

Temporal Transcriptomic and Lipidomic Analysis Reveals Multi-Omics Dynamic Profiles of Brassica napus Seed Germination

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Abstract

Background

Lipids constitute a major component of *Brassica napus* seeds. During seed germination, lipid mobilization serves as a critical energy source for seedling establishment, which directly impacts germination vigor and the subsequent growth potential of seedlings. Although lipid mobilization is essential for rapeseed germination, the time-dependent coordination between transcriptional regulation and lipid conversion remains largely unclear, and the corresponding molecular regulatory network still needs to be systematically explored.

Results

We conducted an integrated transcriptomic and lipidomic analysis on dry *B. napus* seeds and germinating seeds at 6, 12, 24, and 48 hours after imbibition. The results revealed distinct stage-specific characteristics of gene expression and lipid metabolism during germination. In the early imbibition stage, differentially expressed genes (DEGs) were primarily enriched in biological processes related to water transport, stress response, and signal transduction, whereas significant changes in lipid metabolism were observed to be relatively delayed. During the initiation of germination, triacylglycerols (TAGs) underwent rapid degradation, accompanied by a significant up-regulation of genes involved in the β -oxidation and gluconeogenesis pathways. In the late germination stage, genes responsible for membrane lipid synthesis were sharply up-regulated, which induced extensive membrane lipid remodeling.

Conclusion

This study is the first to depict the dynamic multi-omics profile during *B. napus* seed germination, systematically illustrating the global molecular and metabolic features of *B. napus* seeds at different imbibition stages. It also clarifies the expression patterns of characteristic genes and lipid metabolites at each stage of germination. These findings further deepen our systematic understanding of the regulatory mechanisms governing *B. napus* seed germination, and provide valuable theoretical support as well as candidate gene resources for the breeding of high-yield and high-quality *B. napus* varieties.

Background

Seed germination, a key early event in the plant life cycle, directly governs crop emergence, seedling uniformity, and subsequent growth potential, exerting profound effects on the final yield and quality of crops^[1, 2]. As an important oilseed crop worldwide, *Brassica napus* L. seeds are rich in lipids, serving as a vital source of edible vegetable oil, animal feed, and bioenergy^[3, 4]. In contrast to cereal crops, stored lipid mobilization and metabolism serve as a major source of energy for *B. napus* seed germination^[5, 6]. Dynamic lipid degradation, transport and transformation processes are key prerequisites for successful seed germination and the seamless establishment of seedlings^[7]. Uncovering the regulatory mechanism of lipid metabolism during seed germination can thus provide a theoretical basis for the genetic improvement and accelerated breeding of oilseed crops.

Triacylglycerols (TAGs) represent the primary storage lipid in *B. napus* seeds and play two core roles during germination. On one hand, stored lipids are catabolized via a cascade of metabolic reactions to generate energy substrates, which supply the energy required for seed imbibition, radicle emergence, and early seedling growth^[8]. On the other hand, intermediate metabolites from lipid metabolism act as precursors for the synthesis and remodeling of cell membranes. These processes are critical for maintaining cellular structural integrity and fluidity, which in turn ensures the proper execution of essential physiological processes such as cell division and differentiation.^[9]

Current understanding of the molecular regulatory mechanisms underlying seed germination remains partial, with several core pathways and key genes identified to mediate this process. In the hormone signaling pathway, GA promotes the expression of germination-related genes and breaks seed dormancy, while ABA represses germination^[10–12]. In the lipid metabolic pathway, lipid hydrolysis, β -oxidation and gluconeogenesis act synergistically to convert stored lipids into energy and carbon sources, thus fueling seed germination^[13, 14].

In this study, we set multiple consecutive time points and integrated transcriptomic and lipidomic analyses to characterize the dynamic temporal and synergistic changes in gene expression and lipid metabolic profiles during *B. napus* seed imbibition and germination. We further identified core genes and characteristic lipid molecules associated with different germination stages, as well as core nodes potentially involved in mediating seed germination. These findings may provide a preliminary theoretical basis for the genetic improvement of seed germination traits in *B. napus*.

Methods

Plant Materials and Sample Collection

In this study, *B. napus* seeds (cultivar LY80) with a genetically stable background were freshly harvested in the same year, fully dried, and then hermetically stored at 4 °C. Prior to germination, plump, intact seeds of uniform size were disinfected with 75% ethanol, rinsed thoroughly with sterile water for five times, and then placed in Petri dishes lined with double layers of filter paper for germination in a constant-temperature incubator at 25 °C in the dark.

Samples were collected at 0 h (dry seeds), 6 h, 12 h, 24 h and 48 h after seed imbibition, with approximately 0.2 g of mixed seeds designated as one independent biological replicate and three biological replicates prepared for each time point. All samples were snap-frozen in liquid nitrogen and stored at -80 °C for subsequent transcriptomic and metabolomic analyses.

RNA-sequencing (RNA-seq) analysis

In this study, Total RNA was isolated and checked for integrity using an Agilent 2100 Bioanalyzer. cDNA libraries were constructed and sequenced on an Illumina NovaSeq 6000 to generate 150-bp paired-end reads. Clean reads were mapped to the *B. napus* reference genome, and DEGs were identified using DESeq2 with Benjamini–Hochberg correction. Genes with $\text{padj} \leq 0.05$ and $|\log_2(\text{fold change})| \geq 1$ were defined as significantly DEGs in this study. KEGG (Kyoto Encyclopedia of Genes and Genomes) annotations, the enrichment analysis of differentially expressed genes (DEGs) were performed using cluster Profiler (3.8.1).

Lipid Extraction and UHPLC-MS/MS analyses

Total lipids were extracted from rapeseed seeds via a modified MTBE method. In brief, 100 mg liquid nitrogen-ground seed powder was vortexed with 0.75 mL methanol, blended with 2.5 mL MTBE and shaken for 60 min at room temperature. Then 0.625 mL chromatography-grade water was added for phase separation, and the mixture was left standing for 10 min^[15]. The separated aqueous phase was re-extracted with 1 mL of a mixed solvent (MTBE/methanol/water, 10:3:2.5, v/v/v). The combined organic phases were dried and redissolved in 100 µL of isopropanol for storage, and then subjected to analysis by LC-MS/MS. UHPLC-MS/MS analyses were performed using a Vanquish UHPLC system (Thermo Fisher, Germany) and an Orbitrap Q ExactiveTM HF mass spectrometer (Thermo Fisher, Germany). Samples were injected onto a ThermoAccucore C30 column (150×2.1 mm, 2.6 µm) using a 20-min linear gradient at a flow rate of 0.35 mL/min. The column was maintained at 40 °C. Mobile phase A was acetonitrile–water (6:4) with 10 mM ammonium acetate and 0.1% formic acid; phase B was acetonitrile–isopropanol (1:9) with the same additives. The gradient program was: 30% B (0–2 min), 43% B (5 min), 55% B (5.1 min), 70% B (11 min), 99% B (16 min), and re-equilibrated to 30% B at 18.1 min^[16]. Q ExactiveTM HF mass spectrometer was operated in positive[negative] polarity mode with sheath gas :40 psi, sweep gas: 0 L/min, auxiliary gas rate: 10 L/min[7 L/min], spray voltage: 3.5 kV, capillary temperature: 320 °C, heater temperature: 350 °C, S-Lens RF level: 50, scan range: 114–1700 m/z, automatic gain control target: 3e6, normalized collision energy: 22 eV; 24 eV; 28 eV [22 eV–24 eV–28 eV], Injection time: 100 ms, Isolation window: 1 m/z, automatic gain control target (MS2): 2e5, dynamic exclusion: 6 s.

