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Transcriptome analysis provides insight into gamma irradiation delaying quality deterioration of postharvest *Lentinula edodes* during cold storage

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ABSTRACT

To better determine how gamma irradiation (GI) improves abiotic stress resistance, a transcriptome analysis of postharvest *L. edodes* in response to 1.0 kGy GI was conducted, and further the underlying mechanism of GI in delaying quality deterioration over 20 d of cold storage was explored. The results suggested that GI was involved in multiple metabolic processes in irradiated postharvest *L. edodes*. In comparison with the control group, the GI group contained 430 differentially expressed genes, including 151 upregulated genes and 279 downregulated genes, which unveiled characteristic expression profiles and pathways. The genes involved in the pentose phosphate pathway were mainly upregulated and the expression level of the gene encoding deoxy-D-gluconate 3-dehydrogenase was 9.151-fold higher. In contrast, the genes related to other energy metabolism pathways were downregulated. Concurrently, GI inhibited the expression of genes associated with delta 9-fatty acid desaturase, ribosomes, and HSP20; thus, GI helped postpone the degradation of lipid components, suppress transcriptional metabolism and regulate the stress response. Additionally, the metabolic behavior of DNA repair induced by GI intensified by noticeable upregulation. These regulatory effects could play a potential and nonnegligible role in delaying the deterioration of *L. edodes* quality. The results provide new information on the regulatory mechanism of postharvest *L. edodes* when subjected to 1.0 kGy GI during cold storage.

1. Introduction

Lentinula edodes, the "Queen of mushrooms", is the second most cultivated edible mushroom and popular in the global food market because of its delicious taste and nutritional value (Li et al., 2021; Kong et al., 2021; Tao et al.,2021). It is an important food source which is rich in many nutrients including polysaccharide, fiber, fatty acid, essential amino acid, and mineral and vitamin (Tao et al., 2021; Zhang et al., 2022b). Recently, the annual production of *L. edodes* in China reaches over 10 million tons, and it constitutes 90 percent of the total mushroom output in the world (Li et al., 2022).

The fruiting body of *L. edodes* is highly perishable within a few days after harvest due to its high transpiration and respiration, and the absence of protective structure on the surface, leading to typical deterioration phenomena characterized by softening, browning, cap development, water loss, as well as microbial attack during storage, which severely limits its shelf-life and results in great economic losses (Liu et al., 2021; Zhang et al., 2022b; Kong et al., 2021). To maintain its shelf-life and edible quality, several fresh preservation techniques recently reported have been applied to delay quality deterioration and extend the shelf life of fresh *L. edodes*; these techniques include γ-irradiation, ultraviolet-C irradiation, coating with different chitosan complexes, modified atmosphere packaging, negative air ions (Zhang et al., 2022b), pulsed light (Wen et al., 2020), γ-polyglutamic acid hydrogels (Tao et al., 2021), coating with polysaccharides from *Oudemansiella radicata* (Liu et al., 2021), phase change materials (Kong et al., 2021; Li et al., 2021), plasma gas and plasma-activated water (Gavahian et al., 2019), and ozone treatment (Liu et al., 2020). Among the various alternative approaches, γ-irradiation deserves special attention. Food irradiation is performed through the production of ionizing energy,

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which is utilized to preserve food characteristics and the quality of the products; this method is certified by international organizations, such as the World Health Organization (WHO), International Atomic Energy Agency (IAEA) and Food Agriculture Organization (FAO) (Ravindran & Jaiswal, 2019). Low-dose irradiation, which is widely accepted as a safe and typical nonthermal preservation technology in over 60 countries, has been proven to be highly effective in quarantine treatment, inhibition of physiological growth, extension of shelf life, sterilization and disinfection (Ravindran & Jaiswal, 2019). As a phyto-sanitary measure, an irradiation dose of up to 1.0 kGy for fresh fruits and vegetables is permitted. Moreover, this technology is attractive because of its technological advantages, including high throughput, wide flexibility and lack of toxic residues; in addition, the changes in nutritional, functional, and sensory properties of food are minor (Ravindran & Jaiswal, 2019). Furthermore, low-dose irradiation is the only appropriate process that can be applied for foods after their final packaging. In fact, apart from the immobility of irradiation devices, which is likely the limiting factor of industrial applications, low-dose γ-irradiation is a promising approach for food preservation.

Notably, studies have demonstrated that low-dose irradiation exerts considerably beneficial effects on mushrooms during storage and provides several advantages, such as reducing the microbial population (Bisht et al., 2021), stimulating the biosynthesis of phenolic compounds (Tejedor-Calvo et al., 2019) and improving antioxidant activity (Arvanitoyannis et al., 2009; Villa-Rodriguez et al., 2015; Fernandes et al., 2017). Furthermore, it was found that gamma radiation at 1.0 kGy with modified atmosphere packaging greatly improved the storage life of *L. edodes* up to 20 d (Jiang et al., 2010). Our previous results also showed that 1.0 kGy irradiation treatment improved the postharvest quality maintenance of *L. edodes*, mainly by strengthening the antioxidant capacity and retarding water mobility and loss, as well as reducing natural microflora attached to its surface (Shi et al., 2022). This indicated that low-dose γ-irradiation is an effective method of extending the shelf life and improving the postharvest quality of *L. edodes*. To achieve this effect, γ-irradiation, in addition to exerting a bactericidal effect, could act as an abiotic elicitor for the biosynthesis and accumulation of mushroom bioactive molecules; furthermore, it could be implicated in the regulation of key metabolic processes to a great extent. Nevertheless, the underlying mechanisms associated with the transcriptional regulation of postharvest *L. edodes* following irradiation treatment are poorly understood.

While the effects of low-dose irradiation have been examined and applied to fresh *L. edodes* during storage, the relevant metabolic mechanisms have not yet been elucidated. To determine the regulatory mechanism through which 1.0 kGy gamma irradiation prevents quality deterioration of postharvest *L. edodes,* this study investigated a transcriptome analysis of fresh *L. edodes* subjected to 1.0 kGy gamma irradiation after 20 d of storage, and differentially expressed genes (DEGs) were screened to unravel overall changes in gene expression.

2. Materials and methods

2.1. Mushroom preparation and irradiation treatment

Fresh mature fruiting bodies of *L. edodes* at the closed-cap stage were harvested from a mushroom farm in Suizhou, China. in November 2020 and transported to the laboratory at 4 °C within 2 h. Samples were selected for the experiments that exhibited a uniform size and shape, color, appearance and texture and showed no any visible damage or fungal infection. Fresh *L. edodes* (strain L808) were assigned into three groups and packaged in fresh-keeping bags (25 μm thickness; O_2 permeability 7.35×10^{-16} mol m^{-2} s^{-1} Pa^{-1}; water vapor permeability 5×10^{-13} mol m^{-2} s^{-1} Pa^{-1} at 23 °C). There were three technical replicates per group and 30 fruiting bodies per replicate, a total of 27 bags in 3 groups. The 10 fruiting bodies in each bag were merged together as one biological repeat. The experimental groups were set as follows. (1) Gamma irradiation group (marked G): The packed samples were irradiated in insulated boxes containing ice using Hubei Agricultural Products Irradiation Processing Centre (Wuhan, China) with a source intensity of 9.435×10^{15} Bq at a dose rate of 3.6 kGy/h; Amber Perspex 3042 dosimeters (Atomic Energy Research Establishment, Harwell, Oxfordshire, UK) were used to measure the radiation dose. To ensure uniformity of the irradiation dose, the instrument was equipped with a circle suspension conveyor surrounding the center of a ^{60}Co γ-ray source, and six dosimeters were used for each lot to monitor the irradiation process to obtain a dose of 1.0 kGy. (2) Control group (marked C): nonirradiated samples were used as the control. After irradiation, these two groups were stored at 4 °C and 90% RH for 20 d. (3) Fresh group (marked F): The fresh group was fresh *L. edodes*. After sampling, the sporophores were immediately frozen in liquid nitrogen and then stored at − 80 °C for RNA extraction and RNA-Seq experiments. According to our previous findings, postharvest *L. edodes* stored at 4 °C and 90% RH for 20 d showed significant changes in quality.

2.2. RNA extraction and Illumina sequencing

Total RNA from *L. edodes* was extracted from mixed samples of three bags was set as one replicate for each group, and purified using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA), and genomic DNA was removed using DNaseI (Takara, Dalian, China). The integrity of RNA samples was checked by agarose gel electrophoresis. The quality of RNA samples was tested using a 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA) and quantified using a Nanodrop 2000 (NanoDrop Thermo Scientific, Wilmington, DE, USA). A 1 μg high-quality RNA sample (OD260/280 = 1.8 ~ 2.2, OD260/230 ≥ 2.0, RIN ≥ 8.0, 28S:18S ≥ 1.0) was used to construct a sequencing library.

RNA-Seq was performed by technicians at Shanghai Majorbio Biopharm Biotechnology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions (Illumina, San Diego, CA). Three independent biological replicates for each group were prepared. Briefly, transcriptome libraries were prepared using the TruSeqTM RNA sample reparation kit (Illumina, San Diego, CA). mRNA was purified via oligo (dT) magnetic beads and then fragmented by fragmentation buffer. The SuperScript double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) and random hexamer primers were used to construct the cDNA library. Double-stranded cDNA was generated and processed with end-repair, A-tailed and adapter ligation according to the Illumina protocol. Libraries were size selected on 2% low range ultra agarose followed by PCR amplification for 15 PCR cycles and quantified by TBS380 (Invitrogen, Carlsbad, CA, USA). All raw sequence data have been deposited in the NCBI Sequence Read Archive with accession number SRP408314.

2.3. Analysis of RNA-Seq data

The libraries were sequenced using the Illumina NovaSeq 6000 platform. To ensure the quality of information analysis, the raw data were filtered by fastp (https://github.com/OpenGene/fastp) and trimmed using sickle (https://github.com/ najoshi/sickle). Reads of less than 20 bp after processing were discarded. To identify the gene expression signatures from the *L. edodes* libraries, the genome database (https://fungi.ensembl.org/Lentinula_edodes/Info/Index) was used as a reference database. All clean reads were aligned to the reference sequences using HISAT2 (https://ccb.jhu.edu/software/hisat2/index.shtml) software.

Considering the depth and length effects of gene sequencing accounting for fragments, gene expression levels were calculated based on the fragments per kilobase of exon per million mapped reads (FPKM) values within a 95% confidence interval (Trapnell et al., 2010). Finally, the DESeq2 package was used to identify the differentially expressed genes (DEGs) with p value < 0.05 and $|\log_2 FC| \geq 1$. The identified DEGs

were then subjected to enrichment analysis of gene ontology (GO) functions and KEGG pathway analysis.

