

Integrated multi-omics approach to reveal the molecular networks underlying the seed germination of *Polygonatum cyrtoneura*

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Abstract

Background

Polygonatum cyrtoneura (*P. cyrtoneura*), a famous traditional medicinal resource for over two thousand years in China, is in high demand with the vigorous development of the health industry. The rhizome propagation could easily lead to germplasm degradation, severe pests, diseases, etc., and thus, the seed propagation method is a superior alternative to artificially cultivating seedlings on a large scale. However, the seeds of *P. cyrtoneura* need at least two years dormancy period under natural circumstances to undergo morphological and physiological maturation, and germination of seeds has not been fully understood. To reveal the mechanisms of hormone crosstalk and metabolic process regulating germination, transcriptomic, proteomic and plant hormonomics analyses of *P. cyrtoneura* seeds in three different germinating stages (the fresh mature seed, named S1, the emergence of radicle, named S2 and the phase between primary rhizome and cotyledon emergence, named S3) were performed.

Results

Transcriptome analysis revealed that 11,565 differentially expressed genes (DEGs) between S1 and S2, 10,196 between S1 and S3, 7,696 between S2 and S3. Proteome analysis revealed 647 differentially expressed proteins (DEPs) between S1 and S2, 1720 between S1 and S3, and 1475 between S1 and S3. Comprehensive transcriptomic and proteomic analysis indicated that metabolism and plant hormone signal transduction might be the main pathways involved in seed germination of *P. cyrtoneura*. In addition, several proteins, including abscisic acid receptor PYL4 (PYL4) and protein phosphatase 2C (PP2C) bind ABA to form PYL-ABA-PP2C complex, associated with ABA signaling, were down-regulated between S1 and S3. And the sucrose synthase (SUS), involving in the antagonism between ABA and GA, decreased between S1 and S3. While, these proteins such as beta-fructofuranosidase (INV), alpha-xylosidase (α -Xyl), beta-D-xylosidase (β -D-Xyl) and beta-glucosidase (bglX), related to starch and sucrose metabolism, were up-regulated between S1 and S3. Hormone content detection showed that ABA, GAs, IAA, JA and SA decreased significantly, while, the ratio of GAs/ABA increased distinctly from S1 to S3, suggesting that transduction in plant hormones, especially ABA-GA antagonism might regulate seed germination.

Conclusion

The current study showed a possible schematic and a novel insight to explain the dynamic seed germination mechanism of *P. cyrtoneura*, and emphasized that phytohormone cross-talk, ABA signaling and shydrolisis of starch to glucose might play an important role in regulating seed germination.

1. Background

Polygonatum cyrtoneura (*P. cyrtoneura*) belongs to the Liliaceae family and has a wide distribution in southern China [1](Xia et al., 2022). The dried rhizomes of *P. cyrtoneura* have been used as Huangjing (HJ), which is a famous traditional medicine resource for over two thousand years in China, benefiting the lung, spleen, kidney and so on [2, 3]. Recently, with the vigorous development of the health industry in China, HJ has attracted great attention for treating fatigue, diabetes and premature graying of hair. The demand for HJ raw materials is increasing, *P. cyrtoneura* has become endangered due to the uncontrolled excavation of natural resources. At present, rhizome was predominantly used for the asexual propagation in the cultivation of *P. cyrtoneura*. However, this costly method often results in germplasm degradation, low seedling emergence rates, uneven emergence, severe pests and diseases, etc., which affects the yield, quality and promotion of medicinal herbs. Therefore, seed propagation is the most efficient method for growing *P. cyrtoneura* seedlings for large-scale artificial cultivation.

The seed dormancy, caused by the underdeveloped and immature embryo, is a natural mechanism that adjusts to the unfavorable environmental conditions [4–6]. It takes at least two years for *P. cyrtoneura* seeds to germinate after being released from mature capsules under natural conditions [7–9]. Waxy and hard seed coats are made up of lignified cells that prevent water absorption and inhibit germination [10]. These studies had shown that the dormancy of *P. cyrtoneura* seeds might be caused by the immature embryo, which was wrapped in a thick and hard endosperm [10], with a low content of gibberellic acid (GA) and cytokinin (CK), and a high content of abscisic acid (ABA) [7, 11]. In addition, seed germination is physically hindered by lignin, a secondary metabolite of plants [3]. As a result, even under otherwise suitable germination conditions, these dormant seeds are unable to germinate. While, treatment with appropriate concentrations of exogenous GA3 could stimulate the germination of dormant seeds [12, 13]. Moreover, the study showed that differentially expressed genes (DEGs) related plant hormones play a critical role in regulating dormancy and germination of *P. cyrtoneura* seeds. And, these DEGs were significantly regulated in phenylpropanoid metabolism, flavonoid biosynthesis, starch and sucrose metabolism pathways [3]. Therefore, seed germination of *P. cyrtoneura* may be a complex process, in which many physiological and biochemical reactions take place, including signal transduction, reactivation of metabolism, and redox homeostasis regulation. However, seed germination by *P. cyrtoneura* remains poorly understood, and elucidating the mechanism may be helpful in breaking dormancy and reducing breeding time.

A combined transcriptomic and proteomic analysis is crucial for identifying the regulatory networks governing seed germination genes and proteins [14]. These studies on seed germination had revealed that DEGs and differentially expressed proteins (DEPs) were highly enriched in metabolic pathways related to amino acids, lipids, carbohydrates, secondary metabolites, glycolysis and gluconeogenesis [15, 16]. However, transcriptional changes do not always translate into proteins, and there is a poor correlation between mRNA and protein expression.

It was very difficult to raise seedlings and utilize resources of *P. cyrtoneura* because of seed dormancy and low germination rate. In the present study, the seeds of *P. cyrtoneura* were incubated with wet sand to investigate seed germination, and three different stages of the seed (the fresh mature seed, named S1,

the emergence of radicle, named S2 and the phase between primary rhizome and cotyledon emergence, named S3) were used to perform transcriptomic, proteomic and plant hormonomics analyses. The study of *P. cyrtanema* seed germination revealed regulation of seed germination by transcription and translation, and phytohormone cross-talk triggered seed germination.

2. Results

2.1 RNA-seq denovo assembly and function annotation of *Polygonatum cyrtanema*

To detect gene expression information during seed germination, nine samples (S1-1, S1-2, S1-3, S2-1, S2-2, S2-3, S3-1, S3-2, S3-3) were sequenced using Illumina HiSeq. A total of 197,611 unigenes and 207,157 transcripts were detected, and the lengths of unigenes ranged mostly from 200 to 300 bp (Fig. 1B and Table S1). The number of unigenes were 775,597(88.45%), 53,754 (61.27%), 47,394 (54.02%), 43,817 (49.95%), 40,423 (46.08%), 40,112 (45.72%), and 30,132 (3.35%) annotated in the NR, Swiss-Prot, Pfam, KOG, GO, KEGG and COG database, respectively (Fig. 1C and Table S1). The functional information of homologous sequences in related species showed that the unigenes sequences had 19.35%, 6.40%, 5.68%, 2.21%, 2.18%, 1.63%, 1.24%, 1.16%, 0.78%, 0.75%, and 58.63% similarity with *Asparagus officinalis*, *Fusarium sp.*, *Rhizoctonia solani*, *Elaeis guineensis*, *Neonectria ditissima*, *Phoenix dactylifera*, *Ensete ventricosum*, *Nothophytophthora sp.*, *Fusarium oxysporum*, *Fusarium ambrosium*, and other genomes, respectively (Fig. 1D).