Lipidomic data analysis

The raw data files generated by UHPLC-MS/MS were processed using the Lipidsearch. Principal components analysis (PCA) and Partial least squares discriminant analysis (PLS-DA) were performed at metaX (a flexible and comprehensive software for processing metabolomics data). We applied univariate analysis (t-test) to calculate the statistical significance (P-value). The metabolites with VIP > 1 and P-value < 0.05 and fold change ≥ 2 or FC ≤ 0.5 were considered to be differential metabolites. Volcano plots were used to filter metabolites of interest which based on Log₂(FC) and -log₁₀(P-value) of metabolites. For clustering heat maps, the data were normalized using z-scores of the intensity areas of differential metabolites and were plotted by Pheatmap package in R language. The correlation between differential metabolites were analyzed by cor () in R language (method = pearson). Statistically significant of correlation between differential metabolites were calculated by cor.mtest() in R language. P-value < 0.05 was considered as statistically significant and correlation plots were plotted by corplot package in R language.

RNA Extraction and Quantitative RT-PCR

Total RNA was extracted from 0.2 g rapeseed powder with RNAiso Plus. RNA purity was quantified using a NanoDrop 200, and 2 µg RNA was reverse-transcribed into cDNA. qRT-PCR was performed with Actin as the internal reference. Three biological and technical replicates were set, and relative expression levels were determined via the comparative 2^{−ΔΔCt} threshold method^[17].

Results

Seed morphology and water content of after imbibition

To clarify the temporal characteristics of *B. napus* seed imbibition and germination, we divided the process into sequential stages based on morphological observation and water content determination (Fig. 1). Dry seeds had a

water content of 6.82%, which increased rapidly to 28.57% within 6 h, with slight seed swelling but intact seed coat and no radicle protrusion. From 6–12 h, water absorption slowed, reaching 39.24% at 12 h (Fig. 1C), accompanied by continued swelling, softened seed coat and embryo expansion without seed coat rupture. Between 12–24 h, water content stabilized at 35.11%–42.38% (Fig. 1D), with radicle protrusion and slight seed coat cracking. After 24 h, water content remained stable, with rapid radicle elongation to at 48 h, hypocotyl stretching and complete seed coat rupture (Fig. 1F). Temporal characteristics of seed imbibition lay a fundamental foundation for subsequent omics detection and analysis.

Global Dynamic Changes of Transcriptome During Seed Imbibition and Germination

To systematically explore the transcriptional dynamics during *B. napus* seed imbibition and germination, transcriptome sequencing was performed on seed samples at 0 h, 6 h, 12 h, 24 h, and 48 h after imbibition, with three biological replicates per time point. Quality assessment revealed that the Q30 score for all sequencing libraries exceeded 97.36%, and the GC content ranged from 46.29% to 47.04%, indicating high sequencing accuracy and reliability, which met the requirements for subsequent bioinformatics analysis (Table 1). Meanwhile, transcriptome sequencing reads were aligned to the *B. napus* reference genome. The Total_map ($\geq 91.75\%$) and Unique_map ($\geq 84.59\%$) of all samples fell within a reasonable high-value range with good alignment efficiency, demonstrating high reliability of the sequencing data and its suitability for subsequent analyses. Principal Component Analysis (PCA) was adopted to assess the overall differences and repeatability of transcriptomic patterns among samples at different germination stages. The results showed that samples of the same time point were closely clustered together, reflecting good reproducibility of biological replicates. Notably, samples of different time points were clearly separated along the first principal component (PC1, explaining 63.71% of the total variation) (Figure S1), which indicated that the transcriptional profiles changed significantly with the progression of seed imbibition and germination, and the samples were well distinguished by the time sequence, laying a foundation for the subsequent analysis of differential gene expression.

Table 1
Quality and genome alignment statistics of transcriptome data

Sample	Clean_reads	Error_rate	Q20	Q30	GC_pct	Total_map	Unique_map
Im0R1	68807008	0.01	99.44	97.65	47.04	63748432(92.65%)	58491777(85.01%)
Im0R2	71868122	0.01	99.39	97.47	46.67	66516572(92.55%)	60790905(84.59%)
Im0R3	71232200	0.01	99.36	97.47	46.73	65579636(92.06%)	60256477(84.59%)
Im6R1	75799360	0.01	99.39	97.48	46.96	69910950(92.23%)	65035249(85.8%)
Im6R2	74211060	0.01	99.39	97.54	46.76	68278888(92.01%)	63402952(85.44%)
Im6R3	75960730	0.01	99.37	97.36	46.92	69885929(92.0%)	64409897(84.79%)
Im12R1	75916102	0.01	99.42	97.54	46.45	69896072(92.07%)	65302548(86.02%)
Im12R2	76203644	0.01	99.38	97.49	46.29	69916393(91.75%)	65546390(86.01%)
Im12R3	74881814	0.01	99.42	97.54	46.38	68977426(92.12%)	64707700(86.41%)
Im24R1	75852410	0.01	99.41	97.47	46.69	70150592(92.48%)	66070476(87.1%)
Im24R2	74776434	0.01	99.42	97.6	46.64	69196973(92.54%)	65068678(87.02%)
Im24R3	76766098	0.01	99.45	97.53	46.69	71101624(92.62%)	66878307(87.12%)
Im48R1	73803716	0.01	99.44	97.58	46.99	68631698(92.99%)	64762903(87.75%)
Im48R2	71675682	0.01	99.41	97.53	46.96	66525494(92.81%)	62620014(87.37%)
Im48R3	74436288	0.01	99.41	97.5	47.02	69119002(92.86%)	65291130(87.71%)

In this study, differentially expressed genes (DEGs) among different time points were identified using the criteria of $|\log_2(\text{fold change})| \geq 1$ and adjusted P-value < 0.05 . The number of DEGs varied with imbibition time: compared with the 0 h dry seed control, the numbers at 6 h, 12 h, 24 h, and 48 h exhibited an increasing trend(Fig. 2A)(Table S1). A Venn diagram analysis was performed to clarify the overlap of DEGs among different time points(Fig. 2B), and the results showed that only 404 DEGs were commonly expressed across all four comparison groups (Im6h vs Im0h, Im12h vs Im6h, Im24h vs Im12h, Im48h vs Im24h), indicating that most DEGs were specifically expressed at different germination stages. Hierarchical clustering analysis of all DEGs showed that transcriptional patterns were highly similar between dry seeds and seeds at 6 h of imbibition, whereas the clustering results at all other time points differed considerably from one another, which further confirms that transcriptional changes are time-dependent during imbibition(Fig. 2C).

