expression of the DEGs encoding beta-fructofuranosidase (INV) and sucrose synthase (SUS), UDP-glucose was converted to trehalose, 1,3-β-glucan, cellulose, cellulose and cellobiose through significant upregulation of the DEGs encoding trehalose 6-phosphate phosphatase (otsB), glucan endo-1,3-beta-glucosidase 1/2/3 (GN1_2_3), sucrose-phosphate synthase (E2.4.1.14), beta-glucosidase (bglX), endoglucanase (E3.2.1.4), beta-glucosidase (bglB), and glucan endo-1,3-beta-glucosidase 4 (GN4), they were further converted to D -glucose and α- D-glucose 1-phosphate, and the increase in the levels of D-fructose, α,α-trehalose, and α- D-glucose 1-phosphate in the metabolome soaked with GA3 also confirms that, D-glucose and α-D-glucose 1-phosphate enter the energy conversion pathway to provide energy for metabolism and seed germination in the later stages. In this study, TCA cycle was significantly enriched, with 3 enzymes with differential genes, 2 enzymes with upregulated differential gene expression, and 1 enzyme with downregulated differential gene expression.

## Discussion

Germination rate reflects the dynamic relationship between the seed germination rate and time. Germination potential is an indicator that reflects and explains the rate of seed germination and accurately reflects whether or not the seed has germinated in an orderly manner in the soil. The germination index is an indicator of whether the germination rate is stable and consistent. In general, the higher the seed germination potential and germination index, the higher the seed germination rate. Light and temperature are also two key factors affecting seed germination. To explore the effect of GA3 on perilla seed germination, we used germination conditions that take into account the consistency of light and temperature with the interaction between GA3. Throughout the germination process, GA3 treatment significantly increased the germination rate of perilla seeds, and the greatest effect of GA3 treatment on breaking perilla seed dormancy and promoting germination was observed at a GA3 concentration of 200 mg/L (Fig. 1).

Among the many factors that contribute to seed dormancy and germination, endogenous hormones are considered the most important, with ABA and GA playing important roles in the regulation of seed dormancy and germination[28,46]. In this study, we analyzed the hormone-related metabolic pathways related to dormancy release of Perilla seeds and found significant enrichment of metabolic pathways related to plant hormone synthesis and signal transduction. We mapped hormone-associated metabolic pathways (Fig. 6A, B). In Arabidopsis, GA treatment or mutations causing increased GA signaling downregulate the transcript levels of GA3ox1, GA20ox1, GA20ox2, and GA20ox3, while GA treatment upregulates the transcript levels of the GA catabolism genes GA2ox1 and GA2ox2. GA defects or mutations that reduce GA signaling lead to the upregulation of GA3ox and GA20ox expression and the downregulation of GA2ox expression[47]. In particular, the GA3ox1 and GA3ox2 enzymes for biosynthesis and the GA2ox2 enzymes for catabolism play key roles in GA signaling during seed germination. In this study, the DEGs encoding GA20ox in GA3 soaked seeds were downregulated, in natural dormancy released seeds were upregulation, it may be due to the increase of GA3 content in the seeds caused by GA3 soaking. The DEGs encoding GA2ox were significantly upregulated. GA2ox may promote the conversion of GA3 to GA4, promotes the transduction of GA4 signaling. GA4 are the first steps in catalyzing the biosynthesis pathway of GA, and the absence of GA4 can lead to a lack of GA in plant seeds and prevent germination[48]. This finding is also consistent with the change of GA4 content in the dormancy released seeds. Early perception and signaling of GA involves three main components: the GID1 (GA receptor) and GID2 (F-box) proteins are positive regulators[49,50], while the DELLA proteins are negative regulators. Binding of GA to GID1 promotes the interaction of GID1 with DELLA repressors[51]. The DEGs encoding GID1 were downregulated in GA3 soaked