2.2 Identification and analysis of differentially expressed genes (DEGs)

Figure 2A showed that the correlation coefficients of sample replicates were very high, suggesting that the sequencing data were reliable. The number of unigenes identified at S1, S2 and S3 was 56,606, 71,097 and 54,818 respectively, and only 21,290 unigenes were common in the three groups (Fig. 2B). There were 11,565 DEGs between S1 and S2, 10,196 between S1 and S3, 7,696 between S2 and S3 (Table S2). Figure 2C showed the number of DEGs up-regulated and down-regulated. According to KEGG enrichment analysis, these DEGs enriched 339, 340, and 321 pathways, among which 30 were significantly enriched (Fig. 2E). Out of the 30 pathways spanning seed germination, phenylpropanoid biosynthesis, stilbenoid, diarylheptanoid and gingerol biosynthesis, flavonoid biosynthesis, plant hormone signal transduction, toll-like receptor signaling pathway, NF-kappa B signaling pathway, starch and sucrose metabolism exhibited the greatest enrichment. Therefore, the results indicate that plant hormone signal transduction pathways, starch metabolism pathways, and sucrose metabolism pathways might play a role in seed germination.

2.3 Identification and analysis of differentially expressed proteins (DEPs)

Analysis of seed proteome profiles was conducted. A total of 4228 proteins were detected, and the distribution of protein abundance in three different stages of seed germination had nearly the same general shape (Fig. 3A and Table S3). These identified proteins were then analyzed for GO enrichment. In Fig. 3B, cell was the most enriched subclass among cellular components, followed by cell part, organelle, and membrane. Catalytic activity ranked as the top subclass in the category of molecular function, followed by binding and localization. In the biological process category, metabolic process topped the list, followed by cellular process, and biological regulation. The results showed that 647 proteins (449 up- and 198 down-regulated) between S1 and S2, 1720 (1044 up- and 676 down-regulated) between S1 and S3, and 1475 (848 up- and 627 down-regulated) between S1 and S3, were differentially expressed (Fig. 3C). The KEGG annotations indicated that hydroxyindole-O-methyltransferase, UDP-glucuronosyl and UDP-glucosyl transferase, aspartyl protease and FOG:RRM domain in between S1 and S2, FOG:RRM domain, aspartyl protease, serine/threonine protein kinase and UDP-glucuronosyl and UDP-glucosyl transferase between S1 and S3, FOG:RRM domain, aspartyl protease, glyceraldehyde 3-phosphate dehydrogenase and iron/ascorbate family oxidoreductases between S2 and S3, were significantly enriched, respectively (Fig. 3D).

2.4 Conjoint analysis of transcriptomics and proteomics

An integrated transcriptomic and proteomic analysis was performed to investigate the correlation between transcript and protein levels. The expression between genes and proteins were shown in Fig. 4A, Fig. 4B, Fig. 4C and Table S4. There were 240 translational changes, 76 opposite changes, 220 homodirectional changes between S1 and S2, 735 translational changes, 131 opposite changes, 646 homodirectional changes between S1 and S3, 888 translational changes, 124 opposite changes, 333 homodirectional changes between S2 and S3.

KEGG enrichment analysis was then performed on these three responsive modules. In Fig. 4D, aspartyl protease was the most enriched subclass among homodirectional category, followed by β -fructofuranosidase, UDP-glucuronosyl and UDP-glucosyl transferase. molecular chaperone (small heat – shock protein Hsp26/Hsp42) ranked as the top subclass in the opposite category, followed by chitinase and predicted chitinase. In the translational category, FOG:RRM domain topped the list, followed by β -fructofuranosidase (invertase) and arylacetamide deacetylase. According to the KEGG enrichment analysis of *P. cyrtonema*, genetic information processing (ribosome, protein processing in endoplasmic reticulum, spliceosome, RNA transport), metabolism (starch and sucrose metabolism, cyanoamino acid metabolism, anthocyanin biosynthesis, phenylpropanoid biosynthesis, stilbenoid, diarylheptanoid and gingerol biosynthesis, amino sugar and nucleotide sugar metabolism, ubiquinone and other terpenoid-quinone biosynthesis), and signal transduction (plant hormone signal transduction) might be the main pathways involved in seed germination.

2.5 Phytohormone levels

A dynamic profile of ABA, IAA, JAs, SAs, GAs, CKs and IBA was detected at stages S1, S2, and S3 of *P. cyrtonema* seeds to monitor phytohormone levels. (Fig. 5B, Fig. 5C and Table S5). The ABA, JA and total

GAs levels exhibited the same patterns, which decreased significantly from S1 to S2, and increased at S3 stage. The IAA and SA content were down-regulated across three stages, decreased significantly from S1 to S3, respectively. While, the level of CKs varied insignificantly and maintained at a low level, and IBA was not detected in these samples. The ratio of GAs/ABA increased significantly from S1 to S2, and maintained at a high level at S3.

2.6 The phytohormones associated genes/proteins

Across seed germination, 483 unigenes and 72 proteins are implicated in plant hormone signal transduction. (Fig. 5A and Table S6). But among them, only 41 DEPs were identified. Several phytohormone signal transduction-related genes showed a consistent trend of changes in mRNA and protein expression. For example, the genes encoding transport inhibitor response 1 protein (TIR1) and GH3 auxin-responsive promoter (GH3) related auxin, abscisic acid receptor PYL2 (PYL2), abscisic acid receptor PYR1 (PYR1) and gibberellin regulated protein (GRP) related ABA and NADH oxidase (Nox) related JA related brassinosteroid, all exhibited increased transcripts and protein abundance between S1 and S2 (Table S6). Other genes/proteins between S2 and S3, such as the genes encoding nucleoside diphosphate kinase (NDK), abscisic acid receptor PYR1 (PYR1), PYL2, 2-oxoglutarate-dependent dioxygenase (2OGD) and gibberellin receptor GID1 (GID1) showed opposite transcription levels (Table S6). The dynamic profiles of hormone in three different stages of seed germination were shown in Fig. 5B, Fig. 5C and Table S5. Interestingly, the ABA, JA, SA, IAA and total GAs level decreased significantly from S1 to S2 or S3. In general, the up- and down-regulation of these genes/proteins could result in an imbalance of phytohormones during seed germination.

2.7 Multiple molecular networks mediated by differentially abundant proteins

During seed germination, multiple metabolic levels were modulated by partial differentially abundant genes and proteins, resulting in a possible schematic representation of molecular networks. In Fig. 6 and Table S7, 163 proteins were shown to describe the KEGG categories, including organismal systems, signal transduction, cellular processes, genetic information processing, energy and carbohydrate metabolism, lipid and nucleotide metabolism, amino acid metabolism, and biosynthesis of other secondary metabolites, in the seed germination.

3. Discussion

It has been reported that the dormancy in *P. cyrtoneura* seeds typically is complex, requiring sequential the physiological after-ripening to break dormancy of shoot and root [3, 7, 8, 9]. It usually takes 2–3 years for seeds to germinate under natural circumstances, and the survival rate of seedlings is low [7, 9]. *P. Cyrtoneura* seed germination was comprehensively studied through transcriptomic and proteomic analyses, and global changes in mRNA and protein levels were observed (Fig. 2, Fig. 3 and Fig. 4). Furthermore, according to previous studies, there was little correlation between transcriptomic and

proteomic data, with less than 20% of proteins correlated at the transcriptional level [17–22]. The KEGG annotations showed that stilbenoid, diarylheptanoid and gingerol biosynthesis, phenylpropanoid biosynthesis, flavonoid biosynthesis and plant hormone signal transduction for DEGs (Fig. 2C), but aspartyl protease, FOG:RRM and β -fructofuranosidase for DEPs (Fig. 3D), were significantly enriched, respectively. In fact, many DEPs implicated in metabolic and signal pathways had been identified through proteome studies, which provided a molecular explanation for seed germination since these proteins were directly involved in biological activity.