Figure 2. Analysis of gene expression in *B. napus* seeds at different imbibition stages. (A) Statistics of the number of significantly altered transcripts between successive stages in rapeseed seeds after imbibition. (B) Venn diagram analysis showing the overlap of differentially expressed genes (DEGs) between successive stages in rapeseed seeds after imbibition. (C) Hierarchical clustering heatmap of expressed genes at different stages in *B. napus* seeds after imbibition.the ordinate shows the FPKM-normalized values of differentially expressed genes; the redder the color, the higher the expression level, and the bluer the color, the lower the expression level.

To reveal the biological roles of DEGs during seed imbibition and germination, we performed GO functional classification and KEGG pathway enrichment analysis for DEGs identified at each sampling stage. GO enrichment analysis revealed stage-specific enrichment of DEGs during seed imbibition: DEGs at 6 h were mainly enriched in water transport, water stimulus response and cell wall organization-related biological processes; 12 h DEGs in protein folding, ribosome biogenesis and RNA processing; 24 h DEGs in lipid catabolism and energy metabolism; and 48 h DEGs were significantly enriched in cell division, cell elongation and hormone-mediated signaling pathways (Figure S2). KEGG pathway enrichment analysis also highlighted distinct metabolic and signaling dynamics across stages. At 6 h, the most significantly enriched pathways were MAPK signaling, ABC transporters and phenylpropanoid biosynthesis, reflecting early stress responses and secondary metabolism (Fig. 3A). By 12 h, ribosome biogenesis, porphyrin metabolism and sulfur metabolism dominated, indicating active protein synthesis and fundamental metabolic activation (Fig. 3B). At 24 h, starch and sucrose metabolism, glutathione metabolism and DNA replication were prominent, supporting the transition to active growth (Fig. 3C). By 48 h, intensive carbon metabolism, including starch and sucrose metabolism and pentose and glucuronate interconversions, along with phenylpropanoid biosynthesis and secondary metabolite production, was observed, underpinning cell expansion and organogenesis (Fig. 3D). Collectively, these results illustrate a dynamic transcriptional and metabolic reprogramming process that drives seed imbibition and the initiation of germination.

Global Dynamic Changes of Lipidome During Seed Imbibition and Germination

To systematically characterize the lipidomic dynamics during *B. napus* seed imbibition and germination, lipidomic profiling was performed on the same seed samples as transcriptomic analysis, with three biological replicates per time point. A total of 1733 lipid molecules were identified across all samples, belonging to 15 major lipid classes, among which Triacylglycerol (TG) accounted for the highest proportion (16.40%), followed by Phosphatidylcholine (PC, 12.47%) and Diacylglycerol (DG, 9.82%) (Fig. 4A). PCA was conducted to evaluate the similarity of lipidomic profiles among samples, and the results showed that biological replicates of the same time point were closely clustered. Samples of different germination stages were clearly separated along PC1, which explained 46.62% of the total variation, suggesting significant temporal changes in lipid composition during seed imbibition and germination (Fig. 4B). Additionally, KEGG pathway annotation of all identified metabolites revealed that lipid metabolism was the most enriched pathway, containing 1253 annotated metabolites, followed by global and overview maps and the digestive system, highlighting the central role of lipid metabolism during seed imbibition and germination (Fig. 4C). Lipidmaps annotation demonstrated that glycerophosphocholines (GP01, PC) were the most abundant lipid subclass, followed by glycerophosphoethanolamines (GP02) and glycerophosphates (GP10) (Fig. 4D). Glycerolipids and sphingolipids were also detected, confirming the dominance of phospholipids in membrane structure and the role of glycerolipids as storage and energy sources.

The hierarchical clustering heatmap (Fig. 5A) revealed distinct temporal patterns in lipid metabolite abundance across imbibition stages, with clear clusters of lipids showing increasing or decreasing abundance over time, reflecting dynamic lipid remodeling during seed imbibition and germination. Differential lipids are enriched in different imbibition stages (Table S2). K-means clustering (Fig. 5B) further grouped these differential lipids into four subclusters: Subcluster 1 (110 metabolites) showed continuous accumulation, Subcluster 2 (450 metabolites) gradually increased and peaked at 24 h, Subcluster 3 (126 metabolites) remained relatively stable, and Subcluster 4 (99 metabolites) exhibited a sustained decrease, consistent with the breakdown of storage lipids

to fuel germination; together, these findings illustrate the coordinated, stage-specific lipid metabolic changes that underpin seed imbibition and germination, aligning closely with our transcriptomic observations.

To dissect the temporal dynamics of lipid metabolism during seed imbibition and germination, we performed KEGG pathway annotation of lipid metabolites across four key time points. The analysis revealed a progressive activation of lipid metabolism: at 6 h (Figure. 6A), lipid metabolism was moderately represented (210 metabolites) alongside active glycan biosynthesis and core metabolic networks, reflecting the initial phase of water uptake and stress response; by 12 h (Figure. 6B), lipid metabolism activity temporarily declined (15 metabolites) as the seed prioritized core metabolic maintenance; at 24 h (Figure. 6C), lipid metabolism reactivated (18 metabolites) to support membrane repair and early growth; and by 48 h (Figure. 6D), it emerged as the most dominant pathway (92 metabolites), which aligns with transcriptomic observations of enhanced lipid catabolism to provide energy and metabolic precursors for the transition to active germination. This temporal progression demonstrates a tightly coordinated metabolic response that underpins seed imbibition and germination, with strong consistency between lipidomic and transcriptomic data.

WGCNA analysis of DEGs

To identify the potential regulatory network related to lipid metabolism and seed germination in *B. napus*, weighted gene co-expression network analysis (WGCNA) was performed. Genes with low expression were filtered out, and the remaining genes were used to construct a scale-free network. A total of 10 distinct co-expression modules were identified by hierarchical clustering (Fig. 7A). Module–trait correlation analysis showed that the MEblue module was strongly positively correlated with the catabolism of energy-storing substances such as lipids and carbohydrates, while The black module was associated with growth processes including membrane lipid biosynthesis (Fig. 7B). The inter-module correlation heatmap illustrated the relationships among the identified co-expression modules. Most modules exhibited weak to moderate correlations with one another, indicating that they participate in distinct biological processes (Fig. 7C).

qRT-PCR Validation of Transcriptomic Data

To confirm the credibility of transcriptome data, eight genes involved in lipid metabolism were selected for qRT-PCR detection (Fig. 8). These genes exhibited either significant upregulation or downregulation across imbibition stages, including those showing marked decreases in early imbibition (BnaA08G0155600ZS, BnaA03G0274900ZS) and those displaying increases over time (BnaA05G0136600ZS). The qRT-PCR expression profiles of these genes were fully consistent with their transcriptomic profiles, a strong correlation that supports the robustness of our transcriptomic analysis and the validity of the stage-specific transcriptional changes observed during seed imbibition.

Discussion

The Early Stage of Seed Germination is an Active Transcriptional Preparation Stage

In the rapid imbibition stage (0–6 h), DEGs identified by transcriptomic analysis were mainly enriched in biological processes such as water transport, response to water stimulus, and cell wall organization. The early imbibition stage was accompanied by initial lipid metabolic remodeling; however, transcriptional regulation at this stage

appeared to focus more on laying a structural and environmental foundation for subsequent lipid metabolism and seed germination, rather than on active lipid catabolism. The activation of these DEGs may be closely associated with the rapid water absorption and swelling of *B. napus* seeds, a physiological process that is particularly critical for this oilseed crop. Unlike cereal crops, *B. napus* seed germination relies predominantly on stored lipids for energy supply, and the normal progression of early imbibition may directly determine whether subsequent lipid mobilization and metabolism can proceed smoothly^[17–19]. Specifically, genes related to water transport may promote the rapid penetration of water into seed cells, which not only helps break the dormant state of dry seeds but also may activate the activity of hydrolases that are prerequisites for subsequent lipid hydrolysis^[20, 21].