seeds, but the DEGs encoding GID1 in natural dormancy released seeds were upregulated. The trend of changes in the regulatory genes of DEllA protein was the same as that of GID1. The DEGs encoding the transcription factor phytochrome interacting factor 3 (PIF3) at the end of the GA signaling pathway were all significantly upregulated, regulating seeds to release dormancy. Therefore, we speculate that GA4, GA20ox, GA2ox, GID1, DELLA, and PIF3 plays an important role in the dormancy release of perilla seeds. Their changing trends in different groups may be due to different ways of releasing dormancy. In the carotenoid biosynthesis pathway, 9-cis-epoxycarotenoid dioxygenase (NCED) and abscisic-aldehyde oxidase (AAO3) regulatory genes were significantly downregulated, the genes regulating (+)-abscisic acid 8'-hydroxylase (CYP707A) and abscisate beta-glucosyltransferase (AOG), the convertase of Abscisate into 8'-hydroxyabscisate and abscisic acid glucose ester, were significantly upregulated, this may be related to the increase of abscisic acid content during seed placement. PP2c regulatory genes were significantly downregulated, and the regulatory genes of SnRK2 were significantly upregulated. Under the combined action of synthesis and metabolism, the effect of abscisic acid was reduced, promoting the release of seed dormancy. The changes of GA4, GA3 and ABA content, reduced the ratio of ABA/GA in perilla seeds, and promoted the release of perilla seed dormancy. This finding is also consistent with studies showing that the spatiotemporal balance of gibberellins and abscisic acid plays a crucial role in seed biology, favoring dormancy at relatively high ABA/GA ratios and vice versa at relatively low ratios[30]. ABA is involved in the induction and maintenance of seed dormancy, whereas IAA is also a positive regulator of seed germination[38].

IAA is an essential phytohormone that regulates seed dormancy and germination. In this study, combined transcriptome and metabolome analysis revealed enrichment of the tryptophan metabolism pathway, and the regulatory genes of amiE and ALDH related to the synthesis of indoleacetic acid were upregulated, promoting the synthesis of auxin. In terms of seed dormancy release, the IAA level was detected with an increasing trend during seed imbibition and after ripening in Arabidopsis, pea, and wheat[52–54]. With the exception of the downregulated DEGs encoding SAUR, all DEGs encoding other enzymes related to IAA metabolism were upregulated, promoting IAA signaling transduction. These findings indicate that IAA synthesis and metabolic activities are vigorous during perilla seed germination, and we hypothesize that IAA play important roles in breaking perilla seed dormancy and promoting seed germination. The change of IAA content confirms its important role in seed dormancy release and germination.

Other plant hormones, such as CTK, ETH, BR, and JA, are also involved in regulating seed dormancy, primarily by mediating the ABA/GA balance[8]. CTK, ETH, and BR positively regulate seed dormancy release in dicot species, and they function by modulating ABA synthesis, catabolism, and signaling transduction[55,56]. But our research did not find a correlation between these hormones and the dormancy release of perilla seeds. Our analysis revealed that the DEGs encoding IPT associated with CTK synthesis were upregulated and in CTK metabolism the DEG encoding ARR-A was upregulated, encoding AHP was downregulated, and the DEG encoding AHK2_34 had different expression patterns in GA3 soaked seeds and natural dormancy released seeds. The DEGs encoding CYP90B and CYP92A6, related to brassinolide synthesis, were significantly upregulated, the DEGs encoding BSK and CYCD3, the signaling regulator of brassinolide, were significantly upregulated. Ethylene related regulatory genes had different expression patterns. Salicylic acid related regulatory genes were essentially downregulated. The upregulation of jasmonic acid metabolism regulatory genes were upregulated. The functions of these genes in seed dormancy release need further investigation.

In this study, the combined transcriptome and metabolome analysis enriched the starch and sucrose metabolic pathways, which we hypothesized to be the major energy supply pathways during the lifting of dormancy and the initiation of germination in perilla seeds, and therefore we mapped the starch and sucrose metabolic pathways associated with the dormancy released in perilla seeds (Fig. 5). Starch and Sucrose are stored in plant seeds that can be converted to reducing sugars and plays an important role in seed germination[57], Sucrose can also act as a transport compound that is produced in the endosperm and moves into the embryo[7]. In the study, by upregulation of the DEGs encoding ENPP1_3, glgc, and WAXY, UDP-glucose synthesized starch, which is further converted into maltose. The changes of Maltose content also confirmed this. We found that the expression of regulatory genes of UDP-glucose metabolism and catabolism-related enzymes TPS was significantly up-regulated to promote their conversion to Trehalose. UDP glucose is also converted into 1,3-beta-Glucan, Cellulose, and Cellodextrin/Cellulose. The above substances were further converted into D-glucose and enter the energy supply.