There are a number of metabolic and structural changes that occur during seed germination, such as the mobilization of active reserves, the repair of DNA templates, the de novo synthesis of proteins, rapid division of cells and so on [23]. It is well known that the germination process is regulated by a complex metabolic and signaling network that is highly sensitive to phytohormone changes [23]. In *P. cyrtonema* seed germination studies, signal transduction, carbohydrate, lipid and amino acid metabolism, as well as secondary metabolite synthesis, have been identified as pathways involved in germination [15, 16, 24]. In present study, a total of 116 key proteins were identified across the seed germination of *P. cyrtonema*. Among them, 54 proteins were related to metabolism, 41 to signal transduction, 21 to organismal systems, 19 to genetic information processing, and 9 to cellular processes, respectively (Fig. 5).

A variety of metabolisms are activated in cells during seed germination, providing essential nutrients and energy to seedlings [23]. For example, a large amount of starch in the endosperm is gradually degraded, serving as a primary source of ATP and precursor for anabolic reactions in plant embryos [25]. In the study, several key proteins related to the metabolism of starch and sucrose, such as beta-fructofuranosidase (INV), alpha-xylosidase (α -Xyl), beta-D-xylosidase (β -D-Xyl) and beta-glucosidase (bglX) associated with seed germination, exhibited increased abundance between S1 and S3. The seeds of *P. cyrtonema* contained a significant amount of starch in endosperm, which was converted into glucose by hydrolysis to provide the primary source of energy for seedling emergence [3]. It had reported that INV, α -Xyl, β -D-Xyl and bglX were the major enzymes related to hydrolyze starch into glucose [26, 27]. Especially, the α -Xyl and β -D-Xyl weakened the endosperm cell wall by modulating the structure of xyloglucan during seed germination [28, 29]. And the decomposition of starch could promote seed sprouting [30]. Thus, these proteins, which were related to metabolism, might play crucial role in seed germination.

Signal transduction, such as the plant hormone signal transduction, controlled environmental responses during seed dormancy and germination. Plant hormones, including ABA, IAA and GA, were key regulators for seed germination [31, 32]. For instance, ABA promotes seed dormancy and inhibits seed germination by suppressing the loosening and expansion of cell wall [33]. In this study, the content of ABA is higher in S1 (Fig. 5B and Table S5), which might be due to the seed embryo was surrounded by a thick and hard endosperm, blocking its transport. While, it decreased significantly and maintained at a low level in the process of physiological after-ripening. Several key proteins involved in ABA signaling, such as the abscisic acid receptor PYL4 (PYL4), protein phosphatase 2C (PP2C) and serine/threonine-protein kinase SAPK3-like (SNRK2), exhibited decreased abundance between S1 and S3. The binding of ABA to PYL4

causes PP2C to lose the phosphatase activity, and the inhibition of PP2Cs enables activation of SnRK2 which consequently phosphorylated ABA-responsive element binding factors to induce the expression of ABA-related genes [34–37], such that the seed germination might be inhibited in S1. While, the PP2C phosphatases inhibit SNRK2 in S3, such that the ABA signal transduction pathway is in the “off” state and seed might start to germinate [34, 38]. Thus, ABA may play a major role in the regulation of *P. cyrtonema* seed germination.

It has been proven that IAA and JA work synergistically with ABA to regulate seed dormancy and germination [39–41]. In this study, the contents of IAA and JA were down-regulated from S1 to S2, which aligned with the decreasing trend of ABA (Fig. 5C and Table S5). In addition, 11 proteins, including auxin-binding protein (ABP), transport inhibitor response 1 protein (TIR1), GH3 auxin-responsive promoter (GH3), 2-oxoglutarate-dependent dioxygenase (2OGD), nucleoside diphosphate kinase (NDK), RT (Reverse transcriptase), jasmonate ZIM domain-containing protein (JAZ), 3-hydroxyacyl-CoA dehydrogenase (HAD), abscisic acid receptor PYL2 (PYL2), NADH oxidase (Nox), 12-oxophytodienoate reductase (OPR), related to IAA and JA signaling were identified during seed germination (Fig. 5A). For example, GH3 up-regulated between S1 and S3, which could conjugate plant hormones such as IAA and JA to amino acids for degradation or storage to down-regulate the levels of biologically active hormones [42]. And, JAZ, acting as a critical regulator to modulate JA-responsive gene expression [43], down-regulated between S1 and S3, such that it may lead to decrease of JA. It had been also reported that the auxin signaling pathway controlled the expression of the JAZ gene through stimulation of ABA signaling, enabling molecular interplay between IAA, JA and ABA signaling [39, 44]. Therefore, IAA and JA action depends on the ABA signaling pathway, suggesting that IAA, JA, and ABA play interdependent roles in seed dormancy and germination.

SA has suppressive or promotive effects on seed germination, based on the proper concentration and the used conditions [45]. The SA level decreased significantly across the three stages in our study (Fig. 5C and Table S5), suggesting that seeds germination of *P. cyrtonema* might promote with the reduction of SA. While, the PR (pathogenesis-related protein), suggested to play a role in host SA-mediated defense signaling [46], increased significantly abundances from S1 to S2 /S3 (Fig. 5A). Additionally, PR-3 was reported to mediate ABA-dependent salt stress signals that affect seed germination in arabidopsis [47]. The result may suggest that unusual SA mediated PR-ABA regulation to *P. cyrtonema* seed germination.

Conversely, GAs promote seed germination by antagonizing the inhibitory effect of ABA, which induces the enzymes to accelerate cell division, cell enlargement, endosperm degradation and so on [48, 49]. The total content of GAs decreased significantly, but the ratio of GAs/ABA increased significantly from S1 to S2 or S3 (Fig. 5B and Table S5). Besides, 3 proteins, including gibberellin receptor GID1 (GID1), 2-oxoglutarate-dependent dioxygenase (2OGD), GRAS domain family (GRAS), related to GAs accumulation and signaling were identified across the seed germination of *P. cyrtonema*. For instance, GID1 was related to seed dormancy-breaking, with the crosstalk between GA and ABA signaling [50]. Moreover, sucrose synthase (SUS), considered to associated with the ABA-GA antagonism [51], decreased significantly abundances from S1 to S2 /S3 (Fig.). SUS was differentially regulated by ABA and GA [51], which could

promote storage substance degradation during seed germination [52]. The result indicates that GAs may break the dormancy and promote seed germination of *P. cyrtoneura* through ABA-GA antagonism regulation.

There are a series of organismal systems, such as the environmental adaptation, imbibition, circulatory system, which related to activate metabolic and synthetic processes, in the seed cells. For instance, phytochromes, principally phytochrome B (PHYB) and phytochrome A (PHYA), enhanced seed germination by receiving red and far-red light as photoreceptors [53]. PHYA promoted seed germination by utilizing very low light intensities, which influenced the auxin-associated genes differentially [54]. In this study, the PHYA protein increased abundance between S1 and S2 or S3, with up-regulating the expression of auxin-associated elements (transport inhibitor response 1 protein, TIR1; GH3 auxin-responsive promoter, GH3). The results indicated that hormones might be directly or indirectly involved in the PHYA signaling.