Potential Temporal Regulation of TAG Degradation and Energy Supply Conversion

Data from both transcriptomics and lipidomics indicated that there may be a precise temporal coordination mechanism between biochemical pathways such as β -oxidation and gluconeogenesis during *B. napus* seed germination, and this mechanism might ensure the continuous supply of energy and carbon skeletons required for seed germination and seedling establishment. As the main storage lipid in *B. napus* seeds, triacylglycerols (TAGs) serve as the core energy source during germination, the germination and early seedling establishment of *B. napus* are highly dependent on the efficient mobilization and metabolic conversion of TAGs, making the putative precise temporal regulation of TAG degradation particularly critical^[22–24]. Our results showed that the stepwise degradation of TAGs and their subsequent metabolic conversion are likely regulated by temporal programs at both the transcriptional and metabolic levels, which was well illustrated by the dynamic changes in KEGG pathway annotations across different imbibition stages. Specifically, at the early imbibition stage (0–6 h), transcriptomic analysis indicated that DEGs were mainly enriched in water transport and cell wall organization processes, while lipidomic profiles and KEGG annotations (Fig. 3A) showed only initial lipid metabolic remodeling without active TAG degradation. This suggests that the transcriptional regulation at this stage prioritizes the establishment of a stable physiological environment for subsequent TAG mobilization, rather than immediate energy supply conversion^[25].

3.3 Enhancing Seed Germination Quality to Improve Yield

Seed germination is the foundational stage of the plant life cycle, directly determining crop emergence rate, seedling uniformity, and subsequent growth potential, which in turn affects final crop yield and quality^[26, 27]. For *B. napus*, an important oil crop and alternative protein source worldwide, the efficiency of seed germination and early seedling establishment is not only closely related to its yield and product quality but also plays a vital role in maintaining the stability of global oil supply and food security, especially under the context of increasing population pressure and deteriorating environmental conditions^[3, 28, 29]. For *B. napus*, an important oil crop and alternative protein source worldwide, the efficiency of seed germination and early seedling establishment is not only closely related to its yield and product quality but also plays a vital role in maintaining the stability of global oil supply and food security, especially under the context of increasing population pressure and deteriorating environmental conditions^[28]. From the perspective of rapeseed breeding practice, enhancing seed germination quality through molecular breeding strategies is a direct and effective approach to improve yield. The core genes and characteristic lipid metabolic markers identified by integrated multi-omics analysis can be used as potential molecular targets for breeding rapeseed varieties with superior germination performance^[29]. Enhancement of seed germination quality can effectively elevate the field emergence rate, optimize the population structure, and

improve the efficiency of resource utilization, thereby increasing the per unit area yield of *B. napus* without the need for cultivated land expansion.

Conclusions

This study systematically presents the global molecular and metabolic characteristics of *B. napus* seed germination at different imbibition stages via integrated transcriptomic and lipidomic analyses, clarifying the expression patterns of characteristic genes and lipid metabolites in each stage. It further deepens the systematic understanding of the regulatory mechanisms underlying *B. napus* seed germination, and provides theoretical support and candidate gene resources for high-yield and high-quality rapeseed breeding.

Abbreviations

TG: Triacylglycerol

PC: Phosphatidylcholine

DG: Diacylglycerol

CL: Cardiolipin

Cer: Ceramide

PE: Phosphatidylethanolamine

PI: Phosphatidylinositol

PG: Phosphatidylglycerol

PA: Phosphatidic acid

FA: Fatty acid

PS: Phosphatidylserine

MGDG: Monogalactosyldiacylglycerol

DGDG: Digalactosyldiacylglycerol

PMt: Phosphatidylmethanol / Phytomelatonin

PFAA: Perfluoroalkyl acid / Polyunsaturated fatty acid

LPC: Lysophosphatidylcholine

SQDG: Sulfoquinovosyldiacylglycerol

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

Declarations

Acknowledgements

Not applicable

Author Contributions:

BZ conceived the project and research plans; BZ, JW and LM performed the experiments; BZ, LM and HL analyzed the data; BZ wrote the article; BZ, JD and WS critically revised the manuscript.

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Availability of data and material

All transcriptomic and lipidomic data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) public databases to ensure free and open access for all researchers. The transcriptomic sequencing data are available in the Sequence Read Archive (SRA) database under the accession number [SRR37226466, SRR37226465, SRR37226464, SRR37226463, SRR37226462, SRR37226461, SRR37226460, SRR37226459, SRR37226458, SRR37226457, SRR37226456, SRR37226455, SRR37226454, SRR37226453, SRR37226452]. All data can be accessed freely upon publication to facilitate related research on *B. napus* seed germination and lipid metabolism, and to provide support for rapeseed genetic improvement and breeding.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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Figures