2.4. Validation of RNA-Seq data through qRT-PCR

There were three biological replicates in qRT-PCR analysis. qRT-PCR analysis was carried out following a protocol (Shanghai Majorbio Biopharm Technology Co., Ltd., Shanghai, China). The qRT-PCR template cDNA library was constructed from 5 μg of total RNA by TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, Beijing, China). Primer sequences of genes used for qRT-PCR analysis were designed and are listed in Table S1. qRT-PCR was conducted in a total reaction volume of 20 μL containing 0.2 μM relevant primers, 50 ng of cDNA template, and 1 × PerfectStart Green qPCR Supermix (TransGen, Beijing, China). All qRT-PCR were performed in duplicate on an Applied Biosystems QuantStudio 3 & 5 (ThermoFisher Scientific, Waltham, MA, USA). β-tubulin (*Btu*) was used as an internal reference to normalize any differences in gene expression (Xiang et al., 2018). The $2^{-\Delta\Delta CT}$ method was employed to determine the relative expression level of DEGs.

2.5. Statistical analysis

SPSS software was used to analyze the data. Statistical differences were performed with Duncan's test at a significance level of *P* less than 0.05. Three biological replicates and technical replicates were conducted for each sample.

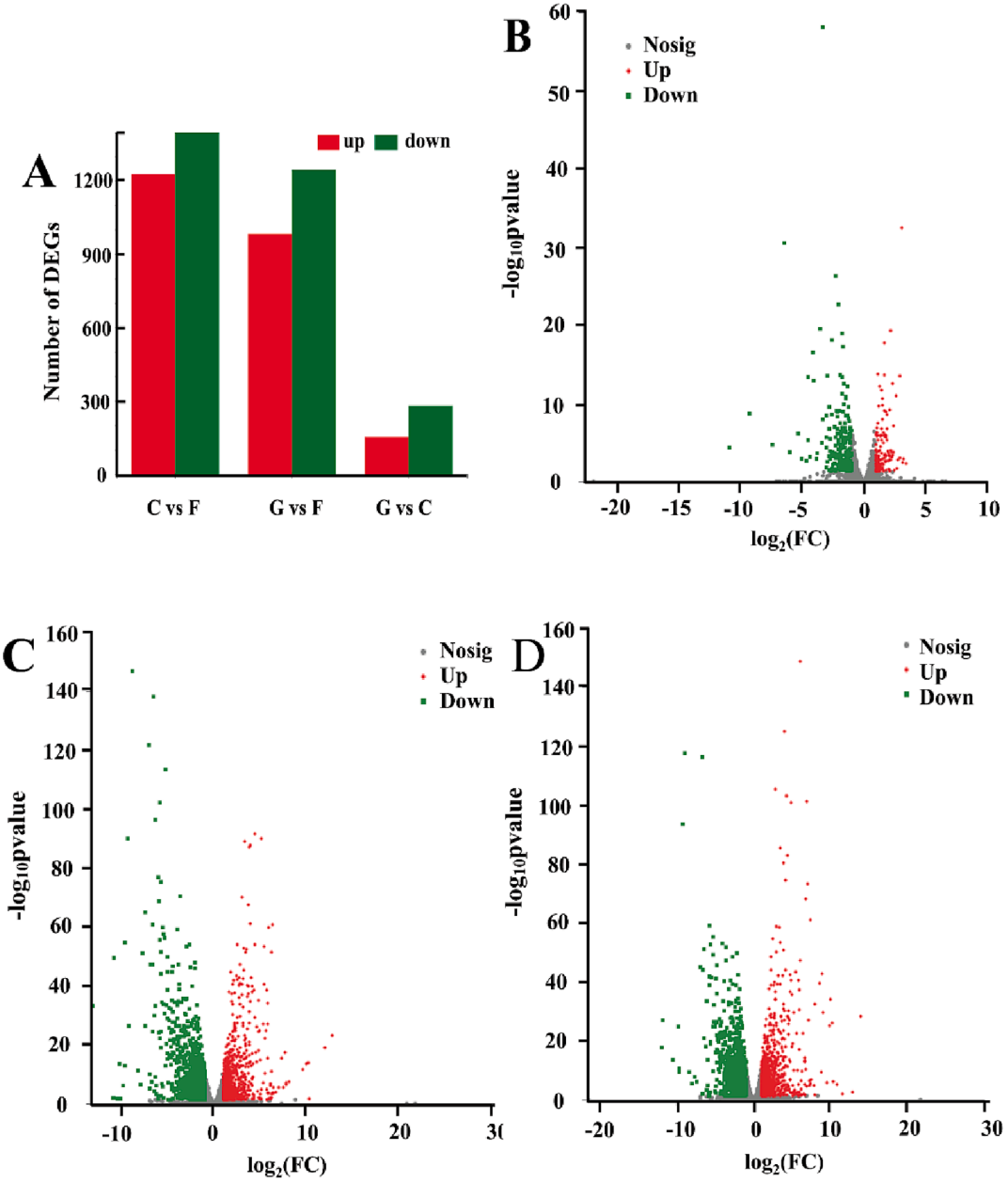


Fig. 1. Transcription expression analysis of fresh *L. edodes* in G/C, C/F and G/F groups. Number of DEGs in three groups (A), volcano plots of DEGs in G/C(B), G/F (C) and C/F (D) groups. The up-regulated genes were indicated by red dots, the down-regulated genes were indicated by green dots represent, and non-significant genes were indicated by gray dots.

3. Results

3.1. Transcriptome of postharvest L. Edodes RNA libraries

A total of 9 samples with biological replicates were sequenced. After the original sequencing sequence underwent a quality pretreatment, 76.38 GB of clean reads with Q30 values greater than 95.32% was obtained, and the average G + C content was 48.67% (Table S2). The minimum number of clean reads obtained by filtering was 45.96 M, the minimum total number of clean bases acquired from each sample was 6.85 GB, and the maximal percentage of error rate was 0.0239%. All indicators showed that the sequence obtained from the samples was highly reliable and could be used for subsequent bioinformatics analysis.

3.2. Identification of DEGs

A total of 11,120 genes were obtained, and 3185 DEGs were identified in three comparison groups, including G vs. C (G/C), G vs. F (G/F) and C vs. F (C/F) (Fig. 1A). Among them, 430 DEGs were marked in the G/C comparison group, including 151 upregulated genes and 279 downregulated genes (Fig. 1B). Concerning the G/F comparison group, 2228 DEGs were found, including 983 upregulated genes and 1245 downregulated genes (Fig. 1C). Regarding the C/F comparison group, 2624 DEGs were characterized, including 1226 upregulated genes and

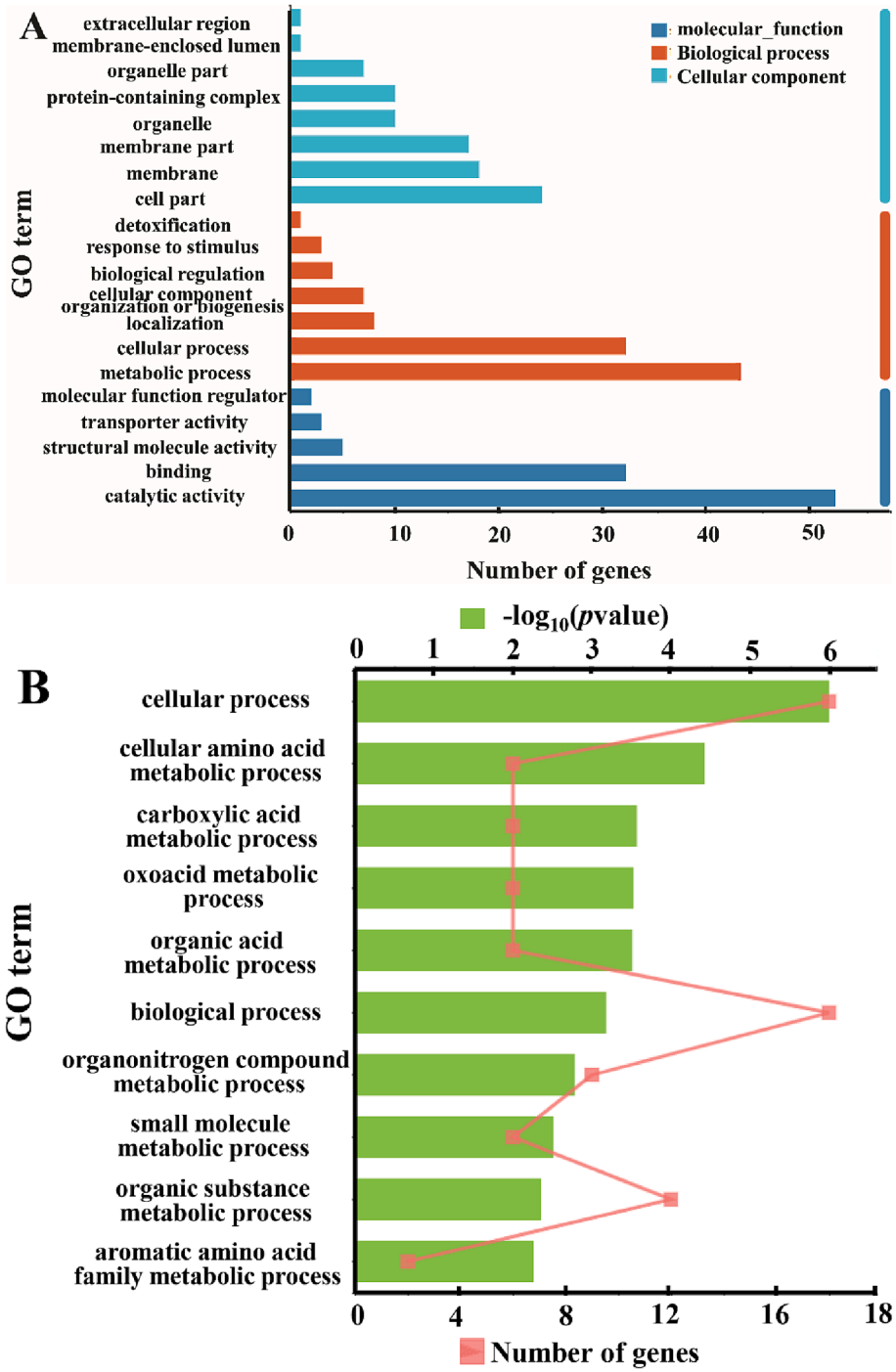
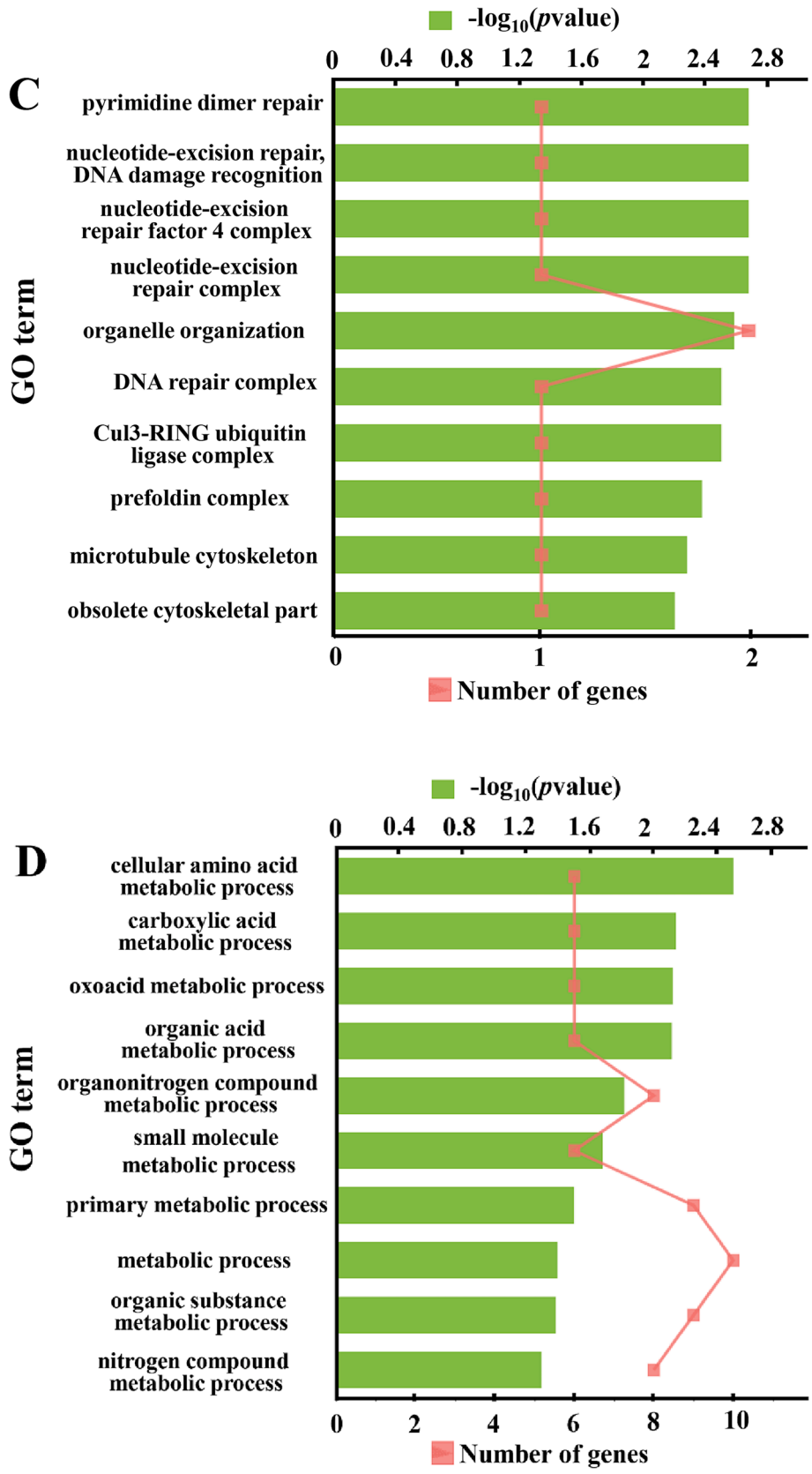