It is believed that genetic information processing, including transcription, translation and folding, is associated with germination. For example, Translation occurred early in germination because translation inhibitors inhibited both Arabidopsis and rice seed germination [55, 56]. The WRKY transcription factor (WRKY), which was one of the major plant protein superfamilies, played an important role in the regulation of translation associated with seed dormancy, seed germination, plant responses to ABA [57, 58], and so on. In this study, WRKY increased abundance between S1 and S2 or S3, while it decreased abundance between S2 and S3. It had proved that WRKYs were positive regulators in ABA-responsive signalling networks, but which were negative in seed germination [59], conversely. We found that ABA facilitates seed germination and dormancy by the WRKY transcription factor.

In the early stages of seed germination, the embryonic cells elongated, including the protrusion of radicles and the elongation of shoots [23]. These cellular changes are associated with cell division, cell differentiation, apoptosis and so on. Serine/threonine protein phosphatase type 2A (PP2A), comprised a catalytic (PP2Ac), a scaffolding (A), and a regulatory (B) subunit, is key components in the regulation of developmental programs and control of cell metabolism in plants [60, 61]. For instance, sprout growth is accelerated by overexpression of serine/threonine-protein phosphatase 2A regulatory subunit B (PPP2R3) [60]. In this study, PPP2R3 increased abundance between S1 and S2 or S3, which played a basic housekeeping role in plant cells. Our results suggested that PPP2R3 accelerated seed germination by mediating cell metabolism.

4. Conclusion

In this study, the seed germination of *P. cyrtoneura*, which usually takes 2–3 years for seeds to germinate under natural conditions, was comprehensively analysed by combining technologies of transcriptome, proteome and plant hormone. An integrated transcriptomic and proteomic analysis revealed changes in several important KEGG categories, including organismal systems, signal transduction, cellular processes, genetic information processing, energy metabolism, carbohydrate metabolism, lipid

metabolism, nucleotide metabolism, amino acid metabolism and biosynthesis of other secondary metabolites. A possible schematic presentation of molecular networks modulated by differentially abundant proteins was given to clarify the changes across the seed germination (Fig. 6 and Table S7). The results suggested that genetic information processing (ribosome, protein processing in endoplasmic reticulum, spliceosome, RNA transport), metabolism (starch and sucrose metabolism, cyanoamino acid metabolism, anthocyanin biosynthesis, phenylpropanoid biosynthesis, amino sugar and nucleotide sugar metabolism, ubiquinone and other terpenoid-quinone biosynthesis, stilbenoid, diarylheptanoid and gingerol biosynthesis), and signal transduction (plant hormone signal transduction) might be the main pathways participated in *P. cyrtoneura* seed germination. In addition, the possible potential regulation of plant hormone signal transduction, starch and sucrose metabolism in seed germination were discussed. Several key proteins, including PYL4, PP2C, SNRK2 and SUS, decreased between S1 and S3. The PYL4 mediated the activity of PP2C and SnRK2 to regulate the state of ABA signal transduction pathway, and SUS took part in the antagonism between ABA and GA. On the other hand, the proteins, such as INV, α -Xyl, β -D-Xyl and bglX, involved in starch and sucrose metabolism, increased. These proteins helped to weaken the endosperm cell wall and hydrolyze starch into glucose. Moreover, the contents of ABA, GAs, IAA, JA and SA decreased significantly, suggesting that phytohormone cross-talk might regulate seed dormancy and germination. While the ratio of GAs/ABA increased significantly from S1 to S2 or S3, indicating that ABA-GA antagonism might play a role in seed germination. The integrative multilevel omics analysis revealed that starch and sucrose metabolism, as well as plant hormone signal transduction, might be important for seed germination, but future studies will be needed to verify it. In conclusion, the study provides an understanding of seed germination mechanisms in *P. cyrtoneura*.

5. Methods

5.1 Germination of seed

The plant material was identified as *Polygonatum cyrtoneura* Hua by Professor Chengzi Yang, College of Pharmacy, Fujian University of Traditional Chinese Medicine. And the plants had been cultivated for five years in the garden of medicinal plants at Fujian University of Traditional Chinese Medicine (26°4'39.48"N, 119°10'54.27"E). We collected the seeds from these plants of *P. cyrtoneura* without artificial pollination in October 2018. These seeds were removed the pulp, washed and incubated with wet sand in an incubator. In order to better understand how *P. cyrtoneura* seeds germinate, three germination stages (the fresh mature seed, named S1, the emergence of radicle, named S2 and the phase between primary rhizome and cotyledon emergence, named S3) were chosen for investigation. Three independent biological replications were performed in this study. Tissue samples were stored at - 80 °C until used after being frozen in liquid nitrogen.

5.2 RNA-Seq analysis

The total RNA of seed was extracted utilizing the Trizol method. As shown in Fig. 1A, concentration and integrity of total RNA were determined, respectively. RNA sequencing was conducted by Biomix Biotech

Co, Ltd. (Beijing, China). The reference genome information was from the Liliaceae *Polygonatum* (<http://ensemblgenomes.org/>). Adaptors, poly-N and low-quality bases were removed to obtain clean reads. NCBI non-redundant protein sequences (NR), euKaryotic Ortholog Groups (KOG), Clusters of Orthologous (COG), Gene Ontology (GO), manually annotated and reviewed protein sequence database (Swiss-Prot), Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to annotate gene function. Fragments per kilobase of transcript per million fragments mapped (FPKM) were used to represent gene expression levels.

5.3 Sample iTRAQ labeling, LC-MS/MS and bioinformatics analysis

These samples for iTRAQ labeling, LC-MS/MS and bioinformatics analysis were conducted according to Chen et al [62]. As shown in Fig. 1A, the iTRAQ labeling of peptides was conducted using an iTRAQ 4-plex kit (Applied Biosystems, USA). The LC-MS/MS data were compared with the Liliaceae *Polygonatum* Reference Proteome. With the DESeq R package, the DEGs and DEPs across the seed germination were identified. A GO enrichment analyse was carried out in Cytoscape using the BiNGO plugin based on a FDR of 0.05 for DEGs and DEPs. The KEGG enrichment analysis were performed for DEGs and DEPs in the KOBAS software.

5.4 Quantification of endogenous phytohormones

The quantification of endogenous phytohormones was performed by MetWare (Wuhan, China). To measure the phytohormones concentrations of ABA, indole-3-acetic acid (IAA), jasmonic acid (JA), methyle jasmonate (MeJA), salicylic acid (SA), methyle salicylic acid (MeSA), gibberellin 1 (GA1), gibberellin 3 (GA3), gibberellin 4 (GA4), gibberellin 6 (GA6), gibberellin 7 (GA7), gibberellin 8 (GA8), gibberellin9 (GA9), gibberellin 12-ald (GA12-ald), gibberellin 15 (GA15), gibberellin 19 (GA19), gibberellin 20 (GA20), gibberellin 24 (GA24), gibberellin 29 (GA29), gibberellin 34 (GA34), gibberellin 44 (GA44), gibberellin 51 (GA51), gibberellin 59 (GA59), cis-zeatin-riboside (CZR), trans-zeatin-riboside (TZR), 6-(gamma,gamma-dimethylallylamino) purine (iP), 6-(gamma,gamma-dimethylallylamino) purine riboside (iPR), 3-indolebutyric acid (IBA), the seeds (approximately 1 g for each sample) were ground up in liquid nitrogen and extracted in acetonitrile. By using ESI-UPLC-MS/MS, phytohormones were detected and quantified in each sample after internal standards were added before grinding.