## Conclusion

The molecular mechanism of Perilla seed dormancy release caused by GA3 was analyzed by transcriptomic and metabolomic. The results revealed that plant hormone signal transduction, starch and sucrose metabolism and citric acid cycle (TCA cycle) were significantly enriched pathways closely related to perilla seed dormancy release. Perilla seeds release their dormancy by enhancing the expression of GID1, PIF3, SnRK2, IAA, ARR-A, GH3, MKK4_5, otsB, GN1_2_3, glgC, WAXY, inhibiting the expression of DELLA, PP2C, glga, bglX, and GN4, and regulating the content of GA4, ABA, IAA, sucrose, maltose, trehalose, and α-D-glucose 1-phosphate. IAA plays an important role in breaking seed dormancy and promoting seed germination. The energy required for breaking seed dormancy and germination of perilla seeds is mainly provided through sucrose metabolism. Citric acid cycle (TCA cycle) is the main energy supply transformation pathway for seed germination. These findings provide a basis for further understanding the mechanism of seed dormancy release by GA3 and provide a valuable reference for breeding Perilla varieties with high germination rates.

## Materials and methods

### Perilla seed materials and treatment conditions

The seeds of Perilla frutescens were collected from Lianhe village (106°65′24″E, 26°62′09″N, 1055 m above sea level), Changshun County, and Qiannan Prefecture, with the permission of the Guizhou Academy of Agricultural Sciences in China. The sample plant name code M1201 was Perilla frutescens var. frutescens.

The two groups of Perilla seeds were soaked in 200 mg/L $GA_3$ solution and water for 8 h, and the other groups of seeds were left at room temperature to release dormancy naturally. Afterward, the seeds were naturally dried, after which a standard germination test was performed. The $GA_3$-soaked seeds are represented by GA1, GA2, GA3, the water-soaked seeds are represented by WA1, WA2, WA3, and the natural dormancy-release seeds are represented by CK1, CK2, CK3. Most seeds in the GA and CK groups presented exposed radicles and released dormancy, and the WA was not relieved. Approximately 0.1 g of seeds from each sample was taken as the test material for transcriptome and metabolomic analysis. Three independent biological replicates were performed for each group.

### Transcriptome data analysis

RNA was extracted from seeds by standard extraction method, and the quality of the RNA samples was controlled by Agilent 2100 bioanalyzer. After the construction of the library, first use Qubit2.0 fluorometer for preliminary quantification, then use Agilent 2,100 bioanalyzer to detect the insert size of the library, and qRT-PCR was used to accurately quantify the effective concentration of the library (the effective concentration of the library was greater than 2 nM) to ensure the quality of the library. After the library was qualified, carry out Illumina sequencing.

Use fastp software[58] to control the sequencing raw data of each sample, use HISAT2 software[59] to compare the clean data reads after quality control to the reference genome, and then Qualimap RNA-seq software[60] was used to evaluate the comparison. The featureCounts software[61] in the subread software package was used to calculate the gene count, the formula in the reference documents of FPKM and TPM was calculated, the genes with the detection rate (the proportion of count was not 0) less than 0.25 was screened out, the R language DESeq2 package[62] and edgeR package[63] were used to calculate the corrected expression, the correlation coefficient between the two samples was calculated, and the repeatability of biological experiments in the sample group was evaluated. The gene expression value (TPM) of all the samples were analyzed by PCA. The differential genes were screened with the standard of log2 (Fold Change) ≥ 1.00 and padj ≤ 0.05 by using DESeq2 software. The R language clusterProfiler package[64] was used to conduct GO function enrichment analysis and KEGG pathway enrichment analysis on the differential gene set.