Fig. 2. GO annotations (A) and enrichment analysis of up-regulated DEGs related to BP (B), CC (C), and MF (D), and down-regulated DEGs related to BP (E), CC (F), and MF (G) in G/C groups.



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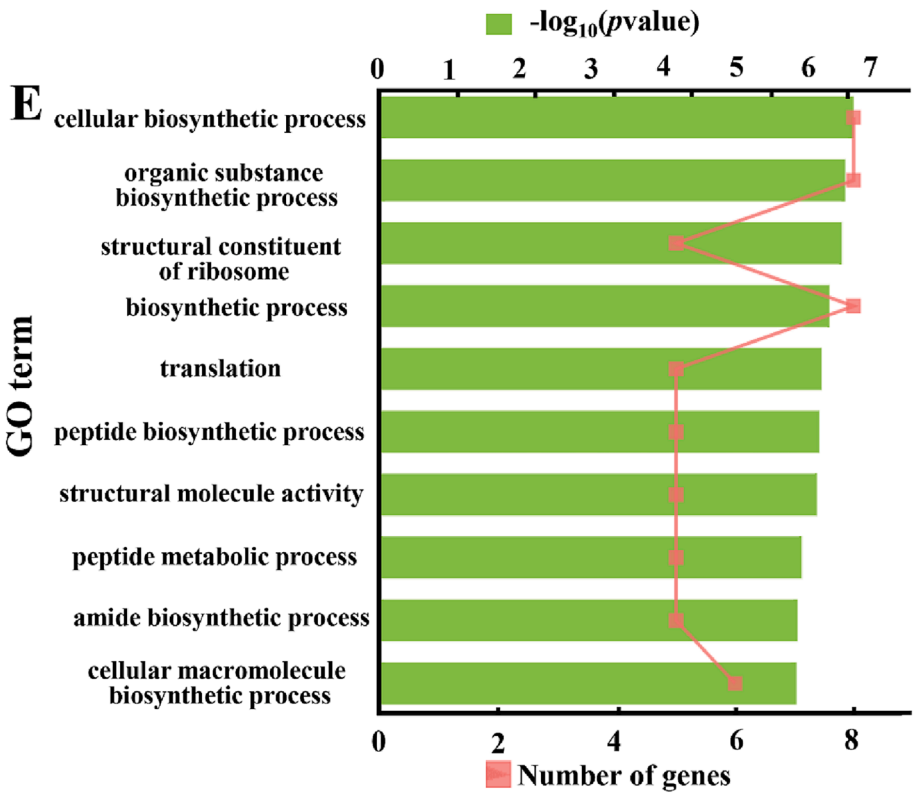
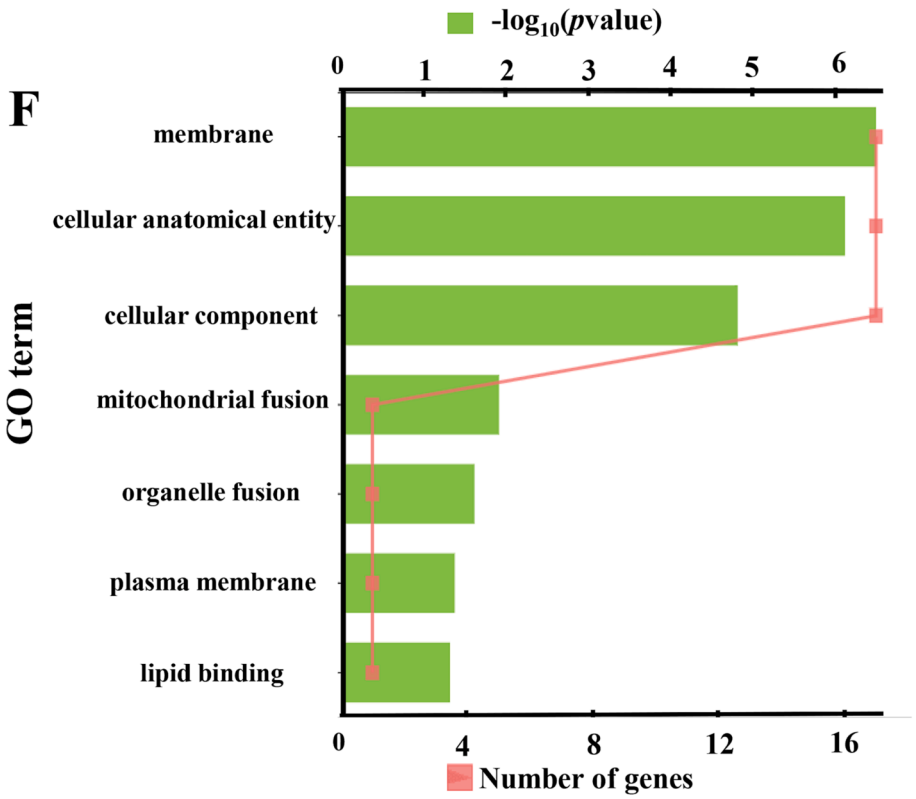
1398 downregulated genes (Fig. 1D). In general, postharvest quality deterioration is closely related to alterations in gene expression (Zuo et al., 2021). From the overall trend, it was found that the number of downregulated DEGs was much larger than the number of upregulated DEGs in the three groups, indicating that the performance of gene expression irreversibly tended to decrease during postharvest storage of





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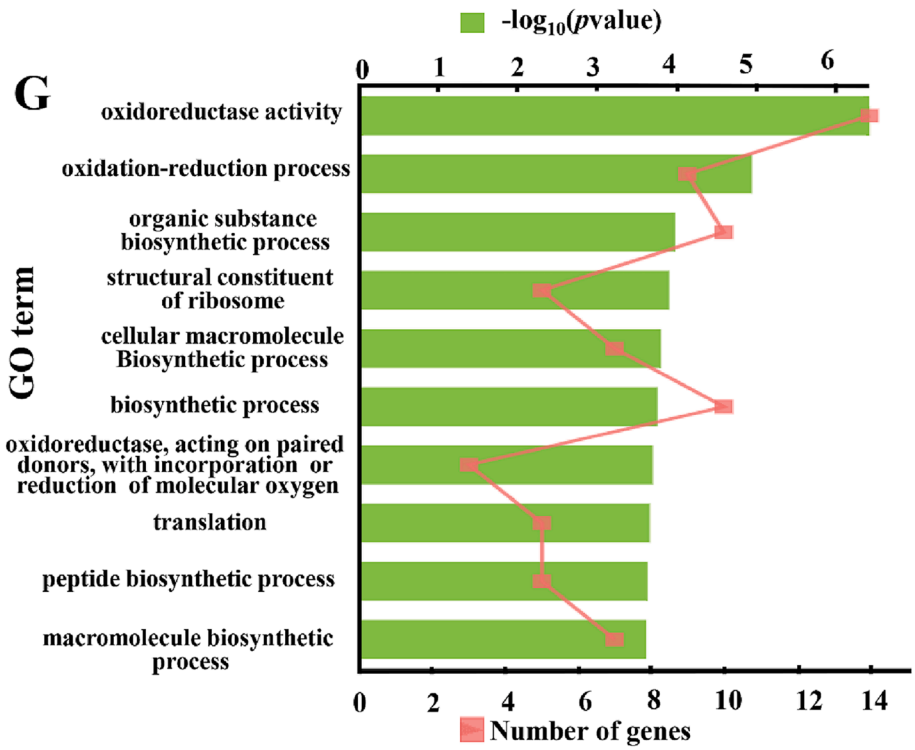
fresh *L. edodes*, which is related to the accelerated progression and mushroom senescence.

A larger number of DEGs was observed in the C/F group than in the G/F group. This suggested that a variety of physiological processes were suppressed in postharvest *L. edodes* after irradiation treatment during cold storage. As we previously reported, low-dose irradiation (≤1.0



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kGy) could retain the freshness and prolong the shelf life of fresh mushrooms (Shi et al., 2022). Therefore, an in-depth exploration of the DEGs in the G/C comparison group (listed in Tables S3–S6) would be helpful for investigating the mechanism of irradiation preservation at the transcription level.

3.3. GO annotation and enrichment analysis of DEGs

GO annotations were available in the G/C comparison group for 281 DEGs (Table S3), including biological process (BP), cellular component (CC) and molecular function (MF), as shown in Fig. 2A. The top 10 GO terms associated with the largest number of DEGs were "catalytic activity" (GO:0003824, 52 DEGs), "metabolic process" (GO:0008152, 43 DEGs), "cellular process" (GO:0009987, 32 DEGs), "binding" (GO:0005488, 32 DEGs), "cell part" (GO:0044464, 24 DEGs), "membrane" (GO:0016020, 18 DEGs), "membrane part" (GO:0044425, 17 DEGs), "protein-containing complex" (GO:0032991, 10 DEGs), "organelle" (GO:0043226, 10 DEGs), and "localization" (GO:0051179, 8 DEGs). The DEGs in the G/C comparison group were divided into two groups (upregulated and downregulated genes). In general, the vast majority of GO terms were associated with more downregulated genes than upregulated genes after 20 d of storage.