Abbreviations

S

germination *Polygonatum cyrtoneura* seed

ABA

Abscisic acid

GA

gibberellic acid

CK

cytokinin
iP
N6-(Δ^2 -isopentenyl)adenine
iPR
N6-(Δ^2 -isopentenyl) adenosine
CZR
cis-Zeatin-riboside
TZR
trans-Zeatin-riboside
JA
jasmonic acid
MEJA
methyle jasmonate
SA
salicylic acid
MESA
methyle salicylic acid
IAA
indole-3-acetic acid
DEGs
Differentially expressed genes
DEPs
Differentially expressed proteins
NR
non-redundant protein sequences
KOG
euKaryotic Ortholog Groups
COG
Clusters of Orthologous
GO
Gene Ontology
Swiss-Prot
manually annotated and reviewed protein sequence database
KEGG
Kyoto Encyclopedia of Genes and Genomes
FDR
False Discovery Rate
GO-BP
Gene ontology of biological process
iTRAQ

Isobaric tags for relative and absolute quantification

Declarations

Ethics approval and consent to participate

The authors declare that the study complies with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

All the authors declare that there is no conflict of interest.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary files. The sequencing data associated with transcription profiles in this study have been deposited in NCBI SRA database with accession number PRJNA1013739 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1013739>). The mass spectrometry proteomics data have been deposited to the ProteomeXchange database via the iProX partner repository with accession number PXD045963 (<http://proteomecentral.proteomexchange.org/cgi/GetDataset>).

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Authors' contributions

FYC and WX conceived and designed the study. HLX, ZMC, RYH, NY, MTP, SMF, LHC and YYH performed the experiments. YSW analyzed high-throughput sequencing data. HLX, YSW, YN and FYC analyzed the data as a whole and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

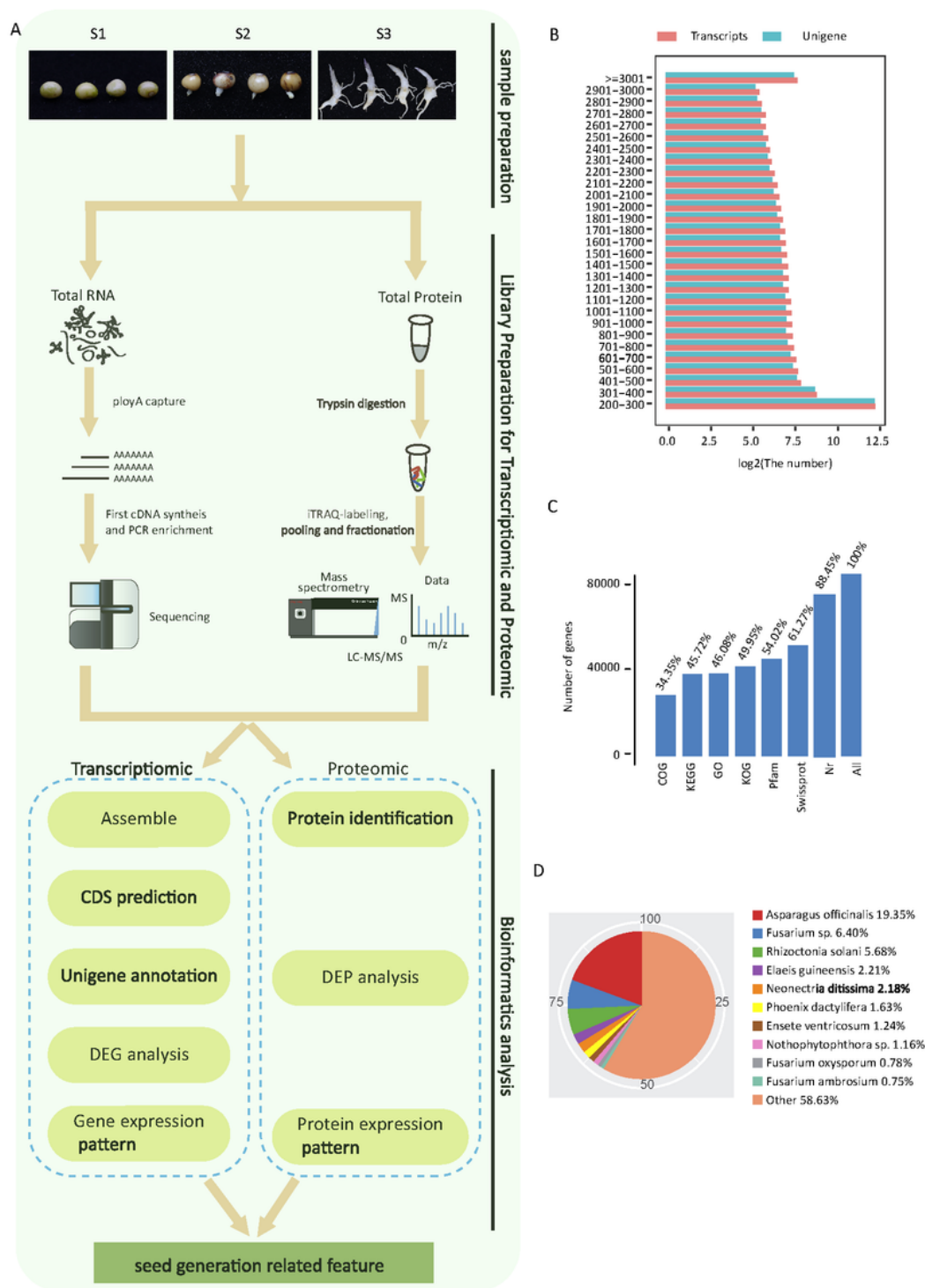
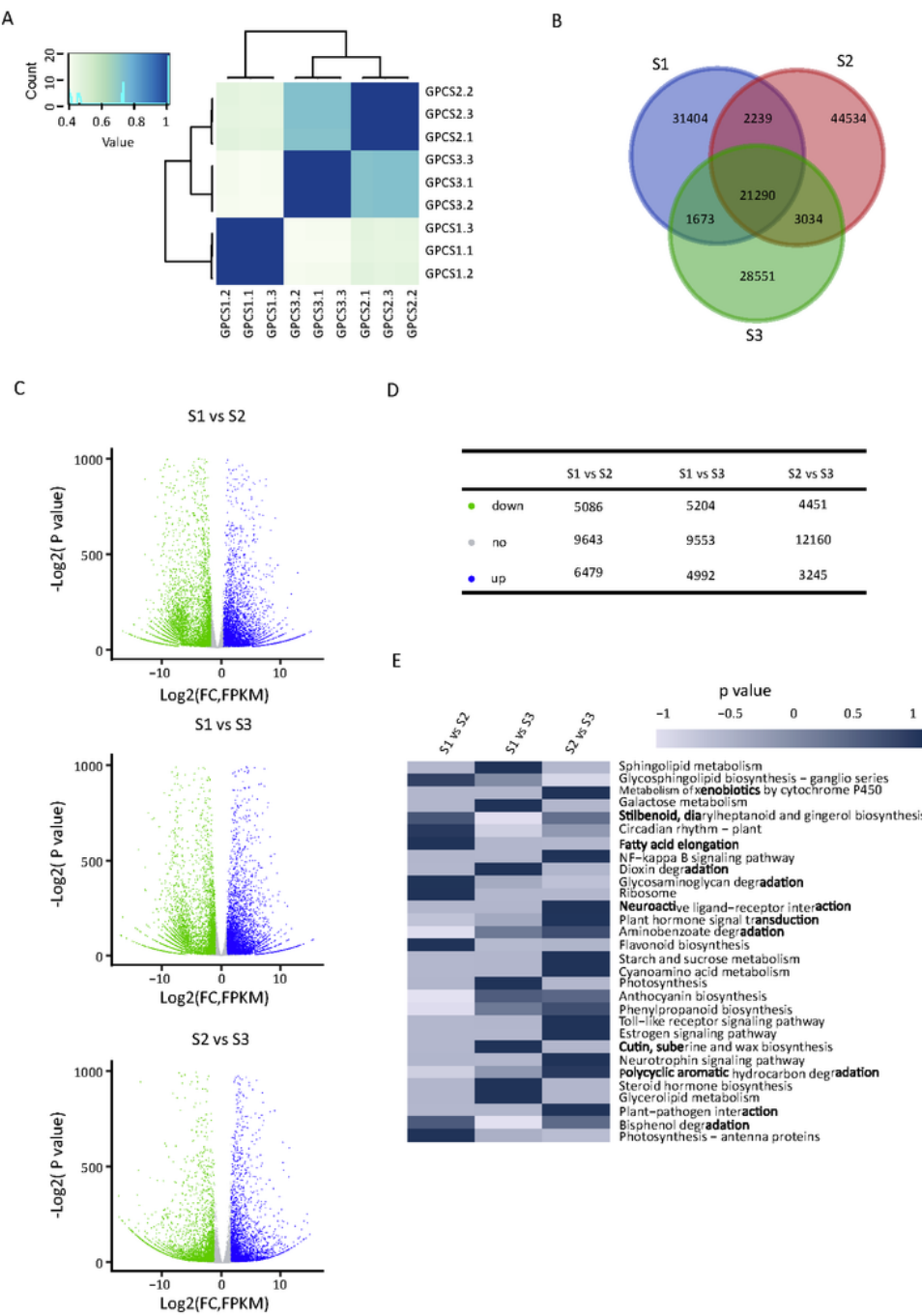