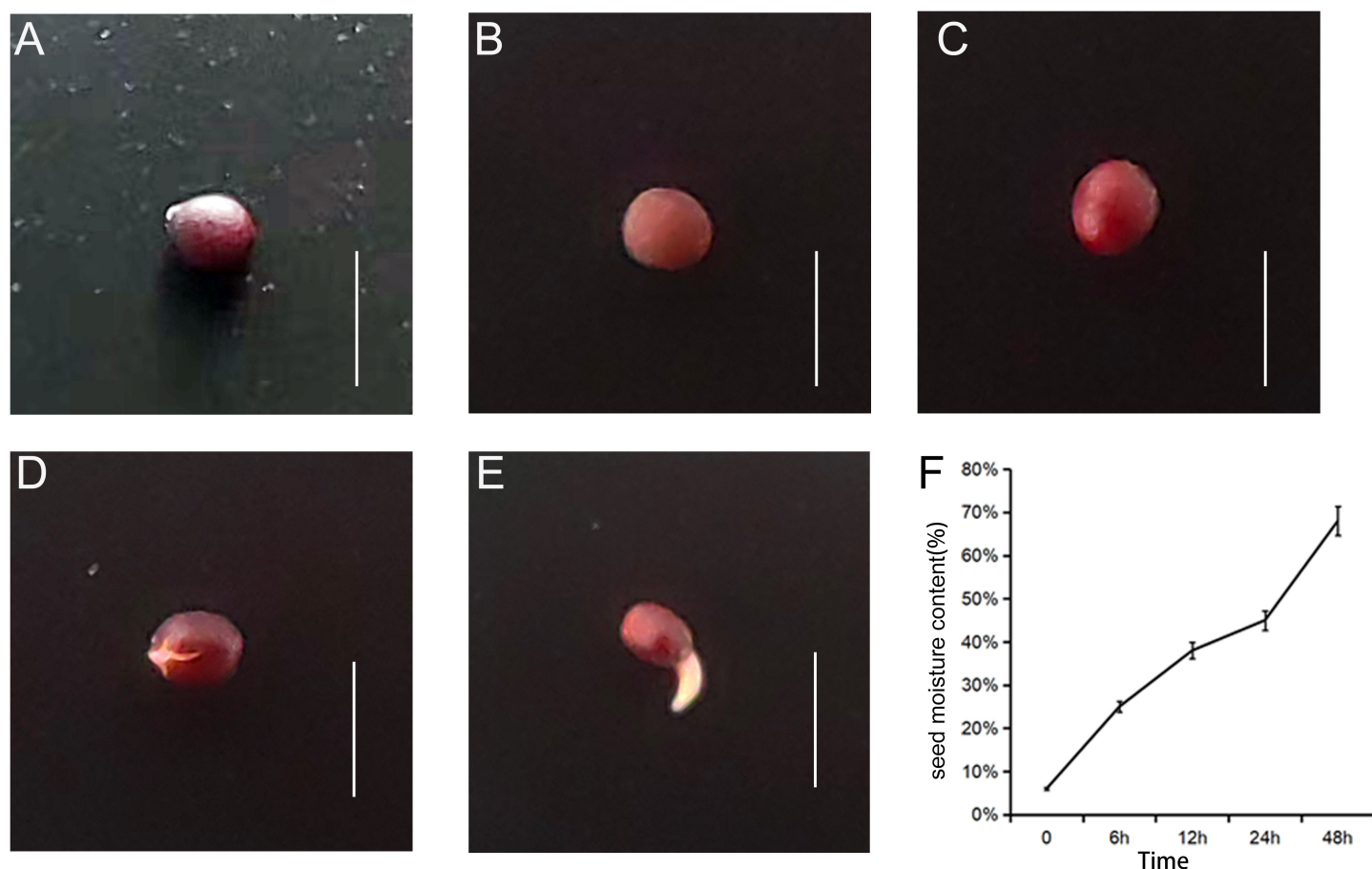


Figure 1

Morphological characteristics of *B. napus* seeds at different imbibition stages. (A), (B), (C), (D) and (E) represent the morphology of seeds after 0 h, 6 h, 12 h, 24 h and 48 h of imbibition, respectively. (F), Comparison of water content in *B. napus* seeds at different imbibition stages.

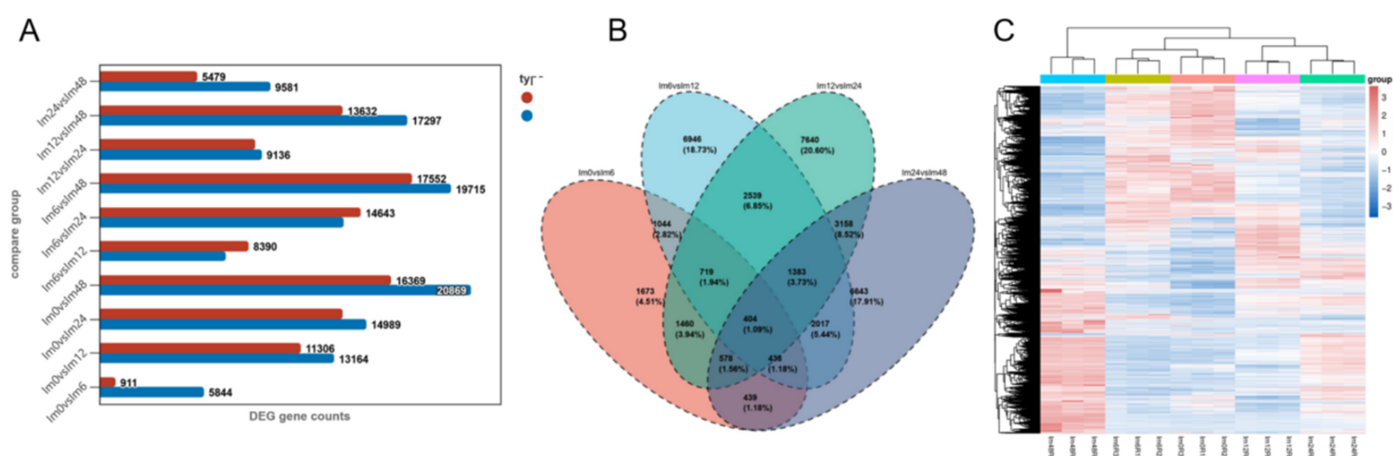


Figure 2

Analysis of gene expression in *B. napus* seeds at different imbibition stages. (A) Statistics of the number of significantly altered transcripts between successive stages in rapeseed seeds after imbibition. (B) Venn diagram analysis showing the overlap of differentially expressed genes (DEGs) between successive stages in rapeseed seeds after imbibition. (C) Hierarchical clustering heatmap of expressed genes at different stages in *B. napus* seeds after imbibition. the ordinate shows the FPKM-normalized values of differentially expressed genes; the redder the color, the higher the expression level, and the bluer the color, the lower the expression level.