GO enrichment analysis is a bioinformatics method utilized to annotate genes and gene products to terms in specific ontologies. (Song et al., 2018). GO enrichment analysis of the DEGs was also performed by sorting 10 items from large to small based on the -$\log_{10}$(*p*value). According to the GO enrichment analysis, the functions of the DEGs were characterized in many aspects (Table S4). The upregulated DEGs involved in BP were mainly enriched in response to cellular process, cellular amino acid metabolic process, carboxylic acid metabolic process, oxoacid metabolic process, organic acid metabolic process, biological process, organonitrogen compound metabolic process, small molecule metabolic process, organic substance metabolic process, and aromatic amino acid family metabolic process (Fig. 2B). The upregulated DEGs involved in CC were mainly enriched in response to pyrimidine dimer repair, nucleotide-excision repair, DNA damage recognition, nucleotide-excision repair Factor 4 complex, nucleotide-excision repair complex, organelle organization, DNA repair complex, Cul3-RING ubiquitin ligase complex, prefoldin complex, microtubule cytoskeleton, and obsolete cytoskeletal part (Fig. 2C). The upregulated DEGs involved in MF were mainly enriched in response to cellular amino acid metabolic process, carboxylic acid metabolic process, oxoacid metabolic process, organic acid metabolic process, organonitrogen compound metabolic process, small molecule metabolic process, primary metabolic process, metabolic process, organic substance metabolic process, and nitrogen compound metabolic process (Fig. 2D).

The downregulated DEGs involved in BP were mainly enriched relative to cellular biosynthetic process, organic substance biosynthetic process, structural constituent of ribosome, biosynthetic process, translation, peptide biosynthetic process, structural molecule activity, peptide metabolic process, amide biosynthetic process, and cellular macromolecule biosynthetic process (Fig. 2E). The downregulated DEGs involved in CC were mainly enriched relative to membrane, cellular anatomical entity, cellular component, mitochondrial fusion, organelle fusion, plasma membrane, and lipid binding (Fig. 2F). The downregulated DEGs involved in MF were mainly enriched relative to oxidoreductase activity, oxidation–reduction process, organic substance biosynthetic process, structural constituent of ribosome, cellular macromolecule biosynthetic process, biosynthetic process, oxidoreductase activity acting on paired donors with incorporation or reduction of molecular oxygen, translation, peptide biosynthetic process, and macromolecule biosynthetic process (Fig. 2G).

3.4. KEGG annotation and enrichment analysis of DEGs

Generally, 98 DEGs were annotated in the KEGG analysis and mapped to 55 pathways in the G/C comparison group (Table S5). The number of DEGs involved in the two levels categories of KEGG was investigated. These pathways belonged to 18 second-level KEGG categories and 5 first-level KEGG categories in Fig. 3A. The results indicated that the number of downregulated DEGs added up to 68 greatly exceeded the number of 30 upregulated DEGs.

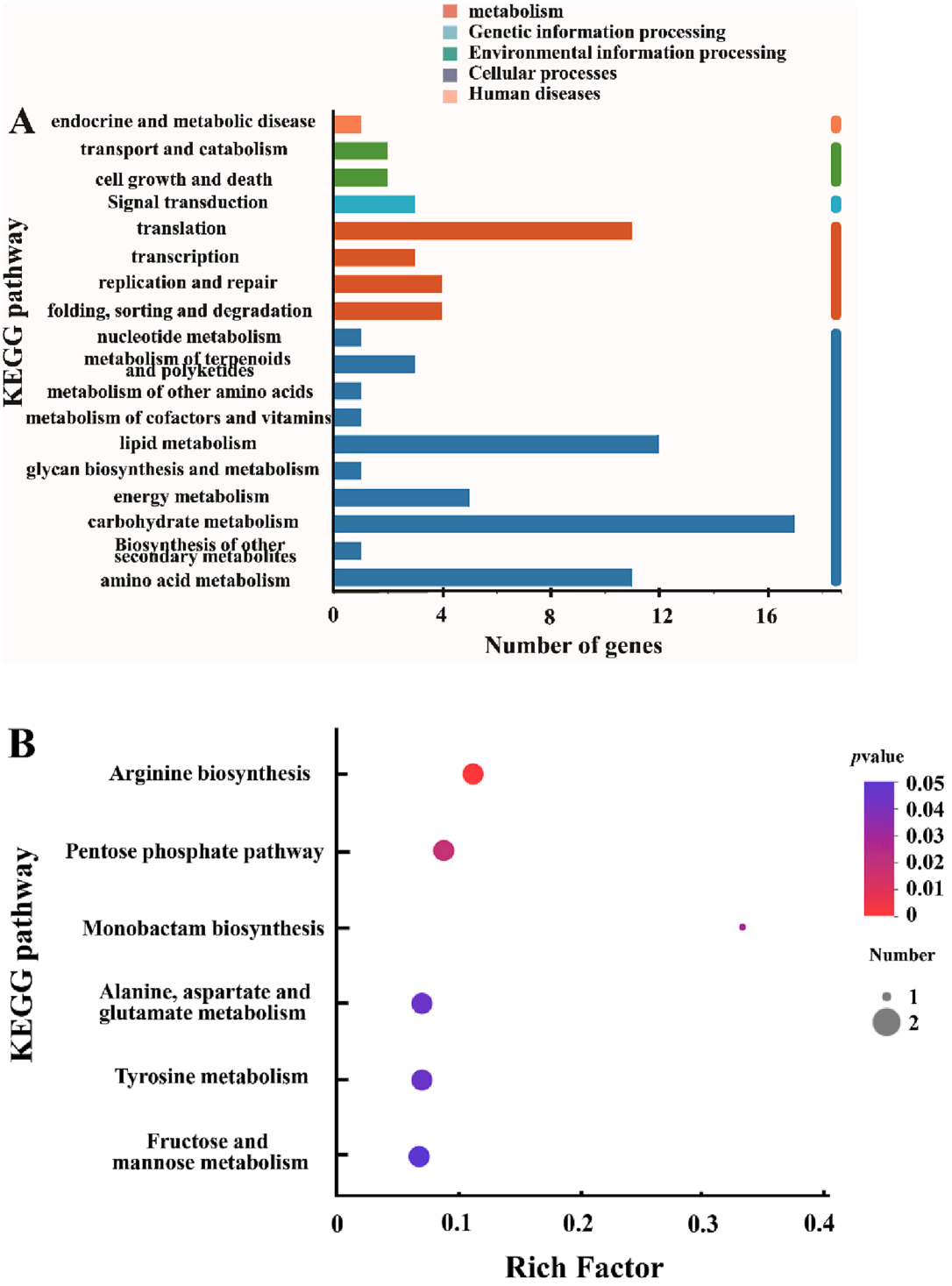
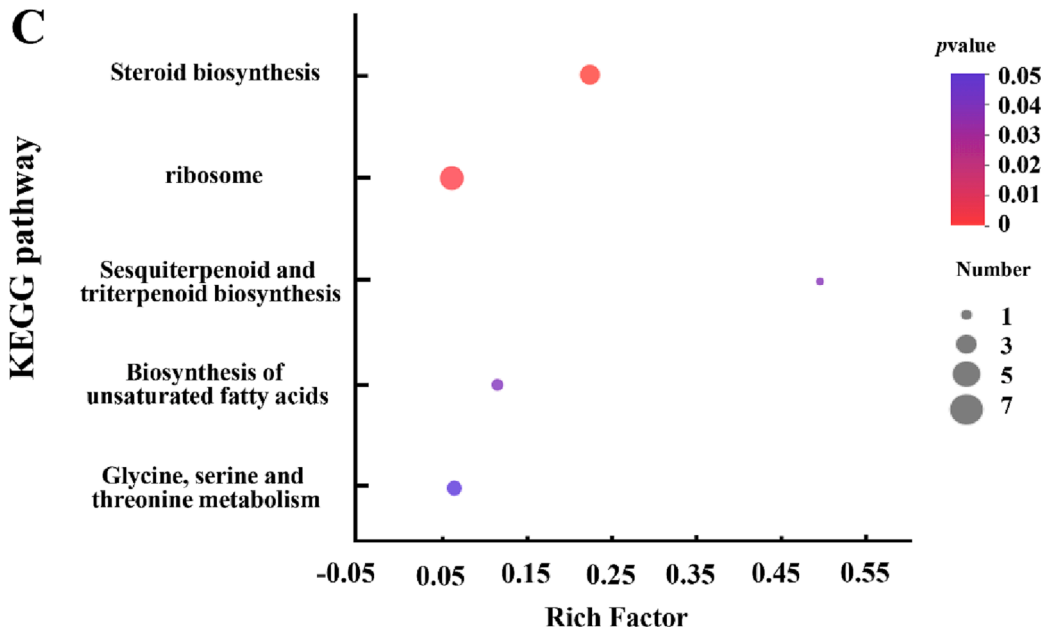


Fig. 3. KEGG annotations (A) and the top enrichment analysis of upregulated DEGs (B) and downregulated DEGs (C) in G/C group.

The first-level category of "metabolism" was related to most pathways and DEGs (Table S5), which mainly involved "carbohydrate metabolism", "amino acid metabolism", and "lipid metabolism". In the first-level category of "genetic information processing", the DEGs were annotated in "transcription", "folding, sorting and degradation", "replication and repair", and "translation". In the first-level category of "environmental information processing", "signal transduction" was responsible for the only pathway (map04011). For the first-level category of "cellular processes", the two subcategories "cell growth and death" and "transport and catabolism" were prominent.

KEGG enrichment was also performed to emphasize the most prominent pathways involved in upregulated and downregulated DEGs (Table S6). We screened the pathways with a number of DEGs greater than 2 and sorted the pathways according to the rich factor. The larger the rich factor is, the greater the degree of enrichment. In this study, the upregulated DEGs in the G/C comparison group were enriched in arginine biosynthesis (map00220), the pentose phosphate pathway (map00030), monobactam biosynthesis (map00261), alanine, aspartate



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and glutamate metabolism (map00250), tyrosine metabolism (map00350), and fructose and mannose metabolism (map00051) (Fig. 3B). On the other hand, the downregulated DEGs in the G/C comparison group were mainly enriched in steroid biosynthesis (map00100), ribosome (map03010), sesquiterpenoid and triterpenoid biosynthesis (map00909), biosynthesis of unsaturated fatty acids (map01040), and glycine, serine and threonine metabolism (map00260). The results showed that the most represented metabolic pathway was amino acid metabolism in the second-level category, followed by carbohydrate metabolism; in addition, the former was significantly enriched by both the upregulated DEGs and downregulated DEGs (Fig. 3C).