Figure 1

Schematic overview of transcriptomic and proteomic analysis for *P. cyrtonema*. (A) Transcriptomic and proteomic analysis for *P. cyrtonema*. Up, middle and down plots indicated the phenotype of three different stages of seed germination, the library preparation and bioinformatics analysis for transcriptomic and proteomic. (B) Length distribution of assembled transcripts and unigenes from *P. cyrtonema*. (C) The

number of assembled unigenes annotated by different public data such as COG, KEGG, GO, KOG, Pfam, Swissprot and Nr. (D) Species distribution of assembled unigenes annotated by Nr.



unigenes in three different stages of seed germination. (C) Volcano Plot indicated the differentially expressed assembled unigenes during different stages of seed germination. (D) Table indicated the number of differe up-regulation, down-regulation and non-regulation unigenes. Blue, green and gray points indicated the up-regulation, down-regulation and non-regulation unigenes respectively. (E) KEGG enrichment analysis was performed using all differentially expressed assembled unigenes.

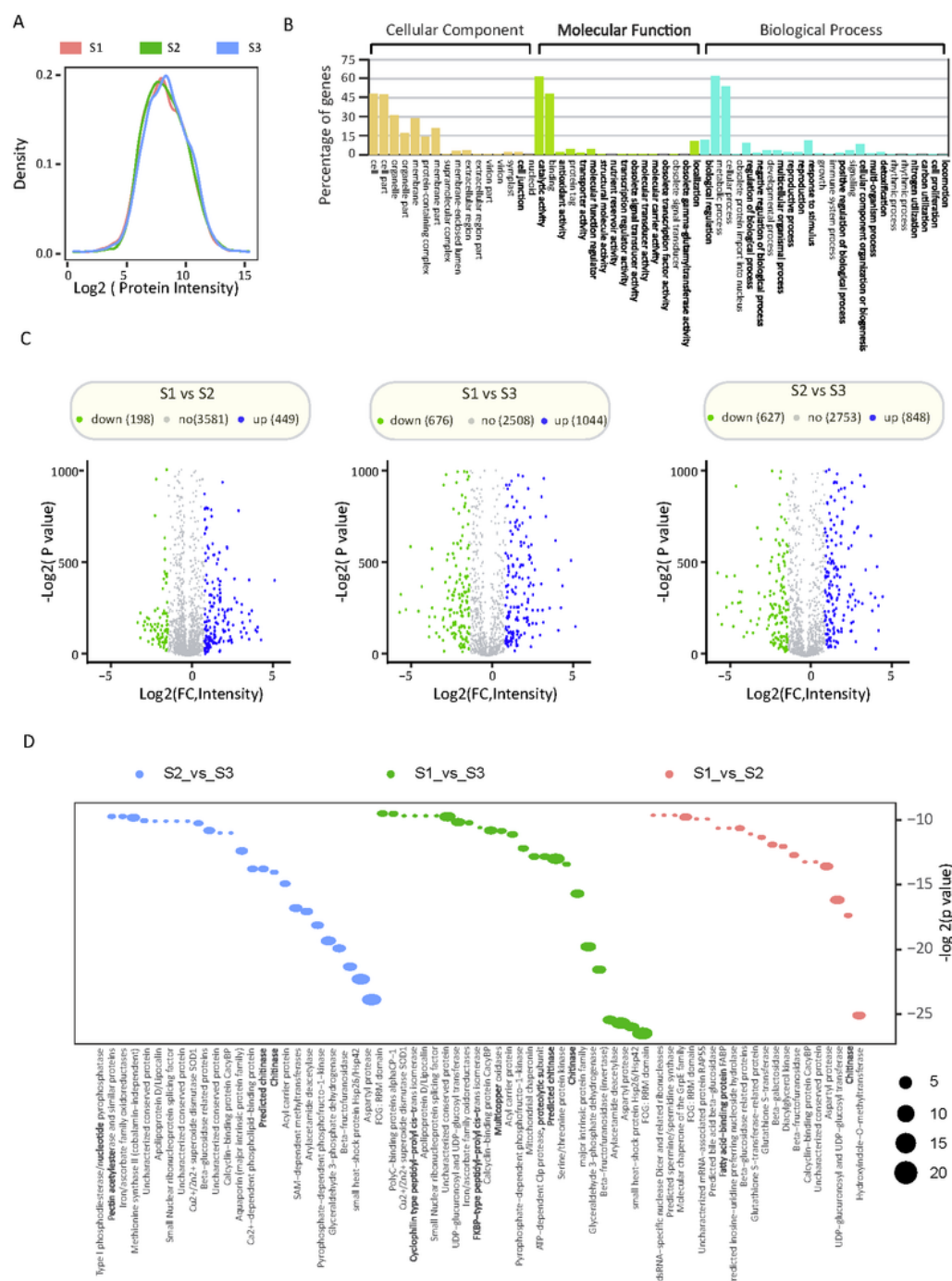


Figure 3

Differentially abundant proteins during different stages of seed germination. (A) The abundance of detecting proteins in three different stages of seed germination. (B) GO enrichment analysis all detecting proteins was performed using the biological process, cellular component and molecular function terms. (C) The differentially expressed proteins (DEPs) different stages of seed germination. Up indicated the number of up-regulation, down-regulation and non-regulation unigenes. Volcano Plot (down) showed the DEPs. Blue, green and gray points indicated the up-regulation, down-regulation and non-regulation unigenes respectively. (D) KEGG enrichment analysis was performed using DEPs after pairwise comparison such as S1 and S2, S1 and S3 and S1 and S3.

colours indicated nine responsive groups (fold change ≥ 2 and q-value ≤ 0.01). (D) KEGG enrichment analysis was performed using three responsive modules such as homo-directional changes (red bars), opposite changes (yellow bars) and translational changes (blue bars).