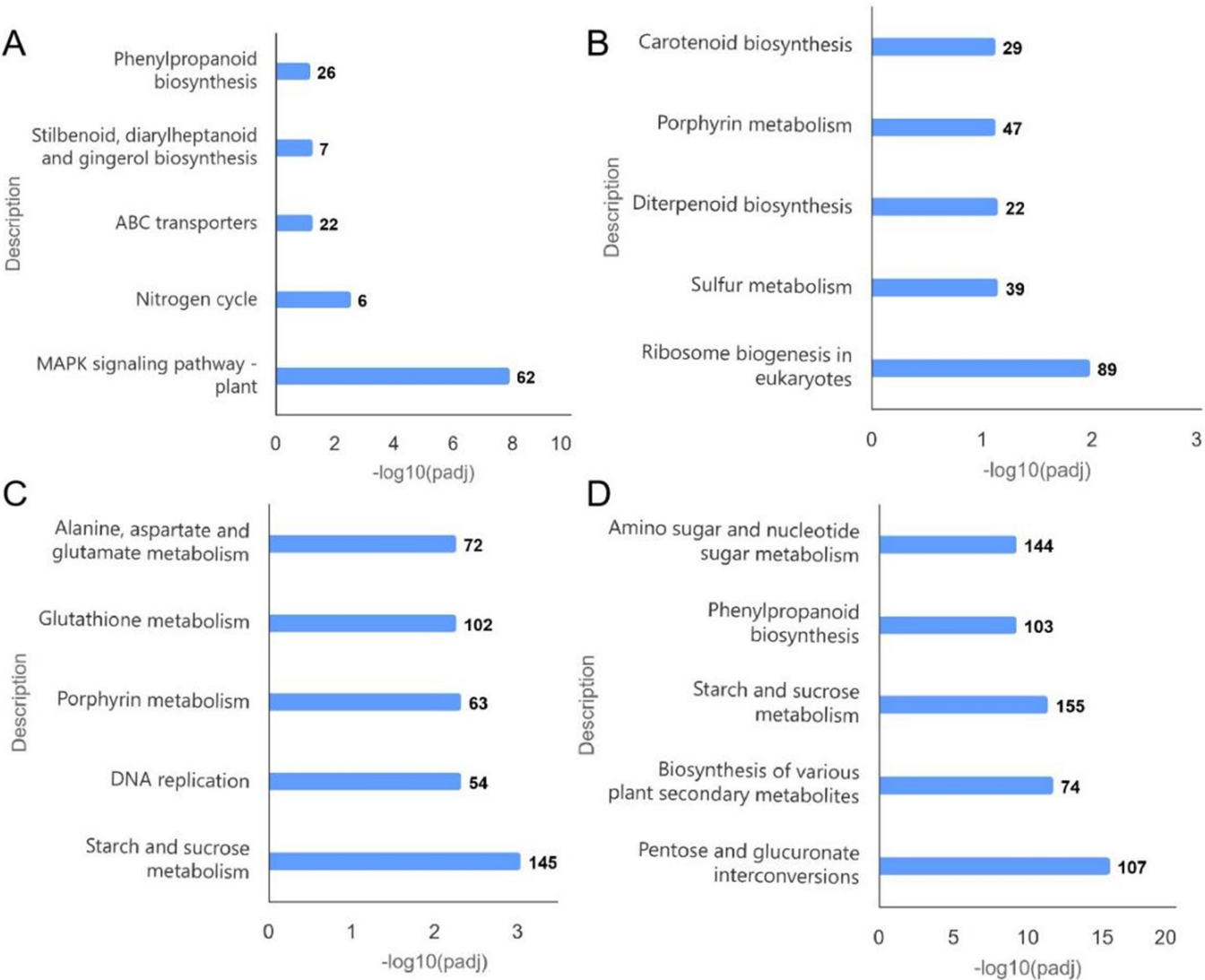


Figure 3

KEGG enrichment analysis of DEGs at different imbibition stages in *B. napus* seeds.(A), (B), (C), and (D) represent the KEGG pathway enrichment results of DEGs at 6 h, 12 h, 24 h, and 48 h of seed imbibition, respectively.

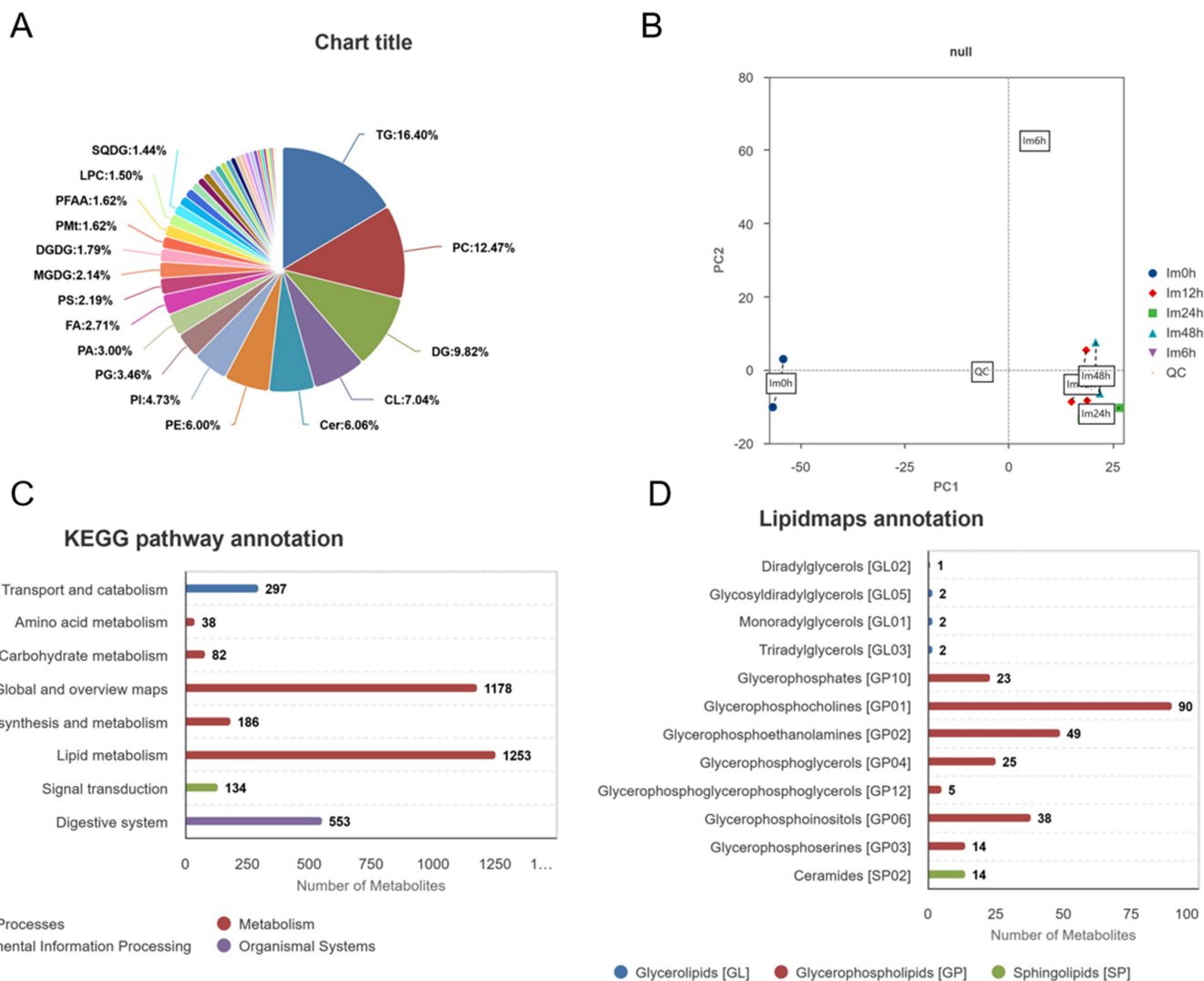


Figure 4

lipidomics analysis of *B. napus* seeds after imbibition. (A) Variations in lipid subclass levels in *B. napus* seeds during imbibition. (B) PCA analysis of all lipidomic samples from *B. napus* seeds after imbibition. (C) KEGG analysis of the identified lipid compounds. (D) Annotation of the eight major lipid classes and their subclasses for the identified lipids using the lipid maps database.