3.5. Validation of transcriptome data by qRT-PCR

The 15 selected DEGs responsible for carbohydrate metabolism, protein and amino acid metabolism, fatty metabolism, and biochemical reactions in organelles, such as ribosomes, nucleus, endoplasmic reticulum, and mitochondria, were confirmed by qRT-PCR analysis. We selected 11 DEGs that were found all downregulated from our RNA-Seq in the G group compared to the C group. Among them, *FET3_5*, *HSP20*, *NDUFA4*, *CBH2*, *glgB*, *ERG5*, *ERG3*, *PSMC3*, *CHDH*, *RPL7A*, and *ogt* were responsible for enzymatic browning, heat shock protein, NADH dehydrogenase, cellulase, starch and sucrose metabolism, steroid biosynthesis, proteasome, glycine, serine and threonine metabolism, ribosome, and DNA methylation, respectively. On the other hand, 4 downregulated DEGs were investigated including *gdhA, gntK, RAD16,* and *XPC* involved in NADP-specific glutamate dehydrogenase, Gluconokinase, and DNA repair protein, respectively. The results indicated that the expression levels of the selected genes showed a high relevance to the transcriptome data, confirming the reliability of the data acquired from transcriptome analysis in Fig. 4.

4. Discussion

Postharvest quality deterioration seriously affects the desirability of fresh *L. edodes* and leads to economic losses for producers and sellers. Compared with other traditional preservation methods, the application of food irradiation technology can play an important role in improving postharvest quality and extending shelf life due to its high efficiency. Most previous research has concentrated on the role of irradiation as a potential nonthermal decontamination strategy to ensure the safety of fresh fruits and vegetables, including *L. edodes* (Arvanitoyannis et al., 2009; Bisht et al., 2021). For example, microbiological analysis plays an essential role in promoting the postharvest performance of *L. edodes* to a large extent because irradiation helps reduce the spoilage of organisms, such as *Pseudomonas* (Jiang et al., 2010). Additionally, some research has illustrated that appropriate irradiation doses positively affect the physicochemical properties and physiological metabolism of mushrooms, such as *Agaricus bisporus* (Benoît et al., 2000), *Hypsizygus marmoreus* (Xing et al., 2007), *Pleurotus nebrodensis* (Xiong et al., 2009), *shiitake mushrooms* (Jiang et al., 2010; Akram et al., 2013), *Tuber melanosporum* truffles (Rivera et al., 2011), *Lactarius deliciosus* (Fernandes et al., 2012a), *Macrolepiota procera* (Fernandes et al., 2013), wild mushrooms (*Boletus pinophilus* and *Clitocybe subconnexa*) (Fernandes et al., 2016), and *Boletus edulis* (Fernandes et al., 2017). However, research in this area is scarce, especially research on the detailed preservation effect of the technology on *L. edodes* at the transcription level.

Modern omics technologies for RNA sequence analysis (RNA-seq) are a powerful tool that can reveal the mechanism of gene regulation in an accurate and comprehensive manner (Wu et al., 2021; Zuo et al., 2021). Many studies have been performed using RNA-seq to elucidate the interaction between physiological changes, preservation processes and regulatory mechanisms during postharvest storage of fruits and vegetables (Sakamoto et al., 2009; Trapnell et al., 2010; Song et al., 2018; Yoo et al., 2019; Zhu et al., 2020; Wen et al., 2022). However, only limited research has been conducted on the application of RNA-seq to investigate postharvest mushrooms. For instance, *de novo* RNA-seq technology was utilized to explore genes and signal pathways related to cold-induced transformation of mycelium to primordium in *Flammulina. Velutipes* (Wu et al., 2018). Likewise, Zuo et al. (2021) revealed the metabolic mechanism of nanocomposite packaging material (Nano-PM) in delaying the quality deterioration of *F. velutipes* during storage by transcriptome analysis. Similarly, Wang et al. (2020) performed a transcriptome analysis and identified key genes involved in the two lignifications in stored king oyster mushrooms at the molecular level. For *L. edodes,* transcriptome analysis has also been for signaling and metabolic pathways (Wang et al., 2018), comparative transcriptome analysis of two developmental stages in the mycelium and a mature fruiting body of *L. edodes* (Song et al., 2018), candidate genes related to

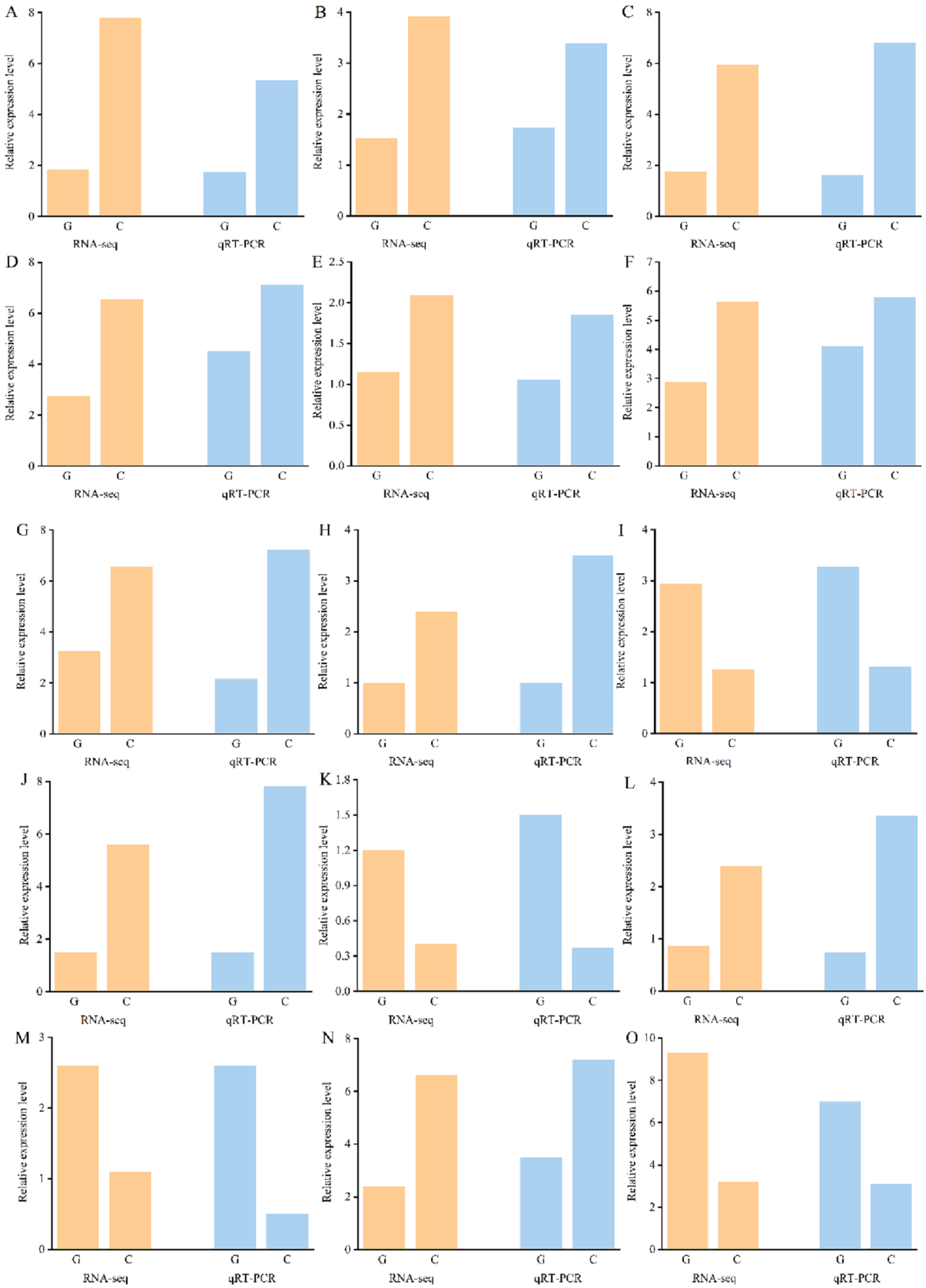

Fig. 4. The expression levels of 15 DEGs related to *FET3_5*(A), *HSP20* (B), *NDUFA4*(C), *CBH2* (D), *glgB*(E), *ERG5*(F), *PSMC3*(G), *CHDH*(H), *gdhA*(I), *ERG3*(J), *gntK*(K), *RPL7A*(L), *RAD16*(M), *ogt*(N), and *XPC*(O) between RNA-Seq and qRT-PCR between G group and C group at the end of storage. β-tubulin (*Btu*) was used as the reference gene, and the qRT-PCR validation was determined by $2^{-\Delta\Delta CT}$ as expressed. Each bar represents mean ± SE of individual samples.

light-induced brown film formation and mycelium browning (Yoo et al., 2019), genetic architecture (Zhang et al., 2021a), formation of abnormal fruiting bodies (Yan et al., 2021) and adaptive evolution during fruiting body growth (Zhang et al., 2022a); however, these analyses mainly focused on the processes of development from mycelium to fruiting bodies rather than on postharvest quality deterioration.

Recently, a growing scientific interest has emerged to better investigate the effects of irradiation on the biological mechanisms in postharvest mushrooms. Thus, this study mainly investigated the transcriptional regulation of irradiated *L. edodes* as well as the expression level of major related genes involved during storage. The effects of irradiation on the gene expression levels of postharvest *L. edodes* were associated with a series of defined subsets of metabolic pathways and gene families. Therefore, these genes representing biological significance according to the annotation features of DEGs, expression levels, and the relevance of postharvest metabolism and processes will be further discussed and explored (Tables S7–S11).