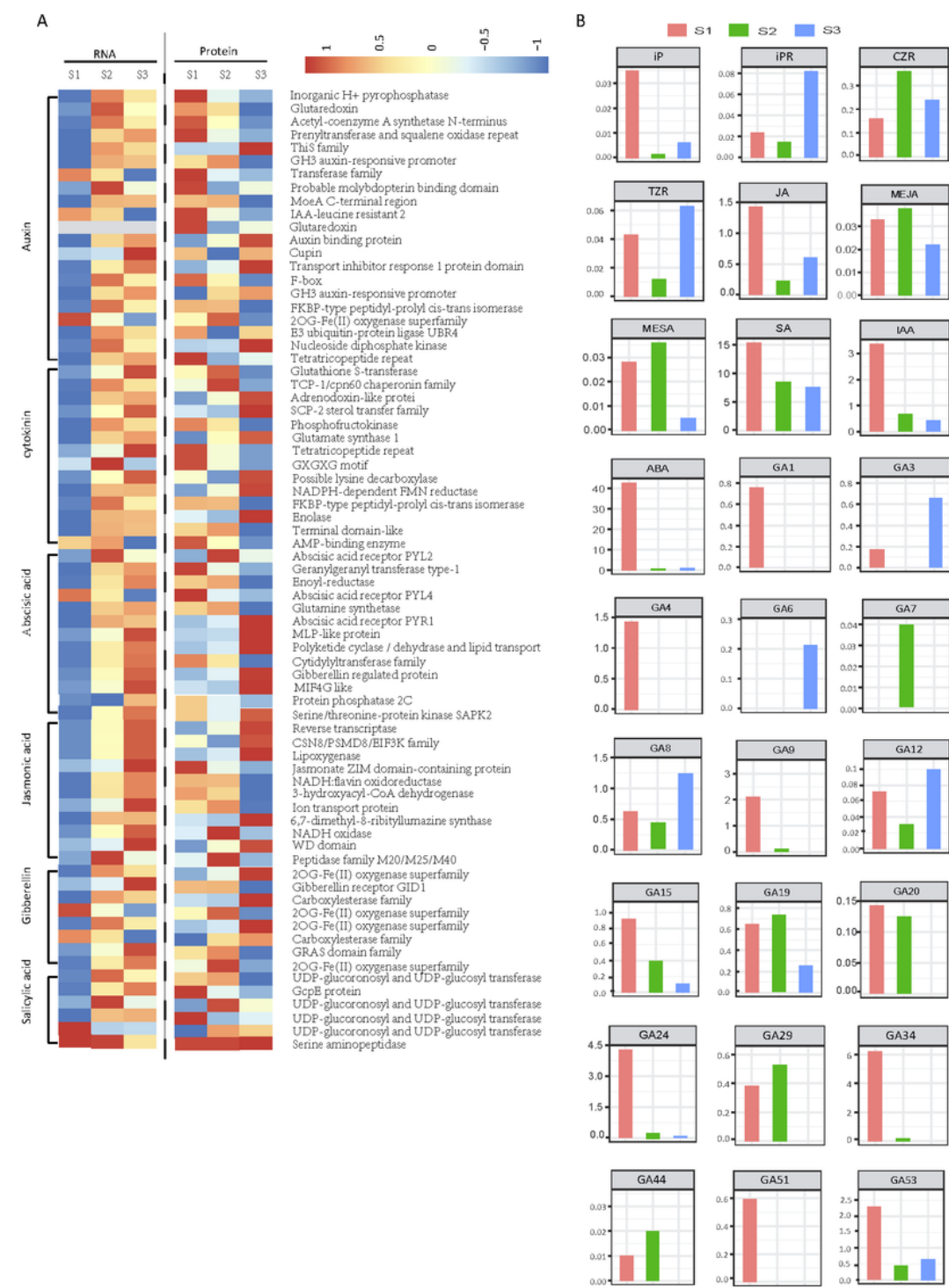


Figure 5

Dynamic changes of hormone related unigenes at transcriptional and translational levels and hormone distribution. A, Heatmap showed the transcriptional and translational levels of seven hormone related unigenes three different stages of seed germination. B, C, hormone distribution in three different stages of seed germination. CZR, cis-zeatin-riboside; TZR, trans-zeatin-riboside; JA, jasmonic acid; MeJA, methyle jasmonate; GA1, gibberellin1; GA3, gibberellin3; GA4, gibberellin4; GA6, gibberellin 6; GA7, gibberellin7; GA 8, gibberellin 8; GA9, gibberellin9; GA12-ald, gibberellin12-ald; GA15, gibberellin15;GA19, gibberellin19; GA20, gibberellin20; GA24, gibberellin24; GA29, gibberellin29;GA34, gibberellin34; GA44, gibberellin44; GA51, gibberellin51; GA59, gibberellin59; IAA, indole-3-acetic acid; SA, salicylic acid; MeSA, methyle salicylic acid; ABA, abscisic acid; iPR, 6-(gamma,gamma-dimethylallylamino) purine riboside; iP, 6-(gamma,gamma-dimethylallylamino) purine.