### Validation of transcriptomic data for real-time quantitative reverse transcription PCR (qRT-PCR)

To verify the accuracy of our transcriptomic data, fifteen DEGs involved in Perilla seed dormancy release were selected for qRT-PCR, and specific primers for these genes were designed with Primer 6.0 (Table 1). Reverse transcription amplification was carried out with Goldenstar RT6 cDNA Synthesis Mix, the Qingke reverse transcriptional kit. The cDNA product obtained by reverse transcription was diluted 4 times and used as a qPCR template × T5 Fast qPCR Mix (SYBR Green I) for amplification, and the components of the amplification system were as follows: 2 × T5 Fast qPCR Mix (SYBR Green I) 10 μL, 10 μM primer F 1 μL, 10 μM primer R 1 μL, template (cDNA) 1 μL, and ddH2O 7 μL, for a total of 20 μL. The above amplification system was amplified according to the following amplification procedure and then fused: pre-denatured 95 °C for 2 min for 1 cycles; in the cycle stage, 95 °C 15 s 40cycles, 60 °C 20 s, 72 °C 20 s read fluorescence here; At the melting stage, 95 °C 5 s 1cycles, 65 °C 1 min 1cycles, 95 °C 0.05 °C/s heating 1cycles continuously collected fluorescence for 5 times/℃, 50 °C 30 s 1cycles. All the samples were examined in triplicate with four technical replicates. The relative expression of genes was calculated by the 2-DDCt method using actin as the reference gene[65,66].

### Metabolite extraction and metabolomic data processing and analysis

Accurately weigh 200 mg (± 1%) of sample in 2 mL EP tube, and add 0.6 mL 2-chlorophenylalanine (4 ppm) methanol (−20 °C), vortex for 30 s; Add 100 mg glass beads and grind the samples by a high-throughput tissue grinder for 90 s at 60 Hz.Room temperature ultrasound for 30 min and ice for 30 min; centrifuge at 4 °C for 10 min at 12,000 rpm, and the supernatant was filtered through 0.22 μm membrane to obtain the prepared samples for LC–MS; Take 20 μL from each sample to the quality control (QC) samples; Use the rest of the samples for LC–MS detection[67–69]. The XCMS package of R (v3.1.3) is used for peak identification, peak filtration, and peak alignment to obtain the data matrix, and then the precursor molecules in the positive and negative ion mode are obtained. Based on R language MetaboAnalyst R package[67], QC quality control, batch calibration, data standardization, metabolite content statistics, PCA, screening of characteristic metabolites, correlation analysis and path analysis of characteristic metabolites (enrichment analysis, topology analysis, path map) were carried out. DIMs and DEGs were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) databaseand an automated annotation server[70]. Hierarchical clustering of key DIMs in different groups using ComplexHeatmap 2.12.0[71]. Gene-metabolite pairs with Pearson's correlation coefficients > 0.9 in pathways associated with seed aging between cultivars were used to construct the transcript-metabolite network.

## Data availability

The RNA-seq datasets generated during the current study have been submitted to the NCBI Sequence Read Archive under the accession number PRJNA1080333 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1080333?reviewer=5afc4h1td9qod0g17quislksvm, accessed on 04 Dec 2024).

Received: 18 March 2024; Accepted: 18 February 2025
Published online: 05 March 2025