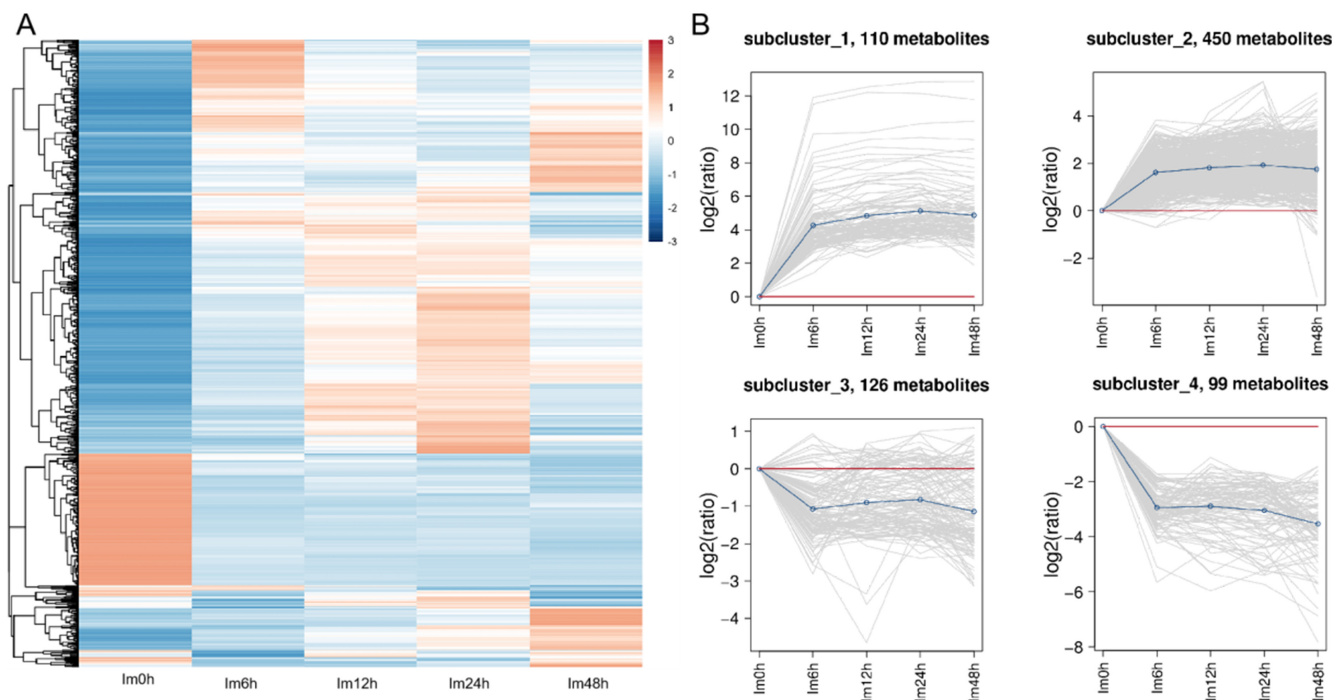


Figure 5

Differential analysis of lipid compounds in *B. napus* seeds at different imbibition stages. (A) Clustering analysis of all differential lipid compounds in *B. napus* seeds after imbibition. (B) K-means analysis of differential lipid compounds in *B. napus* seeds after imbibition

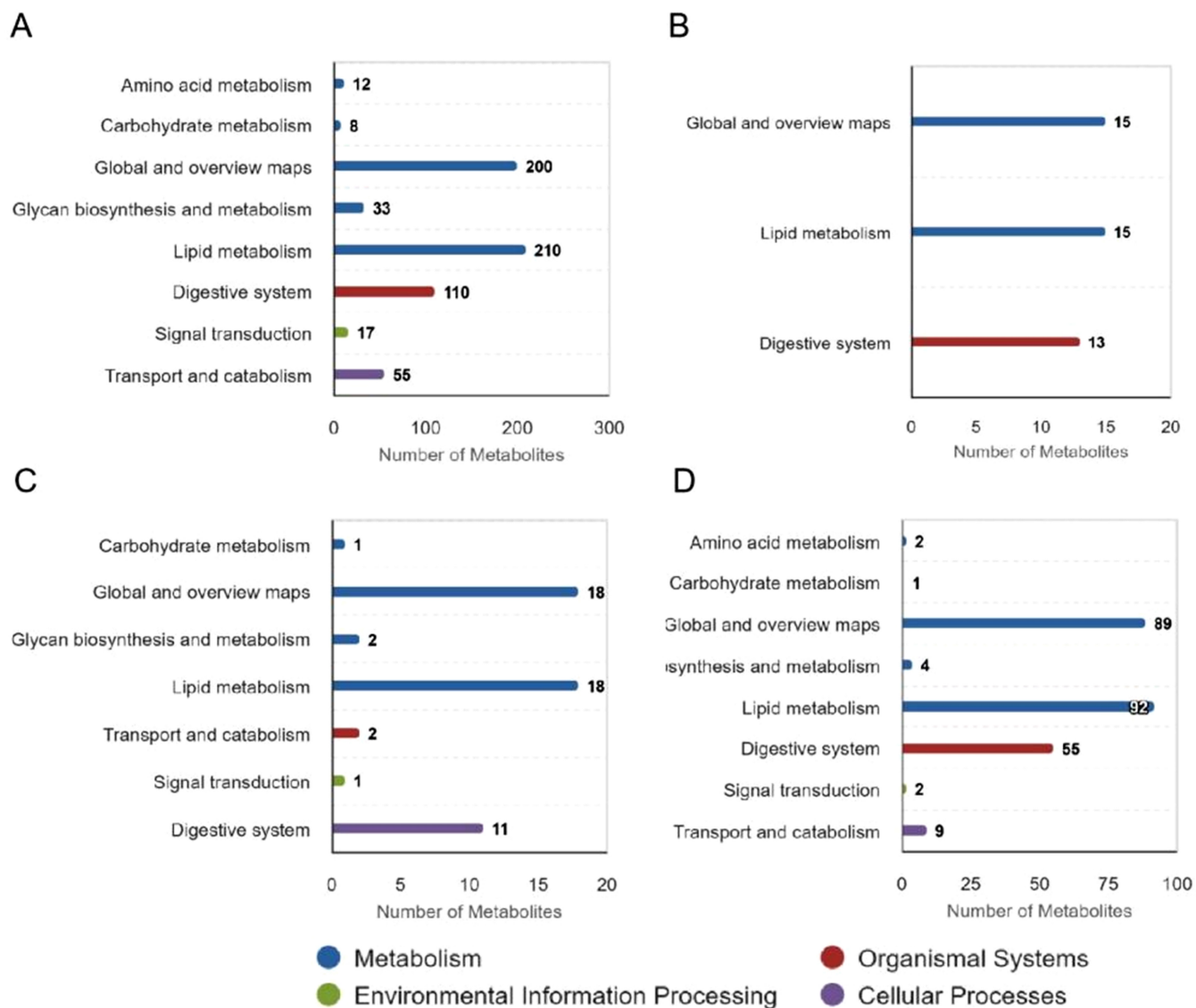


Figure 6

KEGG enrichment of differentially expressed lipids at different stages of seed imbibition. (A), (B), (C), and (D) represent the KEGG pathway enrichment results of differentially expressed lipids at 6 h, 12 h, 24 h, and 48 h of seed imbibition, respectively.