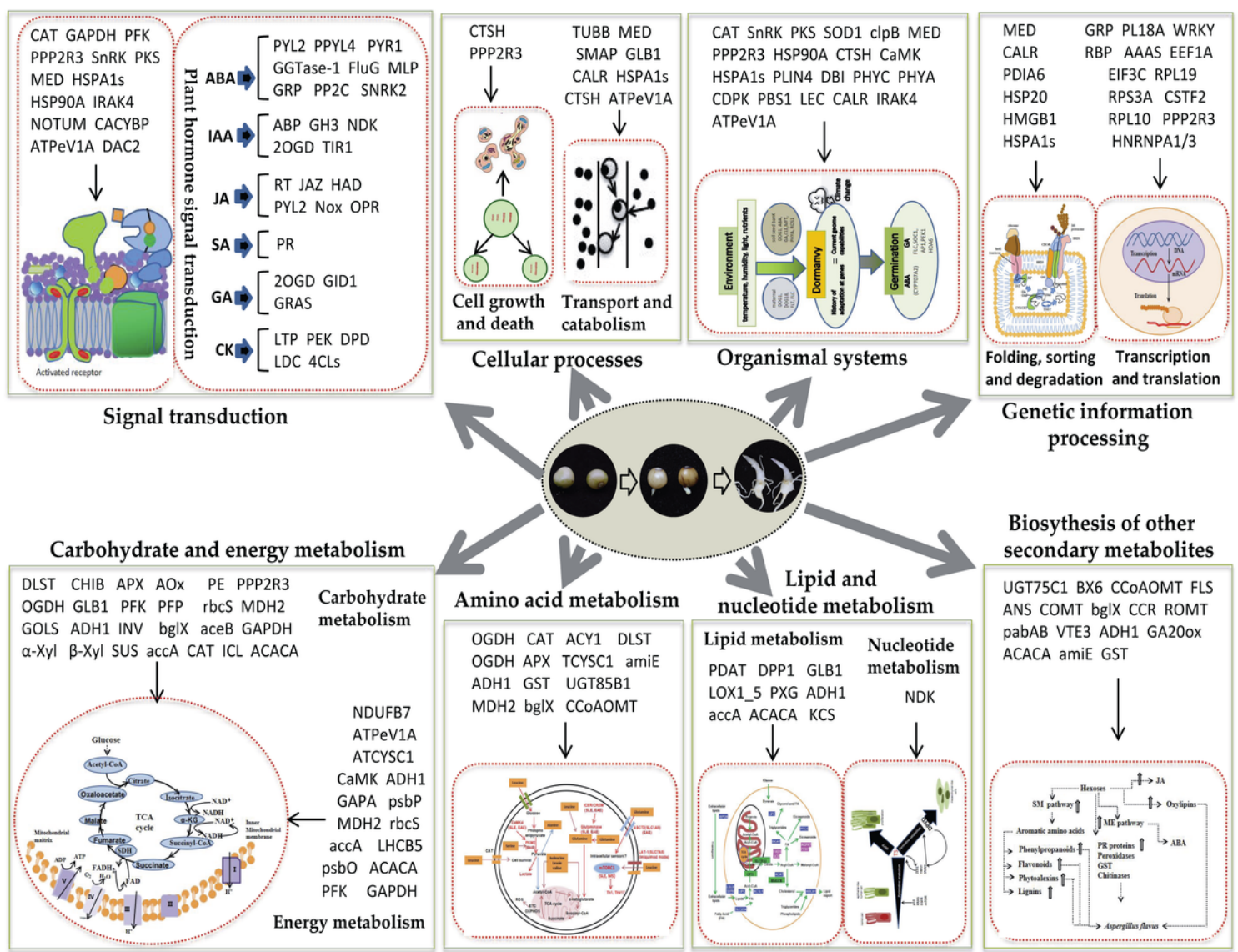


Figure 6

Schematic presentation of molecular networks mediated by partial DEGs/DEPs across seed germination of *P. cyrtonema* (not all the identified genes/proteins are included). CTSH: cathepsin H; PPP2R3: serine/threonine-protein phosphatase 2A regulatory subunit B; TUBB: tubulin beta; SMAP: stromal membrane-associated protein; MED: RNA polymerase II transcription mediator; HSPA1s: heat shock 70kDa protein; GLB1: beta-galactosidase; CALR: calreticulin; ATPeV1A: V-type H⁺-transporting ATPase subunit A; CAT: catalase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PFK: 6-phosphofructokinase; SnRK: SNF1-related protein kinase regulatory subunit beta-1-like; PKS: SNF1-related protein kinase regulatory subunit gamma-1-like; HSP90A: molecular chaperone HspG; IRAK4: interleukin-1 receptor-associated kinase 4; NOTUM: O-palmitoleoyl-L-serine hydrolase; CACYBP: calcyclin binding protein; DAC2: voltage-dependent anion channel protein 2; PYL2: abscisic acid receptor PYL2; PPYL4: abscisic acid receptor PYL4; PYR1: abscisic acid receptor PYR1; GGTase-1: geranylgeranyl transferase type-1; FluG: protein fluG; MLP: MLP-like protein 423; GRP: Gibberellin regulated protein; PP2C: protein phosphatase 2C; SNRK2: serine/threonine-protein kinase SAPK3-like; ABP: auxin-binding protein; GH3: GH3 auxin-responsive promoter; NDK: Nucleoside diphosphate kinase; 2OGD: 2-oxoglutarate-dependent dioxygenase; TIR1: Transport inhibitor response 1 protein; RT: Reverse transcriptase; JAZ: jasmonate ZIM domain-containing protein; HAD: 3-hydroxyacyl-CoA dehydrogenase; Nox: NADH oxidase; OPR: 12-oxophytodienoate reductase; PR: pathogenesis-related protein; GID1: gibberellin receptor GID1; GRAS: GRAS domain family; LTP: lipid-transfer protein; PEK: Phosphofructokinase; DPD: Dihydropyrimidine dehydrogenase; LDC: lysine decarboxylase; 4CLs: 4-coumarate:CoA ligase-like protein; HSP20: HSP20 family protein; PDIA6: protein disulfide-isomerase A6; HMGB1: high mobility group protein B1; RBP: RNA-binding protein HNRNPA1/3: heterogeneous nuclear ribonucleoprotein A1/A3; WRKY: WRKY transcription factor; AAAS: aladin; EEF1A: elongation factor 1-alpha; EIF3C: translation initiation factor 3 subunit C; RPL19: large subunit ribosomal protein L19e; RPS3A: small subunit ribosomal protein S3Ae; RPL18A: large subunit ribosomal protein L18Ae; RPL10: large subunit ribosomal protein L10e; CSTF2: cleavage stimulation factor subunit 2; DLST: dihydrolipoamide succinyltransferase; OGDH: 2-oxoglutarate dehydrogenase; MDH2: malate dehydrogenase; ATCYSC1: L-3-cyanoalanine synthase/cysteine synthase; ACY1: aminoacylase; amiE: amidase; CCoAOMT: caffeoyl-CoA O-methyltransferase; ADH1: alcohol dehydrogenase class-P; UGT85B1: cyanohydrin beta-glucosyltransferase; bglX: beta-glucosidase; GST: glutathione S-transferase; APX: L-ascorbate peroxidase; UGT75C1: anthocyanidin 3-O-glucoside 5-O-glucosyltransferase; BX6: 2,4-dihydroxy-1,4-benzoxazin-3-one-glucoside dioxygenase; FLS: flavonol synthase; ANS: anthocyanidin synthase; COMT: caffeic acid 3-O-methyltransferase; CCR: cinnamoyl-CoA reductase; pabAB: para-aminobenzoate synthetase; VTE3: MPBQ/MSBQ methyltransferase; GA20ox: gibberellin-44 dioxygenase; ACACA: acetyl-CoA carboxylase / biotin carboxylase 1; CHIB: basic endochitinase B; AOx: L-ascorbate oxidase; PFP: diphosphate-dependent phosphofructokinase; INV: beta-fructofuranosidase; GOLs: inositol 3-alpha-galactosyltransferase; α -Xyl: α -Xylosidase; β -Xyl: β -Xylosidase; PE: pectinesterase; SUS: sucrose synthase; accA: acetyl-CoA carboxylase carboxyl transferase subunit alpha; aceB: malate synthase; rbcS: ribulose-bisphosphate carboxylase small chain; ICL: isocitrate lyase; GAPA: glyceraldehyde-3-phosphate dehydrogenase; psbP: photosystem II oxygen-evolving enhancer protein 2; psbO: photosystem II oxygen-evolving enhancer protein 1; LHCB5: light-harvesting complex II chlorophyll a/b binding protein 5; NDUFB7: NADH dehydrogenase (ubiquinone) 1 beta subcomplex

subunit 7; CaMK: calcium/calmodulin-dependent protein kinase; PDAT: phospholipid:diacylglycerol acyltransferase; DPP1: diacylglycerol diphosphate phosphatase / phosphatidate phosphatase; LOX1_5: linoleate 9S-lipoxygenase; PXG: peroxygenase; KCS: 3-ketoacyl-CoA synthase; PLIN4: perilipin-4; DBI: diazepam-binding inhibitor; PHYC: Phytochrome C; PHYA: phytochrome A; PBS1: serine/threonine-protein kinase PBS1; LEC: leafy cotyledon1.

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