## References


1. Oracz, K. & Karpiński, S. Phytohormones signaling pathways and ROS involvement in seed germination. *Front. Plant Sci.* **7**, 864 (2016).
2. Finch-Savage, W. E. et al. Seed dormancy and the control of germination. *New Phytol.* **171**(3), 501–523 (2006).
3. Nelson, S. K., Tohru, A. & Steber, C. M. Biology in the dry seed: Transcriptome changes associated with dry seed dormancy and dormancy loss in the arabidopsis ga-insensitive sleepy 1–2 mutant. *Front. Plant Sci.* **8**, 2158 (2017).
4. Baskin, C. C. & Baskin, J. M. Seeds: Ecology, biogeography, and evolution of dormancy and germination. *Crop Sci.* **40**(2), 5–64 (2014).
5. Linkies, A. et al. *The Evolution of Seeds* (IEEE, 2010).
6. Tognacca, R. S. et al. Alternative splicing regulation during light-induced germination of *Arabidopsis thaliana* seeds. *Front. Plant Sci.* **10**, 1076–1076 (2019).
7. Li, B. et al. Integrated analysis of the transcriptome and metabolome revealed candidate genes involved in GA3-induced dormancy release in Leymus chinensis seeds. *Int. J. Mol. Sci.* **22**, 4161 (2021).
8. Miransari, M. & Smith, D. L. Plant hormones and seed germination. *Environ. Exp. Bot.* **99**(3), 110–121 (2014).
9. Graeber, K. et al. Cross-species approaches to seed dormancy and germination: conservation and biodiversity of ABA-regulated mechanisms and the Brassicaceae DOG1 genes. *Plant Mol. Biol.* **73**, 67–87 (2010).
10. Nambara, E. Abscisic acid and the control of seed dormancy and germination. *Seed Sci. Res.* **20**(2), 55–67 (2010).
11. Penfield, S., Li, Y., Gilday, A. D., Graham, S. & Graham, I. A. Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. *Plant Cell* **18**, 1887–1899 (2006).
12. Penfield, S., Li, Y., Gilday, A. D., Graham, S. & Graham, I. A. Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. *Plant Cell* **18**(8), 1887–1899 (2006).
13. Dave, A. et al. Regulation of arabidopsis thaliana seed dormancy and germination by 12-oxo-phytodienoic acid. *J. Exp. Bot.* **67**(8), 2277–2284 (2016).
14. Fu, X. et al. The Arabidopsis mutant sleepy1gar2–1 protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates. *Plant Cell* **16**, 1406–1418 (2004).
15. Ueguchi-Tanaka, M. et al. Gibberellin receptor and its role in gibberellin signaling in plants. *Annu. Rev. Plant Biol.* **58**(1), 183–198 (2007).
16. Mitchum, M. G. et al. Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. *Plant J.* **45**, 804–818 (2010).
17. Jiang, Z., Xu, G., Jing, Y., Tang, W. & Lin, R. Phytochrome B and REVEILLE1/2-mediated signaling controls seed dormancy and germination in Arabidopsis. *Nat. Comm.* **7**, 12377 (2016).
18. Claeys, H., Bodt, S. D. & Inzé, D. Gibberellins and DELLAs: central nodes in growth regulatory networks. *Trends Plant Sci.* **19**(4), 231–239 (2014).
19. Cutler, S. et al. Abscisic acid: emergence of a core signaling network. *Annu. Rev. Plant Biol.* **61**, 651–679 (2010).
20. Finkelstein, R. Abscisic acid synthesis and response. *Arabidopsis Book* **11**(e0166), e0166–e0166 (2013).
21. Bewley, J. D. Seed germination and dormancy. *Plant Cell* **9**(7), 1055–1066 (1997).
22. Seo, M. et al. Regulation of hormone metabolism in Arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. *Plant J.* **48**, 354–366 (2006).
23. Yang, L. et al. Expression patterns of ABA and GA metabolism genes and hormone levels during rice seed development and imbibition: A comparison of dormant and non-dormant rice cultivars. *J. Genet. Genom.* **41**, 327–338 (2014).