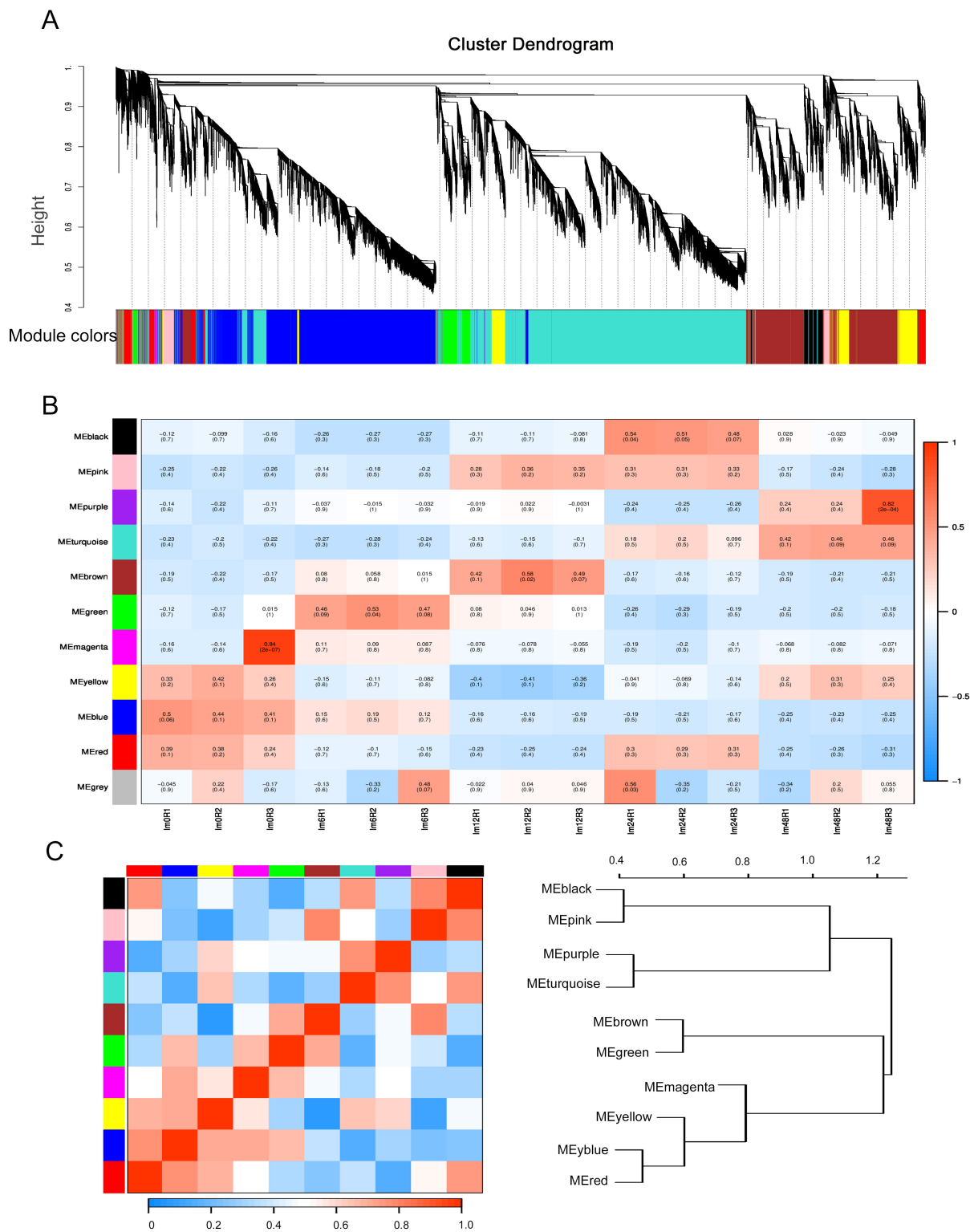


Figure 7

Analysis of WGCNA in *B. napus*. (A) Gene clustering dendrogram and module division. (B) Number of genes in each module and correlation coefficients between modules and lipid metabolic traits. Red and blue in the color scale represent positive and negative correlations, respectively. (C) Bar plot of module significance (MS) for lipid metabolism-related traits.

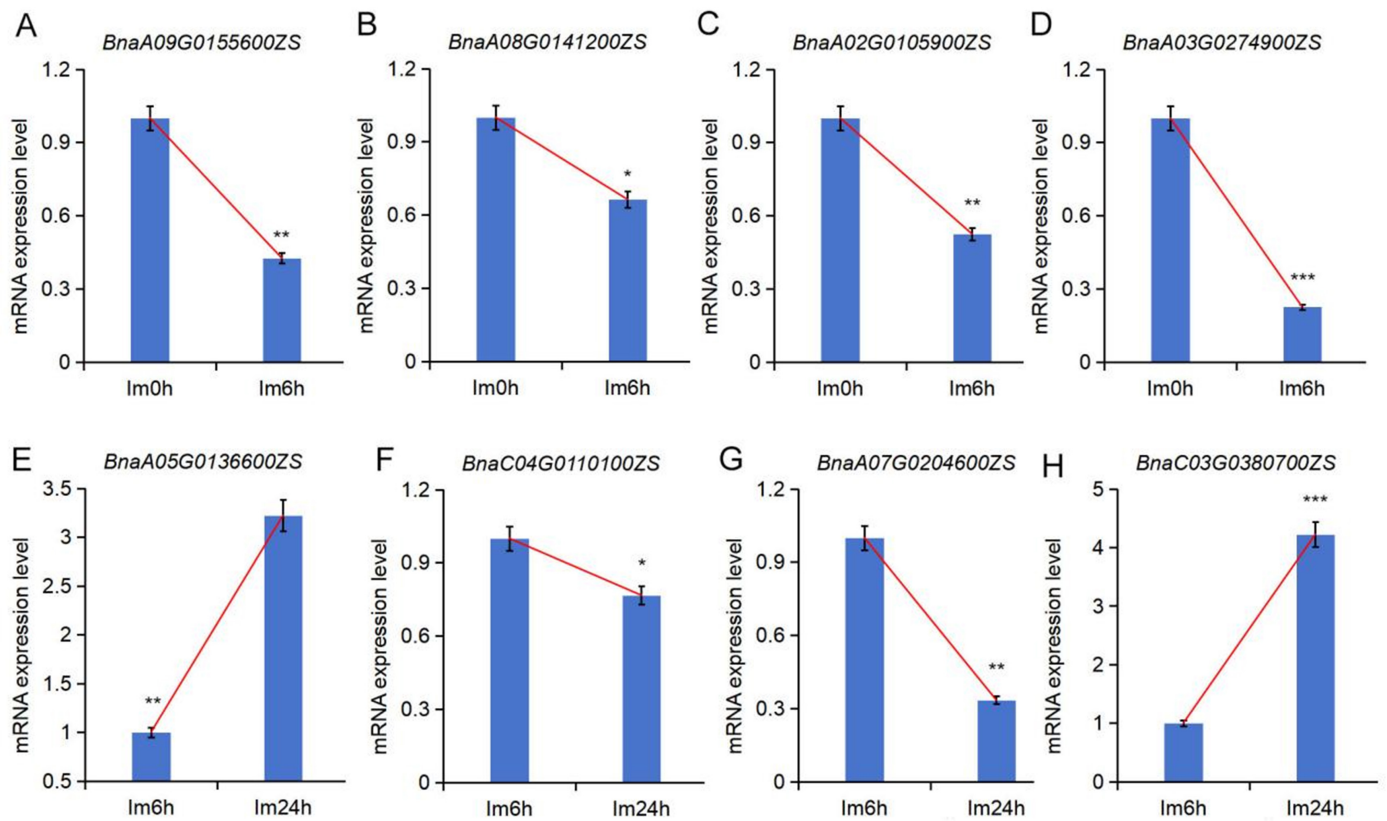


Figure 8

Expression level analysis of differentially expressed genes from the transcriptome in *B. napus* seeds after imbibition. Data are the means $\pm$ standard error ($n = 3$). Asterisks indicate the P value determined using a Student's t test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Supplementary Files

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