4.1. Carbohydrate metabolism

4.1.1. Cell wall-degrading enzymes

Cell wall degradation is among the most substantial issues that affects the postharvest quality of *L. edodes* (Sakamoto et al., 2017). Postharvest *L. eddoes* becomes softer during storage, which is closely associated with the degradation of cell wall components, such as glucan and chitin. Likewise, the dominant nutritional component of mushroom dry matter is carbohydrates (Yadav & Negi, 2021). Previous studies have shown that the degradation of cell wall components related to cell wall-related enzymes during the storage of *L. edodes* could be a common phenomenon (Sakamoto et al., 2009 & 2017). Thus, carbohydrate

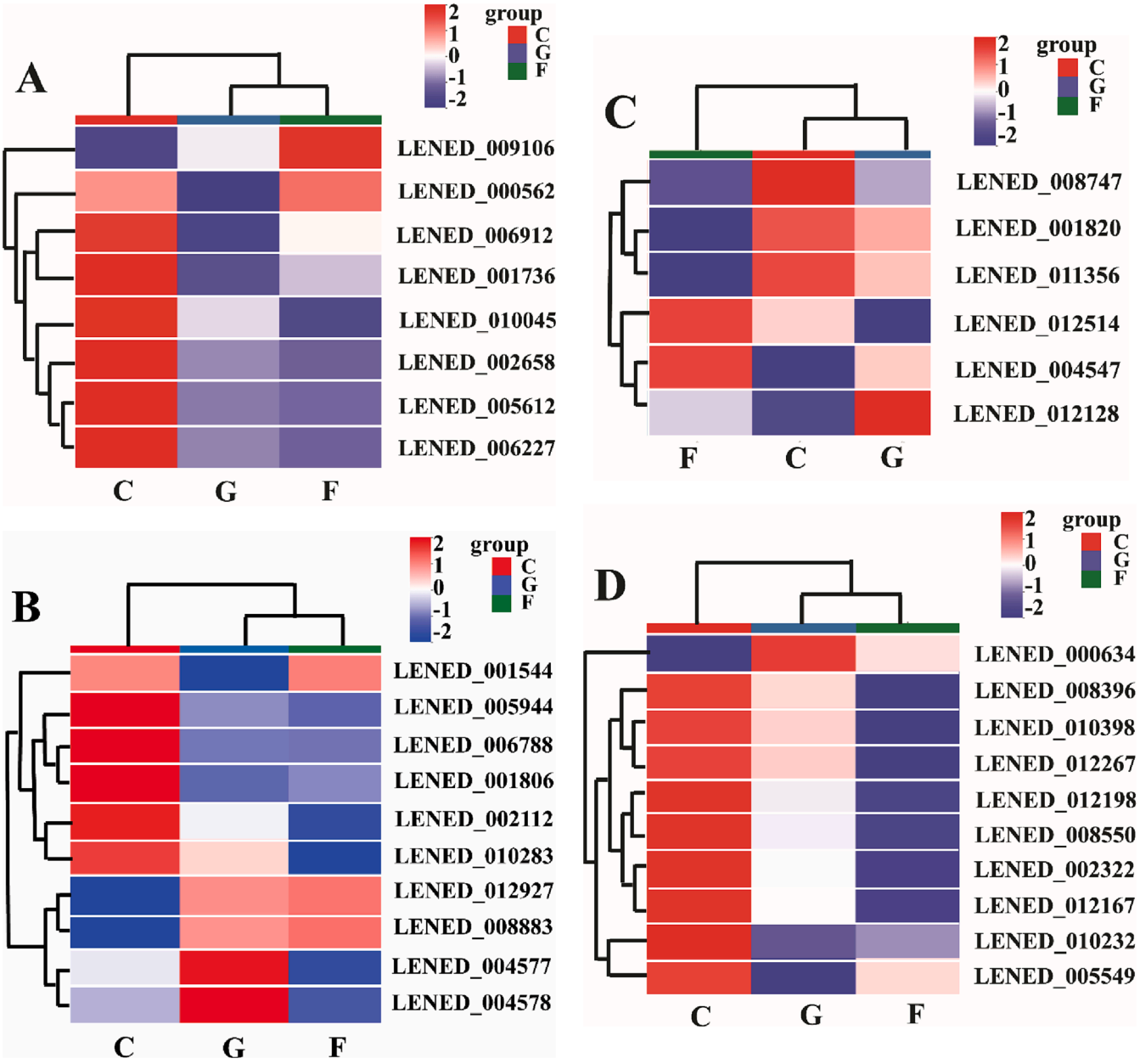

Fig. 5. Heat-map analysis of DEGs related to the transcription analysis for cell wall degrading enzymes (A), EMP-TCA-ETC-PPP pathways (B), proteins and amino acid metabolism (C), fatty metabolism (D), ribosomes (E), and DNA damage and repair (F) in G, C and F groups. The rows in the heat map represent genes, and the columns indicate the different samples. The colors of heat-map cells indicate scaled expression levels of genes across different samples. The scale ranging from blue (low) to red (high) indicates the expression levels of the FPKM values.

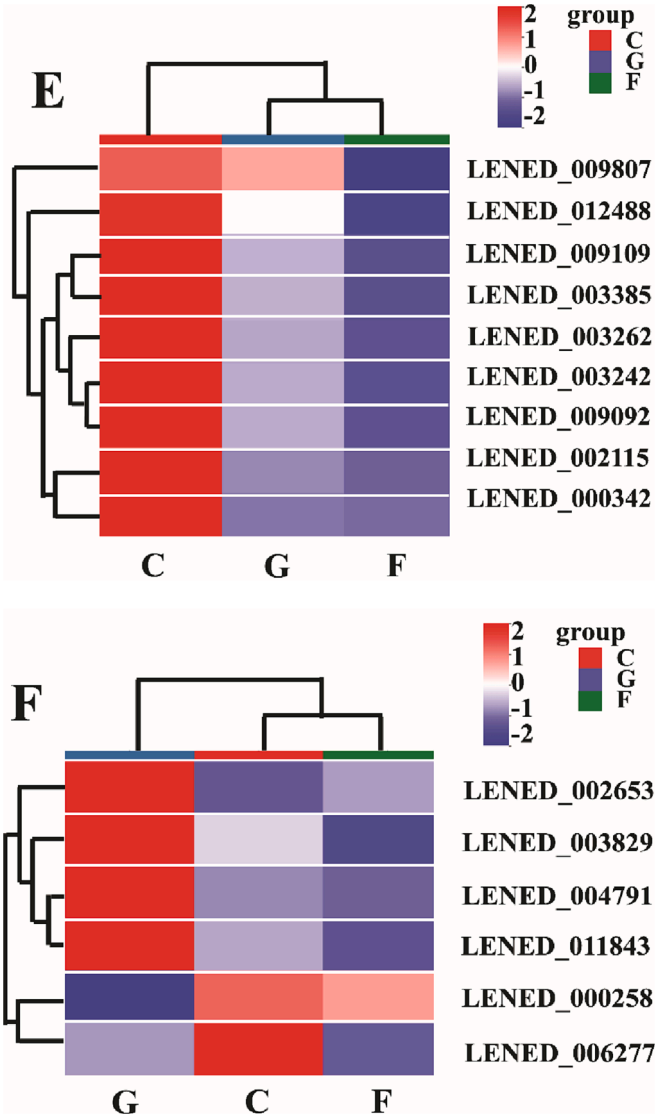

Fig. 5. (*continued*).

metabolism may be increasingly implicated in the degradation of cell wall components (Yan et al., 2021). Recent studies have shown that carbohydrate-active enzymes (CAZymes) play vital roles in fungal cell wall remodeling (Krizsán et al., 2019). For example, hydrolase activity is essential for cellular and subcellular movement (Song et al., 2018). In particular, glycoside hydrolase cell wall-degrading enzymes synergistically coordinate for fast fruiting body autolysis. Presently, catalytic activity, hydrolase activity and oxidoreductase activity on glycosyl bonds were upregulated in postharvest *L. edodes* without the intervention of alien fresh-keeping methods (Sakamoto et al., 2017).

In this enrichment experiment, when CAZyme-related DEGs were mapped to KEGG pathways, 15 DEGs were mapped in the "carbohydrate metabolism" and "glycan biosynthesis and metabolism" pathways, which were related to the synthesis and degradation of carbohydrates (Tables S3, S6, S7). As shown in Fig. 5A, the related genes of CAZyme were identified, and their specific expression levels were analyzed. The genes encoding the key enzymes, including the 1,4-alpha-glucan-branching enzyme (LENED_000562), β-glucan synthesis-associated protein KRE6 (LENED_006912), cellulase (LENED_001736), cell wall alpha-1,3-glucan synthase (LENED_010045), glycoside hydrolase family 27 protein (LENED_002658), endoglucanase-7 (LENED_005612), and chitinase (LENED_006227), were downregulated by 0.497-, 0.453-, 0.471-, 0.461-, 0.324-, 0.377-, and 0.233-fold compared to the 2.894-fold upregulation of glycoside hydrolase family 28 protein (LENED_009106). These results indicated that pathways related to cell wall-degrading enzymes were mainly dominated by downregulated DEGs in the G/C comparison group during storage. Among the KEGG subclassifications, the number of downregulated DEGs was greater in the G group than in the C group, consistent with the GO enrichment analyses. Additionally, carbohydrate metabolism was enriched in *L. edodes* in KEGG metabolic pathways, similar to the previous report by Song et al. (2018), as determined by comparative transcriptome analyses between mycelia and mature fruiting bodies. Basically, the cell wall of mushrooms is a multilayered complex network formed chiefly of chitin and β-glucan (Feofilova, 2010; Vetchinkina et al., 2017; Wen et al., 2022). Increased glucanase and chitinase activity usually causes the cell wall in the *L. edodes* fruiting body to degrade after the mushroom is harvested (Sakamoto et al., 2017). As reported previously, the gene expression of chitinase in postharvest *L. edodes* followed an upregulated trend during storage (Sakamoto et al., 2009). In our study, glycoside hydrolase and chitinase, as well as cellulase, may be inhibited when fresh *L. edodes* is exposed to irradiation according to decreased expression levels. Furthermore, the preservation effects on glucan and chitin as the main components of the cell wall caused by irradiation treatment help retain the texture of the *L. edodes* fruiting body. In other words, delayed degradation of these macromolecular carbohydrates, which are

frequently degraded rapidly if postharvest measures are not introduced or adopted, could slow energy and material consumption in fresh *L. edodes*.

4.1.2. EMP-TCA-ETC-PPP pathways

Postharvest mushrooms usually undergo metabolism by several pathways, including Embden-Meyerhof-parnas (EMP), tricarboxyfic-acid-cycle (TCA) accompanied by reactive oxygen with electron-transport chain (ETC), etc., typically manifesting a high respiration rate. As shown in Fig. 5B, the related genes in the EMP, TCA and ETC pathways were confirmed, and their characteristic expression levels were analyzed. The expression of aldehyde dehydrogenase (LENED_005944) involved in EMP and NADP-dependent malic enzyme (LENED_002112) in TCA was 0.374- and 0.423-fold lower, respectively. at the transcription level.

Reactive oxygen species (ROS) are key signaling molecules in the cell component that activate the defense response to abiotic stress acclimation (Zuo et al., 2021). As the main rate-limiting enzyme in the ETC accompanying ROS production, NADH dehydrogenase regulates ROS generation (Wu et al., 2021). In this study, the gene related to NADH dehydrogenase (LENED_010283) in oxidative phosphorylation was downregulated 0.290-fold in response to irradiation at the gene level.

Notably, the pentose phosphate pathway (PPP) was also important, as confirmed by the 2.603-, 2.340-, and 9.151-fold upregulation of carbohydrate kinase (LENED_012927), aldolase (LENED_008883) and deoxy-D-gluconate 3-dehydrogenase (LENED_004578), respectively. Among the DEGs, deoxy-D-gluconate 3-dehydrogenase was highly expressed and showed the higher transcript levels in the G group compared with the C group. In fact, the PPP can supply NADPH and intermediate products to metabolic processes under abiotic stress and prevent oxidative stress (de Freitas-Silva et al., 2017). Therefore, the PPP was enhanced by irradiation to accommodate reductant and metabolic intermediates for biosynthetic reactions. In addition, gamma irradiation has been reported to immediately stimulate the accumulation of ROS (Marcu et al., 2013). Thus, the improved PPP in this study could be a response to irradiation and could attenuate oxidative injury by NADPH owing to irradiation. This trend was similar to the findings of Hu et al. (2021) regarding fresh walnuts following irradiation treatment. Therefore, in our study, the intensified PPP could be a response resulting from irradiation-induced stress.