24. Shu, K. et al. ABI4 regulates primary seed dormancy by regulating the biogenesis of abscisic acid and gibberellins in Arabidopsis. *PLoS Genet.* **9**, e1003577 (2013).
25. Liu R, Lu J, Xing J et al. Transcriptome and metabolome analyses revealing the potential mechanism of seed germination in Polygonatum cyrtonema. (2020).
26. Siobhan, M. B. & McCourt, P. Hormone cross-talk in seed dormancy. *J. Plant Growth Regul.* **22**, 25–31 (2003).
27. Tsuchiya, Y. Thermoinhibition uncovers a role for strigolactones in Arabidopsis seed germination. *Plant Cell Physiol.* **53**(1), 107–117 (2012).
28. Shu, K. et al. Two faces of one seed: Hormonal regulation of dormancy and germination. *Mol. Plant* **9**(001), 34–45 (2016).
29. Wang, W. et al. Transcriptome profiling provides insights into dormancy release during cold storage of Lilium pumilum. *BMC Genom.* **19**, 196 (2018).
30. Holdsworth, M. J., Bentsink, L. & Soppe, W. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytol.* **179**(1), 33–54 (2008).
31. Liu, L. et al. Salinity inhibits rice seed germination by reducing α-amylase activity via decreased bioactive gibberellin content. *Front. Plant Sci.* https://doi.org/10.3389/fpls.2018.00275 (2018).
32. Zhen, X. et al. Salicylic acid inhibits gibberellin-induced alpha-amylase expression and seed germination via a pathway involving an abscisic-acid-inducible WRKY gene. *Plant Mol. Biol.* **64**(3), 293–303 (2007).
33. Kaneko, M. et al. The alpha-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium. *Plant Physiol.* **128**(4), 1264–1270 (2002).
34. Ma, Z. et al. Glyoxylate cycle and metabolism of organic acids in the scutellum of barley seeds during germination. *Plant Sci.* **248**, 37–44 (2016).
35. Jia, Y. J. et al. Effects of different oligochitosans on isoflavone metabolites, antioxidant activity, and isoflavone biosynthetic genes in soybean (glycine max) seeds during germination. *J. Agric. Food Chem.* **67**(16), 4652–4661 (2019).
36. Takatoshi, W. et al. Planteose as a storage carbohydrate required for early stage of germination of Orobanche minor and its metabolism as a possible target for selective control. *J. Exp. Bot.* **11**, 3085 (2015).
37. Zhuang, W. et al. Metabolic changes upon flower bud break in Japanese apricot are enhanced by exogenous GA4. *Hortic. Res.* **2**(1), 10 (2015).
38. Peiretti, P. G. Fatty acid content and chemical composition of vegetative parts of perilla (Perilla frutescens L.) after different growth lengths. *Res. J. Med. Plant* **5**(1), 72–78 (2011).
39. Asif, M. Health effects of omega-3,6,9 fatty acids: perilla frutescens is a good example of plant oils. *Oriental Pharm. Exp. Med.* **11**(1), 51–59 (2011).
40. Yamazak, M. et al. Differential gene expression profiles of red and green forms of Perilla frutescens leading to comprehensive identification of anthocyanin biosynthetic genes. *FEBS J.* **275**, 3494–3502 (2008).
41. Keiko, Y. S. et al. Molecular and biochemical characterization of a novel hydroxycinnamoyl-CoA: Anthocyanin 3-O-glucoside-6″-O-acyltransferase from Perilla frutescens. *Plant Cell Physiol.* **41**(4), 495–502 (2000).
42. Ito, M., Honda, G. & Sydara, K. Perilla frutescens var. frutescens in northern Laos. *J. Nat. Med.* **62**, 251–258 (2008).
43. Zhang, T. Y. et al. RNA sequencing and coexpression analysis reveal key genes involved in α-linolenic acid biosynthesis in Perilla frutescens seed. *Int. J. Molecular ences* **18**(11), 2433 (2017).