Cytochromes are involved in oxidation stress (Sakamoto et al., 2017). Cytochrome enzymes could manifest the level of aerobic respiratory metabolism. Among them, cytochrome P450 enzymes play important roles in fungal biosynthesis due to their broad substrate scope, high frequency of involvement and tremendous catalytic versatility (Zhang et al., 2021b). As early as 2000, Muraguchi and Kamada (2000) reported that *eln2,* encoding a novel kind of cytochrome P450 enzyme in *Coprinus cinereus*, was responsible for stipe elongation. Then, it was found that cytochrome P450 was related to fruiting body development in *L. edodes* (Akiyama et al., 2002). Sakamoto et al. (2017) found that cytochromes involved in the reduction of oxidation stress were upregulated in postharvest *L. edodes*. As important oxidoreductases that regulate both oxidation processes and highly diversified and sophisticated structural modifications, P450 monooxygenases are involved in many essential cellular processes (Črešnar & Petrič, 2011). In this study, the expression of genes (LENED_001806) related to cytochrome P450 decreased 0.322-fold in response to gamma irradiation during storage. Therefore, irradiation suppressed the oxidation process by down-regulating the expression of P450-related genes. In fact, NADH is mostly generated by the EMP-TCA pathway and can be utilized to evaluate the intensity of this pathway (Chen et al., 2020). However, in our study, irradiation weakened the metabolism of EMP-TCA by downregulating the transcription level of some key enzymes, which then reduced respiration metabolism. Although PPP can provide NADPH to supply energy, it seemed nondominant.

4.2. Proteins and amino acid metabolism

Mushrooms, including *L. eddoes,* are also a good source of proteins (Yadav & Negi, 2021). Generally, a decrease in protein content is an important indicator of mushroom tissue senescence (Xiong et al., 2009; Marçal et al., 2021). Similar results for *L. edodes* reported by Sakamoto et al. (2017) showed that protein degradation was related to upregulated protease after harvest. Recently, Wen et al. (2022) investigated the changes in *Tricholoma matsutake* fruiting bodies during cold storage and reported that three pathways related to protein degradation were predominated by the upregulated DEGs, involving "ubiquitin mediated proteolysis" (map04120), "proteasome" (map03050), and "phagosome" (map04145). As shown in Fig. 5C, the related genes in the protein and amino acid metabolism pathways were verified, and their specific expression levels were investigated. The expression level of the "proteasome" gene (map03050; LENE D_008747) was downregulated 0.422-fold in *L. eddoes* subjected to irradiation (Tables S5, S6 and S10). Generally, metabolic enzymes, including proteases, have been previously reported to be active in mushrooms after harvesting (Zhang et al., 2013). Therefore, our results indicated that irradiation treatment inhibited the increase in protease activity by lowering its expression level. The enriched DEGs in the KEGG pathway were mainly involved in glycine, serine and threonine metabolism (LENED_001820 and LENED_011356), proteasome (LENED_008747), phenylalanine, tyrosine and tryptophan biosynthesis (LENED_012514), alanine, aspartate and glutamate metabolism (LENED_004547) and arginine and proline metabolism (LENED_012128); the DEGs in the first two metabolism pathways were downregulated, whereas the ones in the remaining pathways showed opposite trends.

4.3. Fatty metabolism

Fatty metabolism is also a suitable indicator of extending shelf-life conditions (Cardoso et al., 2019). In our study, the majority of down-regulated DEGs were enriched and classified in the steroid biosynthesis pathway (Table S10), possibly associated with sterol metabolism. As shown in Fig. 5D, the related genes in the fatty metabolism pathway were recognized, and their typical expression levels were inspected. The downregulation expression levels of two genes encoding lanosterol 14-alpha-demethylase (LENED_012267) and lathosterol oxidase (LENED_002322) were 0.476 and 0.245, respectively. These genes participate in ergosterol biosynthesis and might function in the irradiation response. As one of the important lipophilic compounds in mushrooms, ergosterol can stabilize the membranes of fungal cells against biotic and abiotic stresses, such as heat stress (Zhang et al., 2022a). Moreover, Cardoso et al. (2019) supported that gamma irradiation treatment contributed to higher ergosterol contents in *Agaricus Bosporus*. In our study, the transcription level of the gene encoding lipoxygenase (LENED_012167, Table S11) was expressed at levels 0.416-fold lower in the G group than in the C group; a reasonable explanation is that lipoxygenase is the easiest enzyme to be inactivated by irradiation (Arvanitoyannis et al., 2009).

In addition, we found a 0.466-fold increase in the expression level of LENED_008396 encoding delta 9-fatty acid desaturase (Table S10 and S11). Previous studies have demonstrated that the delta 9-fatty acid desaturase (in *Pleurotus eryngii* subsp. *tuoliensis*) adapted to chilling or cold tolerance by modifying lipid composition (Fu et al., 2016). Similarly, it could be inferred in our study that alterations in the composition of membrane lipids in fresh *L. edodes* may contribute to stabilizing the membrane structure. Radiation tolerance in fungi can trigger lipid remodeling in the membrane system and alter related gene transcription levels in lipid metabolism. Thus, it can be speculated that irradiation may postpone the degradation of lipid components and membrane lipid peroxidation by modifying the gene expression of lipid-regulating enzymes.

4.4. Ribosomes

Ribosomes are highly related to gene expression processes and stress responses. Studies evaluating the expression change of genes encoding ribosomal proteins and their roles mostly involve various structural changes and several stress-response reactions, such as cap expansion and responses to drought, wounding, salinity, cold, and pathogenic stresses (Krizsán et al., 2019; Yan et al., 2021). As shown in Fig. 5E, the related genes in ribosome metabolism were discriminated, and their representative expression levels were explored. In our study, pathways related to "ribosome" were entirely enriched in the KEGG analysis with 7 downregulated DEGs, including 60S ribosomal protein L9-A (LENED_000342), 60S ribosomal protein L23-A (LENED_002115), 60S ribosomal protein L1-B (LENED_003262), 40S ribosomal protein S2 (LENED_003385), 60S ribosomal protein L36-B (LENED_009109), 60S ribosomal protein L8 (LENED_003242) and 60S ribosomal protein L5-B (LENED_009092), the expression levels of which were 0.449, 0.443, 0.384, 0.473, 0.429, 0.322 and 0.350, respectively. Our results revealed that the DEGs involved in ribosomes were mostly downregulated in the G group compared with the C group, which was also confirmed by KEGG enrichment analysis (Fig. 3C).

A report on the storage of *Pleurotus tuoliensis* fruitbodies by Wu et al. (2021) showed that proteins correlated with the ribosome were mainly responsible for the translation process, primarily involving ribosome biogenesis and the ribosome's constituent proteins. In addition, ribosome metabolism is related to the synthesis and decomposition of new proteins and the reuse of amino acids (Wen et al., 2022). Irradiation treatment could inhibit physiological and metabolic changes associated with ribosomes in cells to a great extent. This feature further highlights the importance and effect of postharvest treatment (including gamma irradiation) on ribosome metabolism after the preservation of *L. edodes*.

4.5. DNA damage and repair

Existing evidence has highlighted that mushroom senescence is essentially an oxidative process involving the degradation of cellular structures and macromolecules (Liu et al., 2013). Ionizing radiation induces oxidative DNA damage resulting from ROS due to water radiolysis in cells (Kim et al., 2019). DNA damage is known to be caused by ROS and metabolic byproducts or the stress response (Wu et al., 2021). DNA repair and replication play a positive regulatory role in nucleic acid synthesis in purine metabolism, and DNA damage initiated by ROS can be repaired by the base excision repair pathway (D'Amico & Vasquez, 2021). As shown in Fig. 5F, the related genes in DNA damage and repair metabolism were screened, and their expression levels were examined and estimated. DEGs, including DNA repair protein rad16 (LENED_003829), DNA repair protein rhp42 (LENED_002653), DNA replication licensing factor mcm10 (LENED_004791), and methylated-DNA–protein-cysteine methyltransferase (LENED_ 011843), were upregulated by 2.161-, 2.617-, 2.903- and 3.256-fold at the transcription level in the G group compared with the C group, while deoxycytidylate deaminase (LENED_000258) and DNA polymerase alpha catalytic subunit (LENED_ 006277) were downregulated 0.412- and 0.326-fold. This result showed that upregulation rather than downregulation dominated the process of DNA repair. Wang et al. (2017) found that irradiation stimulation activated the defense systems of these blueberry fruits, repairing the cell membranes during cold storage. Similarly, this phenomenon observed in our study possibly occurred due the stress response of chromosomes in *L. edodes* induced by irradiation treatment.

On the other hand, ionizing radiation frequently results in various epigenetic alterations, which are highly linked with DNA methylation and histone modification (Kim et al., 2019). DNA methylation levels are influenced by the transcription levels of DNA methyltransferases to a great extent, and the generation and maintenance of DNA methylation regulated by DNA methyltransferases exhibit different regulatory mechanisms (Yang et al., 2021). Guo et al. (2022) found that elevated DNA methylation was positively correlated with the extended shelf life of "Kyoho" grapes, while another study illustrated that the lower methylation levels of the PyMYB10 promoter in pear improved its transcription, absolutely stimulating the accumulation of anthocyanins (Qian et al., 2014). A recent study also showed that the expression of DNA methyltransferases manifested a downward trend under cold and drought stresses in tea plants, indicating that DNA methyltransferases play a significant role in the response to abiotic stresses (Zhu et al., 2020). Compared with other plants, mushrooms have been less investigated with respect to DNA methylation patterns. In this study, DNA methyltransferase (LENED_011843) was found to be upregulated 3.256-fold in response to irradiation. This pattern of gene regulation is inconsistent with those that occur during stress responses in tea (Yang et al., 2021) and potato (Xiong et al., 2022). Notably, DNA methylation in postharvest mushrooms has not been previously reported. Therefore, the detailed regulatory mechanism of DNA methylation in irradiated *L. edodes* needs to be further elucidated.

4.6. Other metabolism

Heat shock proteins (HSPs) are usually involved in the stress response and are a group of molecular chaperons in physiological states, and their expression can be induced when cells are exposed to various stresses. The evolution of HSPs is beneficial to cells, helping them adapt to adverse environments (Yan et al., 2021). Small HSPs have attracted considerable attention, as reported in previous studies. Hsp20, which is an essential part of small HSPs, has mechanisms to prevent protein aggregation induced by abiotic stress, such as exposure to thermal drive in molecular chaperones, enabling apoptosis to some extent (Wu et al., 2021; Bruey et al., 2000). Although the profiles of HSPs have also been reported in a variety of stresses, such as heat shock and freezing, glucose or nitrogen starvation desiccation, high ethanol concentrations, and toxic substances (Wu et al., 2021), research concerning the relationship between irradiation and HSPs remains rare. In this study, the expression level of *HSP20*, which is involved in cellular components, was substantially downregulated in G samples compared with C samples (LENED_003931, 0.357-fold; Tables S10 and S11); thus, introducing irradiation could inhibit the stress response of heat shock proteins during the deterioration process of fresh *L. edodes*. Likewise, Tereshina (2005) reported that fungi, similar to plants, can respond to stress factors, such as oxidative stress, through *HSP* expression. Additionally, the gene was also found in Bailinggu mushrooms in response to cold stress (Fu et al., 2016).