44. Sa, K. J. et al. Identification of genetic variations of cultivated and weedy types of perilla, species in Korea and Japan using morphological and ssr markers. *Genes Genom.* **35**, 649–659 (2013).
45. Zhang, Y. et al. Incipient diploidization of the medicinal plant Perilla within 10,000 years. *Nat. Commun.* **12**(1), 5508 (2021).
46. Weitbrecht, K., Müller, K. & Leubner-Metzger, G. First of the mark: Early seed germination. *J. Exp. Bot.* **62**, 3289–3309 (2011).
47. Yamaguchi, S. Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* **59**, 225–251 (2008).
48. Xian, B. S. et al. The ABI4-RGL2 module serves as a double agent to mediate the antagonistic crosstalk between ABA and GA signals. *New Phytol.* **241**(6), 2464–2479 (2024).
49. Mcginnis, K. M. et al. The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. *Am. Soc. Plant Biol.* **15**, 1120–1130 (2003).
50. Strader, L. C. et al. Recessive-interfering mutations in the gibberellin signaling gene SLEEPY1 are rescued by overexpression of its homologue, SNEEZY. *Proc. Natl. Acad. Sci. U. S. A.* **101**(34), 12771–12776 (2004).
51. Urbanova, T. & Leubner-Metzger, G. Gibberellin and seed germination. In *Annual Plant Reviews, Volume 49: The Gibberellins* (eds Hedden, P. & Thomas, S. G.) 253–284 (Wiley, 2016).
52. Preston, J. et al. Temporal expression patterns of hormone metabolism genes during imbibition of *Arabidopsis thaliana* seeds: A comparative study on dormant and dormant accessions. *Plant Cell Physiol.* https://doi.org/10.1093/pcp/pcp121 (2009).
53. Ayele, B. T., Ozga, J. A., Wickramarathna, A. D. & Reinecke, D. M. Gibberellin metabolism and transport during germination and young seedling growth of pea (*Pisum sativum* L.). *J. Plant Growth Regul.* **31**, 235–252 (2012).
54. Liu, A. et al. Regulation of wheat seed dormancy by after-ripening is mediated by specific transcriptional switches that induce changes in seed hormone metabolism and signaling. *PLoS ONE* **8**, e56570 (2013).
55. Corbineau, F. et al. Ethylene, a key factor in the regulation of seed dormancy. *Front. Plant Sci.* **5**(539), 539 (2014).
56. Menghan, S. et al. Ethylene regulates post germination seedling growth in wheat through spatial and temporal modulation of ABA/GA balance. *J. Exper. Bot.* **71**, 1985–2004 (2020).
57. Major, L. B. J. et al. Mechanisms leading to excess alpha -amylase activity in wheat (*Triticum aestivum*, L) grain in the U.K. *J. Cereal Sci.* **33**(3), 313–329 (2001).
58. Chen, S. & Zhou, Y. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 (2018).
59. Kim, D., Langmead, B. & Salzberg, S. L. HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods* **12**, 357–360 (2015).
60. Okonechnikov, K., Conesa, A. & García-Alcalde, F. Qualimap v2: advanced quality control ofhigh throughput sequencing data. *Bioinformatics* https://doi.org/10.1093/bioinformatics/btv566 (2015).
61. Yang, L., Smyth, G. K. & Wei, S. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **7**, 923–930 (2013).
62. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**(12), 550 (2014).
63. Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**(1), 139 (2010).
64. Yu, G. et al. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS J. Integr. Biol.* **16**(5), 284–287 (2012).
65. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods Companion Methods Enzymol.* **25**, 402–408 (2001).
66. Chen, S. et al. Transcriptome analysis reveals common and distinct mechanisms for sheepgrass (Leymus chinensis) responses to defoliation compared to mechanical wounding. *PLos One* **9**(2), e89495 (2014).
67. Ponnusamy, K. et al. Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *J. Med. Microbiol.* **60**(6), 817–827 (2011).
68. Ng, J. S. Y. et al. Development of an untargeted metabolomics method for the analysis of human faecal samples using cryptosporidium-infected samples. *Mol. Biochem. Parasitol.* **185**(2), 145–150 (2012).
69. Sangster, T. et al. A pragmatic and readily implemented quality control strategy for HPLC-MS and GC-MS-based metabonomic analysis. *Analyst* **131**(10), 1075–1078 (2006).
70. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
71. Eon, J. E. et al. A pathogen-responsive gene cluster for highly modified fatty acids in tomato. *Cell* **180**(1), 176–187 (2020).


## Author contributions

He Wen and Sen Yang conceived and designed the research. Zhiwei Shang, Xingyue Li and Ping Guo conducted the experiments. Shunbo Yu and Heng Zhang contributed new reagents. Shimei Yang and He Wen analyzed the data. He Wen and Sen Yang wrote the manuscript. All the authors read and approved the manuscript. He Wen and Sen Yang contributed equally to this work.

## Funding

This research was funded by Guizhou Provincial Key Technology R&D Program (No. Qian Kehe Support [2023] General 059, No. Qian Kehe Support [2023] General 067, No.Qian Science and Technology Service Enterprise [2021] No. 13); Tongren City Science and Technology Plan Project (Research in Tong City {2023}73); Guizhou Academy of Agricultural Sciences technolog- ical innovation (2022) No.12; Qian Academy of Agricultural Sciences after the national foundation subsidy (2021) No. 29; Guizhou Academy of Agricultural Sciences Germplasm Resources (2023)No.15. The founder has no role in the study design; in the data collection, analysis, and interpretation; or in writing the manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-91039-3.

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