For *L. edodes*, enzymatic browning can seriously degrade its commodity value or make it worthless. The browning of mushrooms is governed by enzymatic activities. As one of the genes associated with discoloration reactions, laccase is a multicopper-containing oxidase with varied functions, including pigmentation, polymerization/depolymerization of lignin, wound healing, sclerotization, morphogenesis, sporulation, melanin formation, fruiting body formation, and endospore coat protein synthesis (Nagai et al., 2003; Sakamoto et al., 2017). This enzyme, ubiquitous in mushrooms, characteristically catalyzes the oxidation of phenolic compounds to produce quinones (Wu et al., 2021). After harvest, the increase in laccase frequently leads to melanin synthesis, a typical deterioration phenomenon characterized by mushroom browning, especially in *L. edodes* (Nagai et al., 2003). Laccases, as well as manganese peroxidases, are mainly responsible for lignin degradation and directly result in the softening of fruitbodies. Moreover, Fernández-Fueyo et al. (2012) confirmed that laccase can oxidize phenols and reduce molecular oxygen to water. In this study, genes encoding laccase 10 (LENED_003084, 0.496-fold) were downregulated by irradiation. Thus, it is suggested that laccase in *L. eddoes* treated with irradiation is the key enzyme responsible for the browning process.

4.7. Postharvest metabolic characteristics of fresh L. Edodes under irradiation

At present, much research is being performed on the development of irradiation preservation technologies for fruits and vegetables, and this research mainly involves physico-chemical, nutritional, microbiological nutritional value and bioactive properties (Fernandes, et al., 2012b; Villa-Rodriguez et al., 2015; Marçal et al., 2021; Bisht et al., 2021). However, the irradiation-induced stress response in mushrooms, especially *L. edodes*, has not been well characterized through transcriptome analysis. The lack of an in-depth explanation of this stress response may be ascribed to the following two aspects. First, the exploration of the irradiation mechanism of postharvest mushrooms is very complex and multifaceted and involves diverse factors, such as the radiation source, irradiation dose, kind and features of mushrooms, radiation sensitivity and tolerance, and biological effects of irradiation. Second, mushrooms are classified as a completely different kingdom than plants, which is noteworthy but often neglected (Gil-Ramírez et al., 2016). A very large number of studies in the literature deal with the effects and mechanisms of postharvest abiotic stresses, such as irradiation on fruits and vegetables; in contrast, very few studies have investigated the regulatory mechanisms for fresh mushrooms in response to irradiation. Therefore,

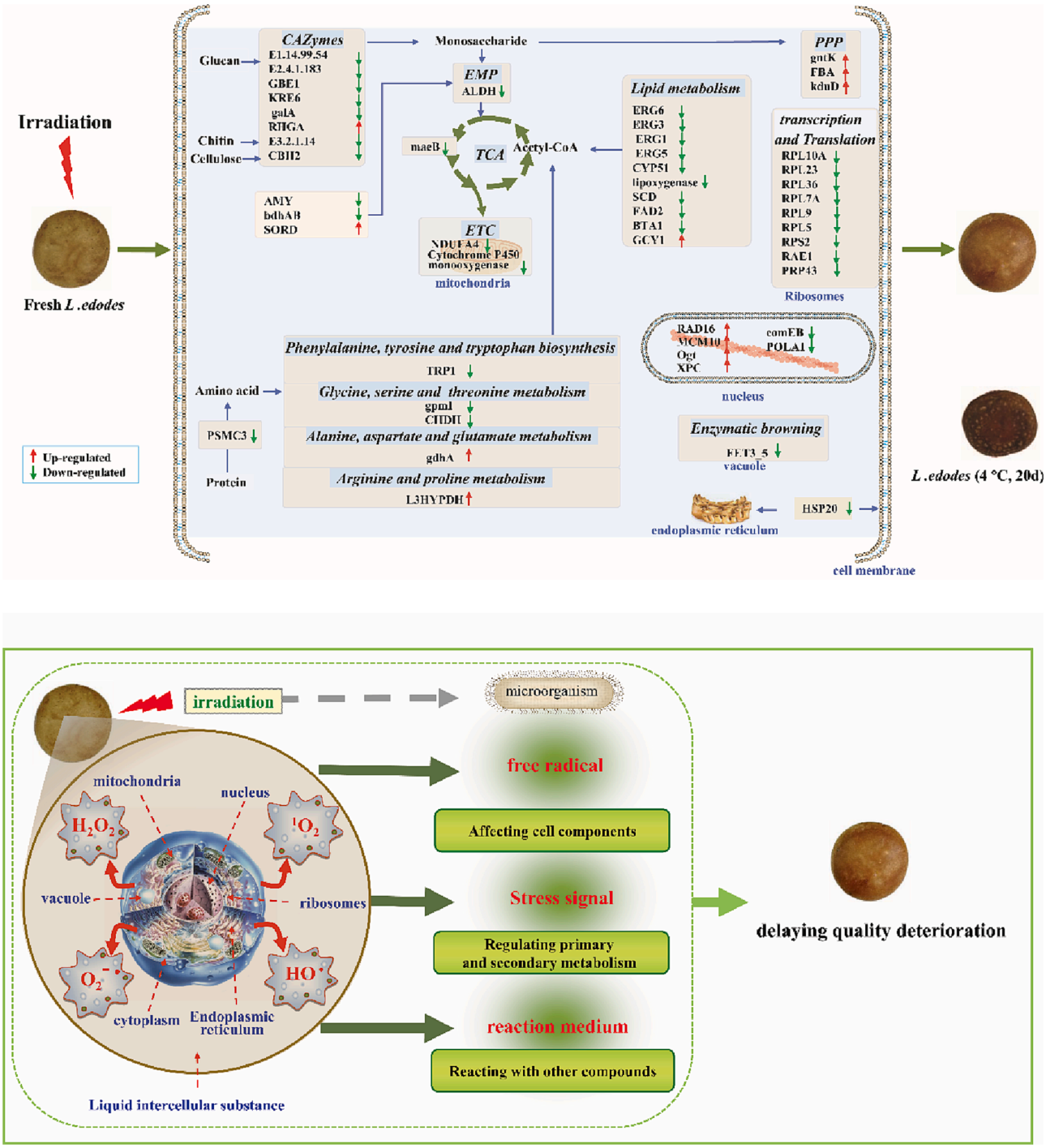

Fig. 6. The regulation mechanism of DEGs related to the major metabolic process in fresh *L. edodes* subjected to 1.0 KGy irradiation (A); The proposed model for 1.0 kGy irradiation maintaining the postharvest quality of fresh *L. edodes* during cold storage (B).

it is necessary to explore the mechanism by which irradiation preserves mushrooms based on the features and patterns of irradiation and mushroom properties. In this study, combined with previous literature reports and our study, the key DEGs were screened and summarized to reveal the irradiation-mediated metabolic pathways (Fig. 6A). These metabolites not only offer substrates and energy to cope with substantial defense responses but also serve the multiple roles of signaling molecules to actively perform in response to irradiation stimulation.

In fact, gamma rays penetrate the body of mushrooms, involving direct and/or indirect effects, as well as inhibiting or killing microorganisms. In the former, gamma rays directly affect organic matter, leading to alterations in molecules such as nutritional and functional components, taste and flavor substances, and enzymes. In the latter, irradiation occurs through water radiolysis, and subsequently, diverse products react with compounds around water molecules (Villa-Rodriguez, et al., 2015). Kuan et al. (2013) noted that indirect effects are dominant when biomacromolecules are surrounded by liquid solution. Bisht et al. (2021) further stated that fresh produce is mainly composed of water. Similarly, the principal ingredient of fresh *L. edodes* is water, containing as high as approximately 88% moisture content. In addition, it is important to note that the application of food irradiation cannot have a negative effect on taste, flavor, color, nutritive value and other features, which limits the use of irradiation to preserve fresh produce to a large extent. Thus, in our study, the indirect effect characterized by water radiolysis is the main mechanism. Briefly, upon irradiation, the occurrence of free radicals caused by water radiolysis plays an important role in the response of *L. edodes* cells to abiotic stress. A hypothetical model speculating the irradiation-regulated metabolic processes in extending the shelf life of fresh *L. edodes* is proposed in Fig. 6B. The modulation of intracellular redox homeostasis and appropriate responses to low-dose irradiation in fresh *L. edodes* relied on the fine-tuning of reactive oxygen species to some extent. Free radicals and reactive species not only affect cell components, such as DNA, carbohydrate proteins, and lipids, but also serve as stress signals to regulate metabolic processes (Bisht et al., 2021; Villa-Rodriguez, et al., 2015). In addition, free radicals caused by irradiation can influence other compounds, such as ergosterol, enzymes, and tocopherols (Fernandes et al., 2013; Cardoso et al., 2019). Overall, metabolic stress responses are motivated by irradiation treatment, which unequivocally influences the evolution of the postharvest quality of fresh *L. edodes*. Despite all this, the transcriptional regulation of physiological metabolism in *L. edodes* subjected to irradiation was complex during storage. Further research should be performed, including research on the effect of irradiation on secondary metabolites (bioactive compounds) and pathways, the interaction of irradiation and antioxidant enzymes, and the accurate tolerance of mushrooms against irradiation; this research would provide better scientific knowledge on irradiation and promote the development of irradiation preservation technology.

5. Conclusion

Our research is the first report on the stress response of postharvest *L. edodes* following 1.0 kGy GI treatment using RNA-Seq and qRT-PCR. The results indicated that 1.0 kGy irradiation resulted in more downregulated genes than upregulated genes. Among them, some unique metabolic events and processes of related genes deserve attention. Compared to the familiar metabolic pathways related to carbohydrate, protein and amino acid and fatty metabolism, our results indicated that GI mainly regulated energy metabolism by enhancing PPP pathways and inhibiting EMP, TCA and ETC; these pathways are involved in the upregulated expression of the gene encoding deoxy-D-gluconate 3-dehydrogenase, a typical biomarker specific to 1.0 kGy irradiated *L. edodes*. Furthermore, lower expression of the gene encoding delta 9-fatty acid desaturase as well as lipoxygenase was responsible for delaying the degradation of lipid components. GI also regulated the expression of genes closely related to ribosomes, DNA repair, heat shock proteins, etc., and further delayed the quality deterioration of *L. edodes*, which validated and expanded our previous research at the transcriptional level. Additionally, irradiation could affect enzymatic browning by regulating laccase, which would be an effective strategy for controlling the discoloration reaction of postharvest *L. edodes*. The study contributed to more in-depth knowledge of the role of GI treatment in mushrooms.

CRediT authorship contribution statement

Hong Gao: Conceptualization, Writing – original draft. **Shuang Ye:** Writing – original draft. **Yani Liu:** Methodology. **Xiuzhi Fan:** Methodology. **Chaomin Yin:** Resources. **Ying Liu:** Data curation. **Jingyu Liu:** Data curation. **Yu Qiao:** Conceptualization. **Xueling Chen:** Visualization. **Fen Yao:** Investigation, Validation. **Defang Shi:** Writing – original draft, Writing – review & editing, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2023.100172.

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